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Phytochemical study of extracts from the leaves of *Senna alata* (L.) Roxb. (Leguminosae-Caesalpinioideae) and *Lantana cumara* L. (Verbenaceae), two main plants traditionally used to treat skin infections in Benin

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Abstract

In Benin, two medicinal plants are commonly used by rural populations to treat skin infections. Very few investigations are carried out on them against skin infections, despite their increased use. The present study is carried out on these two anti-infectious plants, namely: *Senna alata* (L.) Roxb. (Leguminosae-Caesalpinioideae) and *Lantana cumara* L. (Verbenaceae). The objective of this study is to characterize the groups of chemicals contained in the two herbal drugs. The phytochemical tests carried out on the two (02) plant extracts revealed that the different drugs contain Anthocyanins, Quinone Derivatives, Coumarins, Free Anthracenics, Saponosides, Triterpenes, Mucilages and Reducing Compounds which gave them the antibacterial properties. The best yield is obtained with the aqueous extract.

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Introduction

Human infectious diseases represent a major public health problem in the world and especially in developing countries where they are endemic (Khabbaz *et al*., 2014). Nearly fourteen million deaths are recorded each year from infectious diseases and over 90% of these deaths occur in developing countries (Fonkwo, 2008). Currently, the emergence of skin infections is mainly due to the advent of Acquired Immune Deficiency Syndrome (AIDS), the misuse of strippers and the overuse of drugs. In addition to these difficulties, there are the crucial phenomena of resistance to antibiotics and especially the constantly increasing prices of available drugs (Ouattara *et al*., 2013). In addition, the recurrence of the outbreaks of this infection sometimes means that families neglect the fight against infection. Added to this is the inadequacy or nonexistence of health infrastructure (Zerbo, 2011). These populations are therefore turning to plants to find remedies to heal themselves. Medicinal plants are therefore a source of local medical care. Indeed, the use of plants for therapeutic purposes is reported in ancient Arabic, Chinese, Egyptian, Hindu, Greek, Roman literature (Anonymous, 1974). As a result, the World Health Organization has encouraged research on medicinal plants since 1978 to improve, secure and reduce the cost of medical products (WHO, 2002). No less than 170,000 bioactive molecules have been identified from plants to date (WHO, 2002). Plants are still the primary reservoir for new drugs (Fouchet *et al*., 2000). In Africa, the therapeutic power of plants was known by our ancestors and our parents empirically (Nacoulma, 1996). Thus we did not know anything about the chemical composition of drugs used every day by many populations for health care. The exploitation of herbal medicine heritage cannot remain static and be limited to the collection of traditional recipes (Vanhaelen, 2002). It is therefore opportune to develop studies that can explore new areas of knowledge that refer not only to the forms of the ordering of natural knowledge but also to the study of chemical compounds in plants (Pousset, 2006). Some work in the sub-region has already initiated the phytochemistry of medicinal species used in various regions. In Benin, bioactive molecules have been identified in certain medicinal plants which are effective and non-toxic in animal experiments (Sènou *et al*., 2016a, 2016b; Tchogou *et al*., 2017; Agbogba *et al*., 2019).

In the context of infectious diseases, significant pharmacological and phytochemical work has been carried out on medicinal plants from the Ivory Coast (Kouamé *et al*., 2004; Zirihi *et al*., 2005; Békro *et al*., 2007; Zirihi *et al*., 2007; N'Guesan *et al*., 2009. Kamanzi (2002) Bioassays were carried out on 104 species of medicinal plants. An evaluation of the antibacterial, antifungal, antimalarial and antitrypanosome activities was carried out; cytotoxicity was studied. Regarding phytochemical investigations, the author researched the bioactive principles of the plants studied; five (05) prenulated isoflavonoids have been isolated among which vogeline A, vogeline B and vogeline C, had never been described. Previous studies have revealed that Ficus platyphylla has antibacterial, anti-nociceptive, anti-inflammatory properties, and gastrointestinal activities in rodents (Amos *et al*., 2001; Amos *et al*., 2002; Kubmarawa *et al*., 2009). Thus, it is to promote anti-infectious medicinal plants by relying on traditional medicine that we include the studies we have carried out on *Senna alata* (L.) Roxb. (Leguminosae-Caesalpinioideae) and *Lantana cumara* L. (Verbenaceae), two plant species commonly used to treat skin infections in Benin. It will be a question of characterizing the chemical groups contained in these plants which will make it possible to explain their anti-infective effects obtained.

