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Original Article

ANTI-INFLAMMATORY, ANALGESIC AND ANTIOXIDANT ACTIVITIES OF HYDRO-ACETONIC EXTRACT FROM *WISSADULA AMPLISSIMA* VAR. ROSTRATA (SHUM. AND THONN.) R. E. FRIES

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ABSTRACT

Objective: The present study aims to investigate anti-inflammatory, analgesic and antioxidant effects of the hydroacetonic extract (HAE) from *Wissadula amplissima* whole plant and to determine its polyphenol and flavonoid contents.

Methods: Extraction was achieved by soaking defatted *W. amplissima* with aqueous acetone solvent. Acute toxicity and pharmacological investigations were performed *per os* and intraperitoneally on Naval Medical Research Institute (NMRI) mice. The carrageenan-induced paw edema and the acetic acid-induced writhing tests were used to assess the *in vivo* anti-inflammatory and analgesic activities. Inhibition of xanthine oxidase and lipoxygenase were carried out *in vitro* using endpoint spectrophotometric assays at 295 nm and 234 nm. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power **(**FRAP), lipid peroxidation and desoxyribose degradation assays were used to point out antioxidant activities of extract. Aluminium chloride colorimetric method was used to determine total flavonoids content when and total phenolic content was measured by the method Folin-ciocalteu reagent.

Results: *W. amplissima*'s HAE exhibited a slight acute toxicity with no lethality observed. Significant ($p<0.05$) anti-inflammatory and analgesic activities were recorded as well as strong inhibitory activities on lipoxygenase and xanthine oxidase. *W. amplissima*'s HAE also demonstrated interesting antioxidant activities particularly in preventing lipid peroxidation. Polyphenol and flavonoid contents were respectively estimated to 64.33±1.34 mg Gallic Acid Equivalent (GAE) and 35.17±0.77 mg Quercetin Equivalent (QE) per 100 mg of *W. amplissima*'s HAE.

Conclusion: HAE of *W. amplissima* exhibited a promising anti-inflammatory and analgesic potential compared to the reference (acetylsalicylic acid, Hydrocortisone, paracetamol) and regarding the inhibition of lipoxygenase and lipid peroxidation. The anti-inflammatory and analgesic activities seem to be related to the antioxidant and enzymes inhibitors compounds of the plant extract.

Keywords: Anti-inflammatory, Antioxidant, Lipoxygenase, Xanthine oxidase, *Wissadula amplissima*

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INTRODUCTION

Populations of all the continents use plants for their various needs in terms of shelter, clothing, food, flavours and fragrances as not the least, medicines [1]. The last decade has witnessed a growing number of populations who rely on traditional remedies derived from medicinal plant and tremendous progress in medicinal plant research. This worldwide trend towards the use of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant for the treatment of minor ailments to more complex diseases.

Malvaceae botanical family consists of dicotyledonous plants with at least 2300 species in 200 genera [2]. *Wissadula amplissima* a member of this family is present throughout Africa from Cape Verde to Senegal and Eritrea to South Africa [3]. In the central region of Burkina Faso, species belonging to this family are well known and used by traditional practitioners to treat several diseases. Ethnopharmacological studies report the use of this species in folk medicine to treat vomiting, severe diarrheas, mucosa, skin and bronchus inflammation; inflammatory diseases, fever and allergies. The roots of the plant are used to treat anemia, hemorrhages and as a fortifying [4].

Inflammation is a complex defensive system of the body against any aggression. It involves in pathogenesis of diseases including infections, cancer, diabetes, atherosclerosis, arthritis. Several mediators such as cytokines (TNF α, IL6, IL 1β), free radical (reactive oxygen species (ROS), prostaglandins (PGE2) and leukotriene are released during inflammation response. Lipoxygenase (LOX) involved leukotriene synthesis play an important role in inflammation maintenance [5].

Previous biological investigations have demonstrated that methanol extract of *Wissadula amplissima* leaves possess antioxidant properties (2, 2–diphenyl-1-pricrylhydrazyl method) and anti-inflammatory capacity using carrageenan-induced paw edema in chicks [6].

