Academic City University College – Accra Ghana
IEEE Area Six (6) Coordination Centre
Tony Blair Institute for Global Change
Trinity University, Lagos, Nigeria
Harmarth Global Educational Services
FAIR Forward – Artificial Intelligence for All
Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH
Society for Multidisciplinary & Advanced Research Techniques (SMART)

Accra Bespoke Multidisciplinary Innovations Conference (ABMIC)

Investigations of the Chemical Composition and Biological Activities of the Essential Oil of the Leaves of Tapinanthus truncatus

Badmos, S.T & Olaoluwa, O.O.
Organic Unit, Department of Chemistry,
University of Ibadan, Ibadan- Nigeria
Email: badmos1995@gmail.com
Phone: +234 8103085220

ABSTRACT

Tapinanthus truncatus (Loranthaceae) is a species of mistletoes that is widely used in folk medicine for the treatment of various diseases like cancer, diabetics and infertility. The leaves (300g) were hydro-distilled using an all-glass Clevenger set-up and the essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS) technique. The essential oil of the leaves of *T. truncatus* was subjected to cytotoxicity and antioxidant bioassays. The cytotoxicity assay was carried out employing Brine Shrimp Lethality Test (BSLT) while the anti-oxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity method. Sixty-four and Sixty-nine compounds were identified in the leaves' essential oil. The major constituents in the leaves were 4-methyl-2-pentanone (4.79%) and n-hexadecanoic acid (18.52%). LC_{50} value obtained from Brine Shrimp Lethality Test (BSLT) was 7.223 $\mu g/ml$ while IC_{50} values obtained from the anti-oxidant assay showed that the essential oil of the leaves (IC_{50}= 14.44 mg/mL) is moderately active when compared with the standards used; BHA (IC_{50}= 7.89 mg/mL) and Vit. C (IC_{50}= 3.63 mg/mL). This study shows that *T. Truncates* leaves contain pharmacological constituents which are responsible for their wide use in ethnomedicine.

Keywords: *Tapinanthus truncatus*, *Artemia salina*, Essential oil, Lethality Test,.Antioxidant

Proceedings Citation Format
Badmos, S.T. & Olaolwa, O.O.c(2022): Investigations of the Chemical Composition and Biological Activities of the Essential Oil of the Leaves of Tapinanthus truncatus. Proceedings of the 31st Accra Bespoke Multidisciplinary Innovations Conference. University of Ghana/Academic City University College, Ghana. 1st – 3rd June, 2022. Pp 113-120, www.isteams.net/ghanabespoke2022. dx.doi.org/10.22624/AIMS/ABMIC2022P13

1. BACKGROUND TO THE STUDY

Mistletoe belongs to the kingdom Plantae, subkingdom Tracheobionta, superdivision Spermatophyte, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Santales, and has roughly 1400 species worldwide. Loranthaceae is the largest family of mistletoes, with 75 genera and over 900 species (Judd *et al*., 2002). *Tapinanthus, Agelanthus, Loranthus, Globimetula*, *Phragmanthera*, and *Englerina* are the six major genera found in Nigeria. *Tapinanthus*, on the other hand, is significantly more common in the Nigerian savanna (Omolaja and Gamaye, 1998). Mistletoes, as parasitic plants are capable of penetrating the living tissue of another plant's stems and branches and extracting the necessary resources for their survival (Fernanda *et al.,* 2018). Mistletoe, is known in Yoruba as "afomo", in Igbo 'apari' while in Hausa it is called 'kauci' and 'children's matches' in Eastern Cameroon presumably because of the match-like shape of the flower (Oluwole *et. al*., 2013).

1.1 Statement of Problem

Extensive research has been done on other members of the *Tapinanthus* family. However, *Tapinanthus truncatus*, to the best of our knowledge has not been worked on. This research therefore, would further bring to the limelight the hidden treasures present in the leaves of this plant which can provide a baseline for future drug development.

1.2 Objective

The objective of this work is to analyze the essential oil constituents, screen the essential oil of the leaves for anti-oxidant and toxicological activities

2. METHODOLOGY

2.1 The Research Design

2.1.1 Extraction of Essential oil

Essential oil from the leaves of the *Tapinanthus truncatus* was obtained by Hydro-distillation method using all-glass Clevenger Set-up. 300 g of the leaves of the samples were placed into 5 L round bottom flasks and water was added until the samples were fully immersed. The flask was then placed on a heating mantle fitted with a all-glass Clevenger apparatus. The extraction lasted for about 3 h. The extraction process was repeated for the weighed samples for another 3 h (Oloyede, 2016).

