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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF MISTLETOE (*Tapinanthus globiferus* A. Rich, Tiegh.) ON SELECTED CLINICAL ORGANISMS USING TWO DIFFERENT SOLVENTS

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ABSTRACT

The study investigated the qualitative and quantitative phytochemical constituents and antimicrobial activities of the leaf extracts of Mistletoe plant (*Tapinanthus globiferus*). Chloroform and ethyl acetate were used in extracting the bioactive components of the leaves, while crude extract was obtained by squeezing fresh leaves to obtain the extract. The agar well diffusion method was used in investigating the antimicrobial activities of the extracts. The result of the phytochemical test showed that tannin, phenol, alkaloids, flavonoids and saponin were present in the leaf extracts of *T. globiferus* at varying intensity. The quantitative analysis showed that tannin content of the extracts ranged from 2.70 – 7.01%. The phenol, alkaloids, flavonoids and saponin content ranged from 3.18 – 5.01%, 2.65 – 6.19%, 2.85 – 8.46% and 2.63 - 5.85% respectively. The antimicrobial study showed that the chloroform extract of *T. globiferus* gave an inhibition of 10.67 mm (*Salmonella enterica*), 17.33 mm (*Pseudomonas aeruginosa*), 15.33 mm (*Staphylococcus aureus*), 15.33 mm (*Penicillium digitatum*) and 11.00 mm (*Rhizopus stolonifer*). Similarly, ethyl acetate extract gave an inhibition of 12.67 mm for *S. enterica*, *P. aeruginosa* (12.00 mm), *S. aureus* (9.33 mm) *P. digitatum* (14.00 mm) and *R. stolonifer* (18.67 mm), while crude extract gave the inhibition of 11.67 mm (*S. enterica*), *P. aeruginosa* (7.00 mm), *S. aureus* (13.67 mm) *P. digitatum* (8.33 mm) and *R. stolonifer* (0 mm). The control (Gentamicin and Ketoconazole) gave zone of inhibition that ranged from

35 – 46 mm against the test organisms by Gentamicin whereas Ketoconazole showed zone of inhibition greater than 43 mm for all the test organisms.

KEYWORDS: Phytochemical, Antimicrobial, Qualitative, Quantitative, Extract, Inhibition, Microorganisms.

INTRODUCTION

The use of plant as a source of medicine to treat pathogenic diseases predate history; nearly all culture and civilization from ancient time to present day, have used herbal medicine to treat infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal or medicinal plant products as sources of novel compounds to fight the ever increasing problems (Adwan *et al.,* 2006).

The primary benefits of using plant derivative medicine are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Amos *et al.,* 2005).

Mistletoe is one of those plant species that has been utilized for its therapeutic values. It is a semi-parasitic plant, that is found growing on branches of most trees such as, Citrus tree (*Citrus grandis and Citrus sinensis*), Sheer butter (*Vitllaria paradoxa*) Umbrella tree (*Terminalia mantaly)*, Cocoa (*Theobroma cacao*), Cashew (*Anacardium occidentale*), Bush mango (*Irvingia gabonensis)*, Guava (*Psidium guajava*) Mango (*Mangifera indica*), Neem (*Azadirachta indica*), Sugar apple (*Annona squamosal*), and Kola tree (*Cola nitida*) etc. Plants of these species (parasitic plants), are known all over the world to cause damages to their host and pose serious threats to various plantations by paralyzing cultivated and tended plants (Deeni and Sadiq, 2002). In addition, they are agent of diseases and therefore affect the host physiology leading to reduction in growth, survival and reproduction (Shaw *et al.,* 2004).

Despite their destructive nature to their host, the plant possesses some active ingredients that can inhibit the growth of microorganisms, and therefore are used medicinally for the treatment of various ailments. (Orhue *et al*., 2014). For example, mistletoes are used ethno-botanically by the Hausa and the Fulani ethnic groups of Northern Nigeria in the treatment of many human and animal diseases such as dysentery, diarrhea, stomach ache and cancer (Deeni and Sadiq, 2002). In Burkina Faso, the decoctions of this species have been

traditionally used to treat cardiovascular diseases and asthma etc. (Boly *et al.,* 2016; Carrie *et al.,* 2014).