In the best of our knowledge, there is no scientific information concerning the *in vitro* anti-inflammatory activities using the enzymatic method, *in vivo* anti-inflammatory activities using carrageenan-induced paw edema in mice, the toxicity and analgesic activities of this species.

The aim of this present study was to investigate anti-inflammatory (*in vivo* and *in vitro*), analgesic and antioxidant effects of the hydroacetonic extract (HAE) from *Wissadula amplissima* whole plant and to determine polyphenol and flavonoids contents.

MATERIALS AND METHODS

Plant materials

The whole plant of *Wissadula amplissima* var. Rostrata (Shum. and Thonn.) R. E. Fries (Malvaceae) was harvested in January 2013 at Gampela (25 km, East of Ouagadougou, Burkina Faso). Botanical identity was assessed by Professor Jeanne Millogo-Rasolodimby from laboratoire de biologie et ecologie vegetale (University of Ouagadougou, Burkina Faso) where a voucher specimen (CI: 16884) was deposited. Plant material was dried at room temperature, pulverized and stored in an airtight bag until use.

Animals and housing

Naval medical research institute (NMRI) mice (7 to 8 w old, 25 to 35g body weight), provided by the animal housing facility of the

University of Ouagadougou, were used. Mice were kept in an environmentally controlled breeding room (20-25 °C, 75% humidity, 12 h photoperiod), fed with standard laboratory food and water *ad libitum*. Mice were fasted 17 h before experiments. Investigation at animals was conducted in accordance with the official journal of european committee in 1991 and approved by the institutional committee regarding the care and use of animals for the experimental procedure in 2010; CEE509 [7, 8].

Chemicals

Chemicals were from analytical grade. Acetic acid, acetylsalicylic acid, allopurinol, carrageenan, dimethylsulfoxide, 2,2-diphenyl-1-picrylhydrazyl, hydrocortisone, lecithin, 15-lipoxygenase (EC 1.13.11.12), paracetamol, quercetin, sodium chloride, xanthine and xanthine oxidase (EC 1.1.3.22) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, desoxyribose, ferric trichloride, hydrochloric acid, potassium hexacyanoferrate, thiobarbituric acid and trichloroacetic acid were supplied by Labosi (Paris, France). Boric acid, ethylenediamine tetraacetic acid, gallic acid, linoleic acid, sodium carbonate, sodium phosphate dibasic, sodium phosphate monobasic, sodium tetraborate, trichloroacetic acid and all solvent used were sourced from Prolabo (Paris, France).

Extract preparation

Powdered plant material (50 g) was defatted with petroleum ether (500 ml) in a soxhlet extractor and then soaked (48 h, 25 °C, continuous stirring) in acetone/water (80/20, 500 ml). Extract was filtrated, concentrated in a vacuum evaporator (Büchi Rotavapor R-200) and lyophilized (Telstar Cryodos 50) to get 44.7 g of hydro acetone extract (HAE). HAE was stored at 4 °C until use.

Acute toxicity test

Acute toxicity was carried out to determinate lethal dose (LD_{50}). NMRI mice orally received 500, 1000, 2000, 3000, 5000 mg/kg and intraperitoneally 1000 and 1500 mg/Kg of body weight (bw). After the administration of extract, the animals were observed for 2 to 72 h. Then morbidity or mortality and changes in behavior have been noted [9].

Anti-edematous activity

Anti-edematous activity was evaluated according to Winter *et al.* [10]. Seven groups of six mice were randomly used. HAE of *Wissadula amplissima* was administrated *per os* at 2 different doses (200 and 300 mg/kg bw) and intraperitoneally at 150 mg/kg bw to sample groups. Control groups received only vehicle (Water *per os*, 0.9 % NaCl ip) while reference groups received reference compounds (acetylsalicylic acid at 150 mg/kg bw *per os*, hydrocortisone at 10 mg/kg bw ip). One hour later, 50 µl of carrageenan (1% in 0.9 % NaCl) was injected under the aponeurosis plantar of the hind paw at each mouse. Paw edema volume was measured at time intervals of 1 h, 3 h and 5 h after carrageenan injection using plethysmometer (model Ugo Basil, n °7141, Italy). The anti-edematous activity was evaluated in the percentage of reduction of the edema in treated mice compared to negative control using the following formula:

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

A represents the average difference paw edema volume in the negative control group and B represents the average difference of paw edema volume in treated groups of mice.

