



Comparative Phytochemical Screening and Antibacterial Activity of *Tapinanthus globiferus* (Mistletoe) Growing on *Azadirachta indica*, *Olea europaea* and *Albezia chevelieri*

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Abstract

Tapinanthus globiferus is mistletoe which grows on many plants. Mistletoes have been used in traditional medicine for treatment of diseases. Their extracts have been reported to demonstrated potent biological activities. *Tapinanthus globiferus* from *Azadirachta indica*, *Olea europaea* and *Albezia chevelieri* were extracted with the aid of a soxhlet extractor using n-hexane and methanol. The extracts were screened for the presence of phytochemicals using standard procedures. The antibacterial activity of the extracts were evaluated against gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *P. aeruginosa*) bacterial isolates using agar diffusion method. The preliminary phytochemical screening revealed the presence of saponins, tannins, flavonoids and cardiac glycosides in all the three samples. Anthraquinones were absent in all three samples. Alkaloid was found to be present in *Tapinanthus gloiferus* growing on *Azadirachta indica*. The susceptibility test of the methanol extracts showed zone of inhibition with the range of 8-20 mm. The hexane extracts did not show any activity. The result of the MIC (minimum inhibition concentration) of the methanol extract was 7.5 mg/mL and the MBC (minimum bactericidal concentration) was 15 mg/mL. The result of this study indicates a little difference in the chemical composition of various *Tapinanthus globiferus* growing on each of the plants. This may be attributed to the fact that the chemistry *Tapinanthus globiferus* depends on the host plant. Since they grow on different host plants, they may contain slightly different chemical constituents. The antibacterial activity demonstrated may be due to the presence of these phytochemicals.

Keywords: *Phytochemical Screening; Antibacterial Activity; Tapinanthus globiferus; Azadirachta indica; Olea europaea; Albezia chevelieri.*

1.0 INTRODUCTION

A medicinal plant is defined as any plant which contains substances that can be of therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs (Safowora, 1982; Borokini and Omotayo, 2012). A plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically proven (Bako *et al.*, 2005). Plants are a great source of medicines, especially in traditional medicine. They are useful in the treatment of various diseases (Bako *et al.*, 2005). According to World Health Organization (WHO, 2001), about 80 % of the world's population use medicinal plants in the treatment of diseases and in African countries, the percentage is higher. It has been estimated also that up to 90 % of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs due to the high cost of modern medicines (WHO, 2002). It has been reported in recent times that 50 % of top pharmaceutical products sold are linked to natural product research (Falodun *et al.*, 2007).

Mistletoe is known as *kauci* (Hausa), *awuruse* (Igbo) and *afomo ashana* (Yoruba). It is a hemiparasitic plant (i.e. partial parasite) that is found on various host plant. They are partially parasitic in the sense that though they are attached to the host plant from which they get nutrient and water, they have green leaves which carry out photosynthesis and manufacture food for the plant. Besides the host plant is considered as important as the mistletoe since distinction is made between host and not between mistletoe,

2.0 MATERIALS AND METHOD

2.1 Plant Collection and Identification

Fresh samples of *Tapinanthus globiferus* growing on *Azadirachta indica*, *Olea europaea* and *Albezia cheveleri* were collected in January, 2015 by Dr. H.E. Mshelia within Sokoto metropolis. The samples were identified by Mal. Auwali (Taxonomists) at the Department of Biological Sciences Usman Danfodiyo University, Sokoto. Voucher specimens with voucher number UDUH|ANS|0076 were

implying that the biology of the mistletoe is not clearly understood (Adeshina *et al.*, 2013).

