
# Journal of Medicinal Plant Research

Volume 10 Number 12, 25 March, 2016

ISSN 1996-0875


Academic Journals

# ABOUT JMPR

**The Journal of Medicinal Plant Research** is published weekly (one volume per year) by Academic Journals.

**The Journal of Medicinal Plants Research (JMPR)** is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peer reviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

**Contact Us**


Editorial Office: jmpr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JMPR

Submit manuscript online http://ms.academicjournals.me/

# Editorial Board

Journal of Medicinal Plants Research

Table of Contents: Volume 10 Number 12 25 March, 2016

## ARTICLE

academicJournals

Vol. 10(12), pp. 149-157, 25 March, 2016
DOI: 10.5897/JMPR2016.6044
Article Number: 56A41D257906
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Potency of extracts of selected plant species from Mbeere, Embu County-Kenya against *Mycobacterium tuberculosis*

**Sospeter Ngoci Njeru[1,2]* and Meshack Amos Obonyo[3]**

[1]Department of Medicine, Kisii University, P. O. Box, 408-40200, Kisii, Kenya.
[2]Stem Cell Aging Research group, Fritz Lipmann Institute (FLI) – Leibniz Institute for Age Research, D-07745, Jena, Germany.
[3]Department of Biochemistry and Molecular Biology, Egerton University, P.O Box. 536-20155 Egerton, Kenya.

Received 3 January, 2016; Accepted 19 February, 2016

**Tuberculosis is a serious chronic infectious disease affecting large global population. While efforts to control tuberculosis have intensified, they are challenged by rapid drug resistance development. For this reason, prospecting for compounds with potential antituberculous activity have been stepped up. The current study was done in a participatory appraisal manner to identify ten plants commonly used for management of "persistent coughs". Bioassays were conducted against *Mycobacterium tuberculosis* (H37Rv ATCC 27294) using the BACTEC MGIT™ 960 system. This was followed by assay of toxicity of the extracts towards Vero cells (ATCC CCL-81). Six extracts showed remarkable antitubercular activity. Four extracts had complete inhibition (0 GU- Growth Units) of *Mycobacterium tuberculosis*. The extracts were tested for their general antimicrobial activity and found to be broad spectrum antimicrobials. The highest activity against *Escherichia coli* (15.3 mm) was by *Cissampelos pareira,* while *Mangifera indica* yielded the highest activity against *Staphylococcus aureus* (11.7 mm) and *Candida albicans* (12.0 mm). In addition, six crude methanolic extracts were found to be within the acceptable toxicity limit ($CC_{50}$<90 µg/ml). The observed activity is attributable to phytochemicals in the extracts, including: phenols, terpenoids, flavonoids and anthraquinones. These findings could partly explain observed "positive" treatment outcome by indigenous people using these plant formulations.**

**Key words:** Antibacterial activity, antituberculous activity, BACTEC MGIT™ 960 system, cytotoxicity, flavonoids, phytochemicals, terpenoids, Vero cells.

## INTRODUCTION

Tuberculosis is a very serious chronic infectious disease affecting a large part of the population worldwide. Millions of people have died as a result of infection by the pathogen tubercle bacillus (Snider et al., 1994). Particularly of concern today is that the disease has spread to cover both developing and industrialized nations

*Corresponding author. E-mail: hicogn@gmail.com. Tel: +49 15219228417.

and this is accompanied by widespread emergence of drug-resistant strains of the pathogen. For example, the World Health Organization (WHO) estimates that, out of 9.6 million people who developed tuberculosis (TB) in 2014, 480,000 (5%) had multidrug resistant (MDR; resistance against isoniazid and rifampicin) (WHO, 2015). According to the WHO, the incidence of new tuberculosis infections have been steadily dropping (about 2% annually) over the last two decades when the disease was declared a global emergency (WHO, 2013). WHO TB Global Report (2015), also reported that, 9.6 million people are estimated to have fallen ill with TB in 2014 globally: 5.4 million men, 3.2 million women and 1.0 million children. 12% of the 9.6 million new TB cases in 2014 were also HIV-positive (WHO, 2015), highlighting the dangerous synergy between TB and HIV/AIDS.

In humans, tuberculosis is primarily caused by *Mycobacterium tuberculosis* (MTB) although to a lesser extent, other members of the *Mycobacterium* complex (*Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti*) have been implicated in pathogenesis (Sreevatsan et al*.,* 1997). Without a known reservoir outside man, inhalation of aerosolized droplets containing infectious *M. tuberculosis* remains the predominant route of infection thus making pulmonary tuberculosis the prevalent form of infection (Glickman and Jacob, 2001). The tubercle bacilli are characterized by slow growth, dormancy, intracellular pathogenesis, genetic homogeneity, a complex cell envelope containing mycolic acid in their cell wall which makes them acid fast with a distinctively slow rate of division (~24 h) (Wheeler and Ratledge, 1994; Cole et al., 1998; Lawn and Zumla, 2011). These attributes enable it to persist in latent state for extensive periods of time, but perhaps more important accounts for the chronic phase of the disease. From a clinical perspective, the persistence imposes lengthy treatment regimens and presents a formidable obstacle to intervention (Cole et al*.,* 1998).

As a result of prolonged duration of therapy, there is the associated adverse toxicity as well as poor patient compliance to the treatment regimen. The poor compliance is often a cause for selection of drug resistant strains of tuberculosis which have been reported recently (multidrug resistant and extensively drug resistant tuberculosis) (Mariita et al., 2010a). The pathogen *M. tuberculosis* has been reported to rapidly develop resistance to several classes of antibiotics and this is largely attributable to it highly hydrophobic cell envelope which acts as a permeability barrier to most conventional drugs (Cole and Telenti, 1995). Additionally, other potential resistance determinants encoded in tuberculosis genome include: hydrolytic or drug modifying enzymes (beta-lactamases, aminoglycoside acetyl transferases) and many potential drug–efflux systems (14 members of the major facilitator family and numerous ABC transporters) (Brennan and Draper, 1994).

The aforementioned are some of the most important factors that has turned attention to tuberculosis necessitating continuous effort to counter its impact. This therefore means that several different classes and combinations of drugs continue to be developed and tested on tuberculosis. However, these efforts have further escalated cost of therapy. Continued attempts to scale this cost barrier have resulted in patients accessing alternative/traditional herbal therapy as they seek other options. It is believed that the answer to tuberculosis is hidden in the forest of the plant kingdom because this is one of the places where the untapped promise for treatment of infectious diseases lies. This is believed to be true especially in the context of developing countries where there is little or no access to modern health services (Mann et al., 2007; Idu et al., 2010). The current study builds upon previous efforts in prospecting for anti-tuberculosis activities among some traditional plants used in Eastern and Southern Africa (Tabuti et al., 2009; Earl et al., 2010; Mariita et al., 2010a). However, an important addition is that prior to conducting bioassays the main practitioners also known as herbalists were interrogated from whom identities of plants which have been used to manage “persistent coughs” was established. This was in an effort to contribute to prospecting efforts but perhaps more importantly to establish the safety of these plants. Therefore, an important prerequisite was the determination of toxicity levels of the plant extracts.

## RESEARCH DESIGN

The current study employed both descriptive and laboratory research designs. The descriptive research was carried out in a participatory rural appraisal manner. In order to gather first hand data from the respondents (herbalists) which was helpful in establishing the identity of various medicinal plants used in the management of “persistent coughs” and other respiratory tract diseases. Thereafter, on the basis of information gathered, extraction and bioassays were conducted to determine the possible impact of the respective plant metabolites on *M. tuberculosis* and Vero cells.

### Sample collection, preparation and phytochemical assay

Ten medicinal plants earlier identified by herbalists as useful in management of persistent coughs were used in this study (Table 1). None of these listed plants is an endangered species and were collected in open community field hence no prior permission was required. The geographical coordinates for the collection points were around 0°46'27.0"S 37°40'54.9"E; -0.774156 and 37.681908 (Kathuri village, Mbeere in Embu county, Kenya). These plants were later identified by a plant taxonomist in Egerton University (Njoro, Nakuru, Kenya) where voucher specimens were deposited and their numbers are recorded (Table 1). All plant parts were chopped into small pieces (about 2 to 3 cm) and air-dried under a shade at room temperature (23±2°C) to constant weight. The dry specimens were separately ground to powder in a mechanical grinder and separately macerated in methanol (50 g powder in 200 ml) for 48 h. Afterwards, the extracts were then filtered using a filter

**Table 1.** List of plants and parts used.

| Botanical name | Family name | Voucher No. | Part(s) used |
|---|---|---|---|
| *Aspilia pluriseta Schweinf.* | Asteraceae | NSN2 | Roots |
| *Euphorbia ingens* E.Mey. ex Boiss. | Euphorbiaceae | NSN3 | Roots |
| *Gnidia (Lasiosiphon) buchananii* Gilg | Thymelaeaceae | NSN5 | Roots |
| *Mangifera indica* L. | Anarcardiaceae | NSN6 | Bark |
| *Cissampelos pareira* L. | Menispermaceae | NSN7 | Roots |
| *Dichrostachys cinerea* (L.) Wight and Arn | Fabaceae | NSN8 | Roots |
| *Dalbergia melanoxylon* Guill. & Perr. | Fabaceae | NSN9 | Bark |
| *Indigofera lupatana* Baker F | Fabaceae | NSN1 | Roots |
| *Acacia ataxacantha* DC | Fabaceae | NSN10 | Roots |
| *Lonchocarpus eriocalyx* Harms | Fabaceae | NSN12 | Barks |

paper (Whatman's No. 1) and the filtrate concentrated *in vacuo* using a rotary evaporator (Büch Rotavapor R205, Switzerland), after which, products were allowed to air dry and their percentage yields recorded (Table 2). Once dry, the plant extracts were stored in air tight sample bottles at -20°C until next use. Standard procedures were employed for screening of the major classes of plant secondary metabolites in the extracts including: alkaloids, anthraquinones, terpenoids, phenolics and flavonoids (Harborne, 1984).

