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Full Length Research Paper

Phytochemical screening and evaluation of cytotoxic, antimicrobial and cardiovascular effects of *Gomphrena globosa* L. (Amaranthaceae)

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***Gomphrena globosa* L. (Amaranthaceae) is used in folk medicine in the treatment of high blood pressure and other diseases. To confirm this popular use, ethanol extract from leaves of *G. globosa* L. was prepared by maceration and analyzed by some phytochemical and biological assays, including cardiovascular activity. The phytochemical screening detected the presence of saponins, alkaloids, reducing sugars and coumarins. The extract did not induce either lethality in the brine shrimp (*Artemia salina* Leach) bioassay or antimicrobial activity. The negative results for cytotoxic and antimicrobial assays indicated possible low cytotoxicity to the extract. In other hand, it promoted a hypotensive activity by significant reduction in arterial blood pressure without change heart rate, confirming the therapeutic use as antihypertensive for this plant.**

Key words: *Gomphrena*, Amaranthaceae, antihypertensive, cytotoxicity, *Artemia salina.*

INTRODUCTION

The knowledge about medicinal plants often symbolizes the only therapeutic option for many communities and ethnic groups. The comments about the popular use and efficacy of medicinal plants contribute significantly to the dissemination of therapeutic properties of plants, commonly used by medical effects they produce, although chemical constituents sometimes are not known (López, 2006). Phytochemical screening provides a better choice of material to be studied and gives the possibility of adapting extracts fractionation techniques and isolation and characterization of pure substances, according to the constituents previously detected by qualitative tests. Thereby, it facilitates the subsequent work of isolation and purification of the constituents biologically actives (Farnsworth, 1966). Specimens of

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Abbreviations: Gg-EE, Ethanol extract from leaves of *Gomphrena globosa* L.; **LC_{50},** lethal concentration that induced 50% from death; **MAP,** mean arterial pressure; **HR,** heart rate; **ATCC,** American type culture collection.

Artemia salina Leach (brine shrimp), a marine microcrustacean, are being used as target organisms to detect bioactive compounds in plant extracts and the toxicity test against these animals has shown a good correlation with antitumor activity (McLaughlin and Rogers, 1988). This bioassay provides an advantage in the standardization and quality control of botanical products, and in evaluation of new drugs (Lieberman, 1999; Citó et al., 2003).

The species *Gomphrena globosa* L. (Amaranthaceae) is popularly known in Brazil as "perpétua" or "perpétua-roxa". Its leaves and flowers have application in folk medicine, and have been used in the treatment of hypertension, diabetes, kidney problems (Agra et al., 2007; Lans, 2006), hoarseness, cough, bronchitis and other respiratory diseases, mainly due expectorant action (Camejo-Rodrigues et al., 2003), and reproductive problems, due estrogenic activity (Lans, 2007). Extracts of aerial parts of some species of the *Gomphrena* genus have the following biological activities reported: larvicide (Dadang and Ohsawa, 2001) for *G. globosa*; antimicrobial for *G. martiana* and *G. boliviana* (Pomilio et al., 1992), antitumor to *G. martiana* (Pomilio et al., 1994) and estrogenic to *G. demissa* Mart. (França et al., 2008). Additionally, for species from *Gomphrena* genus, chemical identifications were cited: betacyanins (Heuer, 1992; Minale, 1967), hydroxycinamamides (Martin-Tanguy et al., 1992), and flavonoids, including flavones (Buschi, 1980; Pomilio, 1994) and flavonols (Bouillant, 1978).

The aim of this study was to characterize the phytochemical profile, assess the toxicity against brine shrimp (*A. salina* Leach) bioassay and determine the antimicrobial and cardiovascular effects of ethanol extract from leaves of *G. globosa* L.

MATERIALS AND METHODS

Botanical identification

G. globosa L. was cultivated and collected from the garden of medicinal and aromatic plants center (NUPLAM) from Federal University of Piauí, Brazil. The botanical identification was carried out at Graziela Barroso Herbarium of Federal University of Piauí, Brazil (voucher specimen no. 20901).

