ANDERSON ASSAID SIMÃO

COMPOSIÇÃO QUÍMICA, EFICÁCIA E TOXICIDADE DE PLANTAS MEDICINAIS UTILIZADAS NO TRATAMENTO DA OBESIDADE

LAVRAS-MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, para a obtenção do título de Doutor.

Orientadora

Dra. Angelita Duarte Corrêa

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APROVADA em 04 de setembro de 2013.

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Orientadora

LAVRAS – MG

2013

À memória do meu amado pai, por tudo
que pode fazer por mim, por me mostrar
sempre o caminho certo.

Ao meu lindo e pequeno Cauã, por me
trazer tantas alegrias e motivação.

Aos meus irmãos, Andréia e Luís Fernando, pela
amizade e apoio.

À minha mãe, por sempre confiar e me amar.

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RESUMO

A prevalência de obesidade tem aumentado, em taxas alarmantes, em todo o mundo, e vem se tornando o maior problema de saúde na sociedade moderna, evidenciando a necessidade de adjuvantes para auxiliar em seu tratamento. As plantas medicinais são ricas fontes de compostos bioativos com potencial de utilização terapêutica, podendo estes ser uma alternativa viável para o desenvolvimento de medicamentos eficazes e seguros para auxiliar no tratamento da obesidade. O objetivo neste estudo foi avaliar a utilização das plantas medicinais *Aloe vera* (L.) Burm., *Simaba ferruginea* St. Hil., *Baccharis trimera* (Less.) DC, *Garcinia cambogia* Desr. e *Tournefortia paniculata* Cham. e do fitoterápico Moder diet elaborado com a combinação destas plantas, na forma de simulado, no tratamento da obesidade. Foi determinada a composição química das referidas plantas, ensaios de inibição de enzimas digestivas, avaliação do potencial antioxidante e ensaio *in vivo* com a planta que mostrou maior presença de fitoquímicos com propriedades farmacológicas, visando sua caracterização terapêutica (potencial para prevenção e tratamento da obesidade) e toxicológica. Os resultados indicaram a presença de substâncias de interesse farmacológico, como os compostos fenólicos, saponinas e fibras alimentares, em todas as plantas, e o cálcio, na *G. cambogia* e *S. ferruginea*. Foi observada inibição das enzimas α-amilase (*T. paniculata*) e α-glicosidase (*T. paniculata, A. vera* e *B. trimera*) após simulação de fluído gástrico; e potencial antioxidante, *in vitro*, em todas as plantas, com a *T. paniculata apresentando* os mais altos teores de compostos fenólicos e vitamina C. O fitoterápico simulado não causou inibição das enzimas digestivas e mostrou baixa atividade antioxidante. Diante desses resultados, a *T. paniculata* foi escolhida para realização do ensaio biológico, sendo administrada por gavagem na forma de farinha e extrato aquoso, a ratos *Wistar*, submetidos à dieta hipercalórica, durante 42 dias, para avaliação dos parâmetros: peso corporal, consumo alimentar, glicemia, lipídios, peroxidação lipídica e toxicidade (genotoxicidade e histologia). A farinha e o extrato aquoso das folhas de *T. paniculata* foram eficazes na redução da gordura hepática, glicose e triacilgliceróis séricos e não apresentaram potencial genotóxico, nas condições avaliadas. O extrato aquoso reduziu o consumo alimentar e a peroxidação lipídica. Conclui-se que as plantas estudadas mostram grande diversidade de fitoquímicos com potenciais para serem utilizadas em preparações farmacológicas com possíveis benefícios a saúde. Entre as plantas, a *T. paniculata* apresenta os mais elevados teores de fitoquímicos (compostos fenólicos, saponinas e vitamina C), efeito inibidor sobre as enzimas α-amilase e α-glicosidase e atividade antioxidante, em ensaios *in vitro*. Os resultados do ensaio biológico sugerem as folhas de *T. paniculata* como alternativa terapêutica no tratamento da obesidade.

Palavras-chave: Obesidade. Plantas Medicinais. Antioxidantes. Enzimas digestivas. Toxicidade. Fitoterápico.

ABSTRACT

The prevalence of obesity has increased worldwide at alarming rates, and has become a major health problem in modern society, highlighting the need for adjuvants to aid in its treatment. Medicinal plants are rich sources of bioactive compounds with potential therapeutic use, and may be a viable alternative for the development of safe and effective drugs to aid in the treatment of obesity. The objective of this study was to evaluate the use of the medicinal plants *Aloe vera* (L.) Burm., *Simaba ferruginea* St. Hil., *Baccharis trimera* (Less.) DC, *Garcinia cambogia* Desr. and *Tournefortia paniculata* Cham., as well as of the phytotherapic Moder diet, prepared from the combination of these plants for the treatment of obesity, performing the chemical characterization of the constituents of the plants, as well as inhibition assays of digestive enzymes, evaluation of the antioxidant potential and the bioassay with the plant that shows the highest amount of phytochemicals with pharmacological properties, in order to perform their therapeutic (in the prevention and treatment of obesity) and toxicological characteristics. The results indicated the presence of substances of pharmacological interest, especially phenolic compounds, saponinas, dietary fiber in all plants; and calcium in *G. cambogia* and *S. ferruginea*. Enzymatic inhibition was detected for α-amylase (*T. paniculata*) and α-glucosidase (*T. paniculata, A. vera* and *B. trimera*) after simulation of gastric fluid; and *in vitro* antioxidant potential in all plants, with emphasis on *T. paniculata*, which presented the highest levels of phenolic compounds and vitamin C. The simulated phytotherapic caused no inhibition of digestive enzymes and showed a low antioxidant activity. Given these results, *T. paniculata* was chosen for the bioassay, and was administered by gavage in the form of flour and water extract to *Wistar* rats subjected to a high calorie diet for 42 days, for the evaluation of the following parameters: body weight, food intake, glycemia, lipids, lipid peroxidation and toxicity (genotoxicity and histology). The flour and the aqueous extract of *T. paniculata* leaves were effective in reducing liver fat, glucose, triacylglycerols, and showed no genotoxic potential, in the evaluated conditions. The aqueous extract reduced food intake and lipid peroxidation. It was possible to conclude that the studied plants show a great diversity of phytochemicals with potential to be used in pharmaceutical preparations with possible health benefits. Among the plants, *T. paniculata* presents the highest contents of phytochemicals (phenolic compounds, saponins and vitamin C), inhibition on the enzymes α-amylase and α-glucosidase and antioxidant activity, in *in vitro* assays. The results of the bioassay suggest that *T. paniculata* leaves may be a therapeutic alternative in the treatment of obesity.

Keywords: Obesity. Medicinal Plants. Antioxidants. Digestive enzymes. Toxicity. Phytotherapic.

SUMÁRIO

PRIMEIRA PARTE

APRESENTAÇÃO

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos, os quais se encontram no item artigos.

As referências bibliográficas referem-se somente as citações que aparecem nos itens introdução e referencial teórico.

Cada artigo está estruturado de acordo com as normas das revistas científicas escolhidas para a submissão ou publicação do mesmo.

1 INTRODUÇÃO

A utilização de plantas medicinais na prevenção e tratamento de doenças é uma prática muito antiga e apresenta uma importância histórica nas transformações da terapêutica, tanto como fonte de matérias-primas farmacêuticas, como fonte de substâncias ativas isoladas e utilizadas como protótipo de fármacos e, mais recentemente, na forma de medicamentos fitoterápicos.

O crescente aumento das enfermidades e a falta de opções, acesso, segurança e eficácia dos tratamentos para as doenças levaram muitas pessoas a buscarem nas plantas medicinais opções para combate destas. Um importante exemplo dessa prática se dá no tratamento da obesidade, um dos principais problemas que a área de saúde enfrenta atualmente, e devido às proporções que vem alcançando tem mobilizado grandes esforços na produção do seu conhecimento. Sua incidência independe de fatores sócio-econômicos, idade e suas consequências vão desde o desenvolvimento de doenças debilitantes até a morte, afetando diretamente a qualidade de vida dos indivíduos.

Estima-se que haja mais de um bilhão de adultos acima do peso no mundo, e destes, 400 milhões estejam clinicamente obesos (TUCCI; BOYLAND; HALFORD, 2010), o que levou a doença à condição de epidemia global. No Brasil, de acordo com o Ministério da Saúde, aproximadamente 50% da população apresenta excesso de peso, e 15,8% obesidade, e estimativas apontam que, em 2025, o Brasil será o quinto país do mundo em número de obesos (BRASIL, 2012b).

A obesidade causa problemas psicológicos, frustrações e predispõem o organismo a uma série de doenças, em particular doenças cardiovasculares, alguns tipos de câncer, diabetes e hipertensão arterial, causando gastos econômicos tanto pelo governo como pela sociedade.

Entre a comunidade científica, a classe médica e as diversas organizações internacionais de saúde, há um consenso de que as principais causas da obesidade são: dieta inadequada rica em carboidratos e gorduras saturadas, sedentarismo e falta de atividades físicas regulares. Além da questão de saúde, há também a questão da estética que vem sendo muito valorizada nos últimos anos o que levou muitas pessoas a buscarem opções para tratamento da obesidade.

Das opções disponíveis para tratamento desta enfermidade as mais empregadas são o uso de dietas balanceadas, práticas regulares de exercícios físicos e os tratamentos medicamentosos, que vão desde inibidores de lípases a anorexígenos.

Devido aos efeitos colaterais, ineficácia e elevado custo dos medicamentos tradicionalmente utilizados no tratamento da obesidade, a utilização de plantas medicinais está sendo amplamente explorada, tanto pela população, devido ao fácil acesso, baixo custo, não exigência de prescrição médica e crença de ausência de efeitos tóxicos, como pela indústria farmacêutica que vê nessas plantas uma alternativa viável para o desenvolvimento futuro de medicamentos que induzam a redução de peso de forma eficaz e segura (MAYER et al., 2009; PARK; LEE; SUNG, 2005). Estudos mostram que vários produtos naturais, incluindo extratos e compostos isolados de plantas, estão sendo utilizados para a redução do peso corporal e prevenção da obesidade (RAYALAM; DELLA-FERA; BAILE, 2008; SIMÃO; CORRÊA; CHAGAS, 2012; SOUZA et al., 2011).

A importância das plantas medicinais deve-se a sua contribuição como fonte natural de fármacos e por proporcionar grandes chances de obtenção de moléculas protótipos devido à ampla diversidade de seus constituintes (YUNES; CALIXTO, 2001). No entanto, inúmeras plantas são usadas em preparações fitoterápicas sem que seja realizado um controle de qualidade e seguridade

adequados, uma vez que a literatura científica apresenta, para a maioria das plantas medicinais, a presença de substâncias tóxicas e/ou composição química variável (CAPASSO et al., 2000).

Além disso, algumas indústrias farmacêuticas que visam somente o lucro pregam a existência de alguns produtos milagrosos, fazendo propaganda enganosa e na maioria das vezes sem se preocupar com a saúde pública, oferecendo produtos ditos naturais, mas que na maioria das vezes tem em suas formulações a adição de substâncias não declaradas, havendo ainda, em alguns casos, a ausência de estudos com os constituintes presentes nas formulações.

Um importante exemplo dessa prática é o uso do fitoterápico Moder diet, composto pela combinação de extratos das plantas *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr. e *Tournefortia paniculata* Cham. (marmelinho), que tem sido comercializado livremente no Brasil, com o suposto efeito inibidor de apetite e redutor de peso, sem que houvesse comprovações científicas para garantir seu uso seguro. No entanto, a Agência Nacional de Vigilância Sanitária (ANVISA), proibiu a venda desse fitoterápico por falta de estudos que atestassem sua eficácia bem como a ausência de riscos durante seu uso (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA - ANVISA, 2009).

Recentemente, o uso do Moder diet teve um alto crescimento, refletido no surgimento de inúmeros casos de perda de peso e pela sua divulgação principalmente em sites na internet e em academias. No entanto, devido aos efeitos surpreendentes proporcionados por esse emagrecedor, há uma preocupante possibilidade de adulterações, principalmente por anorexígenos e riscos toxicológicos pelo uso continuo, ressaltando a importância de estudos com as plantas que fazem parte de sua composição para comprovação de seus efeitos terapêuticos e toxicológicos.

Diante da busca de alternativas terapêuticas de menor custo, fácil acesso e segurança comprovada para o tratamento da obesidade, bem como a falta de informações científicas sobre o fitoterápico Moder diet e as plantas que o constitui, faz-se necessária uma investigação detalhada a respeito de seus constituintes químicos, possível toxicidade decorrente do uso contínuo e elucidações sobre o mecanismo de ação.

1.1 Objetivo geral

Determinar a composição química das plantas que compõem o fitoterápico Moder diet, realizar ensaios de inibição de enzimas digestivas, medir o potencial antioxidante e ensaio biológico com a planta que mostrar maior presença de fitoquímicos com propriedades farmacológicas, visando sua caracterização terapêutica (potencial para prevenção e tratamento da obesidade) e toxicológica.

1.2 Objetivos específicos

a) Realizar a triagem fitoquímica, determinar a composição centesimal e mineral e o teor de alguns compostos bioativos das plantas que constituem o fitoterápico Moder diet.
b) Realizar ensaios de inibição de enzimas digestivas na presença de fluido gástrico simulado com as plantas que constituem o fitoterápico Moder diet e com a mistura equivalente ao fitoterápico.
c) Avaliar o potencial antioxidante das plantas que constituem o fitoterápico Moder diet e da mistura equivalente ao fitoterápico.
d) Avaliar os efeitos antiobesidade da planta que mostrar maior presença de fitoquímicos com propriedades antiobesidade, inibição de enzimas digestivas e

atividade antioxidante, em ratos *Wistar*, submetidos à dieta hipercalórica, por meio dos parâmetros: peso corporal, consumo alimentar, glicemia, lipídios, peroxidação lipídica e toxicidade (genotoxicidade e histologia).

2 REFERENCIAL TEÓRICO

2.1 Epidemiologia: relevância do problema

Em função das consequências causadas pela obesidade e da velocidade de sua disseminação em todo mundo, esta vem sendo considerada uma epidemia mundial, com mais de um bilhão de adultos com excesso de peso, dos quais, 400 milhões apresentam obesidade clínica, atingindo tanto países desenvolvidos como os em desenvolvimento, entre eles o Brasil (TUCCI; BOYLAND; HALFORD, 2010; WORLD HEALTH ORGANIZATION - WHO, 2010). Na América, a obesidade vem aumentando, para ambos os gêneros, tanto em países desenvolvidos quanto nos em desenvolvimento. Na Europa, verificou-se na ultima década um aumento da obesidade entre 10% e 40%, na maioria dos países. Em alguns países da região Oeste do Pacífico, como a Austrália, o Japão, Samoa e China, também se nota a elevação da prevalência da obesidade. No continente africano, a obesidade é ainda relativamente incomum, sendo que sua prevalência é mais elevada na população urbana em relação à população rural (WANDERLEY; FERREIRA, 2010).

No Brasil, a análise de cinco inquéritos populacionais, realizados pelo Ministério da Saúde - Estudo Nacional sobre Despesas Familiares (ENDEF), realizado entre 1974-1975; a Pesquisa Nacional sobre Saúde e Nutrição (PNSN), de 1989; a Pesquisa sobre Padrões de Vida (PPV), desenvolvida em 1996-1997; e a Pesquisa de Orçamentos Familiares (POF), de 2002-2003 e de 2008-2009 – permitiu avaliar a emergência da obesidade e verificar seus principais determinantes. De acordo com esses estudos, a obesidade e o sobrepeso estão aumentando de forma acelerada na população brasileira, principalmente entre os adultos, enquanto a prevalência de desnutrição declina de forma acelerada nas últimas décadas. Dados do Instituto Brasileiro de Geografia e Estatística (IBGE)

revelam que o sobrepeso na população de 20 anos ou mais, no sexo masculino saltou de 18,5% em 1974-1975 para 50,1% em 2008-2009. No sexo feminino, o avanço foi de 28,7% para 48%, no mesmo período (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2009).

Em relação às crianças e adolescentes, os dados sobre aumento da obesidade também são preocupantes. Para colocar a epidemia da obesidade entre crianças nos Estados Unidos em perspectiva, o número de crianças obesas ultrapassa o número combinado de crianças afetadas pelo vírus da Aids, câncer, diabetes infantil e fibrose cística (FREEDMAN et al., 2007). No Reino Unido, 16% das crianças são obesas e outras 14% estão acima do peso.

No Brasil, o excesso de peso nessa parte da população cresce de forma acelerada: em 1974, o excesso de peso atingia 4,9% de crianças entre 6 e 9 anos de idade e 3,7% entre os adolescentes de 10 a18 anos. Já no período entre 1996 e 1997, observou-se 14% de excesso de peso na faixa etária de 6 a 18 anos de idade (WANG et al., 2002). Dados divulgados pelo POF (2008-2009) indicam que 33,5% das crianças brasileiras de 5 a 9 anos estão acima do peso, sendo que 16,6% do total de meninos são obesos e 11,8% das meninas estão com sobrepeso (IBGE, 2009). A prevalência de obesidade nessa faixa etária é preocupante devido ao alto risco da criança obesa se tornar um adulto obeso, apresentando variadas condições mórbidas associadas.

Estudos recentes mostram que quase metade da população brasileira está acima do peso. Os resultados da última pesquisa de Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (VIGITEL), promovida pelo Ministério da Saúde, mostram que o excesso de peso e a obesidade aumentaram nos últimos seis anos no Brasil. De acordo com o estudo, a proporção de pessoas acima do peso no Brasil avançou de 42,7%, em 2006, para 48,5%, em 2011. No mesmo período, o percentual de obesos subiu de 11,4% para 15,8%. O estudo mostra também que o aumento das porcentagens de

pessoas obesas e com excesso de peso atinge tanto a população masculina quanto a feminina. Em 2006, 47,2% dos homens e 38,5% das mulheres estavam acima do peso ideal. Agora, as proporções subiram para 52,6% e 44,7%, respectivamente (BRASIL, 2012b).

Estimativas da WHO apontam que em 2015 o número de obesos e de pessoas com sobrepeso no mundo ultrapassará mais que o dobro dos números atuais passando a ser de 700 milhões e 2,3 bilhões, respectivamente. Já para o Brasil (idade de 15 a 100 anos) estima-se que 66,5% das mulheres e 60,3% de homens apresentarão sobrepeso e obesidade (WHO, 2010), evidenciando a necessidade urgente de alternativas seguras e eficazes para conter o avanço dessa enfermidade.

2.2 Obesidade: conceito e classificação

A obesidade pode ser definida, de forma geral, como uma doença resultante do acúmulo anormal ou excessivo de gordura sob a forma de tecido adiposo, sendo consequência do balanço energético positivo e que resulta em prejuízos à saúde, podendo levar a morte (SOCIEDADE BRASILEIRA DE ENDOCRINOLIGA E METABOLOGIA - SBEM, 2010).

As medidas antropométricas comumente utilizadas para diagnosticar a obesidade são o índice de massa corporal (IMC), a medida da circunferência abdominal e a razão cintura quadril, sendo a primeira a mais utilizada (WHO, 2010). O IMC é calculado dividindo-se o peso do indivíduo (em kg) pelo quadrado de sua altura (em metros) (SBEM, 2010). É o padrão utilizado pela WHO, que identifica o peso normal quando o resultado do cálculo do IMC está entre 18,5 e 24,9 kg/m^2. A faixa de IMC entre 25 e 29,9 kg/m^2 é denominada de sobrepeso ou excesso de peso. Valores de IMC acima de 30 kg/m^2 indicam obesidade. A obesidade é classificada em três diferentes graus. De 30 a 34,9

kg/m^2 tem-se a obesidade grau I, de 35 a 39,9 kg/m^2 a obesidade grau II e acima de 40 kg/m^2 obesidade grau III (obesidade mórbida).

Sobrepeso e obesidade são fatores de risco para uma série de doenças. Quando comparados a indivíduos com peso normal, os obesos têm mais propensão a desenvolver problemas como hipertensão, doenças cardiovasculares, diabetes tipo 2, entre outros (SBEM, 2010; WHO, 2010).

Além do grau de gordura, outro fator que interfere nos riscos associados ao excesso de peso é a sua distribuição regional no corpo. Quando o excesso de gordura ocorre na região abdominal (obesidade androide), os riscos associados á outras doenças são bem maiores do que quando a gordura é distribuída de forma uniforme e periférica (Obesidade ginecoide) (WHO, 2010).

2.3 Etiologia

A etiologia da obesidade não está totalmente esclarecida, mas existe na literatura um consenso que ela não se caracteriza como uma desordem singular, mais sim como um grupo heterogêneo de condições com múltiplas causas, que, em última análise, refletem no fenótipo obeso (ASSOCIAÇÃO BRASILEIRA PARA O ESTUDO DA OBESIDADE E DA SÍNDROME METABÓLICA - ABESO, 2009).

Muitas são as causas que podem conduzir a obesidade, entre elas podem ser citados os fatores genéticos, ambientais, sociais, econômicos, culturais e as desordens endócrinas (SBEM, 2010). Associados ao ganho de peso estão também fatores como mudanças em alguns momentos da vida (casamento, separação, viuvez); determinadas situações de violência; fatores psicológicos (depressão, ansiedade e estresse); tratamentos medicamentosos (com psicofármacos e corticoides); suspensão do hábito de fumar; redução drástica de prática de exercícios, entre outras (BRASIL, 2006).

Das várias causas da obesidade, o determinante mais imediato do acúmulo excessivo de gordura é o balanço energético positivo, que acontece quando a quantidade de energia consumida é maior que a quantidade gasta (HENRY; PANDIT, 2009). Este é totalmente favorecido pelas dietas atuais que apresentam alta densidade energética, são ricas em gorduras saturadas, açúcares simples, e tem baixos teores de carboidratos complexos e fibras, e pelo aumento da inatividade física, configurando o estilo de vida ocidental contemporâneo (POPKIN; GORDON-LARSEN, 2004).

As explicações para o aumento da obesidade tem sido um pouco controversas. Alguns estudos têm demonstrado diferenças genéticas entre pessoas obesas e não obesas, enquanto outros estudos se concentram em fatores como mudanças no estilo de vida, tais como maus hábitos alimentares e inatividade física (PERUSSE; BOUCHARD, 1999; SUNDQUIST; JOHANSSON, 1998).

2.4 Obesidade e suas co-morbidades

Associa-se a obesidade algumas das mais prevalentes doenças da sociedade moderna. O excesso de gordura corporal aumenta o risco de desenvolver diabetes, doenças coronarianas, câncer, hipertensão arterial, dislipidemias, doenças do trato digestivo, doenças pulmonares, complicações ortopédicas, entre outras, podendo levar até a morte (DANIELS, 2009; GUH et al., 2009).

A obesidade também atinge crianças e adolescentes que vêm sendo cada vez mais suscetíveis ao excesso de peso, sendo a obesidade infantil um fator para a obesidade adulta. Nessa faixa etária a obesidade está relacionada a doenças crônicas como hipertensão e diabetes, além de dislipidemias. Outras co-

morbidades associadas são problemas ortopédicos, infecções fúngicas de pele e esteatose hepática (DANIELS, 2009).

O excesso de peso está associado com o aumento da morbidade e mortalidade e este risco aumenta de acordo com o ganho de peso. Comparando-se com indivíduos de peso normal, pessoas obesas têm 20% a mais de chance de morrer por todas as causas; possuem o risco duas vezes maior de falecer por diabetes; têm 40% a mais de chance de desenvolver disfunções na vesícula biliar e 25% a mais de doenças coronarianas. Em homens com 40% acima do peso desejável (obesidade grau II), a mortalidade por todas as causas é 55% maior, apresentam 70% a mais de chance de desenvolver doenças coronarianas, e o risco de morte por diabetes é quatro vezes maior do que entre pessoas de peso normal (BLUMENKRANTZ, 1997).