**Antitubercular and antimicrobial activity screening**

The test organism *M. tuberculosis* H37Rv ATCC 27294 was sourced from the Kenya Medical Research Institute (KEMRI), Nairobi. Prior to its use, the *M. tuberculosis* was revived on Lowenstein Jensen (LJ) slants for 14 days at 37°C following standard procedures (Gupta et al., 2010; Mariita et al., 2010a). The efficacy of the plant extracts against *M. tuberculosis* was carried out using the BACTEC MGIT 960 system. This is a fully automated, high volume, non-radiometric instrument that offers continuous monitoring of culture growth. The dry extract from each plant was first dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 g/ml. Growth supplement (0.8 ml) containing a mixture of oleic acid, bovine albumen, dextrose and catalase (OADC) was added to five 7 ml BBL™ MGIT™ tube labeled growth control, streptomycin, isonaizid, rifampicin, and ethambutol to provide essential substrates for rapid growth of *Mycobacteria*. 100 µl of BBL™ MGIT™ streptomycin, isonaizid, rifampicin, and ethambutol (SIRE) prepared aseptically according to the manufacturers' instruction was added to corresponding labeled BBL™ MGIT™ tube followed by addition of 0.5 ml of 1% *Mycobacterium* suspension. *Mycobacterium* suspension was prepared by pipetting 0.1 ml Middlebrook 7H9 Broth containing *Mycobacterium* adjusted to 0.5 McFarland standard into 10 ml sterile saline aseptically. The BACTEC MGIT™ 960 system was then loaded following the manufacturer's instructions and incubated at 37°C (Becton and Company, 2007). These served as the positive control (streptomycin at 1.0 µg/ml, isoniazid at 0.1 µg/ml, rifampicin at 1.0 µg/ml and ethambutol at 5.0 µg/ml whereas DMSO was used as a negative control). The procedure was repeated using plant extracts at 1.0 g/ml in place of SIRE. The general antimicrobial activity using *Escherichia coli* (ATCC 2592), *Staphylococcus aureus* (ATCC 25923) and *Candida albican* (ATCC 90028) was assayed by standard disc diffusion method according to Ayo et al. (2007), Mbaveng et al. (2008), Ngoci et al. (2012) and Mwitari et al. (2013).

**Cytotoxicity screening**

The toxicity of the plant extracts was assayed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay; a colorimetric assay based on the ability of mitochondrial enzyme (Succinate dehydrogenase) to reduce the yellow water soluble MTT into an insoluble colored compound called formazan (which can be measured Spectrophotometrically). Since only metabolically active cells can reduce MTT, the level of activity is usually directly proportional to the measure of the cell viability (Denizot and Lang, 1986). The test cell line used was Vero cells from African green Monkey Kidney cells (*Cercopithecus aethiops* epithelial cell line; ATCC CCL-81) (Mosmann, 1983). The cells were cultured in a T-75 flask containing Minimum Essential Medium (MEM) Eagle's Base supplemented with 15% Fetal Bovine Serum (FBS), 2.62 g/L $NaHCO_3$, 20 mM L-glutamine, 10 ml/L Penstrep 0.5 mg and Fungizoid. The cells were maintained at 37°C in 5% $CO_2$ until they attained confluency when they were harvested by trypsinization. Trypsin was inactivated within 1 min of action by addition of 8 ml of growth media and the cell crumps broken gently by sucking and releasing the cell suspension using a pipette. The harvested cells (2 ml) were then transferred into a 50 ml vial and topped up to 50 ml mark using growth media. Cell suspension (100 µl) at $1 \times 10^5$ cell/ml was seeded into two rows of wells A-H in a 96-well micro-titer plate for one sample. The cells were then incubated in 100 µl of MEM at 37°C and 5% $CO_2$ for 48 h to form a confluent monolayer. The growth medium was then aspirated off and replaced with 100 µl of maintenance medium. Afterwards, the Vero cells were exposed to increasing concentrations of respective plant extracts (from 2.0 to 500 µg/ml) and incubated at 37°C for 48 h. This was followed by a further incubation period of 4 h in 10 µl of 5 mg/ml MTT solution after aspirating off the plant extracts. Later, this was followed by addition of 100 µl acidified isopropanol (0.04 N HCl in isopropanol). The well plate was gently shaken for 5 min to dissolve the formazan and then Optical Density measured using ELISA Scanning Multiwell Spectrophotometer (Multiskan Ex labssystems) at 562 and 690 nm as reference. Rows of cells containing medium without plant extracts were included as negative control. Cell viability (%) was calculated at each concentration as follows (Ngeny et al., 2013).

$$\text{Cell viability (\%)} = \frac{OD_{\text{sample } 562} - OD_{690}}{OD_{\text{control } 562} - OD_{690}} \times 100$$

**Statistical analysis**

GraphPad Prism (version 6.04) and Ms Excel 2010 data sheet were

**Table 2.** Crude extract´s percentage yields and antituberculous activity.

| Plants | % Yield | PC-GU | GC-GU | ME-GU | R/S | DH |
|---|---|---|---|---|---|---|
| *Aspilia pluriseta* | 8 | 0 | 400 | 0 | S | 9.14 |
| *Euphorbia ingens* | 8 | 0 | 400 | 0 | S | 9.20 |
| *Gnidia (Lasiosiphon) buchananii* | 4 | 0 | 400 | 1 | S | 9.15 |
| *Mangifera indica* | 4 | 0 | 400 | 0 | S | 9.16 |
| *Cissampelos pareira* | 6 | 0 | 400 | 56.5 | S | 5.23 |
| *Dichrostachys cinerea* | 4 | 0 | 400 | 0 | S | 5.22 |
| *Dalbergia melanoxylon.* | 4 | 0 | 400 | 223 | R | 5.19 |
| *Indigofera lupatana* | 4 | 0 | 400 | 200 | R | 6.7 |
| *Acacia ataxacantha* | 4 | 0 | 400 | 400 | R | 8.4 |
| *Lonchocarpus eriocalyx* | 4 | 0 | 400 | 400 | R | 8.11 |

ME: Methanolic extract; GU: average growth units (n=2); R: Resistant; S: Sensitive; GC: Growth/negative control; PC: Positive control of streptomycin at 1.0 µg/ml, isonaizid at 0.5 µg/ml, rifampicin at 1.0 µg/ml and ethambutol at 5.0 µg/ml; DH: Days and Hours the BACTEC machine ran to yield results recorded.

used to analyze the data. The extract material from each plant was expressed as percentage yield and results presented in bar graph. Cytotoxicity using Vero cells was expressed as $CC_{50}$ values, which is the concentration that kills 50% of the Vero cells. This was determined by Regression Analysis and results presented in bar graphs. A particular extract was considered cytotoxic if it had $CC_{50}$ less than 90 µg/ml (Irungu et al., 2007). Unpaired t test was used to analyze antimicrobial activity. Diameters of zones of inhibition were expressed as mean±standard error of mean (SEM) and $p<0.05$ was used to test the level of significant difference between the test sample and the positive drug control.

## RESULTS

The different crude extract yields are recorded and presented in (Table 2). The highest yield was 8% (4/50 g), while the lowest was 4% (2/50 g).

The interpretation of the antituberculous result data was based on the method previously described by Mariita et al. (2010b) and Lawson et al. (2013). When the growth unit (GU) of the growth control reached 400 (usually in 4 to 13 days), the GU values of the extract-containing vials were evaluated. If the GUs value of the extract-containing tube to be compared was ≥100, the strain was considered to be resistant (R) to the extract; while if the GU of the extract-containing tube was <100, the strains were considered to be sensitive (S) to the extract. Based on this, six plants crude methanol extracts were found to have considerable antituberculous activity. These were: (i) *Aspilia pluriseta*, (ii) *Euphorbia ingens*, (iii) *Gnidia buchananii*, (iv) *Mangifera indica*, (v) *Cissampelos pareira*, and (vi) *Dichrostachys cinerea*. Four of these extracts had similar GU as the positive control SIRE (Table 2). At the same time *Acacia ataxacantha* and *Lonchocarpus eriocalyx* extracts were inactive against MTB (Table 2), while another two, *Indigofera lupatana* and *Dalbergia melanoxylon* had an average sensitivity of 200 and 223 GU, respectively.

The extracts from *I. lupatana, A. pluriseta, G. buchananii*, and *M. indica* were cytotoxic having $CC_{50}$ <90 (Table 3). While *I. lupatana* had no antituberculous activity, the other 3 plant extracts were active against *M. tuberculosis.* The rest of plant extracts were not cytotoxic indicating that they were tested within the acceptable toxicity limits. All plant extracts except *L. eriocalyx* had varying broad spectrum antimicrobial activity ranging from diameters of zones of inhibition of between 6.0 and 15.3 mm (Table 5). The activity was concentration dependent and the lowest MIC and MBC recorded was of 117 µg. The activity was either cidal or static as recorded (Table 5). However, there was significant difference ($P< 0.05$) between the activity of the test extract and the positive control drugs (Gentamycin, Oxacillin and Nystatin).

Phytochemical results demonstrated that all plant extracts had phenols and terpenoids in varying quantities, while flavonoids were found to be present in three plant extracts. Anthraquenones were found only on one plant extract while alkaloids were absent in all extracts (Table 4).

## DISCUSSION

A participatory approach was used to identify medicinal plants for use in this study. While there have been previous studies undertaken on antibacterial activities on some of these plants used in the current study (Khalil, 2003; Abubakar, 2009; Sripathi and Sankari, 2010; Aworet-Samseny et al., 2011; Ighodaro et al., 2012), to the best of our knowledge, there is little scientific information to confirm their antituberculous activity. Though Cateni et al. (2003) hypothesized about antimycobacterial activity of *Euphorbia* species and *C. pareira* has been implicated by Antoun et al. (2001) as having antituberculous activity, no proper antituberculous

**Table 3.** Cytotoxicity results of methanolic extracts.

| Plant | $CC_{50}$ (µg/ml) |
|---|---|
| *Aspilia pluriseta* | 24.51 |
| *Euphorbia ingens* | 105.55 |
| *Gnidia (Lasiosiphon) buchananii* | 76.24 |
| *Mangifera indica* | 88.61 |
| *Cissampelos pareira* | 179.02 |
| *Dichrostachys cinerea* | 201.22 |
| *Dalbergia melanoxylon.* | 120.04 |
| *Indigofera lupatana* | 60.37 |
| *Acacia ataxacantha* | 90.39 |
| *Lonchocarpus eriocalyx* | 201.87 |

**$CC_{50}$**: Concentration that kills 50% of the cells. The plant extracts were two-fold serial diluted to varying concentrations ranging from 3.90625 to 500 µg/ml. CC50 values ≤90 µg/ml were considered to be cytotoxic (Irungu et al., 2007).

