PHYTOCHEMICALS AND ANTIMICROBIAL EVALUATION OF *Tapinathus bangwensis* Leaves

Abstract

Background: The leaves of *Tapinathus bangwensis* have been used in the treatment of infectious and non-infectious diseases by the herbalist. This instigated evaluation of extract and fractions of *Tapinathus bangwensis* leaves for antimicrobial activity against some pathogenic organisms and identifications of the phytoconstituents.

Methods: The standard phytochemical methods and GC-MS were used to identified the phytoconstituents of extract and fractions. The antimicrobial activity was determined using agar dilution method.

Results: The phytochemical analysis revealed the presence of saponins, flavonoids, tannins, terpenoids and steroidal glycosides in the extract whereas n-hexane fraction contains terpenoids only, ethyl acetate contains flavonoids, tannins, terpenoids, saponins and n-butanol contains saponins, tannins and cardiac glycosides. The GC-MS analysis identified fatty acids, phthalic acid esters, saturated and unsaturated hydrocarbons in the extract and fraction. Most of the compounds identified possess antimicrobial, anticancer, antioxidant and cytotoxicity effects. However, the antimicrobial activity showed that *Escherichia coli* alone was susceptible to the extract with mics of 5 mg /ml. *Escherichia coli, salmonella typhi, streptococcus pneumoniae, proteus mirabilis, and candidia albicans* were susceptible to the n-hexane fraction which showed good activity with MIC range of 2.5-5 mg/ml. *Escherichia coli, staphylococcus aureus and candida albicans* were susceptible to ethyl acetate fraction with MIC range of 2.5-5mg/ml and *Escherichia coli and candida albicans* were susceptible to butanol fraction. with MIC range of 2.5-5mg/ml. *klebsiella pneumoniae* was not susceptible to the extract and any of the fractions.

Conclusion: The findings provide justification for the use of *Tapinathus bangwensis* leaves as antimicrobial agent. Hence, the phytochemicals if isolated can serve as a template for the development of antimicrobial agent.

Keywords: Antimicrobial activity, minimum inhibitory concentrations, GC-MS, Phytochemicals and Tapinathus bangwensis

1. Introduction

The unprofessional use of antibiotics promotes the development of antibiotic resistance among infectious microbial strains. This eventually leads to a very serious side effect and increase financial burden in the treating diseases caused by multi-resistant pathogenic organisms. Consequently, there is need for alternative antimicrobial agents from medicinal plants with the goal to discover new chemical structures which can overcome the above anomaly. Many medicinal plants have been used in treating infectious diseases because of their antimicrobial potentials, which are due to secondary metabolites present in the plant as reported by Djeussi [1] and Medina [2]. Medicinal Plants are rich in varieties of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found to demonstrate good antimicrobial properties in in-vitro bioassay model [3].[4]. *Tapinanthus bangwensis* (Loranthaceae) is a hemiparasitic plant widely distributed in Africa, America and Asia [5]. It is an evergreen parasitic plant, leaves are of a yellow-green color, and the berries are whitish, opaque and sticky. This plant grows on a variety of host plants which can be edible or non-edible [6]. *Tapinanthus bangwensis* leaves is often used in folk medicine for the treatment of a variety of ailments such as diabetics, hypertension, syphilis, asthma, epilepsy, cancers of the ovary and breast, AIDS [7],[8],[9],[10]. Previous phytochemical studies on Tapinanthus genus have revealed the presence of a variety of secondary metabolites including saponins, triterpenoids, flavonoids [11], [12]. Keeping in mind the various medicinal uses of *Tapinanthus bangwensis,* the present study was designed to carry out GS-MS analysis and antimicrobial activities evaluation of extract and fractions of *Tapinathus bangwensis* leaves.

2. Materials and Methods

2. 1 Plant Material

The leaves of *Tapinanthus bangwensis* used for the research work were harvested from branches of *Parkia biglobosa* tree located behind Julius Eze Auditorium in the Enugu State University of Science and Technology Agbani in January 2020. It was identified by Mr. Felix Nwafor, a taxonomist in charge of the herbarium unit of the department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria. A voucher specimen with No PCG/411/A/112 was deposited at the herbarium of the department.