2.5 Impactos econômicos

Pesquisas mostram que há mais de um bilhão de adultos com excesso de peso no mundo (TUCCI; BOYLAND; HALFORD, 2010), sendo que este quadro implica em altos gastos econômicos tanto pelos órgãos governamentais como pelos indivíduos afetados. Nos Estados Unidos, país com maior número de obesos no mundo, o levantamento do *Centers for Desease Control and Prevention* (CDC) realizado entre os anos de 1998 e 2006 revelaram que os custos com a obesidade aumentaram de 6,5% para 9,2% (CENTERS FOR DISEASE CONTROL AND PREVENTION - CDC, 2012). Já em países em desenvolvimento, os gastos variam de 2 a 6% do custo total com a saúde. Se levar em conta que uma pessoa obesa desenvolve uma série de outras doenças em consequência do excesso de peso, por exemplo: diabetes tipo 2, doenças cardiovasculares, entre outras, os verdadeiros custos com essa epidemia podem ser muito maiores.

No Brasil, os gastos diretos com essa doença, incluindo internações, consultas e medicamentos, passam de um bilhão de reais por ano, que equivale a 12% do total de gastos anuais do Sistema Único de Saúde (SUS) com internações. Mundialmente, o Brasil ocupa a sexta posição entre os países com problemas com a obesidade (GIGANTE et al., 2009).

2.6 Tratamento

A obesidade deve ser reconhecida como enfermidade e tratada como tal. Seu tratamento deve ser complexo e multidisciplinar e sua escolha deve ser baseada na gravidade do problema e na presença de complicações associadas.

Várias opções estão disponíveis para tratamento do sobrepeso e da obesidade, mas fundamentalmente os caminhos a seguir devem passar pela redução da energia ingerida e pelo aumento do gasto calórico.

A maioria dos tratamentos passa por dietas balanceadas, práticas de exercícios físicos com frequência, terapia medicamentosa e cirurgia bariátrica.

O tratamento medicamentoso inclui diversos agentes promotores de perda de peso. Estes estão divididos em três categorias: os que diminuem a fome ou modificam a saciedade; os que reduzem a digestão e absorção de alimentos e os que aumentam o gasto energético (NONINO-BORGES et al., 2006).

No Brasil, existem, alguns medicamentos registrados para tratamento da obesidade como a sibutramina, dietilpropiona (anfepramona), femproporex, mazindol e orlistat. Estes são indicados apenas em casos em que o IMC for maior ou igual a 30, ou maior ou igual a 25 acompanhados de fatores de risco em casos que o tratamento com dietas, exercícios físicos e mudanças comportamentais não provocam efeitos. Contudo, há muitas controvérsias sobre a utilização destes, pelos escassos estudos sobre seus efeitos a longo prazo e pelos seus efeitos colaterais, como doenças cardíacas, hipertensão, disfunção

sexual, entre outras. Outra questão associada ao tratamento medicamentoso, é que infelizmente o tratamento na maioria dos casos só traz benefícios em curto prazo, este é frequentemente associado com ganho de peso após a cessação do uso das drogas (MAHAN; SCOTT-STUMP, 2008).

Devido à gravidade da obesidade e os efeitos colaterais do tratamento medicamentoso, a utilização de produtos naturais teve alto crescimento nos últimos anos, principalmente pelo fato que a população acredita que esse tipo de tratamento não acarreta efeitos colaterais, não necessita de prescrição médica, além de apresentar baixo custo.

Alguns dos produtos naturais utilizados são a aloína (*Aloe vera*), alcachofra (*Cynara scolynmus*), boldo (*Peumus boldus*), carqueja (*Baccharis* sp.), cáscara-sagrada (*Rhamnus purshiana*), centella-asiática, extrato de citrin (*Garcinia* sp.), clorela (*Chorella pyrenoidosa*), espinheira-santa (*Maytenus ilicifolia*), espirulina (*Spirulina maxima*), *Fucus* sp., guaraná (*Paullinia cupana*), ginseng-falso *(Pfaffia paniculata*), glucomanan (*Amorphophallus konjac*), jurubeba (*Solanum paniculatum*), maracujá (*Passiflora alata)* e sene (*Cassia angustifolia*). Seu uso também vem sendo estimulado por propagandas de diversos meios de comunicação (Internet, Revistas não científicas leigas, entre outros), além de muitas farmácias de manipulação que tem comercializado estas plantas na fórmula de cápsulas.

Outra prática bastante comum utilizada pela população e pelas indústrias de fitoterápicos é o uso da combinação de plantas visando obter um maior espectro de efeitos, no entanto, a maioria desses produtos não tem comprovação científica sobre sua eficácia e segurança, necessitando de novos estudos.

As plantas podem auxiliar no tratamento da obesidade de forma direta ou indireta. Na forma direta, estas podem atuar estimulando o metabolismo e na modulação do apetite. Já na forma indireta, elas atuam no tratamento da obesidade associada a outras patologias. Nesse caso, podem-se citar plantas com

ação diurética e aquelas que agem como depressores do sistema nervoso central (MORO; BASILE, 2000).

O uso de produtos naturais vem ganhando bastante crédito, principalmente depois da implantação do Programa Nacional de Plantas Medicinais e Fitoterápicos (PNPMF), que visa inserir com segurança, eficácia e qualidade, plantas medicinais, fitoterápicos e serviços relacionados a fitoterapia no Sistema Único de Saúde (SUS) (BRASIL, 2006).

Outra vantagem do uso de produtos naturais, baseia-se no fato que mesmo uma diminuição modesta, de 5 a 10% do peso corporal já traz benefícios à saúde (SBEM, 2010). Estudos realizados na China, Canadá, nos Estados Unidos e em vários países europeus mostraram que mais da metade das pessoas com excesso de peso conseguiram reduzir a incidência de diabetes por meio da redução de peso e da prática de exercícios físicos (ORGANIZAÇÃO PAN-AMERICANA DA SAÚDE - OPAS, 2003). Assim, a busca e uso de plantas medicinais para tratamento da obesidade aumentaram nos últimos anos.

Infelizmente, a maioria das plantas medicinais utilizadas para tratamento da obesidade, é usada sem orientação médica, o que é motivo de preocupação já que, para muitas delas, há poucos estudos científicos ou estes são até mesmo inexistentes, o que demonstra a necessidade de uma ampla caracterização dessas plantas, para verificação de sua eficácia e segurança.

2.7 Fitoterápico Moder diet e seus componentes

Emagrecedor natural composto de extratos de ervas, o Moder diet se caracteriza como um composto vegetal usado para auxiliar o organismo na perda de peso e na prevenção da obesidade. Atua na redução do apetite e ansiedade, acelera o metabolismo eliminando gorduras localizadas e flacidez. Leva a perda

de 6 a 8 kg por mês, com alegação de não apresentar efeito colateral nem contra indicação (WIKIPEDIA, 2010).

Em sua composição estão presentes extratos de calunga (*Simaba ferruginea*), para inapetência, é tônica usada para combater anemia e fraqueza em geral; carqueja (*Baccharis trimera*), combate o mal funcionamento do intestino, é antidiabética, anti-reumática e diurética; marmelinho (*Tournefortia paniculata*), antibiótico das vias urinárias; babosa (*Aloe vera*), revitaliza e combate a flacidez, antienvelhecimento; *Garcinia cambogia*, combate a obesidade, utilizada como inibidor de apetite e também para bloquear a absorção e síntese de gordura.

Produzido pelo laboratório Erusmed, o Moder diet está na mira da ANVISA, que pela resolução 3.691/2009, determinou a suspensão da fabricação, distribuição, comércio e uso do medicamento, pelo fato do fitoterápico não ter registro, nem tão pouco estudos que comprovem sua eficácia e/ou toxicidade (ANVISA, 2009).

Vendido como remédio natural, na forma de cápsulas, esse fitoterápico pode apresentar misturado com suas ervas anorexígenos, hormônios tireoidianos e outras substâncias de uso proibido ou só recomendado com prescrição médica. Além disso, as misturas das ervas também não são recomendadas. Elas podem ser incompatíveis e causar a chamada interação medicamentosa, com efeitos imprevisíveis, necessitando assim de estudos para comprovação científica de seus efeitos.

Entre as espécies vegetais presentes na composição do Moder diet, a *Aloe vera* é a mais estudada, estando presente nas listas RENISUS (espécies vegetais que apresentam potencial de gerar produtos de interesse ao SUS (BRASIL, 2009)), ANVISA (plantas medicinais com medicamentos fitoterápicos derivados simples registrados na ANVISA (CARVALHO et al., 2008)), LMFRS (plantas medicinais com medicamentos fitoterápicos derivados

presentes na lista de medicamentos fitoterápicos de registro simplificado (Instrução Normativa 5/08) (BRASIL, 2008)) e SUS (plantas medicinais com medicamentos fitoterápicos derivados oficializados para uso no SUS (RENAME) (BRASIL, 2012a)). Em seguida, destacam-se a *Baccharis trimera* presente na lista RENISUS e registrada como DV (espécies que podem ser comercializadas na forma de drogas vegetais industrializadas (Resolução da Diretoria Colegiada 10/10) (BRASIL, 2010)), e a *Garcinia cambogia* presente na lista da ANVISA (TEIXEIRA, 2013). As espécies *Simaba ferruginea* e *Tournefortia paniculata* estão ausentes em todas as listas e seu uso se baseia apenas em descrições de uso popular.

No que se refere ao tratamento da obesidade escassos estudos são encontrados para essas plantas, o que revela a necessidade de estudos que poderão ajudar no entendimento farmacológico e/ou tóxico do uso interno dessas plantas no combate a obesidade.

2.7.1 *Aloe vera*

Aloe vera (L.) Burm. (babosa), é uma espécie de planta pertencente ao gênero *Aloe* e família Liliaceae, nativa do norte da África (BACH; LOPES, 2007). É uma planta utilizada para diversos fins medicinais, sendo as principais aplicações relacionadas a problemas da pele como acne, psoríase, hanseníase e queimaduras, tendo ampla utilização na indústria de cosméticos (VALVERDE et al., 2005). Relatos macedônicos, egípcios, chineses e até mesmo na bíblia demonstram que era comum o uso dessa planta na antiguidade para diversas finalidades (BACH; LOPES, 2007).

As folhas dessa planta são constituídas de um tecido parenquimático rico em polissacarídeos (mucilagem), o que lhe confere uma consistência viscosa (baba). Seus princípios ativos são encontrados nessa mucilagem e esta é

constituída de tecidos orgânicos, vitaminas (A, B e C), enzimas (lipases, proteases), sais minerais e aminoácidos, dos quais alguns são importantíssimos para o homem (BOUDREAU; BELAND, 2006; HE et al., 2005). A seiva obtida de sua casca é rica em aloína, alantonína e antraquinonas. Essas substâncias lhe confere propriedades cicatrizantes, antibacteriana, antifúngica e antivirótica (VALVERDE et al., 2005). As atividades biológicas relatadas para esta espécie vegetal são comumente atribuídas a uma ação sinérgica dos compostos nela contidos, em vez de apenas uma substância química (TALMADGE et al., 2004).

Quanto ao seu potencial tóxico, estudos realizados com extratos aquosos de *A. vera* mostram que em doses usuais (40 mL L^{-1}), o extrato aquoso desta planta não induz mutagenicidade sobre células de *Allium cepa*, enquanto que em doses superiores às utilizadas popularmente, o extrato foi capaz de induzir citotoxicidade e mutagenicidade quando avaliado sobre o mesmo tipo celular. Nesse estudo, os autores também verificaram efeito antimutagênico (mutagenicidade induzida pelo paracetamol) do gel de *A. vera* sobre linfócitos humanos utilizando o teste do micronúcleo (STURBELLE et al., 2010), o que também foi verificado por Stanic (2007), em estudo sobre a ação antimutagênica do gel de *A. vera* sobre os efeitos induzidos por metanosulfonato etílico.

Capacidade antioxidante foi observada por Yun, Juan e Qiuhui (2003), que analisando a *A. vera* em várias idades, constataram que na idade de quatro anos essa planta mostra maior potencial antioxidante que em idades inferiores, o que demonstra que a fase de crescimento tem papel vital na composição antioxidante do gel de *A. vera*. Já Rajasekaran, Sivagnaman e Subramanian (2005), observaram que na administração oral do extrato alcoólico de *A. vera* (300 mg kg^{-1}) para ratos diabéticos, os altos níveis da peroxidação lipídica e de hidroperóxidos nos tecidos desses ratos diabéticos foram revertidos para níveis quase normais depois do tratamento com o extrato dessa planta. O tratamento também resultou num aumento significativo nos níveis de glutationa reduzida,

superóxido dismutase, catalase, glutationa peroxidase e glutationa-S-transferase no fígado e nos rins de ratos diabéticos, resultados que evidenciam a propriedade antioxidante do extrato de *A. vera*.

Em relação à obesidade, um estudo desenvolvido com o extrato alcoólico da *A. vera*, administrado oralmente, nas concentrações de 200 e 300 mg kg^{-1} de peso corporal em ratos com alimentação normal, ratos com sobrecarga de glicose e com hiperglicemia induzida por estreptozotocina, demonstrou que o extrato promoveu a manutenção na glicemia por controlar a ação das enzimas que metabolizam os carboidratos (RAJASEKARAN et al., 2004), o que também foi observado por Noor et al. (2008). Mohamed (2011) e Rajasekaran et al. (2006) observaram que a administração oral de *A. vera* resultou em uma diminuição significativa nos níveis de colesterol e triglicérides, em experimento realizado com ratos, o que demonstra que o extrato liofilizado de *A. vera* pode ser utilizado como auxiliar no tratamento da obesidade.

No mercado norte americano há produtos a base de *A. vera* que prometem a cura de diabetes, câncer e até da tuberculose. Contudo, as propriedades antibacterianas e antiinflamatórias relatadas para esta planta ainda não foram comprovadas em humanos acometidos por estas doenças.

De acordo com a legislação brasileira somente cosméticos e medicamentos fitoterápicos de uso tópico podem ser fabricados em escala industrial a partir desta planta. Produtos de uso interno na forma de alimentos, como suco e isotônico, comercializados em outros países, não estão autorizados no Brasil pela falta de pesquisas que comprovem a segurança de ingestão. No que se refere ao tratamento da obesidade escassos estudos são encontrados.

Diante da escassez de estudos sobre seus constituintes químicos e efeitos de seu uso interno, estudos mais detalhados poderão ajudar no entendimento farmacológico e/ou tóxico do uso interno dessa planta no combate a obesidade.

2.7.2 *Baccharis trimera*

Pertencente à família Asteraceae, a *Baccharis trimera* (Less.) DC, conhecida popularmente como carqueja, é uma planta perene nativa da Amazônia, sendo encontrada nas regiões tropicais, incluindo Brasil e outros países da América do Sul. Na medicina popular são atribuídas a *B. trimera* funções no uso interno como estomática, anti-reumática, anti-helmíntica, tônica para o fígado e no tratamento de diabetes, gastroenterites, anorexia, gripe, resfriado, além de ter ação digestiva, diurética e redutora de peso. Pelo efeito diurético, auxilia no emagrecimento e no controle de diabetes (OLIVEIRA et al., 2005). No uso externo a *B. trimera* é indicada para tratamento de feridas e ulcerações (VERDI et al., 2005). Na indústria da cerveja podem ser usadas como substitutas do lúpulo.

Flavonoides e terpenoides são considerados os grupos de maior ocorrência nessa planta. Encontrando-se também outros compostos como as saponinas (SOUZA et al., 2011) e óleos essenciais (AGOSTINI et al., 2005). Dos muitos efeitos biológicos da *B. trimera* já relatados, podem ser citados o antioxidante (OLIVEIRA et al., 2003), gastroprotetor (BAGGIO et al., 2003), antiúlcera (DIAS et al., 2009), hipoglicemiante (BARBOSA-FILHO et al., 2005), entre outros.

Quanto ao seu efeito tóxico, o principal já relatado é o de indução de aborto, comprovado experimentalmente em animais (VERDI et al., 2005). Baixa toxicidade e efeitos antimutagênicos foram observados por Nakasugi e Komai (1998), em frações metanólicas isoladas de *B. trimera*. Baggio et al. (2003) analisando extratos aquosos e hidroalcoólicos de folhas de *B. trimera* e Dias et al. (2009) analisando o extrato bruto liofilizado desta planta não observaram efeitos citotóxicos em ratos *Wistar*, o que também foi observado por Peron et al. (2008) ao avaliar células da medula óssea de ratos *Wistar*, tratados *in vivo*, via

gavagem, com extrato aquoso desta planta, nas concentrações de 6,85 e 68,50 mg mL^{-1}. Já Pinho et al. (2010) avaliando a mutagenicidade *in vivo* e *in vitro* do chá de *B. trimera,* nas doses de 20 g L^{-1} (dose usual) e 200 g L^{-1} (10 vezes mais concentrado), pelo teste de *Allium cepa* L. e o de aberrações cromossômicas em linfócitos humanos relataram a necessidade de moderação durante seu consumo.

Rodrigues et al. (2009) analisando extratos aquosos de *B. Trimera,* administrados por gavagem nas doses de 200, 500 e 2.000 mg kg^{-1} camundongo, durante três dias, não observaram efeito genotóxico em amostras de sangue e fígado desses animais pelo teste cometa. Entretanto, o extrato aquoso mostrou potencial antigenotóxico (danos oxidativos induzidos pelo peróxido de hidrogênio) em amostras de sangue desses ratos pelo teste do cometa, o que pode ser devido ao potencial antioxidante demonstrado, *in vivo,* pelo extrato aquoso desta planta, possível responsável por proteger moléculas de DNA contra danos oxidativos induzidos por radicais livres.

Em relação ao potencial antioxidante desta planta, Pádua et al. (2010) registraram bom potencial antioxidante *in vitro e in vivo* para o extrato hidroetanólico de *B. trimera* nos neutrófilos de ratos Fisher. Já Mendes, Tabach e Carlini (2007) relataram que o extrato hidroalcoólico de *B. trimera* apresenta moderada capacidade antioxidante, não sendo muito eficiente como antiúlcera em ratos (indução de úlcera por stress). Porém, Dias et al. (2009), analisando diferentes extratos de *B. trimera*, observaram que alguns extratos mostraram atividade antiúlcera sendo capazes de reduzir lesões em ratos *Wistar* (indução de úlcera por ácido clorídrico em etanol).

No que se refere ao tratamento da obesidade, Souza et al. (2011) constataram alta atividade inibitória de extratos metanólicos de *B. trimera* sob algumas enzimas digestivas (lipase e amilases), mostrando que a utilização desses extratos pode ser útil para auxiliar no tratamento dessa enfermidade.

No Brasil, a *B. trimera* está entre as plantas medicinais mais comercializadas, e estudos mais detalhados sobre seus constituintes químicos e sobre seu modo de atuação no tratamento da obesidade podem contribuir para um uso mais seguro dessa planta.

2.7.3 *Garcinia cambogia*

Garcinia cambogia Desr. é uma pequena árvore, pertencente à família Gutiferaceae, originária das florestas do Camboja, sul da África e Polinésia, amplamente cultivada na Índia e em países do extremo oriente. As partes da planta mais utilizadas são a casca seca e a polpa dos frutos, sendo o fruto a parte que apresenta o componente capaz de promover perda de peso (SANTOS et al., 2007).

O ácido hidroxicítrico (AHC) encontrado na casca dos frutos é a substância responsável pela perda de gordura e pelas propriedades saciantes da *G. cambogia* (SANTOS et al., 2007). Atribui-se ao AHC as seguintes ações para promover perda de peso:

a) Bloqueador de lipídios – quando ingeridos em excesso, os carboidratos são metabolizados e armazenados como lipídios, durante esse processo ocorre a participação da enzima citrato liase. O AHC liga-se a essa enzima inibindo-a, impedindo o armazenamento de lipídios.

b) Redutor de apetite – o controle de apetite é feito a partir de uma maior síntese de glicogênio feita pelo AHC, que ao bloquear a citrato liase direciona as calorias que não são armazenadas na forma de lipídios para formação de glicogênio, dessa forma, quando as reservas de glicogênio estão altas, os receptores de açúcar no fígado são estimulados e enviam sinal de saciedade ao cérebro, sem estimular o sistema nervoso central. Outro modo de atuação está

relacionado à capacidade de estimular a liberação de serotonina, um neurotransmissor diretamente envolvido no controle de apetite.

Estudos realizados com extratos de *G. cambogia* demonstraram a redução de adiposidade visceral em camundongos tratados com dieta rica em lipídios (KIM et al., 2008), propiciando uma atividade semelhante a leptina na redução dos níveis sanguíneos de insulina e melhora do metabolismo da glicose (HAYAMIZU et al., 2003a), e em humanos a redução da gordura abdominal típica da obesidade, sugerindo seu uso na prevenção e redução do acúmulo de gordura visceral (HAYAMIZU et al., 2003b). Já em condições de dietas ricas em carboidratos o AHC isolado foi eficaz na redução da síntese de novos lipídios em humanos (KOVACS; WESTERTERP-PLANTENGA, 2006).

Quanto a sua toxicidade, Saito et al. (2005) observaram que dietas contendo *G. cambogia* (com teores de ácido hidroxicítrico maiores que 10,10 g kg^{-1} de ração) administradas a ratos Zucker obesos, revelaram-se altamente tóxicas para os testículos desses animais e causaram a diminuição da espermatogênese após 13 semanas de tratamento. Já para ratos *Wistar*, extratos etanólicos de *G. cambogia* administrados durante seis semanas, apresentaram efeitos tóxicos no sistema reprodutivo dependentes da dose, sendo identificadas distorções nas células do epitélio germinativo (KAYODE et al., 2007).

Para humanos, não foram constatados efeitos adversos dos extratos de *G. cambogia* administrados na forma de comprimidos, contendo 1.000 mg de ácido hidroxicitrico dia^{-1}, durante 16 semanas (HAYAMIZU et al., 2003b), e durante 12 semanas, não sendo observadas alterações nos níveis séricos dos hormônios sexuais testosterona, estrona e estradiol (HAYAMIZU et al., 2008).

Apesar do AHC apresentar toxicidade relativamente baixa, o amplo espectro de ações e a escassez de informações sobre seus efeitos em longo prazo, tornam necessários estudos mais detalhados sobre possíveis interferências em alguns parâmetros fisiológicos.

2.7.4 *Tournefortia paniculata*

A *Tournefortia paniculata* Cham., tradicionalmente conhecida como marmelinho, é um arbusto pertencente à família Boraginaceae. Sua origem é atribuída às regiões mediterrâneas e aos Estados Unidos da América, ocorrendo amplamente nos trópicos e subtrópicos (MORAES; SOUSA, 2007). No Brasil, suas folhas são usadas na medicina popular como diurético e antiinflamatório das vias urinárias e em casos de litíase renal (BERTOLUCCI et al., 2000). Escassos estudos são encontrados sobre seus constituintes químicos, necessitando assim de ampla caracterização química e funcional.

2.7.5 *Simaba ferruginea*

Simaba ferruginea St. Hil, popularmente conhecida como calunga, calunga ferruginea, ou fel da terra, com sinonímia científica de *Quassia ferrugiena* D. Dietr, é uma árvore nativa do cerrado seco do Brasil central, pertencente à família Simaroubaceae. Sua casca tanto do tronco quanto do rizoma, apresenta substâncias de gosto amargo, que são empregadas na medicina popular para tratamento de febre, cicatrização de feridas, dores reumáticas, úlceras gástricas, obesidade, e outros transtornos, principalmente na forma de chás (LORENZI; MATOS, 2002; MARCELO, 2002). Embora várias plantas pertencentes à família Simaroubaceae tenham sido usadas na medicina tradicional para tratamento de várias doenças, apenas alguns produtos químicos e farmacológicos têm sido relatados sobre o gênero *Simaba* (MUHAMMAD et al., 2004; TOMA et al., 2002), o que releva a necessidade de estudos sobre sua composição química e atividade farmacológica visando comprovações de sua eficácia, até então relatada apenas na medicina popular.

2.8 Inibidores de enzimas digestivas

Inibidores de enzimas digestivas são substâncias químicas (a maioria proteínas) presentes nos tecidos vegetais, como sementes, raízes e outros e em animais como, por exemplo, na clara de ovo. Essas substâncias, quando ingeridas, inibem a ação de enzimas, como por exemplo, as amilases, lipases e tripsina, que são muito importantes para o metabolismo normal do organismo humano, impedindo assim, a absorção de carboidratos, lipídios e proteínas, o que pode acarretar perda de peso, podendo ser uma ferramenta importante para auxiliar no tratamento da obesidade (GENOVESE; LAJOLO, 2001).

