



Phytochemical Screening and *in Vitro* Anti-Inflammatory Activity Evaluation of the Combined Methanol Leaf Extracts of *Ageratum conyzoides* and *Cytratus cymbopogon* with a Trial Formulation of a Pharmaceutical Suppository

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Abstract

From Literature review 50,000 plant species worldwide have successfully been used for medicinal purposes and amongst these, almost 13% are flowering plants. The practice is now increasing due to increased global health challenges. In Cameroon, the use of plants as a source of treatment for malaria, typhoid, pain, infections and many other diseases is very current. Besides the Asteraceae family, we have other families such as the Poaceae or the Gramineae found in Cameroon which was localised in the Maroua locality where the following species was identified *i.e.* (*Cymbopogon citratus*, *Pennisetum glaucum*, *Zea mays* and *Sorgum bicolor*), *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, anti-filarial, anti-fungal and anti-inflammatory properties amongst others. Meanwhile, *Ageratum conyzoides* in Cameroon is a local remedy for skin diseases and wound healing, also the leaves when crushed in water are given as an emetic and are also applied intra vaginally for uterine complications and are used in the treat-

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ment of pneumonia. The voucher specimens of *Ageratum conyzoides* and *Cymbopogon citratus* were harvested in the (Camrail camp of “cite de la paix”) in the month of November 2020 at 10:20 a.m. Plant stems were then immediately separated from the leaves and other parts after harvesting to optimize the drying process. There after standard procedures for pre-treatment of plant materials were followed. After which the extracts were filtered using a 0.45 millipore whatmann filter paper associated with cotton wool. Then, the extracts were concentrated using a rotary evaporator at 60°C with 102 rotations per minute with percentage yields of 19.13% for *Ageratum cornyzoides* and 8.83% for *Cymbopogon citratus*. Phytochemical examinations of the stem extracts were carried out by applying the standard methods. Hence, the presence or absence of various phyto-constituents was determined. The anti-inflammatory activity of the combined extract was studied by using inhibition of albumin denaturation technique with a few modifications at the level of concentration of the solutions. Six suppositories were formulated for each of the extracts. Phytochemical screening revealed the presence of alkaloids, phenols tannins and saponins in methanol extracts of *Ageratum cornyzoides* and *Cymbopogon citratus*. The efficiency of the combined extract increase significantly with an increase in concentration but was less efficient and potent than Aspirin. This is also confirmed by the IC₅₀ value of aspirin (IC₅₀ 350.1 g/ml) which is greater than that of the combined extract (IC₅₀ 292.7 g/ml). The low efficiency and potency of the combined extract is probably due to steric hindrance which prevents the pharmacophore group from acting. The dose-dependent efficiency could be due to polyphenols. Studies also done on terpenoids have been reported to have anti-inflammatory properties. Thus the anti-inflammatory properties could either be due to the presence of polyphenols or terpenoids or both.

Subject Areas

Phytotherapy, Physiology, Galenic Pharmacy

Keywords

Ageratum cornyzoides, *Cymbopogon citratus*, Anti-Inflammatory, Polyphenols, Pharmacophore, Suppositories

1. Introduction

The history of plant's use for mankind is as old as the start of the human race. Initially, people used plants for their nutritional purposes but after the discovery of medicinal properties, this natural flora became a useful source of disease treatment and health improvement across various human communities [1]. From Literature review 50,000 plant species worldwide have successfully been used for medicinal purposes and amongst these, almost 13% are flowering plants [2]. Egyptian papyruses showed that coriander and castor oil were useful for medicinal applica-

