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Comparative study of *in vitro* antioxidant and anti-inflammatory potentials of two *malvaceae* used in folk medicine in Burkina Faso

Abstract

The purpose of this study was to evaluate and compare the *in vitro* antioxidant and anti-inflammatory potentials of *Wissadula amplissima* (L.) and *Abutilon grandifolium* (Willd.) Sweet, two malvaceae used in the traditional medicine in Burkina Faso. Aqueous, ethanolic and hydroacetonic extracts of these species were used for different assays. Total phenolic and total flavonoid contents were determined using spectrophotometric methods. Antioxidant capacities of the extracts were screened using four methods: DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAOC) and anti-lipid peroxidation assay. The different extracts were also assayed on xanthine oxidase, lipoxygenase and cyclooxygenases 1 and 2 to establish *in vitro*, their anti-inflammatory potentials. Results obtained revealed that the two Malvaceae species were rich in phenolic and flavonoid compounds. These compounds may have antioxidant and anti-inflammatory properties, as in this study we have highlighted the antioxidant capacities and the anti-inflammatory potential of the extracts from the two species. *W. amplissima* and *A. grandifolium* can be important sources of bioactive molecules with antioxidant and anti-inflammatory properties.

Keywords: antioxidant, anti-inflammatory, *abutilon grandifolium*, *wissadula amplissima*

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Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, α-diphenyl-β-picrylhydrazyl; TAOC, total antioxidant capacity; GAE, gallic acid equivalent; QE, quercetin equivalent; LPI, lipid peroxidation inhibition; AAE, ascorbic acid equivalent; ROS, reactive oxygen species; NOS nitrogen oxygen species; XO, *xanthine oxidase*; LOX, *lipoxygenase;* COX 1, *cyclooxygenase 1*; COX 2, *cyclooxygenase 2*

Introduction

Inflammation is a vital immunological process which is indispensable to animal's survival.[1] Persistent inflammation state can lead to chronic inflammation that can contribute to many common diseases[2] from infectious to physiological dysfunctioning.[3,4] Reactive oxygen species (ROS) and nitrogen oxygen species (NOS) are produced during inflammatory processes, and also contribute to amplifying oxidative stress phenomenon.[5] Oxidative stress and inflammation are two linked pathophysiological processes.[6] Regulation of reactive species has proven to attenuate the inflammation.[7] Modern drugs used to treat different forms of inflammatory diseases are known to cause severe side effects,[8] therefore it is urgent to find a new source of efficient drugs with minimum side effects.

The global use of medicinal plants predates the introduction of antibiotics and other modern drugs.[9] Plants have an innate ability to biosynthesize a wide range of metabolites capable to attenuate ROS and NOS induced oxidative damage as well as to interact with different physiological mediators and effectors of inflammation in animal's bodies.[10]

Several ethnomedicinal surveys carried out on plants belonging to the Malvaceae family have reported their traditional uses in the treatment of diverse ailments associated with the inflammatory process.[11,12] *In vitro* and *in vivo* investigations also support their efficiency in the modulation of different aspects of inflammation process.[13,14] The present study was designed to compare the antioxidant potential and inflammatory enzymes inhibition activities of *W. amplissima* and *A. grandifolium* two species belonging to the Malvaceae family used in the folk medicine of Burkina Faso.

Material and methods

Collection and authenticity of plant material

Whole plants of *Wissadula amplissima* (L.) var. Rostrata (Schumach & Thonn) R. E. Fries (*Malvaceae*) and *Abutilon grandifolium* (Willd.) Sweet (Malvaceae) were harvested in January 2013 in Gampèla (25km, East of Ouagadougou, Burkina Faso). Professor Jeanne Millogo-Rasolodimby from the laboratory of Biologie et ecologie végetale (University.of Joseph KI-ZEBO, Burkina Faso) assessed the botanical identity of the plants, then voucher specimens (CI: 16884 and CI: 16885) corresponding respectively to *Wissadula amplissima* and *Abutilon grandifolium* were deposited in the university's herbarium. Plant materials collected for different tests were dried at room temperature, pulverized and stored in an airtight bag until use.

