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Phytochemical and Antimicrobial Study on the Leaf Extract of *Tapinanthus dodoneifolius* (Van Teigh) Loranthaceae

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Leaves of *Tapinanthus dodoneifolius* is used as traditional medicine for the treatment of menstrual pain, wound, gonorrhea, and control of diarrhea and dysentery which was extracted by cold maceration in methanol. The concentrated methanol extract was subsequently partitioned with petroleum ether, chloroform, ethylacetate and n-butanol. Agar well Diffusion method was used to

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determine the antimicrobial activities of the extracts against *Staphylococcus aureaus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiela pneumonia, Salmmnella typhi*, *Streptococcus pyogenes*, *Candida krusei*, *Candida tropicalis,* and *Candida albican* at different concentrations. Antimicrobial sensitivity test indicated that, the extract inhibited the growth of all microorganisms to varying degrees except *Pseudomonas aeruginosa* and *Candida krusei* respectively. The *in vitro* antimicrobial screening revealed that, the extracts exhibited diverse activities against different microbe's concentrations ranging from 2.5mg/ml-5mg/ml. The plant extract was found to contain flavonoids, terpenoids, saponins, tannins and cardiac glycosides which may be responsible for its antimicrobial properties. The result justifies the traditional use of the plant leaf in the treatment of menstrual pain, sexual transmitted diseases, wounds and control of dysentery and diarrhea.

Keywords: Tapinanthus dodoneifolius; phytochemistry; antimicrobial activity.

1. INTRODUCTION

Biologically active compounds from natural sources are of interest as possible new drugs for infectious diseases [1]. Nature has been a source of medicinal agent since time immemorial. The importance of herbs in the management of human ailment cannot be over emphasized. Plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [2]. Herbal medicine in developing countries is commonly use for the traditional treatment of health problems [3].

T. dodoneifolius, is a hemi-parasitic plant that grows attached to and within the branches of a tree. African mistletoe as it is locally called is a parasitic savannah plant widely used in Northern Nigeria and other cities within the region. The parasitic plant is widely use in Northern Nigeria as a folk remedy to control chronic diarrhea and dysentery [4].

T. dodoneifolius is used in Ayurvedic medicine as an Emmenagogue while the stem extract is use orally to stimulate uterine contraction in pregnant women [5]. The aqueous infusion of the leaf extract with *Tamarindus indicus* is used as an analgesic agent to alleviate menstral pain [6]. The hot aqueous extract of the leaf mixed with trona is taken as a remedy for the treatment of sexually transmitted diseases. The decoction of the leaf and stem bark extract of the plant is use as an antihelmintic as well as an antispasmodic agent. In recent years, multiple drug resistance in both human and plant pathogenic microorganism have developed due to the indiscriminate use of commercial antimicrobial drugs commonly use in the treatment of infectious disease [7,8]. *T. dodoneifolius* has not been well investigated in terms of antimicrobial properties. The aim of this study is to evaluate the leaves of *T. dodoneifolius* in terms of antimicrobial activities against wide range of associated microorganisms including fungi and bacteria.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant *T. dodoneifolius* (Van teigh) Loranthaceae was collected from a farm land on a host plant *Parkia biglobosa* (Fabaceae) commonly known as locust bean tree as a parasitic plant in Zaria Kaduna state, Nigeria in the month of Feb. 2014. The plant was authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen, No. DC 1638 was deposited.

2.2 Preparation of Plant Material and Extraction

The fresh plant material was carefully separated into the leaves, stem bark. The leaves part of the plant was air dried at room temperature for two weeks. The leaves were made into powder using pestle and mortar subsequently referred to as the powdered material. The powdered leaves material (500g) was extracted with petroleum ether 60-80c^0 (3x500ml) to exhaustion using cold maceration. The defatted marc was air dried at room temperature and exhaustively extracted at room temperature with methanol (4x500ml). The solvents from each extract were recovered *in-vacuo* to afford an oily light green and dark brown gummy mass referred to as petroleum ether and methanol extract coded as P_E and M_E. 20g of the methanol extract was suspended in water (500ml) and sequentially partitioned with Ethylacetate (3x400ml) and n-butanol (4x400ml).