2.2 Preparation of the plant extract

The leaves were harvested, rinsed with clean tap water and then dried under shade for 2 weeks. It was pulverized into coarse powder suing mechanical grinder. The powdered sample was stored in a cool dry cupboard awaiting further procedures.

Cold maceration method was used for the extraction, a 400 g of the pulverized plant material was weighed out and transferred into a glass container with lid. A 2 L of methanol was poured into the container and made air tight with the lid. The contents were agitated intermittently and kept for 72 hours at room temperature. The extract was filtered and concentrated using rotary evaporator under pressure to obtain methanol extract.

Fractionation of the Extract

Liquid-liquid extraction (LLE) commonly known as solvent extraction or partitioning was used fractionation based on their relative solubility in two different immiscible liquids and separate into layers when shaken together. Selection of these solvent was based on polarity order. The dry methanol extract (50 gm) was dissolved in 200 mL of 20 % methanol-water and the resulting mixture (i.e., the aqueous layer) partitioned with *n*-hexane (2 x 500 mL), ethyl acetate (3 x 500 mL) and *n*-butanol (1 x 500 mL) using separating funnel to obtain *n*-hexane, ethyl acetate and *n*-butanol fractions respectively. Each of the fractions were concentrated using rotary evaporator under pressure. The dried extract and fractions were stored in the refrigerator till further analysis.

Phytochemical Screening of Extract and Fractions

The phytochemical screening was carried out by standard phytochemical methods as described by Tiwari and others [13].

GC-MS Profiling of Extract and Fractions

The GC-MS analysis was carried out using Shimadzu system and Gas chromatograph interfaced to a mass spectrometer instrument under these working conditions: Column Elite-1 fused silica capillary column (30m x 0.25mm 1D), an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2µl was employed (Split ratio of 10:1) injector temperature of 250^0C; ion-source temperature 280^oC. The oven temperature was programmed from 110^0C for 2 min with an increase of 10^0C/min to 220^0C then 5^0C/min to final temperature of 280^0C/min. The contents of phytochemicals present in the test samples were identified based on comparison of their retention time (min), peak area, peak height and mass spectral patterns with those spectral databases of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

Antimicrobial Evaluation of Extract and Fractions

The microorganisms used for this study were clinical isolates of *staphylococcus aureus, proteus mirabilis, Escherichia coli, klebsiella pnuemoniae, salmonella typhi and candidas albicans*. These test organisms

were selected based on their role in so many bacterial and fungal infections decimating the general population. The bacteria were precultured from stock into broth while fungal inoculum was prepared from the culture grown on agar medium containing 1.5% agarose gel.All microorganisms used in this study were obtained from clinical laboratory stock of Adonai Biomedical Laboratory Research Centre, Nsukka Enugu State

Agar Dilution Method

The minimum inhibitory concentration (MIC) of extract and fractions were evaluated by agar dilution methods. 51.2g of nutrient agar was dissolved in 320ml of water and then subdivided into 20 bijou bottles. The bottles were sterilized by heating in an autoclave at 121^0C for about 15 minutes and allowed to cool. A 40 mg of the extract was weighed and transferred into sterile test tube, 5 ml of dimethylsulphoxide (DMSO) was added for complete dissolution of the extract to afford a stock solution of 20 mg/ml while for the fractions 20mg each was weighed to afford stock of 10mg/ml. From the stock solutions, two-fold serial dilutions were carried out to produce 20, 10, 5 and 2.5 mg/ml for extract and 10, 5, 2.5 and 1.25 mg/ml for the fractions respectively. 1ml each of the antimicrobial agents from the prepared concentrations were incorporated into the Molten agar at concentration of 2.5 – 20 mg/ml for extract and 1.25 – 10 mg/ml for different fractions. The antimicrobial agents and the Molten agar were mixed thoroughly and poured into corresponding plates. When the media solidified and dried in oven for 30 minutes at 50 ^{0}C. Each microorganism was streaked on the section of the plate as labelled appropriately before incubation at 37 ^{0}C for 24 h (bacteria) and 25 ^{0}C for 48 h (fungi). Following the incubation, the plates were observed for the presence or absence of any visible microbial growth.The MIC were recorded as the lowest concentration of the antimicrobial agents without absence of visible growth.