2.1.2 Gas Chromatography (GC/MS) Analysis

The model of GC is agilent technologies 7890 coupled with mass spec. 5975 agilent technologies, the principles behind the analysis is separation techniques, the mobile phase is helium gas and the stationary phase is the column of model agilent technologies HP5MS of length 30 m, internal diameter of 0.320 mm and thickness of 0.25 micro metre. The oven temperature, the initial temperature is 80 degrees held for 2 mins at 12 degrees per minute to the final temperature of 240 degrees held for 6 minutes, the scan ranges is 50 to 550, the interface temperature between GC and MS is 250 degrees Celsius, the volume of sample injected is 1 microlitre

2.1.3 Brine Shrimp Lethality Test

The Brine Shrimp Lethality Test was carried out by the method described by Aboaba *et al*., (2010) and Oloyede (2016). The essential oil was prepared in sea water into vials at 1000, 100, and 10 µg/mL (each test in triplicate). The essential oil had been previously dissolved in 2 mL of Dimethyl sulfoxide (DMSO) since they are not soluble in water and 0.5 mL each of the dose level was introduced in a test-tube to which 4 mL of sea water added. Ten brine shrimps per test tube were added to each concentration and made up to 5 ml seawater to make 1000-10 µg/mL of final concentration of extract. After 24 hrs, the number of deaths over the number of total shrimps (survivors) was counted and recorded.

2.1.3.1 Brine shrimp nauplii exposure to extracts

The essential oil was prepared at three concentration levels namely, 1000, 100, 10 ug/mL. Ten newly hatched brine shrimp nauplii were used per test tube, in which they were then exposed to the various concentrations of the essential oil. A negative control, DMSO not exceeding 0.05% was included for the dispersing the oil in the sea water. After 24 h, the number of surviving nauplii was determined in order to generate the lethality data. After triplicate analysis, Finney probit computer programme was used to determine the LC_{50} (Concentration at which 50% of the nauplii died).

2.1.4 Antioxidant Assay

Radical Scavenging Activities (RSA) of *T. truncatus* leaves essential oil was determined using the method described by Amiri (2011) and Oloyede (2021) with minor modifications. Stock solution (10 mg/mL each) of the essential oil and standard antioxidant BHA and Vitamin C were prepared in Methanol. Dilutions are made to obtain concentrations ranging from 5 mg/mL to 0.0625 mg/mL. Diluted Solutions (0.5 mL each) were mixed with 3 mL of freshly prepared 0.1mM DPPH methanol solution and allowed to stand for 30 mins in the dark at room temperature for any reaction to take place. Ultraviolet (UV) absorbance of these solutions was recorded on a UV spectrometer at 517 nm using a blank containing the same concentration of oil, BHA, or Vitamin C without DPPH.

Inhibition of free radical DPPH in percent (I%) was calculated. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} were reported as means ± SD of triplicates

3. DATA PRESENTATION

3.1 Chemical Constituents of the Essential Oils

3.1.1 GC-MS Result of *T. truncates* Leaves Essential oil

Table 1 shows the chemical composition of essential oils of *T.truncatus* leaves. Sixty-four compounds were identified from the GC MS analysis.

