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Ethnomedicinal and Pharmacognostical Investigation on Three Medicinal Asteraceae Species from Burkina Faso: *Bidens Engleri* O. E. Schulz, *Acanthospermum hispidum* DC. and *Ageratum conyzoides* L.

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Abstract

Aims: This study aimed to collect data on ethnomedicinal uses and pharmacognostic evaluation of *Bidens engleri*, *Ageratum conyzoides* and *Acanthospermum hispidum*, three species of Asteraceae family commonly found in Burkina Faso. **Methods:** Ethnomedicinal data were collected through questionnaires, interviews and discussions with local language practitioners. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging and lipoxygenase inhibitory assays were used to determine antioxidant and anti-inflammatory activities, respectively. Phenolics were quantitatively and qualitatively determined using spectrophotometric, histochemical and thin layer chromatography methods. **Results:** Surveys have shown many uses of the three species to treat various pathologies. Histochemical and phytochemical studies characterize the alkaloids, flavonoids, tannins, coumarins, sterols, triterpenes and saponosides in these three plants. *Bidens engleri* had the highest contents of total phenolics (19.38 ± 0.35 mg GAE / 100 mg), total flavonols (1.47 ± 0.08 mg QE / 100 mg) and tannins (16.79 ± 0.72 mg TAE/ 100 mg). However, *Acanthospermum hispidum* possessed the highest total flavonoid contents (6.07 ± 0.12 mg QE / 100 mg). Caffeic acid and rutin were identified in the methanolic extract of *Acanthospermum hispidum* and rutin in that of *Ageratum conyzoides* using thin-layer chromatography. *Bidens engleri* showed the best DPPH

radical scavenging activity with an IC_{50} of 14.97 ± 0.06 µg/mL and the best inhibition of lipoxygenase (70.59 ± 4.16 %) at the concentration of 100 µg/mL. **Conclusions:** These scientific results could justify the various traditional uses of these plants in Burkina Faso in the treatment of oxidative, inflammatory, microbial and parasitic diseases.

Keywords
Asteraceae, ethnomedicinal, phenolics, thin layer chromatography, antioxidant, anti-inflammatory.

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1. INTRODUCTION

There is a growing interest in medicinal products derived from plants, partly because of the low economic availability for African people of chemical synthetic drugs and secondly because of adverse side effects and microbial resistance [1]. Many plant species are traditionally used to treat opportunistic infections related to HIV-AIDS, malaria, bacterial and fungal infections and chronic diseases [2]. Too, the criteria that local people use for selecting medicinal plants have been a recurrent topic in pharmacology and ethnobotany [3]. It is therefore useful to undertake research on the bioactive molecules of medicinal plants in order to justify and secure their use [4]. *Bidens engleri* O.E. SCHULZ, *Acanthospermum hispidum* DC. and *Ageratum conyzoides* L. are annual aromatic Asteraceae known and used for their medicinal properties in Burkina Faso. *Bidens engleri* is traditionally used to treat tonsillitis, tooth decay, myalgia, urticaria, allergies, prostate tumors, cramps, intestinal parasitosis, bronchitis, inflammations and respiratory diseases, as well as malaria and also serves as an insecticide and larvicide [5], diabetes mellitus, hypertension and old wounds [6].

Acanthospermum hispidum DC., is used as analgesic, antibacterial, depurative, cholagogue, diuretic, emollient and against convulsions, tonsillitis, diphtheria, abdominal disorders (shighellosis, typhoid), headache, dysentery, jaundice, against hemorrhoids, wounds, rheumatism, leprosy and scorpion stings [7]. This plant is also antimicrobial, antimalarial, antiviral, anthelmintic, arthritis, rheumatism [8].

Ageratum conyzoides L. is used to treat inflammations, liver diseases, dental flares, burns, uterine disorders, pain in pregnant women, jaundice, hiccups, dizziness, costal pain, pneumonia, tachycardia, snakebites and diarrhea, dermatitis, children's fever, inflammations, scarring [7]. This plant is also used to treat pain, fever and inflammatory chronic diseases such rheumatoid arthritis [9].

These data come from etnomedicinal allegations obtained in other regions of the world. In Burkina Faso, there is little data on these species and in particular on the pharmacognosic analysis of these three species of the asteraceae family used in traditional medicine in Burkina Faso.

This study was undertaken for a better ethnobotanical and pharmacognosic knowledge of these three species of Asteraceae used in traditional medicine in Burkina Faso.

2. MATERIALS AND METHODS

2.1. Ethnomedicinal survey and plant material

Ethnomedicinal data were collected through questionnaires, interviews and discussions with local language practitioners (Moore) and included *Bidens engleri* O. E. SCHULZ, *Acanthospermum hispidum* DC., *Ageratum conyzoides* L., *Tridax procumbens* L., *Laggera aurita* (Lf) DC., *Vernonia galamensis*; six aromatic asteraceae family plants prevalent in Burkina Faso. The survey was conducted under presentation of a note notified by the Director of our laboratory at the University to practitioners of traditional medicine. These people surveyed were motivated most often by cola. The questions in the survey were mainly based on plant knowledge, different uses, diseases treated, parts used, forms and modes of administration. The plants were selected for the rest of the study, based on their frequency of medicinal uses. For each disease cited by traditional medicine practitioners to be treated by each plant, the frequency of use was calculated through the ratio of the number of practitioners who know and use the plant to the total number of practitioners surveyed.