Reagents and chemicals

All chemicals were analytical grade. Sulfuric acid, Dimethyl sulfoxide (DMSO), petroleum ether, acetone, methanol, ethanol,

Xanthine oxidase from bovine milk (EC 1.17.3.2.), Xanthine, hydrochloric acid (HCl), Lipoxygenase from glycine max (soybean) (EC 1.13.11.12.), linoleic acid, boric acid (H_3BO_3), gallic acid, quercetin, sodium hydrogenophosphate (Na_2HPO_4, $12H_2O$), sodium bicarbonate ($NaHCO_3$), L-α-lecithin, aluminium trichloride ($AlCl_3$), ferric trichloride ($FeCl_3$), Folin Ciocalteu reagent (FCR), potassium dihydrogenophosphate (KH_2PO_4), potassium hexacyanoferrate ($K_3[Fe(CN)_6]$), sodium phosphate (Na_3PO_4), trichloroacetic acid (TCA), thiobarbituric acid (TBA), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), α, α-diphenyl-β-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA), COX (ovine/human) inhibitor screening assay kit was purchased form, Cayman Chemical Compagny, (New York, NY, USA), Ascorbic acid was supplied from Labosi (Paris, France).

Samples preparation

Powdered plant material (50g) of each species was defatted with petroleum ether (500mL) in a Soxhlet extractor before soaked (48h, 25°C, continuous stirring) with 500mL of ethanol, mixed acetone–water (80/20 v/v) and water. Hydroacetonic and aqueous extracts were filtrated, concentrated in a vacuum evaporator (Büchi Rotavapor R 200) and lyophilized (Telstar Cryodos 50). The ethanolic extracts after filtration and concentration were left under a hood (Captair Chem, erlab) until the residual organic solvent were evaporated. The different samples were then stored at 4°C until use.

Phytochemical quantification

Determination of total phenolics content: Total polyphenol content in the different extracts was determined according to the method described by Singleton et al.[15] Briefly, the extracts of *W. Amplissima* and *Abutilon grandifolium* (25µL, 100µg/mL in methanol) were mixed with Folin Ciocalteu reagent (105µL, 0.2 N) and 5min later sodium bicarbonate (100µL, 75g/L) was added. After a 1 hour incubation, the absorbance of each mixture was recorded at 760nm 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 (0-100mg/L). Polyphenol content was expressed as mg of gallic acid equivalent per 100mg of extract (mg GAE/100mg).

Determination of total flavonoids content: The total flavonoids content was estimated according to the method of Dowd as adapted by Arvouet–Grant et al.[16] Briefly, the extracts of *W. Amplissima* and *A. grandifolium* (75µL, 100µg/mL) were mixed with aluminium trichloride (75µL, 2% in methanol). Absorbances were subsequently read at 415nm after 10min of incubation against a blank with a UV/visible spectrophotometer (Epoch, BioTeck instruments, USA). A standard calibration curve (Y=0.0068X+0.0225; R^2 = 0.9945) was plotted using quercetin (0-150mg/L). Total flavonoid content was expressed as mg of quercetin equivalent per 100mg of extract (mg QE/100mg).

Antioxidant capacity evaluation

DPPH radical scavenging assay: DPPH radical scavenging activity was assayed as described by Velázquez et al.[17] Briefly, 200µL of freshly prepared DPPH solution (0.02mg/mL in methanol) was mixed with 100µL of different extracts of *W. amplissima* and *A. grandifolium* (from 100 to 25µg/mL in methanol). After shaking, the mixture was incubated for 15min in darkness at ambient temperature and absorbance measured at 517nm against a blank (methanol) with a microplate reader. Inhibition of DPPH radical was calculated as follows:

$$DPPH\ free\ radical\ Inhibition\ \%: \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} X100$$

Abs_{Blank} and Abs_{Sample} are respectively the absorbances of the blank and the absorbance of the sample. The concentration of extract that scavenges 50 % of DPPH free radicals (IC_{50}) was graphically determined. Gallic acid and Quercetin were used as positive controls.