Extraction and phytochemical screening

Leaves (211.6 g) were dried at room temperature and then powdered. The powder was exhaustively extracted with 99.8% ethanol by maceration at room temperature (Gg-EE). The ethanol extract solvent was evaporated to dryness under reduced pressure and lyophilized to yield 34.7 g (16.4%). Evaluation of main phytochemicals groups from Gg-EE was carried out according to Farnsworth (1966) by application of qualitative tests: saponins, steroids and terpenoids, alkaloids, flavonoids, catechins, tannins and polyphenols, reducing sugars, lactones, coumarins and quinones. In all tests, results were observed and compared with published data in respect of the presence or absence of each phytochemical group and therapeutic uses.

Establishment of toxicity in brine shrimp bioassay

The toxicity bioassay on *A. salina* Leach was performed according to the methodology proposed by Meyer et al. (1982), with some modifications (Nunes et al., 2009). From 20 mg of Gg-EE, 1, 10, 100 and 1000 µg/mL solutions were prepared in triplicate. Then, 10 specimens with 48 h of hatching in sea water and distilled water (1:1) were placed in each tube and three negative control tubes (saline solution and DMSO 1%). Appropriate volumes of the saline solution in tubes were added until 5 mL of saline solution containing 10 nauplii each to afford the final sample concentrations. After 24 h, the number of deaths was counted and results were tabulated and analysed.

Evaluation of antimicrobial activity

The Gg-EE was subjected to antibacterial assay using the agar diffusion plate method as described by Alves and colleagues (2000), with some changes and guided by NCCLS/CLSI (2005). Tests were performed against following standard microorganisms: *Staphylococcus aureus* ATCC 25923, *Staphylococus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. In addition, four multiple-drug resistant clinical isolated microorganisms were also tested: methicillin-resistant *S. aureus* MRPI 98 (strain typed as belonging to the Brazilian epidemic clone – BEC; Soares et al., 2000); methicillin-resistant *S. epidermidis* MRSE H111 (methicillin-resistant); *E. faecalis* 7426 (strain vancomycin resistant and positive for gene *van*A; Soares et al., 2000) and *Stenotrophomonas maltophilia* EM 012004 (strain only sensible to Trimethoprim + Sulfametoxazole).

The microorganisms were cultured overnight at 35°C in Mueller-Hinton Broth (Difco) before use. Suspension of bacterial strains with optical density of McFarland 0.5 ($1x10^8$ CFU/mL) were made in isotonic sodium chloride 0.9% solution. After inoculation and drying for 10 min, four wells (6 mm diameter) were made and aseptically filled up with different concentrations (1,10, 50 and 100 mg/mL) of Gg-EE, whereas equal volume of dimethyl-sulfoxide (DMSO) was used as negative control and Mueller-Hinton agar only as positive control for microbial growth. All plates were incubated at 35°C for 24 h. The antibacterial activity was measured at the diameter (mm) of clear zone of growth inhibition, and it was interpreted as: 9 to 13mm, moderate activity; > 13 to 17 mm, active; > 1 7mm, very active. Each test was performed in triplicate.

Effects of Gg-EE on cardiovascular parameters

Male dogs (*Canis familiaris)* (5 to 10 kg) were adequately anesthetized with thiopental solution (25 mg/kg, i.v.). Then, inferior cava vein through femoral vein was cannulated for administration of Gg-EE. To measure the mean arterial pressure (MAP) and heart rate (HR) before and after application of substances, a heparinized catheter inserted into abdominal aorta through femoral artery of each animal was coupled to a pressure measurement device (AVS Projects, São Paulo, SP, Brazil). After cardiovascular parameters

Table 1. Main phytochemical groups identified from Gg-EE.

Phytochemicals groups	Qualitative tests	Result
Saponins	Foam test	+
Steroids and Terpenoids	Liebermann-Buchard reaction	-
Alkaloids	Dragendorff, Bouchardat and Bertrand reactions	+
Flavonoids	Shinoda reaction	-
Catechins	Na_2CO_3 reaction	-
Tannins e Polyphenols	Ferric chloride 1 %	-
Reducing sugars	Benedict reaction	+
Lactones	Baljet reaction	-
Coumarines	NaOH/Ethanol (w/v), UV	+
Quinones	NH_4OH reaction	-

Legend: (+) Present; (-) Absent.

