

June 30, 2021

Ethnopharmacological Study of Anti-Inflammatory Plants from the Markets of Man, Côte d'Ivoire

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Received Date: 07-June-2021 Accepted Date: 25-June-2021 Published Date: 30-June-2021

Abstract

In the search for plants that can fight against inflammatory; we have launched an ethnopharmacological survey on the markets of Man city in Côte d'Ivoire. Three markets in the city were visited for this purpose: big market, market of Gbapleu and that of Zélé. This choice is justified by an impressive number of medicinal plants sellers in these markets. The survey interviewed 73 herbalists based on an questionnaire sheet. These investigations revealed 20 species of plant used in traditional medicine, in the treatment of inflammation. They are administrated by decoction-way as a drink and by anal route. In comparison with the calculated frequency of citations, two plants specie look important. They are: Alchornea cordifolia (Fc = 7.49%) and Rauwolfia vomitoria (Fc = 8.01%). All vendors of medicinal plants specie know them. In addition, a phytochemical screening was performed to assess the scientific basis of the empirical use of these two most common plants. The tests revealed that these plants contain sterols, polyterpenes, polyphenols, flavonoids, saponosides and alkaloids. So, maybe anti-inflammatory effect could be linkable to the presence of these chemical groups. Then, these two plants specie could be of scientific interest for the fight against inflammatory diseases.
Keywords: Plants, Ethnopharmacology, Inflammation, Man, Côte d'Ivoire

1. INTRODUCTION

For centuries, humans have used plants (Ta Bi *et al.*, 2020). The need to remain healthy led to the creation of traditional medicine all over the world. However, in the West, the birth of synthetic molecules, have opened the way to modern or conventional medicine. That convinced peoples that herbal medicine is over. Surrounded by chemistry, the classical medicine has overpassed the other therapeutic approaches, especially traditional medicine (Doh, 2015). Nevertheless, in recent decades, there has been a resurgence of interest in the use of medicinal plants, even in developed countries. The reason is that, in certain localities of developing countries, like in Côte d'Ivoire, there are less and less medical centers (TA Bi and N'Guessan, 2021). Sometimes, they hold very far from the inhabitations, obliging people to focus on surrounded medicinal plants specie. In addition, the unavailability of drugs, the high cost of health services and the high cost of pharmaceutical products, are factors that encourage populations, generally poor, to turn out to traditional medicine, their only hope (N'Guessan, 2008). Moreover, despite the progress in the medical domain, the treatments of modern medicine are based on the use of chemical products that can have negative consequences on the health of patients. It is the case of steroidal and non-steroidal anti-inflammatory treatments which cause cardiovascular problems for sick people (Heymonet, 2013; Nurich, 2015). The use of plants with anti-inflammatory properties would therefore be desirable to avoid these serious consequences of pharmaceutical products. So, in perspective of researching new therapies, we have conducted this study focusing on medicinal plants with anti-inflammatory effect and on people engaged in this trade throughout the markets of Man (Côte d'Ivoire). This study is therefore a contribution to the search for plants specie with anti-inflammatory potential. It reveals the