Result

The percentage yield of extract was 10.8 %. Among the solvent fractions, ethyl acetate gave the highest yield (25.37 %) while n-hexane fraction gave the lowest yield (17.23 %) and n- butanol fraction gave (25.1 %) as shown in Table 1.

Table 1: percentage yields of extract and fractions

Extract/Fractions	*Mass of pulverized sample / extract (gram)*	*Mass of extract / fractions recovered (gram)*	*% Yield*
Extract	500	54.05	10.8
n-hexane	30	5.17	17.23
Ethyl acetate	30	7.61	25.37
n-butanol	30	7.53	25.1

Phytochemical Screening Extract and Fractions

The results of the phytochemical screening are present in varying amount in extract and each of the fractions. Most phytoconstituents were present in high amount in extract and ethyl acetate fraction than in n-hexane and n-butanol fractions as shown in Table 2.

Table 2: Phytoconstituent of Extract and Fractions

Phytoconstituents	*Extract*	*n-hexane fraction*	*Ethylacetate fraction*	*n-butanol fraction*

Reducing sugars	++	-	++	+
Flavonoids	++	-	++	++
Terpenoids	++	+++	+++	-
Saponins	+++	+	+++	+++
Tannins	+++	-	+++	++
Alkaloids	-	-	-	-
Cardiac glycosides	++	-	+	+
Fat&oil	+++	+++	+	-

Key: +: Low colour intensity; ++: Moderate colour intensity; +++: High colour intensity;

and -: no colour change noticed

GCMS profiling of extract and fractions

The chemical constituents identified in extract and fractions: fatty alcohol, unsaturated hydrocarbon, phthalic acid ester, saturated hydrocarbon, benzoic acid ester, fatty acid and citric acid ester using the molecular formula, molar mass and pear area as shown in table 3.

Table 3: GCMS profiling of extract and fractions

Name of compound	***Molecular formula***	***Molecular mass***	***Nature of compound***	***Peak area %***
Hexane fraction				
1. *1-octadecene*	**C18H36**	**253**	Unsaturated hydrocarbon	**1.11**
2. *Tri ethyl citrate*	**C12H20O7**	**276**	Citric acid ester	**0.59**
3. *Phthalic acid, isobutyl octyl ester*	**C20H30O4**	**334**	Phthalic acid ester	**14.22**
4. *Bis (2-ethylhexyl) phthalate*	**C24H38O4**	**391**	Phthalic acid ester	**58.45**
5. *1-heptacosanol*	**C27H56O**	**397**	Fatty alcohol	**0.40**
6. *1-nonadecene*	**C19H38**	**267**	Unsaturated hydrocarbon	**0.79**
7. *Behenic alcohol*	**C22H46O**	**327**	Fatty alcohol	**1.44**
8. *Tributyl (methoxy)*	**C13H30OSi**	**230**	Silicon based	**7.34**