tions, cosmetics and preservatives through thousands of recipes [3]. The practice is now increasing due to increased global health challenges [1]. Because of the accelerated international, national and local interest in recent years, the demand for medicinal and aromatic plants have increased manifold and the pharmaceutical industries view plant wealth as a source of income [2]. In Cameroon, the use of plants as a source of treatment for malaria, typhoid, pain, infections and many other diseases is very current. Besides the Asteraceae family, we have other families such as the Poaceae or the Gramineae found in Cameroon which was localised in the Maroua locality where the following species was identified *i.e.* (*Cymbopogon citratus*, *Pennisetum glaucum*, *Zea mays* and *Sorghum bicolor*) [4], where by *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, anti-filarial, antifungal and anti-inflammatory properties with various other effects like antimalarial, anti-mutagenic, anti-mycobacterial, antioxidants, hypoglycaemic and neurobehavioral properties [5]. Whereas, *Ageratum conyzoides* has a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. Like the case of Cameroon. In Cameroon, it is a local remedy for skin diseases and wound healing, also the leaves when crushed in water are given as an emetic and are also applied intra vaginally for uterine complications and are used in the treatment of pneumonia [6].

2. Material and Methods

Materials used were obtained from the laboratory of the research institution and the characterization of study was limited to the type of activities carried out.

2.1. Characterisation of Study

This was an experimental study that took place in the following laboratories; the chemistry laboratory of natural substances in the Faculty of Medicine and Pharmaceutical sciences of the University of Douala (FMPS); for the assistance in concentration of the plant extract: the pharmacy technology laboratory of the University Institute of the Gulf of Guinea (IUG); for the phytochemical screening and anti-inflammatory activity.

2.2. Materials

2.2.1. Material for Phytochemical Screening

Test tubes for holding solutions; Plant extracts; Reactive (methanol, acetic anhydride, acetone, wagner reagents, iron (III) chloride 2% and 0.1%, magnesium fillings, copper (II) solution, sulphuric acid and hydrochloric acid at 25%), dragendorff reagent, chloroform, naphthol, distilled water.

2.2.2. Material for Anti-Inflammatory Activity

Glass tubes; Albumin (egg white); Phosphate Buffer Solution (Psb); Distilled water; Aspirin tablet; Micropipette; UV-vis Spectrophotometer; Round bottom flask (100 mL).

2.2.3. Material for Suppository Formulation

Suppository moulds; Excipient e.g. (cocoa butter); Spatula; Electronic balance; Heating mantle/water bath; beaker; Round bottom flask; Sheet of paper; Refrigerator; Aluminum foil.

3. Methods

3.1. Preparation of Plant Extracts

The preparation of the plant extracts commenced from the harvesting procedure onto obtaining the methanol extracts of the separate samples.

3.1.1. Harvesting and Treatment of Voucher Specimen

The voucher specimens of *Ageratum conyzoides* and *Cymbopogon citratus* were harvested in the (Camrail camp of “cite de la paix”) in the month of November 2020 at 10:20 a.m. Plant stems were then immediately separated from the leaves and other parts after harvesting to optimize the drying process. Thereafter standard procedures for pre-treatment of plant materials were followed. The basic steps include cleaning, air drying under shade at room temperature, grinding into fibers and storage in an airtight container at appropriate temperature (room or refrigerated) [7].

3.1.2. Extraction

After preparing the plant material and transforming it into fibres using a craft mill, leaves of *Ageratum conyzoides* and *Cymbopogon citratus* were blended and mixed with 2.5 L of methanol each, for a period of 72 hours at room temperature following an extraction process called maceration which is suitable for preserving thermo-labile compounds. Occasional stirring was done twice a day within the 72 hours to facilitate extraction by increasing diffusion, and removing concentrated solution from the sample surface while bringing new solvent to the menstruum for more extraction yield [8]. The extracts were then filtered using a 0.45 millipore whatmann filter paper associated with cotton wool. Then after, the extracts were concentrated using a rotary evaporator at 60 °C and 102 rotations per minute.

$$\text{Percentage yield (\%)} = \frac{\text{Mass of dried extract}}{\text{Mass of total plant fibres}} \times 100 \quad (1)$$

3.2. Phytochemical Screening

Phytochemical examinations of the stem extracts were carried out applying the standard methods. Hence, the presence or absence of various phyto-constituents was determined [9].