The therapeutic values of mistletoes cannot be over emphasized, as this research work is focus on determining the bioactive constituents of mistletoe plant using two different solvent as well as its crude form. The antimicrobial activity of these extracts on selected clinical organisms will also be investigated.

MATERIALS AND METHODS

Study area

The research work was carried out at Emery Research laboratory located at Ahiaeke Ndume Ibeku in Umuahia North LGA of Abate State.

Collection of plant Materials and Identification

The fresh leaves of Mistletoe *T. globiferus* (Plate 3.1) were collected from an umbrella tree (*Terminalia mantaly*), (Which Serves As The Host For This Parasitic Plant) in the vicinity of National Root Crops Research Institute Umudike, Abia State at Dr. Ikoro's residential compound and were identified by the Taxonomist in the Herbarium unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umuahia Abia State, Nigeria. The fresh and tender leaves of the plant were used for the phytochemical analysis.

Plate 3.1: Shows mistletoe plant leaf *T. glabiferius* collected from *Terminalia mantaly* (Host tree).

Preparation of plant material

The leaves of the plant were harvested and washed under running tap to remove dirt. The leaves were divided into two parts; one part was oven-dried at 40°C for 48hrs and then ground into a fine powder using an attrition milling machine. The powdered sample were weighed and stored in two separate sample bottles.

Extraction of plant material

Maceration method of extraction according to Nagappan (2012) was employed for the extraction. Chloroform, ethyl acetate were used for extraction from the powdered leaves, while the remaining fresh leaves were squeezed to obtain the crude extract. Twenty grams of each powdered plant material was macerated in 200 ml of chloroform and ethyl acetate into a glass bottle and covered such that the level of the solvent was above that of the plant material. The macerated mixtures were then left to stand over-night at room temperature. The extracts were filtered out from the macerated mixture using a muslin cloth. The chloroform and ethyl acetate extracts were concentrated, labeled and allowed to stand at room temperature to permit evaporation of residual solvents. The crude extract was used as collected.

Phytochemical analysis

Qualitative analysis of the leaf extracts

Alkaloids

The extract from *T. globiferus* (1 ml) was treated with a few drops of Wagner's reagent. The red-brown precipitate indicated the presence of alkaloids (Salehi-Surmaghi *et al*., 1992).

Flavonoids

The presence of flavonoids was estimated by Shinoda test. 1ml of the extracts was treated with a few drops of dilute NaOH solution. The appearance of yellow colour indicated the presence of flavonoids (Somolenski *et al*., 1972).

Tannins

One ml of the extract from each solvent was treated with 1ml of alcoholic $FeCl_3$ reagent. Blue colour indicated the presence of tannins (Segelman *et al*., 1969).

Saponins

The presence of saponins was determined by Frothing test. The extract of each plant (1ml) was vigorously shaken with distilled water (1ml) and was allowed to stand for 10 minutes

and classified for saponin content as follows: no froth indicate absence of saponins and stable froth indicated the presence of saponins (Kapoor *et al*., 1969).

Phenol

The 1 ml of each extract of each plant was treated with 1 ml of dilute HCl and 1 ml of alcoholic $FeCl_3$ reagent. Blue colour indicated the presence of phenol

Quantitative phytochemical analysis

Determination of alkaloids

The gravimetric method of Haborne (1973) was used in determination of alkaloids. One gram of each extract was dispersed in 50 ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for four hours before it was filtered. The filtrate was evaporated to one quarter of its original volume. Concentrated NH_4OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH_4OH solution, the filtering was done with a weighed filter paper. The precipitate in filter paper was dried in the oven at 60^0C for 30 minutes and reweighed. By weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample weighed analyzed.