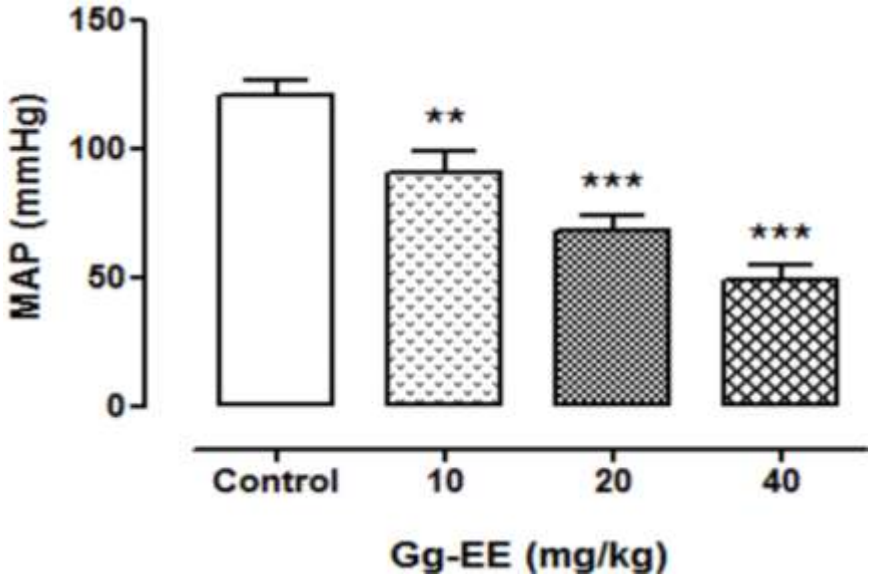

Figure 1. Hypotensive effect in anaesthetized dogs before (control) and after acute administration of Gg-EE (10, 20 and 40 mg/kg). MAP (mean arterial pressure) values are mean ± SEM of six experiments. **p<0.01, ***p<0.001 vs. control.

had stabilized, the MAP and HR were recorded before (control) and after administration of successive doses of Gg-EE (10, 20 and 40 mg/Kg). Successive injections were separated by a time interval sufficient to allow full recovery of haemodinamic parameters.

All experimental procedures were performed in accordance with the National Research Council's guidelines and approved by the Animal Research Ethics Committee of the Federal University of Piauí. Values are shown as mean ± standard error of the mean (s.e.m). Experimental results are expressed as percentage decreases in arterial blood pressure and heart rate. ANOVA one-way followed by Student-Newman-Keuls post-test were used in the data analysis and results were considered significant when p<0.05. GraphPad™ Prism 5.0 (GraphPad Software, Inc., CA, USA) was used to perform the data analysis.

RESULTS

The phytochemical screening of *G. globosa* ethanol extract (Gg-EE) detected the presence of saponins, alkaloids, reducing sugars and coumarins (Table 1). In turn, the extract did not induce mortality in the *A. salina* bioassay. Then, it was not possible to calculate the LC_{50}. The Gg-EE did not promote antimicrobial activity against microorganisms tested. In other hand, it induced a significant reduction in arterial blood pressure (Figure 1) without change heart rate (Figure 2), confirming hypotensive activity for this extract.

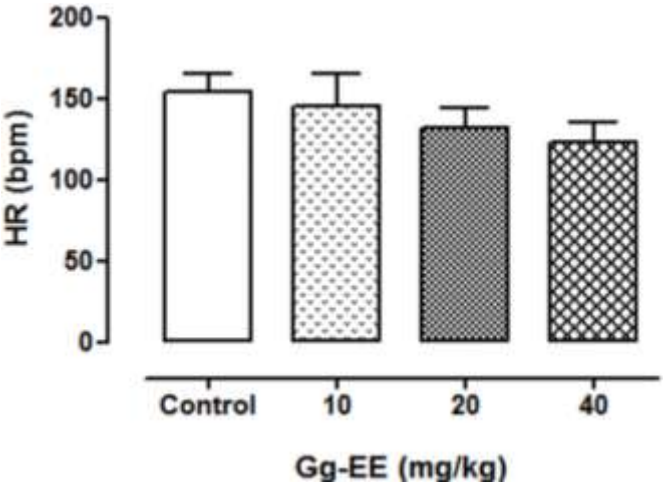

Figure 2. Bradycardic effect in anaesthetized dogs before (control) and after acute administration of Gg-EE (10, 20 and 40 mg/kg). HR (heart rate) values are mean ± SEM of six experiments.