Ferric reducing antioxidant power (FRAP) assay

The method described by Lamien–Meda et al.[18] was used to assess the ferric reducing antioxidant power of the different extracts. Briefly, 100µL (1mg/mL in methanol) of each tested sample was mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250µL of potassium hexacyanoferrate solution (1% in water). After 30 min of incubation (50°C), 250µL of trichloroacetic acid (10% in water) was added and the mixture was centrifuged (2000 g for 10 min). The supernatant (125µL) was mixed with water (125µL) and 25µL of fresh $FeCl_3$ solution (0.1 % in water) and then absorbance was read at 700nm with a microplate reader. Ferric reduction potential of extracts was expressed in micromole ascorbic acid equivalent per gram (µmol AAE/g) of raw material

Lipid peroxidation inhibition assay

The method described by Jaishree et al.[19] was used to evaluate the anti-lipid peroxidation capacity of the different samples. Briefly, 100µL of each extract (from 100 to 25µg/mL in methanol) were added to 100µL of lecithin (10 mg/mL in phosphate buffer 10 mmol, pH 7.4), $FeCl_3$ (100 µL; 40mmol) and ascorbic acid (100µL; 20 mmol) for 1 hour incubation at 37°C. HCl (1mL, 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. Inhibition of lipid peroxidation was calculated as follows:

$$Lipid\ peroxidation\ inhibition\% = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100$$

Abs_{Sample} and Abs_{Sample} are respectively the absorbance of the blank and sample reactions. IC_{50} (Concentration inhibiting 50 % of lipid peroxidation) was graphically determined.

Total antioxidant capacity (TAOC) assay

The TAOC evaluation was based on the formation of the phosphomolybdenum complex following the method described by Pietro et al.[20] Briefly, each sample (100µg/mL; final concentration) was mixed with 3mL of reagent solution (H_2SO_4; 0,6 M, sodium phosphate 28mM and ammonium molybdate 4mM). A blank consisting of 4 mL of each tested sample was used. The mixtures obtained are incubated at 95°C for 150min. After a cooling period to ambient temperature, 200 µL of each tested mixture was taken and placed in the microplate well. TAOC are expressed in ascorbic acid equivalent (AAE) after absorbance recording against a standard curve (y =0,0594x+0,156; R^2 =0, 9983) plotted with ascorbic acid (0-150mg/L).

Inflammatory Enzymes inhibition assay

Xanthine oxidase inhibition: Xanthine oxidase inhibition potential was assessed according to the method developed by Ferraz Filha et al.[21] with slight modifications. In brief, 20µL of enzyme solution (0.28U/ml in phosphate buffer pH 7.5) was mixed with 20µL of a

sample (100µg/mL in DMSO), 60µL of phosphate buffer (1/15 M; pH 7.5) and then incubated at room temperature for 2min. The reaction was initiated by the addition of 100µL of the substrate (150µM of xanthine in phosphate buffer) and the velocity was recorded for 3min at 295 nm with a microplate reader. DMSO was used as negative control while quercetin was used as a reference inhibitor (positive control). Percentage of xanthine inhibition was calculated according to the equation:

$$\%of\ \ inhibition = \frac{Vo_{control} - Vo_{sample}}{Vo_{control}} \times 100$$

$Vo_{control}$: Enzymatic activity without inhibitor, Vo_{sample} : Enzymatic activity in presence tested sample or quercetin.

15-Lipoxygenase inhibition assay

The method described by Malterud & Rydland[22] was used to assess the inhibition of lipoxygenase, with slight modifications. Briefly, 100µL of enzyme solution (200U/mL) prepared in a boric acid buffer (0.2 M; pH 9.0) was mixed with 25µL of extract (1mg/mL in DMSO) and then incubated at room temperature for 3min. The reaction was then initiated by the addition of 125µL of the substrate (250µM of linoleic acid) and the velocity was recorded for 3min at 234nm with a microplate reader. DMSO was used as control while quercetin and ibuprofen were used as reference compounds. Percentage of lipoxygenase inhibition was calculated according to

the equation: $\frac{Vo_{control} - Vo_{sample}}{Vo_{control}} \times 100$

$Vo_{control}$:Enzymatic activity without inhibitor, Vo_{sample} : Enzymatic activity in the presence of the tested sample.

Cyclooxygenases (COX-1 and COX-2) inhibition assay

The inhibition of COXs was performed using a commercially available colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemical Company, New York, NY, USA). All the inhibitors were dissolved in an appropriate solvent. The COX activity was evaluated using N, N, N', N'-tetramethyl-phenylenediamine (TMPD) as a co-substrate, with arachidonic acid. The TMPD oxidation was monitored spectrophotometrically at 590 nm. The inhibition percentage that was induced by 100 µg/ml of the sample was calculated.