A presença desses inibidores em produtos naturais tem sido bastante estudada para a busca de medicamentos baseados no mecanismo de inibição enzimática que ocasiona alterações benéficas no metabolismo, se apresentando como uma excelente alternativa para o desenvolvimento seguro e eficaz de drogas antiobesidade (BHUTANI; BIRARI; KAPATI, 2007).

Muitas pesquisas demonstram a eficácia, a importância e o potencial de uso de inibidores dessas enzimas no tratamento da obesidade e comorbidades associadas e reforçam a necessidade da busca por novas fontes desses inibidores: amilases (OBIRO; ZHANG; JIANG, 2008; UDANI et al., 2009), glicosidases (KWON; APOSTOLIDIS; SHETTY, 2006) e lipases (SHARMA; SHARMA; SEO, 2005; SOUZA et al., 2011). Assim, inibidores de enzimas digestivas que ajudem a limitar a absorção intestinal de carboidratos e gorduras na fase inicial podem revelar-se úteis como auxiliares no tratamento da obesidade.

2.8.1 Amilases

Amilases são algumas das enzimas produzidas por animais, plantas e microrganismos que participam da hidrólise de carboidratos de forma a

permitirem a sua utilização como fonte de energia. Para que essa energia possa ser utilizada pelos seres vivos, os carboidratos precisam ser hidrolisados a monômeros e absorvidos. Dessa maneira, estes podem participar dos processos catabólicos intracelulares, liberando a energia que armazenam (WANG et al., 2008).

A principal forma de armazenamento de carboidratos em plantas, inclusive naquelas utilizadas na alimentação pelos humanos, é o amido, um complexo de reserva nutritiva, formado por dois tipos de polímeros de glicose, a amilose e a amilopectina, sendo a amilose um polímero não ramificado de glicose unidas por ligações glicosídicas α-1,4, e a amilopectina, um polímero ramificado possuindo cadeia principal de glicose unidas por ligações glicosídicas α-1,4 interligadas por ligações α-1,6 (SORENSEN et al., 2004).

Desta forma, devido à complexidade do amido, são necessárias muitas enzimas para sua degradação completa e consequente absorção e utilização como fonte energética. Estas são divididas em grupos de acordo com seu mecanismo de ação em: endo-amilases, exo-amilases e enzimas de desramificação.

As endo-amilases, conhecidas como α-amilases (1,4-glucano-4-glucanohidrolases), classificadas pela sigla E.C 3.2.1.1, clivam as ligações glicosídicas α-1,4 presentes na parte interna do amido, convertendo-o em açúcares simples para que possam ser completamente digeridos pelo organismo. A hidrólise exercida por esta enzima ocorre em diversos passos, começando com a ligação do substrato a enzima, seguida da separação do polímero, e um processo hidrolítico para libertação de diversas moléculas menores (ANTUNES, 2008). Os produtos resultantes da digestão pela α-amilase são uma mistura de maltose, maltotriose e unidades ramificadas de glicose.

A inibição da α-amilase pode ter amplo campo de aplicação, como por exemplo, no tratamento adjuvante da obesidade e diabetes tipo 2, pois sua

inibição pode dificultar a digestão dos carboidratos reduzindo assim a absorção e consequentemente a disponibilidade desses açúcares, promovendo ou apoiando a perda de peso (CHOKSHI, 2007). Outro campo de aplicação tem sido na agricultura no controle de insetos e pragas.

Diversos tipos de compostos orgânicos são conhecidos pela sua atividade inibitória sobre a amilase. Os inibidores não proteicos geralmente são moléculas de baixo peso molecular como os compostos fenólicos e açúcares, enquanto que os proteicos são classificados de acordo com sua estrutura, sendo que destes um grupo é encontrado em fungos e os outros seis são oriundos de vegetais superiores (SILVA, 2008). Inibidores de amilase já foram identificados em várias espécies de plantas, sendo que a descoberta de novas plantas com potencial inibitório pode se tornar um forte aliado para auxiliar no tratamento da obesidade e suas complicações.

Outro grupo de enzimas utilizadas para degradação do amido são as exo-amilases, representadas pela α-glicosidase, que cliva as ligações glicosídicas α-1,4 externas da amilose, amilopectina e polissacarídeos relacionados (OTA et al., 2009).

Os inibidores de α-glicosidases também são agentes de grande interesse terapêutico, uma vez que inibindo a degradação de açúcares para absorção pelo organismo apresentam atividade contra a obesidade (KANDRA et al., 2005). Outras aplicações desses inibidores são o tratamento de infecções virais, tumores, osteoartrites, entre outras doenças.

Alguns fármacos como a arcabose e o miglitol são utilizados como inibidores de α-glicosidases, e utilizados no tratamento da obesidade, porém, além desses, poucas opções são encontradas na terapêutica, justificando a necessidade de estudos para obtenção de novas fontes de inibidores dessa enzima.

2.8.2 Lipases

As lipases são descritas como triacilglicerol lipase (E.C.3.1.1.3), que atuam sobre ligações ésteres presentes em triglicérides, liberando ácidos graxos e glicerol, constituindo uma classe especial de carboxilesterases. Elas hidrolisam principalmente triglicérides de cadeia longa, ou seja, com cadeia acila com mais de 10 átomos de carbono. Essas enzimas são de grande importância, não apenas por atuarem sobre substratos insolúveis em água, mas também por catalisarem reações diferentes, como as de hidrólise, esterificação, alcoólise e aminólise (MUKHERJEE, 2003). Essa diversidade de propriedades propicia a utilização das lipases em diferentes campos de aplicação.

Entre as lipases, se tem a pancreática, que é a principal enzima lipolítica sintetizada pelo pâncreas e desempenha papel importante na digestão eficiente dos triglicérides. Sua atuação ocorre pela remoção dos ácidos graxos, preferencialmente nas posições 1 e 3 dos triglicérides ingeridos na dieta, liberando 2-monoacilglicerol e ácidos graxos de cadeia longa saturados e poli-insaturados como produtos lipolíticos, sendo responsável pela hidrólise de 50 a 70% do total de gorduras ingeridas na dieta (MUKHERJEE, 2003; SHI; BURN, 2004).

A inibição da lipase pancreática se mostra como uma opção bastante promissora para auxiliar no tratamento da obesidade, já que pode limitar o impacto nutricional da absorção de lipídios, sendo um dos mecanismos mais estudados para a determinação e eficácia potencial de produtos naturais como agentes antiobesidade. Agentes antilipase agem por meio da redução ou do bloqueio da digestão de lipídios, impedindo a absorção destes pelo trato-gastrointestinal e, portanto, imitam o efeito da redução da ingestão de alimentos.

Um dos principais medicamentos usados para tratamento da obesidade, o orlistat, atua por meio de inibição de lipases, e seu sucesso tem estimulado

muitas pesquisas para identificação de novos inibidores dessas enzimas. Entre essas pesquisas, cita-se a busca por produtos naturais com ação inibitória sobre a lipase, que tem sido alvo de muitos estudos, principalmente pela descoberta em plantas de moléculas inibidoras pertencente a diversas classes de compostos como as saponinas, lectinas e alguns compostos fenólicos (BIRARI; BHUTANI, 2007; SHARMA; SHARMA; SEO, 2005; SOUZA et al., 2011).

2.8.3 Tripsina

Tripsina (E.C.3.2.21.4) é uma enzima digestiva produzida pelo pâncreas na forma de seu zimogênio enzimaticamente inativo (tripsinogênio) e levado até o intestino, onde por ação enzimática da enteroquinase e/ou tripsina se torna ativa e realiza a digestão das proteínas, liberando peptídeos (BARCELOS, 2004). Pertence à classe das endopeptidases, ou seja, é uma proteína que hidrolisa ligações peptídicas distantes dos extremos C e N-terminal. Além da digestão intestinal, atua em processos de lise celular de organismos invasores, coagulação sanguínea, fertilização, entre outros.

A tripsina é susceptível, em vários graus, a alguns inibidores naturais de proteases, podendo esses inibidores ser de natureza proteica ou não proteica (SCHACHTER, 1980). O bloqueio da ação dessa enzima resulta em aumento excessivo da concentração plasmática de colecistoquinina, e desta forma, o pâncreas é continuamente estimulado a liberar mais enzima, provocando hipertrofia pancreática (SILVA; SILVA, 2000). Embora a hipertrofia pancreática seja frequentemente relatada em animais de laboratório e supostamente desencadeada pela presença de inibidores de proteases, não existem evidências de efeitos deletérios em seres humanos, mesmo no caso de leguminosas consumidas cruas, como no caso do amendoim, que

comprovadamente possui inibidores de tripsina, não havendo qualquer relato de efeito nocivo à saúde (SGARBIERI, 1987).

Alguns estudos mostram que a inibição da digestão de proteínas pode ser benéfica para a saúde na medida em que diminui o valor calórico total de uma refeição e abranda a digestão da mesma levando a uma sensação de saciedade pela passagem do bolo alimentar através do trato gastrointestinal, podendo contribuir para perda de peso. Porém, como os aminoácidos obtidos da digestão das proteínas são substâncias essenciais para o organismo, a inibição da tripsina também pode acarretar problemas ao organismo, sendo considerada como um efeito antinutricional (MCDOUGALL et al., 2005).

2.9 Radicais livres e antioxidantes

Radicais livres (RL) são espécies químicas capazes de existência independente, que contenham um ou mais elétrons desemparelhados, com meia vida curtíssima. Dessa forma, eles são altamente reativos, sendo capazes de atacar qualquer biomolécula, se caracterizando como moléculas destrutivas (BIANCHI; ANTUNES, 1999).

Os radicais mais importantes presentes nos organismos vivos são radicais hidroxila ($HO^{\bullet}$), ânion superóxido ($O_2^{-\bullet}$), peroxila ($ROO^{\bullet}$), alcoxila ($RO^{\bullet}$) e óxido nítrico ($^{\bullet}NO$). Existem outras espécies reativas nos organismos que não são radicais livres, mas podem induzir reações radicalares, a exemplo do oxigênio singlete ($^1O_2$), peróxido de hidrogênio (H_2O_2) e ácido hipocloroso (HOCl) (YILDIRIM; MAVI; KARA, 2001).

Esses radicais podem ser gerados por fontes endógenas ou exógenas. Por fontes endógenas, originam-se de processos biológicos que normalmente ocorrem no organismo, tais como redução de flavinas e tióis; resultado da atividade de oxidases, cicloxigenases, lipoxigenases, desidrogenases e

peroxidases; presença de metais de transição no interior da célula e de sistemas de transporte de elétrons. Essa geração de RL envolve várias organelas celulares, como mitocôndrias, lisossomos, peroxissomos, núcleo, retículo endoplasmático e membranas. As fontes exógenas incluem tabagismo, poluição do ar, solventes orgânicos, anestésicos, pesticidas e radiações (SOARES, 2002; YILDIRIM; MAVI; KARA, 2001).

Os Radicais livres desempenham um papel importante no organismo onde apresentam funções contra organismos infecciosos, ajudam na manutenção do sistema imunológico, entre outras (HALLIWELL, 2011). Geralmente, o metabolismo equilibra sua presença no corpo, mas o problema é que, quando se formam em excesso, sob certas condições anormais, cria-se uma situação que se denomina estresse oxidativo (BARREIROS; DAVI; DAVI, 2006). Atualmente, sabe-se que o estresse oxidativo está associado a vários processos de toxicidade celular, tais como danos na estrutura de proteínas, peroxidação lipídica nas membranas, alteração do DNA e inativação de enzimas (BIANCHI; ANTUNES, 1999).

Dessa maneira, estão envolvidos com várias patologias, como doenças do trato gastrointestinal, como a úlcera gástrica e intestinal; doenças relacionadas ao sistema nervoso, como a síndrome de Down e o mal de Alzheimer; doenças cardiovasculares, como a aterosclerose e câncer. O conjunto de danos causados ao organismo pode resultar em envelhecimento precoce, podendo ser a causa ou o agravante dessas doenças (HALLIWELL; GUTTERIDGE; CROSS, 1992). Além de ser a causa de várias doenças, os RL também causam danos a alimentos, afetando nesses, a qualidade nutricional, cor, aroma, sabor, textura, e são responsáveis pela formação de compostos potencialmente tóxicos e antinutricionais para o organismo.

Com a descoberta dos efeitos deletérios causados pelos RL no organismo humano e nos alimentos surgiu um grande interesse pelo estudo de

substâncias conhecidas como antioxidantes. Antioxidantes são substâncias capazes de retardar ou inibir a oxidação de substratos oxidáveis e têm como principal função proteger os constituintes celulares e manter o estado redox celular (HALLIWELL, 2001). Eles previnem os efeitos deletérios da oxidação, inibindo o início da lipoperoxidação, sequestrando RL e/ou quelando íons metálicos; protegem organismos aeróbicos do estresse oxidativo (RODRIGUES et al., 2003). Apesar de os antioxidantes doarem elétrons para determinados substratos, eles não se tornam RL, pois eles são estáveis em ambas às formas.

O organismo possui um sistema enzimático (endógeno) para combater os RL, sendo composto por diversas enzimas, destacando-se a superóxi-dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx); sendo o primeiro a agir, ele evita que o peróxido de hidrogênio e radicais como o superóxido se acumulem (HALLIWELL, 2011).

A SOD tem papel fundamental na defesa do organismo contra as espécies reativas de oxigênio, pois atua na remoção do radical superóxido. Já a CAT decompõe o peróxido de hidrogênio em excesso, um produto tóxico do organismo, em água e oxigênio molecular, o que também é feito pela enzima GPx, porém, ela atua nessa reação somente quando o peróxido de hidrogênio está presente em baixas concentrações (BARREIROS; DAVI; DAVI, 2006; BIANCHI; ANTUNES, 1999; ROVER JÚNIOR; HOEHR; VELLASCO, 2001).

Mesmo apresentando alta capacidade de defesa do organismo, as defesas antioxidantes endógenas não são infalíveis, não protegendo totalmente os componentes celulares. Dessa forma, é bem estabelecido que antioxidantes obtidos de fontes exógenas são indispensáveis para a defesa apropriada contra os RL e, assim, apresentam importante papel na manutenção da saúde

Em algumas pesquisas há relatos de que muitos vegetais, frutas e plantas medicinais apresentam, em sua constituição, compostos com ação antioxidante, entre os quais se pode citar as vitaminas C e E, compostos fenólicos,

carotenoides e alguns minerais (DJERIDANE et al., 2006). Muitos destes fitoquímicos possuem uma capacidade antioxidante que está associada com uma menor ocorrência de algumas doenças humanas.

A vitamina C atua como antioxidante pelo fato que o ascorbato pode doar átomos de hidrogênio para reduzir outro composto. Ela atua na fase aquosa como excelente antioxidante sobre os RL, mas não é capaz de agir nos compartimentos lipofílicos para inibir a peroxidação dos lipídios.

Nos compostos fenólicos, o potencial antioxidante é atribuído às propriedades redox dos grupos hidroxil e a sua relação com diferentes partes da estrutura química que pode ser determinada por cinco fatores: reatividade como agente doador de hidrogênio e elétrons, estabilidade do radical flavanoil formado, reatividade frente a outros antioxidantes, capacidade de quelar metais de transição e solubilidade e interação com as membranas (BARREIROS; DAVI; DAVI, 2006). Estes compostos podem agir tanto na etapa de iniciação como na propagação do processo oxidativo e os produtos intermediários formados por sua ação são relativamente estáveis, devido à ressonância do anel aromático que apresentam (RAMALHO; JORGE, 2006; SOARES, 2002).

Os carotenoides agem *in vivo* no combate aos RL, atuam como desativadores do oxigênio singleto e tripleto ou como sequestradores dos radicais peroxila. Eles são capazes de interromper as reações RL que podem oxidar lipídios insaturados e proteger o DNA contra ataques desses radicais. A capacidade de exterminar RL dos carotenoides se dá pela sua estrutura contendo duplas ligações alternadas, que tem a capacidade de acomodar cargas ou elétrons desemparelhados.

Os carotenoides atuam como antioxidantes lipofílicos e juntamente com a vitamina C e os compostos fenólicos (antioxidantes hidrofílicos) formam uma forte defesa contra os RL por atuarem em diferentes compartimentos das células.

Assim, esses compostos mostram potenciais para prevenir e combater os RL e as diversas doenças causadas por eles.

3 CONSIDERAÇÕES FINAIS

A prevalência de obesidade tem aumentado, em taxas alarmantes, em todo o mundo, e vem se tornando o maior problema de saúde na sociedade moderna, evidenciando a necessidade de adjuvantes para auxiliar em seu tratamento. As plantas medicinais são ricas fontes de compostos bioativos com potencial de utilização terapêutica, podendo estes ser uma alternativa viável para o desenvolvimento de drogas eficazes e seguras para auxiliar no tratamento da obesidade.

Neste trabalho observou-se que as plantas estudadas, *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr. e *Tournefortia paniculata* Cham. (marmelinho), mostram grande diversidade de fitoquímicos com potenciais para serem utilizadas em preparações farmacológicas com possíveis benefícios a saúde. Entre as plantas, a *T. paniculata* é a que apresenta os mais elevados teores de fitoquímicos (compostos fenólicos, saponinas e vitamina C), alta inibição sobre as enzimas α-amilase e α-glicosidase e atividade antioxidante, em ensaios *in vitro*.

A avaliação dos efeitos terapêuticos e toxicológicos da *T. paniculata* revelou que as folhas desta planta apresentam potencial antiobesidade e antioxidante, pois causam redução nos níveis de glicose, triacilgliceróis e gordura hepática (farinha e extrato aquoso) e do consumo alimentar e peroxidação lipídica (extrato aquoso), e não causam efeito genotóxico, nas condições avaliadas. Porém, um estudo mais detalhado do potencial medicinal e toxicológico assim como a caracterização dos fitoquímicos presentes na *T. paniculata* ainda se faz necessário para melhor compreensão de seus mecanismos no tratamento da obesidade, além de outras possíveis aplicações terapêuticas.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1

CHEMICAL COMPOSITION OF MEDICINAL PLANTS USED AS AUXILIARIES IN THE TREATMENT OF OBESITY

Anderson Assaid Simão; Angelita Duarte Corrêa

Submetido para a Revista Bioscience Journal.
Artigo redigido conforme norma da revista. Este artigo é uma versão preliminar, considerando que o conselho editorial da revista poderá sugerir alterações para adequá-lo ao seu estilo próprio.

ABSTRACT: Medicinal plants are rich sources of bioactive compounds with potential therapeutic use, and the knowledge about the composition of the compounds present in their extracts is critical to ensure the scientific proof of the efficiency and safety of their use. The objective of this study was to perform phytochemical screening, determine percent and mineral compositions, as well as the content of some bioactive compounds in the medicinal plants *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja),

Garcinia cambogia Desr. and *Tournefortia paniculata* Cham. (marmelinho), aiming to find substances of pharmacological interest, in order to aid in the treatment of obesity and other diseases. Important substances with therapeutic potential, especially phenolic compounds, saponins and dietary fiber, were found in all plants, as well as calcium, in *G. cambogia* and *S. ferruginea*. It was possible to conclude that the studied plants show a great diversity of phytochemicals with potential to be used in pharmaceutical preparations with possible health benefits. However, recommending the use of these plants regarding possible risks and benefits for human health is premature, and additional studies of toxicity, efficiency and safety are necessary, particularly in relation to the saponins found in all plants and to the high levels of phenolic compounds in *T. paniculata* (36.19 g 100 g^{-1} dry matter).

KEYWORDS: Phytochemical screening. Percent composition. Minerals. Bioactive compounds. Medicinal plants. Obesity.

INTRODUCTION

The prevalence of obesity has increased steadily, and is considered an important public health problem in developed countries and a global

epidemic by the World Health Organization (WORLD HEALTH ORGANIZATION - WHO, 2010). Its treatment is essential, as it is associated with various diseases, such as diabetes, some cancers, cardiovascular diseases, among others (GUH et al., 2009).

Despite being one of the oldest diseases known to man, pharmacological options for the treatment of obesity are still limited and have many side effects (MAHAN; SCOTT-STUMP, 2008). Given the above, the use of medicinal plants is being widely explored, both by the population, due to easy access, low cost, no need for prescriptions, a belief in the absence of toxic effects, and by the pharmaceutical industry, which sees these plants as a viable alternative to the future development of drugs that induce weight reduction effectively and safely (MAYER et al., 2009). Studies show that several natural products, including extracts and compounds isolated from plants, are used for reducing body weight and preventing obesity (SOUZA et al., 2011; SIMÃO et al., 2012).

Various medicinal plants, such as *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr. and *Tournefortia paniculata* Cham. (marmelinho), are used in the treatment of obesity (SOUZA et al., 2011;

SIMÃO et al.; 2012; 2013). However, most of these plants have no scientific evidence of their efficacy and safety in the treatment of this disease, and their use is solely based on popular beliefs, highlighting the need for scientific studies to elucidate the chemical constituents of these plants, in order to help in the pharmacological/toxic understanding regarding their use in the treatment of obesity and other diseases.

This study aimed to perform phytochemical screening, determine the chemical and percent compositions, as well as the content of some bioactive compounds from the medicinal plants *A. vera*, *B. trimera*, *S. ferruginea*, *G. cambogia* and *T. paniculata*, used as auxiliaries in the treatment of obesity, in order to find substances of pharmacological interest and ensure the use of these plants by the population in industrialized and phytotherapic formulations.

MATERIAL AND METHODS

Sample collection and preparation

B. trimera and *T. paniculata* leaves, as well as the stem bark of *S. ferruginea*, were acquired in the municipal market of Belo Horizonte, Minas Gerais, in January 2011, and transported to the laboratory. *B.*

trimera and *T. paniculata* leaves were washed with tap and distilled water, and then placed together with the stem bark obtained from *S. ferruginea* in forced air circulating ovens to dry for 48 hours, at a temperature of ±35°C. After drying, the leaves and the bark were ground in a Wiley-type mill, and the flours were stored in hermetically sealed flasks until the analyses. The commercial powder of *A.vera*, obtained by lyophilization of the plant mucilage, and of *G. cambogia*, obtained by spray drying, were acquired from FLORIEN, a distributor of pharmaceutical raw materials.

Analyses

Phytochemical screening

Flours from medicinal plants (FMP) were subjected to phytochemical screening, and specific reagents were used for each chemical group, with the use of chemical reactions that result in the development of color and/or precipitate, characteristic for each class of substances (MATOS, 1997). Organic acids were analyzed, as well as reducing sugars, alkaloids, anthraquinones, azulenes, carotenoids, catechins, depsides and depsidones, coumarin derivatives, steroids and

triterpenoids, flavonoids, cardiotonic glycosides, sesquiterpene lactones and other lactones, polysaccharides, proteins and amino acids, saponins and tannins.

Percent composition

Moisture contents were determined in an oven at 105ºC, until constant weight. The ether extract was determined using a Soxhlet continuous extractor. The crude protein was measured by the Kjeldahl method, using the conversion factor of 6.25 (N x 6.25). Ash and fixed mineral residue were obtained from a defined quantity of samples by incineration (550°C) in a muffle furnace, thus determining the percentage of residue. Total, soluble and insoluble dietary fiber were determined by the enzymatic method. The non-nitrogen extract was determined by the difference between 100 and the sum, in dry matter, of the ether extract, protein, ash and total dietary fiber. These analyzes of percent composition were performed using the methodology described by the Association of Official Analytical Chemists - AOAC (2005).

Mineral composition

In order to quantify the minerals (Fe, Zn, Mn, Cu, Ca, Mg, P, K and S), the samples were subjected to a nitropercloric digestion in digester blocks with temperature control. P and S were determined by colorimetry, K by flame photometry and Ca, Mg, Cu, Mn, Zn and Fe by atomic absorption spectrophotometry. For all analyzes, the procedures described by Malavolta et al. (1997) were used.