Tapinanthus globiferus is mistletoe of the family *Loranthaceae*. It is a woody, spreading shrub with blackish, smooth stem made rough by the presence of lenticels. The leaves are opposite but sometimes alternate. Mistletoe attached to lime trees has been used as medicinal herbs for the treatment of tonsillitis and otitis media (Osadebe *et al.*, 2004). Ethanol extract of *Tapinanthus globiferus* has been proven in the study carried out by Osadebe *et al.* (2010) to contain compounds that may be capable of scavenging free radicals and also a good raw material for the development of drugs useful for the treatment of chronic inflammatory diseases. Mistletoe plant has also shown broad spectrum antimicrobial properties against certain bacterial and fungi of farm animals (Deen and Sadiq, 2002). Bassey (2012) found out that the host of the mistletoe plant influences the chemical composition of the plant. Methanolic leave extract of *Tapinanthus globiferus* showed anti-trypanosomal activity by suppressing the development of parasiteamia (Abedo *et al.*, (2013). Previous studies have focused on the anti-diabetic, potential antitumor and anticancer activities (Osadebe *et al.*, 2010 and Sadika *et al.*, 2003). Recently some species of mistletoes *Plicosepalus* and *Phragmanthera* genus have been proved to inhibit fatty acid synthesis with subsequent reduction of body weight in rat (Wanga *et al.*, 2008; Waly *et al.*, 2012). The purpose of this work was to evaluate the phytochemical constituents and antibacterial activities of *Tapinanthus globiferus* leaves extracts parasitizing on three different host plants.

deposited at the herbarium for reference purpose.

2.2 Preparation and Extraction of Samples

The leaves of the various *Tapinanthus globiferus* were shade dried and ground to powder using pestle and mortar. They were stored in separate polythene bags until required for use. The powdered plant samples (150 g) were extracted first with hexane with the aid Soxhlet apparatus for 3 hours. The marcs (residues) were allowed

to dry and weighed. The marcs were then extracted with methanol as described above. The extracts were concentrated by direct exposure to air and their percentage yields were calculated.

2.3 Preliminary Phytochemical screening

Phytochemical screenings of the extracts for the presence of secondary metabolites were carried out using standard procedures as described by Trease and Evans (2000) and Safowora, 1982.

2.4 Antibacterial Screening of the Extracts

2.4.1 Test Organisms

Standard laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usman Danfodiyo University, Sokoto.

2.4.2 Preparation of Culture Medium

The nutrient broth was prepared according to the manufacturer's specification and sterilized by autoclaving at 121 °C.

2.4.3 Susceptibility Test

The agar diffusion method was used. The nutrient agar was prepared according to the manufacturer's specification and then sterilized at 121°C. This was allowed to cool and then transferred to sterilize petri-dishes (the petri-dishes were sterilized at 170 °C for 1hour) and allowed to set. After setting, the petri-dishes were flooded with 1ml of the standardized culture of various dilutions of the test bacteria and the excess were drained using Pasteur pipette. Wells measuring 4 mm in diameter were bored into the inoculated petri-dishes using cork borer. The wells were sealed by a drop of melted nutrient agar and were filled with 0.05 mL each of 15 mg/mL, 20 mg/mL, 25 mg/mL and 30

mg/mL of hexane extract. The procedure described above was repeated with the methanol extract. The plates were allowed to stand for pre-diffusion time for 2 hours and then incubated for 24 hours at 37 °C. The zone of inhibition was measured to the nearest millimeter using a metric rule. The readings were taking in triplicate and the means recorded. Similarly, the plate of amoxicillin (10 mg/mL) was used as the positive control and the zone of inhibition was also determined.

2.4.4 Determination of minimum inhibitory concentration (MIC)

The nutrient broth (2 mLs) were prepared and distributed into 10 test tubes. The first tube contains 2 mLs of double strength nutrient broth and the other 9 tubes contain 2 mL of single strength nutrient broth. Into the first test tube, 2 mLs of the extract each containing 30 mg/mL (This is the concentration at which zone inhibition was observed) was added and mixed properly. This was serially diluted until the last test tube. The test organism (0.5 mL) containing 10^5 cfu/mL was added to the test tubes and inoculated at temperature of 37 °C for 24 hours.

2.4.5 Determination of minimum bactericidal concentration (MBC)

The MBC was determined from the MIC and the concentration just before it. 20 mLs of nutrient agar was prepared and poured into a sterilized petri-dish and allowed to cool. This was inoculated with the test organism (i.e. the organism that gave the highest zone of inhibition). The plate was divided into 10 portions. A loopful of the first test tube from MIC was collected and transferred into the first portion of the petri-dish. This was repeated in like manner until the last test tube. The plates were incubated at 37 °C for 24 hours.