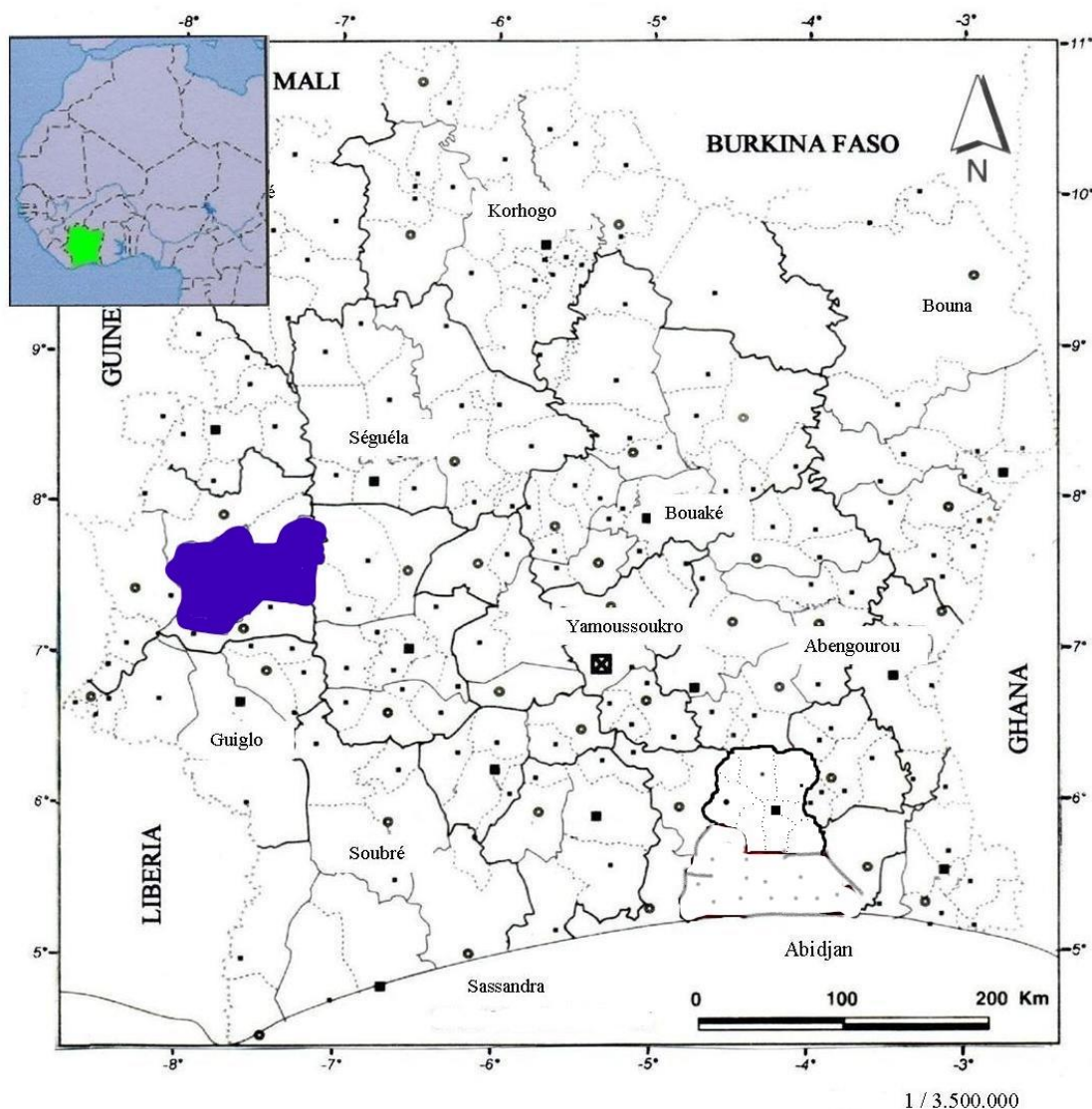
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plants used by the population of Man against inflammatory diseases and the phytochemical compounds that could be responsible of this empirical use.

2. MATERIAL AND METHOD

2.1. Study area

The study took place in the city of Man, Côte d'Ivoire (Figure1).



Man in Côte d'Ivoire

Côte d'Ivoire in Africa

Figure 1: Localization of Man in Côte d'Ivoire, a country of West Africa

2.2. Material

2.2.1. Survey material

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We used a biological material composed of all the plants specie encountered during the market investigations, as well as the different parts (leaves, flowers, fruits, roots and stems) that constitute them. Then, we needed a questionnaire to collect data and a camera to make pictures. We also needed a computer and appropriate software for the statistical analysis.

2.2.2. Phytochemical screening material

Biological material

The phytochemical screening concerned the most used plants specie for the treatment of inflammation problems.

Technical material

It concerns the usual laboratory material such as glass, oven, electrical scale, water heater and so on.

Chemical material

Solvents and reagents

The chemical material was composed of distilled water and several reagents. Stiasny's reagent was used for the search of catechetical and gallic tannins. Bornstraëgen's reagent was used for the search for quinone substances. The characterization of the alkaloids was performed with Burchard's reagent and Dragendorff's reagent.