Determination of tannins

The Follin–Dennis spectrophotometric method was used. The method was described by Pearson (1976). One gram of each extract was dispersed in 10 ml of distilled water and agitated. This was left to stand for 30mins at room temperature being shaken every 5 min. At the end of the 30mins, it was centrifuged and 2-5 ml of the supernatant was dispersed into a 50 ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50 ml flask.

A 1.0 ml follin-dennis reagent was measured into each flask, followed by 2.5 ml of saturated to mark in the flask (50 ml) and incubated for 90 minutes at 150 m temperature the absorbance were measured at 250 nm in a Gen-way model 6000 electronic spectrophotometer, readings were taken with the reagent blank at zero. The tannin content was given as follows.

Determination of flavonoid

Flavonoid in the test extracts was determined by the acid hydrolysis gravimetric method of Harborne (1973). One gram of the plant sample was mixed with diluted 1 ml HCl solution to

form a ratio 1:10 w/v. The mixture was boiled for 30 minutes. The boiled extract was allowed to cool and filtered through What-man No 42 filter paper. A portion of the extract (20mls) was measured with a beaker and treated with ethyl acetate to precipitate the flavonoid. The precipitate was measured by filtering with a weighed filter paper and determined by weight difference.

Determination of saponin

The method used was described by AOAC (2000). About 5.0 g of dry ground sample was weighed inside extractor thimble and transferred into the soxhlex extractor chamber fitted with a condenser and a round bottomed flask. Some quantity of acetone, enough to cause a reflux was poured into the flask; the sample was exhaustively extracted of its lipid and interfering pigments for 3 hours by heating the flask on a hot plate and the solvent distilled off. This is the first extraction. For the second extraction, a pre-weighed round flat bottomed flask is fitted unto the soxhlex apparatus (bearing the sample containing thimble) and methanol poured into the flask, the methanol should be enough to cause the reflux, the saponin is then exhaustively extracted for 3 hours by heating the flask on a hot plate after which the difference between the final and initial weights of the flask represents the weight of saponin extracted.

Determination of phenol

Phenol was determined by spectrophotometric method described by Pearson (1976). One gram of the flour sample was added into a test tube and 10 millilitres of methanol was added to it and shaken thoroughly, the mixture was left stand for 15 minutes before being filtered using What-man No 42 filter paper. One milliliter of the extract was placed in a test tube and 1ml follin Dennis reagent in 5ml of distilled water was added and colour was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760nm wavelength. The phenol content was calculated thus.

Antimicrobial assay

The antimicrobial assay was done using the agar well diffusion method. One gram for each extracts were dissolved in 2 ml Dimethyl Sulphoxide (DMSO) to get a concentration of 500 mg/ml, other lower concentrations (200 mg/ml and 100 mg/ml) were prepared by diluting serially with DMSO. The various bacterial and fungal isolates were standardized using the 0.5 McFarland turbidity standards. These standardized strains were inoculated onto the surface of sterile plates of Mueller Hinton Broth (MHB). Cork borer (6 mm) was used to

make wells on the inoculated sterile plates. Each concentration of the extracts was introduced into designated wells. These were allowed to be absorbed into the agar, and then incubated at 37^0C for 24h. The antimicrobial activities were determined by the width of the zone of growth inhibition (Bauer, 1998).

Minimum inhibitory concentrations

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration or the highest dilution of an antimicrobial agent that prevents visible growth after 18-24 hours of incubation. It was determined by making dilution of various concentrations (200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml) of the chloroform, ethyl acetate and crude extracts in test tubes. 1ml stock solution of the plant extract 100 mg/ml was diluted in sterile test tube containing 0.95ml of Mueller Hinton Broth (MHB) to obtain further dilution. Serial dilution techniques were employed by transferring 1 ml from the first test tube to the second test tube and from the second to the third. This was continued to the seventh test tube from where 1ml was discarded to give concentration of 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml. Another test tube was also prepared with MHB (control) of the test tube which was inoculated with standard suspension (50 µl) of the test organisms and incubated at 37°C overnight.