**Table 4.** Phytochemical tests.

| Plant | Test for | | | | |
|---|---|---|---|---|---|
| | Phenols | Terpenoids | Flavonoids | Anthraquinones | Alkaloids |
| *Aspilia pluriseta* | + | +++ | + | - | - |
| *Euphorbia ingens* | + | ++ | - | - | - |
| *Gnidia (Lasiosiphon) buchananii* | + | + | - | - | - |
| *Mangifera indica* | + | ++ | - | - | - |
| *Cissampelos pareira* | + | ++ | ++ | + | - |
| *Dichrostachys cinerea* | + | + | - | - | - |
| *Dalbergia melanoxylon.* | + | ++ | - | - | - |
| *Indigofera lupatana* | + | + | - | - | - |
| *Acacia ataxacantha* | + | ++ | - | - | - |
| *Lonchocarpus eriocalyx* | ++ | + | + | - | - |

+, Low concentration of phytochemicals; ++, Medium concentration; +++, High concentration of phytochemicals and -, Absence of phytochemicals.

studies on the target plants has been undertaken using the pathogenic MTB strain.

Cytotoxicity of plant extracts is crucial in determining safety, particularly in the context of TB therapy that often entails lengthy treatment regime (Zaleskis, 2006). A major output of the current study is the identification of six plants methanolic crude extract that demonstrate antituberculous activity with accompanying data on their toxicity levels showing that the used concentration was still within the acceptable toxicity margin. This is particularly important as these extracts become promising candidates for further testing in intracellular assays. However, even the other tested extracts (Table 3) that show cytotoxicity should not be disqualified as drug candidates as structural modification can be undertaken to improve on their safety. They can also be looked upon as possible candidates for cancer treatment (Ngeny et al., 2013).

In addition to antituberculous activity, the plant extracts had broad spectrum activity as they inhibited growth of Gram positive, Gram negative bacteria and a fungus. The inhibition zones were dose dependent (Figure 1). Variation was observed with the microbial strain tested indicating selectivity in the activity of the extracts. Gram positive strain (*S. aureus*) was more susceptible to the extract often yielding higher zones of inhibition than Gram negative (*E. coli*) (Table 5) and fungal strain (*C. albicans*). This corroborates previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda, 2006; Mohamed et al., 2010; Ngoci et al., 2012). The higher sensitivity of Gram-positive bacteria has been attributed to their outer peptidoglycan layer which is not an effective permeability barrier as compared to the outer phospholipid membranes of Gram-negative bacteria (Trombetta et al., 2005; Tomczykowa et al., 2008; Kaur and Arora, 2009;

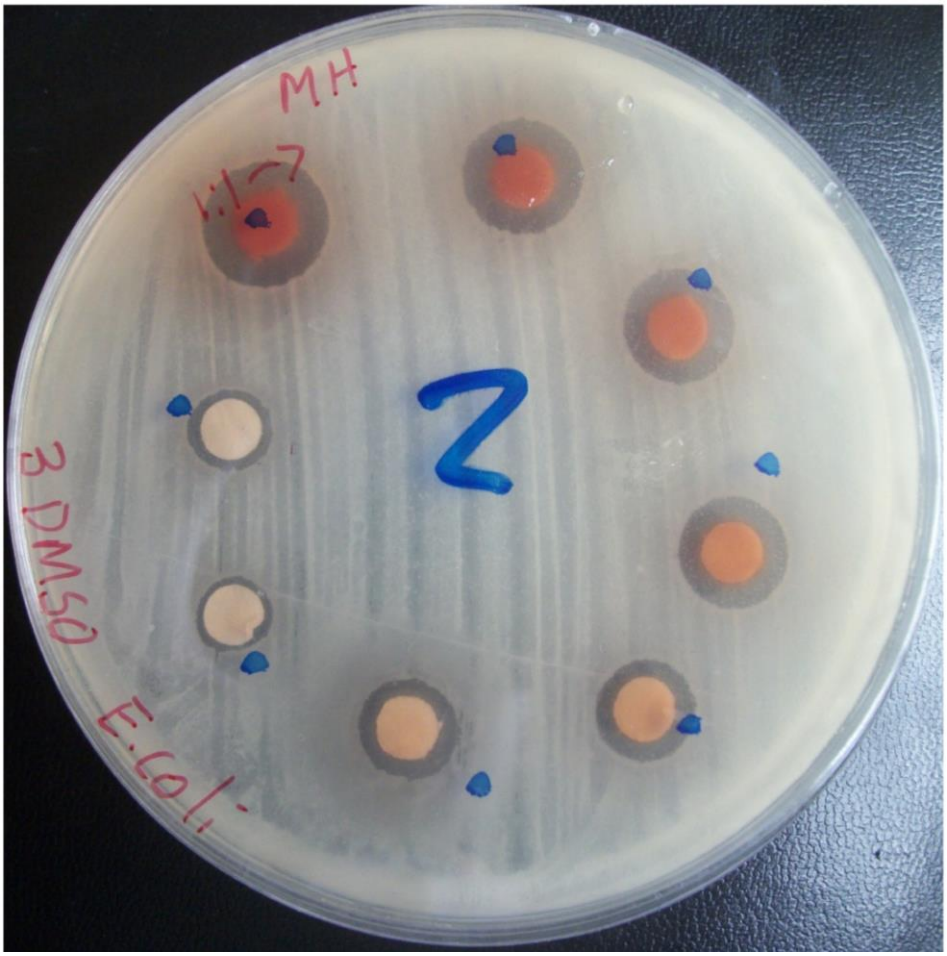


**Figure 1.** Zones of inhibition of *Mangifera indica* methanolic extract against *E. coli*. The extract was serially diluted and zones of inhibition diameter decreased as concentration decreased demonstrating a concentration dependent activity.

Ngoci et al., 2012).

Although the concentrations of the extract fractions were in the range of 100 times more than the standard drugs (positive controls in both antituberculous and general antimicrobial sensitivity testing), they showed marked anti-microbial activity as evidenced by their zones of inhibition and zero GU. This could be due to the fact that the active components in the extract comprise only a fraction of the total extract used. Therefore, the concentration of the active components in the extract could be much lower than the standard antibiotic used. It is important to note: if the active components were isolated and purified, they would probably show higher antibacterial activity than those observed in this study.

The activity observed in the plant extracts in the current study may be associated with the group of phytochemical compositions tested in the extracts. For example, the current study established the presence of terpenoids in all extracts in varying concentrations and flavonoids in *A. pluriseta* and *C. pareira*. Other studies have shown that flavonoids have antituberculous activity and they function mechanistically by inhibiting *de novo* fatty acid biosynthesis in *Mycobacteria*, inhibiting mycolic acid biosynthesis, proteasome inhibition, topoisomerase inhibition, inhibition of phosphatidyl-inositol 3-kinase, induction of cell cycle arrests, accumulation of p53 or enhanced expression of c-fos and c-myc genes (Yuan et al., 2009; Mariita et al., 2010b). Flavonoids have also been shown to have antimicrobial activity and to act by complexing microbial proteins and disrupting microbial membranes (Cowan, 1999; 2003; Al-Bayati and Al-Mola, 2008; Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009; Ngoci et al., 2011, 2012). On the other hand, terpenoids have been shown to have antibacterial activity (Cowan, 1999), although the mechanism of action is not well understood but it is thought to involve membrane disruption and inhibition of protein synthesis (Cowan, 1999; Mariita et al., 2010b). *C. pareira* was found to have terpenoids, flavonoids and anthraquinones and this is in agreement with earlier study by Ngoci et al.

**Table 5.** Antimicrobial activity.

| Plant | ZID in mm at ($15 \times 10^3$ µg) | | | MIC (µg) | | | MBC (µg) | | | Effect (BS/BC) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.C | S.A | C. A | E.C | S.A | C. A | E.C | S.A | C. A | E.C | S.A | C. A |
| *Aspilia pluriseta Schweinf.* | 11.6±1.2 | 9.0±0.6 | 7.7±0.3 | 234 | 1875 | 3750 | >15000 | 1875 | 3750 | BS | BC | BC |
| *Euphorbia ingens* | 11±1.7 | 10±0.9 | 11.7±0.3 | 234 | 234 | 117 | >15000 | 7500 | 117 | BS | BC | BC |
| *Gnidia (Lasiosiphon) buchananii* | 6.7±0.3 | 7.3±0.3 | 6.7±0.3 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *Mangifera indica* | 11.0±0 | 11.7±0.3 | 12.0±0.6 | 234 | 117 | 1875 | 468 | 117 | 1875 | BS | BC | BC |
| *Cissampelos pareira* | 15.3±0.3 | 11.0±1.0 | 11.7±0.3 | 234 | 117 | 468 | >15000 | 117 | 468 | BS | BC | BC |
| *Dichrostachys cinerea* | 6.0±0 | 6.0±0 | 6.0±0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *Dalbergai melanoxylon.* | 12.0±0.6 | 11.0±1.0 | 11.7±0.3 | 117 | 117 | 468 | >15000 | >15000 | 937 | BS | BS | BS |
| *Indigofera lupatana* | 7.0±0 | 6.3±0.3 | 6.7±0.3 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *Acacia ataxacantha* | 8.3±0.7 | 6.7±0.3 | 7.0±0.6 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *Lonchocarpus eriocalyx* | 6.0±0 | 6.0±0 | 6.0±0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Positive standard | 22.0±0 | 24.7±1.3 | 16.3±0.9 | NT | NT | NT | NT | NT | NT | BC | BC | BC |
| Negative Control | 6.0±0 | 6.0±0 | 6.0±0 | NA | NA | NA | NA | NA | NA | NA | NA | NA |

ZID: Zone of inhibition diameter taken in mm (mean±SEM, n=3); MIC: minimum inhibitory concentration; MBC; minimum bactericidal concentration; EC: *E. coli*; SA: *S. aureus*; CA: *C. albican*; BS: bacteriostatic effect; BC: bactericidal effect; NA: not applicable; No inhibition zone observed; NT: not tested; Positive Standard: Gentamycin (10 µg) for *E. coli*, Oxacillin (10 µg) for *S. aureus* and Nystatin (100 µg) for *C. albican*; Negative control: A disc loaded with 15 µl of DMSO. Unpaired t test analysis demonstrated that there was significant difference in zones of inhibition ($P< 0.05$) between the test sample mean and positive control drug means.

(2014), though alkaloids that tested positive then, tested negative in this particular study. Phytochemical differences on what other scientist have published and what we tested from same plant can be associated with the great diversity of plants bioactive compounds. This diversity of bioactive compounds from same plant species is influenced by; genetic characteristics, environmental factors such as climate, altitude and soil type; the period when collection took place, the treatment after collection and existence of a distinct phenotype of a particular species (also known as chemical races). This diversity can either be in regard to presence and absence of certain phytochemicals or be in the levels of concentration of a certain phytochemical in a plant sample. Therefore, the observed activity in the current study could also be attributed to the presence of flavonoids and terpenoids which have been shown to have capacity to traverse the highly hydrophobic tubercle envelop (Edwards and Ericsson, 1999; Rao et al., 2010).