Phenolic compounds

The extraction of phenolic compounds was carried out with 50% methanol, under reflux for three consecutive times, at 80°C, and the extracts were collected, evaporated up to 25 mL and submitted to phenolic compound measurement, using the Folin-Denis reagent, and tannic acid as a standard (AOAC, 2005).

Oxalic acid

For the measurement of oxalic acid, the method developed by Loures and Jokl (1990) was employed; according to this method, the

oxalic acid was hot extracted with hydrochloric acid, precipitated and quantified by titration of calcium oxalate with potassium permanganate.

Nitrate

Nitrate was extracted with distilled water at 45°C. In the dosage, a complex is formed by the nitration of salicylic acid under highly acidic conditions, then the reading at 410 nm is performed in basic solutions (pH greater than 12). The absorbance of the material was directly proportional to the present amount of nitrate, without the occurrence of interference of ammonium, nitrite, or chlorine ions. Potassium nitrate was used as a standard (CATALDO et al., 1975).

Trypsin inhibitor

Trypsin inhibitors were extracted with 0.1 mol L^{-1} NaOH under magnetic stirring. After centrifugation at 10,000 x g for 60 minutes, an aliquot of the supernatant was used in the enzyme assay (KAKADE et al., 1974). The trypsin activity was determined according to the methodology proposed by Erlanger et al. (1961), in which 200 µL of the plant extracts and 200 µL enzyme were incubated in a water bath at 37°C for four time

periods after the addition of 800 µL of the substrate benzoyl-DL-arginine-p-nitroanilide (BApNA) prepared in TRIS buffer (tris(hydroxymethyl)aminomethane) at 0.05 mol L^{-1}, pH 8.2 plus 20 mmol L^{-1} $CaCl_2$. The reaction was stopped by adding 200 µL of 30% acetic acid, and the product was read in a spectrophotometer at 410 nm.

Trypsin inhibition was obtained from the determination of the line slopes (absorbance vs. time) of the activity assays of the control enzyme (without plant extract), and enzyme + inhibitor (with plant extract). The slope of the line is due to the formation speed of the product per minute of reaction, and the presence of the inhibitor causes a reduction in the slope. Absorbance values were converted to nmol using the molar extinction coefficient of BApNA determined by Erlanger et al. (1961).

Saponins

The extraction of saponins was performed with ethanol under stirring at room temperature for 60 minutes. The total saponin content was determined by the reaction of saponins with anisaldehyde, and digitonin was used as a standard (BACCOU et al., 1977).

Statistical analysis

Data are the average of three replicates ± standard deviation and were statistically evaluated by analysis of variance, and the means were compared using the Scott Knott test ($P < 0.05$) with the aid of the R software (R Development Core Team, 2011).

RESULTADOS E DISCUSSÃO

The phytochemical screening is characterized by the identification of the chemical compounds present in plant material. The results of the phytochemical screening in the FMPs used as auxiliaries in the treatment of obesity are shown in Table 1.

The results indicate the presence of different metabolic groups of pharmacological interest in the studied plants, such as tannins (*G. cambogia* and *T. paniculata*), which prevent lipid peroxidation, degradation of nucleotides and accelerate the healing process (PIETTA et al., 2000; MONTEIRO et al.; 2005); depsides and depsidones (*B. trimera*, *S. ferruginea* and *T. paniculata*), which present antioxidant (HIDALGO et al., 1994), antiviral (NEAMATI et al., 1997), antitumor, analgesic and

Table 1. Phytochemical screening of medicinal plants.

Constituents	*Aloe vera*	*Baccharis trimera*	*Simaba ferruginia*	*Garcinia cambogia*	*Tounerfotia paniculata*
Organic acids	(-)	(-)	(-)	(-)	(+)
Reducing sugars	(+)	(+)	(+)	(+)	(+)
Alkaloids	(-)	(-)	(-)	(-)	(-)
Anthraquinones	(-)	(-)	(-)	(-)	(+)
Azulenes	(+)	(+)	(+)	(-)	(-)
Carotenoids	(-)	(+)	(-)	(-)	(+)
Catechins	(-)	(-)	(-)	(+)	(+)
Depsides and depsidones	(-)	(+)	(+)	(-)	(+)
Coumarin derivatives	(-)	(-)	(-)	(-)	(-)
Steroids and triterpenoids	(-)	(+)	(+)	(-)	(+)
Flavonoids	(-)	(+)	(+)	(-)	(+)
Cardiac glycosides	(-)	(-)	(-)	(-)	(-)
Sesquiterpene lactones and other lactones	(-)	(-)	(-)	(-)	(+)
Polysaccharides	(+)	(+)	(-)	(-)	(-)
Proteins and amino acids	(+)	(+)	(+)	(+)	(+)
Saponins	(+)	(+)	(+)	(+)	(+)
Tannins	(-)	(-)	(-)	(+)	(+)

The sign (+) indicates presence and (-) indicates absence of the metabolite.

antipyretic properties (OKUYAMA et al., 1995); carotenoids (*B. trimera* and *T. paniculata*), with properties associated with various physiological actions, may be precursors of vitamin A, and are implicated in protective mechanisms against oxidative damage in cells, cardiovascular diseases, cataract and macular degeneration, besides contributing to the improvement of the immune system (SIMÕES et al., 2007); triterpenoids (*B. trimera*, *S. ferruginea* and *T. paniculata*), with medicinal properties, and great potentialities in biological activities, such as anti-inflammatory, bacterial, fungicidal, antiviral, analgesic, cardiovascular, antitumor, etc. (SIMÕES et al., 2007), among other groups of metabolites.

Alkaloids and cardiac glycosides were not detected in any of the analyzed plants. The preliminary phytochemical screening provides an overview of the chemical groups in the plants, but further studies are needed to determine the concentration and characterization of these substances.

The results of the phytochemical screening are in agreement with other studies with these plants, which also showed the presence and absence of these metabolites, such as the study by Vásquez et al. (1996),

with the *A. vera* gel and its extracts, which recorded the presence of saponins and absence of tannins, flavonoids and alkaloids; those by Rodrigues et al. (2009), with *B. trimera*, who observed the presence of flavonoids, saponins and absence of alkaloids, anthraquinones, coumarins and cardiac glycosides; those by Moraes & Souza (2007), with *T. paniculata* leaves, which reported the presence of flavonoids, tannins and absence of alkaloids, and those by Subhashini et al. (2011), with *G. cambogia*, who found saponins, tannins, sugars, proteins and absence of flavonoids, but the result for the alkaloids test was positive. In the literature consulted, there were no studies on the phytochemical screening of *S. ferruginia*.

The results of the chemical composition in the FMPs are shown in Table 2. Regarding ether extract, the contents found were low, and *T. paniculata* had the highest content (3.88 g 100 g^{-1} dry matter - DM). Ether extract was not detected in *A. vera* and *G. cambogia*. The contents of crude protein were also relatively low, and the ash contents were very high in *G. cambogia* (34.18 g 100 g^{-1} DM).

The highest content of dietary fiber (DF) was found in *B. trimera* (66.26 g 100 g^{-1} DM), and the lowest in *A. vera* (10.97 g 100 g^{-1} DM).

Table 2. Percent composition, in g 100 g^{-1} dry matter, of medicinal plants.

Constituents	*Aloe vera*	*Simaba ferruginea*	*Baccharis trimera*	*Garcinia cambogia*	*Tournefortia paniculata*
Ether Extract	ND[2]	1.97±0.11[c]	2.49±0.20[b]	ND	3.88±0.21[a]
Crude Protein	1.54±0.11[d]	8.96±0.11[b]	7.15±0.22[c]	1.78±0.25[d]	10.78±0.35[a]
Ash	3.30±0.08[e]	8.53±0.04[b]	6.18±0.05[c]	34.18±0.24[a]	3.61±0.01[d]
Insoluble Fiber	5.20±0.18[d]	45.20±0.43[c]	64.67±0.50[a]	3.09±0.18[d]	46.71±0.80[b]
Soluble Fiber	5.77±0.33[b]	3.74±0.22[c]	1.60±0.07[d]	30.11±1.19[a]	1.16±0.05[d]
Total Fiber	10.97±0.42[d]	48.94±0.62[b]	66.26±0.53[a]	33.08±1.02[c]	47.87±0.86[b]
NNE[1]	84.18±0.51[a]	31.60±0.64[c]	17.93±0.60[d]	30.96±1.17[c]	33.86±0.11[b]

Data are the mean of three replicates ± standard deviation. Same letter in rows do not differ by the Scott-Knott test ($P < 0.05$). [1]NNE: Non-nitrogen extract. [2]ND: Not detected. Moisture contents in the flours from medicinal plants, in g 100 g^{-1}: *Aloe* = 8.53; *Simaba* = 8.42; *Baccharis* = 8.56; *G. cambogia* = 3.94; *Tournefortia*: 9.90.

The high contents of soluble DF shown by *G. cambogia* (30.11 g 100 g^{-1} DM), as well as insoluble DF found in *B. trimera* and *T. paniculata*, 64.67 and 46.71 g 100 g^{-1} DM, respectively, should also be mentioned.

Epidemiological studies suggest that dietary fibers are capable of preventing obesity and weight gain, besides contributing to the reduction of the risk for developing diabetes, cardiovascular diseases, among others (LIU et al., 2003).

In general, the soluble DF helps in the treatment of obesity, because it slows gastric emptying, glucose absorption, and reduces cholesterol in blood serum (RIQUE et al., 2002; MELO; LAAKSANEM, 2009). On the other hand, the insoluble DF accelerates intestinal transit and increases feces weight (RIQUE et al., 2002). Thus, the presence of these fibers in the composition of the analyzed plants can be characterized as a strong ally in the treatment of obesity.

The non-nitrogen extract or glicidic fraction consists mainly of sugars. Thus, the highest content was found in *A. vera* (84.18 g 100 g^{-1} DM), followed by *T. paniculata*, *S. ferruginea* and *G. cambogia*.

Besides the influence exerted by macronutrients in the development of obesity, micronutrients, especially minerals, have received much attention, due to the influence on body weight control.

Table 3 shows the mineral contents of FMPs used as auxiliaries in the treatment of obesity. It is important to highlight the high levels, in mg 100 g^{-1} DM, of potassium (1,009.44) and manganese (47.07) in *A. vera*, of potassium (1,383.13), calcium (2,660.70) and iron (55.67) in *S. ferruginea*, of potassium (2,336.69) and iron (39.98) in *B. trimera* and of calcium (7,273.23), sulfur (999.38) and iron (73.69) in *G. cambogia*.

Minerals play important roles in the human body, and one of them is regulation of metabolism. The absence of some minerals can cause metabolic problems, such as slowing in metabolism, which may lead to weight gain. In addition, some minerals can participate in the digestion of carbohydrates, fats and proteins, and can act as aids in weight reduction.

The high levels of calcium shown by *G. cambogia* and *S. ferruginea* can be extremely effective for the treatment of obesity, since calcium intake is involved in the regulation of body weight (ST-ONGE, 2005).

Table 3. Mineral composition, in g 100 g^{-1} dry matter, of medicinal plants.

Mineral	*Aloe vera*	*Simaba ferruginea*	*Baccharis trimera*	*Garcinia cambogia*	*Tournefortia paniculata*
P	44.40±0.00^{c}	83.84±6.31^{a}	83.84±6.31^{a}	76.44±0.00^{b}	44.40±0.00^{c}
K	1,009.44±6.31^{c}	1,383.13±27.48^{b}	2,336.69±49.31^{a}	742.59±69.31^{d}	495.75±12.82^{e}
Ca	845.45±6.31^{c}	2,660.70±16.68^{b}	612.42±0.00^{d}	7,273.23±33.46^{a}	381.06±23.10^{e}
Mg	258.74±6.31^{b}	163.79±0.00^{c}	142.17±0.00^{d}	419.88±6.01^{a}	147.98±6.41^{d}
S	258.74±27.51^{e}	731.60±10.92^{b}	630.65±12.63^{c}	999.38±63.32^{a}	527.19±23.54^{d}
Cu	13.12±0.21^{a}	3.10±0.32^{c}	7.81±0.76^{b}	3.38±0.38^{c}	2.04±0.27^{d}
Mn	47.07±0.49^{a}	5.24±0.11^{e}	13.47±0.27^{c}	18.75±0.11^{b}	7.00±0.17^{d}
Zn	1.57±0.23^{d}	2.20±0.16^{c}	3.84±0.00^{b}	6.51±0.15^{a}	1.40±0.05^{d}
Fe	2.58±0.38^{e}	55.67±0.16^{b}	39.38±1.44^{c}	73.69±0.65^{a}	11.51±0.43^{d}

Data are the mean of three replicates ± standard deviation. Same letter in rows do not differ by the Scott-Knott test ($P < 0.05$). Moisture contents in the flours from medicinal plants, in g 100 g^{-1}: *Aloe* = 8.53; *Simaba* = 8.42; *Baccharis* = 8.56; *G. cambogia* = 3.94; *Tournefortia*: 9.90.

Variations in the contents of circulating calcium can affect food intake. High calcium contents decrease food intake and it is assumed that this effect is due to the greater availability of calcium to ion channels. Several studies show that obese patients submitted to diets with high contents of calcium present a reduction in body fat (HEANEY, 2003; MOORE et al., 2004; ZEMEL et al., 2004).

Bioactive compounds, present in plants, act in health maintenance and in the reduction in the risk of disease; however, depending on the concentration, these compounds can cause damage to health, highlighting the need for characterization studies of these compounds in plant extracts. The bioactive compounds in FMPs used as auxiliaries in the treatment of obesity are shown in Table 4.

Phenolic compounds were found in all plants, and *T. paniculata* showed the highest contents (36.19 g 100 g^{-1} DM), and *G. cambogia* (0.09 g 100 g^{-1} DM) and *A. vera* (0.15 g 100 g^{-1} DM) showed the lowest. The maximum dose of phenolic compounds suggested for humans is about 1 g day^{-1} (SCALBERT et al., 2005); thus, with only 3 g of *T. paniculata,* the daily limit is reached. These plants are not used in food, but for the treatment of obesity. If consumed in small quantities, only *T.*

Table 4. Contents of bioactive compounds, in dry matter, of medicinal plants.

Medicinal plant	Phenolic compounds (g 100 g^{-1})	Oxalic acid (g 100 g^{-1})	Nitrate (g kg^{-1})	Trypsin inhibitor (TIA mg^{-1})[1]	Saponins (g 100 g^{-1})
Aloe vera	0.15±0.02[d]	ND[2]	0.77±0.01[d]	ND	0.07±0.00[d]
Simaba ferruginea	1.62±0.03[c]	0.97±0.09[a]	0.81±0.02[c]	0.17±0.02[c]	0.13±0.00[c]
Baccharis trimera	4.03±0.21[b]	0.91±0.03[a]	2.68±0.12[b]	6.26±0.32[b]	0.54±0.03[b]
Garcinia cambogia	0.09±0.01[d]	ND	0.16±0.01[e]	ND	0.07±0.00[d]
Tournefortia paniculata	36.19±0.91[a]	0.93±0.02[a]	6.96±0.13[a]	22.01±2.40[a]	1.00±0.09[a]

Data are the mean of three replicates ± standard deviation. Same letter in columns do not differ by the Scott-Knott test ($P < 0.05$). [1]TIA: trypsin inhibitor activity, in nmol min^{-1} mg^{-1}. [2]ND: Not detected. Moisture contents in the flours from medicinal plants, in g 100 g^{-1}: *Aloe* = 8.53; *Simaba* = 8.42; *Baccharis* = 8.56; *G. cambogia* = 3.94; *Tournefortia*: 9.90.

paniculata can offer some kind of health risk, due to the high contents of phenolic compounds presented.

The contents of phenolic compounds in the leaves of *B. Trimera* (4.03 g 100 g^{-1} DM) were higher than those observed in other studies with this plant, whose levels ranged from 0.045 to 2.67 g 100 g^{-1} DM (FREITAS et al., 2004; SOUZA et al., 2011; OLIVEIRA et al., 2012). These differences may be due to the different forms of preparing the plant (maceration and infusion) and to the use of other extragents, such as ethanol, ethyl acetate, butanol, among others.

For *A. vera*, the contents exceeded that recorded by Moniruzzaman et al. (2012), which was 0.0008 g 100 g^{-1} DM. In the same study, the authors also found that the leaves of *A. vera* have higher phenolic contents than the gel, indicating the use of the leaves as antioxidants. On the other hand, for *G. cambogia*, the contents were lower than that reported by Subhashini, Nagarajan & kavimani (2011), which was 7.5 g pyrocatechol 100 g^{-1} DM, and also than those recorded by Jantan et al. (2012) in 22 methanol extracts of different parts (leaves, trunks , bark and fruits) of nine *Garcinia* species, with contents ranging from 0.44 to 6.28 g gallic acid 100 g^{-1} DM. The different results are

probably due to the pattern used in the dosage, different extragents, different species, plant parts used and even the origin of the samples. For *S. ferruginea* and *T. paniculata*, no records were found in the literature on the phenolic content of these plants.

Some phenolic compounds, such as tannins, can inhibit certain digestive enzymes, such as amylase and trypsin, resulting in weight loss, helping in the treatment of obesity (MONTEIRO et al., 2005). They also have multiple biological effects, such as antioxidants, anti-allergic, anti-inflammatory, anti-bacterial, anti-thrombotic, vasodilating and cardioprotective (BALASUDRAM et al., 2006), showing a broad field of application for the phenolics of these plants.

There was no statistical difference between the plants *B. Trimera*, *S. ferruginea* and *T. paniculata* regarding the content of oxalic acid, and it was not detected in *A. vera* and *G. cambogia*. Oxalic acid contents higher than 10 g are considered toxic to health; therefore, the contents of this substance found in these plants offer no risk to health. The toxic effect of oxalic acid in the body has been associated with the reduction in the bioavailability of some essential minerals, such as calcium, and the main consequences are hypocalcemia and rickets,

besides affecting the absorption of iron, magnesium and zinc (SIENNER, 2005).

Nitrate was also found in all plants, with contents ranging from 0.16 to 6.96 g kg^{-1} DM. The acceptable daily intake of nitrate is 5 mg kg^{-1} body weight (WHO, 2003). The excessive consumption of this compound can lead to cyanosis, through the formation of metmyoglobin and neoplasms from the formation of N-nitroso compounds (FAQUIN; ANDRADE, 2004). Taking the example of a 60-kg person, they could ingest 300 mg nitrate, and this value would only be reached with great quantities of the analyzed plants. Therefore, the contents of nitrate found in these medicinal plants are not able to cause risk to health.

T. paniculata showed the highest potential for trypsin inhibition (22.01 trypsin inhibitor activity in nmol min^{-1} (TIA) mg^{-1} DM), followed by *B. trimera* (6.26 TIA mg^{-1} DM), and *S. ferruginea* (TIA 0.17 mg^{-1} DM). The presence of trypsin inhibitors was not detected in the plants *A.vera* and *G. cambogia*. Souza et al. (2011) observed the presence of trypsin inhibitors in aqueous and methanolic extracts of *B. trimera* leaves, corroborating the results of this study, in which trypsin inhibition was observed for *B. trimera*; however, these authors expressed their results in

percentage of trypsin inhibition, thus making it impossible to make comparisons with the activity observed in this study, which was expressed in TIA mg^{-1} DM. The presence of trypsin inhibitor, particularly in *T. paniculata*, shows its specificity to inhibit the proteolytic enzymes, which can lead to a decrease in protein digestibility, decreasing weight gain in animals.

Saponins were found in all the studied species, and *T. paniculata* showed the highest contents (1.00 g 100 g^{-1} DM). The contents of saponins recorded in this study for *B. trimera* (0.54 g 100 g^{-1} DM – ethanol extract) are within the range reported by Souza et al. (2011) for this plant, which ranged from 0.23 (aqueous extract) to 0.75 (methanol extract), in g 100 g^{-1} DM. In the same study, these authors also found no hemolytic effect in tests conducted with extracts of this plant, results that indicate a low toxicity of the saponins present in these leaves.

Among the side effects that saponins can cause, there are changes in reproduction and growth, as well as the reduction in nutrient absorption, due to changes in the permeability of cell membranes (FRANCIS et al., 2002), highlighting the need for studies to verify the toxicological potential of this phytochemical present in plant extracts.

However, saponins may aid in the treatment of obesity, because they can inhibit digestive enzymes and act on lowering cholesterol in human plasma, by forming micelles in the small intestine with bile acids, thus preventing their reabsorption (PEREIRA; CARDOSO, 2012), and this phytochemical may be responsible for possible anti-obesity properties in the plants analyzed in this study.

In the literature consulted, few studies related to the presence of the bioactive compounds determined in this paper were found for the analyzed plants, reflecting the concern about the possible harmful effects they may cause, highlighting the need for studies with these plants.

CONCLUSION

The plants presented high levels of substances with potential to cause weight loss, such as dietary fibers, some minerals, phenolic compounds, saponins and trypsin inhibitors. However, it is premature to recommend the use of these plants regarding the possible risks and benefits for human health, and additional studies of toxicity, efficacy and safety are necessary, particularly in relation to the saponins found in all plants, and to the high levels of phenolic compounds in *T. paniculata*.

However, the knowledge of these chemical constituents of plants contributes to their better use, either by the population or by the pharmaceutical industry, resulting in a greater use and economic value.

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ARTIGO 2

Inhibition of digestive enzymes by medicinal plant aqueous extracts used to aid in the treatment of obesity

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ABSTRACT

The objective of this work was to perform digestive enzyme inhibition assays with aqueous extracts of the medicinal plants *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga),

Baccharis trimera (Less.) DC (carqueja), *Garcinia cambogia* Desr., *Tournefortia paniculata* Cham. (marmelinho) and with the aqueous extract of the combination of those plants in the proportions 1:1,5:0,5:1,5:0,5, used as aids in the treatment of obesity. The α-amylase, α-glycosidase, lipase and trypsin enzyme inhibition analyses were conducted in the presence and absence of a simulated gastric fluid. In the absence of the simulated gastric fluid the enzymes underwent variable inhibition by the plant extracts, except for lipase, that did not undergo any inhibition. In the presence of the simulated gastric fluid, only the α-amylase and α-glycosidase enzymes were inhibited by the plant extracts. The combination of the plants did not cause inhibition in any of the evaluated digestive enzymes. Those results reveal that the aqueous plant extracts aloe, carqueja and marmelinho present potential as adjuvant in the treatment of obesity and of other dyslipidemias, because they inhibit α-amylase (marmelinho) and α-glycosidase (aloe, carqueja and marmelinho) after the gastric digestion simulation; the same cannot be said of the plants in combination.

Key words: α-amylase, α-glycosidase, lipase, trypsin, medicinal plants, obesity.

INTRODUCTION

Obesity is a chronic disease resulting from the excessive accumulation of body fat, which causes health damage in adults, adolescents and children, both in developed and in developing countries, with significant losses not only in the quality of life, but also in longevity.

The prevalence of obesity has been increasing at alarming rates throughout the world, and has become a major health problem in modern society. Approximately 1.6 billion are overweight, of which 400 million are obese, leading the disease to a global epidemic status (Tucci et al., 2010).

Obesity causes psychological problems, frustration, unhappiness and it predisposes the organism to a series of diseases, in particular cardiovascular diseases, some types of cancer, diabetes and hypertension, causing an increase of

economical costs for governments as well as for society (Bray, 2004).

Weight loss strategies and obesity treatments usually involve a combination of dietary changes, increase of physical activity, behavioral therapy, pharmacotherapy, and, in extreme cases, surgery (Celleno et al., 2007). Another widely used option are the natural products that have had a considerable increase in their consumption in recent years, mainly for the fact that the population believes they are ingesting medicines that do not cause harm to health and present low cost.

Research on therapeutic alternatives, mainly with medicinal plants, has been gaining space and importance in the pharmaceutical industry, being revealed as a quite promising option for the discovery of new phytomedicines and phytotherapeutics, due to the high number of still unstudied plant species, representing a vast field of substances to be discovered (Viegas Jr et al., 2006).

