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(RESEARCH ARTICLE)

Comparative secondary metabolite compositions and anti-microbial properties of n-hexane and ethyl-acetate fractions of *Nelsonia campestris*

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Abstract

Nelsonia campestris is a medicinal plant commonly used to treat measles caused by morbillivirus. This study assessed the phytochemical composition, in vitro antimicrobial activity of n-hexane and ethyl-acetate fractions of *Nelsonia campestris*. Phytochemical composition was determined using standard procedures. Antimicrobial test against six microorganisms, including *K. pneumonia, S. aureus, B. subtilis, E. coli, P. aeruginosa* and *S. typhi* was investigated using agar diffusion method while minimum inhibitory concentrations were determined by broth micro dilution method. Results revealed the presence of alkaloids, phenols, tannis, flavonoids, saponins and steroids in both n-hexane and ethyl-acetate fraction. Glycoside was present in n-hexane fraction but absent in ethyl-acetate fraction while volatile oil and anthraquinone were completely absent. Quantitatively, n-hexane fraction had higher phenol (57.12±0.01 mg/100g), tannins (19.76±0.04 mg/100g), and saponins (104.78±0.05 mg/100g) while ethyl-acetate had higher flavonoid (27.85±0.50 mg/100g) and alkaloids (16.62±0.01 mg/100g). The ethyl-acetate fraction was highly active against all test organisms with inhibition diameters in the range of 7.50±0.56 mm (*P. aeruginosa*) to 21.50±0.32 mm (*E. coli*) The MIC ranges between 2.5-20 mg/mL while the MBC ranged between 5.0-30 mg/ml for the ethyl-acetate fraction. The n-hexane fraction was active only against *P. aeruginosa* and *B. subtilis* with inhibition diameters in the range of 12.50±0.10 mm-17.55±1.00 mm. This study has shown that the n-hexane and ethyl-acetate fractions of *Nelsonia campestris* contain some useful potential bioactive principles that are inhibitory to some pathogenic organisms. The ethyl-acetate fraction was more active than n-hexane fraction and thus could be considered as a natural source of antimicrobials for therapeutic purposes.

Keywords: *Nelsonia campestris*; Secondary metabolite; Antimicrobial; N-hexane and ethyl-acetate fractions

1. Introduction

Microbial infections remain a threat to millions of lives globally as they are ranked the second leading cause of death across the world and the third leading cause of death of economically developed countries [1]. Currently there is an increased problem of antibiotic resistance due to microbial resistance [2]. The uses of antibiotics are widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes [3]. This has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages [4]

Plants are free gifts of nature available to man for various pharmacological uses. The World Health Organization (WHO) recognizes traditional medicine, particularly plant medicine, as an important alternative healthcare delivery system for most of the world's population [5]. They are source of active chemical compounds such as alkaloids tannins, flavonoids, sugars phenols, coumarins, terpenoids, saponins, etc useful to both Man and animals. In Africa,

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traditional medicine, especially plant medicine, provide many citizens with affordable healthcare services [6-8]. Since prehistoric times man has used plants for various purposes and will continue to do so as long as life continues on this planet [9].Traditional medicine is either the mainstay of health care delivery or serves as a complement to it across the world [10]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Many plant leaves have antimicrobial principles such as tannins, essential oils tannins, flavanoids and other aromatic compounds [11].

Nelsonia campestris (Lam.) Spreng of the family Acanthaceae, is a small trailing delicate herb with long underground root and aerial spreading stem with plenty of leaves. The traditional healers use the root, fruit, and leaves of the plant for different disease conditions [12]. This plant, known as "Isa bakaggi," by the traditional practitioners of Nupe speaking people of Niger state, Nigeria, is used to treat measles caused by morbillivirus of the Paramyxovirus family, with concurrent development of other opportunistic infections such as pneumonia, conjunctivitis, diarrhea, and infection of the otitis media [13]. It is also used traditionally for the treatment of skin infections such as chicken pox, fevers, constipation and gastric ulcer, in addition to measles and lots of others. Studies has documented the antibacterial and safety profile of the crude extract of this plant [12-14]. However, scientific literature on antimicrobial properties of solvent fractions of this plant is currently unavailable, the present study therefore evaluated the secondary metabolites compositions and *in vitro* antimicrobial profile of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* leaf.

2. Material and methods

2.1. Plant sample

Fresh samples of *Nelsonia campestris* was obtained from the premises of Federal University of Technology, Minna, Bosso Campus, Niger State Nigeria. A taxonomic authentication of the plant was carried out by Botanist at the Department of Biological science, Federal University of Technology, Minna. Nigeria.

2.2. Sources of microorganisms

The microbial isolates used were collected from the Laboratory of Microbiology Department Federal University of Technology, Minna, Nigeria. The microorganisms include: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonie, Escherichia coli*, and *Salmonella typhii*. These organisms were obtained and identified by standard microbiological methods. The organisms were maintained on agar slants at 4oC and sub-cultured for 24 hours before use.