Analgesic activity

The analgesic activity was evaluated according to the acetic acid-induced writhing test as described by Sawadogo *et al.* [11] with minor modifications. Six groups of 6 randomly selected mice were used. HAE of *Wissadula amplissima* was administrated *per os* at 2 different doses (200 and 300 mg/kg, bw) and intraperitoneally at 150 mg/Kg by to sample groups. Control groups received only vehicle (Water *per os*, 0.9 % NaCl ip) while reference group received paracetamol (100 mg/kg bw *per os*). One hour after drug administration, mice received 0.6% of acetic acid injection (10 ml/kg bw). A number of writhing was recorded within 5 to 20 min after acetic acid injection. The analgesic effect was evaluated according to the following formula:

$$\% \text{ Inhibition} = \frac{\text{Number of Writhes}_{(\text{Control})} - \text{Number of Writhes}_{(\text{Sample})}}{\text{Number of Writhes}_{(\text{Control})}} \times 100$$

Lipoxygenase (LOX) inhibitory activity

The inhibition of lipoxygenase was assayed according to the spectrophotometric method described by Lycklander and Malterud [12] with slight modifications. Briefly, 100 µl of enzyme solution (200 U/ml) prepared in boric acid buffer (0.2 M; pH 9.0) was mixed with 25 µl of HAE of *Wissadula amplissima* (1 mg/ml in DMSO) and then incubated at room temperature for 3 min. Reaction was then initiated by the addition of 125 µl of the substrate (250 µM of linoleic acid) and the velocity was recorded for 3 min at 234 nm with a microplate reader (Epoch, BioTeck instruments, USA). DMSO was used as a control while quercetin and ibuprofen were used as reference compounds. Percentage of lipoxygenase inhibition was calculated according to the equation:

$$\% \text{ Inhibition of lipoxygenase} = \frac{V0_{\text{Control}} - V0_{\text{Sample}}}{V0_{\text{Control}}} \times 100$$

$V0_{\text{Control}}$: Enzymatic activity without inhibitor, $V0_{\text{Sample}}$: Enzymatic activity in presence of HAE or reference compounds.

Xanthine oxidase (XO) inhibitory activity

Xanthine oxidase inhibition was assessed according to the spectrophotometric method reported by Filha *et al.* [13] with slight modifications. In brief, 20 µl of enzyme solution (0.28 U/ml in phosphate buffer pH 7.5) was mixed with 20 µl of HAE of *Wissadula amplissima* (1 mg/ml in DMSO), 60 µl of phosphate buffer (pH 7.5) and then incubated at room temperature for 2 min. The reaction was initiated by the addition of 100 µl of the substrate (150 µM of xanthine in phosphate buffer) and the velocity was recorded for 3 min at 295 nm with a microplate reader (Epoch, BioTeck instruments, USA). DMSO was used as a control while allopurinol was used as reference inhibitor. Percentage of xanthine inhibition was calculated according to the equation:

$$\% \text{ Inhibition of xanthine oxidase} = \frac{V0_{\text{Control}} - V0_{\text{Sample}}}{V0_{\text{Control}}} \times 100$$

$V0_{\text{Control}}$: Enzymatic activity without inhibitor, $V0_{\text{Sample}}$: Enzymatic activity in presence of HAE or allopurinol.

DPPH radial scavenging assay

DPPH radical scavenging activity was evaluated as described by Kadam *et al.* [14]. Briefly, 200 µl of freshly prepared DPPH solution (0.02 mg/ml in methanol) was mixed with 100 µl of HAE of *Wissadula amplissima* (from 100 to 25 µg/ml in methanol). After shaking, the mixture was incubated for 15 min in darkness at room temperature and absorbance measured at 517 nm against a blank (methanol) with a microplate reader (Epoch, BioTeck instruments, USA). Inhibition of DPPH radical was calculated as following:

$$\% \text{ Inhibition of DPPH radical} = \frac{Abs_{\text{Blank}} - Abs_{\text{Sample}}}{Abs_{\text{Blank}}} \times 100$$