The solvent were recovered using rotary evaporator to afford Ethylacetate (E_t), n-butanol (N-but) and residual aqueous extract (Aq) respectively [9].

2.3 Phytochemical Screening

The petroleum ether, methanol extract and various partitioned fraction of the methanol extract were subjected to phytochemical screening using standard protocols [5,10].

3. ANTIMICROBIAL ASSAY

3.1 Microorganism

The microorganisms of *Staphylococus aureus, Bacillus subtitis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Candida krusei, Candida tropicalis* and *Streptococcus pyogenes* were obtained from the medical microbiology Department of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The microorganisms were checked for purity and maintained on slants of nutrient agar.

The control drugs use on this experiment include (i) Sparfloxacin, (ii) Ciprofloxacin and (iii) Fluconazole.

3.2 Preparation of Extract

Three concentrations of 1000mg/ml, 750mg/ml and 500mg/ml of the petroleum ether, methanol, ethylacetate, n-butanol and residual aqueous extract were prepared in distilled water. 10% solution of Dimethyl Sulphoxide (DMSO) in water was use to dissolve petroleum ether extract. Another solution of 10% (DMSO) in water was use as a control to ascertain the growth of microorganism [11].

3.3 Susceptibility Test

The agar well diffusion method as described by [12]. Sensitivity test for organisms to various extracts were adopted. Inoculation of the prepared plates with the microorganism was done using sterilized wire-loop to transfer a loopful of the microorganism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. The plates were incubated at 37°C for 24hrs after which they were examined for zones of inhibition of growth.

3.4 Minimum Inhibitory Concentration (MIC)

Mc-farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Nutrient broth was prepared and 10ml was dispensed into test tube. The minimum inhibition concentrations of the extracts were carried out using the broth dilution method. Mueller Hinton broth was prepared and 10mls was dispensed into test tube, the broth was sterilized at 121^{o}c for 15mins and allowed to cool. Normal saline was prepared, 10mls was dispensed into sterile test tubes and the test microbes were inoculated and incubated at 37^{o}c for 6hrs. Dilution of the test microbes in the normal saline was done until the turbidity marched that of the Mc-farland's scale by visual comparison, at this point the test microbe has a concentration of about 1.5 x 10^{8}cfu/ml [13,14]. Two fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 10mg/ml 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml. The initial concentration was obtained by dissolving 0.8g of the extracts in 10mls each of the sterile broth. Having obtained the different concentrations of the various extract in the broths, 0.1ml of the standard inoculums of the test microbe was then inoculated into different concentrations of the extracts in the broth. Incubation was made at 37^{o}c for 24hrs, after which the test tubes were observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration [15]

3.5 MBC/MFC

The Minimum Bactericidal Concentration and Minimum Fungicidal Concentration were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared and poured into sterile Petri dishes, this was allowed to cool and solidify. The contents of the MIC in the serial dilutions were then sub-cultured onto the prepared medium. Incubation was made at 37^{o}c for 24hrs after which each plate was observed for colony growth. The plate with the lowest concentration of the extract without colony growth is considered the MBC for the Bacteria and MFC for the fungi [16].

Table 1. Phytochemical screening of the leaf extract of *Tapinanthus dodoneifolius*

Constituets	Test	Observation	Inference
Carbohydrate	Molisch	Red colour	+
General test	Barfoed's	Red. ppt	+
Monosaccharide	Fehlings	Red. ppt	+
Reducing sugar			
Tannins	Lead ethanoate	White ppt	+
	Methanol's	Red. ppt	+
	Iron (III) chloride	Blue-black	+
Saponins	Frothing	Persist frothing	+
Sterols	Liebermann-Burchad	Blue-green	+
	Salkowski	Red ring at interphase	+
Terpenoids	Liebermann-Burchard	Brown ring with brown interphase	+
Alkaloids	Dragendoff's	-	-
	Mayer's	-	-
	Wagner's	-	-
Flavonoids	Shinoda	-	+
	Ferric chloride	-	+
	Sulphuric acid	-	-
Cardiac glycoside	Keller-Kilanis	Reddish brown	+
	Legal's	Deep red colour	+