Other chemical products

In addition to reagents, various chemicals have been used for the characterization of chemical groups. The search for catechetical tannins required the use of sodium acetate. While the characterization of gallic tannins was performed with sodium acetate and ferric chloride. For the research of sterols and polyterpenes, acetic anhydride and sulfuric acid were used. The characterization of the flavonoids, needed sulfuric alcohol, magnesium shavings, and isoamyl alcohol. The alcoholic solution of ferric chloride (FeCl_3) at 2%, and alcohol at 90°C respectively made it possible to identify polyphenols and alkaloids. The characterization of quinone substances was carried out with chloroform, twice diluted ammonia and hydrochloric acid.

2.3. Method

2.2.1. Ethnopharmacological investigation

The investigations relating to anti-inflammatory plants specie, were carried out during the fall November 2019 to March 2020, nearer vendors of medicinal plants. They are established in three markets, choosen in Man city: the big market, the market of Gbapleu and that of Zélé. This choice was justified by an impressive number of sellers of medicinal plants in these markets. As an approach used, we visited herbalists at their places of work. They were interviewed through a questionnaire. The information demanded for, concerned the herbs used to treat inflammatory diseases, the different organs used, the technical of preparation and the administration modality. We bought samples of plants to check their botanical names.

2.2.2. Collection of plant material and botanical identification

Specimen of plants specie mentioned by the herbalists were collected from the nature using conventional equipment. Pictures of them were taken too. Then the botanical identification was performed using the herbarium of the National Center for Floristic of Félix Houphouët-Boigny University in Abidjan. . We also had recourse to the systematic works of Aké-Assi (2001; 2002).

2.2.3. Data analysis

Frequency of citations

The index of the frequency of citation was used to detect the most encountered plants specie. It is calculated as follow:

$$F_c = \frac{n}{N} \times 100 \quad (\text{Fah } et \text{ al.}, 2013)$$

n is the number of persons cited the plant species

N is the total number of persons questioned

Hierarchical clustering

From the calculated frequencies of citation (F_c) of plants specie, a hierarchical clustering (HC) was performed to bring out cluster dendrograms by following the Ward's method which consider the euclidian distance. The code Bayer was established to rename the plants specie [8]. This code is composed of five letters, where the three first are initials of the gender and the two last letters are initials of the species. For example, a plant called *Nauclea latifolia* is referred to as *Naula*. The analysis of data needed the use of the software SPSS 20.0.

2.3.4. Phytochemical screening

Preparation of the aqueous extract

The aqueous extract of the leaves of the two most cited plants specie, was obtained from a decoction-way, by operating as in previous studies (Aké-Assi E., 2015; N'Guessan *et al.*, 2009). The leaves were harvested, rinsed with tap water and dried under shade. This method involves boiling 500 g of dry leaves of each species in 3 liters of water for 30 minutes in a 5-liter cooking pot. The process produced 2 liters of extracts. One liter was sufficient for the phytochemical screening. Thus, 250 ml of each filtrate were concentrated to 25 ml on a sand bath, which led to 2 aqueous extracts (Ext1, Ext2).

Tri-phytochemical characterization tests

The phytochemical screening is a set of tests for the detection of large groups of chemical compounds occurring in the plant or in one of the organs. The detection of these chemical groups was based on the principle that they can interact from a particular environment, with appropriate reagents and induce visible color variations to the naked eye. In the case of this study, these tests were carried out with the aqueous extracts according to the classical method of characterization of chemical groups described before, in similar works (Bekro *et al.*, 2007; Kolling *et al.*, 2010).

Search for sterols and polyterpenes

The chemical test set up by Liebermann allowed to bring out the chemical groups. We evaporated to dryness, without carbonized the residue, in a capsule on the sand bath, 5 ml of the solution. The residue was subsequently dissolved in 1 ml of acetic anhydride and the resulting solution was decanted into a test tube. Finally, we added 0.5 ml of sulfuric acid in the test tube and observed the solution. The sight of a purple ring at the interphase, turning to blue and then green, indicated a positive reaction.

Search for polyphenols

The reaction with ferric chloride (FeCl_3) made it possible to characterize the polyphenols. We mixed a drop of alcoholic ferric chloride solution (2%) with 2 ml of each solution. Ferric chloride reacts with polyphenolic derivatives and let appearing a blackish blue, more or less dark green color, indicating the presence of polyphenols.