Abs_{Blank} and Abs_{Sample} are the absorbances of the blank and sample reactions. IC_{50} (Concentration scavenging 50 % of DPPH radicals) was graphically determined. Gallic acid and Quercetin were used as positive controls.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to Lamien–Meda *et al.* [15]. HAE of *Wissadula amplissima* (100 µl, 1 mg/ml in methanol) was mixed with 250 µl of phosphate buffer (0.2 M, pH 6.6) and 250 µl of potassium hexacyanoferrate solution (1% in water). After 30 min of incubation (50 ° C), 250 µl of trichloroacetic acid (10% in water) was added and the mixture was centrifuged (2000 g for 10 min). The supernatant (125 µl) was mixed with water (125 µl) and 25 µl of fresh $FeCl_3$ solution (0.1 % in water) and then absorbance was read at 700 nm with a microplate reader (Epoch, BioTeck instruments,

USA). Ascorbic acid was used to plot a calibration curve (R^2 = 0.99). Reducing power was expressed as mg ascorbic acid equivalent per gram of HEA (mg AAE/g). Gallic acid and quercetin were used as positive controls.

Lipid peroxidation inhibition assay

Inhibition of lipid peroxidation was determined following the method described by Kulkarni *et al.* [16]. Briefly, 100 µl of HAE of *Wissadula amplissima* (from100 to 25 µg/ml in methanol) were added to 100 µl of lecithin (10 mg/ml in phosphate buffer 10 mmol, pH 7.4), $FeCl_3$ (100 µl; 40 mmol) and ascorbic acid (100 µl; 20 mmol) for 1 hour incubation at 37 °C. HCl (1 ml, 0.25N) supplemented with 15% TCA and 0.375 % TBA was added to the mixture, incubated for 15 min at 100 °C and centrifuged (3000 rpm for 10 min). The absorbance of the supernatant was read at 532 nm against a blank (methanol) with a microplate reader (Epoch, BioTeck instruments, USA). Inhibition of lipid peroxidation was calculated as following:

$$\% \text{ Inhibition of lipid peroxidation} = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100$$

Abs_{Blank} and Abs_{Sample} are the absorbances of the blank and sample reactions. IC_{50} (Concentration inhibiting 50 % of lipid peroxidation) was graphically determined. Quercetin was used as a positive control.

Desoxyribose degradation assay

The ability of HAE of *Wissadula amplissima* to prevent the degradation of the desoxyribose was determined using the method described by Houghton *et al.* [17]. Briefly, freshly prepared 2-desoxyribose (100 µl, 28 mmol in phosphate buffer 50 mmol, pH 7.4), EDTA (500 µl; 1.04 mmol), $FeCl_3$ (100 µl; 100 mmol), H_2O_2 (100 µl, 1.0 mmol) and ascorbic acid (100 µl, 1.0 mmol) was mixed and incubated at 37 °C for 1 hour with HAE (100, µl; 1 mg/ml). Thiobarbituric acid (1 ml of 1% aqueous solution) and trichloroacetic acid (1 ml of 2.8 % aqueous solution) was added to the mixture and incubated at 100 °C for 20 min. The resultant mixture was centrifuged (3000 g for 10 min). Absorbance of the organic layer containing thiobarbituric acid reactive substances was measured at 532 nm against a blank (methanol) with a microplate reader (Epoch, BioTeck instruments, USA). Inhibition of Desoxyribose degradation was calculated as following:

$$\% \text{ Inhibition of desoxyribose degradation} = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100$$

IC_{50} (Concentration inhibiting 50 % of desoxyribose degradation) was graphically determined. Quercetin was used as a positive control.

Polyphenol and flavonoids content

Total polyphenol content was determined according to Singleton *et al.* [18]. HAE of *W. Amplissima* (25 µl, 100 µg/ml in Methanol) was mixed with Folin Ciocalteu Reagent (105 µl, 0.2 N) and 5 min later with sodium bicarbonate (100 µl, 75 g/l). After 1-hour incubation, absorbance was measured at 760 nm against a blank with a microplate reader (BioTeck instruments, USA). A standard calibration curve (Y = 0.005X+0.00968; R^2 = 0.99) was plotted using Gallic acid (0-100 mg/l). Polyphenol content was expressed as mg of Gallic acid equivalent to 100 mg of extract (mg GAE/100 mg).

