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Full Length Research Paper

Phytochemical and antibacterial activities of leaf extracts of *Nepeta cataria*

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The phytochemical screening of the dichloromethane and methanolic extracts showed presence of flavonoids (flavones and flavonols), coumarins and glycosides. These secondary metabolites are considered to be responsible for the antimicrobial activity against *Staphyococcus aureus*, *Kliebsiella pneumoniae* and *Salmonella typhii.* The TLC analysis of the dichloromethane extract revealed presence of 3 components with R_f values 0.23, 0.46 and 0.69 using the solvents chloroform: methanol in the ratio of 20: 1.

Key words: *Nepeta cateria*, flavonoids, glycosides, bacteria, dichloromethane.

INTRODUCTION

The main use of plants is for nutritional purposes; however, they are also used at various religious functions, for economic purpose and more importantly for medicinal purposes. Herbal medicine has been shown to be effective and over 60% of the Nigerian population depends on traditional medicine for their healthcare. There are reports of the use of different parts of plants traditionally in Nigeria for the treatment of a wide range of diseases such as fever, dysentery, septic sores, rheumatism, diarrhea, stomach ache, vomiting, skin diseases, etc, (Sofowora, 1982; Olafimihan, 2002). Moreover, orthodox medication is largely unavailable and where available, it is not cheap. Also, pathogenic microorganisms are acquiring multiple resistances to existing antibiotics at an alarming rate globally (Egah et al., 1999). All these factors necessitated the search for alternative medicine of natural origin for the treatment of microbial infections and traditional folk medicine is being used as a guide. Many herbs used in folklore for the treatment of diseases in different parts of the world are being screened for antimicrobial activities and the results obtained from these scientific studies so far have rationalized the tradomedical use of many plants and plant parts (Abo et al., 1999; Elegami et al., 2001; Islam et al., 2001).

Pharmacological and chemical investigations of medicinal plants have provided important advances in the therapeutic approach to several pathologies, as well as extremely useful tools for the theoretical study of physiology and pharmacology.

Nepata cataria L. (catnip) belongs to the botanical family called lamiaceae (labiatae); a perennial herb native of Eurasia and widely naturalized in North America and also some parts of Africa for example Nigeria, etc. (Okezie and Agyakwa, 1987). It is commonly called catnip while the Yorubas and the Urhobos (Nigeria) call it *logbojodu/Egbeowopolo* and *obeigbugugbu*, respectively.

N. cataria L. is an erect-growing plant which can reach a height of one meter, has pubescent leaves and a spike-like inflorescent with purple-spotted flowers. The leaves are square-shaped and lack stipules. Its flowering tops are attractive to bees and are harvested during full bloom and allowed to dry in the shade for preservation of color and fragrance (Akerele, 1991).

It is used locally for treating kidney diseases, flatulence and dysmenorrhoea. It could also be used as a stomach tonic and an enema (Kafaru, 1994).

In this study, the phytochemical screening of plant leaf extract was carried out with a view to identifying the

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presence of the various chemical components that are responsible for the antimicrobial activity of the plant leaves.

MATERIALS AND METHODS

Plant collection and identification

The plants were collected from a traditional herbalist farm in Lagos, Nigeria and brought to Ladoke Akintola University of Technology for identification in the Department of Pure and Applied Biology.

Sample preparation and extraction.

The leaves only were collected and air dried in the laboratory for two weeks and ground into fine powder using a sterilized mechanical grinder. 1.0 kg each of the fine powder was placed in a flask and covered with redistilled n-hexane, dicloromethane and methanol, respectively and allowed to stand for 48 h; after which it was filtered and the filtrate concentrated using a rotary evaporator.

Phytochemical screening of extracts

Identification by chemical test: The method used is that described by Harborne (1973). The extracts were screened for the presence of steroids, flavonoids, tannins, coumarins, alkaloids and glycosides.

Test for flavonoid

1 ml of 10% NaOH was added to 3 ml of the extract/fraction. A yellow coloration observed in each extract/fraction tested indicated the presence of flavonoid in the extract/fraction.

Test for steroids

Salkovoski test; 5 drops of concentrated sulphuric acid was added to 1 ml of the extract/fraction. Observation of red coloration indicates the presence of steroids.

Test for saponins

Frothing test: 2 ml of the extract/fraction in a test tube was vigorously shaken for about 2 min. The observation of frothing indicates presence of saponins.