- ❖ Detection of alkaloids: Wagner’s Test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.
- ❖ Detection of phenols: Ferric Chloride Test: Extracts were treated with 3 - 4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

- ❖ Detection of tannins: Ferric Chloride Test: To the alcoholic extract a few drops of 1% neutral ferric chloride solution was added, formation of blue, green or brownish green color indicated the presence of Tannins.
- ❖ Detection of flavonoids: Shinoda Test: To the alcoholic solution of alcoholic extract, a few fragments of magnesium ribbon and concentrated hydrochloric acid were added. The appearance of magenta colour after a few minutes indicates the presence of flavonoids.
- ❖ Test for saponins: Foam Test: Small amount of alcoholic extract was shaken with little quantity of water, if the foam produced persisted for 10 minutes; it indicated the presence of saponins [10].
- ❖ Detection of terpenoids: Salkowski test: The extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive results of the presence of terpenoids [11].
- ❖ Detection of sugars: Molisch test: Added 2 - 3 drops of alpha-naphthol reagent was added to 2 ml of extract in the test tube. The test tube was inclined very gently and a few drops of concentrated sulphuric acid along the side of the test tube was added. A violet colour ring indicated the presence of carbohydrates in the solution.

3.3. *In Vitro* Anti-Inflammatory Activity

Laboratory assays to assess the ability of extracts to inhibit inflammatory processes were carried out using the protein denaturation method from egg white.

3.3.1. Inhibition of Albumin Denaturation Assay

The anti-inflammatory activity of the combined extract was studied by using inhibition of albumin denaturation technique done according to the method described by Mizushima *et al.*, with some few modifications at the level of concentration of the solutions [12].

❖ PREPARATION OF COMBINED EXTRACT (*AGERATUM CORNIZOIDES PLUS CYMBOPOGON CITRATUS*) AND ASPIRIN SOLUTION

Extracts of *Ageratum cornizoides*, *Cymbopogon citratus* and aspirin were weighed to get the same mass of 0.0096 g; with *Ageratum cornyzoides* and *Cymbopogon citratus* constituting each half of the desired weight for the mass of the extract. The mass of extract was dissolved in few drops of dimethylsulfoxide (DMSO) to have a uniform distribution; equal masses of dissolved extracts and aspirin was diluted in 12 mL of distilled water to have the highest concentration of 800 µg/mL; a serial dilution was made to obtain solutions of decreasing concentration.

❖ PROCEDURE OF ANTI-INFLAMMATORY ACTIVITY

A reaction mixture (5 mL) consisting of 0.2 mL of egg albumin (from fresh hen's egg); 2.8 mL of PBS (pH 6.4); 2 mL of varying concentrations of the combined extracts (50, 100, 200, 400 and 800 µg/mL) was incubated at 37°C in a biochemical oxygen demand for 15 minutes and then heated at 70°C for 5 minutes.

A similar volume (2 mL) of distilled water served as control; after cooling, their absorbance was measured at 660 nm using the vehicle as blank; aspirin was used as a reference drug and treated similarly for determination of absorbance [13]. The percentage Inhibition of denatured proteins was calculated using the formula:

$$\text{Denaturation \% inhibition} = (V_t/V_c - 1) \times 100\% \quad (2)$$

where:

V_t: The sample absorbance value;

V_c: The distilled water used as negative control.

The IC₅₀ value was determined from the dose response curve by drawing the percentage inhibition values against the varying concentrations.

3.3.2. Formulation of Suppository

Before formulating suppositories with the dried extracts, we proceeded with just the excipients in order to calculate the displacement factor of the dried extracts that served as principal active substance. After this, size separation was performed for size uniformity assurance of the dried extracts, then we progressively added 1/3 of the excipient and then triturated to obtain a homogeneous mixture. The remaining excipient was placed in a beaker then heated using a heat source at a temperature not less than 45°C. After the fusion of the excipient, we then added the content into the mortar and the mass was gently stirred (constantly) to homogenise the mixture before filling the wells of the molds [14].