For the treatment of obesity, molecular targets, such as enzymes and receptors present in natural products, have been

studied in order to search for medicines based on the enzymatic inhibition mechanism that causes beneficial alterations in the metabolism, being an excellent alternative for the safe and effective development of anti-obesity drugs (Bhutani et al., 2007).

In this context, the enzymes α-amylase and α- glycosidase are responsible for carbohydrate processing, operating in the breakdown of starch and in the absorption of monosaccharides by the enterocytes (Kandra, 2003; Ota et al., 2009). Inhibitors of those enzymes, present in plants, offer a promising strategy to aid in the treatment of obesity, hyperglycemia associated to type 2 diabetes and hypertension, through the reduction of the starch breakdown and of the glucose absorption in the intestine (Kwon et al., 2006).

Besides those, lipase, involved in the lipid metabolism, is also shown as an interesting target of inhibitors, because its inhibition promotes reduction in triglyceride absorption, causing a decrease of caloric use and weight loss. On the other hand, trypsin inhibition, involved in protein digestion, unlike the other inhibitions, is characterized as having a harmful effect because it impedes the complete absorption of amino acids, which are of fundamental

importance for the organism, present in foods (Friedman and Brandon, 2001).

Much research has demonstrated the effectiveness, importance and the potential use of those enzyme inhibitors in the treatment of obesity and associated comorbidities and they reinforce the need for the search of new sources of those inhibitors: amylases (Obiro et al., 2008; Udani et al., 2009), glycosidases (Kwon et al., 2006) and lipases (Sharma et al., 2005; Souza et al., 2011). As such, digestive enzyme inhibitors that help limit the intestinal absorption of carbohydrates and fats in the initial phase may prove to be useful as aids in the treatment of obesity.

Various medicinal plants such as *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., and *Tournefortia paniculata* Cham. (marmelinho) are used as aids in the treatment of obesity. However, studies related to the presence of enzymatic inhibitors in the extracts of those plants that can participate or even be responsible for their anti-obesity properties, are scarce in the literature.

Based on the above, the objective in this work was to conduct assays of digestive enzyme inhibition with aqueous extracts of aloe, calunga, carqueja, *G. cambogia* and marmelinho and with the aqueous extract of the combination of those plants, so that these plants can be used as aids in the treatment of obesity.

MATERIALS AND METHODS

Sample collection and preparation

The plants *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja) and *Tournefortia paniculata* Cham. (marmelinho) were acquired in the municipal market of Belo Horizonte, Minas Gerais, Brazil, and transported to the Biochemistry Laboratory in the Chemistry Department of Federal University of Lavras (UFLA). The marmelinho and carqueja leaves were washed under running water and distilled water and soon afterwards placed together with the bark obtained from the calunga trunk in forced-air ovens for drying for 48 hours, at temperatures ranging from 30°C to 35°C. After drying, the leaves and the bark were ground in a Willey type mill and the flours stored in

hermetically sealed flasks until the analyses. The commercial powder of *Aloe vera* (L.) Burm. (aloe) (mucilage) and that of *Garcinia cambogia* Desr. (fruit) were acquired from FLORIEN, a pharmaceutical supply distributor.

The flour of the plants were mixed for the elaboration of a simulated phytotherapeutic, from the combination of aloe, calunga, carqueja, G. *cambogia* and marmelinho in the proportion 1:1,5:0,5:1,5:0,5, respectively; the same combination is used in the elaboration of the phytotherapeutic known by the trade name 'Moder Diet'.

Moisture determination

The moisture determination was carried out in triplicate in the medicinal plant flours (MPF) according to the Association of Official Analytical Chemists - AOAC (2005) method, that consists of the water loss by dehydration, at temperatures ranging from 100°C to 105°C.

Extract preparation

The MPF were mixed with distilled water in the proportion 1:25 (p/v), and placed in a horizontal agitator at room temperature for 1 hour. Soon afterwards, the mixture was filtered in filter paper and used as inhibitors in the enzymatic analyses.

Enzyme obtention

For the assays, the pancreatic porcine α-amilase type VI (SIGMA) was used, as well as the pancreatic porcine trypsin and the porcine lipase type II (MERCK). The α-glycosidase was obtained from fresh porcine duodenum provided by the Amimal Science Department of UFLA, that was triturated in blender with Tris-HCl 0.5 mol L^{-1}, pH 8.0 buffer at 4°C, for extraction of the enzymes from the enterocyte membranes and processed in mixer until complete homogenization. The homogenate was filtered in nylon mesh and centrifuged for 10 minutes, at 2,500 x g, at 4°C. The supernatant was collected and used as an enzymatic extract (Souza et al., 2011).

α-amylase activity

The α-amylase activity was determined according to the methodology proposed by Noelting and Bernfeld (1948). Thus, 50 µL of the plant extracts and 50 µL of α-amylase were pre-incubated for 20 minutes, in a water bath at 37°C. The substrate was the 1% starch prepared in Tris 0.05 mol L^{-1}, pH 7.0 buffer with 38 mmol L^{-1} NaCl and 0.1 mmol L^{-1} $CaCl_2$. After addition of 100 µL of the substrate, the mixture was incubated for four periods of time. The reaction was interrupted adding 200 µL of 3.5 dinitrosalicylic acid and the product read in spectrophotometer at 540 nm.

α-glycosidase activity

The α-glycosidase activity was determined according to Kwon et al. (2006), using 5 mmol L^{-1} p-nitrophenyl-α - D-glucopyranoside in a 0.1 mol L^{-1} pH 7.0 citrate-phosphate buffer as substrate. In the assay, 50 µL of the plant extracts and 100 µL of enzyme were incubated in a water bath, at 37°C, for four periods of time, after addition of 50 µL of the substrate. The reaction was

interrupted adding 1.000 µL of 0.05 mol L^{-1} NaOH and the product was read in a spectrophotometer, at 410 nm.

Lipase Activity

In each analysis, the mixture of 100 µL of lipase, 50 µL of the plant extracts and 50 µL of 4 mmol L^{-1} *p*-nitrophenyl laurate substrate in Tris-HCl 0.05 mmol L^{-1} pH 8.0 buffer containing 0.5% Triton-X100 was incubated for four periods of time. The reaction was stopped, transferring the tubes to an ice bath and adding 1.000 µL of Tris-HCl 0.05 mmol L^{-1} pH 8.0 buffer. The *p*-nitrophenol, of yellow coloration, a product of the lipase action on *p*-nitrophenyl palmitate, was read in a spectrophotometer at 410 nm (Souza et al., 2011).

Trypsin activity

The trypsin activity was determined according to the methodology proposed by Erlanger (1961); in which 200 µL of the plant extracts and 200 µL of enzyme were incubated in a water bath, at 37°C, for four periods of time, after addition of 800 µL of

p-benzoyl-DL-arginine-p-nitroanilide substrate (BApNA) prepared in 0.05 mol L^{-1}, pH 8.2 TRIS (tris(hydroxymethyl)aminomethane) buffer with 20 mmol L^{-1} $CaCl_2$. The reaction was interrupted adding 200 µL of 30% acetic acid and the product read in a spectrophotometer at 410 nm.

Determination of Inhibition

The enzyme inhibition was obtained from the determination of the slopes of the straight lines (absorbance x time) of the control enzyme (without plant extract) and enzymes + inhibitor (with plant extracts) activity assays The slope of the straight line is due to the speed of product formation per minute of reaction and the presence of the inhibitor causes a decrease in that inclination. From that inclination, the absorbance values were converted into µmol of product through a standard glucose curve for the amylase and of *p*-nitrophenol for glycosidase and lipase, while, for the trypsin, the of BApNA molar extinction coefficient determined by Erlanger (1961) was used.

Preparation of simulated gastric fluid

With the objective of simulating the digestion process in the stomach *in vitro*, enzymatic activity assays in the presence of a simulated gastric fluid were also carried out. For such, the plant extracts were incubated with the simulated gastric fluid prepared according to The United States Pharmacopeia - USP (1995), for 1 hour in a water bath at 37°C. After that period, they were neutralized with sodium bicarbonate salt to physiological pH and only then were the inhibition activity determination assays conducted.

RESULTS AND DISCUSSION

The digestive enzyme inhibition is a promising alternative for obesity treatment, mainly by the fact that they act on the small intestine, without acting on the central nervous system, where the usual anorexigens act. The results of the enzymatic inhibition by the medicinal plant aqueous extracts are presented in Table 1.

α-amylase was only inhibited by the marmelinho extract that presented an inhibition potential of 2,907.13 μmol min^{-1} g^{-1} dry

Table 1. Inhibition of digestive enzymes by aqueous extracts of medicinal plants given in enzyme inhibition units (EIU[1]).

Medicinal plant	α-amylase	α-glycosidase	Lipase	Trypsin
Aloe	nd[2]	1.23±0.05	nd	nd
Calunga	nd	nd	nd	nd
Carqueja	nd	0.58±0.03	nd	10.38±0.81
Garcinia cambogia	nd	nd	nd	nd
Marmelinho	2,907.13±7.64	35.46±0.58	nd	176.68±7.05
Phytotherapeutic[3]	nd	nd	nd	nd

Data are average of triplicates ± standard deviation. Moisture content of medicinal plant flour in g 100 g^{-1}: aloe = 8.53; calunga = 8.42; carqueja = 8.56; *Garcinia cambogia* = 3.94; marmelinho = 9.90. [1]One EIU is equal to 1 μmol min^{-1} g^{-1} of dry matter. [2]nd: inhibition not detected. [3]Phytotherapeutic: elaborated from the combination of aloe, calunga, carqueja, *Garcinia cambogia* and marmelinho in the proportion 1:1,5:0,5:1,5:0,5, respectively.

matter - DM. This potential is considered high when compared to Pereira et al. (2010), who analyzed different extraction conditions (solvents, times and temperatures) of α-amylase inhibitors in white bean, found inhibition between 36.88 and 66.89 μmol min^{-1} g^{-1} DM. Souza et al. (2011), analyzing the inhibitory potential of different carqueja extracts, also did not detect inhibition of α-amylase.

α-glycosidase was inhibited by the aloe, carqueja and marmelinho extracts, and this latter plant presented an inhibition potential of 35.46 μmol min^{-1} g^{-1} DM, a potential quite superior to that of the other plants that inhibited α-glycosidase . The inhibitory potential of marmelinho in the present work outperforms that verified by Pereira et al. (2011), who, analyzing commercial samples of *Hoodia gordonni*, a plant used as an aid in the treatment of obesity, found inhibition between 10.40 and 16.70 μmol min^{-1} g^{-1} DM. However, the potentials of aloe and carqueja are lower than those of *Hoodia gordonni*. The inhibition of those enzymes induces carbohydrate tolerance, extends gastric emptying, causes satiation and weight loss; all of which are effects that can be useful in the treatment of obesity (Chen et al., 2008).

Inhibition of lipase involved in the lipidic metabolism, was not detected for any of the aqueous extracts of the medicinal plants analyzed. Studies described in the literature show that, in the alcoholic extracts of plants, mainly methanol, lipase inhibitors have been isolated (Sharma et al., 2005; Sugimoto et., 2009). Those studies suggest that organic compounds soluble in methanol present some structural characteristics that have the capacity to bond and inhibit the pancreatic lipase.

For trypsin, a high inhibition was observed: 176.68, μmol min^{-1} g^{-1} DM, (marmelinho) and 10.38 (carqueja). Trypsin inhibitors present in the diet can cause a growth rate reduction in animals accompanied by a protein digestibility decrease which may lead to weight loss (MacDougall et al., 2005). The combination of the plants did not cause inhibition under any of the digestive enzymes analyzed.

For the ingestion of the medicinal plant extract, the passage through the gastrointestinal tract can lead to structural modifications in the inhibitors due to the acid pH of the stomach, causing their deactivation. Considering the expressive inhibition of

α-amylase, α-glycosidase and trypsin in the presence of the plant extracts, they were submitted to a test of possible gastric fluid action on the extract inhibitory activity (Table 2).

It was observed that there was a 14% decrease of the marmelinho extract inhibitory potential on the α-amylase in the presence of the simulated gastric fluid. For the α-glycosidase, the simulated fluid provoked a reduction in the inhibitory potential of the plant extracts of 60% (aloe), 32.8% (carqueja) and 27% marmelinho). However, even with the decrease of enzyme inhibition potential by the plant extracts, the results did not cease to be significant. The carqueja and marmelinho extracts lost their activity on the trypsin enzyme, which indicates that the trypsin inhibitors present in these extracts are not resistant to the passage through the gastric fluid, that can lead to modifications in the inhibitor molecule due to the acidic pH of the stomach or the presence of proteinases, inactivating it.

The inhibition results suggest that aloe, carqueja and mainly marmelinho might be a good source of α-amylase and α-glycosidase inhibitors, which could be used as aids in the

Table 2. Inhibition of digestive enzymes by aqueous extracts of medicinal plants after exposure to simulated gastric fluid given in enzyme inhibition units (EIU[1]).

Medicinal plant	α-amylase	α-glycosidase	Lipase	Trypsin
Aloe	nd[2]	0.49±0.02	nd	nd
Calunga	nd	nd	nd	nd
Carqueja	nd	0.39±0.04	nd	nd
Garcinia cambogia	nd	nd	nd	nd
Marmelinho	2,512.55±8.54	25.90±1.12	nd	nd
Phytotherapeutic[3]	nd	nd	nd	nd

Data are average of triplicates ± standard deviation. Moisture content of medicinal plant flour in g 100 g^{-1}: aloe = 8.53; calunga = 8.42; carqueja = 8.56; *Garcinia cambogia* = 3.94; marmelinho = 9.90. [1]One EIU is equal to 1 μmol min^{-1} g^{-1} of dry matter. [2]nd: inhibition not detected. [3]Phytotherapeutic: elaborated from the combination of aloe, calunga, carqueja, *Garcinia cambogia* and marmelinho plants in the proportion 1:1,5:0,5:1,5:0,5, respectively.

treatment of obesity that can be caused by an elevated level of carbohydrates in the diet. The resistance of the inhibitors, when passing through the simulated gastric fluid, is a strong indication that those results will repeat in *in vivo* assays.

CONCLUSIONS

The aloe, carqueja and marmelinho aqueous plant extracts present potential as adjuvant in the treatment of obesity and of other dyslipidemias, because they inhibit the α-amylase (marmelinho) and the α-glycosidase (aloe, carqueja and marmelinho) after the gastric digestion simulation; the same cannot be said of the plants in combination (phytotherapeutic).

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ARTIGO 3

Antioxidants from Medicinal Plants Used in the Treatment of Obesity

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ABSTRACT

Aims: The objective of this work was to quantify phenolic compounds, flavonoids, vitamin C, total carotenoids, β-carotene and lycopene and to measure the antioxidant activity in the medicinal plants *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., and *Tournefortia paniculata* Cham. (marmelinho) and of the phytotherapic made with the combination of these plants.
Place and Duration of Study: Chemistry Department of Federal University of Lavras – UFLA, Brazil between June 2011 and September 2012.
Methodology: Phenolic compounds, flavonoid, vitamin C, total carotenoids and β-carotene and lycopene contents were quantified by UV-Vis spectrophotometry and antioxidant activity by ABTS and β-carotene/linoleic acid methods.
Results: High contents of phenolic compounds were found in marmelinho (36.19 g 100 g^{-1}dry matter – DM), followed by carqueja (4.03 g 100 g^{-1}DM) and calunga (1.62 g 100 g^{-1}DM); of flavonoids in marmelinho (480.30 mg 100 g^{-}

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^{1}DM) and carqueja (173.68 mg 100 g^{-1}DM); of vitamin C in marmelinho (652.80 mg 100 g^{-1}DM) and *G. cambogia* (127.63 mg 100 g^{-1}DM); and of carotenoids in marmelinho (23.16 mg 100 g^{-1}). The antioxidant activity, in µmol trolox g^{-1}, by the ABTS method, was considered moderate in the aqueous (728.80) and ethanolic (731.06) marmelinho extracts, and weak for the other plants. However, by the β-carotene/linoleic acid method, the aqueous and ethanolic marmelinho extracts show great antioxidant potential at all tested concentrations (above 80% inhibition), and those of carqueja, calunga and the ethanolic of the phytotherapic, at the concentrations of 40,000 and 20,000 mg L^{-1}, also showed good antioxidant potencies (over 60% inhibition).
Conclusion: Those five species of plants showed antioxidant activity with potential for use in pharmaceutical and food preparations, with possible health benefits.

Keywords: Phenolic compounds; Flavonoids; Vitamin C; Carotenoids; Antioxidant potential.

1. INTRODUCTION

In recent years, the effects of antioxidants have been investigated in relation to illnesses. The research has tried to explain the benefits of those substances for the prevention and treatment of various types of diseases [1,2].

Antioxidants are substances that combat free radicals, which are extremely reactive species that cause the oxidation of various biomolecules (lipids, proteins and nucleic acids) present in our organism causing diverse pathologies, such as cancer, neurodegenerative disorders, cardiovascular diseases, diabetes and other chronic diseases, and the free radicals may be the cause or the aggravating factor of their general picture [3]. Thus, research seek alternatives to reduce the harmful effects of free radicals and improve the body's antioxidant capacity, as a form of treatment and prevention of diseases and their complications.

In view of the epidemiologic growth of these diseases, different foods and plants are studied by their substances, which are capable of neutralizing the effects of free radicals, such as phenolic compounds and vitamin C (hydrophilic antioxidants), vitamin E and carotenoids (lipophilic antioxidants), and certain minerals, such as zinc and selenium [4].

In that context, plants that are popularly known as having some therapeutic purpose, and that have not been the object of studies proving their effects, have become the central objective of research that seeks the development of new pharmaceuticals to aid in the treatment of diseases.

Aloe vera (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., *Tournefortia paniculata*

Cham. (marmelinho) are plants used, isolated or associated together, as aids in the treatment of obesity [5,6]. When associated, they are used in an attempt to obtain better results, as is the phytotherapic Moder diet, prepared from the combination of these plants to help in the treatment of obesity, with few studies related to their antioxidant properties which, if proven, could help in the treatment of various other diseases, some directly related to obesity, such as diabetes, cardiovascular diseases, hypertension, among others, which can be caused or worsen by free radicals [7].

Based on the above, the objective of this work was to quantify the phenolic compounds, flavonoids, vitamin C, total carotenoids, β-carotene and lycopene and to measure the antioxidant activity of the medicinal plants aloe, calunga, carqueja, *G. cambogia* and marmelinho and of the phytotherapic made from the combination of these plants, with the purpose of evaluating the possible use of these plants to combat free radicals and consequently in the treatment of various illnesses caused by them.

2. MATERIAL AND METHODS

2.1 Sample collection and preparation

Baccharis trimera (Less.) DC (carqueja) and *Tournefortia paniculata* Cham. (marmelinho) leaves, and the trunk barks of *Simaba ferruginea* St. Hil. (calunga) were acquired in the municipal market of Belo Horizonte, Minas Gerais, Brazil, in January 2011, in three replicates, and transported to the Biochemistry Laboratory in the Chemistry Department of Federal University of Lavras (UFLA). The marmelinho and carqueja leaves were washed under running water and distilled water and soon afterwards placed together with the bark obtained from the calunga trunk in forced-air ovens for drying for 48 hours, at temperatures ranging from 30°C to 35°C. After drying, the leaves and the bark were ground in a Willey type mill and the flours stored in hermetically sealed flasks until the analyses. The commercial powder of *Aloe vera* (L.) Burm. (aloe) (mucilage) and that of *Garcinia cambogia* Desr. (fruit) were acquired from FLORIEN, a pharmaceutical supply distributor. The aloe powder was obtained from the lyophilization of the plant mucilage, while that of *G. cambogia* from drying by spray dryer.

The flour of the plants were mixed for the elaboration of a simulated phytotherapeutic, from the combination of aloe, calunga, carqueja, *G. cambogia* and marmelinho in proportions of 1:1,5:0,5:1,5:0,5, respectively; the same combination is used in the elaboration of the phytotherapeutic known by the trade name ‘Moder Diet’.

2.2 Moisture determination

The moisture determination was carried in the medicinal plant flours according to the Association of Official Analytical Chemists - AOAC [8] method, that consists of the water loss by dehydration, at temperatures ranging from 100°C to 105°C.

2.3 Phenolic compounds

The extraction of the phenolic compounds was carried out with 1 g of sample in 50 mL of 50% methanol, under reflux three consecutive times, at 80°C and the extracts collected, evaporated up to 25 mL and submitted to phenolic compound measurement, using the Folin-Denis reagent, and tannic acid as a standard [8].

2.4 Total flavonoids

The total flavonoid contents were measured using the same extracts of the phenolic compound analyses, using the aluminum chloride colorimetric method, with catechin used as a standard [9].

2.5 Vitamin C

The extraction of the ascorbic acid was carried out with 0.5 g of sample in 50 mL of oxalic acid and 0.1 g of diatomaceous earth, under agitation, for 15 minutes. After filtration (Whatman N^o 40), the vitamin C in the extract was dosed, using ascorbic acid as a standard [10].

2.6 Total carotenoids

For the determination of total carotenoids, the extraction was made according to Higby [11], using 0.5 g of sample in a 40 mL extraction solution of isopropyl alcohol:hexane 3:1. The content was transferred to a 125 mL separation funnel wrapped in aluminum, where the volume was completed with distilled water. It was left at rest for 30 minutes, followed by washing of the material and the discard of the aqueous phase. This operation was repeated three more times. The content was filtered with cotton sprayed with anhydrous sodium sulphate 99% to a 25 mL volumetric flask wrapped with aluminum, where 5 mL of acetone were added and the volume completed with hexane. The readings were made at 450 nm and the results expressed in mg 100 g^{-1}, calculated by the formula:

Total carotenoids = $(A_{450} \times 100)/(250 \times L \times W)$, where:
A_{450} = absorbance, L = cuvette width in cm, and W = ratio of the mass of the original sample and the final dilution volume in mL.

2.7 β-carotene and lycopene

For the determination of the β-carotene and lycopene, the same extract was used as in the total carotenoid analysis, in which those extracts were taken for absorbance readings in a spectrophotometer at four wavelengths: (453, 505, 645 and 663 nm) [12]. For the calculations of the β-carotene and lycopene concentrations, the following equations were used:

β-carotene (mg 100 g^{-1}) = 0.216 A_{663} – 1.22 A_{645} – 0.304 A_{505} + 0.452 A_{453}.
Lycopene (mg 100 g^{-1}) = - 0.045 A_{663} + 0.204 A_{645} + 0.372 A_{505} – 0.0806 A_{453}.

2.8 Antioxidant activity

2.8.1 Extract preparation

The extraction of the antioxidants was conducted using two extragents: water (1:25, w/v) and ethanol (1:25, w/v). For each extragents the samples were maintained under agitation for 1 hour and soon afterwards, filtered in filter paper. All of the extractions were carried out in three replicates, protected from light and subsequently submitted to antioxidant activity (AA) detection process by the methods described below.

2.8.1 β-carotene/linoleic acid method

Starting from the raw extracts of the samples (40,000 mg L^{-1}) dilutions in 20,000 and 10,000 mg L^{-1} were prepared. The methodology used was developed by Rufino et al. [13], with modifications.

For the preparation of the β-carotene/linoleic acid solution system, 50 µL of β-carotene diluted in chloroform (20 g L^{-1}) were used, to which 40 µL of linoleic acid were added, as well as 530 µL of tween 20 (emulsifier) and, for solubilization, 1 mL of chloroform. In a flask covered with aluminum for protection against light, the chloroform was evaporated in a rotary-evaporator and 100 mL of oxygen saturated water (distilled water treated with oxygen for 30 minutes) were added, and the combination was agitated until that the solution system presented a yellow-orange coloration. In test tubes, 2,5 mL of that solution system were added to 0.2 mL of each dilution of the sample used for the test. Control tubes were made containing 2.5 mL of the solution system with 0.2 mL of BHT (butyl-hydroxytoluene - synthetic antioxidant), quercetin and rutin, which are flavonoids with proven antioxidant action all at the concentration of 200 mg L^{-1}. In laboratory tests, it was found that the concentration of 200 mg L^{-1} of BHT is the one that provides the greatest protection for the system, when compared to others; therefore, its use is suggested. After homogenization, their readings were taken in a spectrophotometer at 470 nm, using water for calibration of the spectrophotometer; this was considered to be the reading at

time zero (initial). The tubes were placed in a water bath, at 40°C and readings were taken after 2 hours.

