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PHYTOCHEMEICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF EPIPHYTIC NEEM LEAVES TAPINANTHUSDODONEIFOLIUS EXTRACTS AGAINST SOME SELECTED CLINICAL ISOLATES

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ABSTRACT

The laboratory scale experiment was conducted to determine the phytochemical constituents and antibacterial activities of epiphytic neem leaves (Tapinanthusdodoneifolius) extracts on some selected clinical isolates. The samples were collected using polythene bags to avoid unnecessary contamination of the plants, and it was collected from the BUK old site garden. The phytochemical screening and antibacterial test was carried out in the Chemistry and Biology laboratory respectively in BUK. The result obtained showed that carbohydrate, glycosides, steroids, alkaloids, phenol, saponins and falvonoids are present in the ethanolic extract, however chloroform extract showed a presence of glycosides, phenols and carbohydrates only. Furthermore there

Introduction:

Medicinal plants have a long history of use and that is widespread in both developing and developed countries. According to reports of the World Health Organization, 80% of the world's population relies mainly on traditional therapies which involve the use of plant extracts or their active substances (WHO, 1993). Microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmad et al., 1998). Furthermore, antibiotics

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was no significance difference between the ethanolic extracts against bacterial isolates ($p<0.05$).

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are sometimes associated with side effects (Cunha, 2001), whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002). It is known that more than 400, 000 spp. of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006). Some plant decoctions are of great value in the treatment of diarrhoea or gastrointestinal disorder, urinary tract infections, skin infections, infertility, wound and cutaneous abscesses (Ergene et al., 2006).

The tree, Azadirachta indica of the family Maliaceae; popularly known as neem tree or darbejiya (Hausa) is an evergreen tree, native to the Southeast Asia and found in most tropical countries. It has been in use since ancient times, to treat a number of human ailments and also as household pesticide (Chattopadhyayet al., 1993; Chattopadhyay 1996; Chattopadhyay and Bandyopadhyay, 2005). Extracts from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthosis and respiratory disorders (Ketkar and Ketkar, 1995). Every part of the neem tree has been used as traditional medicine for house-hold remedy against various human ailments from antiquity.

Aims and Objectives

This research is aimed at studying the antibacterial activity and phytochemical properties of epiphytic neem leaves Tapinanthus dodoneifolius extracts on some selected clinical isolates.

Objectives of this research work are:

1. To determine the phytochemical compound in epiphytic neem leaves extracts Tletoe
2. To compare the antibacterial activity of epiphytic neem leaves and its mistletoe against some gastrointestinal isolate.

Materials and Methods

Collection of plant materials

The epiphytic neem leaves of Tapinanthus dodoneifoliussample was collected from Bayero University Kano old campus garden area.

Identification of Plant Material

The collected leaves were identified and authenticated in Herbarium section, department of Plant Biology, Bayero University Kano. Voucher number given used for the purpose of this study
(BUKHAN 0255)

Preparation of the plant materials

The collected sample were washed thoroughly with running tap water and finally with sterile distilled water and then air dried on a sterile blotter under shade for 2 weeks. The dried leaves was grinded into powder using clean motar and pestle.

Sample Extraction

The extraction was carried out using percolation technique according to the method demonstrated by Alade and Irobi (1993). 200gms of the air dried powdered form of the epiphytic neem leaves of Tapinanthus dodoneifoliuswas soaked in 250ml of ethanol and chloroform aqueous for three days. The mixture was stirred every 24 hours using a sterile glass rod. The mixture was filtered through Whatman filter paper No. 1 (Whatman, UK). The concentrated ethanolic and chloroform aqueous

filtrates, obtained in water bath at 30°C, the dried plant extracts obtained was weighed and labeled, stored at 4°C for further use.

Qualitative Phytochemical screening of the extracts

Test for Saponins

2ml of the extracts was vigorously shaken with distilled water and allowed to stand for a while. A persistent frothing was formed which is indicates the present of saponins (Sofowora, 1984).

Test for alkaloids

0.5g of the extracts was stirred with 5ml of 1% HCL on steam bath. The solution was cooled and filtered.1ml of the filtrate was treated separately with drops of Mayer's, dragendroff's and wagner's reagents; and formation of dirty reddish brown precipitated was formed respectively which indicates the presence of alkaloid (Elemy et al., 1994).

Test for steroids

Libermann-Bruchard's test was used to detect the presence of steroids. Acetic anhydride (5 ml) was added to 5 ml of each extract in a test tube. Conc. H_2SO_4 (1ml) was added carefully down the side of the test tube. A pink colour appeared in the chloroform and methanolic extracts which later changed into a blue-green colour indicating the presence of steroids (Umesh et al., 2010)

Test for tannins

2ml of extract was treated with 3 drops of 5% ferric chloride. A dark black colored precipitated was formed in a very dark solution, which gives a green-black to blue black colouration on dilution which indicates the presence of tannins (Sofowora, 1984).