Search for Flavonoids

The search for flavonoids was carried out from the reaction to Cyanidin. It consisted in evaporating to dryness in a capsule, 2 ml of each solution before cooling them. The residue is taken up in 5 ml of a half hydrochloric alcohol. The solution is decanted into a test tube, in which we have added 2-3 chips of magnesium and observed a release of heat. A pink-orange or sometimes purplish color was obtained. Finally, we added 3 drops of isoamyl alcohol, which intensifies the coloring when flavonoids are present.

Search for tannins

The search for catechetical tannins was carried out using Stiasny's reagent. Five (5) ml of each extract was evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80 ° C for 30 min. The observation of a precipitate in large flakes characterized the catechetical tannins. For the gallic tannins, we filtered the previous solution (5 ml of extract and 15 ml of Stiasny's reagent). The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl₃ would cause the appearance of an intense blue-black color, a sign of the presence of gallic tannins.

Search for free or combined quinone substances

The detection of free quinone substances was performed with the reagent of Bornstraëgen. For the combined quinone substances, we carried out a preliminary hydrolysis. The experiment consisted in hydrolyzing the solutions to characterize all the quinone substances, like the free quinone substances and the compound quinone substances. For that, we evaporated 2 ml of each solution to dryness in a capsule and triturated the residue in a fifth into 5 ml of hydrochloric acid. Thereafter, we kept the solution obtained in a boiling bath-water for 30 min. The process led to cooling, extracting the hydrolysate with 20 ml of chloroform in a test tube, collecting the chloroform phase in another tube and adding 0.5 ml of half-diluted ammonia. The sight of a color ranging from red to purple indicated the presence of quinones.

Search for alkaloids

The characterization of the alkaloids was established from the reagent of DRAGENDORFF (reagent to potassium iodobismuthate) and that of BURCHARD (iodine-iodide reagent). We evaporated to dryness in a capsule, 6 ml of each solution, taken up the residue in 6 ml of alcohol at 60 °C and distributed the alcoholic solution in 2 test tubes. After this step, we added 2 drops of DRAGENDORFF reagent to the first tube and 2 drops of BURCHARD reagent to the second tube. In the first tube, the sight of a precipitate or an orange coloration indicated the presence of alkaloids. In the second tube, the observation of a precipitate or a white-cream coloration was evidence of a positive reaction.

Search of saponosides

The saponosides were detected by assessing the abundance of foams after stirring plant extract solutions. We put 15 ml of each extract into a test tube, stirred vigorously for 10 seconds and let to calm for 10 min. The persistence of the foam at a height of 2 to 3 cm, exhibited of the presence of the saponosides.

3. RESULTS

3.1. Plants specie listed from the survey

The ethnopharmacological investigations through markets of Man brought out 73 herbalists and 20 plant species. Two parts of plants are used in the treatment of inflammation: the leaves, the branches. Sometimes, it is the whole plant which is used. The technical of preparations are the decoction and the kneading. The modes of administration for the decoction is the oral route by drinking and the pastes are taken by anal route. All information is recorded in Table 1.

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Table 1: List of inventoried plants and ethnopharmacological characteristics

Scientific names of plant species and families	Parts used for treatment	Technical of preparation	Technique of administration
<i>Agave decipiens</i> (Agavaceae)	Leaf	Decoction	Drink
<i>Alchornea cordifolia</i> (Euphorbiaceae)	Leaf	Decoction	Drink
<i>Boerhavia diffusa</i> (Nyctaginaceae)	Leaf	Kneading	Purging dough
<i>Cajanus cajan</i> (Fabaceae)	Leaf	Decoction	Drink
<i>Cassia occidentalis</i> (Caesalpiniaceae)	Leaf	Decoction	Drink
<i>Chromolaena odorata</i> (Asteraceae)	Leaf	Decoction	Drink
<i>Cnestis ferruginea</i> (Connaraceae)	Leaf	Decoction	Drink
<i>Ficus sur</i> (Moraceae)	Leaf	Kneading	Purging dough
<i>Hoslundia opposita</i> (Lamiaceae)	Leaf	Decoction	Drink
<i>Microdesmis keayana</i> (Pandaceae)	Leaf	Decoction	Drink
<i>Momordica charantia</i> (Cucurbitaceae)	Whole plant	Kneading	Purging dough