silane			ether	
				12.76
9. *Trimethylsilyl methyl stearate*	**C22H46OOSi**	**371**	Silicon based Fatty acid	
10. *1,2-benzenedicarboxylic acid, butyl -1-octyl ester*	**C20H30O4**	**334**	phthalic acid ester	**0.94**
Ethyl acetate fraction				
1. *Phthalic acid, isobutyl octyl ester*	**C20H30O4**	**334**	Phthalic acid ester	**10.34**
2. *Dibutyl phthalate*	**C21H32O4**	**349**	Phthalic acid ester	**2.22**
3. *Bis (2-ethylhexyl) phthalate*	**C24H38O4**	**391**	Phthalic acid ester	**54.64**
4. *3-eicosene*	**C20H4**	**281**	Unsaturated hydrocarbon	**0.99**
5. *1-docosene*	**C22H44**	**309**	Unsaturated hydrocarbon	**0.89**
6. *Nonadecane,9-methyl*	**C20H42**	**283**	Unsaturated hydrocarbon	**2.96**
7. *Docosane*	**C22H46**	**311**	**Saturated hydrocarbon**	**1.96**
n-butanol fraction				
1. *1,2-benzenedicarboxylic acid, dipropyl ester*	**C14H18O4**	**250**	Phthalic acid ester	**1.34**
2. *n-hexadecanoic acid*	**C16H32O2**	**256**	Fatty acid	**6.78**
3. *oleic acid*	**C18H34O2**	**282**	Fatty acid	**32.91**
4. *methyl-9,12-heptadecadienoate*	**C18H32O2**	**280**	Fatty acid	**17.10**
methanol extract				
1. *cyclooctane,1,5-dimethyl-*	**C10H20**	**140**	**Unsaturated**	**0.05**

			hydrocarbon	
2. 2-pyrrolidinone,1-methyl	**C5H9NO**	**99**	**Heterocylic compound**	**0.65**
3. Triacetin	**C9H14O6**	**218**	**Triglyceride**	**0.35**
4. 1,2,3-benzenetriol	**C6H6O3**	**126**	**Polyphenolic**	**14.41**
5. 1,2-benzenediol,3-methoxy	**C7H8O3**	**140**	**Phenol**	**0.19**
6. Vanillic acid	**C8H8O4**	**168**	**Phenolic acid**	**0.19**
7. Benzoic acid 4-hydroxy	**C7H6O3**	**138**	**Phenolic acid**	**3.29**
8. Phthalic acid, isobutyl octyl ester	**C20H30O4**	**334**	**Phthalic acid ester**	**12.47**
9. Benzoic acid 3,4,5-trihydroxy-methyl ester	**C8H8O5**	**184**	**Phenolic acid ester**	**0.91**
10. n-hexadecanoic acid	**C16H32O2**	**256**	**Fatty acid**	**2.52**
11. 2-butynoic acid 4-cyclohexyl-4-oxo-ethyl ester	**C10H12O3**	**208**	**Butynoic acid ester**	**0.39**
12. Triethyl citrate	**C12H20O7**	**276**	**Citric acid ester**	**0.70**
13. Diisooctyl phthalate	**C24H38O4**	**390**	**Phthalate ester**	**25.01**
14. 7-oxooctanoic acid	**C8H14O3**	**258**	**Fatty acid**	**7.01**
15. 1-docosene	**C22H44**	**309**	**Unsaturated hydrocarbon**	**0.80**
16. Trans -3-undecene-	**C11H14**	**146**	**Unsaturated hydrocarbon**	**0.69**

1,5-diyne				
17. Benzoic acid,3,4,5-trihydroxy methyl ester	**C8H8O5**	**184**	**Phenolic acid methyl ester**	**0.54**

Antimicrobial Evaluation of Extract and Fractions

The minimum inhibitory concentration (MIC) of the extract was 10mg/ml against only *E. coli*. For the fractions, it ranged from 2.5-5 mg/ml for n-hexane against *E. coli, S. typhi, P. mirabilis and C. albicans*, 2.5 mg/ml for ethyl acetate against *S. aureus, E. coli and C. albicans* and 2.5-5 mg/ml for n-butanol fraction against *E. coli and C. albicans* as shown in Table 4.

Table 4: Minimum inhibitory concentrations (mg/mL) of extract and fractions

Test organisms	*Extract*	*n-hexane fraction*	*Ethyl acetate fraction*	*n-butanol fraction*
E. coli	10mg/ml	2.5 mg/ml	2.5 mg/ml	2.5 mg/ml
S. aureus	ND	ND	2.5 mg/ml	ND
S. typhi	ND	2.5 mg/ml	ND	ND
K. pneumoniae	ND	ND	ND	ND
P. mirabilis	ND	5 mg/ml	ND	ND
C. albicans	ND	2.5 mg/ml	2.5 mg/ml	5 mg/ml