4. Results and Discussion

4.1. Results of Percentage Yield of Methanol Extracts of Both Plants

Data showing the amount of extract obtained relative to the initial plant material weight.

Amount of water lost for each plant during drying.

Ageratum conyzoides:

Fresh *Ageratum* mass = 300 g; Dried *Ageratum* mass = 60 g; Blended dried *Ageratum* mass = 56 g; Dried methanolic extract of *Ageratum* yield mass = 29.75 g; Amount of water lost in *Ageratum* = (fresh *Ageratum* mass – dried *Ageratum* mass) 300 g – 60 g = 240 g.

Cymbopogon citratus:

Fresh *Cymbopogon* mass = 655 g; Dried *Cymbopogon* mass = 170 g; Blended dried *Cymbopogon* mass = 174 g; Dried methanolic extract of *Cymbopogon* yield mass = 15.45 g; Amount of water lost in *Cymbopogon* = (fresh *Cymbopogon* mass – dried *Cymbopogon* mass) 655 g – 170 g = 475 g;

$$\text{Percentage water loss for each plant} = \frac{\text{Fresh plant}}{\text{Dry plant}} \times 100 \quad (3)$$

$$\textit{Ageratum conyzoides}: \frac{60 \text{ g}}{300 \text{ g}} \times 100 = 20\%$$

$$\text{Cymbopogon citratus: } \frac{170 \text{ g}}{655 \text{ g}} \times 100 = 25.95\%$$

Percentage yield was calculated as from formula 1.

$$\text{Ageratum conyzoides: } \frac{10.71 \text{ g}}{56 \text{ g}} \times 100 = 19.13\%$$

$$\text{Cymbopogon citratus: } \frac{15.45 \text{ g}}{174 \text{ g}} \times 100 = 8.83\%$$

4.2. Results of Phytochemical Screening

Findings indicating which phytochemicals are present in the extracts, supporting their medicinal potential are as follows (see **Table 1**).

Table 1. Results obtained from the phytochemical screening of both plants done separately.

	solvent of extraction	alkaloids	phenols	tannins	flavonoids	saponins	terpenoids
<i>Ageratum cornizoide</i>	methanol	+	+	+	+/-	-	+
	hexane	+	+	-	-	-	+
	aqueous	+	+	+	-	+	+
<i>Cymbopogon citratus</i>	methanol	+	-	+	-	+	-
	hexane	+	-	-	+	+	+
	aqueous	-	+	+	+	-	-

+ = positive; - = negative; +/- = colouration expected was not attained but a colour change was observed.

4.3. Results of Anti-Inflammatory Activity of Aspirin and Extracts

Data demonstrating the extracts' ability to reduce inflammation in vitro, compared to controls are presented in **Table 2** and **Figures 1-3** below.

Table 2. Tabulated representation of the percentage inhibition of the control sample (Aspirin) alongside the inhibitory concentrations of the combined extracts with each concentration respected as shown on the table.

Concentration (µg/ml)	Concentration of <i>Cymbopogon citratus</i> (µg/ml)	Concentrations of <i>Ageratum conyzoides</i> (µg/ml)	% Inhibition of Aspirin	% Inhibition of the combined concentrations
800	400	400	103.9495	84.81272
400	200	200	91.18485	64.02685
200	100	100	85.38274	23.21580
100	50	50	82.45114	13.23249
50	25	25	79.27524	6.799593

The curves in **Figure 1** and **Figure 2** show the inhibition of denatured heat-induced proteins by aspirin and combined extract of *Cymbopogon citratus* and *Ageratum conyzoides* respectively. The comparison curve in **Figure 3** reveals that the efficiency of the combined extract increases significantly with an increase

in concentration but was less efficient and potent than Aspirin. This is also confirmed by the IC_{50} value of aspirin (IC_{50} 350.1 g/ml) which is greater than that of the combined extract (IC_{50} 292.7 g/ml).