Among these six plants, the three most traditionally used plants according to the ethomedicinal survey were selected for a pharmacognosic evaluation. For this, whole plants of *Bidens engleri* and *Ageratum conyzoides* were harvested in Gampela (15 km from Ouagadougou) and for *Acanthospermum hispidum* DC. the collection took place in the field of the National Museum of Ouagadougou. The three plants were authenticated by the botanist Professor Jeanne

MILLOGO-RASOLODIMBY from the University of Ouagadougou. Two herbarium samples from each plant were deposited in the herbarium of the University of Ouagadougou under number 01 of 08/09/09 for *Bidens engleri* O. E. SCHULZ, 02 of 08/09/09 for *Acanthospermum hispidum* DC. and 03 of 08/09/09 for *Ageratum conyzoides* L.

The harvested plants were then completely dried in the sun and reduced to powder for different extractions.

2.2. Histochemical study

Sections were made in fresh stems, leaves and roots of Biden's engleri, *Acanthospermum hispidum* and *Ageratum conyzoides*. Carmino-green tissue dye was used for identification of tissue constituents, Lugol for alkaloids, $FeCl_3$ for tannins and NaOH for flavonoid detection. For double carmino-green staining, the sections were soaked in bleach for 20 minutes, then rinsed 3 times with distilled water. They were then soaked in acetic acid for 5 minutes, then in carmino-green. Finally washed with distilled water, they were observed between blade and coverslip in a drop of glycerine. The material was photographed using a Canon power Shot A 650 digital camera coupled to a Motic BA 200 optical microscope.

2.3. Extraction

Crude plant extracts were prepared by Soxhlet extraction method.25 g of crude powder of each plant was loaded into the thimble and extracted with 250mL of methanol.

The temperature of the heating mantle was set at the boiling point of methanol (65 °C.) for 6 hours. After extraction, the extracts are collected, evaporated with Rotavapor and then weighed at 4 ° C. The yields of the extractions were determined by the following formula:

$$\text{Yield} = \frac{\text{mass of extracted residue}}{\text{mass of vegetable powder}} \text{ X } 100$$

2.4. Characterization of the main phytochemical groups

The methanolic extracts were subjected to various tube tests to characterize the main groups of phytochemicals. The tube test procedures as described by Ciulei [10]were used for all of these tests. Thus, the identification of alkaloids was done by the Dragendorff test; tannins and polyphenols by the $FeCl_3$ test; flavonoids by the Shibata test; steroids and triterpenes by the Libermann-Buchard test; anthracenosides by the Bornträger test, saponosides by saponosides test and coumarins by coumarins test.

2.5. Comparative thin layer chromatography

Thin layer chromatography of the methanolic extracts was performed to identify groups of compounds or individual compounds according to the protocol described by Wagner and Bladts [11]. Silica gel coated plates (F254) served as a support. The migration was carried out using three systems of eluants.

System 1: n-hexane / ethyl acetate / acetic acid (6 / 5 /1; v / v / v);

System 2: ethyl acetate / formic acid / glacial acetic acid / water (10 /1.1 / 1.1 / 2.6; v / v / v / v).

The two systems were used for the separation of flavonoids and phenolic acids in the methanolic extracts of plants. The Chromatograms were observed under 254 nm and 365 nm; UV lights before and after a spraying with the NEU reagent (Diphenylboryloxyethylamine + polyethylene glycol (PEG)). Caffeic acid and the rutin were used as standards, respectively, for phenolic acids and flavonoids identification.

A third system (Ethyl Acetate / Formic Acid / Glacial Acetic Acid (7 / 1.1 / 1.1: v / v / v)) was used for the separation of steroids and triterpenes from these extracts. A mixture of 3% H_2SO_4 in ethanol was used to reveal the seprated sterols and triterpens. The plate was then heated at 110 °C for 10 minutes. After removal, brown and / or blue coloration characterized sterols while purple coloration characterized triterpenes. β-sitosterol was used as standard.

2.6. Total phenolics content

Total phenolics were estimated using the method described by Sombié et al., [12]. It evaluates all the compounds capable of reducing phosphomolybdotungstic acid (Folin-Ciocalteu reagent). The content of the total phenolics was determined using a calibration curve of Gallic acid (0-200 mg/L). The total phenolic content was determined in three independent experiments in quadruplicate. The results were expressed in mg Gallic Acid Equivalent per 100 mg of dried extract (mg GAE/100 mg of extract).

$$C = \frac{c\ x\ D}{Ci}\ x\ 100 \quad \text{Equation (1)}$$

C = Concentration of total phenolics mg GAE/100 mg of extract dry wet

C = Concentration of sample in mg/mL

Ci= Initial concentration of extract

D= Factor of dilution

2.7. Total flavonoid content

The content of flavonoids in extract was determined by the Dowd colorimetric method as described by Sombié et al. [12]. The method evaluates all compounds capable of reacting with aluminum

chloride ($AlCl_3$). Optical densities were read after 10 minutes incubation at 415 nm using a spectrometer CECIL 2041 EC and a calibration curve of quercetin. Total flavonoids content was performed in three independent experiments in quadruplicate and expressed in mg Quercetin Equivalent / 100 mg of extract dry wet (mg QE / 100 mg of extract) by using equation 1

2.8. Total flavonol content

The flavonol content was determined by the method of Abarca et al., [13]. Concentrations were determined after 5 minutes incubation at 425 nm at the CECIL CE 2041 spectrometer against a quercetin curve calibration. Total flavonols content were performed in three independent experiments in quadruplicate. The results were expressed in mg Quercetin Equivalent / 100 mg of extract dry wet (mg QE / 100 mg of extract) by using Equation 1

2.9. Total tannin content

The total tannin content of the samples was determined using the European commission method [14]. The optical densities were read, after 15 minutes of incubation, at 525 nm using a CECIL CE 2041 spectrophotometer and a standard curve of tannic acid. Total tannin content was determined in three independent experiments in quadruplicate.

