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Evaluation of phytochemical and antimicrobial properties of leaf extract of *Tapinanthus sessilifolius* (P. Beauv) van Tiegh

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Leaf extracts of *T. sessilifolius* growing on five different host plants *(Psidium guajava, Citrus lemon, Vernonia amygdalina, Persea americana* and *Jatropa curcas*) were evaluated for antimicrobial activity of the plant. Powdered leaves of *T. sessilifolius* collected from each host plant was divided into two portions. One portion was used for aqueous infusion and the other portion was successively extracted with hexane, ethylacetate and methanol. Infusion of aqueous extract of powdered leaves did not show antimicrobial effect even at the concentration of 1000 and 2000µg/ml on test microorganisms *(Staph. aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Candida albicans)*. However in broth culture, methanolic and hexane extract had MIC range of 62.5-500µg/ml and ethylacetate extract had 250-500 µg/ml. Phytochemical screening of leaf samples of *T. sessilifolius* collected from different host plants showed positive test for hydrolysable tannins, saponins, flavonoids, terpenes, cardiac glycoside, reducing sugars and proteins.LD_{50} concentration was found to be > 1.500 mg/kg for samples from *P. guajava;* 489.89 mg/kg for *J. curcas* and *C. lemon;* and 692 mg/kg for *V. amydalina* in mice.

Keywords: Antimicrobial property, *Candida albicans*, Leaf extract, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Tapinanthus sessilifolius*

African mistletoe, *Tapinanthus sessilifolius* P.Beauv Van Tiegh *(Loranthaceae)* is a semi parasitic plant found growing on a variety of evergreen plants throughout Northern and Southern Nigeria[1]. Unlike true parasite that depends on its host for all nutrients, African mistletoe takes only water and minerals from its host plant[2]. The plant is ever green shrubby epiphytic and grows throughout the year on tree branches of its host with help of suckers. The characteristics of the leaves depend on the host plant that often have no botanical affinity[2]. *T sessilifolius* has clusters of narrowly tubular flowers often brightly coloured called "matches stick /flower". In some parts of Africa, including Nigeria, aqueous extract made from the dried leaves is used as a remedy for hypertension, diabetes, infertility, epilepsy, varicose vein and other metabolic disorders[3-5]. Recently, effect of aqueous extract on gastrointestinal smooth muscle in rabbit has been reported[6]. The present study, therefore, was conducted to evaluate its anti-microbial activity and to study phyto-chemical nature of various extracts of *Tapinanthus sessilifolius.*

Plant material—The leaves of *Tapinanthus sessilifolius* were harvested in the month of November 2000 in Jos, Plateau State of Nigeria. The leaf samples were harvested from different host plants namely, *Psidium guajava, Citrus lemon, Vernonia amygdalina, Jatropha curcas and Persea americana.* They were identified and authenticated by Professor Z.O. Gbile, a consultant taxonomist. The specimens with herbarium voucher number FH1 105336 were deposited at Forestry Research Institute Ibadan and National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria.

Preparation of extract—The leaves of *T. sessilifolius* from different host plants were air dried for 7 days and crushed into coarse powder. The powder thus obtained was successively extracted in soxhlet extractor with hexane, ethylacetate and methanol. For infusion, each plant sample (32g) was soaked in water (1L) for 18 hr, heated up to 70°C, allow to cool, filtered and freeze dried and weighed.

Phytochemical screening—Standard methods were used for phytochemical screening of leaves extract as described earlier[7,8].

Acute toxicity studies—Five groups, each consisting of 5 mice of both sexes were used for the test. Group 1 – 4 were injected (ip) with varying doses (10, 100, 1000 and 2000 mg/kg) of the extract, while group 5 served as control. Signs and symptoms of toxicity over a 24 hr period was observed. Death within this period was recorded. The LD_{50} was estimated using the method of Lorke[9].

Microorganism and media—American typed cultures of *Staphylococcus aureus* ATCC 13709, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 8637 and *Bacillus subtilis* from Ahmadu Bello University Zaria (ABU) were used in the study. Nutrient agar,

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nutrient broth from (oxoid) Ltd. London, UK were used for cultivation, DMSO (BDH England) were used for preparing different dilutions.

Agar diffusion — Agar diffusion technique as described earlier[10, 11] was considered for estimation of zone of inhibition. Wells were made in the 20 ml Nutrient agar plates using cork borer No 4 (8 mm diam.). Surface swabbing inoculation were made with standardized test strain of 10^6 cfu/ml. This wells were then filled with doubling dilutions of the extract starting with 1000- 15.6 µg/ml. This was then allow to stand for 1hr before incubation at 37°C for 24 hr. On each plate a reference standard antibiotic, chloramphenicol (10 µg/ml) was used[12].

MIC determination — Different concentrations of the extracts 1000, 500, 250 125 and 62.5 µg/ml were prepared in nutrient broth. These were then inoculated with 100 µl of the standardized test organism containing 10^6 cfu/ml and incubated at 37°C overnight. The result was read by visual observation for turbidity in the tubes for growth. MIC was determined as the least concentration of extract inhibiting the growth of the test organisms.

