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Quantitative Photochemical Compositions and Antibacterial Activity of *Tapinanthusglobifer us* obtained from Five Different Host Trees

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ABSTRACT

Quantitativephytochemical compositions and antibacterial activity of Tapinanthusglobiferus sourced from Albizzialebbeck, Terminaliamantaly, Terminaliacatappa, Khayasenegalensis and Citrus grandiswas determined. The air-dried leaves ofT. globiferus obtained from the five different host trees were pulverized and the phytochemical constituents were extracted using water as a solvent. The quantitative phytochemical compositions were determined using standard methods. The leaves extracts of the mistletoe species (T. globiferus) were tested on Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, and Escherichia coliIn vitro. Both the Gram-positive and Gram-negative bacterial species tested showed variable sensitivity to the aqueous leaves extracts treatments. The results obtained indicated that the T. globiferus obtained from: A. lebbeck, C. grandis, K. senegalensis and T. catappa and T. mantaly had significantly the highest concentrations of alkaloid, saponin, flavonoid and cyanogenic glycoside, tannin, phenol and phenol and tannin respectively. Also, all the extracts of the T. globiferusexcept the one obtained from T. mantaly and A. lebbeck had some antibacterial activities against the test organisms which increased with an increase in concentrations when compared with standard antimicrobial agent (ciprofloxacin) used as positive control at P<0.05 significant level. In general,T. globiferus from T. catappa had the highest concentration of most compounds while the extracts ofT. globiferusobtained from K. senegalensis showed more antibacterial tendency than those from other host trees.

Key words: Antibacterial; host trees; *Tapinanthusglobiferus*; Quantitative analysis

INTRODUCTION

The compositions and activities of mistletoe are host tree and seasons dependent (Scheer*et al*., 1992, Obatomi*et al.*1994, Wagner *et a*l., 1996 and Osadebeand Ukwueze2004) and are of disease curing specificity, for example, mistletoe grown on Guava, Kolanuts and Citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia (Ekhaise*et al*., 2010). *Tapinanthus globiferus*, wasreported by Bassey (2012) asone of the mistletoes commonly consumed by the people of Akwa Ibom State as a herbal cure for ailments such as hypertension, diabetes, ulcer and heart disease.

Bacteria are listed at first position among the microorganisms causing opportunistic infections (Kone *et al*., 2004), so many antibacterial agents are now used in treating bacterial infections. Their widespread and indiscriminate use lead to development of drug resistance among many virulently pathogenic bacterial species (Berkowitz, 1995).

Most of the currently used antibacterials are associated with adverse effects such as blood cancer, upper gastrointestinal complications, organ damages, toxicity, hypersensitivity,

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immunosuppression and tissue residues thus, posing public health hazard (Calixto, 2000). Also, these synthetic broad spectrum antibiotics are cost prohibitive and are not within the reach of our poor farmers (Calixto, 2000). These disadvantages undermine the therapeutic utility of the currently available antibacterials and hence the need for alternative remedies for the treatment of bacterial infections (Calixto, 2000). Natural plant products have been used for therapeutic purposes since the time immemorial and their use is of a greater demand nowadays (Calixto, 2000). So, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. In our earlier research (Tizhe *et al.,* 2015), *Globimetula braunii* sourced from *A.lebbeck, T. mantaly, T. catappa, K. senegalensis* and *C. grandis*was reported to have some antibacterial activity against *B.subtilis, S.aureus, S.typhi,* and *E. coli*except the one sourced from *T. mantaly* and *A. lebbeck*. This study was aimed at comparatively determining the quantitative phytochemical compositions and antibacterial activity of *T. globiferus* sourced from *A.lebbeck, T. mantaly, T. catappa, K. senegalensis* and *C. grandis* so as to confirm itsphytochemical variations and antibacterial effect.

MATERIALS AND METHODS

Collection of plants material

Fresh leaves of *T. globiferus* growing on *T.catappa, C.grandis,T. mantaly, K. sengalensis*and *A.lebbeck* within Samaru, Zaria Local Government Area of Kaduna State, Nigeria were collected. These were taken to the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria for identification.

Preparation of plant material

The leaves were destalked, washed and air dried at a room temperature. The air dried leaves were pulverized (grinded) into fine powder using wooden pestle and mortar and kept in air tight black nylon until the time for usage.