Test for glycosides

10 ml of 50% sulphuric acid was added to 1 ml of the extract/fraction in a test tube and the mixture was heated in boiling water for 15 min. 10 ml of Fehling's solution was added and the mixture boiled. A brick-red precipitate observed in the extract/fraction indicates the presence of glycosides.

Test for tannins

2 drops of 5% ferric chloride was added to 1 ml of the extract/fraction. A greenish precipitate indicates the presence of tannins.

Test for alkaloids

1 ml of 1% HCl was added to 3 ml of the extract/fraction in a test tube. The mixture was heated for 20 min, cooled and filtered. The filtrate was used for the following tests: (a) 2 drops of Meyer's reagent was added to 1 ml of the filtrate. Observation of a creamy precipitate in the filtrate indicates the presence of alkaloids. (b) 2 drops of Wagner's reagent was added to 1 ml of the filtrate. A reddish brown precipitate observed in the filtrate indicates the presence of alkaloids.

Identification by qualitative thin layer chromatographic analysis

An already coated Merck silica gel plate was obtained and each extract spotted on the plate. Various solvent mixtures were prepared using different ratios in order to determine the appropriate developing solvent mixture. The best solvent mixture obtained was chloroform: methanol in the ratio of 20: 1 which separated components into a wide range of R_f values. The R_f values obtained are given in Table 3. The components were visualized under uv/visible light (254 and 366 nm) and sprayed with the following reagents in order to reveal spots of different groups: Dragendorff's reagent for alkaloids, methanolic potassium hydroxide for coumarins, aluminum chloride for flavonoids and sulphuric acid for steroids and terpenes (Bourrel et al., 1993; Recio et al., 1989; Vanden et al., 1991).

Antibacterial screening

The Bauer et al. (1966) disc diffusion method was used to determine the antibacterial activity. Nutrient agar was used as the mechanism and clinical isolates of *Salmonella typii, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Bacillus subtilis, Proteus vulgaris, Escherichia coli* and *Pseudomonas aeruginosa* were obtained from Baptist Medical Center, Ogbomoso, Nigeria.

Preparation of the medium

Nutrient agar medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min. It was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was measured (4 cm).

Preparation of test samples

1.0 mg of the extract was dissolved in 1.0 ml of redistilled solvents. The activity of Streptomycin was also determined and used as the positive control; 2.5 mg of it was dissolved in 1.0 ml of distill water.

Method

Disc diffusion method of Bauer et al. (1966) was employed. This involved the use of filter paper disc as carrier for the antibacterial agent. Sterilized discs cut from Whatman no. 1 filter paper was impregnated with solution of the antibacterial agent at different concentrations. The solvent was evaporated and the disc dried properly. The nutrient agar medium was inoculated with the test organism and the impregnated disc placed on the surface of the nutrient agar. The antibacterial agent upon contact with the agar diffused into all directions. The ability of the test organism to grow or not in the presence of the test sample was then determined

Table 1. Phytochemical screening of *Nepeta cateria leaf* extracts.

Extract	Alkaloids	Flavonoids	Steroids	Saponins	Tannins	Coumarins	Glycosides
MOH	-	+	-	-	-	+	+
DCM	-	+	-	-	-	+	+
HEX	-	-	-	-	-	-	-

Key: - = absent; + = present, DCM = Dichloromethane; MOH = Methanol; HEX = n-hexane.

Table 2. Antibacterial activity and minimum inhibitory concentration of *Nepeta cateria* leaf extracts (1 mg/ml).

Test organism	Zones of inhibition (mm)				MIC
Bacteria	**HEX**	**DCM**	**MOH**	**STR**	
Bacillus subtillis	00.00	00.00	00.00	22.50	-
Staphylococcus aureus	00.00	24.00*	25.00*	21.00	0.1*
Kliebsiella pneumoniae	00.00	17.00*	17.00*	00.00	0.1*
Corybacterium pyogenes	00.00	00.00	00.00	19.00	-
Proteus mirabilis	00.00	00.00	00.00	00.00	-
Shigella dysenteriae	00.00	00.00	00.00	22.00	-
Escherichia coli	00.00	00.00	00.00	00.00	-
Proteus vulgaris	00.00	00.00	00.00	20.50	-
Pseudomonas aeruginosa	00.00	00.00	00.00	30.00	-
Salmonella typhii	00.00	23.00*	24.00*	26.00	0.1*

Key: MIC = Minimum inhibitory concentration; HEX = n-hexane; DCM = Dichloromethane; MOH = Methanol; ST = *Salmonella typhii;* CP = *Corybacterium pyogenes*; PM = *Proteus mirabilis*; SA = *Staphyococcus aureus*; SD = *Shigella dysenteriae*; EC = E. *coli*; KP = *Kliebsiella pneumoniae*; PV = *Proteus vulgaris*; PA = *Pseudomonas aeruginosa;* * = Mean of quadruplicate determinations.