## Conclusion

Natural products are proven templates for the development of new arsenals of drugs for fighting and management of various diseases, and therefore, they have received considerable attention as potential alternative anti-tuberculosis agents. Our findings preliminarily demonstrate that methanolic extracts of 6 indigenous plants which we worked on had potential in management of tuberculosis while virtually all extracts had varying antimicrobial activity. This demonstrated that there is a degree of reliability in the traditional systems which lead to the identification of these plants. Indeed more work is needed, some of which is already underway to fractionate the plant extracts and possibly identify the specific active components, with a view of deciphering their mode(s) of action.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors would wish to acknowledge: Kenya Medical Research Institute (Dr. Bii C), Kisii University (Prof. Anakalo Shitandi), Egerton University, (Prof. Matasyoh JC, Prof. Ngari SM

and Samwel Nyambati) for the support they gave to the work.

**REFERENCES**


Abubakar EM (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J. Med. Plants Res. 3(7):498-505.

Al-Bayati FA, Al-Mola HF (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. J. Zhejiang Univ. Sci. 9:154-159.

Antoun MD, Ramos Z, Vazques J, Oquendo I, Proctor GR, Gerena L, Franzblau SG (2001). Evaluation of the flora of Puerto Rico for *in vitro* antiplasmodial and antimycobacterial activities. Phytother. Res. 15:638-642.

Aworet-Samseny RRR, Souza A, Kpahé F, Konaté K, Datté JY (2011). *Dichrostachys cinerea* (L.) Wight et Arn (Mimosaceae) hydro-alcoholic extract action on the contractility of tracheal smooth muscle isolated from guinea-pig. BMC Complement. Altern. Med. 11:18.

Ayo RG, Amupitan JO, Zhao Y (2007). Cytotoxicity and anti-microbial studies of 1,6,8-trihydroxy-3-methyl-anthraquinone (Emodin) isolated from the leaves of *Cassia nigricans* Vahl. Afr. J. Biotechnol. 6:1276-1279.

Becton Dickinson Company (2007). BBL MGIT Mycobacteria growth indicator Manual. Maryland, USA. P 123.

Brennan PJ, Draper P (1994). In Tuberculosis: Pathogenesis, protection, and control (ed. Bloom, B. R.) (American Society for Microbiology, Washington DC). pp. 271-284.

Cateni F, Zilic J, Falsone G, Hollan F, Frausin F, Scarcia V (2003). Preliminary biological assay on cerebroside mixture from *Euphorbia nicaeensis*. Farmaco 58:809817.

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393(6685):537-544.

Cole ST, Telenti A (1995). Drug resistance in *Mycobacterium tuberculosis*. Eur. Respir. Rev. 8:701S-713S.

Cowan MM (1999). Plant products as anti-microbial agents. Clin. Microbiol. Rev. 12:564-582.

Denizot F, Lang R (1986). Rapid colorimetric assay for cell growth and survival. J. Immunol. Meth. 89:271-277.

Earl EA, Altaf M, Murikoli RV, Swift S, O'Toole R (2010). Native New Zealand plants with inhibitory activity towards *Mycobacterium tuberculosis.* BMC Complement. Altern. Med. 10:10-25.

Edwards PA, Ericsson J (1999). Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. Annu. Rev. Biochem. 68:157-185.

Glickman MS, Jacobs WR (2001). Microbial pathogenesis review of *Mycobacterium tuberculosis*: Dawn of a discipline. Cell Press 104:477-485.

Gupta VK, Shukla C, Bisht GRS, Kumar S, Thakur RL (2010). Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. Lett. Appl. Microbiol. 52:33-40.

Harborne JB (1984). Phytochemical methods: A guide to modern techniques of plant analysis, 2nd Edition. Chapman and Hall, New York, USA.

Idu M, Erhabor JO, Efijuemue HM (2010). Documentation on medicinal plants sold in markets in Abeokuta, Nigeria. Trop. J. Pharm. Res. 9:110-118.

Ighodaro OM, Agunbiade SO, Omole JO, Kuti OA (2012). Evaluation of chemical, nutritional, antimicrobial and antioxidant-vitamin profiles of *Piliostigma thonningii* leaves (Nigeria species). Res. J. Med. Plant 6:537-543.

Irungu BN, Rukanga GM, Mungai GM, Muthaura CN (2007). In vitro antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya. S. Afr. J. Bot. 73:204-207.

Kaur GJ, Arora DS (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi.* BMC Complement. Altern. Med. 9:30-41.

Khalil IA (2003). Antimicrobial activity of extracts from leaves, stems and flowers of *Euphorbia macroclada* against plant pathogenic fungi. J. Phytopathol. 42:245-250.

Lawn SD, Zumla AI (2011). Tuberculosis. Lancet 378:57-72.

Lawson L, Emenyonu N, Abdurrahman ST, Lawson JO, Uzoewulu GN, Sogaolu OM, Ebisike JN, Parry CM, Yassin MA, Cuevas LE (2013). Comparison of *Mycobacterium tuberculosis* drug susceptibility using solid and liquid culture in Nigeria. BMC Res. Notes 6:215.

Mann A, Amupitan JO, Oyewale AO, Okogun JI, Kolo I (2007). An ethnobotanical survey of indigenous flora for treating tuberculosis and other respiratory diseases in Niger state, Nigeria. J. Phytomed. Ther. 12:1-12.

Mariita RM, Ogol CKP, Oguge NO, Okemo PO (2010a). Antitubercular and phytochemical investigation of methanol extracts of medicinal plants used by the Samburu community in Kenya. Trop. J. Pharm. Res. 9:379-385.

Mariita RM, Okemo PO, Orodho JA, Kirimuhuzya C, Otieno JN, Magadula JJ (2010b). Efficacy of 13 medicinal plants used by indigenous communities around lake Victoria, Kenya, against tuberculosis, diarrhoea causing bacteria and *Candida albicans.* Int. J. Pharm. Technol. 2:771-791.

Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, Bezabih M, Etoa F, Ngadjui BT, Abegaz BM, Meyer JJM, Lall N, Beng VP (2008). Anti-microbial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae). J. Ethnopharmacol. 116:483-489.

Mohamed LT, El Nur BS, Abdelrahman MN (2010). The Antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. EurAsia. J. Biosci. 4:8-16.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. 65:55-63.

Mwitari PG, Ayeka PA, Ondicho J, Matu EN, Bii CC (2013). Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: *Withania somnifera, Warbugia ugandensis, Prunus africana* and *Plectrunthus barbatus*. PLoS ONE 8(6):e65619.

Ngeny LC, Magiri E, Mutai C, Mwikwabe N, Bii C (2013). Antimicrobial properties and toxicity of *Hagenia abyssinica* (Bruce) J.F.Gmel, *Fuerstia africana* T.C.E. Fries, *Asparagus racemosus* (Willd.) and *Ekebergia capensis* Sparrm. Afr. J. Pharm. Ther. 2:76-82.

Ngoci SN, Matasyoh JC, Mwaniki CG, Mwendia CM (2012). Antibacterial activity of methanol root extract of *Indigofera lupatana* Baker F. Eastern J. Med. 17:11-16.

Ngoci SN, Mwendia CM, Mwaniki CG (2011). Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. J. Anim. Plant Sci. 11(1):1364-1373.

Ngoci SN, Ramadhan M, Ngari SM, Oduor PL (2014). Screening for antimicrobial activity of *Cissampelos pareira* L. methanol root extract. Eur. J. Med. Plants 4:45-51.

Parekh J, Chanda S (2006). In-vitro antimicrobial activities of extractsof *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biomed. Res. 9:89-93.

Rao A, Zhang Y, Muend S, Rao R. (2010). Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob. Agents Chemother. 54(12):50-62.

Samy RP, Gopalakrishnakone P (2008). Review: Therapeutic potential of plants as anti-microbials for drug discovery. J. Evid. Based Complement. Altern. Med. 7:283-294.

Snider DE Jr, Raviglione M, Kochi A (1994). In Tuberculosis: Pathogenesis, protection, and control (ed. Bloom, BR) (American Society of Microbiology, Washington DC). pp. 2-11.

Sreevatsan S, Pan X, Stockbauer KE, Connell ND, Kreiswirth BN, Whittam TS, Musser JM (1997). Restricted structural gene polymorphism in the Mycobacterium tuberculosis complex indicates evolutionarily recent global dissemination. Proc. Natl. Acad. Sci. USA 94:9869-9874.

Sripathi SK, Sankari U (2010). Ethnobotanical documentation of a few Medicinal plants in the Agasthiayamalai region of Tirunelveli district, India. Ethnobot. Leaflets 14:173-181.

Tabuti JRS, Kukunda CB, Waako PJ (2009). Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. J. Ethnopharmacol. 127:130-136.

Tomczykowa M, Tomczyk M, Jakoniuk P, Tryniszewska B (2008). Antimicrobial and antifungal activities of the extracts and essential oils of *Bidens tripartite.* Folia Histochem. Cytobiol. 46:389-393.

Trombetta D, Castelli F, Sarpietro M, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G (2005). Mechanisms of anti-bacterial action of three monoterpenes. Antimicrob. Agents Chemother. 49:2474-2478.

Wheeler PR, Ratledge C (1994). In Tuberculosis: Pathogenesis, protection, and control (ed. Bloom, BR) (American Society of Microbiology, Washington DC). pp. 353-385.

World Health Organization (WHO) (2013). Global tuberculosis report 2015. http://www.who.int/tb/publications/global_report/en/

World Health Organization (WHO) (2014). Global Tuberclosis Report. www.who.int/tb/publications/global_report/

Yuan E, Liu B, Ning Z, Chen C (2009). Preparative separation of flavonoids in *Adinandra nitidaleaves* by high-speed counter-current chromatography and their effects on human epidermal carcinoma cancer cells. Food Chem. 115:1158-1163.

Zaleskis R (2006). Adverse effects of anti-tuberculosis chemotherapy. Eur. Respir. Rev. 47-49.

academicJournals

Vol. 10(12), pp. 158-166, 25 March, 2016
DOI: 10.5897/JMPR2015.6005
Article Number: B9048AA57908
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

*Full Length Research Paper*

# Administration of the aqueous extract of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) does not alter obesity induced by high-fat diet in mice

**Luana, M. Cercato[1], Pollyanna, A. S. White[1], Vanessa, S. Batista[1], Luciana, C. Brito[2], Charles dos Santos Estevam[1], Márcio R. V. dos Santos[1] and Enilton A. Camargo[1]***

[1]Department of Physiology, Federal University of Sergipe, (UFS), São Cristóvão, 49100-000, SE, Brazil.
[2]Department of Physical Education, Federal University of Ceará (UFC), Fortaleza, CE, Brazil.