2.10. Antioxidant activity

The DPPH method for antioxidant capacity of extract was evaluated using the method of Velázquez [15]. Each extract of these three plants was prepared at a concentration of 1 mg / mL and then diluted in half cascade to obtain a range of several concentrations. Then, a volume of 0.75 mL of each sample was mixed with 1.5 mL DPPH (20 mg/L). Methanol served as blank. Absorbance of the mixture was read after 15 min of in cubation, at 517 nm using a CECIL CE 2041 spectrophotometer.

The tests were performed in three independent experiments in quadruplicate and the radical scavenging activity was expressed as a percentage inhibition according to the formula:

$$\text{Radical scavenging capacity (\%)} = \frac{\text{Absorbance Blank-Absorbance Sample}}{\text{Absorbance Blank}} \text{ X } 100$$

The radical scavenging capacity of several concentrations of the extract was used to calculate the 50 inhibitory concentration (IC_{50})

2.11. Evaluation of anti-inflammatory activities

The anti-inflammatory activities were determined by measuring the potential of extracts to inhibit lipoxygenase enzyme. The lipoxygenase inhibitory activity of extracts was determined using the spectrophotometric method as described by Malterud and Rydland [16]. Thus, 400 µL lipoxygenase solution (167 U / mL), 100 µL extract solution (at the final concentration of 100 µg / mL) were mixed and incubated for 1 min at 25°C. The reaction was initiated by the addition of 500 µL linoleic acid substrate solution (134 µM) and the variation of absorbance was followed at 234 nm for 3 minutes. The tests were performed in three independent experiments in quadruplicate. The percentage of inhibition was calculated using the following formula:

$$\mathbf{I\% = \frac{E - S}{E} X\ 100}$$

Where E is the activity of the enzyme without inhibitor and S its activity in the presence of the extract.

2.12. Statistical analysis

The results, presented as mean ± standard deviation for triplicate analysis were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. A p value < 0.05 was considered as significant. The statistical analysis was performed using XLSTAT version 7.5.2 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSION

Ethnomedicinal survey and plant material: The etnomedicinal data (local name, parts used and mode of preparation, medicinal uses and frequencies of uses) collected through questionnaire, interviews and discussions among the traditional medicine practitioners in their local language Mooré. Obtained results for all plant species are presented in Table 1. *Bidens engleri* O. E. SCHULZ, *Acanthospermum hispidum* DC. and *Ageratum conyzoides* L. are the most used plants among those surveyed. Etnomedicinal surveys have shown that these plants are mainly used for the treatment of inflammatory and microbial diseases. The parts used are usually whole plants. The decoction and the oral route are respectively the modes of preparation and administration of these medicinal plants. It appears that among the six plants that were the subject of our

etnomedicinal study, *Bidens engleri* O. E. SCHULZ, *Acanthospermum hispidum* DC. and *Ageratum conyzoides* L. are the most commonly used plants in traditional medicine in the central plateau of Burkina Faso (Table 1).

Histochemical study: The histochemical sections (Figure 1) revealed the tissue structure of the plant organs by double carmino-green staining and to locate in situ different groups of secondary metabolites. Thus, carmino-green double staining revealed a pink (parenchyma, collenchyme) and midnight blue (xylem, phloem) stain in the histochemical sections of *Bidens engleri* and *Ageratum conyzoides*. A brown pink (parenchyma, phloem, collenchyme) and green (xylem) color of *Acanthospermum hispidum* is observed in histochemical study (Figure 1). Histochemical differentiation highlights the conducting vessels of the different secretion products highlighted by the different reagents. Tannins, flavonoids and alkaloids were characterized in the organs of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides*. In fact, Lugol causes a brown coloration of the cortical parenchyma, xylem and phloem of the various organs that caracterise alkaloids in the three plants. The 2% NaOH gives a yellow coloration of the cortical parenchyma of the organs of *Ageratum conyzoides*, *Bidens engleri* and *Acanthospermum hispidum* characteristic of flavonoids. Tannins were also characterized in the three plants through the black-blue xylem coloration of the organs of *Bidens engleri* and *Acanthospermum hispidum* followed by the green-black coloration of that of *Ageratum conyzoides* by 3% $FeCl_3$ (figure 1).

Extraction yields: *Bidens engleri* exhibited the best Soxhlet methanolic extraction yield (21.51%), which was substantially equal to that of *Ageratum conyzoides* L. (19.46%). *Acanthospermum hispidum* exhibited the lowest extraction yield (14.61%). The best Soxhlet methanolic extraction yield exhibited by *Bidens engleri* substantially equal to that of *Ageratum conyzoides* show that the compounds contained in *Bidensengleiri* and *Ageratum conyzoides* are more soluble in methanol than those contained in *Acanthospermum hispidum*.

Characterization of main phytochemical groups: The results of tube tests of phytochemical screening of the different methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* are presented in Table 2. This classical phytochemical characterization tests allowed us to highlight polyphenolic compounds in the extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides*. The methanolic extracts color change of the three plants initially green to the orange color reflects the presence of flavonoid aglycones in the methanolic extracts of these plants. The green-black coloration obtained with the methanolic extract of *Acanthospermum hispidum* would reflect the presence of catechin tannins in this plant. These results corroborate those of Gomathi who also identified the tannins in the extract of *Acanthospermum hipidum* [17]. Non-fluorescent result of the test tubes of the methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* compared to control shows the presence genins and heterogenins of coumarins in the methanolic extracts of these plants. The absence of Anthracenosides and alkaloids in the methanolic extracts of these three plantes could certainly be justified by their low content in these plants or be due to the method used. Secondary metabolites are very diverse chemicals derived from primary metabolic products by specific processes. Their role is to protect and defend the plants that contain them against the various external aggressors including micro-organisms, insects and animals. They are very variable from one plant to another and depend on ecologic and climatic ecological conditions [18]. This multitude of compounds in these plants could thus justify their traditional uses.