Key: + = Present, - = Absent

Table 2. Antimicrobial activities of the control drugs with their Zones of inhibition against test organism

Test organism	Sparfloxacin 5 (µg/ml)	Ciprofloxacin 5 (µg/ml)	Fluconazole 5 (µg/ml)
S. aureus	(S) 31	(S) 28	(R) Nil
B. subtilis	(S) 33	(S)37	(R) Nil
E. coli	(S) 35	(S) 32	(R) Nil
K. pneunoniae	(S) 47	(S) 45	(R) Nil
P. aeruginosa	(S) 42	(R) Nil	(R) Nil
S. typhi	(S)27	(S) 25	(R) Nil
C. krusei	(R) Nil	(R) Nil	(S) 41
C. tropicalis	(R) Nil	(R) Nil	(S) 36
S. pyogenes	(S) 46	(S) 40	(R) Nil
C. albican	(R) Nil	(R)Nil	(S)35

4. RESULTS

The result of the antimicrobial screening of the different concentrations of the extracts and fractions on the test isolates are shown on Table 3. The result shows that an increase in the concentration of the extract and the fractions also increases the zone inhibition of the microorganisms. The petroleum ether extract was resistance to *P. aeruginosa*, *S. typhi, C. tropicalis* and *C. krusei* at various concentrations. The susceptibility test shows that, the extracts and the various fractions were resistance to *P. aeruginosa, C. Krusei and C. tropicalis*. The highest zone of inhibition of 34mm was exhibited by 1000 (mg/ml) concentration of the methanol extract against *S. aureus*.

The minimum inhibition concentrations of the extracts and fractions on the test isolates were shown in Table 4. The lowest minimum inhibitory concentration (MIC) was observed against *S. aureus* and *E. coli* with a concentration of 1.25mg/ml. The minimum bactericidal concentration of the extract and fractions at 2.5mg/ml were exhibited by the methanol and n-butanol fraction against *S. aureus* and *E. coli* while the minimum fungicidal concentration of 10mg/ml was exhibited against *C. albican* respectively.

Table 3. Susceptibility result of the extract against test isolate

Test organism	Mean zone of inhibition (mm)														
	1000 (mg/ml)					750 (mg/ml)					500 (mg/ml)				
	P_E	M_E	E_t	N	Aq	P_E	M_E	E_t	N	Aq	P_E	M_E	E_t	N	Aq
Staphylococcus aureus (+)	12	34	18	29	19	10	26	16	23	16	9	22	14	18	10
Bacillus subtilis (+)	17	25	20	19	17	14	20	18	14	12	10	15	12	12	8
Escherichia coli (-)	14	28	15	26	24	10	26	15	19	10	8	22	9	18	16
Klebsiella pneumoniae	18	30	19	28	22	13	28	10	22	16	9	25	18	20	25
Pseudomonas aeruginosa (-)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmonella typhi	0	29	20	26	25	0.0	25	20	23	20	0	24	22	25	21
Candida krusei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Candida tropicalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus pyogenes	10	31	19	17	15	18	26	14	10	8	6	21	13		
Candida albican	5	10	6.0	4.0	5.0	30	8	6	4	2	2.0	7	0.0	0.0	0.0

Key: P_E = Petroleum ether, M_E = Methanol, E_T – Ethylacetate, N = n-butanol and Aq = aqueous residual extract.