After incubation, the turbidity in each tube was checked. The tube that contains the lowest concentration which showed no turbidity i.e. a clear view, was observed to be the MIC of the antimicrobial agent for the organism tested, the lower the MIC, the more susceptible is the test organism.

Statistical analysis

Complete Randomized Design (CRD) was the experimental design used which was replicated three times. The data collected were subjected to Analysis of variance (ANOVA) using SPSS version 20 and the mean separation carried out using Duncan's Multiple Range Test (DMRT).

RESULTS

The results of the phytochemical constituents of chloroform, ethyl acetate and crude extracts of *Tapinanthus globiferus* are presented in Tables 4.1 and 4.2. The qualitative analysis result obtained showed that tannin, phenol, alkaloids, flavonoids and saponin were present in the

leaves of *T. globiferus* at varying intensities in all the solvents used for the extraction (Table 4.1).

The result of the quantitative analysis revealed that *T. globiferus* contains tannins that was 2.70 % for chloroform, 4.80 % for ethyl acetate and 7.01 % for the crude. The percentage of Phenol was 3.18, 4.75 and 5.01 % for chloroform, crude and ethyl acetate respectively. The alkaloids content was 2.65 % (chloroform), 3.12 % (ethyl acetate) and 6.19 % (crude). Flavonoids content was 2.85 % for chloroform 4.25 % for ethyl acetate and 8.46 % for crude extract. The amount of saponin obtained was 2.63 % for ethyl acetate, 3.26 % for chloroform and 5.85 % for crude extract. The phytochemicals obtained showed varying concentrations at different levels depending on the extraction solvent. Crude extract gave the highest percentage (8.46 % for flavonoid) followed by ethyl acetate extract that produced 5.01 % for phenol whereas chloroform extract gave the least value with 2.65 % for alkaloids (Table 4.2).

Table 4.1: Shows the qualitative phytochemical analysis of the leaf extract of *T. globiferus* using chloroform, ethyl acetate and its crude form.

Parameters	Chloroform extract	Ethyl acetate extract	Crude extract
Tannin	+	++	+++
Phenol	+	++	++
Alkaloid	++	+	+++
Flavonoid	+	++	+++
Saponin	+	+	++

+ signifies the intensity of colouration (precipitate)

Table 4.2: Shows the quantitative phytochemical analysis of the leaf extract of *T. globiferus* using chloroform, ethyl acetate and its crude form.

Parameters	Chloroform extract	Ethyl acetate extract	Crude extract
Tannin	$2.70^{c}\pm0.11$	$4.80^{b}\pm0.00$	$7.01^{a}\pm0.16$
Phenol	$3.18^{b}\pm0.20$	$5.01^{a}\pm0.00$	$4.75^{a}\pm0.08$
Alkaloid	$2.65^{c}\pm0.00$	$3.12^{b}\pm0.00$	$6.19^{a}\pm0.16$
Flavonoid	$2.85^{c}\pm0.00$	$4.25^{b}\pm0.18$	$8.46^{a}\pm0.34$
Saponin	$3.26^{b}\pm0.20$	$2.63^{c}\pm0.18$	$5.85^{a}\pm0.18$

Values are mean ± SD. Values on the same row with different superscripts are significantly different ($P<0.05$).