Table 1: Chemical Composition of *T. truncatus* Leaves Essential Oil

S/N	COMPOUND	% COMPOSITION	R.T
1	3-Hexanone	1.59	3.119
2	1-Hexyl-3-methylcyclopentane	0.71	3.146
3	4-methyl-2-pentanone	4.97	3.179
4	3-Hexanol	3.48	3.255
5	2-Nonanol	4.14	3.325
6	1,3-dimethylcyclohexane	2.18	3.379
7	Propyl-cyclopentane	0.37	3.471
8	1-(2,2-Difluorocyclopropyl)-2-methyl indole	0.59	3.649
9	Hexamethyl-cyclotrisiloxane	3.13	3.687
10	2-Naphthalenesulfonic acid	1.53	3.730
11	Ethyl-Cyclohexane	3.95	3.800
12	2-Methyl-5H-dibenz[b,f] azepine	2.45	3.849
13	1,3,5-Trimethylcyclohexane	1.52	4.081
14	1,2,4-trimethylcyclohexane	0.63	4.151
15	2-Methyloctane	1.31	4.308
16	p-xylene	0.54	4.373
17	2,5-Dimethylheptane	0.88	4.421
18	1,2,4-trimethyl-Cyclohexane	0.46	4.475
19	Cis-1-Ethyl-3-methyl-cyclohexane	0.64	4.659
20	m-xylene	0.58	4.751
21	n-Nonane	0.50	4.881
22	Methylene Cyclohexane	0.34	5.167
23	2-methyl-cyclopentadecanone	0.40	5.318
24	3-Octanol	2.99	5.691
25	Propyl benzene	0.38	5.761
26	4-methyl-2-pentanol	3.19	5.853
27	1-ethyl-4-methyl benzene	1.32	5.896
28	Mesitylene	2.15	6.415
29	Decane	2.57	6.534
30	Octamethyl-cyclotetrasiloxane	2.50	6.566
31	1,2,4-Trimethylbenzene	0.74	6.885
32	0-Cymene	0.42	6.928
33	Limonene	0.30	6.993
34	4-methylstyrene	0.75	7.090
35	1-methyl-3-propylbenzene	0.64	7.360

S/N	COMPOUND	% COMPOSITION	R.T
36	1,3-butadienylidebe-cyclohexane	0.31	7.436
37	1-ethyl-3,5-dimethyl-benzene	0.56	7.479
38	2-ethyl-1,4-dimethylbenzene	0.35	7.792
39	1,2,4,5-tetramethyl-benzene	0.50	7.895
40	Undecene	0.64	8.133
41	Cymene	0.26	8.224
42	1,2,3,5-tetramethylbenzene	0.30	8.376
43	1,2,3,4-trimethylbenzene	0.56	8.440
44	2-butyl-1-octanol	0.27	9.634
45	Tridecane	0.80	11.039
46	Copaene	0.33	12.114
47	Valencene	0.32	12.298
48	Tetradecane	0.58	12.363
49	Humulene	0.46	13.124
50	Germacrene D	0.68	13.464
51	d-cadinene	0.36	13.967
52	Valencene	0.45	15.928
53	(E)-2-octadecenal	0.37	16.095
54	Citronellyl acetone	0.99	17.473
55	Untriacontane	0.82	18.261
56	Heptacosane	0.38	18.672
57	Untriacontane	0.62	20.271
58	1-iodo-Eicosane	0.44	20.644
59	Docosane	0.36	20.811
60	Eicosane	0.50	22.108
61	Hentriacontane	0.40	22.437
62	Eicosane	0.35	22.491
63	Methyltris(trimethysiloxy) silane	2.04	28.839
64	Arsenous acid, tris(trimethylsilyl) ester	2.09	29.708

4. DISCUSSION

4.1 GC-MS Analysis

Sixty-four compounds were identified in the essential oil of leaves of *T. truncatus*. These compounds are majorly non-terpene derivatives with a large percentage of hydrocarbons. Atewolara-Odule and Oladosu (2016) reported that the composition of *T. bagwensis* is majorly hydrocarbons. Some of the compounds identified are: Limonene (0.30%), Humulene (0.46%), Valencene (0.45%) and Germacrene D (0.68%). 4-methyl-2-pentanone (4.97%),2-Nonanol (4.14%), 4-methyl-2-pentanone (4.79%), 3-Hexanol (3.48%), and ethyl-cyclohexane (3.95%). Germacrene D is a terpene that has been noted for its aromatherapy benefits with a range of medicinal properties. Plants containing germacrene have been used in traditional medicine in countries around the world (Maggi, *et al.*, 2017).

Limonene is also recognized to have antibacterial, anti-depressant, and anti-cancer effects. It penetrates cell membranes causing other terpenes to be absorbed more rapidly and effectively. Limonene's potent anti-carcinogenic and anti-fungal properties, has also been reported to be the component protecting marijuana smokers from *Aspergillus* fungi and carcinogens found in cannabis smoke (Reichard, 2013).

4. 2 Brine Shrimp Lethality Assay

The result of the brine shrimp lethality assay of the essential oil extracted from the Leaves of *Tapinanthus truncatus* are given below.

Table 2: Brine Shrimp Lethality assay result of *T. truncatus* leaves' essential oil.

