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Phytochemical and Antimicrobial Evaluation of the Potency of *Nepata cateria* Leaves against Some Pathogens

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ABSTRACT

A phytochemical and antimicrobial investigation was undertaken to determine the efficacy of the methanolic extract of the leaves of *Nepata cateria*, locally used for the treatment of diarrhea and dysentery in Gombe State, North Eastern Nigeria. Phytochemical screening confirmed the presence of bioactive secondary metabolites, namely phenols, tannins, terpenoids, glycosides, resins, phlobatannins, alkaloids, saponnins and volatile oils. Carbohydrates, flavonoids and sterols were absent. The leaf extract showed significant antimicrobial activity against *Escherichia coli*, *Shigella dysenterica*, *Streptococcus pyogenes* but had no effect on *Salmonella typhii* and *Staphylococcus aureus*. This correlates with the ethnomedicinal claims, thus providing for the first time, a pharmacological basis for the folkloric use of leaves of *N. cateria* as a cure for diarrhea and dysentery in Gombe State.

Keywords: antimicrobial investigation, ethnomedical claims, dysentery, phlobatannins, *Salmonella typhi*, terpenoids

INTRODUCTION

Plant-based drugs have gained popularity due to their fewer side effects, better patient tolerance, and relatively less expense than synthetic drugs and are therefore more accepted due to a long history of use. Plants are also the source of most natural and synthetic drugs (Clark 1996; Iniaghe *et al.* 2009). Biomolecules of plant origin can serve as alternatives for the control of resistant human pathogens. For instance, *Nepata* species (*Labiateae*) are used in traditional medicine in many countries and have large ethnobotanical effects with diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and carminative properties (Nostro *et al*. 2001; Ghannadi *et al*. 2003). The *Labiateae* contains 6900 genera (Heywood *et al.* 2007) and 7200 species, many of which are aromatic, including many widely used culinary herbs like basil, mint, rosemary, sage, savory, marjoram, oregano, thyme, lavender, and perilla.

Nepeta cataria is famous for inducing a delirious state in felines. Common names include cat wart, catmint, catnip (English), *bunsurun fadama* (Hausa), *kachikachiga* (Fulani) and *wandin* (Tangale). Throughout history, this herb has been used in humans to produce a sedative effect (Tyler 1994). Several disease conditions, including cancer, toothache, and corns have been treated traditionally with this plant (Still *et al*. 1979). It is used traditionally to reduce gas and acts as a digestive (Sherry *et al.* 1979). It is used as a household herbal remedy, being employed especially in treating disorders of the digestive system, and stimulates sweating, thus reducing fever (Chievavier 1996). The herb's pleasant taste and gentle action makes it suitable for treating cold, flu and fevers in children. A diethyl ether extract of *N. cataria* has been shown to have antimicrobial activity against fungi (*Aspergillus* and *Penicillium* spp.) and Gram-positive bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritis* and *Pseudomonas aeruginosa*) (Nostro *et al*. 2001). The oil from *N. cataria* showed antibacterial activity against all the same strains of bacteria tested (Zenasni *et al.* 2008) but was more effective when used in conjunction with elder flower (*Sambucus nigra*) (Chevallier 1996). It is useful in the treatment of restlessness and nervousness in children (Grieve 1984). A leaf infusion is also applied externally to bruises, especially black eyes (Genders 1994). The major compound found in *N. cataria* essential oil is nepetalactone, the stereoisomer 4a-α,7-α,7a-β-nepetalactone and dihydranepetalactone which represents more than 82% of the oil from the plant leaves (Zenansni e*t al.* 2008). *N. cataria* also contains acetic, butyric, nepetalic and valeric acids, citral dipentene, citronella, limonene, tannins, terpene and volatile oils (HDW Inc. 2008).

The plant is said to deter insects such as ants, fleas and beetles (Holton 1979) as well as rats and mice from perching on them (Huxley 1992).

Botanically, *N. cataria* is a perennial, minty-smelling herb, growing upright (Chevallier 1996; Heywood 2007) 30-90 cm tall (Clapham 1962; Chiej 1984). Stems are branching and square and the toothed, heart-shaped leaves covered with downy hairs give the whole plant a grayish-green appearance. Clusters of white or pale-lavender scented flowers grow at the ends of the branches. The plant flowers from July to November and seeds ripen from September to October. The flowers are hermaphroditic and bee-pollinated. The plant grows in light sandy and medium loamy soils and requires well-drained dry or moist soil. It flourishes in acid, neutral and basic soils and grows in very alkaline soil by road sides and near streams, hedgerows, borders of fields, dry barks and waste ground, especially on calcareous and gravelly soils but does not grow in the shade (Clapham 1962; Chiej 1984).

In Gombe, North Eastern Nigeria, the leaves of *N. cataria* are used as an oral decoction and for the treatment of dysentery and diarrhea both in children and adults (pers comm. Baba Hamidu of Kaltugo LGA, Gombe State).

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Research Note

This paper reports on the phytochemicals in the leaves of this indigenous medicinal plant and the potential of the methanolic extract against microbial activity related to its ethnomedicinal disease-relieving properties.