The total flavonoids content was estimated according to Arvouet–Grant *et al.* [19]. HAE of *W. amplissima* (75 µl, 100 µg/in methanol) was mixed with aluminium trichloride (75 µl, 2%in methanol). Absorbance was subsequently read at 415 nm after 10 min incubation against a blank with a microplate reader (Epoch, BioTeck instruments, USA). A standard calibration curve (y = 0.02891X+0.0036; R^2 = 0.99) was plotted using Quercetin (0-100 mg/l). Flavonoid content was expressed as mg of Quercetin equivalent to 100 mg of extract (mg QE/100 mg).

Statistical analysis

Experiments were carried out in triplicate and results expressed as mean±SEM. ANOVA analysis ($p<0.05$) and linear regression were performed with GraphPad Prism 5 and XLSTAT 7.1 software.

RESULTS

Acute toxicity

Any mortality was observed at the highest doses of *W. amplissima* HAE (5000 mg/Kg bw *per os,* 1500 mg/Kg bw intraperitoneally) on mice model. However behavioral signs (prostration, loss of exploration instinct, sleepiness and feed refusal) were observed within two hours following the extract administration *per os* at 5 000 mg/kg bw. Although behavioral signs disappear after 2 d and mice regain their initial vitality. These results point out that HAE did not exhibit any acute toxicity effect on NMRI mice at the doses tested.

Anti-inflammatory activity *in vivo*

The anti-inflammatory activity of *W. amplissima* HEA was assessed *in vivo* through its anti-edematous and analgesic effects as summarized in table 1 and table 2.

Either in oral or intraperitoneal (i. p.) administration, edema induced by carrageenan injection was significantly ($p<0.05$) reduced in a time-dependent manner within 5 h after inflammation, that is not the case of acetylsalicylic acid administrated *per os*. In our experimental conditions, HEA administrated *per os* at 200 and 300 mg/kg bw were less active than acetylsalicylic acid (150 mg/kg bw *per os*) within an hour but were more efficient at the fifth hour. HEA (150 mg/kg; i. p.) exhibited the same effect than hydrocortisone (15 mg/kg) within 3 h, the latter being more effective at the fifth hour. Regarding the analgesic activity, reduction of contortions dose-dependent when HAE is administrated *per os*. Either in oral (200 and 300 mg/kg bw) or intraperitoneal (150 mg/kg bw) administration HAE was more effective than paracetamol (100 mg/kg, bw, *per os*).

Inhibition of lipoxygenase and xanthine oxidase

Table 3 show that HAE possess significant inhibitory activities on both enzymes respectively with 72.80% inhibition for xanthine oxidase (XO) and 68.99% inhibition for lipoxygenase (LOX) when tested at 100 µg/ml (final concentration). Regarding LOX inhibition, HAE is more potent than quercetin (52.97%) but less active than ibuprofen (78.61%). Concerning XO inhibition, HEA is slightly weaker than quercetin (93.63%) and allopurinol (95.49%).

Table 1: Effect of HAE of *W. amplissima* on edema induced in mice by the injection of carrageenan

Samples administration (mg/kg)		Volume of edema (ml)			Inhibition percentage (%)		
		1 h	3 h	5 h	1 h	3 h	5 h
per os	Control (Nacl 0.9%)	0.21±0.04	0.33±0.01	0.36±0,01	---	---	---
	HAE (200)	0.16±0.01	0.19±0.01*	0.12±0.01*	21.66±3.67	38.36±1.22	65.69±2.69
	HAE (300)	0.13±0.03*	0.23±0.03*	0.07±0.04*	38.09±1.52	48.83±5.68	76.06±4.39
	acetylsalicylic acid (150)	0.10±0.01*	0.16±0.01*	0.15±0.01*	48.47±6.81	50.96±2.37	56.47±4.80
i. p.	Control (Nacl 0.9%)	0.22±0.05	0.36±0.03	0.38±0,02	---	---	---
	HAE (150)	0.15±0.03	0.22±0.05*	0.20±0.07*	24.85±1.45	35.75±1.6	41.11±2.00
	Hydrocortisone (15)	0.15±0.05*	0.21±0.01*	0.16±0.02*	24.28±2.50	35.95±5.90	53.51±2.80

Data are expressed as mean values±SEM (n= 6), HAE: Hydro acetone extract of W. amplissima, *Values within the same sub-column harbouring asterisk mark in superscript are significantly different from the control group ($p<0.05$).