Quantitative phytochemical analysis

Alkaloid, saponin, flavonoid, total phenols, cyanogenic glycoside and tannin were quantitatively analysed using standard methods (Spectrophotometric method, Pearson, 1976, Boham and Kocipai-Abyazan, 1994, Obadomi and Ochuko, 2001, Harborne, 1973 and Onwuka, 2005).

Extraction of plant material

Maceration method of extraction was used for the extraction of the plant materials. About 40g of powdered plant material was kept in 250ml conical flask and 200ml of extraction fluid (water) was added. It was then stirred several times with sterile glass rod after which the mouth of the conical flask was covered with aluminium foil and kept for one days at room temperature. After the period of 24 hours, the mixture was filtered through muslin cloth and finally by Whatman number 1 filter paper. The solvent was then removed from the extract by putting the extract in evaporating dish placed on water bath set at temperature of 50^0C. Finally, the residue was then collected and kept in a refrigerator at 4^0C in a specimen bottle pending analysis.

Test organisms

The test organisms which include *B. subtilis, S. aureus, S.typhi* and *E. coli* were clinical isolates of bacteria which were collected from the Department of Microbiology, Ahmadu Bello University, Zaria.

Preparation of the different concentrations of the extracts

The extract of each of the samples was prepared into four (4) different concentrations ranging from 200mg/ml to 25mg/ml (i.e 25, 50, 100 and 200 mg/ml) and each with two (2) replications. The extract concentrations were prepared by weighing 2 g of the extract and dissolve it in 10 ml sterile distilled water (200mg/ml). A serial dilution of the diluted extract (200mg/ml) was carried out into three (3) different labeled bottles to obtain concentrations of 100, 50 and 25 mg/ml respectively.

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Preparation of standard inoculate of the test organisms

The test organisms (inocula) were prepared by streaking the organisms on the freshly prepared nutrient agar plates to obtain discrete bacterial colonies. A colony was then picked and subculture unto sterile nutrient broth and incubated at 37^0C for 18-24hours. After the incubation period, a loopful of broth culture was transferred into bottles containing sterile distilled water to obtain a bacterial cell density of 1.5×10^8 as determined by Mcfarland turbidity standard (scale number one).

Antimicrobial activity assay

The standardized organisms were uniformly streak unto freshly prepared Mueller Hinton Agar with the aid of a sterile swab stick (cotton swabs). Four wells were punched on the inoculated agar plates using a cork borer. The wells were properly labeled according to the different concentrations of the extract prepared. The punched wells were then filled with the extract. The plates were allowed to stay on the bench for 1hour for the extract to diffuse into the agar after which they were incubated at 37^0C for 18-24hours. After the incubation period, the plates were observed for any evidence of inhibition, which appeared as clear zones that were completely devoid of growth around the wells. The diameter of the clear zones was measured with a transparent ruler calibrated in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method.

Serial dilution of the extract was carried out in well labeled test tubes using Mueller Hinton Broth (MHB) as a diluent. The lowest concentration inhibitory to each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth. Each test tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile Mueller Hinton Broth without any organism was used as a control.The tubes were then incubated at 37^0C for 18-24hours. After the incubation period, the tubes were determined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

Determination of Minimum Bacteriocidal Concentration (MBC)

A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test, it was then streaked unto a freshly prepared nutrient agar plates. The plates were then incubated at 37^0C for 18-24hours. After the incubation period, the plates were then examined for the presence or absence of growth.

RESULTS AND DISCUSSION

The results of the quantitative phytochemical compositions of *T. globiferus* obtained from five host tree species (*A. lebbeck*, *T. mantaly*, *T. catappa*, *C. grandis* and *K. senegalensis*) comparatively showed that, the *T. globiferus* obtained from *T. catappa* and *T. mantaly* had significantly ($P<0.05$) the highest concentrations of cyanogenic glycosides, tannin, total phenol and tannin, total phenol respectively compared to *T. globiferus* obtained from other host trees (Table 1). This was an indication that, *T. globiferus* from these host trees might have an antimicrobial tendency than other *T. globiferus* from other host as they possessed compounds which exhibit medicinal and physiological activities (Sofowora, 1993). Similarly, *T. globiferus* from *A. lebbeck*, *C. grandis* and *K. senegalensis* had significantly ($P<0.005$) the highest concentration of alkaloid, saponin and flavonoid respectively compared to that obtained from other hosts (Table 1). It was obvious that this mistletoe from different hosts might differ from one host to another in its antimicrobial tendency as they possessed different concentrations of compounds which

were known to exhibit some pharmacological and physiological importance (Sofowora, 1993; Rao and Rao, 1995; Higdon and Frei, 2003; Encyclopaedia Britannica, 2014). The variations in the concentrations of these compounds in *T. globiferus* sourced from these five host tree species might be attributed to differences in host and source of their nutrients as the contents and activity of mistletoes are host plant dependent (Osadebe et al., 2008; Bassey, 2012).