Materials and methods

Technical equipment

In connection with the phytochemical screening, we used an oven at 33 ° C, to dry the samples which had become wet, before their pulverization using an electric grinder. We used an electric balance for the different weighings, a water bath at 37 ° C. A sand bath was used to evaporate the extracts in porcelain capsules. We had a water heater. This equipment also included spatulas for the collection of drug powders, cotton wool used as a filter, a hood to protect against the powders ejected during the spraying of drugs, a trituration rod and forceps.

Plant material

The phytochemical study required material represented by various drugs (leaves) which were pulverized and used for the preparation of the etheric, methanolic and aqueous extracts (Table 1).

Solvents

The etheric, methanolic and aqueous extracts were obtained by successive extractions with solvents, according to the increasing order of their polarity. In that order, we used petroleum ether, methanol, and distilled water. The compound group detection tests focused on these 3 types of crude extracts.

Reagents

The phytochemical screening required various reagents. The search for catechetical tannins was made possible by Stiasny's reagent and sodium acetate. For the characterization of gallic tannins, we used Stiasny's reagent, sodium acetate and ferric chloride. Acetic anhydride and concentrated sulfuric acid were needed in the research of steroids and polyterpenes. Twice diluted hydrochloric alcohol, magnesium shavings and isoamyl alcohol were used to test for flavonoids. The 2% alcoholic ferric chloride solution allowed the characterization of polyphenols. Bornstraëgen's reagent, chloroform, ammonia diluted twice and hydrochloric acid were used to test for quinone substances. We characterized the alkaloids using 60 ° alcohol, Burchard's reagent (iodine-iodine reagent) and Dragendorff's reagent (potassium iodobismuthate reagent). We used ethanol and distilled water for the extraction of our various extracts.

Phytochemical screening

The various chemical analyzes are carried out in the laboratory by phytochemical screening. This is a qualitative analysis based on coloring and/or precipitation reactions. This is carried out on dry plant drugs according to the methodology described by Houghton & Raman (1998). This methodology was carried out according to standard procedures: the Mayer and Dragendorff tests is for alkaloids, the Fehling test for free reducing sugars, the Fehling test for glycosides, the Liebermann-Burchard test for triterpenoids, the test for Liebermann-Burchard for steroids, foamy test for saponins, Shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, the Guignard test for derivative-free cyanogenetics and the Bornträger test for free anthraquinones.

Preparation of plant extracts

Fifty grams (50 g) of each powdered plant was mixed with 500 ml of solvent [water-ethanol: 4/6, v / v (HE) and distilled water (H2O). The mixture was macerated for 72 hours and filtered three times in succession. Then the filtrate was evaporated to dryness at 40 ° C using a rotary evaporator (Heidolph efficient from Laborota 4000) coupled to a water cooler (300 Julabo FL) to obtain crude extracts.

Data processing

The data were transferred with SPSS 12.0 software where they were subjected to various analyzes.

Results

Phytochemical study

The main families of secondary metabolites sought are Gallic tannins, Catechic tannins, Alkaloids, Flavonoids, Steroids, terpenes, Leucoanthocyanins, Anthocyanins, Mucilages, Heterosides, C-heterosides, Free heterosides, Reducing compounds, Coumarins, Cardiotonic glycosides, Quinones, cyanogenic (Table 2).

This table reveals the richness of the samples of plant materials in phytochemicals (thirteen groups of chemical compounds). From test to test, we note that the two (02) plant materials all contain Alkaloids, Catechetical Tannins, Gallic Tannins, Flavonoids and Steroids. Moreover, the *Lantana cumara* plant alone contains apart from the other compounds it contains, Anthocyanins, Quinone Derivatives, Coumarins and

Free Anthracenes. On the other hand, *Senna alata* contains on its own, the Saponosides, the Triterpenes, the Mucilages and the reducing compounds. We also noticed the absence of Leucoanthocyanins, cyanogenic derivatives, combined anthracenes, O-heterosides, C-heterosides and cardiotonic heterosides in the two different plant materials.

Yield of the different extracts

From Table 3 the yield ranges from 4.81 to 7.97 for the hydro-ethanoic extract and from 1.40 to 2.09 for the hydro-ethanoic extract.

This allows us to say that the best yield (more than double) is obtained with the aqueous extract.

Table 1. List of plants studied, parts used and place of harvest.

Pantes used	Families	Parts used	Shapes used	Harvest location	National herbarium identification number
Senna alata (L.) Roxb.	Leguminosae-Caesalpinioideae	Leaves	Trituration	Adjara(Porto-Novo)/ Benin	YH 511/ HNB
Lantana cumara L.	Verbenaceae	Leaves	Trituration	Abomey-Calavi/ Benin	YH 512/ HNB

Discussion

Phytochemical

The phytochemical study revealed the richness of its two (02) plant materials with active chemical compounds such as Alkaloids, Catechic Tannins, Gallic Tannins, Flavonoids and Steroids could explain the traditional use of these plants to treat skin infections.