Received 11 November, 2015; Accepted 17 February, 2016

**Ethnobotanical surveys have shown that the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) is popularly used to treat obesity and diabetes. However, there is no experimental evidence that confirms such use. The present study investigated the effects of the aqueous extract of the stem bark of *H. speciosa* (AEHS) on the glycemic and adipogenic profiles of obese mice. Mice were divided into four groups that received standard diet (SD), standard diet plus AEHS (SDE), high-fat diet (HD) and high-fat diet plus AEHS (HDE). The administration of AEHS (in a concentration of 0.3 mg.mL$^{-1}$. *ad libitum* in the drinking water) was performed for the last 8 weeks totaling a period of 18 weeks, in which the animals received the diets. Whole body weight, liquid intake and food consumption were measured during the entire experiment. Blood glucose levels, insulin sensitivity, glucose tolerance and adipose pads weight were evaluated. Animals from the HD group presented higher body weight in comparison to animals from the SD group. That was associated with insulin resistance and glucose intolerance, as well as increased blood glucose levels ($p < 0.05$) and weight of adipose tissue pad ($p < 0.05$), when compared to the SD group. The treatment with AEHS did not alter obesity induced by high-fat diet, because no significant difference was observed between the HD and the HDE groups in all of the parameters evaluated. These findings allowed the conclusion that AEHS does not reverse the alterations caused by high-fat diet in mice, what goes against the popular use.**

**Key words:** *Hancornia speciosa,* obesity, high-fat diet, adipose pads, glucose intolerance, insulin resistance

## INTRODUCTION

Obesity can be defined as a multifactorial syndrome consisting of biochemical, metabolic and anatomical alterations, such as increased adipose tissue and body weight (Go et al., 2013). Nowadays, obesity is an important risk factor for several types of diseases that lead to poor quality of life, considerable morbidity and premature death (Flegal et al., 2012). Obesity is increasing at an alarming rate and is considered as

*Corresponding author. E-mail:. enilton.camargo@pq.cnpq.br

a worldwide epidemic condition that affects all age groups. It is a chronic and multifactorial disease and may be a result of endogenous and/or exogenous factors. It is important to mention that the exogenous factors hold the majority of cases related to environmental factors, especially the lack of physical activity and negligible eating habits (Yach et al., 2006).

Disappointing results after lifestyle modification or pharmacotherapy have indicated the need of other treatment modalities to produce better results in terms of weight loss (Abdollahi and Afshar-Imani, 2003). Nevertheless, herbal supplements and diet-based therapies for weight loss are among the most common treatments in complementary and alternative medicine. Equally, a wide variety of these natural and herbal products, including crude extracts and compounds isolated from plants, can be used to induce weight loss and prevent diet-induced obesity. Furthermore, in recent decades, medicinal plant preparations have been widely used in the treatment of obesity (Barnes et al., 2004; Han et al., 2005; Cercato et al., 2015). These plants contain a variety of components that may interfere with the metabolism and oxidation of fatty acids, possibly increasing their lipolysis, thus presenting anti-obesity and antioxidant properties. Some herbs have been investigated and discovered as being useful in the treatment of obesity, diabetes and other chronic diseases (Hasani-Ranjbar et al., 2009, 2010); however various plants used by population have not been considered for the scientific evaluation.

*Hancornia speciosa* Gomes (Apocynaceae) is a tree naturally found in Brazil, where it is distributed throughout the Midwestern, Southeastern, Northern and Northeastern regions, with higher abundance in the areas of coastal plains and plateaus of the Northeast. In popular medicine, *H. speciosa,* or “mangabeira”, is used in various ways: the bark is used to treat dermatoses, liver diseases and diabetes, and is also used as an anti-inflammatory and for weight loss; the roots are used in the treatment of dislocations, rheumatism, and also as stomatic and antihypertensive; the latex and leaves are used as astringent, in the treatment of menstrual cramps, dermatitis, tuberculosis, ulcers, herpes and warts, and in the treatment of diseases affecting the liver; the fruits are used as a food source (Grandi et al., 1989; Rodrigues and Carvalho, 2001; Macedo and Ferreira, 2005; Souza and Felfili, 2006; Conceição et al., 2011; Pasa, 2011; Ribeiro et al., 2012).

It is important to highlight that scientific information related to the popular use of this plant for the treatment of metabolic syndromes, such as obesity and hyperlipidaemia, would be of a great clinical importance. This popular use has been extensively mentioned in ethnobotanical surveys in different regions of Brazil. However, few biological activities have been evaluated (Grandi et al., 1989; Rodrigues and Carvalho, 2001; Macedo and Ferreira, 2005; Silva et al., 2010a, b; Cercato et al., 2015).

Therefore, an intense popular use of *H. speciosa* for several conditions, including body weight loss in obese or overweight people, is observed. Yet, there is lack of research analyzing its therapeutic potential to treat obesity. In this way, this study aimed to verify the beneficial effect of the aqueous extract of the stem bark of *H. speciosa* on the glycemic and adipogenic profiles of obese mice in the high-fat diet model.

## MATERIALS AND METHODS

### Plant material and preparation of the stem bark aqueous extract

For this study, the stem of *H. speciosa* Gomes (Apocynaceae Juss.) was collected in the town of Pirambu-SE, Brazil, in March 2012. The identification of the plant was confirmed by Dr. Ana Paula Prata, from the Federal University of Sergipe, and a voucher specimem was deposited in the Herbarium of the Federal University of Sergipe (ASE30170). For the preparation of the aqueous extract of the stem bark *H. speciosa* (AEHS), 500 g of the stem bark of *H. speciosa* was dried, ground and subjected to extraction by infusion in 5 L of distilled water at 100°C for 30 min. The solution obtained was kept and cooled to room temperature (25°C). It was then filtered with a filter paper of 125 mm to obtain 3.4 L of the final solution. Hence, this solution was lyophilized and 52.5 g was obtained and stored at -20°C for later use. The yield of this extraction was 10.5%.

### Animals for experimentation and experimental conditions

Male Swiss mice (21 to 23 days, 10 to 14 g) were obtained from the Central Animal Facility of the Federal University of Sergipe. After one week of adaptation in the laboratory, animals were randomly divided into 4 groups of 8 animals that received:

1) Standard diet for 18 weeks (SD).
2) Standard diet for 18 weeks and the aqueous extract of the stem bark *H. speciosa* (AEHS) in the last 8 weeks (SDE).
3) High-fat diet for 18 weeks (HD).
4) High-fat diet for 18 weeks and AEHS in the last 8 weeks (HDE).

These animals were maintained on identified polypropylene cages with 4 animals each, with diet and water *ad libitum*. In the groups supplemented with AEHS, the administration was carried *ad libitum* in the drinking water. The temperature remained at the 22±2°C range, with light / dark cycle of 12 h. The Ethics Committee on Animal Research of the Federal University of Sergipe approved the experimental protocol of this study, under the reference number 81/12. During all experimental procedures, the ethical principles for animal testing were adopted, following the National Council for Animal Experiment Control (CONCEA).

### Induction of obesity

For the induction of obesity in mice, a high-fat diet was offered *ad libitum* to animals during 18 weeks (HD and HDE groups), according to White et al. (2013). Control groups received a standard diet (normal lipid content) for the same period (SD and SDE groups). The diets were commercially obtained from

**Table 1.** Composition of diets.

| Ingredients | Standard diet | | High-fat diet | |
|---|---|---|---|---|
| | U (g/kg) | kcal | U (g/kg) | kcal |
| Corn starch | 415.0 | 1 660 | 14.3 | 57.2 |
| Soybean meal | 305.0 | 1 281 | 410.0 | 1 722 |
| Sucrose | 80.0 | 320 | 80.0 | 320 |
| Maltodextrin | 70.0 | 280 | 70.0 | 280 |
| Lard | 0.0 | 0 | 302.0 | 2 718 |
| Soybean oil | 0.0 | 0 | 0.0 | 0 |
| Soybean fatty acid | 50.0 | 350 | 50.0 | 350 |
| Microcrystalline cellulose | 31.7 | 0 | 25.4 | 0 |
| L-cystine | 1.8 | 7,2 | 1.8 | 7.2 |
| Choline chloride | 1.5 | 0 | 1.5 | 0 |
| Buty-hydroxytoluene | 0.014 | 0 | 0.028 | 0 |
| Mix min. mod 50 gps | 35.0 | 0 | 35.0 | 0 |
| Vitamin mix | 10.0 | 40 | 10.0 | 40 |
| Total | 1 000.0 | 3 938 | 1 000.0 | 5 494 |

Standard diet (SD): 73.9% of carbohydrate, 14.8% of protein and 9.8% of lipid. High-fat diet (HD): 26.3% of carbohydrate, 14.4% of protein and 57.6% of lipid.

PragSoluções (São Paulo, Brazil) and their compositions are specified in Table 1.

**Supplementation with the aqueous extract**

The aqueous extract of the stem bark of *H. speciosa* was offered *ad libitum* to mice of groups HDE and SDE, at room temperature, during the 8 weeks of the experiment at a concentration of 0.3 $mg.mL^{-1}$, which resulted in an estimated dose of 200 $mg.kg^{-1}$ based on the daily water consumption of the animals.

**Evaluation of water intake, food intake and weight gain of animals**

The evaluation of both water intake and food consumption was performed daily for each box of animals during the entire period of the experiment. Body weight, in turn, was measured once a week.

**Evaluation of glycemic profile**

***Insulin tolerance test (ITT)***

The blood glucose was measured after 5 h of fasting at the end of the 18 weeks, 3 days before euthanasia. The blood supply obtained from the animal's tail vein was used, using Accu-check® (Roche) glucometer, according to the manufacturer's specifications. The insulin was intraperitoneally injected in the proportion of 0.7 $U.kg^{-1}$ and blood glucose levels were measured after 20, 40 and 60 min post-injection (Ali et al., 2011). The total area under the curve was calculated from 0 to 60 min.