2.3. Sample preparation and extraction

The collected fresh samples of *Nelsonia campestris* were washed with clean-water, dried at room temperature and finally grounded using a grinder mill and dried at room temperature. Extraction of plant materials was performed by weighing 50 g of the powdered plants and extracted by soxhlet extraction using 200 ml each of methanol [13]. The resulting extract was concentrated in a water bath and then fractionated with n-hexane and ethyl-acetate to give n-hexane and ethyl-acetate fractions respectively.

2.4. Qualitative and quantitative screening for secondary metabolites

The plant extract was analyzed for the presence of some secondary metabolite including alkaloids, terpenes, tannins, saponins, phenols, steroids, phlobatannins and flavonoids using standard procedures [15-18]. Quantitative analysis was conducted for flavonoid, alkaloids, total phenol, tannin and saponins using standard procedures [19].

2.5. Antimicrobial activity

The antimicrobial activities of the fractions were evaluated at concentrations of 20, 30, and 40 mg/ml using agar well diffusion method as described by Yusuf *et al.* [20]. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant fractions. The minimum inhibitory concentration (MIC) and Determination of minimum bactericidal concentration (MBC) were evaluated as reported by Tsado et al. [21]. MIC was determined as the lowest concentration of the fractions permitting no visible growth (no turbidity) of the organism. The MBC was determined by sub-culturing the test dilution on fresh solid medium and further incubating at 37^oC for 18-24 hours. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

2.6. Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 21 and presented as means ± SE of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$.

3. Results

3.1. Phytochemical composition

Phytochemical screening of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* revealed the presence of alkaloids, phenols, tannis, flavonoids, saponins and steroids in both n-hexane and ethyl-acetate fraction. Glycoside was present in n-hexane fraction but absent in ethyl-acetate fraction while volatile oil and anthraquinone were completely absent (Table 1). Quantitatively, n-hexane fraction had higher phenol (57.12±0.01 mg/100g), tannins (19.76±0.04 mg/100g), and saponins (104.78±0.05 mg/100g) while ethyl-acetate had higher flavonoid (27.85±0.50 mg/100g) and alkaloids (16.62±0.01 mg/100g (Table 2).

Table 1 Qualitative phytochemical composition of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* extract

Phytochemicals	n-hexane	Ethyl-acetate
Total phenol	+	+
Total flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	+	+
Glycoside	-	+
Volatile oil	-	-
Anthraquinonne	-	-

Key: + means present and – means absent

Table 2 Quantitative phytochemical composition of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* extract

Phytochemicals	n. Hexane (mg/100g)	Ethyl-acetate (mg/100g)
Total phenol	57.12±0.01	54.59±0.01
Total flavonoids	11.06±0.01	27.85±0.50
Tannins	19.76±0.04	12.58±0.02
Alkaloids	12.84±0.00	16.62±0.01
Saponins	104.78±0.05	86.78 ±0.03

3.2. Antimicrobial activities

The Zones of inhibition of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* against *K. pneumoniae, S. typhi*, *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis* are presented in table 3. The zones of inhibition demonstrated by the extracts increased with increasing concentration. The ethyl-acetate fraction was highly active against all test organisms with inhibition diameters in the range of 7.50±0.56 mm (*P. aeruginosa*) to 21.50±0.32 mm (*E. coli*) The MIC ranges between 2.5-20 mg/mL while the MBC ranged between 5.0-30 mg/ml for the ethyl-acetate fraction. The n-hexane fraction was active only against *P. aeruginosa* and *B. subtilis* with inhibition diameters in the range of 12.50±0.10 mm-17.55±1.00mm. However standard antibiotics demonstrated highest activity against all organisms tested with inhibition zone in the range 28.06±0.34 mm-35.07±0.03 mm.

Table 3 Antimicrobial activities of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* showing zones of inhibition of the organisms

Organism	Ethyl-acetate fraction (zone of inhibition MM)			n-hexane fraction (zone of inhibition MM)			Ciprofloxacin (mg/ml)
	20 mg/ml	30 mg/ml	40 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	40
P. aeruginosa	7.50±0.56	11.5±0.56	12.5±0.32	12.50±0.10	13.50±0.23	17.55±1.00	28.06±0.34
B. subtilis	12.50±0.50	14.00±0.40	17.5±0.10	13.00±0.50	12.00±0.55	15.00±0.15	33.00±0.00
K. pneumonia	10.00±0.04	14.0±0.55	16.00±0.10	-	-	-	35.07±0.03
S. aureus	13.00±0.42	16.00±0.07	17.50±0.50	-	-	-	27.50±0.50
E. coli	16.50±0.50	16.00±0.15	21.50±0.32	-	-		28.50±0.50
S. typhi	10.50±0.10	11.50±0.55	13.00±0.50	-	-	-	30.50±0.0