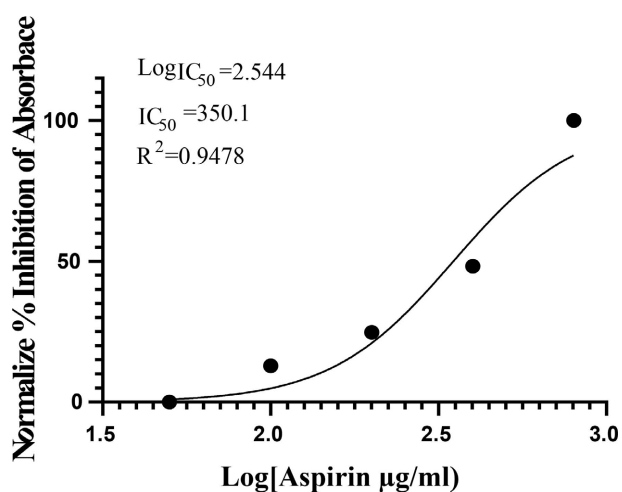


Figure 1. Normalized percentage inhibitions of absorbance of Aspirin with variation in logarithmic concentrations.

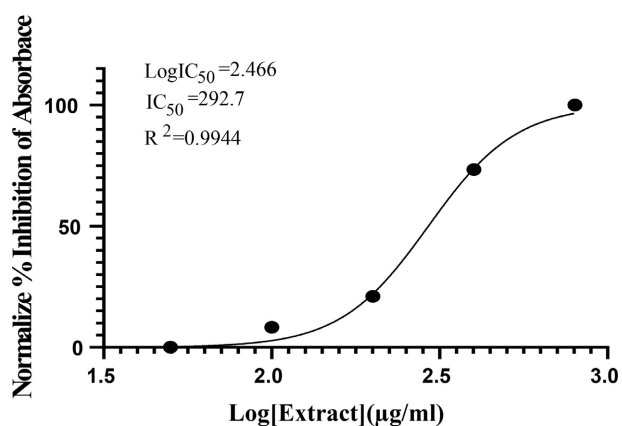


Figure 2. Normalized percentage inhibitions of absorbance of combined extracts with variation in logarithmic concentrations.

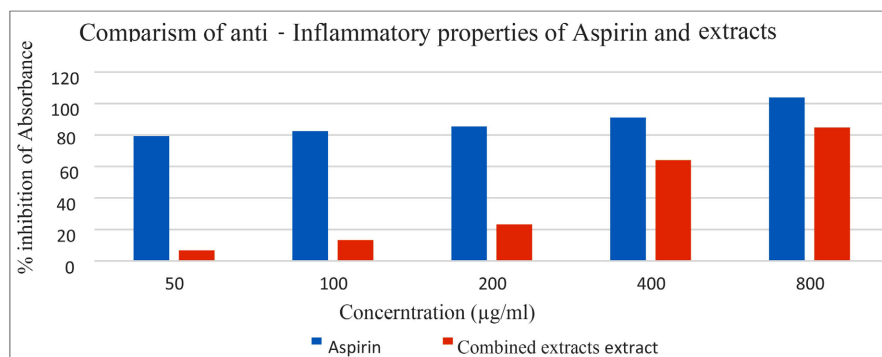


Figure 3. Comparative percentage inhibitions of absorbance of combined extracts and Aspirin with variations of concentrations.

The low efficiency and potency of the combined extract is probably due to steric hindrance which prevents the pharmacophore group from acting [15]. The dose-dependent efficiency could be due to polyphenols [16]. Studies also done on terpenoids have been reported to have anti-inflammatory properties [17]. Thus the anti-inflammatory properties could either be due to the presence of polyphenols and terpenoids.

According to literature, one of the characteristics of several Non-Steroidal Anti-Inflammatory Drugs NSAIDs is their ability to prevent denaturation of heat-treated albumin at physiological pH [18]. These results can also be supported with those of Mizushima *et al.* and Sakat *et al.* which reported valuable anti-inflammatory activity of *Enicostemma axillare* using inhibition of albumin denaturation technique [19].

