PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 9 (4): 358-361, 2010
ISSN 1680-5194

Phytochemical Screening of Solvent Extracts from *Hyptis suaveolens LAM* for Fungal Growth Inhibition

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Abstract: *Hyptis suaveolens* was targeted on the basis of folkloric uses which suggest its toxicity to microbes, coupled with its importance as food to humans. The pulverized plant material was extracted with 96% ethanol and further partitioned using chloroform, distilled water, petroleum ether and methanol. Soluble solvent extracts of the plant were tested for phytochemicals which revealed the existence of alkaloids, flavonols, flavones, flavonones, terpenoids, tannins, aldehydes and ketones and the absence of steroids, saponins and anthraquinones. Antifungal screening exhibited growth inhibition in some instances which exceeded that of griseofulvin antibiotics. The presence of phytochemicals and activity against *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species support ethno-medicinal uses of plant.

Key words: *Hyptis suaveolens*, soluble solvent extracts, phytochemicals, *Aspergillus niger*, *Candida albican*, *Cryptococcus* and *Fursarium* species, griseofulvin, fungal growth inhibition, zones of inhibition and toxic components

INTRODUCTION

A wide range of our recently used medicines had their roots directly or indirectly from plants. Some of these medicines are no longer synthesized in large quantities by competitors because they have shown toxicity to humans and other animals. This has made it possible for more investigations to be carried out on plants so as to enable us know the therapeutic status of newly discovered drugs of plant origin. In this respect, plant based research has made promising results in the fields of anticancer and anti-malarial therapies (De Smet, 1997).

Of the 250,000-500,000 species of existing plants on earth (Borris, 1996), only about 300 species are being used worldwide in the pharmaceutical, food, cosmetics and perfume industries (Robber and Speedie, 1996; Lee *et al*., 1997). A relatively small percentage (1-10 %) of these plants is consumed as food by both humans and other animal species, while more are used for medicinal purposes (Moerman, 1996).

Plant foods contain constituents such as flavonoids, saponins, tannins, phenolics, etc, which have been assessed for their anti-oxidant, anti-mutagenic, anti-carcinogenic and other biological effects (Krishnaswamy and Raghuramulu, 1998). Interestingly, natural product research guided by ethno-pharmacological knowledge has made substantial contributions to drug innovation by providing novel chemical structures and or mechanisms of action (De Smet, 1997).

According to a report by Walker, the medicinal properties of plants could be seen in their response to attacks from insect predators and disease organisms. This is achieved by the accumulation of phytochemicals at the sites of infection of plants, several of which are insecticidal, anti-bacterial, anti-fungal, etc (Walker, 1975; Ameen *et al*., 2005).

MATERIALS AND METHODS

The plant, *Hyptis suaveolens LAM* was randomly collected from the farmland on Navrongo Campus at University for Development Studies, Ghana. It was identified by Dr. Walters M. Kpikpi and Dr. Sarkey, both from Department of Applied Biology, Faculty of Applied Sciences. The entire plant was air-dried at room temperature for three weeks and later ground. The pathogenic fungi: *Aspergillus niger*, *Candida albican*, *Cryptococcus* and *Fursarium* species were collected from the Medical School of University of Legon, Ghana after characterization and identification using microbiological procedures of Cowan and Steel (1965). The solvents and other materials used in this study were of analytical grades bought from Timster laboratory Supplies Limited, Accra, Ghana. These include: 96% ethanol, chloroform, methanol, petroleum ether, distilled water, Dimethylsulphoxide (D.M.S.O), sabouaud dextrose agar, Petri dishes, Whatmann number 1. filter papers, etc.

Extraction procedure: Air-dried, ground plant sample (600 g) was percolated with 2,200 ml of 96% ethanol

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at room temperature for two weeks with intermittent shaking. The percolate was evaporated to dryness at room temperature and a crude extract (ethanol soluble) was obtained. 0.1 g of the crude extract was then transferred in to a vial and kept at the lower compartment of a refrigerator until required for use. The remainder of the crude extract was used in a fractionation process.

Fractionation of crude extract: The crude extract prepared as mentioned above was partitioned between chloroform and distilled water (120, 1:1), using a separating funnel. The chloroform and distilled water soluble fractions were separately evaporated at room temperature to residues. 0.1 g of the chloroform soluble residue and all the distilled water soluble residue were transferred in to two distinct vials and kept at the lower compartment of a refrigerator until required for use. The remainder of the chloroform soluble residue was also partitioned between methanol and petroleum ether (120, 1:1). These solvent soluble fractions were separated and concentrated as the chloroform and distilled water soluble fractions to their respective residues which were transferred in to distinct vials and kept as other residues until required for use.

Qualitative phytochemical evaluation: Phytochemical screening was conducted to determine the presence of natural products in the extracts obtained from the entire parts of *Hyptis suaveolens* LAM.

Alkaloids (Wakama test): 0.2 g of each extract was re-extracted with 1% HCl for 24 h. 2 ml portion of the filtrate was taken and tested for alkaloids by adding drops of Meyer's reagent. Alkaloids formed a picric yellow precipitate with the reagent.