Table 2: Effect of HAE of *W. amplissima* against writhing induced by acetic acid

Samples administration (mg/kg)		Number of Writings	Inhibition percentage (%)
per os	Control	66.27±6.50	---
	HAE (200)	28.66±14.75*	56.74±2.41
	HAE (300)	24.66±4.84*	62.78±6.67
	Paracetamol (100)	35.16±2.13*	46.94±2.78
i. p	Control	74.40±3.79	---
	HAE (150)	47.93±3.67*	47.93±3.67

Data are expressed as mean values±SEM (n= 6), HAE: Hydro acetone extract of W. amplissima *Values within the same sub-column harboring asterisk mark in superscript are significantly different from the control group ($p<0.05$)

Table 3: Inhibition of xanthine oxidase and lipoxygenase by HAE from *W. amplissima*

Samples (µg/ml)	Xanthine oxidase inhibition (%)	Lipoxygenase inhibition (%)
HAE (100)	72.80±1.44^{b}	68.99±1.39^{b}
Allopurinol (100)	95.49±0.47^{a}	nd
Quercetin (100)	93.63±0.36^{a}	52.97±0.71^{c}
Ibuprofen (100)	nd	78.61±0.12^{a}

Data are expressed as mean±SEM (n= 3), HAE: Hydro acetone extract of W. amplissima, $^{a-c}$Within the same column, values with different letter in superscript are significantly different ($p<0.05$).

Antioxidant activity, polyphenol and flavonoid content

The antioxidant potential of the HAE of *W. amplissima* to scavenge DPPH radical, to prevent ferric reduction, lipid peroxidation and desoxyribosse degradation has been compared with those of Quercetin. As shown (table 4), HAE of *W. amplissima* demonstrates an interesting antioxidant potential. Indeed, in the lipid peroxidation model, HAE of *W. amplissima* exhibited the same antioxidant activity than quercetin, our antioxidant reference. Although HAE of *W. amplissima* was less active than quercetin in the other antioxidant model tested, it ferric reducing power was only 1.5 lower than that of quercetin and IC_{50}values recorded in DPPH and desoxyribose assays were lower than 100 µg/ml. Total polyphenol and flavonoid content of HAE from *W. amplissima* were respectively 64.33±1.34 GAE/100 mg of extract and 35.17±0.77 QE/100 mg of extract.

Table 4: Antioxidant activity, polyphenol and flavonoid content of HAE from *W. amplissima*

	Samples	
	HAE	Quercetin
DPPH assay (IC_{50}, µg/ml)	57.33±2.3^{b}	1.35±0.13^{a}
Desoxyribose assay (IC_{50}, µg/ml)	32±2.51^{b}	10.06±0.11^{a}
Lipid peroxidation assay (IC_{50}, µg/ml)	11.9±0.1^{a}	9.13±0.11^{a}
FRAP assay (µmol AAE/g)	120.37±2.52^{b}	187.77±2.27^{a}
Polyphenol content (mg GAE/100 mg)	64.33±1.34	-
Flavonoid content (mg GAE/100 mg)	35.17±0.77	-

Data are expressed as mean±SEM (n= 3), HAE: Hydro acetone extract of W. amplissima, $^{a-b}$: Within the same line, values harboring different letter in superscript are significantly different ($p<0.05$).

DISCUSSION

The present study demonstrated that hydro acetonic extract (HAE) of *W. amplissima* possess potential pharmacological properties including antioxidant, anti-edematous, analgesic and inhibiting enzymes (xanthine oxidase and lipoxygenase) effects. HAE of whole *W. amplissima* was considered as slightly toxic conferring to toxicity scale established by Hodge and Sterner [9], because the LD_{50} was above 5000 mg/kg of body weight.