Essential oil	Total no of Shrimps	No of dead shrimps 1000 ug/mL	% mortality	No of dead shrimps 100 ug/mL	% mortality	No of dead shrimps 10 ug/mL	% mortality	LC_{50} µg /ml
TTL	30	30	100	22	73	18	40	7.223

TTL: *Tapinanthus truncatus* Leaves

The total number of surviving nauplii was the sum of triplicate determinations (n=3) with 10 nauplii in each trial. LC_{50} analysis was carried out with the aid of SPSS statistical package. The LC_{50} values obtained were further tested with Finney Probit Computer Programme. The Toxicity testing criteria based on Clarkson 2004 was used. LC_{50} values greater than 1000 ug/mL were considered non-toxic while values between 500 and 1000 $\mu g/mL$ were considered less toxic, values between 100 and 500 were considered moderately toxic while values less than 100 ug/mL were considered highly toxic. A substance is considered to be cytotoxic if it inhibits vital metabolic processes or it causes disorders in living organisms resulting in perversion of behaviour or death (Abdalla et. al 2021).

The Lethality Concentration (LC_{50}) of *T. truncatus* leaves essential oil was 7.223 $\mu g/mL$ and. This showed that the essential oil is lethal against the brine shrimps. The lethality of the essential oil to the brine shrimp larvae was found to be directly proportional to the concentration of the oil. The activities of the extracts are manifested as toxicity to shrimps by bioactive components present in the extracts. Total mortalities took place at a concentration of 1000 µg/mL in the essential oil of the leaves. This showed that the essential oil from the leaves have a very high toxicity index. Therefore, the essential oil was found to be highly toxic to *Artemia salina* nauplii according to Clarkson's toxicity criteria (Clarkson, *et al.,* 2004).

The high toxicity effects of the essential oil could be attributed to the presence of some compounds that are known to possess cytotoxic properties. Since the essential oil showed high cytotoxic effects, there is need for caution by traditional caregivers regarding the prescription of these plants for therapeutic purposes. However, it will be very imperative to also carry out cancer cell line assay to really ascertain the level of this toxicity in humans.

4.2 Antioxidant Assay

Table 3: Anti-Oxidant Activities of the Essential Oil

Conc. (mg/ml)	TTL (%)	Vit. C (%)	BHA (%)
10	36.6±1.79	77.16±0.69	52.81±4.26
5	32.01±0.27	76.56±0.42	41.97±3.41
2.5	31.39±0.50	52.64±0.27	38.85±3.55
1.25	29.32±0.51	43.39±0.13	35.15±3.08
0.625	26.40±0.68	35.19±0.37	33.44±2.55
$IC_{50\ (mg/ml)}$	14.44	3.63	7.89

1. **Key:** TTL = *Tapinanthus truncatus* Leaves;; Vit. C = Vitamin C; BHA = Butylated hydroxy anisole; IC_{50} = Half maximal inhibitory concentration; % = Inhibition activity.

The antioxidant activity of the essential oil was determined by DPPH method. The essential oil was added to the stable free radical, that is 2,2 diphenyl-1-picryl hydrazyl (deep violet colour) which converted it to 2,2-diphenyl-1-picrylhydrazine with discolouration. The degree of discolouration indicates the free radical scavenging potentials of the essential oil. The Percentage inhibition of the essential oil against DPPH radical ranged from 36% at 10 mg/mL to 26% at 0.625 mg/mL for the stem and 40% at 10 mg/mL to 26% at 0.625 mg/mL for the leaves of *T. truncatus.* The IC_{50} which is the concentration by which 50% of the radicals of DPPH will be scavenged was determined using linear regression analysis. The essential oil of the leaves gave IC_{50} values of 14.44 mg/mL compared with BHA (7.89 mg/mL) and Vit. C (3.63mg/mL). Comparing the scavenging activity results *T. truncatus* leaves' (14.44 mg/mL), BHA (7.89 mg/mL) and Vit. C (3.63 mg/mL), it was observed that the essential oil showed lower activity compared to that of BHA and Vitamin C. Also, the % inhibition of the essential oil obtained from the leaves of *T. truncatus* were lower than the % inhibition of Vitamin C and Butylated Hydrosyl Anisole (BHA).