Table 3. R_f values of fractions.

Sample	R_f values	Solvent system
Dichloromethane extract	0.23 0.46 0.69	Chloroform: Methanol (20:1)

Key: R_f = Retention factor.

within 24 h by measuring the zones of inhibition. The plates were incubated upside down at 37°C. Redistilled solvent was used as the negative control. All test carried out were done in triplicate and the antibacterial activity was expressed as a mean of inhibition diameters (mm) produced by the leaf extracts.

RESULTS

The phytochemicals assay of the leaf extracts of *N. cateria* revealed the presence of flavonoids, coumarins and glycosides in the extracts of methanol and dichloromethane. The test revealed the absence of alkaloids, saponins, tannins and steroids. The result is as presented in Table 1. The result of antibacterial activity of the extracts of *N. cateria* and the standard antibiotic, streptomycin are shown in Table 2. The n-hexane extract was not active against any of the isolates while the methanolic and dichloromethane extracts were potent against the isolates *S. typii, S. aureus and K. pneumonia* and inactive against *C. pyogenes*, *P. vulgaris, E. coli, P. vulgaris, P. aeruginosa, S. dysenteriae and B. subtilis* . The methanolic and dichloromethane extracts compared favourably with the standard antibiotic, streptomycin for *S. typii* and *S. aureus*. The retention factors were obtained using the solvent system: chloroform: methanol (v/v) in the ratio 20:1 as shown in Table 3.

DISCUSSION

The phytochemical screening of the extracts revealed presence of flavonoids, coumarins and glycosides (Table 1). The presence of flavonoids and a certain degree of lipophilicity might be responsible for the toxicity of the

plant leaf extract to the tested microorganisms (Tomas-Berberan et al., 1990). This could be as a result of interactions with the membrane constituents and their arrangements. Other authors showed the presence of terpenes and isomers of nepalactones (Bourrel et al., 1993; Recio et al., 1989; Vanden et al., 1991).

The antibacterial screening of the extracts showed that the dichloromethane and methanolic extracts exhibited antibacterial activity against *S. aureus*, *K. pneumoniae* and *S. typhii* with MIC of 0.1 mg/ml and inactivity against *P. mirabilis, C. pyogenes, S. dysenteriae, E. coli, P. vulgaris* and *P. aeruginosa* (Table 2). This activity was more pronounced against Gram-positive than Gram-negative bacteria. This could be as a result of the morphological differences between these micro-organisms. The Gram-positive bacteria have a thick outer peptidoglycan layer in their cell wall which is not an effective permeability barrier that makes it to be more susceptible to the extract while Gram-negative bacteria possess a thin peptidoglycan layer plus an outer phospholipidic membrane that carries the lipopolysaccharide components which makes the cell wall impermeable to lipophilic solute (Scherrer and Gerhardt, 1971; Prescott et al., 1990). The porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaar, 1985). The antibacterial activity reported by Nostro et al. (2000) showed inhibition against *E. coli*, *P. vulgaris* and *P. aeruginosa,* although the inhibition was reported to be very low. However, it is difficult to give a good comparison of this data with that of literature because of some variables such as environmental and climatic conditions of the plant and the method of extraction and antimicrobial screening. Moreover, there are no standard criteria for the evaluation of the plant of study and therefore the results obtained by different authors vary widely.

The thin layer chromatographic analysis of the dichloromethane and methanolic extracts were similar and gave three spots with R_f values 0.23, 0.46 and 0.69 (Table 3). The three spots gave positive test for the presence of flavones and flavonols but negative for isoflavone, anthocyanins, flavonones and leucoanthocyanins when sprayed with conc. H_2SO_4 and a solution of 10% NaOH, respectively.

Conclusion

The dichloromethane and methanolic extracts showed presence of flavones and flavonols; and these were observed to be responsible for the antimicrobial activity of the extracts. The results obtained might be considered sufficient for further studies aimed at isolating and identifying the single active principle(s) and evaluating the antimicrobial activity.

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