Table 4. Minimum inhibition concentration of the extract against the test isolate

Test organism	P_E (mg/ml)					M_E (mg/ml)					E_T (mg/ml)					N (mg/ml)					Aq (mg/ml)				
	10	5	2.5	1.25	0.125	10	5	2.5	1.25	0.125	10	5	2.5	1.25	0.125	10	5	2.5	1.25	0.125	10	5	2.5	1.25	0.125
S. aureus	-	MI	+	++	+++	-	-	-	MI	+	-	-	MI	+	++	-	-	-	MI	+	-	MI	+	++	+++
B. subtilis	-	MI	+	++	+++	-	-	MI	+	++	-	-	-	MI	+	-	-	MI	+	++	-	-	0x	+	+++
E. coli	-	MI	+	++	+++	-	-	-	MI	+	-	-	MI	+	++	-	-	-	MI	+	-	MI	+	++	+++
K. pneunoniae	-	MI	+	++	+++	-	-	-	MI	+	-	MI	+	++	+++	-	-	MI	+	++	-	MI	+	++	+++
P. aeruginosa	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
S. typhi	NT	NT	NT	NT	NT	-	-	+	++	+++	-	MI	+	++	+++	-	MI	+	++	+++	-	MI	+	++	+++
C. krusei	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
C. tropicalis	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
S. pyogenes	-	MI	+	++	+++	-	-	-	MI	+	-	MI	MI	+	++	-	-	MI	+	++	-	-	MI	+	++
C. albican	-	MI	+	++	+++	-	MI	+	++	+++	-	+	+	++	+++	-	MI	+	++	+++	-	MI	+	++	+++

Key: P_E = Petroleum ether, M_E = Methanol, E_T – Ethylacetate, N = n-butanol and Aq = aqueous residual extract.
NT = Not tested, MI = MIC, (-) = No growth, (+) = turbid (light growth) (++) = moderate growth and (+++) = highly growth

Table 5. Minimum bacteriocidal/fungicidal concentration of the extracts against test microorganism

Test Organism	P_E (mg/ml)					M_E (mg/ml)					Et (mg/ml)					N (mg/ml)					Aq (mg/ml)				
	10	**5**	**2.5**	**1.25**	**0.125**	**10**	**5**	**2.5**	**1.25**	**0.125**	**10**	**5**	**2.5**	**1.25**	**0.125**	**10**	**5**	**2.5**	**1.25**	**0.125**	**10**	**5**	**2.5**	**1.25**	**0.125**
S. aureus	MB	+	++	+++	++++	-	-	MB	+	++	-	MB	+	++	+++	-	-	MB	+	++	MB	+	++	+++	++++
B. subtilis	MB	+	++	+++	++++	-	M	+	++	+++	-	-	MB	+	++	-	MB	+	++	+++	-	MB	+	++	+++
E. coli	MB	+	++	+++	++++	-	-	MB	+	++	-	MB	+	++	+++	-	-	MB	+	++	MB	+	++	+++	++++
K. pneunoniae	MB	+	++	+++	++++	-	-	MB	+	++	MB	+	++	+++	++++	-	MB	+	++	+++	MB	+	++	+++	++++
P. aeruginosa	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
S. typhi	MB	+	++	+++	++++	MB	+	++	+++	++++	MB	+	++	+++	++++	MB	+	++	+++	++++	MB	+	++	+++	++++
C. krusei	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
C. tropicalis	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
S. pyogenes	MB	+	++	+++	++++	-	-	MB	+	++	-	MB	+	++	+++	-	MB	+	++	+++	-	M	+	++	+++
C. albican	MF	+	++	+++	++++	-	+	++	+++	++++	MF	+	++	+++	++++	MF	+	++	+++	++++	MF	+	++	+++	++++

Key: P_E = Petroleum ether, M_E = Methanol, E_T – Ethylacetate, N = n-butanol and Aq = aqueous residual extract.

NT = Not tested, MB = MBC, MF = MFC, (-) = No growth, (+) = Turbid (light growth) (++) = Moderate growth (+++) = highly growth and (++++)=Densely growth

5. DISCUSSION

In this study, the result obtained from the phytochemical studies reveals the presence of saponins, tannins, terpenoids, flavonoids, cardiac glycoside and carbohydrates while alkaloids are found to be absent.