***Glucose tolerance test (GTT)***

At the end of 18 weeks, with 2 days before ITT, D-glucose (1 $g.kg^{-1}$, prepared in saline solution) was administered intraperitoneally to animals submitted to 12 h of fasting and blood glucose levels were measured before and after 5, 15, 30, 45, 60 and 120 min post-injection (Faulhaber-Walter et al., 2011). The total area under the curve was calculated from 0 to 120 min. The blood supply was obtained from the tail vein of the animals and glucose levels were measured using the blood Accu-check® glucometer, according to the manufacturer's specifications.

***Blood glucose***

Blood glucose was measured with the animals fasting for 5 h. For this determination, the blood of the animal's tail vein was collected and glucose levels were measured by using the Accu-check® glucosemeter, according to the manufacturer's specifications.

***Removal of adipose tissue and determination of adiposity index***

After anaesthesia and euthanasia of animals by using inhaled isoflurane (3-5%) and blood collection, a longitudinal incision in the abdomen was performed to remove the periepididymal, perirenal and retroperitoneal adipose pads. Then, adipose tissues were immersed in saline solution, the excess solution was taken up with gauze and tissues were immediately weighted. The adiposity index was obtained by dividing the sum of the animal's pads by the total animal body mass (White et al., 2013).

**Statistical analysis**

The results were presented as Means±Standard Error of Means (SEM) and the comparison between them was performed with one- or two-way analysis of variance (ANOVA) followed by Bonferroni's post-test, as specified in the legends of each figure. Values of $p < 0.05$ were considered significant.

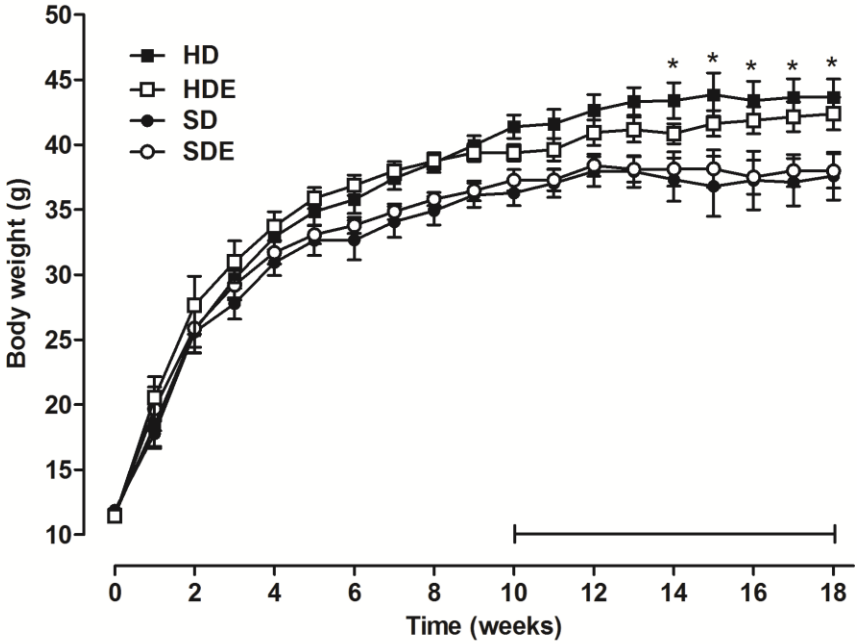


**Figure 1.** Body weight of the groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. The horizontal bar between the 10 and 18th weeks is the period in which the SDE and HDE groups received the treatment with AEHS instead of water. * $p < 0.05$ for HD vs. SD group; Two-Way ANOVA followed by Bonferroni´s post-test.

## RESULTS

Figure 1 shows that at the beginning of the experiment, there was no significant difference in body weight among the groups (10.7±1.2, 11.5±0.5, 11.6±0.2 and 11.5±0.4 g respectively for the SD, SDE, HD and HDE groups). On the 10th week of the experiment, the body weight of the animals did not differ statistically, but a clear tendency for higher values for animals of the HD and HDE groups (41.4±0.9 and 39.4±0.6 g, respectively) was observed in comparison with the animals of the SD and SDE groups (36.3±1.0 and 37.8±0.7 g, respectively). After 14 weeks of treatment with high-fat diet, a significant difference between the HD and SD groups was found ($p < 0.05$, Figure 1). No difference was observed for groups treated with AEHS (SDE or HDE) when compared with their respective control for diets (respectively SD or HD). Both the diet consumption and the liquid intake were measured during the 18 weeks of the experiment. These parameters were not altered in the groups evaluated (data not shown), both before and after the animals that received the standard or high-fat diet were exposed to AEHS (in the last 8 weeks).

Figure 2 shows the weight of adipose pads and adiposity index. The animals of the HD group had significantly higher adipose retroperitoneal ($p < 0.05$; Figure 2A), perirenal ($p < 0.001$; Figure 2B) and periepididymal ($p < 0.05$; Figure 2C) pad weight when compared to the SD group, which resulted in higher adiposity index in the HD group ($p > 0.05$; Figure 2D), when compared to the SD group. The treatment with AEHS lessened the weight pad of the perirenal pad ($p < 0.05$; Figure 2B), without affecting epididymal or retroperitoneal pads (Figure 2A and C). However, this difference did not reflect on the alteration in the adiposity index (Figure 2D), thus indicating no influence of AEHS upon the total fat mass.

At the end of the 18 weeks of the experiment, ITT and GTT were also carried out. Figure 3A shows that glucose levels of mice from the HD group were significantly increased when compared to the animals of the SD group, at 0, 20 or 60 min post-injection of insulin, which was also confirmed by higher values of AUC in the HD group than in the SD group ($p < 0.01$, Figure 3B). The treatment with AEHS, in the last 8 weeks, did not significantly modify the glucose levels or AUC after intraperitoneal injection of insulin, when compared to the respective control for diet.

After a challenge with intraperitoneal injection of glucose, animals from the HD group showed significantly higher levels of blood glucose from 15 to 60 min post-injection in comparison to the SD group (Figure 4A). That

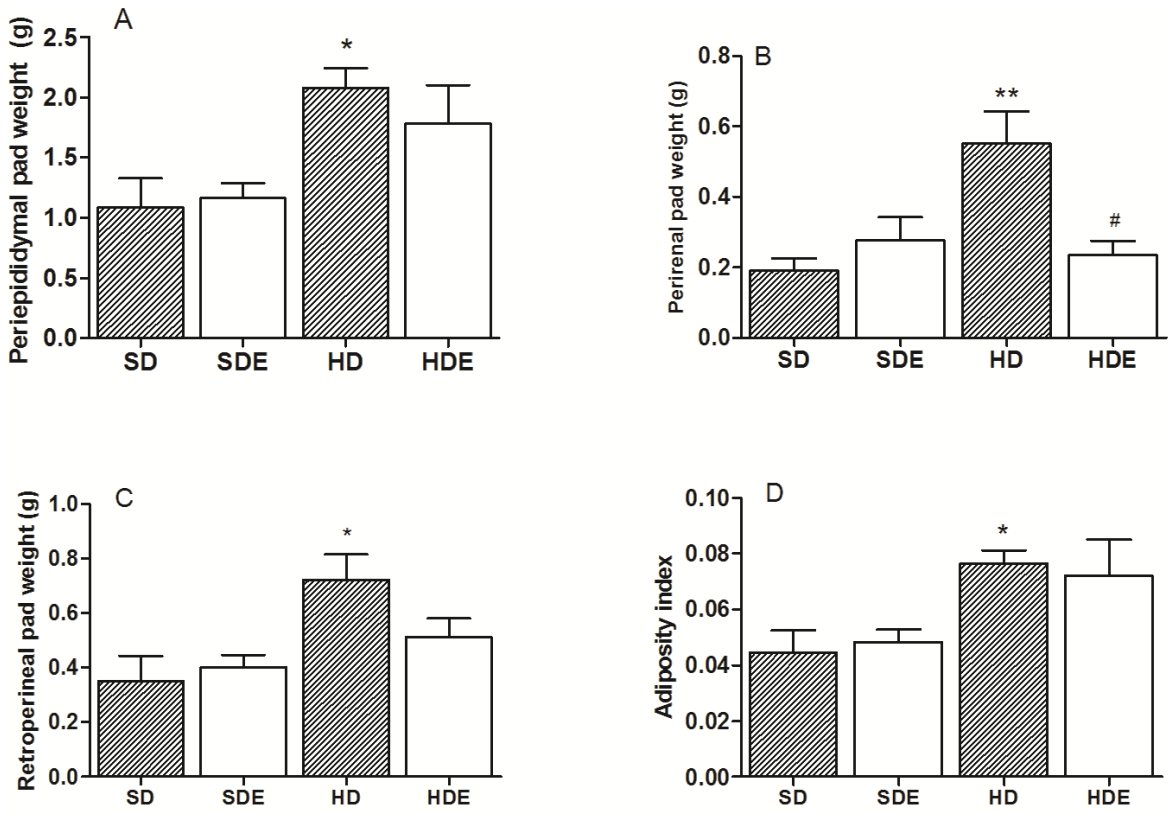


**Figure 2.** Weight of adipose tissue (g) for periepididymal (A), perirenal (B) and retroperitoneal (C) pads and adiposity index (D) of the groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus the aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. * $p < 0.05$ for SD vs. HD groups, # $p < 0.05$ for HD vs. HDE groups. One-way ANOVA followed by Bonferroni's post test.