2.8.2 ABTS method

The methodology used was developed by Re et al. [14], with modifications by Rufino et al. [15]. Four different dilutions of the obtained extracts were conducted for the assays and subsequent construction of the analytical curves.

Analytical curves were made with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and with ascorbic acid, besides tests for comparison with the standards BHT, and with rutin and quercetin, prepared in the concentration of 200 mg L^{-1}.

2.9 Statistical analysis

The data are the average of three replicates ± standard deviation analyzed by one-way analysis of variance (ANOVA) and when this analysis showed a significant difference, the Skott-Knott test ($P<0.05$) was used for the comparison of means. All statistical tests were carried out using R (version 2.15.2) statistical software [16].

3. RESULTS AND DISCUSSION

3.1 Antioxidant substances

The levels of antioxidants in the flours of the medicinal plants and in the phytotherapic are shown in Table 1. Phenolic compounds were found in all the plants, and marmelinho showed the highest contents (36.19 g 100 g^{-1} dry matter - DM) and *G. cambogia* the lowest (0.09 g 100 DM g^{-1}). The phenolic compound content in the phytotherapic was considered relatively high (4.56 g 100 DM g^{-1}) being lower only than those of marmelinho.

The contents of phenolic compounds in the carqueja leaves (4.03 g 100 g^{-1} DM) were higher than those observed in other studies with this plant, whose levels ranged from 0.045 to 2.67 g 100 g^{-1} DM (5,17,18). These differences may be due to the different ways of preparing the plant (maceration and infusion) and to the use of other extraction agents, such as ethanol, ethyl acetate, butanol, among others.

For aloe, the levels exceeded those recorded by Moniruzzaman et al. [19], which was 0.0008 g 100 g^{-1} DM. In the same study, the authors also found that

Table 1. Levels of antioxidants, in dry matter, of medicinal plants and the phytotherapic.

Plants	Phenolic compounds (g100 g^{-1})	Flavonoids (mg100 g^{-1})	Vitamin C (mg100 g^{-1})	Total Carotenoids (mg 100 g^{-1})	β-carotene (mg100 g^{-1})	Lycopene (mg100 g^{-1})
Aloe vera	0.15±0.01 d	ND[1]	13.37±0.60 f	0.54±0.11 e	ND	0.52±0.01 d
Calunga	1.62±0.03 c	55.75±2.76 d	44.40±0.96 d	1.50±0.02 d	ND	0.34±0.04 d
Carqueja	4.03±0.21 b	173.68±3.60 b	19.27±0.38 e	13.67±0.74 b	3.59±0.50 b	1.97±0.15 b
Garcinia cambogia	0.09±0.00 d	ND	127.63±0.70 b	0.21±0.01 e	ND	0.20±0.08 d
Marmelinho	36.19±0.91 a	480.30±4.73 a	652.80±8.66 a	23.16±0.52 a	10.09±0.94 a	3.28±0.36 a
Phytotherapic[2]	4.56±0.20 b	82.10±3.70 c	121.50±1.10 c	4.30±0.70 c	1.36±0.01 c	0.79±0.01 c

Data are the average of three replicates ± standard deviation. Same letters in columns do not differ among themselves by the Scott-knott test (P<0.05). Moisture levels of medicinal plant flour in g 100 g^{-1}: Aloe vera = 8.53; calunga = 8.42; carqueja = 8.56; Garcinia cambogia = 3.94; marmelinho = 9.90. [1]Nd: Not detected. [2]Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in proportion 1:1.5:0.5:1.5:0.5, respectively, these data being obtained by estimation.

the aloe leaves have higher phenolic contents than the gel, indicating the use of aloe leaves as antioxidants. For *G. cambogia*, the levels were lower than those recorded by Subhashiniet al. [20], which was 7.5 g of pyrocatechol 100 g^{-1} DM, and also than those recorded by Jantan et al. [21] in 22 methanol extracts from different parts (leaves, branches, bark and fruits) of 9 *Garcinia* species, with levels ranging from 0.44 to 6.28 g of gallic acid 100 g^{-1} DM and, in both studies, the Folin-Ciocateu method was used. These different results are probably due to the pattern used in the dosage, to the different extractors, different species, plant parts used and even to the origin of the samples.

For calunga and marmelinho, no records about the phenolic content of these plants were found in the literature, so the comparison will be made with other medicinal plants. The contents of phenolic compounds in marmelinho (36.19 g 100 DM g^{-1}) were very high, and superior to those related by Ghimire et al. [22] who, in 24 medicinal plants of Nepal, verified phenolic compounds, in g 100 DM g^{-1}, between 2.38 (*Drymaria cordata*) and 32.12 (*Amamum subulatum*); by Wojdylo et al. [23], who registered contents between 0.07 (*Carum carvi*) and 15.15 (*Echinacia purpurea*) in 32 Polish herbs; and to those of Gan et al. [24] who, in 40 medicinal plants, found contents between 0.04 (*Curcuma* aromatic) and 7.57 (*Sanquisorba officinalis*). The phenolic compounds in marmelinho were surpassed only by *Acacia catechu* Willd with 41.47 g 100 DM g^{-1}, in a study conducted with 133 medicinal plants of 64 different families from India [25], highlighting the high levels of phenolics in marmelinho, and may represent several possible fields for the application of these phenolics, adding value to this plant.

The phenolic compounds act as antioxidants, due to their redox properties, that allow them to act as reducings agents, hydrogen donors and metal chelators. Besides their role as antioxidants, these compounds present a wide spectrum of medicinal properties, such as antiallergic, anti-inflammatory, anti-bacterial and anti-thrombotic, plus present cardioprotective and vasodilator effects [26], showing a broad field of application for the phenolics in these plants.
Marmelinho showed the highest flavonoid content (480.30 mg 100 DM g^{-1}), followed by carqueja (173.68 mg 100 DM g^{-1}) and calunga (55.75 mg 100 DM g^{-1}). Flavonoids were not detected neither in aloe nor *G. cambogia.* However, for the phytotherapic we estimated a flavonoid content of 82.10 mg 100 DM g^{-1}.

The flavonoids in carqueja were higher than those in Borella and Fontoura [27], who recorded levels between 12.43 and 47.50 mg of rutin 100 g^{-1} DM in 8 commercial samples of carqueja leaves. This difference is probably due to the pattern used, besides factors related to cultivation, manure, collection site, plant age, among others. Studies with aloe [19] and with *G. cambogia* [20] showed the presence of flavonoids in the extracts of these plants, differently from the present work, in which flavonoids were not detected. These differences may be due to several factors, such as the environmental, site collection, preparation

and handling of the extracts; besides, tampering and/or forgeries could have occurred during this process.

The flavonoid levels of marmelinho were higher than those related by Sumazian et al. [28] in leaves of 6 vegetables from Malaysia whose levels, in mg 100 DM g^{-1}, ranged from 42 (*Whitania somnifera*) to 405 (*Curcuma tames*), to those of various parts of medicinal plants, in 100 g DM: *Alcea kurdica* flowers (22 mg), *Valerian officinalis* root (110 mg), *Stachys lavandulifolium* flowers (402 mg), and lower in relation to the *Lavandula officinalis* (618 mg) and *Melissa officinalis* leaves (1,000 mg) [29]. For the leaves of 11 plants analyzed by Djeridane et al. [4], the flavonoids of marmelinho surpassed those of 6 plants with levels between 162 (*Ruta montana*) and 454 mg 100 DM g^{-1} (*Globularia alypum*) and they are inferior to those related for 24 medicinal plants from Nepal that presented contents, in mg 100 DM g^{-1}, between 1.353 (*Withania somnifera*) and 10.033 (*Artemesia vulgaris*) [22].

The consumption of foods and plants rich in flavonoids is associated with the risk reduction of various chronic diseases, and their protecting effect is due, partly, to their antioxidant properties and capacity to reduce oxidative stress [30]. Epidemiological data confirm a significant relationship between the high ingestion of flavonoids and the decrease of carcinogenic risk, cardiovascular diseases, myocardial infarction and total LDL concentrations [31,32].

Vitamin C was also found in all the analyzed plants, presenting contents that varied from 13.37 to 652.80 mg 100 DM g^{-1}. The estimated vitamin C level of the phytotherapic (121.50 mg 100 DM g^{-1}) was only lower to those of *G. cambogia* and marmelinho. With the discovery of the antioxidant action of that vitamin, the ingestion of substances with high vitamin C content has been recommended. As such, those plants are shown as good vitamin C sources with potential for use as antioxidants.

The total carotenoid contents varied from 0.20 to 23.16 mg 100 DM g^{-1}. The highest amount total carotenoids, in mg 100 DM g^{-1}, was registered in marmelinho (23.16), followed by carqueja (13.67) and the phytotherapic (4.30). *G. cambogia* presented the lowest carotenoid content. The carotenoids act with lipophilic antioxidants and together with the vitamin C and the phenolic compounds (hydrophilic antioxidants) they form a strong defense against the free radicals as they act in different cell compartments.

β-carotene was not detected in the plants aloe, calunga and *G. cambogia*, while the lycopene was found in all the plants; marmelinho and carqueja showed the highest levels of those substances. The estimated β-carotene content (0.79) and lycopene (1.36) in the phytotherapic were only smaller than that of marmelinho and carqueja.

Studies conducted by Zhao et al. [33] indicate that the supplementation of carotenoids reduces DNA injury and that the combination of carotenoids (β-carotene and lycopene) ingested and reached via diet or in high doses of an individual carotenoid (12 mg) could protect against DNA injury. The β-carotene is highly liposoluble and widely transported with LDL cholesterol (75%) and HDL cholesterol (25%). It possesses an antioxidant function as a free radical scavenger, to reduce the extent of cell nucleus injury and to inhibit lipidic peroxidation induced by the free radical enzyme sources, such as xanthine oxidase [34].

3.2 Antioxidant activity

The results of the (AA) by the ABTS method, calculated by the analytical curve of trolox and by the standard vitamin C curve, in flours of the medicinal plants, the phytotherapic and the three standards are presented in Figure 1. Out of the analyzed plants, marmelinho was the one that showed the highest antioxidant potential, probably due to the higher levels of phenolics, flavonoids, vitamin C and carotenoids in the leaves. For this plant practically no difference in AA between the aqueous and ethanolic extracts, in trolox equivalent, as well as vitamin C. For calunga, carqueja and the phytotherapic, the aqueous extracts showed a higher AA than the ethanolic extracts, both for the trolox equivalent, and for the vitamin C, which can be explained by the fact that the ABTS test shows the best results when in the presence of hydrophilic antioxidants. Aloe and *G. cambogia* did not show AA by the ABTS method, probably due to the low content of phenolic compounds and the absence of flavonoids in the extracts of these plants, which are substances that have strong action in capturing the ABTS radical.

The low antioxidant potential shown by the phytotherapic in the ABTS test, may be due to the lower proportion of marmelinho in the constitution of the phytotherapic (10%), since this plant showed a good antioxidant potential, and also to a negative synergistic action in the combination of these plants.

It was observed that, when compared to the BHT and rutin standards, the antioxidant potential, in trolox and vitamin C equivalents, of the aqueous and ethanolic extracts of marmelinho reached 58% and 54% on average, of the potential of those standards. In relation to quercetin, the potential of marmelinho was much smaller, with only 9.7% of its potential (in trolox equivalent, as well as vitamin C). The antioxidant potential of the extracts of the other plants and of the phytotherapic was considered low. However, as in the extracts of marmelinho, the antioxidant substances are not in the isolated form as are the standards, this average potential of approximately 56% can be considered a good antioxidant potential.

The good antioxidant potential shown by the marmelinho leaves is evidenced when compared to other studies, in which, independently of the extract,

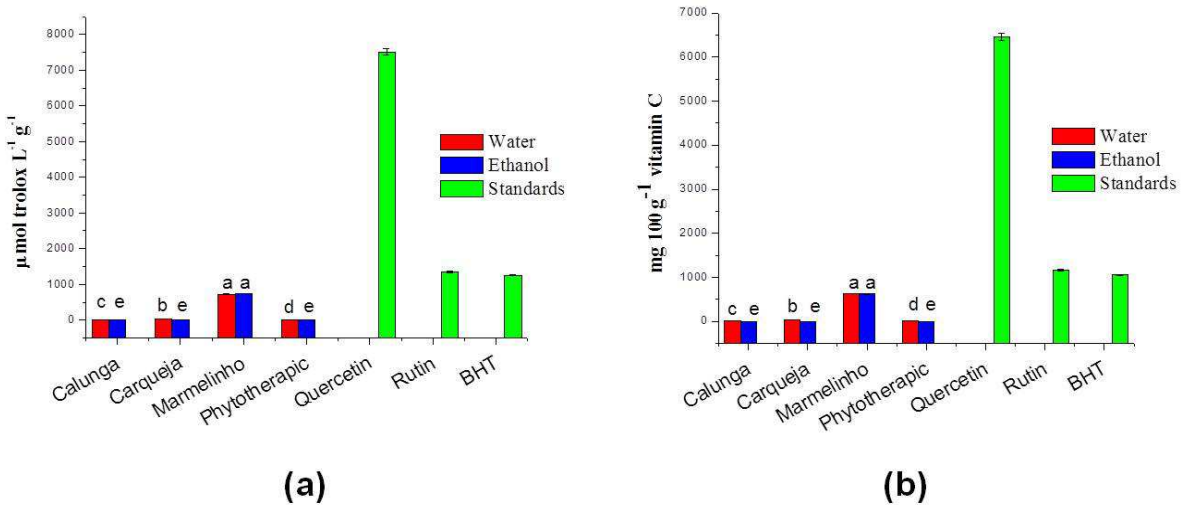

Fig. 1. Antioxidant activity by ABTS method of aqueous and ethanolic extracts of medicinal plants, the phytotherapic and three standards, expressed in (a) µmol trolox L^{-1} g^{-1} and (b) mg g^{-1} de vitamin C.

Data are the average of three replicates ± standard deviation. Same letters in columns do not differ among themselves by the Scott-Knott test (P<0.05). Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in a proportion of 1:1.5:0.5:1.5:0.5 respectively.

surpassed that Wojdylo et al. [23] who in 32 Polish herbs, verified potentials between 0.0045 (*Archangelica officinalis*) and 3.46 (Syzygium aromaticum) µmol trolox g^{-1}. It also surpassed that detected by Bouayed et al. [29] in several parts of medicinal plants, in mg g^{-1} vitamin C: 2.8 (*Alcea kurdica* flowers), 7.36 (*Valerian officinalis* root), 15.4 (*Stachys lavandulifolium* flowers), 19.2 (*Lavandula officinalis* leaves) and 19.3 (*Melissa officinalis* leaves). It is surpassed only by 11 of 132 Indian medicinal plants analyzed by Surveswaran et al. [25] and by two of 40 medicinal plants studied by Gan et al. [24]. Such results were probably due to the presence of different antioxidants, ways and extractors used in the preparation of these plants, which implies a greater antioxidant potential.

The lipidic oxidation inhibition results, by the β-carotene/linoleic acid method for the MPF, the phytotherapic and the three standards after 2 hours of reaction, are shown in Table 2. All the plants demonstrated lipidic oxidation inhibition potential, except the ethanolic extract of *G. cambogia*.

Marmelinho was the plant with the highest antioxidant potential at the analyzed concentrations, and, at concentrations of 40,000 and 20,000 mg L^{-1}, the aqueous and ethanolic extracts showed practically the same antioxidant potential, whereas at the concentration of 10,000 mg L^{-1}, the ethanol extract showed the greatest potential.

The calunga and carqueja extracts (aqueous and ethanolic) at concentrations of 40,000 and 20,000 mg L^{-1} also showed good antioxidant potentials (over 60% inhibition). The phytotherapic showed a great antioxidant potential, especially its ethanolic extract, with an inhibition potential at the concentrations of 40,000 and 20,000 mg L^{-1}, which was only inferior to the antioxidant potential of marmelinho, evidencing the occurrence of a good antioxidant potential by the combination of the plants.

The β-carotene/linoleic acid method shows a better response to antioxidants with apolar character. As marmelinho showed the highest levels of carotenoids (lipophilic antioxidant) and also of other antioxidants, it presented the highest antioxidant potential among the plants analyzed. For calunga, carqueja and the phytotherapic, the greatest inhibition potential of lipid oxidation found in the ethanolic extracts of these plants occurred probably due to the greater removal of antioxidants with apolar character, provided by the alcohol in relation to water. The low antioxidant activity in the aloe and *G. cambogia* extracts probably occurred due to the low levels of carotenoids in the extracts of these plants.

In order to demonstrate the response of polar and apolar antioxidant groups compared to the ABTS and β-carotene/linoleic acid methods, observing Figure 1 (ABTS Test), it is possible to observe that quercetin and rutin, hydrophilic antioxidants, have a greater antioxidant potential than BHT (lipophilic antioxidant), i. e. in this test, polar antioxidant groups showed better responses.

Table 2. **Antioxidant activity of aqueous and ethanolic extracts of the medicinal plants, the phytotherapic and three standards, in % inhibition by the β- carotene/linoleic acid method.**

Plants	Water			Ethanol		
	40,000mg L^{-1}	**20,000mg L^{-1}**	**10,000mg L^{-1}**	**40,000mg L^{-1}**	**20,000 mg L^{-1}**	**10,000 mg L^{-1}**
Aloe vera	50.14±2.33 eA	39.44±0.37 eC	41.73 ± 0.09 dB	16.55± 1.67 eD	12.27± 2,09 eE	14.27±1.10 eE
Calunga	61.97±1.67 cB	58.05±0.85 bB	57.66±0.54 bB	70.75±0.17 dA	64.37±0,59 dA	46.67±3.41 cC
Carqueja	70.91±1.17 bA	60.21±1.32 bB	55.09±7.33 bB	73.53±1.37 cA	68.21±2,26 cA	49.67±0.29 cC
Garcinia cambogia	57.21±1.28 dA	54.13±3.08 cA	20.78 ± 0.28 dB	ND[1]	ND	ND
Marmelinho	99.98±1.10 aA	96.71±1.53 aA	86.08±7.44 aB	100.00±1.40 aA	100.00±0,34 aA	95.71±1.25 aA
Phytotherapic[2]	49.05±0.25 eD	48.41±1.50 dD	43.54±0.61 cE	87.23±0.34 bA	81.22±0,41 bB	56.58±4.15 bC
BHT (200 mg L^{-1})	75.93±1.15					
Quercetin (200 mg L^{-1})	70.48±0.71					
Rutin (200 mg L^{-1})	8.71±1.29					

Data are the average of three replicates ± standard deviation. Lowercase letters in the column compare between plants and uppercase letters on the line compare between concentrations. Same letters do not differ among themselves by the Scott-Knott test (P<0.05).[1]ND: Not detected. [2]Phytotherapic: elaborated from a combination of Aloe vera, calunga, carqueja, Garcinia cambogia and marmelinho in a proportion of 1:1.5:0.5:1.5:0.5 respectively.

Now, by the β-carotene/linoleic acid method (Table 2), BHT shows a greater the antioxidant potential, exceeding that of quercetin and rutin, i. e. nonpolar antioxidants present better results.

When compared to the standards, the marmelinho extracts were higher at all of the tested concentrations, as well as the ethanolic extract of the phytotherapic in concentrations of 40,000 e 20,000 mg L^{-1}, while the carqueja (aqueous and ethanolic) and the calunga (ethanolic) extracts at the concentration 40,000 mg L^{-1} presented the same inhibition potential as the quercetin. In relation to rutin, all the plants showed antioxidant potential above that of this standard. The different results of antioxidant activity observed in the extracts of the plants were probably due to the different antioxidant compounds present in the extracts of these plants, and to the difference principle employed by each antioxidant method used in this work. Given this situation, some plants showed a good antioxidant potential to a method and a weak potential to the other, emphasizing the importance of using more than one technique to reflect the antioxidant capacity of a sample.

Studies performed with these plants confirm this antioxidant potential. Rajasekaran et al. [35] observed that an oral administration of the alcoholic extract of *Aloe vera* gel (300 mg kg^{-1}) to diabetic rats reversed the high levels of lipid peroxidation and hydroperoxides in the tissues of these rats to nearly normal levels. The treatment also resulted in a significant increase in the levels of reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidney of diabetic rats; these results show the antioxidant property of the *Aloe vera* extract.

In vitro studies showed that the *Aloe vera* gel has a potential for capturing free radicals [19,36], which was not observed in the present study, and a potential for combating the inhibition of lipid oxidation [36], corroborating the results of this study. Such differences may be due to several factors, such as environmental, collection site, plant age, preparation and handling of the extracts. Yun et al. [37] analyzing *Aloe vera* at various ages, found that at age four this plant shows a greater antioxidant potential than at ages below, which shows that the development phase plays a vital role in the antioxidant composition of the *Aloe vera* gel.

For carqueja, Pádua et al. [39] reported a good antioxidant potential *in vit*ro and *in vivo* for the hydroethanolic extract of this plant in a study performed with neutrophils of Fisher rats. Mendes et al. [40] reported that the hydroalcoholic extract of carqueja presentes a moderate antioxidant activity. In the same study the authors observed a reduced antiulcer activity in rats (ulcer induction by stress). Dias et al. [41] and Morais et al. [2], analyzing the antioxidant potential of carqueja by the DPPH method, which has the same principle of capturing free radicals as the ABTS method, found a good antioxidant potential for the carqueja extracts. These results differ from those found for carqueja in the

present study, in which it was possible to observe a low antioxidant potential for the extracts (aqueous and ethanolic) by the ABTS method. This difference is probably due to factors such as the collection site, plant age, ways and extractors used in the preparation of the plant.

For *G. cambogia*, Subhashini et al. [20] registered a great antioxidant potential for the aqueous extract obtained from the bark of its fruits by the methods of scavenging free radicals (DPPH), reduced iron (FRAP), total reactive antioxidant potential (TRAP) and lipid peroxidation. Results that differ from those found for the extracts (aqueous and ethanolic) of *G. cambogia* in the present study, when using the ABTS method, and show similar results when it comes to lipid oxidation. The different results among these studies may be due to the way the extracts were prepared, which is an extremely important factor in order to have a good antioxidant activity. In this work, crude extracts were used, while in the work of Subhashini et al. [20], concentrated extracts were used, which can justify these differences. Other factors, such as the geographic origin, plant age, and even tampering and/or forgeries can be considered. In the literature consulted, no studies on the antioxidant potential of calunga and marmelinho were found.

4. CONCLUSION

The studied plants showed an antioxidant potential, and the antioxidant activity by the ABTS method was considered moderate in marmelinho extracts, and weak in the other plants. For the β-carotene/linoleic acid method, the aqueous and ethanolic extracts of marmelinho, carqueja and calunga, and only the ethanolic extract of the phytotherapic presented a good antioxidant potential. Thus, those plants show potential to be used as antioxidant sources in pharmacological and food preparations, with possible health benefits.

CONSENT (WHEREEVER APPLICABLE)

Not applicable.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not applicable.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. T*he study design was by authors AAS, ADC and TRM*, authors FFL and PMBC performed the experiments, authors JMF and RMF managed the literature searches. Authors AAS, JMF and ADC were involved in the writing process of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ARTIGO 4

Effects of the administration of *Tournefortia paniculata* Cham. on *Wistar* rats subjected to a hypercaloric diet: therapeutics and toxicology

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Artigo redigido conforme norma da revista. Este artigo é uma versão preliminar, considerando que o conselho editorial da revista poderá sugerir alterações para adequá-lo ao seu estilo próprio.