Test for carbohydrates

1g of extract was weighed and diluted with 2ml of distilled water. Fehling's solution (A and B) was added and warmed the mixture. A brick- red

precipitated at the bottom of the test tube was formed which indicates the reducing sugars (Brain and Turner, 1975).

Test for flavonoids

2g of the extracts was weighed and placed in a test tube, 10mls of DMSO was added. The mixture was heated, the10g magnesium powder and six drops of concentrated hydrochloric acid was added.

The red colour appeared which is indicates of the presence of flavonoids (Sofowora, 1993).

Test for Glycosides

Keller-Kiliani's test was used to detect the presence of cardiac glycosides. Each extract (0.5 g) was dissolved in 5 ml glacial acetic acid containing 1 drop of 5% ferric chloride solution in a test tube.

The test tube was held at an angle of 45˚and 1 ml of concentrated sulphuric acid was added carefully. All the two extracts showed purple ring at the interface indicating the presence of cardiac glycosides (Inalegwu and Sodipo, 2013).

Test for anthraquinones

Borntrager's test was used to detect the presence of anthraquinones. Extract (0.5 g) was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammoniacal layer indicates the presence of anthraquinones. No colour was observed in all the extracts suggesting the absence of anthraquinones. (Siddiqui and Ali, 1997).

Test organisms

Clinical isolates of bacteria was used for the bioassay studies. The isolates include Escherichia coli, Salmonella typhi, Pseudomonas aeriginosa, and Proteus mirabilis and Staphylococcus aureus. The isolates were obtained

from microbiology laboratory of Murtala Mohammad Specialist Hospital (MMSH), Kano, Nigeria.

Preparation of extract concentrations

This was carried out using standard method described by Cheesbrough (2002). Stock solution of the ethanol extract and chloroform extract was prepared by weighing 0.2g of each and dissolved in 5ml of Dimethyl Sulfoxide (DMSO) in glass vial bottles. This gave an extract concentration of20mg/ml (stock solution). Four different extracts concentrations (10, 5, 2.5 and 1.75mg/ml) was prepared from the stock solution (20mg/ml) using double serial dilution for each extract.

Antibacterial activity of the Extracts

Agar well diffusion method adopted by (Abdullahi et al. 2016) was used to evaluate the antibacterial activity of the crude extracts. Briefly, 0.1 ml of the standard inoculum was inoculated into 90mm sterile Petri plate. Six wells were made on respective agar plate using cork borer of
6mm in diameter size. 0.1ml of methanol and aqueous extracts at different concentrations of;
4000µg/ml, 2000µg/ml, 1000µg/ml, and 500µg/ml was introduced into the respective wells. Then 0.1ml of 30µg/ml of chloramphenicol solution served as a positive control and 0.1 ml of DMSO as a negative control. The plates were allowed to stand on flat bench for 30 minutes to allow diffusion into the agar before incubation at 37^{o}C for 18-24 hours. The experiment carried for each of the extract against each of the test bacteria and mean zone diameter (mm) was recorded. Antibacterial activity evaluated by measuring the diameters of zones of growth inhibition (mm) as described by Cheesbrough (2006). These experiments were replicated for each of the test bacteria.

Results & Discussions

The results of phytochemical screening of chloroform and ethanolic extracts of Tapinanthus dodoneifolius are presented in Table 1. The

phytochemical constituents of ethanolic extract shows the presence of carbohydrates, glycosides, steroids, Alkaloids, phenols, saponins and flavonoids while chloroform extract revealed the presence of glycosides, phenols and carbohydrates present. Tannins and anthraquinones were absent in both extracts. From the results obtained, ethanol extracted seven while chloroform extracted four phytocompounds.

Table 1: Phytochemical constituents of chloroform and ethanolic extracts of Tapinanthus dodoneifolius

Constituents	Ethanol	Chloroform
Carbohydrates	+	+
Glycosides	+	+
Flavonoids	+	
Saponins	+	
Tannins		
Phenols	+	+
Alkaloids	+	+
Steroids	+	-
Anthraquinones		-

Key: + = Present, – = Absent

Table 2 demonstrates the antibacterial activity patterns of different fractions of Tapinanthus dodoneifolius extracts. The result showed that ethanolic extracts at 20, 10, 5 and 2.5mg/ml have antibacterial activity against all the test organisms with the exception of Proteus mirablis which had no bacterial activities against the ethanolic extracts. Statistically no significance difference observedbetween the ethanolic extracts agains the bacterial isolates ($P<0.05$). Ethanolic extract was able to inhibit S. aureus, E. coli, K. pneumoniae and S. typhi and P. aeruginosa. On the other hand the extract did not inhibit P. mirablis. ethanolic extract however, showed the highest activity having inhibition zones ranging from 6.7mg/ml S. aureus to 15.3mg/ml in K. pneumonia The zones of inhibition of the positive control range from 16.5 – 25.00mg/ml. According to Johnson and Case

(Johnson and Case, 1995), zone of inhibition equal to or greater than 16mg/ml is associated with microbial susceptibility. In view of the above, the extract have shown significant antibacterial activity against six bacteria.