MATERIALS AND METHODS

Collection of plant materials

Fresh *N. cateria* leaves were collected in September, 2008 from Gombe State University Campus after flowering. The samples were identified in the Biological Science Department of the University and a specimen of the plant was deposited in their herbarium (forestry herbarium index (FHI) number = 043). The sample (1.2 kg) was air dried in the laboratory before grinding to a fine powder using a pestle and mortar to about 70 mesh sizes and then stored in a dry container.

Methods

1. Extraction

Powdered leaves (140 g) were percolated with distilled methanol (4.5 L) for three-days after which the methanol extract was decanted, filtered, and concentrated by simple distillation to obtain a methanol-soluble fraction ($F_{EG}01$), 35 g in weight.

2. Qualitative chemical tests

All samples were prepared using the following three references: Abulude (2001), Abulude *et al*. (2007) and Ayo *et al*. (2007).

Test for tannins: Two drops of 5% $FeCl_3$ was added to 1 ml of the extract. A dark-green precipitate indicated a positive test.

Test for glycosides: 10 ml of 50% H_2SO_4 was added to 1 ml of extract in a test tube; this mixture was heated in boiling water for 5 min. 10 ml Fehling's solution A and B (5 ml each) was added and boiled. A brick-red precipitate indicated a positive test.

Test for resins: 2.5 ml of copper (II) sulphate solution was added to 2.5 ml of the extract. The resulting solution was shaken vigorously and allowed to separate. A green colour indicated a positive test.

Test for saponins (frothing test): 2 ml of extract was vigorously shaken in a test tube for 2 min. Frothing indicated a positive test.

Test for phlobatannins: 5 cm^3 of distilled water was added to 5 cm^3 of extract solution and boiled with 1% HCl for 2 min. A deep-green colour indicated a positive test.

Test for flavonoids: 2 ml of the extract of solution was heated with 10 ml of ethyl acetate on a water bath and cooled. The layers were allowed to separate and the red ammonia layer that forms indicated a positive test.

Test for sterols (Salkowski test): 2 ml of conc. H_2SO_4 was added 2 ml of extract solution. A red precipitate indicated a steroidal ring.

Test for phenols: Equal volumes of extract solution and $FeCl_3$ were mixed. A deep bluish-green solution confirmed the presence of phenols.

Test for volatile oils: 0.2 g of the extract was mixed with 90% ethanol and 3 drops of ferric chloride were added. The appearance of a green coloration confirmed the presence of volatile oils.

Test for carbohydrates (Fehling test): 5 ml of the mixtures of an equal volume of Fehling solution A and B was added to 2 ml of the extract in a test tube. The resultant mixture was boiled for 2 min. A brick-red precipitate of copper oxide indicated a positive test.

Test for alkaloids: 1 ml of conc. H_2SO_4 was added to 3 ml of the extract, which was then treated with a few drops of Wagner reagent. A reddish-brown precipitate indicated a positive test.

Test for terpenoids (Solkowski test): 0.2 g of the extract sample was mixed with 2 ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface indicated a positive result.

Determination of antimicrobial activity

An antimicrobial test for the extract was carried out in the Microbiology laboratory of the Federal University of Technology, Yola (Fereshteh 2005).

Microorganisms

Clinical isolation of *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysentyrae*, *Streptococcus pyrogenes*, *Salmonella typhii* ($Gram^{+ve}$ = *Staph. aureus*, *Strep. pyrogenes*; $Gram^{-ve}$ = *S. typhi*, *E. coli*, *S. dysentyrae*) were obtained from the Yola Specialist Hospital, Adamawa State, Nigeria where they are cultured for universities and other research organizations. Most of these cause simple opportunistic infectious diseases, e.g., *E. coli* which causes diarrhoeal diseases, urinary infections, wound infections, and bacteraemia; *S. dysenteriae* causes bacillary dysentery and bacteraemia; *S. pyogenes* causes respiratory tract infections; *S. typhii* causes enteric fever, typhoid, and occasionally meningitis, osteomyelitis and arthritis.

Preparation of agar medium

Nutrient agar is a general-purpose medium used for the sensitivity test and as a basis for other media. It was prepared as follows: 2.9 g of nutrient agar was weighed and suspended in 95 ml of distilled water. The mixture was heated on burner for proper and thorough dissolution. The mixture was sterilized by autoclaving at 121°C for 15 min. The solution was allowed to cool to 45°C and then poured into a sterile Petri dish and allowed to set at room temperature and stored in a refrigerator for use. Medium and medium bottles were sterilized by autoclaving for 15 min at 121°C. The wire loop and forceps used for inoculation and introduction of the extract onto the plate respectively were flame sterilized before inoculation of microorganisms in a sterilized environment using acetone.

Preparation of the culture medium

Culture medium was prepared by adding 1.3 g nutrient broth to100 ml distilled water. After heating on a Bunsen burner for complete dissolution, 15 ml of the solution was pipetted into universal bottles which were then sterilized by autoclaving at 121°C for 15 min. The broth solution was allowed to cool to about 45°C and test microbes were in this broth at 37°C for 24 h in an incubator.