5. CONCLUSION

Tapinanthus truncatus (Loranthaceae) is a traditional medicinal plant used for the treatment of various diseases like cancer, diabetics and infertility. Gas-chromatography mass spectrometry analysis of the leaves' essential oil showed hydrocarbons and their derivatives 2-Nonanol (4.14%), 4-methyl-2-pentanone (4.79%), 3-Hexanol (3.48%), and ethyl-cyclohexane (3.95%) as the major constituents. The toxicity of the essential oil determined from Brine shrimp Lethality assay showed highly toxic activities with LC_{50} less than 100 ug/mL.The IC_{50} values obtained from the antioxidant screening showed moderate activity when compared to the standards used. The results observed from the various bioassays show that the leaves of *T. truncatus* contain pharmacologically active constituents which might be responsible for their wide use in ethnomedicine and the high toxicity of the essential oil to brine shrimps implies that the essential oil would possess higher larvicidal, insecticidal and other biological properties.

6. CONTRIBUTIONS TO KNOWLEDGE

Essential oil from the leaves of *T. truncatus* has been characterized which to the best of our knowledge has not been reported before in literature. This report has provided a new insight into the prospects of developing medicines from the plant.

REFERENCES

1. Abdalla, A. A., Mustafa G. I, Ahmed K.E, Al-magboul A.Z, Fadul E. 2021. Cytotoxicity Potential of Essential oil and Extracts of Oleo-Gum resins from Boswellia papyrifera (Tarak tarak) Grown in Different Regions of the Sudan. *Arabian Journal of Medicinal & Aromatic Plants.* (2) 215
2. Aboaba, S.A, Oladosu I.A, Ekundayo O. 2010. Chemical composition, acetyl cholinesterase inhibitory properties and brine shrimp toxicity of essential oil from *Ficus capensis* Thunb from Nigeria. *International Journal of Biological and Chemical Sciences*. 4(5): 1494-1500
3. Amiri, H., 2011. Essential oils Composition and Antioxidant Properties of Three Thymus Species. Evidence-Based Complementary and Alternative Medicine, Article ID 728065.
4. Atewolara-Odule, O.C. and Oladosu I.A. 2016. Comparison of chemical compositions of essential oils from the fresh and dried leaves of Tapinanthusbangwensis (Engl. And K. Krause) Danser [Loranthaceae]. American Journal of Essential Oils and Natural Products. 4(3):31-33
5. Clarkson C, Maharaj V.J, Crouch N.R, Grace O.M, Pillay P, Matsabisa M.G, and Folb P.I. 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of ethnopharmacology*, 92(2): 177-191
6. Fernanda 2018. Mistletoe effects on the host tree tapirira guianensis: insights from primary and secondary metabolites. Springer Nature Switzerland AG 2018.
7. Khan H.; Hussain J., Adnan M., Hussain I., Khan T., Khan A., 2008. Sesquiterpenes: The potent Antioxidants. Pakistan Journal of Scientific and Industrial Research. 51 (6):343-350
8. Maggi, F., Dennis, F., Lupidi, G., Quassinti, L., Bramucci, M., Bruno, M., Casiglia, S. 2017. Kundmanniasicula (L.) DC: a rich source of germacrene D. *Journal of Essential Oil Research*. 29 (6), 437-442
9. Oloyede G. K. (2016). Toxicity, antimicrobial and antioxidant activities of methyl salicylate dominated essential oils of *Laportea aestuans* (Gaud). Arabian Journal of Chemistry 9, S840–S845
10. Oloyede G. K., Ibok M.G. and Ojo T.K. (2021). Chemical Constituents, Antimicrobial and Antioxidant Activities of *Leptoderris brachyptera* (Benth.) Dunn and *Leptoderris micrantha* Dunn Essential Oils Trends in Phytochemical research (TPR) 5(1): 13-23
11. Oluwole, O., Osungunna, M.O, Abimbola, Y. 2013. Phytochemical and antimicrobial screening of Globimetula oreophila (Oliv) Van Tiegh and Phragmanthera capitata (Spreng) Balle. *Int. J. Green Pharm*. 7(2):127-130
12. Omolaja, S.S, Gamaye, A.O. 1998. The incidence of mistletoes on coffee in Ibadan, Nigeria. A paper presented at the 9th Annual Conference of the Botanical Society of Nigeria, University of Ilorin, Nigeria
13. Reichard, Z. 2013. Terpenes: *What Are Terpenoids and What Do These Do*? MedicalJane Publication, USA. 45-53pp.
14. Torres-Matinez R., Garcia-Rodriguez Y.M, Lopez-meza J.E, Ochoa-Zarzosa A., 2017. Antioxidant Activity of the essential oil and its major terpenes of Satureja macrostema (Moc. and Sesse ex Benth.) Briq. *Pharmacogn Mag.* 13 (4) S875-880