Table 1 continued: List of inventoried plants and ethnopharmacological characteristics

Scientific names of plant species and families	Parts used for treatment	Technical of preparation	Technique of administration
<i>Nauclea latifolia</i> (Rubiaceae)	Leaf	Decoction	Drink
<i>Persea americana</i> (Lauraceae)	Leaf	Decoction	Drink
<i>Phyllanthus amarus</i> (Euphorbiaceae)	Whole plant	Kneading	Purging dough
<i>Piliostigma thonningii</i> (Fabaceae)	Branch	Decoction	Drink
<i>Psidium guajava</i> (Myrtaceae)	Leaf	Decoction	Drink
<i>Rauvolfia vomitoria</i> (Apocynaceae)	Leaf	Decoction	Drink
<i>Tectona grandis</i> (Verbenaceae)	Leaf	Decoction	Drink
<i>Vernonia amygdalina</i> (Asteraceae)	Branch	Decoction	Drink
<i>Xanthoxylum gillettii</i> (Rutaceae)	Leaf	Kneading	Purging dough

3.2. Citation frequency

The frequencies of citation were used in a hierarchical clustering analysis which shows cluster dendrogram as indicated in Figure 2. The dendrogram brings out two clusters of plants at an euclidian distance of 3. The first cluster has two plants which are represented among the plant species cited against inflammation in Man. They are *Rauvolfia vomitoria* with FC=8,01 % and *Alchornea cordifolia* for $F_c = 7,49$ %. The other plants compose the second cluster. Their F_c value is lower than 3%. Those plants were rarely encountered during the survey.

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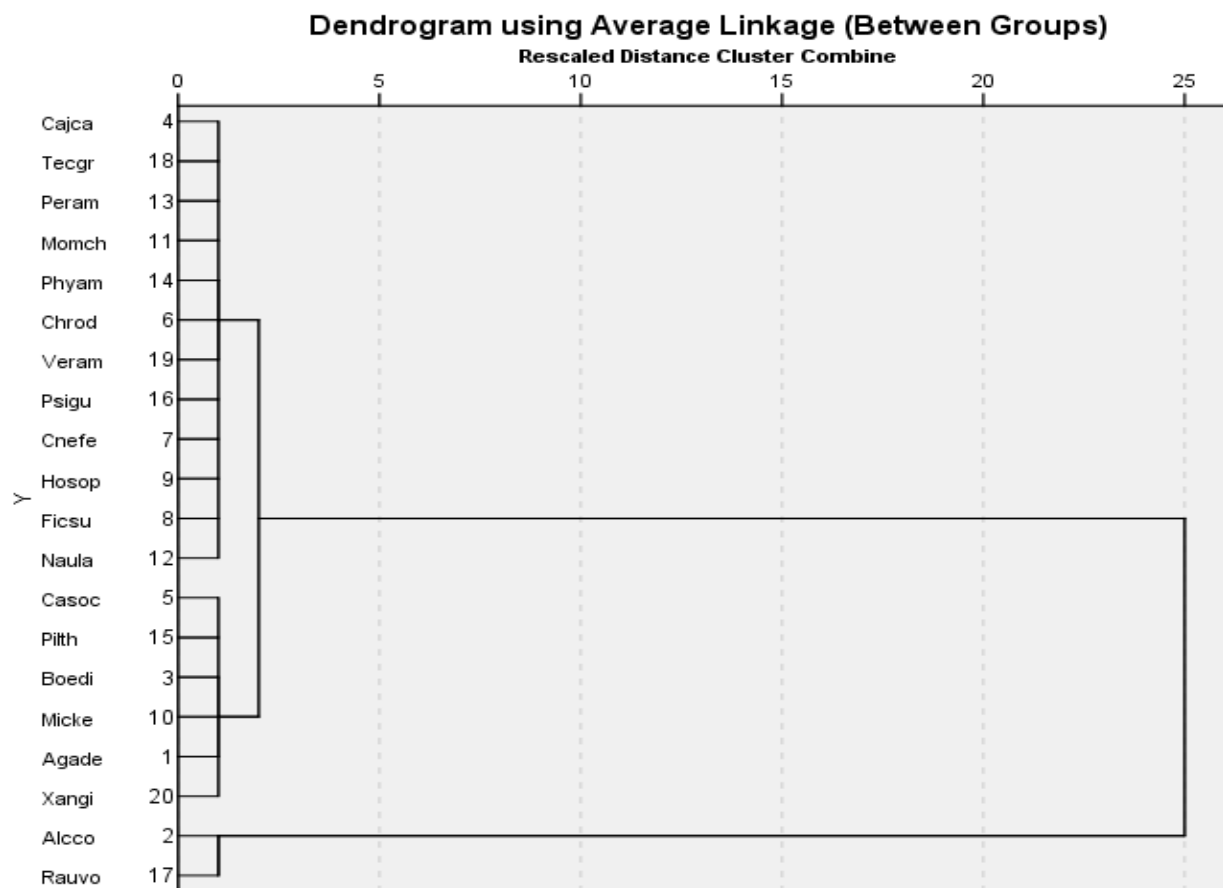