The antimicrobial sensitivity test indicated that, the extract inhibited the growth of *S. aureus, S. pyogenes, B. subtilis, E. coli, K. pneumoniae, S. typhi* and *C. albicans.* This therefore shows that the extract contains substance (s) that can inhibit the growth of some microorganism. Other workers have also shown that, the extracts of the plants inhibit the growth of various microorganisms at different concentrations [17,18,19].

The observed antimicrobial effect on the microorganism could be attributed to the presence of flavonoids, glycosides tannins, saponins, terpenoids and carbohydrate which have been shown to posses antimicrobial activities [20].

Tannins are known to be antimicrobial agent that tends to inhibit the growth of microorganism by precipitating out the microbial protein and thus depriving them of development [21]. In the present study, tannins were detected in the methanol and n-butanol extract fraction of the extract. This can also explain the comparatively better antimicrobial activity of the extract on tested pathogens. This seems further to highlight the limitation of ethylacetate, n-butanol and residual aqueous portion of the extract in traditional management of those ailment which include diarrhea, inflammation of the mouth and throat that were reported to be treated with tannins [21]. Steroids in other hand increases nitrogen level in the body there by producing protein that helps in the production of muscles. Steroids were found to enhance metabolism and thus inhibit the accumulation of fat, adjust disorders like anemia by increasing the production of red blood cells in the treatment of arthritis, asthma, brain injury and other type of cancer. However, steroids plays an important role in the body as it enhance the onset and progression of cardiovascular and liver diseases as well as acne (by stimulating the serum to produce oil). In our present study, steroid were found to be present in petroleum ether, methanol and ethylacetate extract which could be preferred in the management of cardiovascular and liver ailment [22]. The moderate growth inhibition against *E. coli* could also be attributed to the use of the leaf portion for the treatment of diarrhea and dysentery.

The MIC exhibition by the extract against *S. aureus* is of great significant in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections caused by these microbes, especially in the treatment as they develop resistance to known antibiotics. The presence of these metabolites in the plant suggest great potentials for the use of the extract as a source of phytomedicines.

The presence of carbohydrate and reducing sugars in the leaves extract indicate high energy content of the leaves that could be exploited as a source of edible food or raw materials for industries that utilized carbohydrates reducing sugars to produced food, drugs or biodiesel [23].

Flavonoids are ubiquitous in plants they are known to be common part of human diet [24] and could significantly inhibit microbes which are known to be resistance to the conventional antibiotics [12]. Some of the recently isolated flavonoids were reported to exhibit antimicrobial activities [25,26,27]. In addition flavonoids through their free – radical scavenging activity have evoked multiple biological functions which include anti-inflammatory, anti-bactericidal, anti-carcinogenic, Vasodialotory, immune stimulatory, anti-allergic and anti viral functions [28,29]. Consequent upon this, the presence of flavonoids in the methanol and ethylacetate extract in our study has exhibited the therapeutic efficacy of the extract and has accounted for the folkloric use of the plant in the treatment of related ailment especially the inhibitory effect of *S. aureus*.

However, the antifungal activity of saponin has been reported [30,31], though saponins has been detected in the methanol extract but was found not to elicited antifungal activity against the fungi especially *C. krusei* and *C. tropicalis*. This is because the tested concentrations of the solvent extract were not lethal enough to elicit a measurable fungistatic effect. Lastly, the antimicrobial activity result of the various extract is generally known to be conferred by the presence of Phytochemical. Therefore, this study apparently highlighted the scientific basis for the possible use of *T. dodoneifolius* in the trado-medicinal use of methanol and other partition fraction among others as a better antimicrobial potential.

6. CONCLUSION

The antimicrobial activity of the present study suggests the use of *T. dodeneifolius* as a source of herbs. The study also suggest that, the activity or the therapeutic potency of *T. dodoneifolius* leaves extract might be dependent on the constituent metabolite within the polarity of the extract. Therefore, it could be concluded that the results obtained from this study shows that *T. dodoneifolius* possess compounds with antimicrobial properties that can be used as antimicrobial agents in the production of drugs for the therapy of infectious diseases caused by some of the aforementioned pathogens.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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