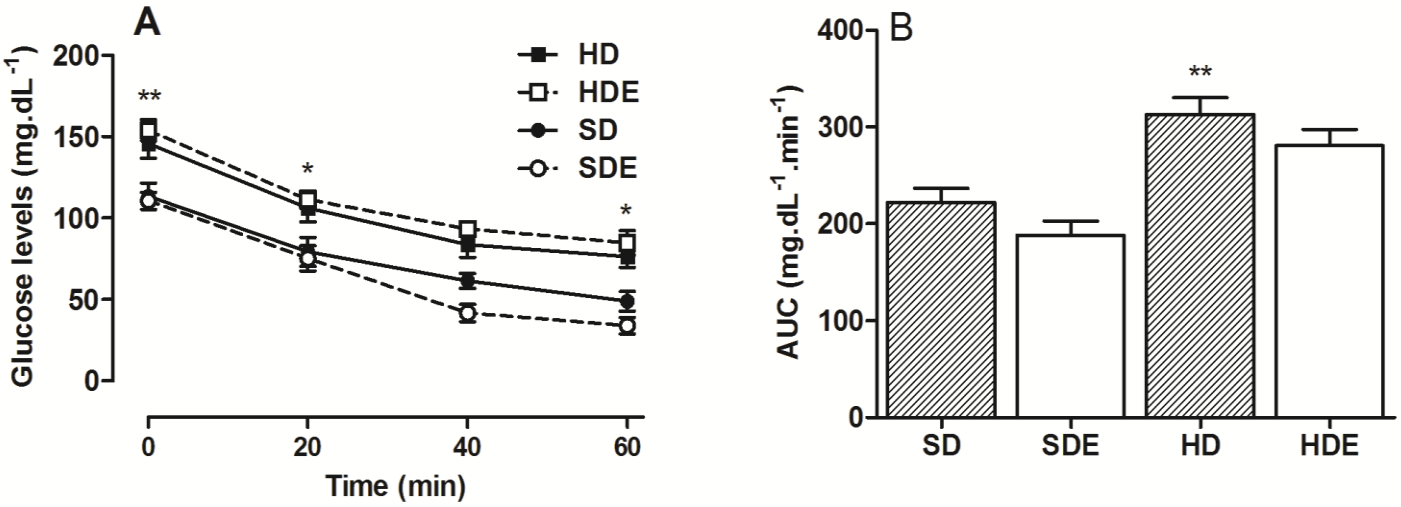


**Figure 3.** Insulin tolerance test (ITT, Panel A) for groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus the aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. Glucose levels ($mg.dL^{-1}$) were measured at 0 (baseline) and 20, 40 and 60 minutes post-intraperitoneal insulin injection. * $p < 0.05$ or ** $p < 0.001$ for HD vs. SD groups. Two-way ANOVA followed by Bonferroni's post-test. (B) Panel B shows the values of area under the curve (AUC) of the same groups. ** $p < 0.01$ for HD vs. SD groups. One-way ANOVA followed by Bonferroni's post-test.

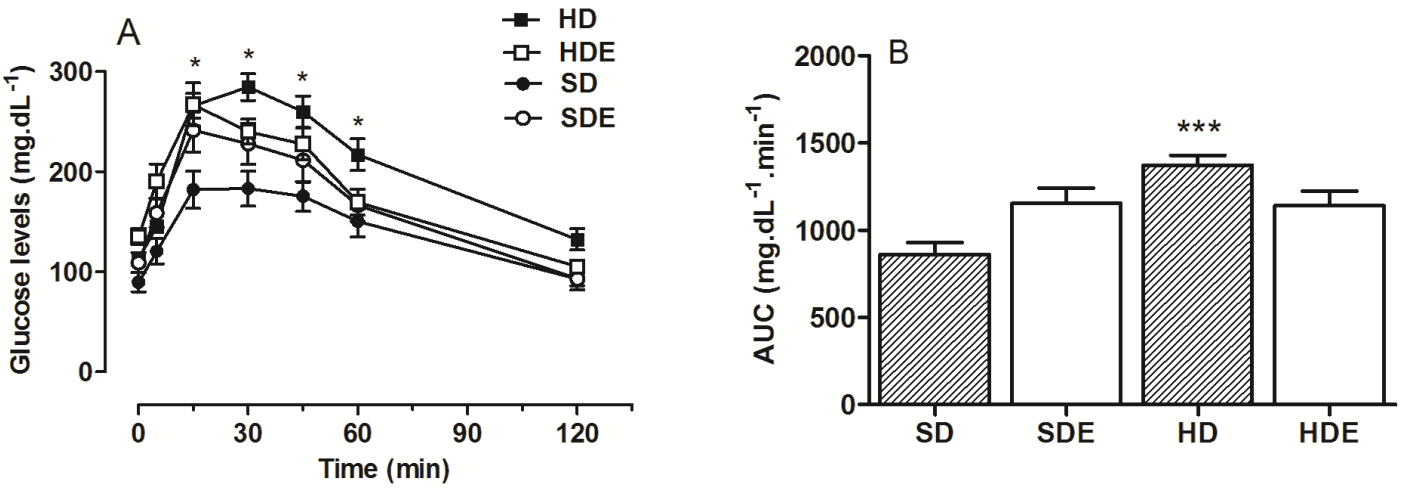


**Figure 4.** Glucose tolerance test (GTT, Panel A) for groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE], N = 8. Glucose levels ($mg.dL^{-1}$) were measured at 0 (baseline) and 20, 40 and 60 minutes post-intraperitoneal glucose injection. * $p < 0.05$ for HD vs. SD groups. Two-way ANOVA followed by Bonferroni's post-test. (B) Values of area under the curve (AUC) of the same groups. *** $p < 0.001$ for HD vs. SD groups. One-way ANOVA followed by Bonferroni's post-test.

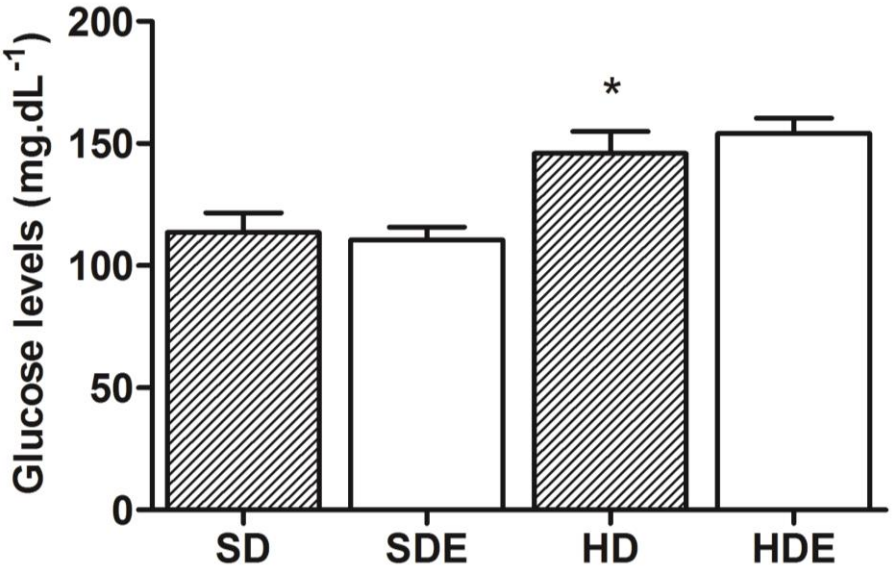


**Figure 5.** Blood glucose levels after 5 h of fasting in the groups treated with standard diet (SD) or high-fat diet (HD) for 18 weeks and the respective groups that received standard diet plus aqueous extract of the stem bark of *H. speciosa* (AEHS) [SDE] or high-fat diet plus AEHS [HDE] after 5 h of fasting (N=8). * $p < 0.05$ for HD vs. SD group. One-way ANOVA followed by Bonferroni's post-test.

resulted in higher AUC ($p < 0.001$) in the HD group than in the SD group (Figure 4B), indicating a glucose intolerance in mice treated with high-fat diet for 18 weeks. However, the treatment with AEHS in the last 8 weeks did not significantly alter the effect of high-fat diet over the glucose intolerance.

Glucose levels were increased in mice from the HD group after a 5 h period of fasting ($p < 0.05$), when compared to the SD group (Figure 5). However, the treatment with AEHS caused no significant change in basal blood glucose levels both in animals submitted to standard or high-fat diet.

## DISCUSSION

Data presented in this study showed that the aqueous extract of the stem bark of *H. speciosa* (AEHS) did not alter the body weight gain, adiposity index, blood glucose levels, sensitivity to insulin and tolerance to glucose in mice fed with high-fat diet, which is consistent with the lack of anti-obesity or favorable glycemic effects of AEHS in the conditions used in the present study. In addition, animals fed with standard diet did not present any change in these parameters.

The hypothesis that the aqueous extract from the stem bark of *H. speciosa* could present such activity was based on the ethnobotanical surveys describing that the population in Brazil uses this medicinal plant to treat obesity or to promote body weight loss (Cercato et al., 2015). That is the case of the study published by Conceição et al. (2011), which described that people from Nova Xantina (MT), Brazil, indicated the use of the infusion or decoction of the bark of *H. speciosa* as anorectic, representing an alternative to appetite control thus reducing food consumption. Other ethnobotanical studies have also demonstrated that people use the bark of *H. speciosa* to lose weight in different regions of Brazil (Grandi et al., 1989; Silva et al., 2010a, b; Santos et al., 2013; Cercato et al., 2015).

In spite of these descriptions, data from the present study failed to show any effect that could corroborate the ethnobotanical description that the bark of *H. speciosa* can be useful both to treat obesity and to produce weight loss. Thus, mice treated with high-fat diet plus AEHS did not show important alteration of the parameters evaluated, and no change was observed in mice treated with standard diet plus AEHS. The model of high-fat diet used in this study was previously standardized (White et al., 2013). This previous study demonstrated a difference in body weight of Swiss mice after ten weeks of exposition to the same high-fat diet utilized in the present study. In fact, a clear tendency for higher values of body weight was found in animals from the HD group on the 10th week, but even eight weeks of treatment with AEHS (~ 200 $mg.kg^{-1}.day^{-1}$) in the drinking water did not modify the body weight and other parameters of mice.

The amount of adipose tissue, measured as the mass of periepididymal, retroperitoneal and perirenal adipose pads, was increased in animals treated with high-fat diet, along with the augmented whole body mass. These results are consistent with the previous study from this study group (White et al., 2013). The treatment with AEHS did not affect the mass of periepididymal or retroperitoneal pads, but interestingly, reduced the weight of perirenal adipose pad. Unfortunately, that was the lower adipose pad and it did not cause a significant effect of AEHS over the adiposity index, which allowed us to conclude that AEHS, in the conditions used in this study, was not effective to promote body weight loss in mice.

It is worthwhile noting that the via of administration chosen in the present study was the drinking water, in order to avoid gavage for eight weeks, which could cause some damage related to the administration that could interfere in the swallowing of mice. One could suggest that the treatment with AEHS in the drinking water could change the liquid intake or the consumption of food, but AEHS promoted alteration of neither the liquid intake (which demonstrates that it was well tolerated by the animals) nor the food intake. Animals continued to consume the same amount of liquid and food that they used to before the AEHS had been introduced. Therefore, there was no significant difference in consumption in grams and absolute consumption in kcal among the groups treated and their respective controls.

Another possibility of bias of the present study could be the dose of AEHS used. The estimated dose was 200 $mg.kg^{-1}.day^{-1}$, which was considered as a dose high enough to cause any possible effect that AEHS could induce, and that could offer biological relevance to the treatment of obesity. Unfortunately, there is no description of how much bark of *H. speciosa* is used by the population in the preparation of decoction or infusion. Besides, other studies have shown that treatment with similar doses of extracts of plants can alter the induction of obesity or other associated conditions. For example, the study from Song et al. (2014) demonstrated that the methanol extract from the stem of *Sasa borealis* (150 mg/kg) reduced the body weight and hepatic steatosis in rats made obese by a high-fat diet consumption. Kim et al. (2014) showed that the treatment with the ethanol extract from the rhizomes of *Boesenbergia pandurata* (200 mg/kg) decreased the whole body and adipose pad weight of C57BL/6J mice submitted to a high-fat diet through activation of AMP-activated protein kinase and regulation of lipid metabolism. However, differences in species of animals, composition of extracts or via of administration do not permit a direct comparison between the studies.