Abstract

Considering that medicinal plants are rich sources of bioactive compounds with potential for therapeutic use, widely used in folk medicine and in the preparation of manufactured drugs, the objective of this study was to evaluate the therapeutic and toxicologic effects of the administration of the flour and aqueous extract of *Tournefortia paniculata*

leaves on *Wistar* rats, subjected to a hypercaloric diet for 42 days. The rats were divided into five groups and were given the following treatments by gavage: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}. The treatments did not significantly alter the weight, but were effective in reducing liver fat, glucose and serum triglycerides. The treatment T1 reduced food consumption and lipid peroxidation. None of the treatments showed genotoxic potential. It is possible to conclude that *T. paniculata* leaves have an anti-obesity potential. However, a more detailed study of the medicinal potential and the characterization of phytochemicals in this plant are still necessary for a better understanding of its mechanisms of action, enabling future applications in the treatment of this pathology or for various therapeutic purposes.

Keywords: obesity, medicinal plants, pharmacology, antioxidants, genotoxicity, phytotherapic.

1 - INTRODUCTION

Due to the consequences caused by obesity and to the speed of its spread throughout the world, it has been considered a global epidemic, with over one billion overweight adults, from which 400 million are clinically obese, reaching both developed countries and countries in development, including Brazil (TUCCI et al., 2010). Its incidence is independent of socioeconomic factors and age, and its consequences range from the development of debilitating diseases to death, directly affecting the quality of life of individuals.

Out of the various causes of obesity, the most immediate determinant of the excessive accumulation of fat is the positive energy balance, which happens when the amount of energy consumed is higher than the amount spent (HENRY; PANDIT, 2009). This is totally favored by current diets, which have a high energy density, are rich in saturated fats, simple sugars, and have low levels of complex carbohydrates and fibers; it is also favored by an increase in physical inactivity, which sets the contemporary Western lifestyle (POPKIN; GORDON-LARSEN, 2004) and contributes to the development of cardiovascular diseases,

some cancers, diabetes, hypertension, among others, significantly affecting the national economy.

Among the options available for the treatment of obesity, the most used ones are: balanced diets, regular physical exercises and drug treatments, ranging from lipase inhibitors to anorectics. However, due to side effects and the high cost of drugs traditionally used in the treatment of this disease, the potential of natural products for the treatment of obesity is being widely explored, and they may be a viable alternative for the future development of effective and safe anti-obesity drugs (MAYER et al., 2009; PARK et al., 2005).

A variety of natural products, including extracts and compounds isolated from plants, are being used for reducing body weight and preventing obesity (RAYALAM et al., 2008; SIMÃO et al., 2012; SOUZA et al., 2012). In this context, plants that are popularly used for therapeutic purposes, which do not have studies showing their effects, are of great value in research aiming for the development of phytotherapics that can help in the anti-obesity treatment and can act in its prevention or treatment, with proven efficacy and safety. Mixtures of phytochemicals or isolated molecules identified from these plants represent an excellent

opportunity for the development of such therapeutics (BIRARI; BHUTANI, 2007).

Tournefortia paniculata Cham., traditionally known as marmelinho, is a shrub belonging to the family Boraginaceae. Its origin is attributed to the Mediterranean regions and the United States, occurring widely in the tropics and subtropics (MORAES; SOUSA, 2007). In Brazil, its leaves are used in folk medicine as a diuretic, anti-inflammatory in the urinary tract and in cases of nephrolithiasis (BERTOLUCCI, 2000).

Recent studies reported that the aqueous extract of *T. paniculata* leaves can act in the treatment of obesity, because of its high inhibitory activity *in vitro* on the enzymes α-amylase and α-glucosidase (SIMÃO et al., 2012). These enzymes are responsible for the processing of carbohydrates from the diet, acting on starch breakdown and on the absorption of monosaccharides by enterocytes (OTA et al., 2009), being a promising strategy to aid in the treatment of obesity, hyperglycemia associated to type 2 diabetes and hypertension, due to the reduction in starch breakdown and to the glucose uptake in the intestine (KWON et al., 2006).

The *in vitro* antioxidant potential was also observed in the aqueous extract of these leaves (SIMÃO et al., 2013) which, if demonstrated *in vivo*, will be characterized as an important tool to aid in the treatment of obesity, since many diseases directly related to obesity, such as diabetes, cardiovascular diseases, hypertension, among others, may be caused or exacerbated by free radicals (VILLAREAL et al., 2005).

Simão et al. (2013) also found high contents of phenolic compounds in *T. paniculata* leaves (36.19 g 100 g^{-1} dry matter); such substances can provide several benefits for the treatment of obesity and other diseases, since they have an inhibitory effect on digestive enzymes, as well as antioxidant and antiglycemic properties, and phenolics may be the substances responsible for the possible anti-obesity effects of these leaves.

Although it is described as a constituent in the composition of the phytotherapic Moder Diet, revoked by National Health Surveillance Agency and still available for purchase over the Internet, *T. paniculata* has no scientific studies which prove its pharmacological and/or toxic effects, and is not present in the official lists of medicinal plants released to the preparation of phytotherapics. Thus, the composition, which is rich

in phenolic compounds, highlights this species as a strong candidate for the development of medicines, although its *in vitro* and *in vivo* pharmacotoxic extensive characterization is still necessary.

In this study, based on *in vitro* results, the potential therapeutic and toxicological effects of the administration of the flour (rich in phenolic compounds) and aqueous extract of *T. paniculata* leaves were evaluated on the metabolism of *Wistar* rats subjected to a hypercaloric diet, in order to determine possible anti-obesity properties, as well as safe and effective doses for the development of therapeutic formulations from this plant.

2 - MATERIAL AND METHODS

2.1 - Sample collection and preparation

T paniculata Cham. (marmelinho) leaves were acquired in the municipal market of Belo Horizonte, Minas Gerais, in January 2011, and transported to the Laboratory. The leaves were washed under running and distilled water and, soon afterwards, they were placed in forced-air ovens for drying for 48 hours, at ±35°C. After drying, the leaves were ground in a Willey type mill, and were then passed through an 80-mesh sieve (0.2

mm), and only the plant material that passed through the sieve was used in the bioassay.

2.2 - Bioassay

2.2.1 - Animals and experimental conditions

The experiment was developed in accordance with the ethical principles in animal experimentation, according to the Law 11,794 of October 2008, and the project was approved by the Ethics Committee on Animal Use of Universidade Federal de Lavras (UFLA - Protocol 014/11).

The experiment was conducted over 10 weeks, using 30 male *Wistar* rats (*Rattus norvegicus*), with initial body weight of approximately 243.15 g. The animals were kept in individual cages at 21ºC, light/dark cycle of 12 hours, with access to distilled water *ad libitum*. During the first four weeks, the rats were given a hypercaloric diet containing 46% commercial food (Biobase Bio-tec Ratos e Camundongos), 46% condensed milk and 8% corn oil (LEVIN et al., 1986). Diets were prepared weekly, in the amount of 1 kg, and stored in a freezer at -24°C to minimize lipid oxidation of polyunsaturated fatty acids.

At the end of four weeks consuming the hypercaloric diet, the animals were weighed, had a body weight of approximately 327.80 g, were divided into five groups of six animals each, and were given the hypercaloric diet and the following treatments for six weeks: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 (positive antioxidant control) - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}

Each treatment was administered daily, diluted with water, and the administration was performed by gavage, using sterilized stainless steel cannula and syringe, at the same hour, by the same individual, with the volume of 1 mL per animal.

For the calculation of the dose for the administration of the aqueous extract and quercetin, the maximum dose of phenolics suggested for humans was considered, that is, 1,000 mg day^{-1} (SCALBERT et al., 2005), as well as the average weight of an adult human, which is 70 kg; thus, it is possible to calculate the dose per gram of body weight, which is equivalent to 14 mg phenolics kg^{-1} animal body weight. On the other

hand, for the administration of the FTL at a dose of 50 and 100 mg, the maximum solubility of this flour in water was taken into account.

For the obtention of the aqueous extract, the FTL was added to water in the ratio 1:25 (w v^{-1}), kept under agitation for 60 minutes at room temperature and then centrifuged at 10,000 x g for 10 minutes. The supernatant was collected and subjected to measurement of phenolic compounds, using the Folin-Denis reagent, with tannic acid as a standard (AOAC, 2005), and the contents of phenolic compounds found in this extract were 16.25 mg mL^{-1} and, in FTL, 40.62 mg 100 mg^{-1}.

On the other hand, for quercetin and FTL, aqueous solutions were prepared at concentrations of 20 and 40 mg mL^{-1}, respectively. The volume administered in each treatment was calculated according to the weight of each rat. Thus, a solution volume corresponding to the dose calculated according to the body weight measured on the day was administered to each animal by gavage. The same was done for the control group, to which only water was administered.

2.2.2 - Body weight and daily food consumption

Body weight and food consumption were recorded daily for the evaluation of the parameters weight gain and food consumption, and food consumption was calculated by the difference between the offered diet and the leftovers.

2.2.3 - End of the bioassay

At the end of the experiment, blood samples were taken from the tail end, after a 12-hour fasting, for the determination of glucose concentrations using a portable glucose meter (Accu-Chek® - Active - São Paulo, Brazil) and reagent strips (Accu-Chek Advantage II).

Then, the rats were anaesthetized with thiopental sodium (1 g of sterile powder dissolved in 40 mL of 0.9% saline solution), intraperitoneally, at a dose of 40 mg kg^{-1}. After the collection of 2 to 4 mL of blood by cardiac puncture, which caused death by exsanguination, a 50-µL aliquot was separated for the evaluation of genotoxicity (comet assay), and the remainder of the blood was centrifuged at 2,500 x g for 10 minutes, for the obtention of the serum, which was kept under refrigeration.

After euthanasia, a necropsy was also performed, with the removal of internal organs (heart, liver, kidneys and pancreas), as well as liver weighing and preparation of histological slides. The liver was divided into three parts: one was intended for the qualitative analysis of the histology of this organ, one for the determination of lipid peroxidation, and one for the determination of ether extract. After removal of the organs, the head, legs, tail and skin were removed, followed by washing of the carcass with salt solution.

2.2.4 - Analysis of liver lipids and carcass

The carcass, as well as part of the liver, were weighed and lyophilized until constant weight. Then, they were crushed in a mortar and subjected to lipid measurement using the Soxhlet method (AOAC, 2005).

2.2.5 - Blood laboratory analyses

The blood serum was used to determine the concentrations of glucose, total cholesterol, HDL cholesterol, triglycerides, activity of the enzymes aspartate aminotransferase (AST), alanine aminotransferase

(ALT) and Gamma glutamyl transferase (GGT). Blood constituents were analyzed by commercial kits (Labtest) and analyzes were carried out with the equipment Automatic Veterinary Biochemical Analyzer Thermoplate.

2.2.6 - Lipid peroxidation

The lipid peroxidation was determined by the formation of Thiobarbituric acid reactive substances (TBARS), according to Winterbourn et al. (1985).

The livers of the animals were weighed and homogenized in a Potter tissue homogenizer, in an ice bath, after the addition of buffered saline in 0.1 mol L^{-1} sodium phosphate (PBS), pH 7.4 (volume equivalent to 4 times the fresh weight of the tissue). The homogenate was centrifuged at 3,000 x g for 10 minutes at 4°C, and the supernatant, kept in an ice bath, was used in the assays. 500-µL aliquots of supernatant were mixed with 500 µL of 25% hydrochloric acid (v v^{-1}), 500 µL of 1% thiobarbituric acid (w v^{-1}, in 0.05 mol L^{-1} NaOH) and 45 µL of 2% BHT (w v^{-1}, in ethanol). The mixture was heated in a boiling water bath for 10 minutes. After cooling in an ice bath, 1.5 mL of butanol were added, and the samples were shaken vigorously, centrifuged at 900 x g for 5 minutes,

and the fraction containing butanol was collected and used for the determination of the absorbance at 532 nm.

The TBARS concentration was calculated from the standard curve of malondialdehyde - MDA (1,1,3,3 tetraethoxypropane). The results were expressed in moles MDA mg^{-1} fresh sample.

2.2.7 - Comet assay

2.2.7.1 - Obtention of nucleoids and electrophoresis

The comet assay was performed according to the methodology described by Singh et al. (1988), with some modifications. An aliquot (15 µL) of each cell suspension containing the treatments was mixed with 100-µL low melting point agarose (0.5% w v^{-1} in PBS), applied to a microscope slide previously coated with normal melting point agarose solution (1% w v^{-1} in PBS), overlaid with a coverslip (24 x 60 mm) and kept at ± 4°C for 5 minutes, until solidification of the agarose. For each treatment/rat, 2 slides were prepared. Then, the coverslips were removed and the slides were immersed in lysis solution (2.5 mol L^{-1} NaCl, 100 mmol L^{-1} EDTA, 10 mmol L^{-1} Tris, 1% Triton X-100, 10% DMSO; pH 10), where they remained for 24 hours at 4°C.

After lysis, the slides were kept for 25 minutes in a freshly prepared electrophoresis solution (1 mmol L^{-1} EDTA, 300 mmol L^{-1} NaOH; pH 13), allowing the loosening of bonds in DNA molecules and the exposure of alkali-labile sites. The electrophoresis was conducted at 25V for 35 minutes. After the electrophoresis, the slides were kept in a neutralization solution (0.4 mol L^{-1} Tris; pH 7,5) for 30 minutes, and then dried and fixed with 100% ethanol. All procedures were performed in the dark.

2.2.7.2 - Staining and analysis

The slides were stained with 45 μL propidium iodide solution (1mg mL^{-1}), overlaid with a coverslip and analyzed in an epifluorescence microscope (Nikon ECLIPSE E400) using a 200x magnification.

In order to measure damage levels in lymphocyte DNA molecules, 100 nucleoids of each slide, 2 slides per treatment/rat (totaling 200 nucleoids per treatment/rat), were counted and classified by the same evaluator, using visual score patterns described by Singh et al. (1988). The cells were classified according to the size of the "tail" and the diameter of the "head" in Class 0: no damage (damage<5%); Class 1: low

damage level (5–20%); Class 2: intermediate damage level (20–40%); Class 3: high damage (40–85%); Class 4: totally damaged (damage> 85%). The average frequency of damage was calculated from the sum of the percentages of damage 1, 2, 3 and 4. The arbitrary units (0-400; where 0 = no damage and 400 = 100% damage) were calculated by the equation (1 x number of nucleoids grouped in class 1) + (2 x number of nucleoids in class 2) + (3 x number of nucleoids in class 3) + (4 x number of nucleoids in class 4), as described by Collins (2004).

2.2.8 - Histopathology

For histopathological analyses, slides containing cuts from the heart, liver, kidneys and pancreas of each rat were prepared. In histological processing, the organs were cut into small pieces, immersed in 10% formalin for 24 hours and kept in 70% alcohol until inclusion in paraffin. 4-μm cuts were made in a rotary microtome, stained with hematoxylin-eosin, mounted on slides/coverslips and analyzed under light microscopy (100x magnification), for the qualitative analysis.

2.3 - Statistical analysis

The experiment was conducted in a completely randomized design. For data on weight gain, the plots were subdivided in a 5 x 7 scheme, that is, five treatments, a seven-week experiment with six replicates and, for data on food consumption, the plots were subdivided in a 5 x 6 scheme, with five treatments, a six-week experiment with six replications. For the other analyzes, five treatments with six replicates were used and, when the analysis of variance showed significance, the comparison of means by the Scott-Knott test ($P < 0.05$) was performed using the Sisvar program (FERREIRA, 2003).

3 - RESULTS AND DISCUSSION

In Figure 1, it is possible to observe body weight and food consumption of animals subjected to different treatments during the six weeks of the experiment. The average weights of the rats showed no significant differences among the treatments (Figure 1). However, the average weight gain of the animals in groups T0, T2, T3 and T4 was 10.20%, 4.57%, 3.07% and 7.11%, respectively, while for the T1 group, a

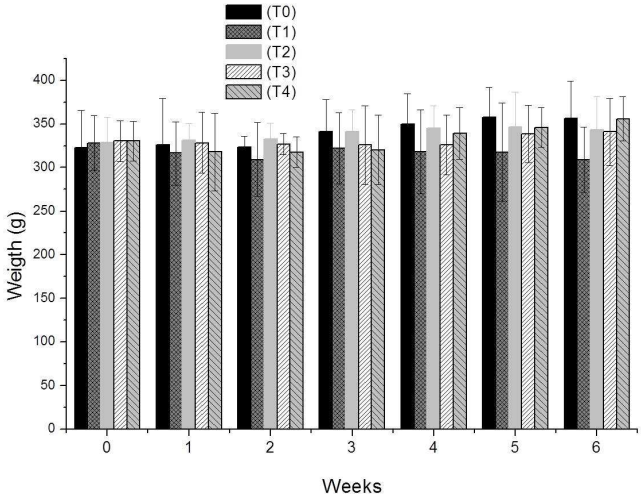

Figure 1. Body weight of *Wistar* rats subjected to the treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}, for six weeks. Data represent the mean of six replications ± standard deviation of the average weight for each week of experiment.

weight loss was observed in the animals, with an average weight gain of 5.80%, although not statistically different.

In another study with the aqueous extract of *T. paniculata* leaves, an *in vitro* inhibition of the enzymes α-amylase and α-glucosidase was observed, and this inhibitory effect is one of the possible mechanisms responsible for weight reduction, considering a lower carbohydrate absorption (SIMÃO et al., 2012). Souza et al. (2012) evaluated the anti-

obesity effects of the methanol extract of *Baccharis trimera* leaves, administered to *Wistar* rats by gavage, and also observed a reduction in the weights of the animals and suggested a possible mechanism of action based on the inhibition of lipases and glycosidases, which were inhibited *in vitro* by the methanol extract of this plant. These reports show that medicinal plants that have digestive enzyme inhibitors in their extracts, as is the case of *T. paniculata*, may be useful for limiting the absorption of fat and carbohydrates of the diet, resulting in weight loss.

Some studies describe the anti-obesity potential of quercetin through mechanisms such as the increase in lipolysis, inhibition of adipogenesis and induction of apoptosis of adipocytes (HSU; YEN, 2006; AHN et al., 2008; FANG, 2008). However, in this study, quercetin (T2) was unable to cause a reduction in body weight, confirming the results of Machha et al. (2007), who also found no decrease in the body weight of *Wistar* rats after the treatment with quercetin, administered by gavage, at a dose of 10 mg kg^{-1} rat for six weeks.

The average daily food consumption by the animals is shown in Figure 2. The treatment T1 showed a significant effect, reducing food consumption.

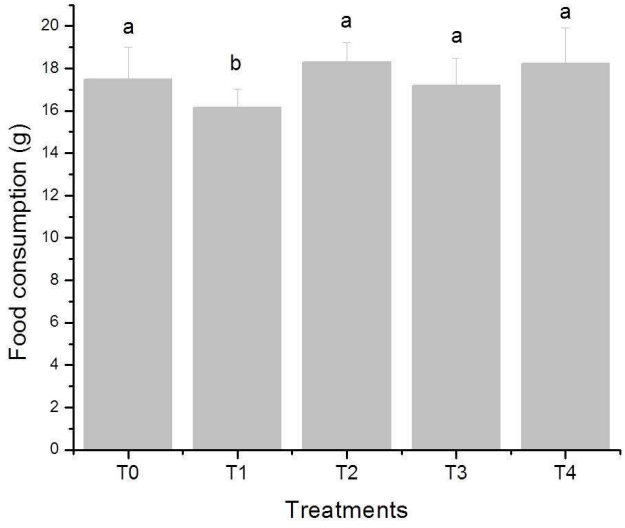

Figure 2. Food consumption of *Wistar* rats subjected to the treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}, for six weeks. Same letters in columns do not differ by the Scott-Knott test ($P < 0.05$).

These results demonstrated that the treatment T1 was effective in reducing appetite, which characterizes an anorexigenic action by an unknown mechanism, and this inhibition may be related to the weight loss observed for this treatment (Figure 1).

Table 1 shows the average weights of the livers, the liver weight/carcass weight ratio and the levels of body and hepatic fat, with no

Table 1. Average liver weight, liver weight/carcass weight, body and hepatic fat of *Wistar* rats subjected to treatments[1] for six weeks.

Parameter	T0	T1	T2	T3	T4
Liver weight (g)	7.71±1.10[a]	7.12±1.39[a]	7.79±1.01[a]	7.95±1.36[a]	8.24±0.74[a]
Liver weight/total body weight (%)	4.49±0.85[a]	4.54±0.70[a]	4.69±0.27[a]	4.82±0.60[a]	4.99±0.40[a]
Hepatic fat (g 100 g^{-1})	13.63±0.84[a]	11.13±1.39[b]	10.63±1.05[b]	8.27±0.99[c]	8.89±1.25[c]
Body fat (g 100 g^{-1})	18.92±1.93[a]	17.24±1.61[a]	17.76±2.74[a]	17.01±1.98[a]	19.35±1.10[a]

Data are the mean of six replicates ± standard deviation. [1]Treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}. Same letters in rows do not differ by the Scott-Knott test ($P < 0.05$).

statistical difference among the treatments for the average weights of the livers, the liver weight/carcass weight ratio and the levels of body fat. The treated groups showed levels of liver fat significantly lower than the group T0 (control), with a reduction of 18.34%, 22.01%, 39.32% and 34.77% for the groups T1, T2, T3 and T4, respectively.

Considering that the liver would be affected by possible antinutritional factors present in *T. paniculata* leaves, damage could reflect on anatomical changes in the treated groups, which did not occur, thus discarding, under the conditions of this experiment, the induction of hypertrophy and hyperplasia.

Body fat has been used as an indicator of obesity in rats. The accumulation of adipose tissue has been associated with various markers of obesity, such as weight gain and body mass and Lee index (NASCIMENTO et al., 2008).

Table 2 presents the results of some biochemical indicators measured in the blood of the animals under study.

The determination of glucose is the initial parameter for the detection of changes and disturbances of the glucose metabolism, such as type 2 diabetes, usually associated with obesity (MOTA, 2009).

Table 2. Biochemical parameters of *Wistar* rats subjected to treatments[1] for six weeks.

Parameters	T0	T1	T2	T3	T4
Caudal capillary glucose (mg dL^{-1})	97.16±2.48[a]	82.5±5.50[b]	84.16±5.38[b]	86.83±7.83[b]	93.66±3.44[a]
Serum glucose (mg dL^{-1})	165.16±17.17[a]	140.34±6.12[b]	168.08±9.34[a]	162.31±8.09[a]	177.96±6.35[a]
Total cholesterol (mg dL^{-1})	56.16±4.11[a]	55.19±5.36[a]	61.48±6.67[a]	59.63±4.26[a]	58.13±4.56[a]
HDL cholesterol (mg dL^{-1})[1]	49.11±5.36[a]	43.77±2.38[a]	47.64±1.27[a]	45.11±3.91[a]	44.33±4.12[a]
Triglycerides (mg dL^{-1})	42.51±1.92[a]	29.23±4.00[b]	31.70±8.73[b]	34.40±3.68[b]	32.80±2.86[b]
AST[2] (U L^{-1})	77.35±0.57[a]	80.45±4.06[a]	74.35±5.14[a]	71.58±5.88[a]	76.12±4.82[a]
ALT[3] (U L^{-1})	59.74±6.32[b]	87.25±16.14[a]	63.17±7.35[b]	56.82±3.15[b]	61.49±4.85[b]
GGT[4] (U L^{-1})	2.55±0.00[a]	2.48±0.07[a]	2.48±0.07[a]	2.42±0.19[a]	2.50±0.06[a]

Data are the mean of six replicates ± standard deviation. [1]Treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}. [2]AST: aspartate aminotransferase. [3]ALT: alanine aminotransferase. [4]GGT: Gamma glutamyl transferase. Same letters in rows do not differ by the Scott-Knott test ($P < 0.05$).

The levels of caudal capillary glucose in the animals subjected to the treatments T1, T2 and T3 were significantly lower than those in group T0 (control), with a reduction of 15.09%, 13.38% and 10.63% in the concentration of glucose, respectively.

For serum glucose, only the treatment T1 was able to significantly reduce the concentration of glucose in relation to the group T0 (control), demonstrating a higher hypoglycemic potential of the aqueous extract of *T. paniculata* leaves, compared to other treatments. This reduction may be due to the inhibition of the enzymes α-amylase and α-glucosidase, reducing the glucose concentration through the lower carbohydrate absorption (SIMÃO et al., 2012), and through the lower food consumption observed for this treatment (Figure 2).