The result of the antimicrobial activities of *T. globiferus* leaf extracts are presented in Table 4.3. The result showed that the chloroform extract of *T. globiferus* gave an inhibition of;

10.67 mm (*Salmonella enterica)*, 17.33 mm (*Pseudomonas aeruginosa*), 15.33 mm (*Staphylococcus aureus*), 15.33 mm (*Penicillium digitatum*) and 11.00 mm (*Rhizopus stolonifer)*. Similarly, ethyl acetate extract gave an inhibition of *S. enterica* (12.67 mm), *P. aeruginosa* (12.00 mm), *S. aureus* (9.33 mm) *P. digitatum* (14.00 mm) and *R. stolonifer* (18.67mm), while crude extract gave the inhibition of *S. enterica* (11.67 mm), *P. aeruginosa* (7.00 mm), *S. aureus* (13.67 mm) *P. digitatum* (8.33 mm) and *R. stolonifer* (0 mm). The control (gentamicin and ketoconazole) gave a ranged of 35 – 46 mm against the test organisms, which was significantly higher (P<0.05) than those of the test extracts. The result showed that there was no significant difference (P>0.05) in the inhibition zone obtained for the extracts against *S. enterica* and *P. digitatum*. There was also no significant difference in the inhibition obtained for chloroform and ethyl acetate extracts against *P. aeruginosa* and *R. stolonifer.*

Table 4.3: The result of Antimicrobial activities of the leaf extracts of *T. globiferus* on some selected clinical organisms.

	Zone of inhibition (mm)				
Plant extracts (mg/ml)	***Salmonella. enterica***	***Pseudomonas aeruginosa***	***Staphylococcus . aureus***	***Penicilium digitatum***	***Rhizopus stolonifer***
Chloroform extract	$10.67^{b}\pm4.04$	$17.33^{b}\pm5.13$	$15.33^{b}\pm4.59$	$15.33^{b}\pm5.03$	$11.00^{b}\pm 2.00$
Ethyl acetate extract	$12.67^{b}\pm3.06$	$12.00^{bc}\pm3.00$	$9.33^{c}\pm2.52$	$14.00^{b}\pm7.94$	$8.67^{b}\pm 1.53$
Crude extract	$11.67^{b}\pm3.06$	$7.00^{c}\pm0.00$	$13.67^{bc}\pm2.52$	$8.33^{b}\pm1.53$	0^{c}
Control (Gentamicin) Ketoconazole	$35.00^{a}\pm0.00$	$40.00^{a}\pm00.00$	$42.00^{a}\pm0.00$	$44.00^{a}\pm0.00$	$46.00^{a}\pm 0.00$

Values are mean ± SD. Values on the same column with different superscripts are significantly different (P<0.05).

Table 4.4 presents the result of the minimum inhibitory concentration (MIC) of the leaf extracts. The result obtained showed that the chloroform extract had an MIC of 6.25 mg/ml for *P. aeruginosa, S. aureus and P. digitatum* as well as an MIC of 25 mg/ml for *S. enterica and R. solonifer.* Ethyl acetate extract had an MIC of 12.5 mg/ml for *S. enterica and P. aeruginosa,* while it also had an MIC of 25 mg/ml for *S. aureus, P. digitatum* and *R. stolonifer.* The crude extract showed an MIC of 12.5 mg/ml for *S. enterica* and *S. aureus*, 25 mg/ml for *P. digitatum*, 100 mg/ml for *P. aeruginosa* and 200 mg/ml for *R. stolonifer* (Table 4.4c).

Table 4.4a: Minimum inhibitory concentration of Chloroform extract of *T. globiferus* leaf on the test organisms.

Test organisms	100	50	25	12.5	6.25
Salmonela enterica	-	-	-	+	+
Pseudomonas aureginosa	-	-	-	-	+
Staphylococcus aureus	-	-	-	-	+
Penicillin digitata	-	-	-	-	+
Rhizopus stolonifera	-	-	-	+	+

Note: + (growth), - (no growth

Table 4.4b: Minimum inhibitory concentration of ethyl acetate extracts *T. globiferus* leaf on the test organisms.

Test organisms	100	50	25	12.5	6.25
Salmonela enterica	-	-	-	-	+
Pseudomonas aureginosa	-	-	-	-	+
Staphylococcus aureus	-	-	-	+	+
Penicillin digitatum	-	-	-	+	+
Rhizopus stolonifera	-	-	-	+	+

Note: + (growth), - (no growth)

Table 4.4c: Minimum inhibitory concentration of crude extracts *T. globiferus* leaf on the test organisms.