Table 2. Results obtained from phytochemical screening.

Compounds sought	Plant species	
	Lantana cumara	*Senna alata*
Alkaloids	++	++
Catechetical tannins	+	+
Gallic tannins	++	++
Flavonoids	+	++
Anthocyanins	+	-
Leucoanthocyanins	-	-
Quinone derivatives	++	-
Saponosides	-	+
Triterpenes	-	+
Steroids	+	+
Cyanogenic derivatives	-	-
Mucilages	-	+
Coumarins	+	-
Reducing compounds	-	+
Anthracene Free	+	-
Combined Anthracene	-	-
O-Heterosides	-	-
C- Heterosides	-	-
Cardiotonic glycosides	-	-

++ : strongly positive reaction; + : positive reaction; - : negative reaction.

On the basis of previous studies, the different chemical compounds (catechic tannins, gallic tannins, flavonoids and steroids, alkaloids and quinone derivatives, mucilages, free anthracenics) could be at the origin of antibacterial activities (Amos, 2001& 2002). Indeed, several authors have shown that the different types of chemical compounds demonstrated in the extracts of these plants have therapeutic effects

(E. N. Frankel, J. Kanner, J. B. German, E. Parks and J. E. Kinsella). The antimicrobial properties of these plants are explained by the presence of these active chemical compounds. The presence of tannins and alkaloids revealed by phytochemical tests would be responsible for the antibacterial activities (Gbogbo *et al*, 2013). Tannins have been reported to exhibit relatively high antibacterial and anticryptococcus (yeast), antiviral, anti-inflammatory, anti-hypertensive, antimutagenic, immunostimulant, anti-tumor and anti-diarrheal activities (Yamasaki *et al.*, 2001) have proven the antibacterial action of several tannins against Staphylococcus aureus. The presence of tannins (catechetical and gallic) in these two plants justifies the use of these plants by populations to fight skin infections. Plant extracts and many other phytochemicals rich in flavonoids have been reported to possess antimicrobial activity (Tim *et al.*, 2005). Due to their structure characterized by the presence of a phenolic group, and other chemical functions, flavonoids are considered very good antimicrobial agents (Harborne and Williams., 2000). Many studies have reported the antimicrobial activities of flavonoids.

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Table 3. Yields of the various extracts obtained.

Type of extract	Name of plant species	Yield (in%)
Hydro-ethanoic extract	*Lantana cumara*	1,75
	Senna alata	2,09
Aqueous extract	*Lantana cumara*	7,97
	Senna alata	8,63

The antifungal activity of flavonoids is also established. A study done on Dianthus caryophyllus showed the effectiveness of the flavonoid glycoside on fungal strains (Galeotti *et al.*, 2008). Moreover, alkaloids which are nitrogenous and basic organic substances endowed with physiological properties are of great therapeutic importance. We could therefore deduce certain compatibility between the properties of the chemical groups found and the therapeutic objectives sought in traditional treatments. These results justify the use of these plants in the traditional treatment of skin infections. The absence in these samples of cardiotonic glycosides and cyanogenic derivatives could reveal the non-toxic character of these plants since cardiotonic glycosides and cyanogenic derivatives are generally toxic compounds (Houngbèmè *et al.*, 2014).

Yield

The best yield is obtained with the aqueous extract, which means that most of the chemical principles of the study plants are polar. In addition, these results are more or less improved compared to previous work (Ali, 2002; Gbaguidi, 2005). This allows us to say that the yields of extractions often depend on the operator and the type of extraction performed. These data compared to those in the literature show that the extraction methods used by the Beninese populations give better yields.

Conclusion

The phytochemical study of *Lantana cumara* and *Senna alata* extracts revealed the presence of the main groups of active chemical compounds such as Alkaloids, Catechic Tannins, Gallic Tannins, Flavonoids and Steroids, Anthocyanins, Quinone derivatives, Coumarins, Free Anthracenics, Saponosides, Triterpenes, Mucilages and Reducing Compounds. The therapeutic effects are induced by these various chemical compounds which constitute the scientific basis of the traditional therapeutic use of the plants studied against skin infections. These bioactive extracts are therefore good candidates for enriching the therapeutic arsenal against skin infections. In addition, we wish to assess the toxicity of these plants and then extend the study in the branch of microbiology to verify the antibacterial activity to justify the traditional therapeutic use of these plants against skin infections. Our work may also contribute to the continuation of work on the *in vivo* study of the activity of these plants on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two germs responsible for skin infections in humans and on the possible isolation of active compounds.

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