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of ethyl-acetate fractions of *Nelsonia campestris*

Test Organisms	MIC (mg/ml)	MBC (mg/ml)
P. aeruginosa	20	30
B. subtilis	20	20
K. pneumonia	2.5	5.0
S. aureus	10	20
E. coli	5	`10
S. typhi	10	20

4. Discussion

Plants are admittedly, a valuable reservoir of bioactive compounds of substantial medical importance [22]. Qualitative phytochemical analysis clearly demonstrated the presence of a number of important active constituents and revealed that both n-hexane and ethyl-acetate fractions of *N. campestris* have similar phytochemical constitution of alkaloids, phenols, tannis, flavonoids, saponins and steroids. The presence of these phytochemicals has been previously reported for crude aqueous extract of *Nelsonia campestris* [13]. Only the glycoside was present in n-hexane fraction but absent in ethyl-acetate fraction. This agrees with the previous study which reported that not all phytochemicals are present in medicinal plants and those present varies with their solvents [23]. Indeed, all kinds of metabolite classes detected into the samples (saponins, flavonoids, tannins, alklaoids and phenols) are well known to have significant inhibitory action against bacteria and fungi [24]. This fact can justify why all fractions showed antimicrobial activity.

Several authors have established that compounds, such as phenols, flavonoids, alkaloids and tannins, have antimicrobial activity [25-27]. These properties arise from polyphenol and tannin capacity to inhibit enzymes and alter membrane characteristics. Enzyme inhibition by phenols results from compound oxidation, possibly through sulfhydryl group reaction with or through nonspecific protein interactions. Flavonoids can cause cell membrane disruption, and form complexes with soluble and extracellular proteins as well as with membrane walls [25-26]. On the other hand, quantitative assays revealed that the extracts have different quantity of the secondary metabolite which can be correlated to their antimicrobial activity.

The antimicrobial activities of n-hexane and ethyl-acetate fractions of *Nelsonia campestris* showed that the fractions had varying degree of antimicrobial activity against the test organisms. On the basis of the results the fractions demonstrated to be more active on Gram-positive than on Gram-negative bacteria. This was not surprising since previous studies have reported that generally plant extracts are usually more active against Gram-positive bacteria than Gram-negative bacteria, and the susceptibility may be due to structural differences in the cell wall of these classes of bacteria. Cells of Gram-negative bacteria are surrounded by an additional outer membrane, which provide them with a hydrophilic surface that functions as a permeability barrier for many substances including natural compounds [28-29]. It was also observed that increase in the concentration of all the extracts yielded their corresponding increase in the zones of inhibition. This linear relationship between the concentrations of extracts and zones of inhibition could be that the extracts were able to diffuse into the inoculated nutrient agar [30]. The ability of the n-hexane and ethyl-acetate fractions of *Nelsonia campestris* to inhibit the growth *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi*, *Staphylococcus aureus* and *E.coli* explains why it is used in folk medicine for the treatment of gastrointestinal and respiratory complications associated with measles.

The extracts were found to be more active with greater zone of inhibition at concentration of 40 mg/ml, this was in accordance with the results of the previous studies, who reported that the higher concentration of antibacterial substance showed appreciable growth inhibition of bacteria [13, 30-31]. Ethyl-acetate fraction was highly active against all test organisms with inhibition diameters in the range of 7.50±0.56 mm (*P. aeruginosa*) to 21.50±0.32mm (*E. coli*) while the n-hexane fraction was active only against *P. aeruginosa* and *B. subtilis* with inhibition diameters in the range of 12.50±0.10 mm-17.55±1.00mm. This result is not surprising since total flavonoids and alkaloids which were the major antimicrobial metabolites are found in higher concentration in the ethyl-acetate fraction than the n-hexane fraction. However, despite the higher zone of inhibition demonstrated by the ethyl-acetate fraction of *Nelsonia campestris*, the zone of inhibitions was lower on all test organism compare to zone of inhibitions demonstrated by standard antibiotics drug (ciprofloxacin) used in this study.

Minimum inhibitory concentration (MIC) is the lowest concentration of an extract that inhibit the visible growth of the test organism after 24hrs incubation [32-33]. In the present study MIC was measured based on turbidity or visible growth show by the organism. The MICs of ethyl-acetate fractions of *Nelsonia campestris* MIC ranges between 2.5-20 mg/ml. This MIC is lower than 80-90 mg/ml previously reported for crude aqueous extract of *Nelsonia campestris* [13], thus indicating higher activity of the fractionated extract compared to the crude extract.

5. Conclusion

This study has shown that the n-hexane and ethyl-acetate fractions of *Nelsonia campestris* contain some useful potential bioactive principles that are inhibitory to some pathogenic organisms. The ethyl-acetate fraction was more active than n-hexane fraction and thus could be considered as a natural source of antimicrobials for therapeutic purpose.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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