Test organisms	200	100	50	25	12.5	6.25
Salmonela enterica	-	-	-	-	-	+
Pseudomonas aureginosa	-	-	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	+
Penicillin digitatum	-	-	-	-	+	+
Rhizopus stolonifer	-	+	+	+	+	+

Note: + (growth), - (no growth)

DISCUSSION

Phytochemical screening of *T. globiferus* revealed the presence of constituents such as alkaloids, saponins, tannins, phenol and flavonoids. These phytochemicals occurred in varying concentration and their concentration also depended on the extraction solvent used. Similar observation was reported by Emaikwu *et al*. (2019) that *T. globiferus* contains tannin, phenol, alkaloids, flavonoids and saponin at different levels depending on the extraction solvent. Crude extract (7.01 %) had the highest tannin content, followed by ethyl acetate extract (4.80 %) while chloroform extract (2.70 %) had the least. There was a significant difference in the tannin content of the extracts. The higher value obtained for the crude

extract shows that tannin in *T. globiferus* is better extracted using the crude form than by the use of solvents.

The phenol content of the extracts ranged from (3.18 %) in the chloroform extract to (5.01 %) in ethyl acetate extract. There was no significant difference in the phenol content of the ethyl acetate extract and the crude extract. The higher value obtained for the ethyl acetate extract shows that phenol was better extracted with ethyl acetate than the other solvents used. This could be attributed to the fact that the extraction potential of solvents increase with increasing polarity i.e. from non-polar to polar solvent (Michael, 2010). Koche *et al*. (2010) also reported that polarity affect the extraction potential of solvents.

Alkaloid content of the extracts ranged from (2.65 %) in chloroform extract to (6.19 %) in the crude extract. There was a significant difference ($P<0.05$) in the alkaloid content of the ethyl acetate extract and the crude extract. The lower value obtained for the chloroform extract showed that chloroform least extracted the alkaloids in *T. globiferus* than the others used in this study. The higher value obtained for the crude extract confirmed the report of Michael (2010) that alkaloids are more extracted using nonpolar solvent than polar solvents.

The flavonoids content of the extract ranged from (2.85 %) in the chloroform extract to (8.46 %) in crude extract. There was a significant difference in the flavonoids content of the extracts. The lower value obtained for the crude extract suggest that flavonoids are better extracted using crude method.

The saponin content ranged from (2.63 %) in ethyl acetate extract to (5.85 %) in crude extract. There was no significant difference in the saponin content of chloroform and crude extract of *T. globiferus*. The higher value obtained for the crude extract compared to the others confirmed the report of Michael (2010) that saponin is less extracted in nonpolar solvent.

Tannins have been described to contain a large number of complex substances that are widely distributed in almost all parts of the plant. They are usually localized in the various parts of the plant such as the leaves, stem, roots and barks (Oyi *et al*., 2001). Phytochemicals are the natural bioactive compounds found in plants. The phytochemicals work with nutrients and fibres to form an integrated part of defense system against various diseases and stress condition (Koche *et al*., 2010). The most important of these bioactive constituents of plants

are alkaloids, tannins, flavonoids and phenolic compound (Pascaline *et al*., 2011). Koche *et al*. (2010) reported that tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes.

Natural phenolic and flavonoids compounds are widespread in the plant kingdom. They are found in leaves, fruits, barks and wood and can accumulate in large amounts in particular organs or tissues of the plant (Nieminen *et al*., 2002). The mode of antifungal action of phenolic and flavonoids extracts might be related to their ability to inactive adhesions, enzymes and cell envelope of microorganisms (Chabot *et al*., 1992 and Cowan, 1999).