Statistical analysis

The results are expressed as mean±SD for triplicate analysis. Data were subjected to one- way analysis of variance (ANOVA), where the differences between results were determined using Tukey's significant test and $P < 0.05$ was considered significant. Statistical analysis was performed using XLSTAT version 7.5.2. (Addinsoft FRANCE) and graphs were plotted using Origin 8.0.

Results and discussions

Antioxidant activities

Plants in adaptation to their environmental conditions produce a wide range of phytochemical compounds, which may be beneficial to humans.[23] Phytoconstituents are responsible for one or more herbal preparation properties.[24] Studied plants extract antioxidant potential are presented in Table 1. The IC_{50} of an extract is inversely proportional to its ability to produce activity. In the present study, the IC_{50} obtained varied from 37.25±2.51 to 81.11±5.01 for DPPH assay and from 11.9±0.1 to 31.9±1.17 for LPI. The best antioxidant activity using DPPH radical savaging method was obtained with aqueous extracts. With the LPI method, hydroacetonic extracts were found to be more effective. A comparison of the extract of each species by type of solvent revealed that extracts from *W. amplissima* possessed the best activities. In this study, quercetin and ascorbic acid were more effective in preventing the production of DPPH radicals. Indeed, quercetin was 3.95 times more antiradical than the aqueous extract of *W. amplissima*; the same observation was found in ascorbic acid, which was 1.44 times more effective on DPPH free radical formation than the best extract. Similarly, in the study of the lipid peroxidation prevention, the most effective extract was less preventive than quercetin and ascorbic acid. The results obtain with FRAP and TOAC assays for the different extracts of *W. amplissima* and *A. grandifolium* follow the same way as that get in the case of DPPH and LPI, with *W. amplissima* extracts being more effective in reducing the ferric ion and the molybdenum VI. *W. amplissima* extracts showed similar reduction power to that of quercetin. These results highlight the antioxidant potential of these plants and justify the usefulness of these plants in the folk medicine.

Anti-inflammatory activities

Several inflammatory diseases treatment is based on the inhibition of enzymes involved in the production of mediators initiating and/or amplifying the inflammatory process.[25] Herbal based remedies with anti-inflammatory activities constitute the foundation of promising curative therapy.[26,27] Table 2 presents results obtained on the inhibition potential of *W. amplissima* and *A. grandifolium* on XO, LOX-15 and COX-1 and COX-2. The anti-inflammatory potential was estimated by the determination of the concentration of each extract that inhibits 50% of the enzyme activity. Hydroacetonic extracts of both plants were more effective in inhibiting the different enzymes compared with ethanolic and aqueous extracts.

Table 1 Comparative antioxidant activities of different extracts from *W. amplissima* and *A. grandifolium* using different methods

Species/ References	Extracts	DPPH IC_{50} (µg/mL)	LPI IC_{50} (µg/mL)	FRAP (mmol AAE/g)	TAOC (mg AAE/g)
W. amplissima	Ethanolic	81.11±5.01	16.06±3.2	1.33±0.44	52.09±1.22
	Hydroacetonic	57.33±2.3	11.9±0.1	1.20±0.52	53.27±2.32
	Aqueous	37.25±2.51	15.39±0.1	1.32±0.52	33.27±2.32
A. grandifolium	Ethanolic	72.07±4.1	31.9±1.17	1.09±0.11	29.56±3.22
	Hydroacetonic	41.15±2.76	21.06±1.31	0.970±0.52	44.92±3.13
	Aqueous	45.04±3.03	26.03±2.9	0.737±0.07	17.27±2.32
Quercetin	-	9.35±1.45	2.25±0.03	1.43±0.14	nd
Ascorbic acid	-	25.82±1.05	7.01±0.31	Nd	nd

nd: not determined

Table 2 W. *amplissima* and A. *grandifolium* inhibiting potential on xanthine oxidase, lipoxygenase and cyclooxygenases comparison