DISCUSSION

The major finding of this study is the ethanol extract from leaves of *G. globosa* L. showed hypotensive effect in anesthetized dogs. The study of Gg-EE cardiovascular effects was motivated by the use of this species in folk medicine to treat high blood pressure (Agra et al., 2007; Lans, 2006). Interestingly, this is the first report of these pharmacological activities for this specie. The hypotensive effect promoted by Gg-EE at doses of 10, 20 and 40 mg/kg was significant, in contrast to the bradycardic effect, which was not observed. This observation indicates that even in blood pressure reduction, heart rate remains unaltered, probably due to the baroreflex response down-regulation, aiming to normalize cardiovascular parameters (Kawaguchi et al., 2007). A decrease in arterial pressure reduces baroreceptor afferent discharge and triggers reflex increases in heart rate, cardiac contractility, vascular resistance, and increased venous return (Lanfranchi and Somers, 2002). Thus, these results represent an important activity for drugs to treat certain types of arterial hypertension.

The phytochemical screening is an important step in the chemical and pharmacological study of a medicinal plant. It may suggest possible pharmacological effects of its extracts or fractions in comparison of identified phytochemicals groups, highlighting a close relationship with its main therapeutic uses. Thereby, results of phytochemical screening are in accordance with the popular use of *G. globosa* L. species in the treatment of hypertension. The presence of saponins in Gg-EE was confirmed by the foam test, observing its formation and continuing for about 20 min. In aqueous solution, saponins form persistent and abundant foam due a lipophilic portion in its chemical structure, called aglycone or sapogenin, and a hydrophilic portion, formed by one or more sugars that provide detergent properties (Farnsworth, 1966; N'guessan et al., 2009). Additionally, reducing carbohydrates were identified by ''Benedict reaction''. These results are consistent with the report of saponosides identified for this species (Heuer et al., 1992; Minale et al., 1967). This group of substances may promote lower blood pressure and cholesterol levels (N'guessan et al., 2009).

The presence of alkaloids was confirmed in Gg-EE by precipitate formation after Dragendorff, Bouchardat and Bertrand reactions (Farnsworth, 1966). Antihypertensive, cardiac depressant, and antitussive pharmacological activities reported for some alkaloids isolated from several species provide evidence for some therapeutic uses of *G. globosa* L. (Cordell et al., 2001; Santos et al., 2006). The identification of coumarins by reaction with an ethanol solution of sodium hydroxide had a positive result under ultraviolet light at 366 nm (Farnsworth, 1966). The hypotensive activity and myocardium relaxant for some coumarins may have related to the *G. globosa* L. use as antihypertensive (Chiou et al., 2001; Chung et al., 1993).

Substances tested by brine shrimp bioassay which leads to death half nauplii at a lethal concentration until 1000 µg/mL (LC_{50}), are considered actives, and thus a good potential for antitumor activity (McLaughlin and Rogers, 1988; Lieberman, 1999). In our studies, Gg-EE did not show cytotoxic activity in this bioassay. Similarly, there was no antimicrobial activity for Gg-EE, in contrast with results from other species of the Gomphrena genus (Pomilio et al., 1992). Several studies have also tried to correlate the toxicity against *A. salina* with other activities such as antifungal, virucidal and antimicrobial (MacBae et al., 1988), parasiticide (Sahpaz et al., 1994), trypanocida (Zani et al., 1995), among others.

In conclusion, Gg-EE showed hypotensive activity, related to its popular use for treating hypertension. Further studies are needed to elucidate what mechanisms are involved in the antihypertensive activity for this species. Although Gg-EE has showed no lethality against various microorganisms, these results indicate low cytotoxicity to the extract. Thereby, the brine shrimp bioassay provides an advantage in the standardization and quality control of botanical products, and in development of new drugs.

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