It has been reported that tannins act as an antifungal agent at higher concentrations by coagulating the protoplasm of the micro-organism (Adekunle and Ikumapayi, 2006). Similarly, the possible mechanism of action of tannins has been linked to interference with energy generation by uncoupling oxidation phosphorylation or interference with glycoprotein of cell surface (Harekrishna *et al*., 2010). Saponins are naturally occurring surface-active glycosides and they have been reported to possess strong antifungal activities in their interaction with membrane sterols (George *et al*., 2002). These secondary metabolites have been found to be present in plants at various parts and levels of growth (Adeshina *et al*., 2010).

For the antimicrobial result obtained, it demonstrated that the control (gentamicin) showed the inhibition greater than 34 mm for all the test organisms. The result shows that ethyl acetate extract showed greater activity against *S. enterica* and *P. digitatum* than the other extracts, while chloroform extract showed higher activity against *P. aeruginosa, S. aureus* and *R. stolonifer* than the other extracts. This shows that the solubilization of bioactive compounds in *T. globiferus* that inhibit microbial growth is relative to the extraction solvent used. The mode of action of solvents (chloroform and ethyl acetate) extracts could be related to their ability to alter membrane properties leading to cell death (Aqil *et al*., 2012). The aqueous extract of *T. globiferus* has been reported to show good inhibitory activity against gram positive and gram negative bacteria (Emaikwu *et al*., 2019). Ndukwe *et al*. (2001) also reported the inhibition of *P. aeruginosa and S. aureus* by *T. globiferus* leaf extracts.

These findings are also in line with the work of Kabiru *et al*. (2017) who reported a similar result that the crude leaf extract and fractions of *T. globiferus* leaves possessed a broad

spectrum antibacterial activity against *S. aureus*, *E. coli, S. pyogenes, P. aureginosa, S. liquefaciens, P. mirabilis and CN. Staph.* at dose dependent manner.

Tari *et al*. (2015) have also reported the wide spectrum antibacterial activity of the extract against multi drug resistance *E. coli*, *Proteus spp.*, *Pseudomonas* spp., *Bacillus* spp. and *Salmonella* spp., isolated from farm animals.

The sensitivity of *S. aureus, S. enterica* and *P. aeruginosa* is very important since they have reportedly shown resistance to antibiotics. *S. aureus* shows resistance to multiple antimicrobial agents leading to therapeutic problems such as methicillin-resistance *S. aureus* (Koche *et al*., 2010), while the *S. enterica* and *P. aeruginosa* is resistant to vancomycin, ampicillin and first generation cephalosporin (Aqil *et al*., 2012). This means that people who consume *T. globiferus* leaves could potentially be safe from diseases caused by these organisms.

The inhibition of the test organisms by the leaf extracts could be attributed to the phytochemicals present in the extracts. Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins as well as to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. There are possible disadvantages of using the agar diffusion method to determine antimicrobial activity is that the antimicrobial effect may be affected by the agar type, salt concentration, incubation temperature and molecular size of the antimicrobial component. Furthermore, it does not distinguish between bactericidal and bacteriostatic effects (Eloff, 2001). *T. globiferus* have been reported in other studies to inhibit the growth of *Staphylococcus spp., P. aeruginosa, Salmonella spp* etc. (Emaikwu *et al*., 2019). *T. globiferus* was also reported to inhibit a host of gram positive and Gram negative bacteria (Ndukwe *et al*., 2001). The higher MIC observed with crude extract against *R. stolonifer* could suggest that the bioactive component needed for the inhibition of the growth of the test organisms were better extracted using non-polar solvent. The antimicrobial activity of the plant extracts against the test organisms could be attributed to the high amounts of the bioactive compounds present in the extracts. The bioactive compounds from plants has been reported to have antimicrobial properties (Lewis and Ausubel, 2006; Adeshokan *et al*., 2007; Oyeleke *et al*., 2008; Udobi *et al*., 2008).

Nevertheless, the antifungal action of the plants extracts against all the test organisms demonstrates that these plants might have broad spectrum therapeutic effect.

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