Some authors have attributed the hypoglycemic effect of plants to the phenolic compounds present in their extracts (FIGUEROA-VALVERDE et al., 2009; URZÊDA et al., 2013), and these compounds, present in *T. paniculata* leaves, can be responsible for the reduction of the glucose concentration observed in the present study.

The blood dosage of lipids and hepatic enzymes provides important parameters in the determination of functional ingredients safety

or final products derived from plants subjected to toxicity assays (PATEL et al., 2008).

The results obtained showed that there was no statistical difference among the groups, regarding serum concentration of total cholesterol and HDL cholesterol. The treated groups did not differ statistically, and triglyceride concentrations were significantly lower than group T0 (control), with a reduction of 31.24%, 25.43%, 19.08% and 22.85% in the concentration for the groups T1, T2, T3, and T4, respectively.

Studies showed a significant reduction in the blood glucose concentration of diabetic rats treated with quercetin (COSKUN et al., 2005; MACHHA et al., 2007), as well as a reduction in triglycerides (RICARDO et al., 2001), and these studies are consistent with the results of this study, in which a reduction in the concentration of glucose and triglycerides was observed for rats treated with quercetin (T2).

Based on the data in Table 2, it was observed that the activities of AST and GGT were statistically equal for all groups. Regarding ALT, the treatment T1 showed an activity significantly higher than those recorded in the other groups, with an increase of 46.04% in activity, compared to

the group T0 (control). Thus, the results of ALT for the treatment T1 suggest that this treatment can cause hepatotoxic effects.

The elevation in serum levels of the ALT enzyme activity caused by the treatment T1 can be probably attributed to a hepatic dysfunction, due to a hepatocyte disruption, resulting from necrosis or changes in the permeability of the cell membrane (KANEKO, 1989). Injury or destruction of hepatic cells release transaminases into the bloodstream (MOTA, 2009), resulting in an increase of their activity.

AST, found primarily in the mitochondria (80%), in organs such as heart, liver, kidneys, pancreas and skeletal muscles, is not released as quickly as ALT, which is essentially hepatic and localized in the cytoplasm, thus justifying the fact that statistical changes were recorded only for the concentrations of ALT in relation to the control group in this study. In addition, ALT is a more sensitive indicator of acute hepatotoxicity than AST (AL-HABORI et al., 2002).

The index of oxidative stress, normally caused by free radicals in the body, was determined by lipid peroxidation, which is considered an important marker of oxidative stress, and also one of the main factors involved in cell damage, caused by these radicals (BALU et al., 2005).

The results of lipid peroxidation (Figure 3) for the treatments T1 and T2 were significantly lower than those in group T0 (control), with a reduction of 34.09% and 18.90%, respectively, in the MDA concentration.

Quercetin (T2) was used as a positive control, because it is a flavonoid with proven antioxidant activity (DUARTE-ALMEIDA et al., 2006; SIMÃO et al., 2013). Through *in vivo* studies, it was possible to observe that quercetin was effective in reducing TBARS in rat livers (GARCÍA-SAURA et al., 2005; YAMAMOTO; OUE, 2006), justifying its use as a reference substance. It has five reactive phenolic hydroxyls and a high resonance stability, and these properties are responsible for its antioxidant potential and possibly for the reduction in the MDA concentration found in this study.

On the other hand, the treatment T1, which has phenolic compounds, may contribute, at least in part, to the antioxidant activity demonstrated in this study, since these compounds have redox properties that allow them to act as hydrogen donor reducing agents. Yet, the complexation of Fe^{+2} to phenolic compounds can reduce the availability of this metal involved in the Fenton reaction, in the initiation and

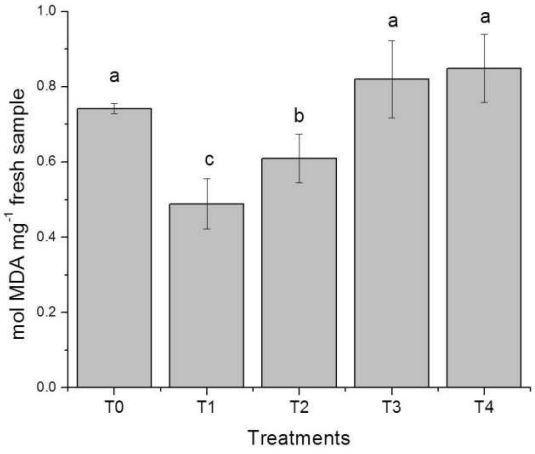

Figure 3. Production of Thiobarbituric acid reactive substances in the hepatic tissue of *Wistar* rats subjected to the treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}, for six weeks. [1]MDA: malondialdehyde. Data are the mean of six replicates ± standard deviation. Same letters in columns do not differ by the Scott-Knott test ($P < 0.05$).

propagation of lipid peroxidation (LIMA et al., 2006), which could explain the reduction in the MDA concentration by the aqueous extract of *T. paniculata* leaves. For this study, the treatment T1 showed better results for antioxidants than the phenolic pattern of quercetin, demonstrating the good antioxidant potential of this plant.

In relation to treatments T3 (50 mg FTL kg^{-1} rat day^{-1}) and T4 (100 mg FTL kg^{-1} rat day^{-1}), containing 20.31 and 40.62 mg phenolics,

respectively, none was able to inhibit the formation of lipid peroxidation products, thus suggesting that the antioxidant substances present in this flour are able to exert antioxidant effects only when they are extracted and concentrated.

It is worth emphasizing that the treatments used in this study, except for the treatment T2 (quercetin), were not subjected to any purification process for the obtention of active ingredients, that is, there were only the flour and the crude aqueous extract of *T. paniculata* leaves, highlighting its therapeutic potential as an antioxidant and hypoglycemic, even when used without any purification process.

For the results regarding the fragmentation of DNA molecules (Table 3), there was a predominance of nucleoids with no damage (class 0), with percentage averages ranging between 43.16% (T1) and 83.05% (T0) and with low damage level (class 1), with averages between 16.32% (T0) and 52.58% (T1). It was also possible to observe (Table 3) a reduced number of nucleoids with intermediate damage levels (class 2), with percentage averages ranging between 0.00% (T4) and 5.29% (T1) and absence of nucleoids with high damage levels (class 3) and totally damaged DNA (class 4) in all treatments.

Table 3. Average nucleoid number per class of comet, frequency of nucleoids with damage and arbitrary units in leukocytes of *Wistar* rats subjected to treatments for six weeks.

Treatments[2]	Class of comet (%)[1]					Frequency of damage[3] (%)	Arbitrary units[4]
	0	1	2	3	4		
T0	83.05±4.28[a]	16.32±3.68[d]	0.98±0.08[b]	0.00±0.00[a]	0.00±0.00[a]	17.30±3.87[d]	18.28±4.20[d]
T1	43.16±16.53[d]	52.58±11.71[a]	5.29±1.18[a]	0.00±0.00[a]	0.00±0.00[a]	57.87±11.86[a]	63.16±13.56[a]
T2	71.30±5.50[b]	28.40±5.11[c]	0.30±0.03[b]	0.00±0.00[a]	0.00±0.00[a]	28.70±5.50[c]	29.00±5.89[c]
T3	59.60±5.86[c]	39.30±4.66[b]	1.13±0.24[b]	0.00±0.00[a]	0.00±0.00[a]	40.76±5.59[b]	41.56±5.95[b]
T4	68.18±9.63[b]	31.83±9.63[c]	0.00±0.00[b]	0.00±0.00[a]	0.00±0.00[a]	31.83±9.63[c]	31.83±9.63[c]

[1]Data represent the mean values obtained in 2 slides, with 100 nucleoids analyzed in each slide, and 4 slides/treatment/rat. 30 rats were used (6 rats/treatment). [2]Treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}. [3]Frequency of damage: sum of the damage of classes 1 through 4; [4]Arbitrary units: (1 x damage in class 1) + (2 x damage in class 2) + (3 x damage in class 3) + (4 x damage in class 4). Same letters in columns do not differ by the Scott-Knott test ($P < 0.05$).

Although the percentages of nucleoids with damage and arbitrary units demonstrate significant differences between the group T0 (control) and the other treatments, these values are related to the damage in class 1 (low damage level) and class 2 (intermediate damage), demonstrating lack of genotoxicity, considering the six weeks of exposure to the treatments, thus suggesting that, in the composition of the evaluated treatments, there are not molecules capable of causing genotoxic and, consequently, mutagenic effects on the DNA under the experimental conditions used.

Some studies have made use of the comet assay to complement the toxicological characterization of plant extracts, investigating the cytotoxic and genotoxic effects of secondary metabolites (phenolic compounds and terpenes), such as those conducted by Pereira et al. (2012), who investigated the genotoxic potential of white bean flour, containing tannins and other phenolic compounds, on human leukocytes, describing the prevalence of low damage levels in their results; the one conducted by Wan-Ibrahim et al. (2010), who evaluated 20 aqueous plant extracts and observed that only two of them, e.g. *Vitex Pinnata* L. and *Quercus infectoria* Oliver, caused damage higher than 50% in the DNA

of human leukocytes, which are attributed to the phenolic content, more specifically to tannic acid; and those by Strange et al. (2009), who analyzed natural products, such as guaco (*Mikania glomerata* Spreng), espinheira-santa (*Maytenus ilicifolia* Mart. ex Reiss) and salvia (*Lippia alba* Mill. NE) and observed cytotoxic and genotoxic activities induced by metabolites, such as coumarins, tannins and terpenes, in the extracts of these plants, highlighting the need for a toxicological characterization of plant metabolites, since they may induce side and/or therapeutic effects dependent on factors such as dose and chronic use.

In Figure 4, images of histological sections obtained from different organs are shown. No macroscopic changes were observed in the organs analyzed, and neither were injuries, or significant microscopic changes. The possible accumulation of fat in the liver of the animals induced by the hypercaloric diet was not observed in the histology, as well as injuries or destruction of hepatic cells that could release transaminases into the bloodstream, causing an increase in their activity. However, the time period of the experiment may not be sufficient to reveal such damage by optical microscopy since, for the treatment T2, there was an increase in the activity of ALT (Table 2) and, for the treated

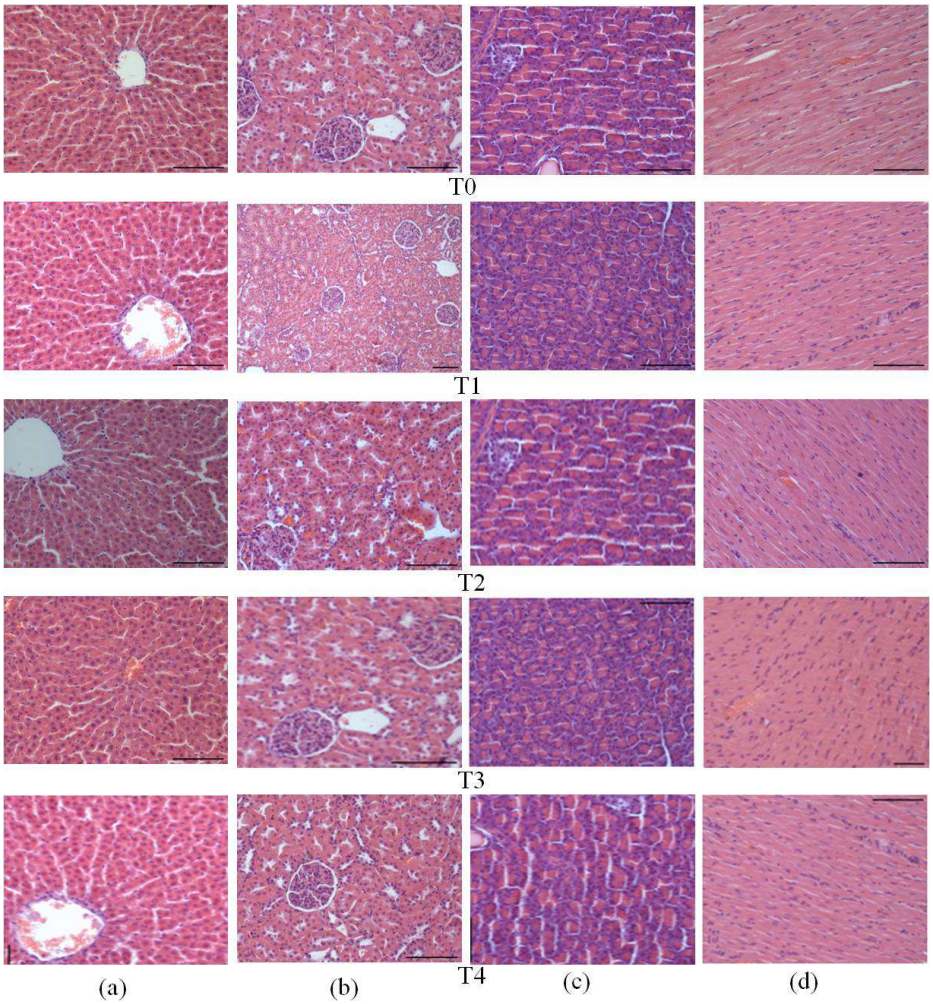

Figure 4. Photomicrographs (reading from left to right) of the liver (a), kidneys (b), pancreas (c) and heart (d) of *Wistar* rats subjected to the treatments: T0 (control) - 1 mL water day^{-1}; T1 - aqueous extract containing 14 mg phenolic compounds kg^{-1} rat day^{-1}; T2 - 14 mg quercetin kg^{-1} rat day^{-1}; T3 - 50 mg flour from *T. paniculata* leaves (FTL) kg^{-1} rat day^{-1} and T4 - 100 mg FTL kg^{-1} rat day^{-1}, for six weeks.

groups, a decrease in the concentration of hepatic fat in relation to the group T0 (control) (Table 1).

The results obtained for *T. paniculata* leaves in this study showed the presence of anti-obesity and antioxidant properties, as well as lack of genotoxicity, showing the high pharmaceutical potential of this plant, which can be widely studied as a source of biomolecules or molecular models for the development of new pharmaceutical and/or cosmetic formulations. These results show prospects of further studies to determine doses and investigate other parameters that define effectiveness and safety.

4 - CONCLUSION

T. paniculata leaves show an anti-obesity potential, since they cause a reduction in serum levels of glucose and triglycerides, as well as in hepatic fat (flour and water extract) and food consumption, and also in lipid peroxidation (aqueous extract), and do not cause genotoxic effects, in the evaluated conditions. However, more detailed studies of the medicinal and toxicological potential, as well as the characterization of the phytochemicals present in this plant, are still necessary to better

understand its mechanisms in the treatment of this pathology, in addition to other possible therapeutic applications.

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APÊNDICE A – ARTIGO 1

APÊNDICE B – ARTIGO 3

APÊNDICE C – ARTIGO 4

Tabela 1A Resumo da análise de variância para composição centesimal e mineral de plantas medicinais.

Parâmetro	FV	GL	QM	CV (%)
Proteína bruta	Tratamentos	4	52,92*	3,80
	Resíduo	10	0,05	
Extrato etéreo	Tratamentos	4	8,40*	8,39
	Resíduo	10	0,02	
Cinzas	Tratamentos	4	510,22*	1,05
	Resíduo	10	0,01	
Fibra total	Tratamentos	4	1284,03*	1,62
	Resíduo	10	0,45	
Fibra insolúvel	Tratamentos	4	2275,40*	1,22
	Resíduo	10	0,16	
Fibra Solúvel	Tratamentos	4	449,17*	6,22
	Resíduo	10	0,32	
Extrato não-nitrogenado	Tratamentos	4	1971,70*	2,10
	Resíduo	25	0,70	
Fósforo	Tratamentos	4	1258,16*	5,99
	Resíduo	10	15,95	
Potássio	Tratamentos	4	1550170*	3,39
	Resíduo	10	1639	
Cálcio	Tratamentos	4	25120662*	0,84
	Resíduo	10	394	

Tabela 1A Continua

Parâmetro	FV	GL	QM	CV (%)
Magnésio	Tratamentos	4	41732*	2,13
	Resíduo	10	23,00	
Enxofre	Tratamentos	4	221384*	5,18
	Resíduo	10	1065	
Cobre	Tratamentos	4	63,624*	5,18
	Resíduo	10	0,09	
Manganês	Tratamentos	4	862,23*	1,47
	Resíduo	10	0,07	
Zinco	Tratamentos	4	13,6588*	4,62
	Resíduo	10	0,02	
Ferro	Tratamentos	4	2650,46*	2,05
	Resíduo	10	0,57	

* Teste de F significativo a 1% de probabilidade.

Tabela 2A Resumo da análise de variância para os compostos bioativos de plantas medicinais.

Parâmetro	FV	GL	QM	CV (%)
Compostos fenólicos	Tratamentos	4	730,76*	4,94
	Resíduo	10	0,17	
Ácido oxálico	Tratamentos	4	0,79*	7,32
	Resíduo	10	0,01	
Nitrato	Tratamentos	4	23,2289*	3,50
	Resíduo	10	0,06	
Inibidor de tripsina	Tratamentos	4	271,30*	19,05
	Resíduo	10	1,17	
Saponinas	Tratamentos	4	0,50*	8,80
	Resíduo	10	0,01	

* Teste de F significativo a 1% de probabilidade.

Tabela 1B Resumo da análise de variância para as substâncias antioxidantes de plantas medicinais.

Parâmetro	FV	GL	QM	CV (%)
Compostos fenólicos	Tratamentos	5	592,13*	4,89
	Resíduo	12	0,14	
Flavonoides	Tratamentos	5	99716,00*	1,74
	Resíduo	12	5,00	
Vitamina C	Tratamentos	5	179983,00*	1,57
	Resíduo	12	7,00	
Carotenoides totais	Tratamentos	5	258,56*	3,69
	Resíduo	12	0,07	
β-caroteno	Tratamentos	5	47,28*	12,28
	Resíduo	12	0,09	
Licopeno	Tratamentos	5	4,36*	14,77
	Resíduo	12	0,03	

* Teste de F significativo a 1% de probabilidade.

Tabela 2B Resumo da análise de variância para a atividade antioxidante pelos métodos ABTS e β-caroteno/ácido linoleico.

Parâmetro	FV	GL	QM	CV (%)
ABTS (Trolox)	Tratamentos	13	201523,00*	2,44
	Resíduo	22	10	
ABTS (Vitamina C)	Tratamentos	11	174651,00*	2,00
	Resíduo	24	5,00	
β-caroteno/ ácido linoleico	Tratamentos	5	13107,10*	7,59
	Resíduo	102	234,40	
β-caroteno/ ácido linoleico	Concentração	2	2502,82*	3,69
	Resíduo	12	4,94	

* Teste de F significativo a 1% de probabilidade.

Tabela 1C Resumo da análise de variância do peso corporal e do consumo alimentar de ratos *Wistar* submetidos aos tratamentos[1], durante seis semanas.

Parâmetro	FV	GL	QM	CV (%)
Peso corporal	Tratamentos	4	3362,76^{ns}	25,53
	Resíduo1	25	7153,62	
	Tempo	6	2144,59*	5,69
	Tratamentos*Tempo	24	449,39^{ns}	
	Resíduo 2	150	355,09	
Consumo alimentar	Tratamentos	4	32,67*	15,31
	Resíduo1	30	7,15	
	Tempo	5	36,79*	13,94
	Tratamentos*Tempo	20	5,68^{ns}	
	Resíduo 2	150	5,93	

* Teste de F significativo a 1% de probabilidade. NS - Teste de F não significativo.
[1]Controle- 1mL de água dia^{-1}; T1- extrato aquoso contendo 14 mg de compostos fenólicos kg^{-1} rato dia^{-1}; T2- 14 mg de quercetina kg^{-1} rato dia^{-1}; T3- 50 mg de farinha de folhas de *Tournefortia paniculata* (FFT) kg^{-1} rato dia^{-1} e T4- 100 mg de FFT kg^{-1} rato dia^{-1}.

Tabela 2C Resumo da análise de variância do peso do fígado, peso do fígado/peso carcaça, gordura corporal e hepática de ratos *Wistar* submetidos aos tratamentos[1], durante seis semanas.

Parâmetro	FV	GL	QM	CV (%)
Peso do fígado	Tratamentos	4	1,00 ^{ns}	14,75
	Resíduo	25	1,31	
Peso fígado/peso corporal total	Tratamentos	4	0,25 ^{ns}	11,73
	Resíduo	25	0,30	
Gordura corporal	Tratamentos	4	21,10 ^{ns}	21,95
	Resíduo	25	14,54	
Gordura Hepática	Tratamentos	4	25,13*	24,89
	Resíduo	25	6,14	

* Teste de F significativo a 1% de probabilidade. NS - Teste de F não significativo.
[1]Controle- 1mL de água dia^{-1}; T1- extrato aquoso contendo 14 mg de compostos fenólicos kg^{-1} rato dia^{-1}; T2- 14 mg de quercetina kg^{-1} rato dia^{-1}; T3- 50 mg de farinha de folhas de *Tournefortia paniculata* (FFT) kg^{-1} rato dia^{-1} e T4- 100 mg de FFT kg^{-1} rato dia^{-1}.

Tabela 3C Resumo da análise de variância de parâmetros bioquímicos e de substâncias reativas ao ácido tiobarbitúrico (TBARS) de ratos *Wistar* submetidos aos tratamentos[1], durante seis semanas.

Parâmetro	FV	GL	QM	CV (%)
Glicose capilar caudal	Tratamentos	4	237,91*	8,28
	Resíduo	25	54,41	
Glicose sérica	Tratamentos	4	1091,94*	10,00
	Resíduo	25	278,64	
Colesterol total	Tratamentos	4	10,57 ^{ns}	11,92
	Resíduo	25	51,03	
Colesterol HDL	Tratamentos	4	166,54 ^{ns}	7,14
	Resíduo	25	16,23	
Triacilglicérides	Tratamentos	4	158,90*	15,75
	Resíduo	25	29,52	
AST	Tratamentos	4	374,83 ^{ns}	20,94
	Resíduo	25	275,05	
ALT	Tratamentos	4	892,25*	20,13
	Resíduo	25	181,98	
Gama GT	Tratamentos	4	0,74 ^{ns}	31,39
	Resíduo	25	0,42	
TBARS	Tratamentos	4	0,11*	9,23
	Resíduo	25	0,00	

* Teste de F significativo a 1% de probabilidade. NS - Teste de F não significativo.
[1]Controle- 1mL de água dia^{-1}; T1- extrato aquoso contendo 14 mg de compostos fenólicos kg^{-1} rato dia^{-1}; T2- 14 mg de quercetina kg^{-1} rato dia^{-1}; T3- 50 mg de farinha de folhas de *Tournefortia paniculata* (FFT) kg^{-1} rato dia^{-1} e T4- 100 mg de FFT kg^{-1} rato dia^{-1}.

Tabela 4C Resumo da análise de variância do número de nucleoides por classe de cometa, frequência de nucleoides com danos e unidades arbitrárias em linfócitos de ratos *Wistar* submetidos aos tratamentos[1], durante seis semanas.

Parâmetro	FV	GL	QM	CV (%)
Dano 0	Tratamentos	4	1322,04*	14,68
	Resíduo	25	91,24	
Dano 1	Tratamentos	4	1082,37*	22,83
	Resíduo	25	59,19	
Dano 2	Tratamentos	4	27,69*	14,10
	Resíduo	25	2,57	
Dano 3	Tratamentos	4	0	
	Resíduo	25	0	
Dano 4	Tratamentos	4	0	
	Resíduo	25	0	
Frequência de danos	Tratamentos	4	1378,64*	23,30
	Resíduo	25	67,66	
Unidades arbitrárias	Tratamentos	4	1719,62*	24,37
	Resíduo	25	80,32	

* Teste de F significativo a 1% de probabilidade.

[1]Controle- 1mL de água dia^{-1}; T1- extrato aquoso contendo 14 mg de compostos fenólicos kg^{-1} rato dia^{-1}; T2- 14 mg de quercetina kg^{-1} rato dia^{-1}; T3- 50 mg de farinha de folhas de *Tournefortia paniculata* (FFT) kg^{-1} rato dia^{-1} e T4- 100 mg de FFT kg^{-1} rato dia^{-1}.