Original Article

PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF *Hyptis specigera LAM* LEAVES

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ABSTRACT

The phytochemical screening of the hexane, chloroform and Ethanol extracts of *Hyptis specigera Lam* leaves revealed the presence of the following plant constituents; alkaloids, saponins, flavonoids, tannins, cardiac glycosides, anthraquinones, as well as carbohydrates. Antimicrobial screening of these extracts against *Staphylococcus aureus*, *Escherichia coli*, *Shigella spp*, *Bacillus subtilis* and *Pseudomonas aeruginosa* indicated that hexane extract had inhibitory effects on two or more test pathogens while ethanol extracts showed a broad spectrum of antimicrobial activity. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the crude extracts were determined for the various organisms using the broth dilution method. The MIC and MBC of the extracts ranged from 1.56-25 mg/ml and 6.25-25 mg/ml respectively. The results from the activity of the crude extracts suggests that *Hyptis specigera Lam* leaves could be used in the treatment of diseases caused by these organisms.

Key words: Phytochemical, antimicrobial, ethnomedicinal, *Hyptis specigera Lam*

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INTRODUCTION

Hyptis specigera Lam, known as black 'beni-seed' or 'black sesame' or 'guardian of mosquitoes' is a common Nigerian and West African plant that belongs to the family of Lamaceae or Labiatea by the Americans (Abdullahi *et al.*, 2003). It is a strong erect aromatic herb growing to about 3m high with terminal inflorescence, dense cylindrical or avoid cylindrical spike to 9cm long of very small white mauve flowers, the stem is erect angled and branched, the leaves are opposite lanceeolate, about 8cm long, 2-3cm wide, acute and serrate, the under surface is dotted with close pellucid glands. The flowers have corolla with purple dots on the lower lip, the fruit is a small capsule and the seeds are small and black. This is an evergreen plant mostly found on road sides, and cultivated land, often on damp sites from Senegal to West Cameroon, possibly native to Brazil. It is now widely naturalised in tropical Africa and Asia (Tapsoba and Dechamp, 2006) and there are three to four species of *Hyptis* in Nigeria including *Hyptis lanceolata pior, Hyptis suavalens poit.*

The plant has been reported to have a lot of uses both medicinally and non-medicinally. In Senegal for instance the crushed plant is applied to head to relieve headaches (Vuksan and Salvia, 2002), while the seeds of the plant is used for eye treatment and as a pain killer (Babu *et al*, 2002). In Burkina Faso, a small quantity of the crushed leaves put in a spoon with water and set aside for while to be used to alleviate tooth pain and also for the relieve of tooth ache (Tapsoba and Dechamp, 2006). Also in Western Cameroon; the leaves are used as cutaneous and subcutaneous against parasitic infection as

well as ceremonial and religious purposes (Babu *et. al.*, 2002; Lino and Deogrocious, 2006).

The leaves are also useful as an antidiarrhoeic and antidysenteric (Buwa and Staden, 2006). In Nigeria, the plant is used for treating diabetes (Akundu, 2001). The leaves are used by Ijaws of South-South Nigeria for curing headache, and as an antimalarial, as well as typhoid fever (Vuksan and Salvia, 2002). The essential oils isolated from aerial parts of the plant are used to rub the chest to relieve chest pain, and on the skin to prevent skin rashes in Northern Nigeria (Abdullahi *et. al.*, 2003). The seeds of the plant have been reported to be useful for reducing post-prandial blood glucose levels, improving endothelial function, coagulation, and fibrinolysis and iron status (Indrayan *et. al.*, 2002).

In some parts of West Africa the plant is used locally as an insect repellent for protecting cowpea seed and other grains during storage (Janveska *et. al.*, 2003). The seeds of the plant as well as the leaves are eaten as vegetable; it is cultivated as a food flavouring agent and also use in cosmetic and perfume (De and James, 2002).The ancient Indians also had notions rooted in superstition about the plant (Bibitha *et. al.*, 2002).

The present study investigated crude extracts of the leaves of *Hyptis specigera Lam* for its phytochemical components which likely are responsible for the reported therapeutic potency. The antimicrobial activity of these extracts against Gram positive and Gram negative bacteria was also studied.

MATERIALS AND METHODS

Plant material

The leaves of *Hyptis specigera Lam* used for this study were collected in the month of September in Tunga Area of Minna Metropolis, Niger State, Nigeria. They were washed with clean water to remove sand and later air-dried and ground to powder using a grinder prior to extraction.

Test for Microorganisms

The microorganisms (*Pseudomonas aeruginosa,Escherichia coli, Staphylococcus aureus,Bacillus subtilis,Shigella* spp) used for the study were obtained from stock cultures in the Department of Microbiology, Federal University of Technology,Minna,Niger State,Nigeria.

Preparation of Plant Extracts

Seventy grams (70g) of powdered plant material was marcerated with n-hexane (200ml) for a period of 24hr. Resulting solution was filtered using sterile filter paper and the filtrate was evaporated to dryness in vacuum, weighed and coded "Hext".The defatted marc was air-dried and extracted with chloroform as well as ethanol using the same principle described above to obtain the chloroform and ethanol extracts.

Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by (Harbone,1992).The hexane, chloroform and ethanol extracts of the plant were evaluated for the presence of alkaloids,saponins,cardiacglycosides,tannins ,steroids,flavonoids,carbohydrate,terpenoids and anthraquinones.