Thin layer chromatography (TLC): The purpose of Comparative Thin Layer Chromatography (TLC) was to identify the flavonoids and phenolic acids contained in the different methanolic extracts of these plants using reference substances. Figure 2i shows the chromatograms (chromatograms I and II) of methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* eluted by system 1: N-hexane / ethyl acetate / acetic acid system (6 /5 /1; v / v / v) and revealed with NEU reagent at 365 nm and in system 2: ethyl acetate / formic acid / glacial acetic acid / water (10 / 1,1 / 1,1 / 2,6, v / v / v) more polar than system 1 to improve the separation of compounds that have not migrated into the system 1. Several compounds have been well separated in the different extracts of these plants. Comparative TLC of steroids and triterpenes study was to identify steroids and triterpenes in the methanolic extracts of *Bidens engleri, Acanthospermum hispidum* and *Ageratum conyzoides*. The chromatograms obtained are shown in figure 2ii (chromatograms III and IV).

Mainly in the methanolic extract of *Ageratum conyzoides*, the blue spots after 365 nm NEU reagent spray by *comparative TLC for Flavonoids and Phenolic Acids* (figure 2i) can be phenolic acids [11] but could not be identified because they do not correspond to the references of phenol acids used. This is also the case of the yellow spot observed in the methanolic

extract of *Acanthospermum hispidum* which could be a flavonoid but does not correspond to any of the reference compounds used. Moreover, a compound of the same color, with the same frontal reference (Rf) has been identified in the methanolic extract of *Acanthospermum hispidum* as caffeic acid. In the methanol extract of *Bidens engleri*, there is a blue zone inconspicuous at the same level which could also be caffeic acid. This compound having same characteristics as caffeic acid would probably tighten it.

In addition, chromatogram of system 2 observed at 365 nm after NEU reagent spraying identified a compound that could be rutin in the methanolic extracts of *Acanthospermum hispidum* and *Ageratum conyzoides*. Indeed, these compounds observed have the same frontal reference (Rf), the same color (yellow) and absorb at the same wavelength (365 nm) as the rutin used as reference compounds.

In sum, the comparative TLC of flavonoids and phenolic acids allowed us to identify in the methanolic extracts of *Acanthospermum hispidum* and *Ageratum conyzoides* a compound that could be rutin. The second compound also identified in the methanolic extract of *Acanthospermum hispidum* could certainly be caffeic acid. Other flavonoids and phenolic acids have also been separated in the extracts of these plants but are different from the reference compounds used.

By, elsewhere, the chromatograms I and II observed respectively in the visible and 365nm highlight several spots (figure 2ii). These spots observed after Libermann reagent spraying and hot plate heating at 110ºC for 10 minutes are characteristic of steroids and triterpenes. In agreement with the tube tests, these observed brown spots would be steroid or triterpenes according to the method described by Wagner & Bladt [11]. Thus, these compounds could justify the use of these plants in traditional medicine for the treatment of microbial and parasitic diseases. With regard to the chromatograms, these three plants could have in common a steroid or triterpene materialized by spots (first spot of each extract) having the same frontal reference (Rf), the same brown and absorbent color at the same wavelengths. In addition to this compound, *Bidens engleri*, *Acanthospermum hispidum*and *Ageratum conyzoides*have in common another steroid or triterpene (figure 2ii, chromatograms IV). The spots observed on the chromatograms (figure 2ii) show that these three plants have many steroids and triterpenes. However, the β-sistosterol used as a reference was not identified in the methanolic extracts of the three plants.

The presence of the steroids and triterpenes characterized in the different methanolic extracts of the three plants by TLC confirms the results of tube tests where the steroids and triterpenes were identified by the Libermann-Buchard test. Thus, these steroids and triterpenes by their anti-inflammatory [19], spasmolytic, hepatoprotective, diuretic, anti-pruriginous, galactagogue and antimicrobial properties [20] in general could justify the different traditional uses of these plants.

Polyphenolic compounds content: Total phenolic, total flavonoids, total flavonol and total tannin contents are shown in Table 3.

Total phenolic contents were 19.38 ± 0.35 mg GAE / 100 mg extract; 16.75 ± 0.23 mg GAE / 100 mg extract and 10.70 ± 0.30 mg GAE / 100 mg extract respectively for *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides*.

The total flavonoid contents of the methanolic extracts of the different plants were 5.35 ± 0.08 mg QE / 100 mg of extract for *Bidens engleri*, 6.07 ± 0.12 mg QE / 100 mg of extract for *Acanthospermum hispidum* and 5.46 ± 02 mg QE / 100mg for *Ageratum conyzoides*. *Acanthospermum hispidum* contains the highest content of total flavonoids.

Bidens engleri exhibited the highest content of flavonols (1.47 ± 0.08 mg QE / 100 mg).

The tannin contentsare 16.79 ± 0.72 mg TAE / 100 mg extract for *Bidens engleri*, 9.34 ± 0.24 mg TAE / 100 mg for *Acanthospermum hispidum* and 6.22 ± 0.46 mg TAE / 100mg for *Ageratum conyzoides*. It is a very high tannin content in the methanolic extract of *Bidens engleri* compared to the extract of the two other plants.

The results of their Polyphenolic compounds content (table 3) show that *Bidens engleri* contains more tannins than *Acanthospermum hispidum*, which also contains more than *Ageratum conyzoides*. These rather interesting levels of tannin in these plants may justify their traditional uses in regenerating tissues in the event of superficial wounds or burns [21], as anti-diarrheal [7] and against oxidation [22]. Total phenolics, total flavonoids, total flavonols and total tannins contents of the methanolic extracts of these three plants shows that *Bidens engleri* contains more polyphenolic compounds than *Acanthospermum hispidum* which also contains more than *Ageratum conyzoides*. This content of secondary metabolites could thus justify the various medicinal uses of these plants.