Edema induced by the carrageenan injection is an animal model assay commonly used to evaluate the anti-inflammatory activity of substances. Carrageenan injection provokes the release of several chemical mediators which are the basis of the inflammatory process. The inflammatory response is biphasic [20, 21]: the first phase lasted one hour that mediated by histamine, serotonin and bradykinin; the second phase (above one hour) is due to the biosynthesis of prostaglandins [22]. HAE of *W. amplissima* whole plant inhibited the edema in a dose-dependent manner and in all these phases. Thus, the extract may have an antagonistic action against histamine, bradykinin, serotonin and the prostaglandins biosynthesis. The highest value of inhibition was obtained at the fifth hour. This suggests that the inhibitor action of the HAE of *W. amplissima* could be acted more on the cyclooxygenases which are implicated in the synthesis of prostaglandins.

The extract was effective as well as it was orally administrated or by intraperitoneal injection. The molecular structure plays a key role in the choice of the way used to administrate a drug. In this study results shown that whatever the administration mode was (orally or intraperitoneally) the HAE of *W. amplissima* was effective. This suggests that bioactive compounds, responsible for the anti-inflammatory properties found in this herbal mixture have been easily absorbed through the intraperitoneal way or via the gastrointestinal mucosa.

Writhing induced by acetic acid injection is a method used to study the peripheral analgesic effect of a substance [23]. Abdominal contortions induced by acetic acid injection were used to evaluate the analgesic effect of HAE of *W. amplissima*. Test showed that the extract had a non-opioid analgesic potential either oral or intraperitoneal administration. The pain caused by the injection of acetic acid is due to the release of serotonin, bradikynin, substance P and prostaglandins ($PGE_{2\alpha}$ et $PGF_{2\alpha}$). These chemical mediators stimulate peripheral nociceptive neurons and induce the vascular permeability [24]. HAE of *W. amplissima* inhibited abdominal contraction in a dose-dependent manner. Analgesic effect of HAE of *W. amplissima* could be related to the inhibition of these chemical mediators.

Lipoxygenases (LOXs) play an important role in the biosynthesis of leukotrienes which are pro-inflammatory mediators involved in the physiopathology of inflammatory diseases. Leukotriene LTB4 is a powerful pro-inflammatory chemotactic agent for a variety of leukocytes; and leukotrienes (C4, D4, E4) are responsible of vascular permeability and smooth muscle contraction [25]. HAE of *W. amplissima* inhibited lipoxygenase; it suggests that extract prevents leukotrienes actions inhibiting LOX.

The enzyme xanthine oxidase (XO) catalyzes the oxidation of hypoxanthine and xanthine to uric acid in the purine catabolic pathway [26]. Uric acid plays a crucial role in pathogenesis hyperuricemia, cardiovascular diseases [27]. HAE of *W. amplissima* whole plant inhibited XO. HAE of *W. amplissima* whole plant was evaluated for its *in vitro* anti-inflammatory activity by inhibition of LOX and XO enzymes. The inhibition of these enzymes is a potential therapeutic to treat asthma, hyperuricemia and gout.

During inflammatory process, excessive production of free radical such as reactive oxygen species (ROS) are released causing cells lysis, pro-inflammatory cytokines (TNFα, IL1β, IL6) production and LOX and cyclooxygenases expression [28]. The inhibitor effect of HAE of *W. amplissima* whole plant against ROS, was evaluated using DPPH, desoxyribose degradation assay, FRAP and lipid peroxidation methods that were widely used to determine the antioxidant capacity of substances. The extract exhibited antioxidant activity with four methods, however, its effect was smaller than quercetin. In this study different methods were used because it takes more than one antioxidant method to assess the mode of action of antioxidants [29]. The antioxidant capacity of *W. amplissima* HAE contributes to reinforce its anti-inflammatory effect.

The results of this study reveal that HAE of *W. amplissima* contains polyphenol and flavonoid compounds which could be responsible of evidenced pharmacological activities.

CONCLUSION

The present study showed that the hydroacetonic extract of *Wissadula amplissima* possesses antioxidant, analgesic and anti-inflammatory (*in vitro* and *in vivo)* effects. Data obtained represent a scientific basis for the traditional use of *Wissadula amplissima* to manage inflammatory diseases. Further bio-guided investigations would help in the isolation of compounds and in the clear elucidation of their mechanism of action.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Authors have no conflict of interest to declare

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