Species/ References	Extracts	XO IC_{50} (µg/mL)	LOX IC_{50} (µg/mL)	COX 1 IC_{50} (µg/mL)	COX 2 IC_{50} (µg/mL)
W. amplissima	Ethanolic	34.16±2.22	33.18±2.43	73.06±2.34	78.51±2. 45
	Hydroacetonic	25.91±1.98	26.95±1.08	50.06±2.34	30.51±2.45
	Aqueous	29.70±2.18	34.15±1.51	53.33±1.92	33.31±2.52
A. grandifolium	Ethanolic	51.67±2.07	66.95±4.13	62.14±2.91	51.01±1.73
	Hydroacetonic	23.52±1.72	39.72±3.04	42.06±1.17	66.68±2.74
	Aqueous	35.41±1.29	55.52±2.50	44.12±2.87	44.31±2.29
Quercetin	-	5.24±0.82	10.62± 2.01	15.89±2.27	9.23±0.42
Ibuprofen	-	nd	22.13±4.12	18.63±0.61	93.33±4.39

nd: not determined

XO inhibition was statistically the same for the ethanolic extract of *W. amplissima* and aqueous extract of *A. grandifolium*. Hydroacetonic extract of *W. amplissima* presented the highest potential to inhibit XO with 25.91±1.98 µg/mL as IC_{50}, while ethanolic extract of *A. grandifolium* was the weakest to inhibit this enzyme.

LOX-15 inhibition was statistically the same for ethanolic and aqueous extracts of *W. amplissima*. The strongest inhibition was obtained with the hydroacetonic extract of *W. amplissima*. Moreover, compared to *A. grandifolium*, it presented the best activity on this enzyme whatever the type of solvent used for the extraction.

COX-1 and COX-2 were differently inhibited by the species studied. For both species, the hydroacetonic extracts were the most effective to inhibit these enzymes. *A. grandifolium* was more effective on COX-1 with 42.06±1.17, while *W. amplissima* was found to be more effective on COX-2 isoform with 30.51±2.45µg/mL as IC_{50}.

The inhibitory activities of the extracts on the different enzymes compared with that of the references used showed that quercetin was more effective than the tested extracts. In the case of ibuprofen, for LOX and COXs inhibitions, the comparison showed that extract was less effective to inhibit LOX and COX-1 isoform, whereas in the COX-2 inhibition extracts are more active than ibuprofen. These results strongly support the use of these plants in folk medicine to threat some inflammation condition.

Phytochemical contents

The results obtained on the total phenolic and total flavonoid contents are shown in Figures 1 & 2. All the extracts contain phenolic compounds (including flavonoids), and the content in these compounds varied according to each solvent used for extraction.

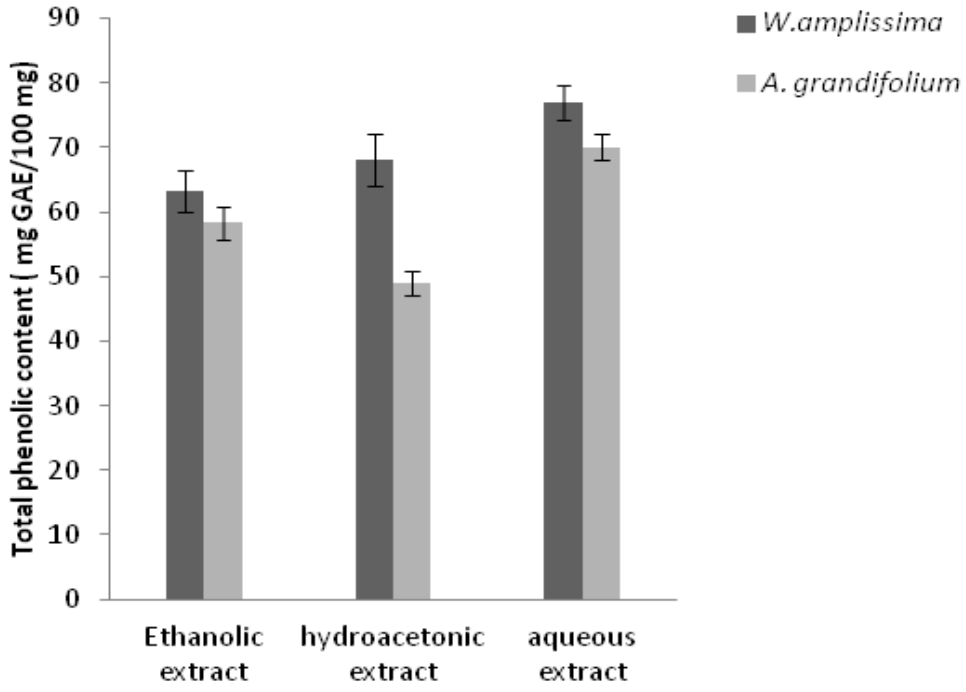

Figure 1 Comparison of total phenolic contents in W. amplissima and A. grandifolium.