Antimicrobial Screening

The Agar dilution method was used to determine the antimicrobial activity of the various extracts against the tested micro organisms. The nutrient agar used to dilute the sample solution to the required concentration was inoculated by surface streaking using sterile wire loop with test organisms. The plates were incubated at 37°C overnight and observed for growth inhibition. Plates that had growth of the test

organism inhibited at 2.0mg/ml were further diluted to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Of Plants Extracts

The MIC of the extracts against the test organisms was determined using the broth dilution method. 0.5cm^3 of each extract solution at a concentration of 100mgcm^{-3} was added to 1.5cm^3 of nutrient broth. The test organisms were inoculated into each test tube mixed thoroughly and was then incubated at 37°C for 24 hours. The tube with the lowest dilution which had no detectable growth was considered as the MIC.

To determine the MBC, a loopful of broth was collected from those tubes which showed no turbidity and inoculated on Nutrient agar. The inoculated agar plates were incubated at 37°C for 24hours. The concentration at which no visible growth was observed was noted as the MBC.

Table 1: Phytochemical analysis of extracts of fresh leaves of *Hyptis specigera* lam

Constituents	Chloroform extract	Ethanol extract	Hexane extract
Alkaloids	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Tannins	-	+	-
Cardiac glycosides	+	+	+
Phlobatannins	-	-	-
Anthraquinones	-	+	-
Steroids and Terpenoids	-	-	-
Carbohydrates	+	+	-

+ : Present - : Absent

RESULTS AND DISCUSSION

The phytochemical screening carried out on the leaves of *Hyptis Specigera* Lam revealed the presence of some of the natural products tested for were not present in the plant material and these include phlobatannins,steroids and terpenoids (Table 1).

Some of the active constituents such as saponins, free anthraquinones and tannins were observed only in the ethanol extract while anthraquinone and tannins were not detected in the chloroform and hexane extract Table (1). Alkaloids and Flavonoids were present in both ethanol and hexane extract .This shows the generality of the component in medicinal plants. Biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties (Kubmarawa *at. al.*, 2008). The presence of some of these compounds had previously been observed by other researchers in related species of the plants

(Babu *et. al.*, 2002; Fabiola *et. al.*, 2002). For instance, the plant showed the presence of alkaloids which supports the report of Edoga *et. al.* (2005) that the plant such as *Hyptis suaveolens* had alkaloid and this

inhibits pathogenic bacteria. The ethanol extract contained tannins and tannins are responsible for colour changes and flavours in food. This further support the use of the plant as food flavouring agents (Edeoga and Gomina, 2000).

Terpenes and steroid were not detected in the plant extracts contrary to report by (Babu *et. al.*, 2002; Edoga *et. al.*, 2005; Buwa and Staden, 2006) that *Hyptis specigera lam* extracts contained steroids and terpenoids. This may be due to soil and environmental factors or it could be that they are being masked by other stronger constituents (Cuneyt and Jolita, 2007).

Table 2:Antimicrobial Property of Extracts of *Hyptis specigera* lam

Organisms	Extracts	Zone of inhibition (mm)
Escherichia coli	Chloroform-	8
	Ethanol	18
	Hexane	28
Bacillus subtilis	Chloroform	Not active
	Ethanol	23
	Hexane	14
Staphylococcus aureus	Chloroform	3
	Ethanol	30
	Hexane	8
Pseudomonas aeruginosa	Chloroform	2
	Ethanol	25
	Hexane	19
Shigella spp	Chloroform	2
	Ethanol	27
	Hexane	8

The results for antimicrobial screening indicated as the diameter of zone of inhibition is given in Table 2. The results showed that all the extracts showed antibacterial activity against the five test organisms. The ethanol extract was found to be the most active against all the test organisms; this could be due to the presence of the bioactive constituents present in the extract. These components have been reported by the investigators to be active against microorganism (Buwa and Staden, 2006). The demonstration of activity against the pathogenic bacteria by the plant is an indication that the plant can be used to develop new antibacterial agents to treat ailments caused by these organisms (Banso and Olutumayin, 2001). The zone of inhibition was measured by the use of a transparent plastic ruler and was observed to be above 14mm for ethanolic extract. It has been found that for Enterobactoriaceae to be regarded as sensitive to any antimicrobial agent, it must produce a zone of inhibition of greater or equal to 14mm. Base on this the plant under study, is therefore an effective anti-microbial agent Barry *et al*, (1985).The organisms are resistant to the chloroform extract.The morphogenetic and phenogenetical variations of the plants harvested at vegetative,full flowering,floral budding,fresh fruiting and mature fruiting stages are factors that contribute to the

difference in activity(Cuneyt and Jolita,2007).

The possession of antimicrobial activity against these bacteria suggest that the extracts probably contain active agents and this provides the basis for their use as a cure for some human ailments such as diarrhea and dysentary.This assertion is also confirmed, as their extracts indicate a relatively moderate number of phytochemical presents.

Table3a:Minimum inhibitory concentrations MIC(mg/ml) of extracts of *Hyptis specigera lam*

Organisms	Chloroform extract	Ethanol extract	Hexane extract
Escherichia coli	1.56	6.25	25
Staphylococcus aureus	1.56	6.25	25

Table3b: Minimum bactericidal concentrations MBC(mg/ml) of extracts of *Hyptis specigera lam*

Organisms	Chloroform extract	Ethanol extract	Hexane extract
Escherichia coli	6.75	25	25
Staphylococcus aureus	6.25	25	6.25

The MIC and MBC of the extracts ranged from 1.56 to 25mg/ml and 6.25 to 25 mg/ml respectively (Table 3 a and b).The low MIC and MBC values recorded are indications of the efficiency of this plant extracts and that the extract has cidal effect on all the test organisms at different concentrations (Edoga *et. al*,2005).

The study on the evaluation of the phytochemical and antimicrobial activity of *Hyptis specigera Lam* leaves indicated that the plant has the potential to generate novel metabolites, since the extracts demonstrated antimicrobial activities. However, further investigation is required on ethanol extract to isolate and elucidate the structure of the compound(s) responsible for the activity and their modes of action.

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