Flavonoids (Willistatter test): To methanol solution of each extract, a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCl. Colors ranging from orange to red indicated flanones, red to crimson indicated flavonols and crimson to magenta indicated flavonones.

Terpenoids and steroids (Liebermann buchart test): A small quantity of each extract was dissolved in trichloromethane and a minimum amount of concentrated sulphuric acid was then added to its content. A blue or green color or a mixture of these two shades was taken as positive test for steroidal compounds, while red, pink or violet color indicates the presence of terpenoids.

Tannins: 0.2 g of each extract was re-extracted with ethanol. The solution obtained was later treated with 5% ferric chloride. A blue-black or blue-green appearance was taken as positive test for tannins.

Saponins: A small portion of each extract was added to 2 ml of distilled water and boiled for 3-5 min. The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins. Froth as high as the aqueous layer was taken as moderate and lower than this as negative for the presence of saponins.

Anthraquinones (Bornstrager-test Kraus modified): About 0.5 g of each extract was boiled for a few minutes with 12 ml of 0.5 M potassium hydroxide and 2 ml of hydrogen peroxide (10 %). The mixture obtained was then cooled, filtered, acidified and extracted with a small quantity of ammonium hydroxide solution. A red color formed in the alkaline layer indicated the presence of anthraquinones.

Aldehydes: To 1 ml of freshly prepared Tollen's reagent in a tilted test tube rinsed with 3 Molar sodium hydroxide solution, one drop of aqueous solution of each extract was slowly added. A silver mirror on the surface of the test tube is a positive test for aldehydes.

Ketones: To 1 ml of aqueous solution of each extract in a test tube, a few drops of 3 molar sodium hydroxide solution was added, followed by a slow addition of 3 drops of iodine solution. The test tube was stopped and shaken vigorously. A positive test resulted from a brown color of the mixture disappearing and a yellow iodo-form solid precipitating out of solution.

Antifungal bioassay: The spreading method of Cruickshanks *et al.* (1980) and dose (agar) diffusion method were used.

Five days old cultures of *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species to be tested were used. 0.1 ml solution of cultures were uniformly spread over the surface of sabouraud dextrose agar with the aid of a sterile inoculating loop. The solvent soluble extracts and griseofulvin antibiotics employed in the test were diluted to obtain different concentrations of 1,500, 1,000 and 500 µg /ml using Dimethylsluphoxide (D.M.S.O). 0.1 ml of various concentrations of the prepared extracts and griseofulvin antibiotics were used to fill holes bored by 5mm improvised cork borer in the inoculated agar. Three plates were made for each extract and organism-griseofulvin, standard drug. The plates were then incubated at 37°C for 24 h. Diameters of zones of inhibition were measured manually in millimeters for the created holes from which sample means were calculated.

RESULTS AND DISCUSSION

From Table 2 results, the crude ethanol extract of *Hyptis suaveolens LAM* did not reveal the presence of alkaloids, flavones, flavonols and flavonones, while subsequent fractions did. This can be explained by masking effect which often occurs when different phytochemicals form a mixture. In this effect, the presence of a particular phytochemical is not noticed because it is being masked or inhibited by other phytochemicals that make up the mixture. In search for phytochemicals from the plant, qualitative analyses of solvent soluble extracts/fractions revealed the presence of alkaloids, flavonoids, terpenoids, tannins, aldehydes and ketones, whereas steroids, saponins and anthraquinones were absent.

Results in Table 1 illustrate the growth inhibitory effect of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin, standard antifungal drug on *Aspergillus niger*. At concentrations of 500, 1,000 and 1,500 µg/ml of the plant extracts/fractions and griseofulvin there was a uniform trend of increase in zones of inhibition. Of all the tested at 500, 1,000 and 1,500 µg/ml concentrations, the distilled water soluble fraction of the plant presented the highest growth inhibitory effect on the isolate. It recorded mean zones of inhibition of 7.5±0.10, 12.0±0.10 and 16.5±0.10. This was closely followed by the chloroform soluble fraction with mean zones of inhibition of 6.0±0.20, 11.0±0.27 and 15.0±0.10. Both the distilled water and chloroform soluble fractions of *Hyptis suaveolens LAM* showed higher growth inhibitory effects on *Aspergillus niger* than the antifungal drug. It is an indication that these soluble fractions contained more toxic components which inhibited the growth of the isolate.

Similarly, from Table 4 results it is observed that the plant extracts/fractions and griseofulvin antibiotics inhibited the growth of Candida albicans in an increasing trend at the concentrations of 500, 1,000 and 1,500 µg/ml. The most toxic soluble fraction of *Hyptis suaveolens LAM* to the isolate is the chloroform soluble fraction with mean zones of inhibition of 5.0±0.10, 10.50±0.10 and 12.50±0.10 in an increasing order at concentrations of 500, 1,000 and 1,500 µg/ml. The methanol soluble fraction of the plant showed the least growth inhibitory effect on Candida albicans just as it did on *Aspergillus niger* with mean zones of inhibition of 1.5±0.10, 3.5±0.20 and 5.50±0.15.