Antioxidant activity through inhibition of the DPPH radical: The study of the anti-radical activity by the 2,2-diphenyl-picrylhydrazyl (DPPH) method allow to determine the ability of the methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and

Ageratum conyzoides to scavenge the free radical DPPH. The inhibition percentages were calculated and the values obtained made it possible to plot the inhibition curves as a function of the concentrations of the extracts. Thus, the 50 inhibitory concentrations (IC_{50}) of methanolic extracts of these plants are shown in Figure 3. In fact, These IC_{50} are 14.97 ± 0.06 µg / mL; 28.17 ± 0.55 µg / mL and 32.07 ± 0.96 µg / mL for *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides*, respectively.

In the reaction medium, this method is based on the reduction of the absorbance at 517 nm when the stable free radical 2,2-diphenyl-picrylhydrazyl (DPPH) reacts with an antiradical [23]. *Bidens engleri* therefore presented the best anti-radical activity followed by *Acanthospermum hispidum*. However, these activities remain below those of standards quercetin and gallic acid. The *Ageratum conyzoides* extract exhibited the lowest anti-radical activity. The ability of a system to trap free DPPH radicals reflects its ability to inhibit lipid peroxidation [24]. Extracts of these plants, particularly from *Bidens engleri*, could be used to trap free radicals in the body and thus inhibit the peroxidation of cell membrane lipids.

Free radicals being responsible for many pathologies, the anti-radical activity of these methanolic extracts, especially of *Bidens engleri*, could indeed justify their traditional use in the treatment of smallpox, wounds, diabetes and liver diseases. Characterized in the methanolic extracts of these plants, the antioxidant potentialities of coumarins [25,26], of Flavonoids [27,28] have been demonstrated.

Anti-inflammatory activity by inhibition of lipoxygenase: The inhibitory powers of methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* on lipoxygenase, an enzyme that controls the formation of leukotrienes, mediators of inflammation were assessed. The percentages of lipoxygenase inhibition obtained (Figure 4) are 70.59 ± 4.16; 49.02 ± 1.7 and 57.84 ± 1.7 respectively for *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* at the concentration of 100 µg/mL (final concentration).

Good inhibition of lipoxygenase was obtained by the methanolic extract of *Bidens engleri*, a moderate inhibition by the methanolic extract of *Ageratum conyzoides* and a low inhibition by those of *Acanthospermum hispidum*. *Bidens engleri* extract is much more active on lipoxygenase inhibition compared to the other two plant extracts at the concentrations used. In view of the percentages of inhibition of quercetin and Gallic acid tested, it is clear that standards quercetin and Gallic acid inhibit better lipoxygenase that our extract. The inhibition of the enzyme by the extracts observed in each case is explained by its ability to occupy the enzymatic sites thus preventing the attachment of the substrate. Lipoxygenase is a metalloenzyme that catalyzes the formation of leukotrienes, which are potent mediators involved in specific inflammatory processes (psoriasis) or allergic reactions by regulation of Langerhans cells [29]. The use of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* would reduce the production of leukotrienes and thus stop inflammation.

Recent studies have highlighted an interaction between lipoxygenase and some diseases such as cancer, heart disease or asthma. Leukotrienes have physiological roles in innate immune responses and pathological roles in inflammatory diseases, such as asthma, allergic rhinitis and atherosclerosis [30]. Anti-leukotriene therapy has proven benefits in the treatment of respiratory disease [30]. The use of natural inhibitors of this enzyme such as extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides* may help to reduce these pathologies associated with inflammation. Indeed, the caffeic acid identified in the mehanolic extract of *Acantospermumhispidum* and *Ageratum conyzoides*is endowed with anti-inflammatory properties [31].

Moreover, rutin identified in the methanol extract of these plants is an inhibitor of the production of prostaglandin [32] by acting on cyclooxygenase responsible for the synthesis of this hormone. In addition, the actions of rutin on the activity of lipoxygenase and the generation of inflammation in human neutrophils have been demonstrated [33].

Table 1: Frequencies, forms of use and diseases treated by plants of the family Asteraceae reported by the ethnobotanical survey.

Botanicalname	Local Name(Mooré)	Ethnomedicinal uses	Parts used / Mode of preparation	Frequencies of use (%)
***Bidens engleri* O. E. SCHULZ**	Kinkirs-sabtulga	Malaria, Jaundice	Whole plant / drink	**96,57**
		Diarrhea	Whole plant / drink	**93,11**
		Smallpox	Stem with leaves / staggering	**88,36**
		Diabetes	Whole plant / drink	**77,34**
		Angina	Whole plant / drink	**75,91**
		The stomach ache	Stem with leaves / spreading	**73,33**
		Cramps, Sprains	Whole plant / friction	**69,44**
		Bronchitides	Whole plant / drink	**49,74**
		Dysentery	Whole plant / drink	**43,29**
		Hernia, prostate	Whole plant / drink	**11,22**
		epilepsy	Whole plant associated with other plants / drink	**11,22**
		No use of the plant		**00**
***Acanthospermum hispidum*DC.**	Nansar-kurkur-gonse or Nansar-kurkurgouanga	Malaria	Whole plant / drink	**97,15**
		Hemorrhoids, diarrhea	Whole plant / decoction /anal way	**95,88**
		vomiting	Whole plant / drink	**92,06**
		The stomach ache	Whole plant / drink	**91,71**
		Jaundice, Angina	Whole plant / drink	**87,19**
		Edema, wounds	Dough of leaves of the plant / spreading	**84,42**
		teething	Leafy stems of plant crushed and infused / anal way	**32,22**
		Facilitates child birth	Leaf stem / decoction /anal way	**20,77**
		scorpion stings	Leaf paste / spreading	**8,69**
		No use of the plant		**00**
***Ageratum conyzoides* L.**	Tougour-taaba	Malaria, Jaundice	Whole plant / drink	**95,35**
		Diarrhea, hot body	Leaf stem / decoction / bath / anal way	**90,57**
		Liver disease, hiccups	Whole plant / drink	**72,85**
		Swelling of the body	Leaf paste / spreading	**48,53**
		snake bite	Leaf paste / spreading ; decoction / drink	**9,79**
		No use of the plant		**00**
Tridax procumbens L.	Baag-yowi	high blood pressure	Whole plant / drink	**26,66**
		icterus	Whole plant / drink	**21,11**
		hepatitis	Whole plant / drink	**18,22**
		Female sterility	Whole plant / drink	**9,77**
		diuretic	Whole plant / drink	**27,65**
		Sores	Stem with leaves / spreading	**23,33**