Table 2: Antibacterial activity of Ethanolic extracts of Epiphytic Neem Leaves (Tapinanthus dodoneifolius

Test bacteria	Zone of inhibition at different concentrations of the extract					Control
	20	10	5	2.5	1.75	
Escherichia coli	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	19.6±0.00
Klebsiella pneumonia	15.3±0.57	14.0±0.00	12.0±0.00	11.0±0.57	0.00±0.00	25±0.000
Proteus mirabilis	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	16.5±0.2
Salmonella typhi	16.0±0.00	13.7±0.57	10.0±0.00	10.0±0.00	8.0±0.00	21.0±0.57
Pseudomonas aeruginosa	12.3±0.57	10.3±0.57	8.0±0.000	0.00±0.00	0.00±0.00	28.2±0.00
Staphylococcus aureus	13.3±0.5	9.0±0.00	8.3±0.57	7.0±0.00	6.7±0.57	19.4±0.0

Table 3 illustrates the antibacterial activity of chloroform extract of Epiphytic Neem Leaves (Tapinanthus dodoneifolius). The result showed that Chloroform extracts at 20, 10, 5 and 2.5mg/ml have antibacterial activity against all the test organisms with the exception of Proteus mirablis which had no bacterial activities against the ethanolic extracts which revealed significance between the extracts ($p<0.05$). Chloroform extract was able to inhibit S. aureus, E. coli, K. pneumoniae and S. typhi and P. aeruginosa. On the other hand the extract did not inhibit P. mirablis. Ethanolic extract however, showed the highest activity having inhibition zones ranging from 6.7mg/ml S. aureus to 15.3mg/ml in K. pneumonia the zones of inhibition of the positive control range from 16.5 – 25.00mg/ml.

Table 3: Antibacterial Activity of Chloroform Extract against Test Organisms (mg/ml)

Isolates	Zone of inhibition at different concentrations of the extract					Control
	20	10	5	2.5	1.75	
Escherichia coli	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	19.6±0.0
Klebsiella pneumonia	15.3±0.57	14.0±0.0	12.0±0.0	11.0±0.57	0.00±0.0	25±0.00
Proteus mirabilis	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.00	0.00±0.0	16.5±0.2
Salmonella typhi	16.0±0.00	13.7±0.0	10.0±0.0	10.0±0.00	8.0±0.00	21.0±0.0
Pseudomonas aeruginosa	12.3±0.57	10.3±0.0	8.0±0.00	0.00±0.00	0.00±0.0	28.2±0.0
Staphylococcus aureus	13.3±0.57	9.0±0.00	8.3±0.00	7.0±0.00	6.7±0.57	19.4±0.0

Table 4, shows the Minimum Inhibitory Concentration (MIC) of different fractions of Tapinanthus dodoneifoliusextracts against the selected clinical isolates. The results showed that all the test organisms have the same MIC of 62.5µg/ml in the petroleum ether extract while P. miravilis, S. aureus and K.pneumonia tested with chloroform extracts have also the same MIC of 62.5µg/ml while E. coli and P. aeruginosa were resistant. Moreover, all the isolates tested with ethanolic extracts of Tapinanthus dodoneifoliusalso have MIC value of 62.5µg/ml with the exception of E.coli,P. earuginosa and K. pneumoniae which all of them revealed resistance to ethanol extract.

Table 4: Minimum Inhibitory Concentration of Chloroform and ethanol extracts of Tapinanthus dodoneifolius

	Extracts concentration (µg/ml)									
	Chloroform					Ethanol				
Isolates	250	125	62.5	31.3	15.6	250	125	62.5	31.3	15.6
P. mirabilis	-	-	-	+	+	-	-	-	+	+
E. coli	-	-	-	+	+	+	+	+	+	+

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S. aureus	-	-	-	+	+	-	-	-	+	+
P. aureginosa	-	-	-	+	+	+	+	+	+	+
K. pneumoniae	-	-	-	+	+	-	-	-	+	+
S. typhi	-	-	-	+	+	-	-	-	+	+

Key: + = Growth not inhibited; - Growth inhibited

Table 5, revealed the minimum bactericidal concentration of Chloroform and ethanol extracts against the test organisms. Minimum Bactericidal Concentration of each isolate was determined at the lowest concentration which inhibits bacterial growth. From the result it showed that E. coli, P. aeruginosa and K. pneumoniae had the same MBC of 125µg/ml while S. aureus and P. mirabilis, have no MBC value across all concentrations of Chloroform extract. However in ethanol extract S. aureus had the MBC value of 250µg/ml while P. mirabilis had the MBC value of 62.5µg/ml and the remaining tests organisms were resistant.