3.0 RESULTS

3.1 Extraction

The mass of the extracts and the percentage yield are presented in Table 1.

Table 1: Mass of the Extracts obtained and Percentage yield.

Extract	mass (g)	Percentage yield (%)
Hexane		
a) <i>Tapinanthus globiferus</i> on <i>Azadirachta indica</i>	13.12	8.75
b) <i>Tapinanthus globiferus</i> on <i>Olea europaea</i>	11.80	7.87
c) <i>Tapinanthus globiferus</i> on <i>Albezia chevelleri</i>	12.20	8.13
Methanol		
a) <i>Tapinanthus globiferus</i> on <i>azadirachta indica</i>	15.58	11.12
b) <i>Tapinanthus globiferus</i> on <i>Olea europaea</i>	16.86	12.04
c) <i>Tapinanthus globiferus</i> on <i>Albezia chevelleri</i>	16.91	12.07

3.2 Phytochemical Analysis

The results of the phytochemical screening of the extracts are presented in Tables 2 and 3, respectively.

Table 2: Phytochemical Screening of the Hexane Extracts.

Secondary Metabolites/Test	<i>Tapinanthus globiferus</i> on <i>Azadirachta indica</i>	<i>Tapinanthus globiferus</i> on <i>Olea europaea</i>	<i>Tapinanthus globiferus</i> on <i>Albezia chevillieri</i>
Alkaloids	N.D	N.D	N.D
a) Mayer's			
b) Wagner's			
c) Drangedorff's			
Anthraquinones	N.D	N.D	N.D
a) Borntrager's			
Saponinns	N.D	N.D	N.D
a) Frothing			
Cardiac glycosides	+	+	+
a) Keller-kiliani's			
Flavonoids	N.D	N.D	N.D
a) NaOH			
b) Shinoda's			
c) FeCl ₃			
Tannins	N.D	N.D	N.D
a) FeCl ₃			
Steroids	+	+	+
a) Salkowski's			
b) Liebermann Buchard's			

Key: N.D= Not Detected; + = Present.

Table 3: Phytochemical Screening of the Methanol Extracts.

Secondary Metabolites/Test	<i>Tapinanthus globiferus</i> on <i>Azadirachta indica</i>	<i>Tapinanthus globiferus</i> on <i>Olea europaea</i>	<i>Tapinanthus globiferus</i> on <i>Albezia chevillieri</i>
Alkaloids	+	N.D	N.D
a) Mayer's			
b) Wagner's			
c) Drangedorff's			
Anthraquinones	N.D	N.D	N.D
a) Borntrager's			
Saponinns	+	+	+
a) Frothing			
Cardiac glycosides	+	+	+
a) Keller-kiliani's			
Flavonoids	+	+	+
a) NaOH			
b) Shinoda's			
c) FeCl ₃			
Tannins	+	+	+
a) FeCl ₃			
Steroids	+	+	+
a) Salkowski's			
b) Liebermann-Buchard's			

Key: N.D= Not Detected; + = Present

3.3 Antibacterial Activity

The results of the antibacterial activity are presented in Tables 4 to 8.

Table 4: Antibacterial activity of the methanolic extract of *Tapinanthus globiferus* Parasitizing on *Azadirachta indica* . Key: - = No inhibition.

Concentration (mg/mL)	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
15	-	10.0	-	-
20	-	11.0	-	-
25	-	14.0	-	-
30	-	16.0	-	-

Table 5: Antibacterial activity of the methanolic extract of *Tapinanthus globiferus* parasitizing on *Olea europaea*.

Concentration (mg/1mL)	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
15	9.0	12.0	8.0	9.0
20	11.0	15.0	10.0	10.0
25	12.0	18.0	11.0	12.0
30	15.0	20.0	14.0	14.0

Key: - = No inhibition

Table 6: Antibacterial activity of methanolic extract of *Tapinanthus globiferus* parasitizing on *Albezia chevelleri*

Concentration (mg/1mL)	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
15	8.0	8.0	-	10.0
20	10.0	11.0	-	11.0
25	12.0	13.0	-	14.0
30	13.0	16.0	-	17.0

Key: - = No inhibition

Table 7: Antibacterial activity of standard antibiotic (Amoxicillin).