Concerning the effects of AEHS over the glycemic profile, it was observed that AEHS did not reverse glucose intolerance and insulin resistance, nor did it normalize the basal blood glucose levels in the HDE group. A study carried out in fifteen traditional communities (non-indigenous) in the Upper Paraguay River Basin and two in the Guaporé Valley collected data about hypoglycemic plants through qualitative approach and with the aid of semi-structured and opened interviews. Among the seventeen identified species, the bark of *H. speciosa* was cited as medicinal and used by community leaders, traditional healers, midwives and other plant users for the treatment of diabetes (Macedo and Ferreira, 2004). Many plants that have been used to reduce blood glucose and that were pharmacologically evaluated have their hypoglycemiant activity confirmed. Among their constituents, the steroid and triterpenoid glycosides are bioactive substances present in many of them (Rao and Gurfinkel, 2000). Some saponins derived

from triterpenoid have hypoglycemic action and their possible effect involves the stimulation of pancreatic β-cells with subsequent secretion of insulin (Ojewole, 2002; Connolly and Hill, 2001).

Studies by Rodrigues et al. (2007), Costa et al. (2008) and Santos et al. (2013) have indicated the presence of organic acids and derivatives, xanthones, proanthocyanidins, volatile compounds, flavonoids, triterpenes and cyclitols in parts of *H. speciosa*. Also, rutin and cyclitol L-(+)-bornesitol were identified in the bark of this plant (Pereira et al., 2012), which are considered primary bioactive compounds. In spite of the presence of triterpenes and other components that could possess a hypoglicemiant activity, this effect was not observed in animals receiving AEHS from groups treated with both standard and high-fat diets, probably due to the difference in the solvents used to extract (ethanol *vs.* water). Interestingly, it was demonstrated that the ethanol extract of the leaves of *H. speciosa* or its dichloromethane fraction reduced the *in vitro* activity of α-glucosidase, and it also enhanced the uptake of glucose in freshly dissociated adipocytes (Pereira et al., 2015). In this study, the treatment of mice with 300 mg/kg of this extract or its dichloromethane fraction reduced glycemia in starch or glucose tolerance tests, which suggests that the ethanol extract of the leaves of this plant may also present some potential to induce a hypoglicemiant activity.

## Conclusions

Altogether, these results demonstrate that the aqueous extract of the stem bark of *H. speciosa* (AEHS) administered to obese mice did not cause alteration in weight gain, insulin resistance, glucose intolerance or hyperglycemia. Data obtained in the present study do not exclude the possibility that preparations from other parts of *H. speciosa* could affect obesity. However, these results from experimental animals disagree with the popular uses demonstrated in the ethnobotanical surveys about the bark of this plant and claim for attention for this use.

## Conflicts of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

The authors sincerely thank “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES; Social Demand Program of Scholarships) and “Conselho Nacional de Pesquisa e Desenvolvimento Científico” (CNPq) for their financial support. EAC and MRVS are beneficiaries of CNPq productivity grants.

## REFERENCES

Abdollahi M, Afshar-Imani B (2003). A review on obesity and weight loss measures. Middle East Pharm. 11:6-10.

Ali TK, Al-Gayyar MM, Matragoon S, Pillai BA, Abdelsaid MA, Nussbaum JJ, El-Remessy AB (2011). Diabetes-induced peroxynitrite impairs the balance of pro-nerve growth factor and nerve growth factor, and causes neurovascular injury. Diabetologia 54(3):657-668.

Barnes PM, Powell-Griner E, Mcfann K, Nahin RL (2004). Complementary and alternative medicine use among adults: United States, 2002. Adv. Data 27(343):1-19.

Cercato LM, White PA, Nampo FK, Santos MR, Camargo EA (2015). A systematic review of medicinal plants used for weight loss in Brazil: Is there potential for obesity treatment? J. Ethnopharmacol. 176:286-296.

Conceição GM, Ruggieri AC, Araújo MFV, Conceição TTMM, Conceição MAMM (2011). Plantas do cerrado: Comercialização, uso e indicação terapêutica fornecida pelos raizeiros e vendedores. Sci. Plena 7(12):99-102.

Connolly JD, Hill RA (2001). Triterpenoids. Nat Prod Rep. 18(5):560-78. http://www.ncbi.nlm.nih.gov/pubmed/11699886.

Costa ES, Hiruma-Lima CA, Lima EO, Sucupira GC, Bertolin AO, Lolis SF, Andrade FD, Vilegas W, Souza-Brito AR (2008). Antimicrobial Activity of Some Medicinal Plants of the Cerrado, Brazil. Phytother. Res. 22:705-707.

Faulhaber-Walter R, Jou W, Mizel D, Li L, Zhang J, Kim SM, Huang Y, Chen M, Briggs JP, Gavrilova O, Schnermann JB (2011). Impaired Glucose Tolerance in the Absence of Adenosine A1 Receptor Signaling. Diabetes 60:2578-2587.

Flegal KM, Carroll MD, Kit BK, Ogden CL (2010). Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 307:91-497.

Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee (2013). Heart disease and stroke statistics-2013 update: A report from the American Heart Association. Circulation 127:e6-e245.

Grandi TSM, Trindade JA, Pinto MJF, Ferreira LL, Catella AC (1989). Plantas medicinais de Minas Gerais, Brasil. Acta Bot. Bras. 3:185-224.

Han L, Kimura Y, Okuda H (2005). Anti-obesity effects of natural products. Stud. Nat. Prod. Chem. 30:79-110.

Hasani-Ranjbar S, Larijani B, Abdollahi M (2009). A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. Inflam. Allergy Drug Targets 8:2-10.

Hasani-Ranjbar S, Nayebi N, Moradi L, Mehri A, Larijani B, Abdollahi M (2010). The efficacy and safety of herbal medicines used in the treatment of hyperlipidemia: A systematic review. Curr. Pharm. Des. 16:2935-2947.

Kim DY, Kim MS, Sa BK, Kim MB, Hwang JK (2012). *Boesenbergia pandurata* attenuates diet-induced obesity by activating AMP-activated protein kinase and regulating lipid metabolism. Int. J. Mol. Sci. 13:994-1005.

Macedo M, Ferreira AR (2005). Plantas hipoglicemiantes utilizadas por comunidades tradicionais na Bacia do Alto Paraguai e Vale do Guaporé, Mato Grosso Brasil. Braz. J. Pharmacogn. 14:5-47.

Ojewole JAO (2002). Hypoglycaemic effect of Clausena anisata (willd) Hook methanolic root extract in rats. J. Ethnopharmacol. 81:231-237.

Pasa MC (2011). Local knowledge and folk medicine: Ethnobotany in Cuiabá, Mato Grosso, Brazil. Boletim do Museu Paraense Emílio Goeldi. Ciênc. Humanas 6:179-196.

Pereira ABD, Veríssimo TM, de Oliveira MA, et al. (2012). Development and validation of an HPLC-DAD method for quantification of bornesitol in extracts from *Hancornia speciosa* leaves after derivatization with *p*-toluenesulfonyl chloride. J. Chromatogr. B

888:133-137.
Rao AV, Gurfinkel DM (2000). The bioactivity of saponins: Triterpenoid and steroidal glycosides. Drug Metabol. Drug Interact. 17:211-235.
Ribeiro SS, De Jesus AM, Dos Anjos CS, da Silva TB, Santos AD, de Jesus JR, Andrade MS, Sampaio TS, Gomes WF, Alves PB, Carvalho AA, Pessoa C, de Moraes MO, Pinheiro ML, Prata AP, Blank AF, Silva-Mann R, Moraes VR, Costa EV, Nogueira PC, Bezerra DP (2012). Evaluation of the cytotoxic activity of some Brazilian medicinal plants. Planta Med. 78:1601-1606.
Rodrigues CM, Rinaldo D, Santos LC, Montoro P, Piacente S, Pizza C, Hiruma-Lima CA, Brito AR, Vilegas W (2007). Metabolic fingerprinting using direct flow injection electrospray ionization tandem mass spectrometry for the characterization of proanthocyanidins from the barks of *Hancornia speciosa*. Mass. Spectrom. 21:1907-1914.
Rodrigues VE, Carvalho DA (2001). Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do Alto Rio Grande-Minas Gerais. Ciênc. Agrotecnologia 25:102-123.
Santos ACB, Silva MAP, Santos MAF, Leite T.R. (2013). Ethnobotanical, chemical and pharmacological survey of Apocynaceae Juss. Species occurring in Brazil. Braz. J. Med. Plants 15:442-458.
Silva MAB, Melo LVL, Ribeiro RV, Souza JPM, Lima JCS, Martins DTO, Silva RM (2010a). Ethnobotanical survey of plants used as anti-hyperlipidemic and anorexigenic by the population of Nova Xavantina-MT, Brazil. Braz. J. Pharmacogn. 20:549-562.
Silva NLA, Miranda FAA, Conceição GM (2010b). Triagem fitoquímica de plantas de cerrado, da área de proteção ambiental municipal do Inhamum, Caxias, Maranhão. Sci. Plena 6(2):1-17.
Song Y, Lee SJ, Jang SH, Ha JH, Song YM, Ko YG, Kim HD, Min W, Kang SN, Cho JH. (2014). *Sasa borealis* stem extract attenuates hepatic steatosis in high-fat diet-induced obese rats. Nutrients 6:2179-95.
Souza CD, Felfili JM (2006). The utilization of medicinal plants in the region of Alto Paraíso of Goiás, GO, Brazil. Acta Bot. Bras. 20:135-142.
White PAS, Cercato LM, Araújo JMD, Souza LA, Soares AF, Barbosa AP, Neto JM, Marçal AC, Machado UF, Camargo EA, Santos MR, Brito LC (2013). Model of high-fat diet-induced obesity associated to insulin resistance and glucose intolerance. Arq. Bras. Endocrinol. Metab. 57:339-345.
Yach D, Stuckler D, Brownell KD (2006). Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. Nat. Med. 12:62-66.

# Journal of Medicinal Plant Research

## Related Journals Published by Academic Journals

- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

academicJournals