4.4. Results of Suppository Formulation from Plant Extract

Outcomes related to the successful preparation of the plant-based suppositories are presented in the following images for each extract obtained (see **Figures 4-9**).



Figure 4. Upper view of frozen suppository of plant extracts of *Ageratum conyzoides* and *Cymbopogon citratus*.



Figure 5. Lower view of frozen suppository of plant extracts of *Ageratum conyzoides* and *Cymbopogon citratus*.



Figure 6. Foiled frozen suppository of *Cymbopogon citratus* extract.



Figure 7. Frozen suppository of *Cymbopogon citratus* extract.



Figure 8. Foiled frozen suppository of *Ageratum conyzoides*.



Figure 9. Frozen suppository of *Ageratum conyzoides*.

5. Discussion

Ageratum conyzoides (extract):

We obtained a mass of 300 g of fresh plant which gave a mass of 29.75 g after extraction and concentration, these result in contrast to those published by [14] notably 250 g of fresh plant and 8 g of extract after concentration were slightly different. Thus the difference in mass of extract after concentration could be explained by the difficulties in obtaining a completely dried mass due to the difference in climatic conditions. We however, obtained persistent oily extract after prolonged drying under normal atmospheric conditions in the absence of a controllable temperature oven (60°C).

Cymbopogon citratus (extract):

Obtaining our powder leaves we got 174 g which gave a mass of 15.45 g crude powder extract after extraction, concentration and drying (percentage yield of 8.88%), this result in contrast to Reddy *et al.* 2016 notably 678 g of powdered leaves and 15.9 g of the powdered extract after concentration and drying (percent-

age yield of 2.35%) [2]. Thus the difference in the percentage yield could be explained by the presence of some systematic errors in the whole process of extraction as well as the difference in the climatic conditions.

Phytochemical screening; In our identification of the different phytochemical constituents in the methanolic dried leaf extract of *Ageratum conyzoides* we could find alkaloids, tannins, flavonoids and the absence of saponins in our extract, we were also able to find in the methanolic dried extract of *Cymbopogon citratus*, alkaloid, tannins, saponins, and the absence of phenols, flavonoids, and terpenoids.

6. Conclusions

We obtained upon extraction a yield of 8.88% for *Cymbopogon citratus* and 53.13% for *Ageratum conyzoides*. After which the phytochemical screening done revealed the presence of Alkaloids, phenols, tanins, saponins, flavonoids and terpenoids in the various methanolic, hexanic and aqueous extracts of *Ageratum conyzoides* and the presence of Alkaloids, tanins, saponins flavonoids phenols and terpenoids in the various methanolic, hexanic and aqueous extracts of *Cymbopogon citratus*.

The formulation of our suppository was done with respect to the normal standards as prescribed by literature review and thus we obtained a unit suppository mass of 2.1 g for *Ageratum conyzoides* and 1.008 g for *Cymbopogon citratus*. These results confirmed not only the possibility of formulating a suppository from these plant extracts that can be used in traditionally ameliorated drugs but also confirmed their anti-inflammatory activity which is slightly matched to that of acetylsalicylic acid (Aspirin).

Acknowledgements

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Data Availability

All data generated or analyzed during this study was included in this Original Research Article.

Authors' Contributions

Tchakouteu Sadjeu Sidoine, Kang Costly Eha-Kang, Takougang Nguondjou Teclaire and Siewe François designed and carried out the study, Siewe François and Kang Costly Eha-Kang, wrote the Original Research Article.

Conflicts of Interest

The authors declare no conflicts of interest.

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Mixed extract samples at different concentrations



Different concentration of the control sample (diclofenac sodium 75 mg)



Figure A1. Phytochemical screening of the methanolic extract of *Cymbopogon citratus* test before.



Figure A2. Phytochemical screening of methanolic extract of *Cymbopogon citratus* test after.



Figure A3. Phytochemical screening of the methanolic extract of *Ageratum conyzoid* test before.



Figure A4. Phytochemical screening of the methanolic extract of *Ageratum conyzoid* test after.