Concentration (mg/mL)	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
10	34.0	36.0	-	37.0

Table 8: MIC and MBC of the Methanol Extract of *Tapinanthus globiferus* parasitizing on *Olea europaea*

Test organism	MIC (mg/mL)	MBC (mg/mL)
<i>S. aureus</i>	15.0	15.0
<i>B. subtilis</i>	7.5	7.5
<i>P. aeruginosa</i>	15.0	15.0

4.0 DISCUSSION

The results of the extraction with hexane and methanol (Table 1) showed the percentage yield of the extracts. It was observed that the percentage yield of methanol extracts were higher than that of the n-hexane extracts. This may be due to the fact that the methanol being a polar solvent has the ability to extract more of the secondary metabolites than n-hexane. The results of the preliminary phytochemical screening of the hexane extracts (Table 2) revealed the presence of cardiac glycosides and steroids. The results of the preliminary phytochemical screening of the methanol extracts (Table 3) revealed the presence of flavonoid, cardiac glycoside, tannins, steroids, saponins in all the three samples. Alkaloids were found to be present only in *Tapinanthus globiferus* parasitizing *Azadirachta indica*. Anthraquinones was not detected in all the three methanol fractions. These results showed that the host on which *Tapinanthus globiferus* were found, influences the phytochemical composition of mistletoe. All *Loranthaceae* are parasites of the xylem tissue and depend on their host for water, nutrient and some carbon compounds (Didier *et al.*, 2008; Minnih *et al.*, 2015). Since the plant they parasite on have medicinal properties there might have been exchange of such properties between the host and the parasite (Minnih *et al.*, 2015). The presence of secondary metabolites in the mistletoes and the host plant has been reported to be responsible for different pharmacological and physiological actions of plant extracts (Cragg and Newman, 2005). The hexane extract of the three samples did not show any antibacterial activity on the test organism at varying concentration of 15, 20, 25, 30 mg/ml.

These may be associated to the inability of the hexane (being a non-polar solvent) to extract the bioactive component that can demonstrate antibacterial activity. The methanol extracts exhibited varying degree of antibacterial activity against the test organisms, with the *Tapinanthus globiferus* parasitizing on *Olea europaea* exhibiting highest activity against *B. subtilis* and *S. aureus* (Table 5). The *Tapinanthus globiferus* parasitizing on *Albezia cheveleri* exhibited highest activity against *P. aeruginosa* and *B. subtilis* (Table 6). *Tapinanthus globiferus* growing on *Azadirachta indica* showed activity only *B. subtilis* and other bacteria were resistance to this sample (Table 4). In the susceptibility test, the methanol extracts showed zone of inhibition ranging between 8 mm to 20 mm. Also, none of the extract demonstrated broad spectrum antibiotic activity. The standard antibiotic (amoxicillin) (Table 7) showed better activity at 10 mg/mL than all the extracts. It can be deduced from the results that the antibacterial activity demonstrated by the extracts were found to be concentration dependent. Generally, the activity demonstrated by the extracts may be due to the presence of flavonoids (Tsuchiya *et al.*, 1996), alkaloids (karou *et al.*, 2005), tannins (Haslam, 1996), steroids (Silva *et al.*, 2003), glycoside (Jacques *et al.*, 2011) and saponins (Soetan *et al.*, 2006). The Minimum Inhibitory Concentration (MIC) of methanol extract of *Tapinanthus globiferus* parasitizing on *Olea europaea* was between 7.5 to 15 mg/mL and the Minimum Bactericidal Concentration (MBC) range was 7.5 to 15 mg/1mL (Table 8). From the result of the MIC and MBC, it showed that the various methanol extracts are bactericidal.

5.0 CONCLUSION

The preliminary phytochemical screening of the leaves extract of *Tapinanthus globiferus* growing on *Azadirachta indica*, *Olea europaea* and *Albezia chevilleri* showed that there is little or no difference in phytochemical constituents

of *Tapinanthus globiferus* growing on one host plant to the other. The methanol extracts showed selective bactericidal activity on the test organisms.

6.0 REFERENCES

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