The antibacterial activity test of *T. globiferus* obtained from *A. lebbeck, T. mantaly, T. catappa, C. grandis*and *K. senegalensis* all showed a variable antibacterial effects on most of the test organisms except the ones obtained from *A. lebbeck* and *T. mantaly* (Table 2). Variations in the antibacterial activities of the aqueous leaf extract of this mistletoe obtained from different hosts might be due to differences in the contents of its phytochemical compositions and host as these affect mistletoe antimicrobial effects (Osadebe*et al*., 2008; Bassey, 2012). Resistibility of test organisms to the aqueous leaf extract of *T. globiferus* from *A. lebbeck* and *T. mantaly* could be due to lack of sufficient compounds of medicinal effect.

Comparatively, the aqueous leaf extract of *T. globiferus* obtained from *K. senegalensis* followed by that obtained from *C. grandis* showed significantly ($P<0.005$) higher inhibition of the entire test organisms compared to the aqueous leaves extracts of *T. globiferus*obtained from the other host plants. On the other hand, the aqueous leaves extracts of *T. globiferus* obtained from *A. lebbeck* and *T. mantaly* showed significantly ($P<0.005$) the least inhibition of all test organisms compared to the rest (Table 3). This outstanding antibacterial activity of *T. globiferus* obtained from*K. senegalensis* might be attributed to the higher concentration of flavonoid observed as analyzed in this study as flavonoid was known to disrupt the functions of viruses and bacteria (Higdon and Frei, 2003). Similar results was reported by Tizhe*et al* (2015) when they tested the antibacterial effect of the aqueous leaf extract of *G. braunii* sourced from the same type of host plants used in this study.

The minimum Inhibitory Concentration (MIC) test of the aqueous leaves extracts of *T. globiferus* obtained from the five host trees as presented in Table 3, indicated that, the aqueous leaf extract of *T. globiferus* obtained from *T. catappa* had an inhibitory effect at 100mg/ml on *S. typhi*. And the minimum inhibitory concentration of the aqueous leaf extract of *T. globiferus* sourced from *K. senegalensis* was 50mg/ml on *S. aureus*, but had no activity on *E. coli, B. subtilis* and *S. typhi*. The trend of events in the Minimum Bacteriocidal Concentration (MBC) test was the same as observed in the minimum inhibitory concentration (MIC) test (Table 4).

CONCLUSION

Tapinanthusglobiferus obtained from the five different host trees showed a significant variation in phytochemical constituents and significant antibacterial activity was shown by the aqueous leaf extract of the*T. globiferus*obtained from *K. senegalensis*. Generally, the phytochemical constituents of *T. globiferus* from *T. catappa* and *T. mantaly* significantly (at $P<0.005$) had the highest concentrations of most compounds than that from other hosts and the aqueous leaf extract of *T. globiferus* from *K. senegalensis* had better antibacterial activity on *E. coli, B. subtilis, S. typhi* and *S. aureus* than that obtained from *C. grandis, T. mantaly, T. catappa*and *A. lebbeck*. Therefore, further research should be conducted so as to find out the active ingredients responsible for the antibacterial activity of the plant extract.

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Table 1: Comparison of the quantitative phytochemical compositions of *Tapinanthus globiferus* obtained from five host plants

Phytochemical Compositions							
Mistletoe species	**Host plant**	**ALK (%)**	**SAP (%)**	**CYA (mg/g)**	**FLA (%)**	**TAN (%)**	**PHE (mg/ml)**
	A. lebbeck	6.10a	10.44cd	3.94c	11.85b	0.09c	0.35c
	T. catappa	1.76b	12.12bc	12.00a	13.70b	1.32a	2.48a
T. globiferus	***T. mantaly***	0.40c	10.00d	3.20c	10.13b	1.28a	2.69a
	C. grandis	1.90b	31.00a	7.07b	7.40c	0.37b	0.80b
	K. senegalensis	0.04c	14.00b	3.13c	22.40a	0.18bc	0.68b
	SE±	0.28	0.58	0.40	1.18	0.06	0.10