		Against the dartre	Fresh leaf / friction	**5,12**
		edemas	Whole plant /staggering	**14,42**
		No use of the plant		**89,55**
		Sores	Whole plant + lemon / drink	**31,11**
		Heartache and liver	The whole plant / incineration then suction	**8,83**
***Laggera aurita* (Lf) DC.**	Kater-tabré	The stomach ache	Whole plant / drink	**35,65**
		Vaginal diseases and STDs	The whole plant / friction	**19,11**
		hemorrhage	Whole plant / drink	**21,44**
		Edemas, Wounds	Powdered whole plant + shea butter / spread	**17,77**
		ulcers	Whole plant / drink	**55,55**
		No use of the plant		**43,21**
		Analgesic	Whole plant / drink	**27,14**
		Againstbacteria	Whole plant / drink	**28,57**
Vernonia galamensis	Kinkiris-siili	Dartre	The whole plant / drink; Leaves paste / friction	**12,85**
		Syphilis	Whole plant / drink	**28,57**
		Against mycosis	Whole plant / drink	**6,57**
		No use of the plant		**58,44**

Table 2 : Results of tube tests

Desired metabolites	*B. engleri*	*A. hispidum*	*A. conyzoides*
Flavonoids	+	+	+
Tannins	+	+	+
Saponosides	+	+	+
Anthracenosides	-	-	-
Coumarins	+	+	+
Sterols/Triterpenes	+	+	+
Alkaloids	-	-	-

(+) = Positive test (-) = Negative test

Table 3: Total Phenolic, flavonoids, Flavonol and Tannin content of *B. engleri*, *A. hispidum* and *A. conyzoides*.

Extract	Total Phenolic (mgEAG/100mg)	Total flavonoids (mgEQ/100mg)	Flavonols (mgEQ/100mg)	Tannins (mgEAT/100mg)
B.engleri	19.38 ± 0.35	5.35 ± 0.08	1.47 ± 0.07	16.79 ± 0.72
A.hispidum	16.75 ± 0.23	6.07 ± 0.12	1.01 ± 0.03	9.34 ± 0.24
A.conyzoides	10.7 ± 0.30	5.46 ± 0.02	1.13 ± 0.04	6.22 ± 0.46

Values are expressed as mean values ± standards deviation ; n = 3 independent experiments in quadruplicate ; GAE : Gallic Acid Equivalent ; QE : Quercetin Equivalents ; QE : Quercetin Equivalents ; TAE : Tanic Acid Equivalent.

Figure 1: Histochemical sections showing tissue structures and different secondary metabolites in Bidens engleri, Acanthospermum hispidum and Argeratum conyzoides.