The preliminary phytochemical screening of the extracts showed the presence of hydrolysable tannins, saponins flavanoids, terpenes, balsam, cardiac glycoside and nutrients such as proteins and carbohydrates (Table 1). The aqueous infusion had no antimicrobial activity against all the test microorganisms even at 1000 and 2000 µg/ml of concentration. However, hexane, ethylacetate and methanolic extracts of *Tapinanthus sessilifolius* parasitic on *Psidium guajava* had activity against all the test microorganisms. Hexane extracts of *Tapinanthus sessilifolius* on *Persea americana* had activity against *S. aureus and B. subtilis* only (Table 2). Minimum inhibitory concentration (MIC) of the extracts of *Tapinanthus sessilifolius* parasitic on *Psidium guajava* has been shown in (Table 3). The secondary metabolites present in the plant could be responsible for some of the observed antimicrobial activity. Normally stress compounds are metabolites produced by plant, which is being used by the plant as a defense against diseases, microbial attack and herbivores. These compounds confer the plant with antimicrobial activity[13]. These compounds also play a role in human nutrition, therapeutics and protection of the plant from both microbial and other foreign bodies.

MIC of hexane and methanolic extracts for *C.albicans* was 62.5, 250 and 500 µg/ml. The hexane, ethylacetate and methanol extracts have the same activity against *B. subtilis* with MIC of 250 µg/ml and against *P. aeruginosa* with MIC of 500 µg/ml. With the current trend of resistance to synthetic antibiotic, the activity shown by *Tapinanthus sessilifolius* parasitic on *Psidium guajava* extracts was a good development. The extract could be further purified and isolated with the targeted goal of obtaining a substance with a more potent antimicrobial activity.

The present results agrees with other report, that secondary metabolites like tanins, flavanoids steroids showed antimicrobial activities[14–16]. The extracts of *T.sessilifolius* parasitic on *Psidium guajava* have been reported to have hypoglycemic activity in rat and rabbits[17]. The fact that this extract also possessed activity against both bacteria and fungi, is a positive development because, this could be used to alleviate the suffering of diabetics that have itchy diabetic feet, implicated by *Candida albicans* as well as diabetic sores infected with bacteria. Further isolations and pharmacological evaluations will be done on the extracts to ascertain other ethnomedical uses.

In all, the methanolic extract of *Tapinanthus sessilifolius* parasitic on *Psidium guajava* showed the highest activity against all microorganisms tested, as indicated by lowest MIC values for the organism when compared with the other extracts.

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Table 1 — LD_{50} and phytochemical screening of *Tapinanthus sessilifolius*

Host plants	% w/w yield	LD_{50} mg/kg body wt	Alka-loids	Tannins		Flavanoids	Terpenes	Balsam	Cardiac glycoside	Protein (% w/w)	Carbohydrate (% w/w)	Total acidity (g citric acid)
				Pseudo	Hydrolisable							
Psidium guajava	21.81	1.500	-	-	+	+	+	+	+	0.586	16.57	0.0063
Citrus lemon	21.43	489.89	-	-	+	+	+	+	+	0.586	15.42	0.009
Jatropha curcas	23.51	489.89	-	-	+	+	+	+	+	0.2	22.96	0.007
Vernonia amygdalina	22.3	692.0		-	+	+	+	+	+	0.39	16.32	0.0063
Persea mericana	23.1	ND	-	-	+	+	+	+	+	0.2	20.20	0.002

(+) – Detected; (-) – Not detected; and (ND) Not determined

Table 2 — Zones of inhibition of leaves extracts of *Tapinanthus sessilifolius* from different host plants using the agar diffusion method

Host plants	Extracts	Total inhibition zone (mm)				
		Sa	Ec	Bs	Ps	Ca
Psidium-	Hexane	(0)	10 (2)	11(3.0)	12(4)	17 (9)
guajava	Ethylacetate	18(10)	17 (9)	19 (11)	5 (7)	12(4)
	Methanolic	(0)	12 (4)	14 (6)	16 (8)	13(5)
Persea americana	Hexane	20 (12)	(0)	18 (10)	(0)	(0)
	Ethylacetate	(0)	(0)	(0)	(0)	(0)
	Methanolic	(0)	(0)	(0)	(0)	(0)
Citrus lemon	Hexane	(0)	(0)	(0)	(0)	(0)
	Ethylacetate	(0)	(0)	(0)	(0)	(0)
	Methanolic	(0)	(0)	(0)	(0)	(0)
Jatropha curcas	Hexane	ND	ND	ND	ND	ND
	Ethylacetate	(0)	(0)	(0)	(0)	(0)
	Methanolic	(0)	(0)	(0)	(0)	(0)
Vernonia amygdalina	Hexane	(0)	(0)	(0)	(0)	(0)
	Ethylacetate	(0)	(0)	(0)	(0)	(0)
	Methanolic	(0)	(0)	(0)	(0)	(0)

Values in parentheses indicate level of anti-microbial activity. (ND) - Not determined
Sa-*Staphylococcus aureus;* Ec-*Escherichia coli;* Bs-*Bacillus subtilis;* Ps-*Pseudomonas aeruginosa* and Ca-*Candida albicans*

Table 3 — Minimum inhibitory concentration (MIC) of the extracts *Tapinanthus sessilifolius* from *Psidium guajava*

Extract fractions	Test organism	MIC (µg/ml)
Hexane	*Escherichia coli* ATCC 8637	500
	Pseudomonas aeruginosa ATCC 27853	500
	Bacillus subtilis ABU, Zaria	250
	Candida albicans ATCC 10231	62.5
Ethylacetate	*Staphylococcus aureus* ATCC 13709	250
	Escherichia coli ATCC 8637	500
	Bacillus subtilis ABU, Zaria	250
	Pseudomonas aeruginosa ATCC 27853	500
	Candida albicans ATCC 10231	500
Methanolic	*Escherichia coli* ATCC 8637	500
	Pseudomonas aeruginasa ATCC 27853	250
	Bacillus subtilis ABU, Zaria	250
	Candida albicans ATCC 10231	62.5

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