NB: Means with the same letter(s) along the column are not significantly different at P<0.05
ALK= alkaloid, **SAP**= saponin, **CYA**= cyanogenic glycosides, **FLA**= flavonoid,
TAN= tannin, **PHE**= phenol, **SE±**= Standard Error

Table 2: The antibacterial activity of the aqueous leaves extract of *Tapinanthus globiferus* obtained from five different trees

		Diameter of zone of inhibition (mm)			
		Test organisms			
Host plant	**Extract conc (mg/ml)**	***S. aureus***	***E. coli***	***B. subtilis***	***S. typhi***
	200	26.50b	28.00b	28.00b	28.00b
	100	22.50bc	21.00c	20.00c	20.50c
K. senegalensis	**50**	19.00c	18.50c	18.00d	17.50c
	25	0.00d	0.00d	0.00e	0.00d
	Ciprofloxacin	40.00a	40.00a	40.00a	45.00a
	SE±	1.97	0.81	1.07	1.92
	200	15.00b	26.00b	12.00b	11.00b
	100	13.00c	23.00c	0.00c	13.00c
C. grandis	**50**	0.00d	20.00d	0.00c	0.00d
	25	0.00d	0.00e	0.00c	0.00e
	Ciprofloxacin	40.00a	40.00a	40.00a	45.00a
	SE±	0.44	0.63	0.00	0.92
	200	0.00b	19.00b	0.00b	19.00b
	100	0.00b	0.00b	0.00b	13.50b
T. catappa	**50**	0.00b	0.00b	0.00b	0.00c
	25	0.00b	0.00b	0.00b	0.00c
	Ciprofloxacin	40.00a	40.00a	40.00a	45.00a
	SE±	0.00	0.44	0.00	0.81
	200	0.00b	0.00b	0.00b	0.00b
	100	0.00b	0.00b	0.00b	0.00b

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T. mantaly	**50**	0.00b	0.00b	0.00b	0.00b
	25	0.00b	0.00b	0.00b	0.00b
	Ciprofloxacin	40.00a	40.00a	40.00a	45.00a
	SE±	0.00	0.00	0.00	0.00
	200	0.00b	0.00b	0.00b	0.00b
	100	0.00b	0.00b	0.00b	0.00b
A. lebbeck	**50**	0.00b	0.00b	0.00b	0.00b
	25	0.00b	0.00b	0.00b	0.00b
	Ciprofloxacin	40.00a	40.00a	40.00a	45.00a
	SE±	0.00	0.00	0.00	0.00

Means with the same letter along the column of each of the host plant are not significantly different at P<0.05.
SE± = Standard error

Table 3: Comparison of the antibacterial activities of the aqueous leaves extracts of *Tapinanthus globiferus* obtained from five different host plants

	Diameter of zone of inhibition (mm)			
	Test organisms			
Host plants	***S. aureus***	***E. coli***	***B. subtilis***	***S.typhi***
Khaya senegalensis	21.60a	21.50a	21.20a	22.20a
Citrus grandis	13.60b	21.80a	10.40b	21.20a
Terminalia catappa	8.00c	11.80b	8.00c	15.50b
Terminalia mantaly	8.00c	8.00c	8.00c	9.00c
Albizzia lebbeck	8.00 c	8.00c	8.00c	9.00c
SE±	0.40	0.22	0.00	0.42

Means with the same letter along the column are not significantly different at P<0.05.
SE± = Standard error

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Table 4: The Minimum Inhibitory Concentrations (MIC) and Minimum Bacteriocidal Concentrations of *T. globiferus* aqueous leaf extract

		Minimum Inhibitory Concentrations (MIC)				**Minimum Bacteriocidal Concentrations (MBC)**			
		Test Organisms				**Test Organisms**			
Host plant	**Mistletoe species**	***S. aureus***	***E. coli***	***B. subtilis***	***S. typhi***	***S. aureus***	***E. coli***	***B. subtilis***	***S. typhi***
K. senegalensis	*T.globiferus*	50	-	-	-	50	-	-	-
C. grandis	*T.globiferus*	-	-	-	-	-	-	-	-
T. catappa	*T.globiferus*	-	-	-	100	-	-	-	100
A. lebbeck	*T.globiferus*	-	-	-	-	-	-	-	-
T.mantaly	*T.globiferus*	-	-	-	-	-	-	-	-

- No inhibition

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