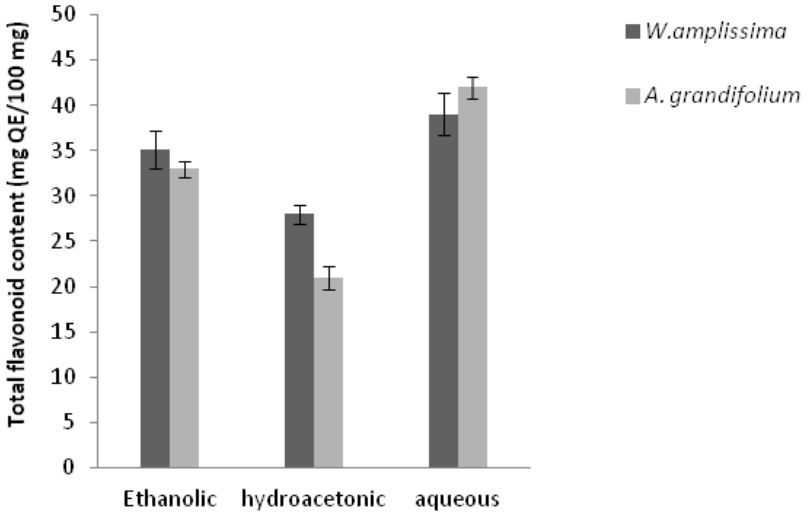

Figure 2 Comparison of total flavonoid contents in W. amplissima and A. grandifolium.

The content of total polyphenols varied from 63.07±3.01 mg GAE/ 100 mg of extract (ethanolic extract) to 77.04±3.26 mg GAE/ 100 mg of extract (aqueous extract) for *W. amplissima*, while for *A. grandifolium* these values were respectively of 57, 49±2.52 mg GAE/ 100 mg of extract and 70.69±1.69 mg GAE/ 100 mg of extract in ethanolic extract and in aqueous extract. Hydro-acetone (80/20 v/v) yielded 1.45 fold more phenolic in *W.amplissima* than in *A. grandifolium*. Under the experimental conditions, significant differences were observed for each compared solvent. Phenolic compounds are metabolites widely distributed in plants. However, their amount varies to each material source [28]. In this study, the major amount of phenolic compounds in both species is hydrophilic kind.

Flavonoids are found in each extract (Figure 2). Comparison in the studied plants revealed that the amount yielded varies according to the extraction solvent. Extraction made with ethanol and acetone, yielded more flavonoids from *W. amplissima* than *A. grandifolium*, whereas when water was the extraction solvent, the amount extracted from *A. grandifolium* was greater than that of *W. amplissima*. Flavonoids constitute one of the largest group of plant phenolic compounds.[29] They are present in all the obtained extract. The highest amount of flavonoids is, as in the case of total phenolics, obtained with water as extracting solvent.

Antioxidant capacities of herbal formulation depend on different parameters such as the phytoconstituents present[30] and the mechanism by which the antioxidant activities are achieved.[31] Differences in antioxidant activities for *W. amplissima* and *A. grandifolium* using different experimental methods may be explained by the fact that depending on plant matrix and the extracting solvent, particular phytomolecules are drained. These molecules are different in type and/or in amount.

Conclusion

The present study highlighted the antioxidant and anti-inflammatory activities and also the polyphenolic compound content of the extracts of *W.amplissima* and *A. grandifolium*. Antioxidant and anti-inflammatory activities of both plants might explain their traditional uses like care given to persons suffering after insect's bites and those affected with skin and respiratory apparatus infections. The comparison of diverse studied activities revealed that *W. amplissima* possesses more therapeutic potential than *A. grandifolium* in terms of antiradical and reduction potentials as well as in the inhibition of enzymes involved in the inflammation process. Results obtained will constitute the basis of the next step of our research, which will focus on the isolation of main active polyphenol compounds.

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Conflicts of interests

Authors have no conflicts of interest to declare.

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