Key: ND - not determined

Discussion

The leaves of *Tapinanthus bangwensis* was screened for the presence of phytochemical constituents and its antimicrobial activities against selected pathogenic microorganisms. The phytochemical tests revealed that extract and the solvent fraction contained various phytoconstituents which could be utilized in the management of some infectious diseases. The yield of the extract and fractions were relatively small which could be attributed to time of harvest, season and solvent used for extraction as shown in table 1. There were different phytochemical compounds identified in methanol extract and fractions using standard methods such as alkaloids, cardiac glycosides, flavonoids, fat&oil, tannins, terpenoids and steroids and saponins in methanol extract. While after fractionation, cardiac glycosides, flavonoids, tannins and saponins were identified in butanol fraction, also alkaloids, cardiac glycosides and flavonoids were identified in ethyl acetate fraction and terpenoids, fat&oil and steroids were identified in non-polar n-hexane fraction. as shown in table 2. The phytochemical results were in agreement with previous work [7] and Similar result except for the absence of cardiac glycosides was previously reported [14] . The GCMS profiling for the extract and fractions recorded 17 compounds in extract and 10 compounds in hexane fraction, 7 compounds in ethyl acetate fraction and 4 compounds in butanol fraction respectively as presented in table 3. The majority of the phytoconstituents in hexane and ethyl acetate fractions were more of phthalic acid acid esters and unsaturated hydrocarbons whereas butanol fraction contained fatty acids. The methanol extract contained various class of organic compounds such as phenolic acid esters, fatty alcohol, unsaturated hydrocarbons and fatty acid esters. Some of the phytoconstituents have been reported to elicit physiological changes and possess therapeutic effects as antibacterial, antioxidants, anticancer and anti-inflammatory as cited in previous research [15]. [16]. Agar dilution method used to

determine the minimum inhibitory concentration of *T. bangwensis* extract and fractions that inhibited the growth of the test microorganisms indicated low significant inhibition in extract and moderate significant inhibition in the fractions. The extract inhibited only growth of *E. coli* with MIC of 10mg/ml as shown in table 4. This could be attributed to other components of the extract that mask the bioactive constituents preventing them from having interaction with some of the test microorganisms. The lesser concentrations and solvent of the extraction might be the challenges that affected the activity of the extract. Previous study on *T. bangwensis* growing on *P. biglobosa* showed that methanol extract inhibited growth of *shingella dysenteriae, salmonella typhimurium, and pseudomonas aeruginosa* at concentration range of 100-250mg/ml with IZD > 10mm whereas chloroform extract inhibited *shingella dysenteriae,salmonella typhimurium, E,coli, salmonella typhi* and *staphylococcus aureus* at concentration range of 100-250mg/ml with IZD > 10mm. from these results it can be established that methanol is not best solvent to extract antimicrobial agents from *T. bangwensis* as reported in other work [17]. The poor activity of methanol extract of *T. bangwensis* was appreciably improved by gradient fractionation with n-hexane, ethyl acetate and n- butanol as solvent. The antimicrobial activity of fractions as showed in table 4 indicated that n-hexane fraction had mic of 5mg/ml for *S. pneumoniae* and *P. mirabilis* and 2.5 mg/ml for *S. typhi*, *E. coli*, and *C. albicans* indicating that the presence of the steroids, terpenoids and other non-polar components of this fraction is responsible for the activity against these microorganisms. The ethyl acetate fraction inhibited *S. aureus*, *E. coli*, and *C. albicans* with mic of 2.5 mg/ml indicating that the components in this fraction have an activity against this microorganism. For the n- butanol fraction the MIC is 2.5 mg/ml against *E. coli* and 5mg/ml against *C. albicans*. The results of the study indicated potentials for the use of the plant part in the development of phytomedicines.

Conclusion

The phytochemical investigation of *T. bangwensis* indicated present of bioactive compounds which account for the observed antimicrobial activity recorded in this study. This have substantiated the traditional uses of *T. bangwensis* leaves in treatment of infectious diseases due to pathogenic microorganisms. Further research is required to isolate the most bioactive constituents for their utilization in the development of newer antibiotics.

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