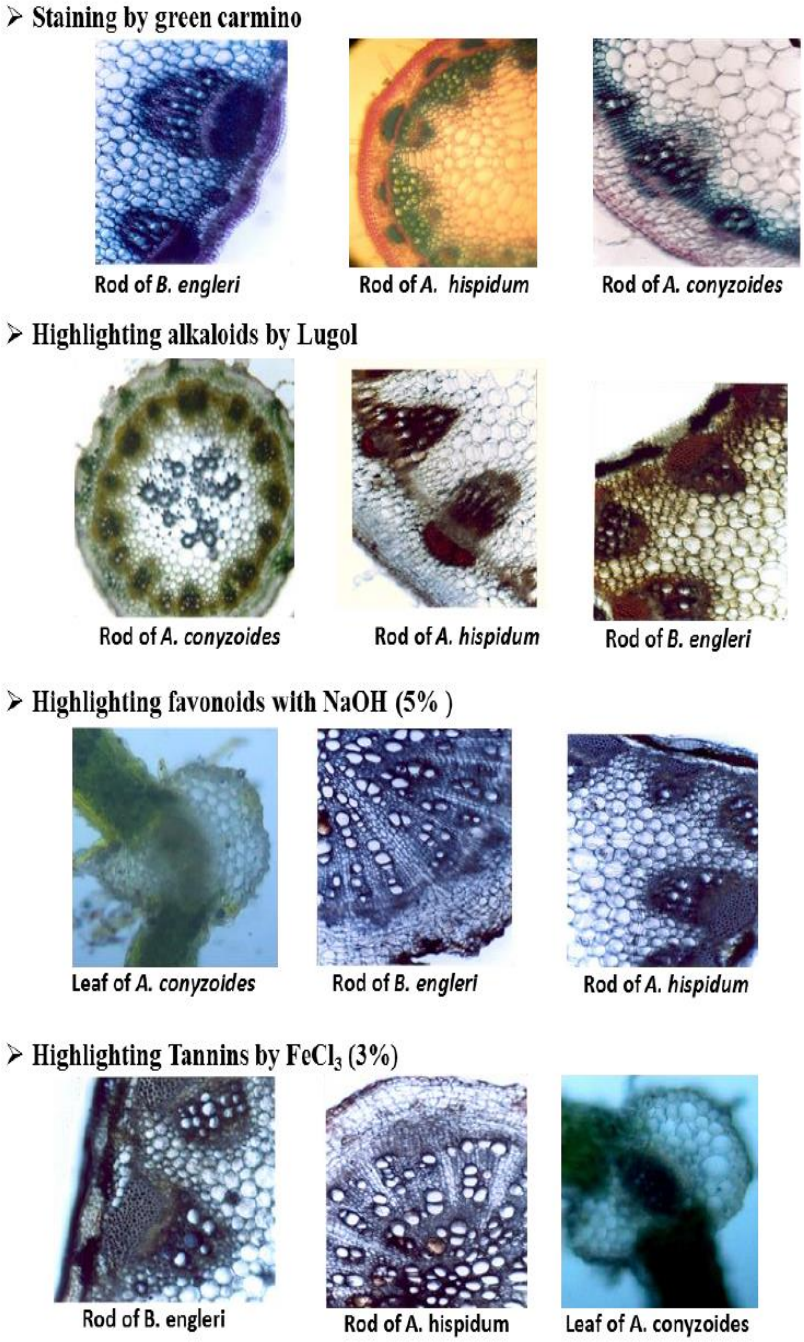


Figure 2: Thin layer chromatography (TLC) of methanolic extracts of the three plants

i: TLC for the detection of flavonoids and phenolic acids revealed at 365 nm. **A** = *B. engleri*; **B** = Quercetin; **C** = *A. hispidum*; **D** = Caffeic acid, **E** = *A. conyzoides*; **F** = Rutine; System 1: (N-hexane / ethyl acetate / acetic acid (6/5/1; v / v / v)); System 2: ethyl acetate / formic acid / glacial acetic acid / water (10 / 1.1 / 1.1 / 2.6: v / v / v / v).

ii: TLC for revealing steroids and triterpenes revealed Libermann reagent observed in the visible (I) and 365nm (II). **A** = *B. engleri*; **C** = *A. hispidum*; **E** = *A. conyzoides* and **G** = β-sistosterol; System 3: Ethyl acetate / formic acid / glacial acetic acid (7 / 1.1 / 1.1 v / v / v).

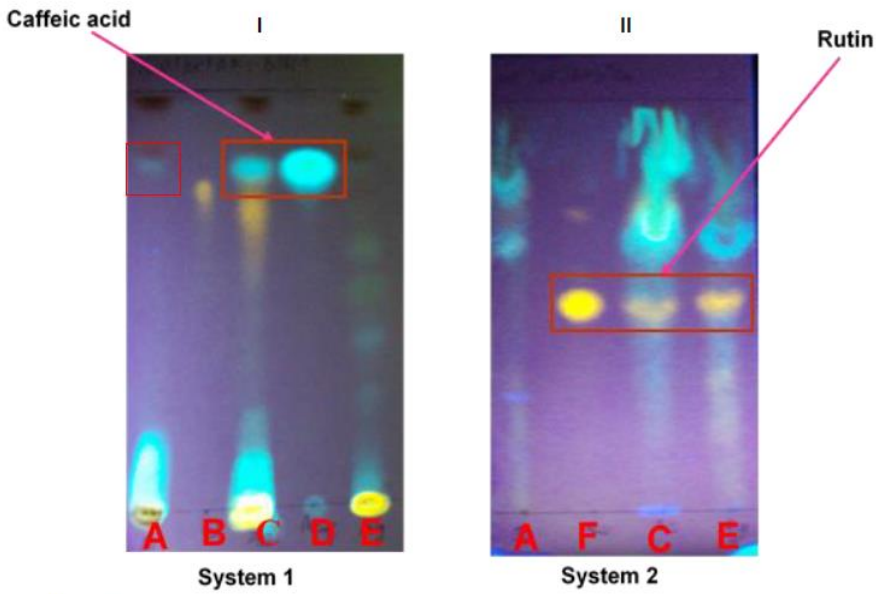


Figure 2i

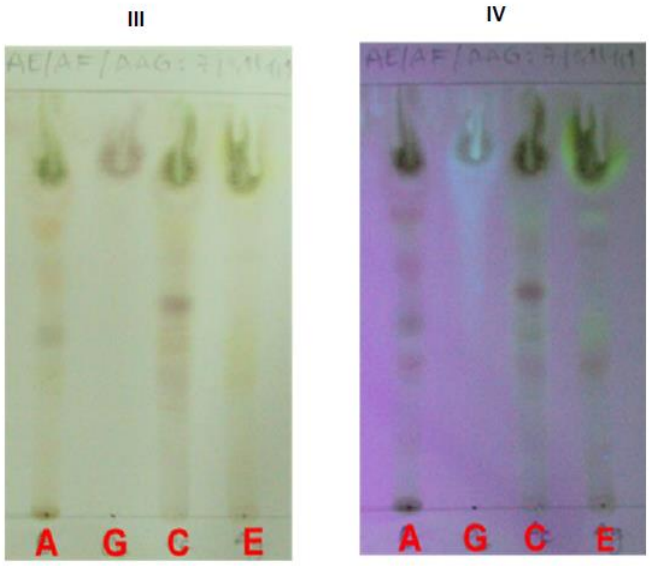


Figure 2ii

Figure 3: IC_{50} (µg / mL) of methanolic extracts of *Bidens engleri*, *Acanthospermum hispidum* and *Ageratum conyzoides*. DPPH, (2,2-diphenyl-1-picrylhydrazyl); n = 3 independent experiments in quadruplicate for the measurement; IC_{50} of antiradical activities of extracts are expressed in µg/mL; IC_{50}, Inhibitory Concentration 50; *, **, ***, ****; $p < 0.05$ from lowest to highest activity and significantly different compared.