The results in Table 3 clearly revealed the resistance to *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics by *Cryptococcus* species. There were no growth inhibitory effects for griseofulvin antibiotics, methanol and petroleum ether soluble fractions at the concentrations of 500, 1,000 and 1,500 µg/ml. It was only at 1,500 µg/ml concentration that the ethanol soluble extract, the distilled water and chloroform soluble fractions of the plant presented mean zones of inhibition of 5.5±0.10, 6.0±0.20 and 9.0±0.10 respectively. This is an indication that these three soluble fractions of *Hyptis suaveolens LAM* contain toxic components that inhibit the growth of *Cryptococcus* species.

Contrary to results in Table 5 and 6 results showed griseofulvin antibiotics and extracts/fractions of *Hyptis suaveolens LAM* to be toxic to Fursarium species with the methanol soluble fraction presenting the least growth inhibitory effect. At concentrations of 500, 1,000 and 1,500 µg/ml, the distilled water soluble fraction recorded the highest mean zones of inhibition of 12.50±0.10, 19±0.27 and 22.50±0.10 respectively. This is closely followed by the chloroform soluble fraction with mean zones of inhibition of 9.0±0.21, 11.05±0.10 and 18.0±0.10.

A comparison of results from Table 3, 4, 5 and 6 revealed that the soluble fraction of *Hyptis suaveolens LAM* with the highest mean zones of inhibition is alternating between the distilled water and chloroform soluble fractions. To some extend, these two soluble fractions are more active against the isolates than the antifungal drug and therefore contain ingredients which could serve as drugs.

Table 1: Texture, color and weight of solvent soluble extracts/fractions obtained from *Hyptis suaveolens LAM*

Extract/fraction	Texture	Color	Weight (g)
EtOH	Sticky	Dark brown	2.40
$CHCl_3$	Sticky	Dark brown	0.60
Distilled H_2O	Sticky	Dark brown	0.90
MeOH	Sticky	Dark brown	0.20
Petroleum ether	Sticky	Dark brown	0.60

Table 2: Phytochemical screening results of solvent soluble extracts/fractions from *Hyptis suaveolens LAM*

Phytochemicals	EtOH	Distilled H_2O	$CHCl_3$	MeOH	Petroleum ether
Alkaloids	-ve	+ve	-ve	+ve	+ve
Flavones	-ve	+ve	+ve	+ve	+ve
Flavonols	-ve	+ve	+ve	+ve	-ve
Flavonones	-ve	+ve	+ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	+ve
Steroids	-ve	-ve	-ve	-ve	-ve
Tannins	+ve	+ve	+ve	+ve	+ve
Saponins	-ve	-ve	-ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve	-ve	-ve
Aldehydes	+ve	+ve	+ve	+ve	+ve
Ketones	+ve	+ve	+ve	+ve	+ve

+ve means phytochemical is present; -ve means phytochemical is absent

Table 3: Growth inhibitory effect of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Aspergillus niger*

Concentration	Zone of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	4.0±0.10	5.5 ±0.20	6.0±0.20	7.5±0.10	0.0±0.0	5.0±0.10
1,000	6.0±0.10	6.0 ±0.10	11±0.27	12.0 ±0.10	0.50±0.01	7.0±0.30
1,500	12.0±0.17	8.0±0.17	15±0.10	16.5 ±0.10	2.0 ±0.20	12.5±0.01

Table 4: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Candida albicans*

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	4.5±0.10	4.0±0.20	5.0±0.10	4.0±0.10	1.5±0.10	5.0±0.20
1,000	10.0±0.20	10±0.10	10.5±0.10	8.0±0.10	3.5±0.20	8.5±0.10
1,500	12.0±0.10	10.5±0.29	12.5±0.10	11.5±0.10	5.5±0.15	11.0±0.10

Table 5: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Cryptococcus* species

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	0	0	0	0	0	0
1,000	0	0	0	0	0	0
1,500	0	5.5±0.10	9.0±0.10	6.0±0.20	0	0

Table 6: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Fursarium* species

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	5.5±0.10	8.0±0.10	9.0±0.21	12.50±0.10	0	9.0±0.20
1,000	7.0±0.17	12±0.10	11.05±0.1	19.0±0.27	3.0±0.10	12.0±0.10
1,500	10.0±0.10	13±0.17	18±0.10	22.5±0.10	6.0±0.10	14.0±0.10

Conclusion: The investigation was able to prove that *Hyptis suaveolens LAM* contained phytochemicals or agents which were effective against *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species. It also went further to explain that bioactive agents of the plant were more effective in inhibiting the growth of isolates than griseofulvin, antifungal drug. These findings justify the ethno-medicinal uses of the plant and could be of interest to pharmaceutical companies.

ACKNOWLEDGEMENTS

Authors are grateful to the Medical School of University of Ghana, Legon, Ghana, for the fungal isolates provided in the investigation.

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