Figure 2: Dendrogram of the hierarchical clustering of listed plants according to their frequencies of citation

3.3. Phytochemical screening

The results of the phytochemical screening are given in Table 2. The tests for the quinonic substances are negative in the two samples. It's also the same with tannins. Both plants contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponosides. However, there is a strong presence of alkaloids and saponosides in both plants and an abundance of flavonoids in the species *Alchornea cordifolia*.

Table 2: Phytochemical screening of the two most common plant species

Chemical groups	<i>Alchornea cordifolia</i>	<i>Rauvolfia vomitoria</i>
Sterols and Polyterpenes	+	+
Polyphenols	+	+
Flavonoids	++	+
Tannins Gal Cat	-	-
Quinonic substances	-	-
Alkaloids B D	++ +	++ +
Saponosides	++	++

Legend

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++: Abundance of the chemical group	Gal: Gallic	B: Burchard
+: Medium presence of the chemical group	Cat : Catechic	D: Dragendorff
-: Absence of the chemical group		

4. DISCUSSION

The ethnopharmacological investigations exhibited 20 plants specie used for the treatment of inflammation. The method of administration of these herbal medicines is mainly the decoction as mentioned previously in Côte d'Ivoire (Ta Bi *et al.*, 2016). Two of the listed plants are regularly encountered by herbalists. They are *Alchornea cordifolia* and *Rauvolfia vomitoria*. The Phytochemical screening of these two plants revealed that they contain various chemical groups including abundant alkaloids, flavonoids and saponins. The use of these two herbs for the treatment of inflammatory diseases could be explained by the presence of these chemical groups, because the alkaloids exert an anti-inflammatory activity thanks to the protopine (Bribi *et al.*, 2015). In addition, flavonoids have antioxidant property (N'Guessan *et al.*, 2014). Their presence could therefore amplify the action of alkaloids. The existence of other chemical groups such as sterols and polyterpenes shows that these two plants could also have an anti-diabetic effect in addition to anti-inflammatory activity because these chemical groups lower the blood glucose level (N'Guessan, 2008). The empirical use of *Alchornea cordifolia* and *Rauvolfia vomitoria* against inflammation may therefore have a scientific basis.

5. CONCLUSION

The ethnopharmacological investigations carried out in the markets of Man, in Côte d'Ivoire, made it possible to inventory 20 plant species used by traditional medicine for the treatment of inflammation. The modes of administration are the decoction to be drunk and pastes to purge. In comparison with the calculated frequencies of citation, two plants specie are very important. They are *Alchornea cordifolia* (Fc = 7.49%) and *Rauvolfia vomitoria* (Fc = 8.01%). These two plants are known by all the vendors of medicinal plants visited during the surveys. They are the most plants used to remove inflammatory diseases nearby the population of Man. A phytochemical screening was performed to assess the scientific basis for the empirical use of these two most common plants. These tests revealed that these plants contain sterols, polyterpenes, polyphenols, flavonoids, saponosides and alkaloids with a strong presence of saponosides, flavonoids and alkaloids. The anti-inflammatory effect could be related to the strong presence of these chemical groups. These two plants could be considered in the research of anti-inflammatory phytomedicines.

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