Preparation of paper discs

Using an ordinary office two-hole puncher, paper disks with an approximate diameter of 6.3 mm were punched out one by one from Whatman #1 filter paper; caution was taken to avoid overlapping of holes since the paper disks had a tendency to curl after punching and these were flattened by spreading them in a single layer on a clean smooth surface then pressed by rolling a bottle repeatedly. The disks were placed in vial then autoclaved for 15 min at 121°C and allowed to cool.

Impregnation of disks

The immersion method (NCCLS 1995) was used. Disks were soaked in the extract solution and then allowed to dry for 2 h.

The dish diffusion test

Sterilized Petri dishes containing the set nutrient agar were inoculated with one microbial species each using a swab stick according to Coyle *et al*. (1984). The plates were labeled. The paper disks impregnated with the extract were placed on the surface of solid

medium, which was previously inoculated with the test bacterium. The inoculated plates containing the extract (paper disks) were incubated for 24 h. The disk diameter and zone of inhibition were observed for proper evaluation.

Experimental design and statistical analyses

The antibacterial activity of the extracts was determined using the agar well diffusion method according to Adeniyi *et al*. (2008). Agar plates used for the sensitivity test were seeded with 0.1 mL of an overnight culture of each bacterial isolate (equivalent to 10^7 – 10^8 cfu/mL). The seeded plates were allowed to set and a standard cork borer 8-mm in diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.1 mL of each extract at a concentration of 0.025 mg/mL. The antibiotic ampicillin (NICHBEN Pharmaceutical Ind. Ltd., Nigeria) at 0.01 g/mL was used as the positive control and distilled water as the negative control. The plates were incubated at 37°C for 24 h after which the diameter of the zones of inhibition were measured. Each treatment was conducted in triplicate as recommended by Fereshteh *et al*. (2005).

RESULTS AND DISCUSSION

The methanolic extract of *N. cataria* leaves contain pharmacologically useful classes of compounds. Tannins, terpenoids, glycosides, resins, phenols, phlobatannins, saponins, alkaloids and volatile oils were present. Carbohydrates, flavonoids, and sterols were absent (**Table 1**). These secondary metabolites can function in a synergistic or antagonistic fashion. Their mixture shows a broad spectrum of biological effects and pharmacological properties. For instance, tannins are known to possess physiological antigen properties, which hasten wound healing and ameliorate inflamed mucus membranes (Tyler *et al.* 1988). They also have haemostatic properties (Awosika 1991). Saponins have an expectorant action which is very useful in the management of upper respiratory tract inflammation, thus being present in plant, is cardiotonic in nature (Finar 1989; Tease and Evans 1989). They also have anti-diabetic properties (Kamel 1991). Alkaloids have analgesic, anti-inflammatory and adaptogenic activity which helps to alleviate pains and develop resistance against diseases and enhances endurance against stress (Guta 1994). The phonological age of the plant, percentage humidity of the harvested material, geographical location, climatic conditions, soil condition, time of harvest, and the method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix 1982).

Table 1 Phytochemical screening of the methanol extract of the leaves of *Nepeta cataria*.

Chemical compounds	Extract
Tannins	+
Saponins	+
Sterols	-
Terpenoids	+
Glycosides	+
Phlobatannins	+
Resins	+
Flavonoids	-
Phenols	+
Alkaloids	+
Essential oils	+
Carbohydrates	-

+ = present; - = absent

Antimicrobial activities of the extract

The methanolic extract of *N. cataria* leaves showed antimicrobial activity against all the test microorganisms except for *S. aureus* and *S. typhii* (**Table 2**), a clear indication that the plant can not treat typhoid, but could treat dysentery and diarrhoea, common opportunistic diseases caused by some of these microorganisms. This is consistent with the activity of secondary plant metabolites of several medicinal plants (Lin *et al.* 2001). This also strengthens the use of the plant by herbalists and Gombe State local doctors for treatment of diarrhea or dysentery and other human ailments. The antibacterial property could be due to the resin and tannin content in the extract, both of which possess appreciable antimicrobial activity (Narayana *et al.* 2001). The result confirms the popular usage of this plant in northern Nigeria (Kumarawa *et al.* 2008) and in the Republic of Mali (Adiaratou *et al.* 2005). Ampicillin (positive control) showed better performance with greater zones of inhibition than the crude methanolic leaf extract.

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Table 2 Antimicrobial activities of the methanol extract of *Nepeta cataria* leaves.

Microorganisms	Extract diameter of zone of inhibition (mm)	Positive control ampicillin diameter of zone of inhibition (mm)	Negative control water diameter of zone of inhibition (mm)
Escherichia coli	8.0	10.0	-
Staphylococcus aureus	NA	8.0	-
Shigella dysentryae	10.5	12.0	-
Streptococcus pyogene	12.0	15.0	-
Salmonella typhii	NA	13.0	-

NA = not active; Concentration of the extract used = 0.025 mg/ml
Concentration of ampicillin = 0.01 mg/ml

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