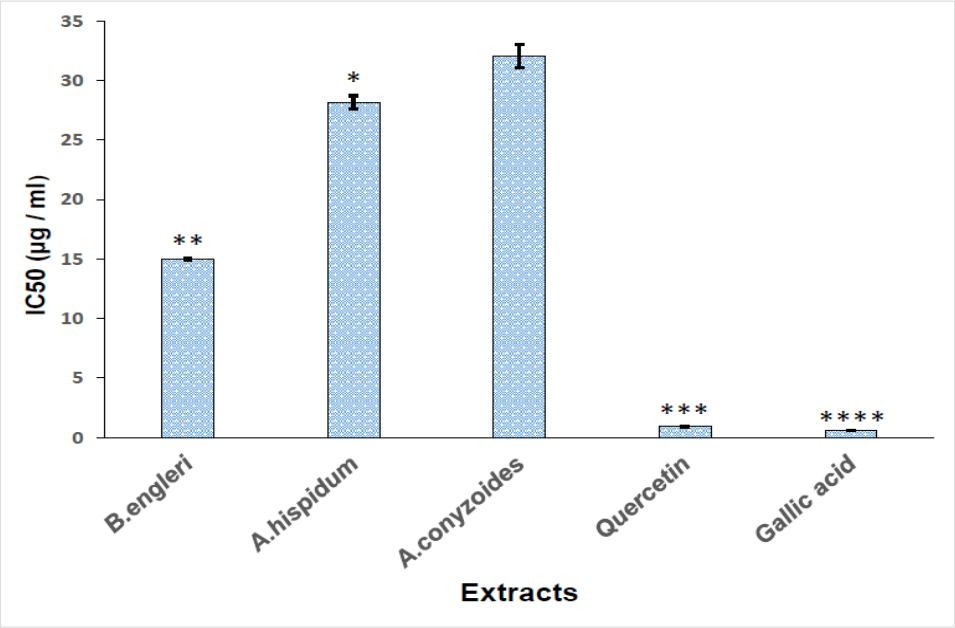
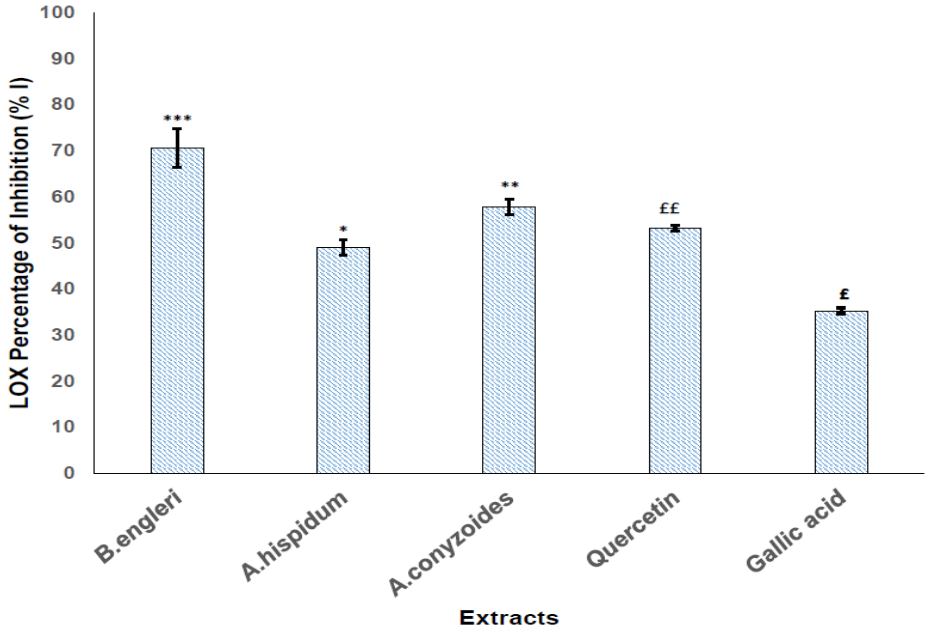


Figure 4: Percent inhibition (% I) of the lipoxygenase by methanolic extracts of three plants.

n = 3 independent experiments in quadruplicate for the measurement of anti-inflammatory activity by lipoxygenase inhibition, expressed as inhibitory percentage, extracts were used at 100 µg / mL; standard Quercetin and Gallic acidwere used at 25 µg / mL. About extract; *, p <0.05 differ significantly compared to *Bidens engleri*; **, p < 0.05 differ significantly compared to *Acanthospermum hispidum*.About standars; £, p < 0.05 differ significantly compared to Quercetin.



4. CONCLUSION

Several phytochemical groups of compounds have been characterized in the methanolic extracts of these three plants. Caffeic acid was found in the methanolic extract of *Acanthospermum hispidum* and *Bidens engleri*. Rutin was identified in the extracts of *Acanthospermum hispidum* and *Ageratum conyzoides.*

The methanolic extract *of Bidens engleri* had the highest contents of total phenolics, flavonols and

total tannins. Only the total flavonoids content is higher in *Acanthospermum hispidum* extract. The methanolic extract of *Bidens engleri* exhibited the best antioxidant but also the best inhibitory activity of lipoxygenase compared to the extracts of the two others plants. This work will contribute to a better knowledge of the phytochemical composition but also of antioxidant and anti-inflammatory activities of these three species of aromatic asteraceae. Moreover, these scientific results could justify the various traditional uses of these plants in Burkina Faso in the treatment of oxidative, inflammatory, microbial and parasitic diseases.

COMPETING INTERESTS

The authors declare no conflict of interests.

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