Table 5: Minimum Bactericidal Concentration of Chloroform and ethanol flower crude extract of Tapinanthus dodoneifolius

	Extracts concentration (µg/ml)					
	Chloroform			Ethanol		
Isolates	**250**	**125**	**62.5**	**250**	**125**	**62.5**
P. miravilis	+	+	+	-	+	+
E. coli	-	-	+	+	+	+
S. aureus	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+
.pneumoniae	-	-	+	+	+	+

CONCLUSION

From the results of this study, it can be inferred that chloroform and methanol extracts of Tapinanthus dodoneifolius exhibited significant antibacterial activity against the test bacteria which could be as a result of the phytochemicals present in the plant. Based on the pharmacological results of the study, it could be confirmed that the extracts contain

chemical constituents of pharmacological significance. The observation that the extracts were effective against the test bacteria suggests the use of crude extract of Tapinanthus dodoneifolius against infection caused by clinical isolates like P. mirabilis, Escherichia coli, S. aureus, S. typhi, P. aeruginosa, and K. pneumonia.It is therefore recommended for the isolation and purification of bioactive compounds in Tapinanthus dodoneifolius tree responsible for the antibacterial activity.

RECOMMENDATION

It is therefore recommended that Further study is should be conducted to isolate and explore individual bioactive compound that is responsible for the antibacterial activity. Further research should be conducted to test the implication of taking the soaked plant parts in water. Aqueous extract of the plant should be used to test its antibacterial properties. Further study should be conducted to ascertain the efficacy, toxicity and suitability of using the extracts in-vivo.

References

Aboughe Angone, S., Bardor, M., Nguema-Ona, E., Rihouey, C., Ishii, T., Lerouge, P., Driouich, A. (2009). Structural characterization of cell wall polysaccharides from two plant species endemic to Central Africa, Fleurya aestuans and Phragmanthera capitata. Carbohydrate polymers 75(1); 104-109.

Adodo, A. (2004). Nature Power, A Christian Approach to Herbal Medicine. Benedictine Publication Nigeria, 3rd Edition, Edo State pgs; 103-111.7th Printing by Generation Press Ltd, Surulere, Lagos.

Adsersen, A. and Adsersen, H. (1997). Plants from Reunion Island with alleged antihypertensive and diuretic effects, an experimental and ethnobotanical evaluation. J. Ethnopharmacol 58; 189-206.

Akanji. M. A., Ayorinde, B. T., Yakubu, M. T., (2009). Anti-lipidaemic potentials of aqueous extract of Tapinanthus globiferus leaves in rats. RPMP Vol 25- Chemistry and Medicinal Value, Eds. V.K. Singh and J. N. Govil pg 1-9.

Akinmoladun, A. C., Obuotor, E. M.,Farombi, E. O., (2010). Evaluation of antioxidant and free radical scavenging capacities of some Nigerian Indigenous Medicinal Plants. Journal of Medicinal Food 13(2); 444-451.

Bannister, P., Graham, L., Strong, Inge A., (2002).Differential accumulation of nutrient elements in some new mistletoes and their hosts. Functional Plant Biology 29 (11); 1309-1318.

Begho, E. R.,Omokhafe, K. O., Omo-Ikerodah, E. E. and Akpaja, E.O. (2007). Some observations on the fruit set and incidence of Mistletoes on rubber trees in Nigeria. Am-Eurasian J. Sustain. Agric. 1(1); 13-18.

Bright, E. O. and Okusanya, B. A.,(1998). Infestation of economic plants in Badeggi by Tapinanthus dodoneifolius (DC) Danser and Tapinanthus globiferus (A. Rich) Van Tiegh.

Nigerian J. of Weed Science 11; 51-56.

Burkill, H.M., (1985), The useful Plants of West Tropical Africa. Royal Botanical Gardens, Kew. Vol.3 (families J-L) pgs; 548-560.

Cebovic, T., Spasic, S., Popovic, M. (2008). Cytotoxic effects of the Viscum album L. extract on Ehrlich tumour cells in vivo. Phytother, Res. 22(8); 1097.

Cunha, B. A. (2001). Antibiotic side effects. Med. Clin. North Am. 85, 149–185. doi: 10.1016/S0025-7125(05)70309-6

Deeni, Y. Y., Sadiq, N. M., (2002). Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (Tapinanthus dodoneifolius (DC) Danser (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. J. Ethnopharmacol. 83(3); 235240.