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PHYTOCHEMICALS IN METHANOLIC LEAF EXTRACTS OF *Tapinanthus globiferous* FROM *Azadirachta indica* AND *Albizia lebbeck*: A COMPARATIVE ANALYSIS OF SOME PHARMACOLOGICAL POTENTIALS

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ABSTRACT
Tapinanthus globiferus Linn is a specie of Viscum album commonly called mistletoe, which is used as medicinal plant. The efficiency of mistletoe species from different host trees were reported and found to depend on the bioactive constituents. Phytochemicals in methanolic leaf extract of Tapinanthus globiferus from Azadirachta indica (neem) and Albizia lebbeck (lebbeck) host trees were analysed using standard laboratory procedures and spectrophotometry methods. Extracts from both trees contained certain elements, amino acids and vitamins A, C and E. The two host trees also revealed varying concentrations of tannins, alkaloids, phenols, flavonoids, total phenolic content (TPC) and scavenge DPPH radicals with an IC_{50} of 28.73(µg/ml) and 0.02(µg/ml) respectively. Potassium (32.80%), manganese (92.33%), copper (70.43%), zinc (30.92%) and most of the amino acids were significantly ($p<0.05$) lowered in the extract from A. indica as compared to A. lebbeck. Flavonoids, alkaloids and total phenolic content were also significantly decreased in A. indica. Calcium (96.95%), Leucine (9.54%) , phenylalanine (18.27%) , and glycine (14.04%), vitamins A, C and E (90.91%, 41.09% and 47.07 %) respectively, were however significantly ($p<0.05$) higher in the A. indica. Manganese, zinc, lysine, isoleucine, tryptophan, and methionine had positive significant correlations ($r = 0.70 - 0.80$, $p<0.05$) with TPC. The extract from A. lebbeck has significantly higher concentrations in 21 (67.74%) of the 31parameters analysed. Therefore, A. lebbeck may be responsible for a more efficient and diverse targeted pharmacological actions than A. indica. Also higher concentrations of vitamins C and E in A. indica could make it a better source of antioxidant vitamin supplement.
Keywords: Tapinanthus globiferus, Azadirachta indica, Albizia lebbeck, Mistletoe, Phytochemicals

INTRODUCTION

Tapinanthus globiferus Linn., (Loranthaceae) is widely distributed in Nigeria and is one of the numerous species of *Viscum album*, which are all commonly referred to as Mistletoe. It is also known as *Kauchi* (in Hausa), *Afomo onisanan*i n (Yoruba) and *Apari / Awushie* in (Igbo) languages of Nigeria (Saleh *et al*., 2015; Tizuhe *et al*., 2016). The *T. globiferus* is a semi parasitic flowering plant that grows on deciduous host trees including *Azadirachta indica (*neem) and *Albizia lebbeck* (lebbeck) and may produce negative effect on the growth and fruiting of their host (Ibe *et al*., 2019). *Tapinanthus* specie is used as medicinal plant to manage metabolic, chronic and several other ailments (Akinmoladun *et al.,* 2017*;* Obatomi *et al.,* 1994). It has been scientifically validated for antimicrobial, antioxidant, anti-diabetes, antihypertensive, anticancer and immunomodulatory as well as the wound healing potentials (Szurpnicka *et al.,* 2020). These activities were attributed to differences in bioactive compounds found in the plant and the host tree (Deeni and Sadiq, 2002; Brahma *et al*., 2016). Antioxidant mechanism is attributed to high phenolic and flavonoid compounds (Pietrzak *et al.,* 2017).

Aqueous leaf extract of *Viscum album* (mistletoe) from Kola nut tree has been reported to contain some mineral elements, amino acids and secondary metabolites (Oseni, 2018). Variation in antimicrobial activities against gram-negative bacterial and fungi, especially the *Candida* species for different solvent extracts of mistletoe plant parts and host trees have also been reported (Brahma *et al*., 2016; Shah *et al*., 2017; Jahagirdar*et al.,* 2018; Szurpnicka *et al.,*

2020). Linolelaidic acid methyl ester and viscotoxins were speculated to be antifungal compounds in the leaf, which could inhibit germination of spores and mycelia growth. Thus, contributing to antifungal effects of the plant. In another study, aqueous leaf extract of a mistletoe from lime tree at 1 μg/mL dose was found to inhibit human parainfluenza virus type 2 (HPIV-2) growth and replication, and suppressed the virus production by 99% in Vero cells (Karagöz *et al.*, 2003). A 0.063 μg/mL of methanolic extract inhibited measles virus growth. Polio, yellow fever and simplex virus-1 (HSV-1) viruses were however shown to be resistant to the same extract (Obi and Shenge 2018). Furthermore, *Tapinanthus bangwensis* was used as antihypertensive and anti-diabetic agents and *Tapinanthus micranthus* was observed to exhibit antimicrobial activities (Osadebe *et al.,* 2013; Osadebe and Ukweze, 2014). The varying pharmacological actions of *Tapinanthus* specie for targeted ailment, which have been reported to depend on accumulated phytochemicals, host plant as well as methods of extraction and the solvent used make this study imperative. Variations in the amount of constituents and effectiveness based on different species and host necessitated the comparative analysis of chemical constituents in *Tapinanthus globiferus* from neem and lebbek trees in this study.

This study aimed at the determination and comparison of chemical constituents in methanolic leaf extract of *T. globiferus* from *A. indica* and *A. lebbeck* trees. The data may provide insights to some of the host-specific and diverse targeted ethno-pharmacological uses.

MATERIALS AND METHODS

Sample Collection and Identification of Plant Material

Leaves *of T. globiferus* were collected from two hosts, neem and *lebbeck* trees at Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria in June 2021. The taxonomic identification and authentication was made by a Botanist at the Department of Plant Biology, Bayero University Kano, Nigeria. Specimen accession number (BUKHAN624) was prepared and deposited in the departmental herbarium.

Preparation of Plant Extract

The leaves were washed under running water, air dried at room temperature (under shade) and pulverized into fine powder using mechanical grinder. A portion of the powdered sample (150 g) was weighed, and dissolved in 500ml of methanol. The solution was shaken for 2 hours, using a digital orbital shaker, left undisturbed for 24 hours then filtered using whatman no 1 filter paper. The resulting filtrate was concentrated in water bath. A 10.85g of the concentrate, which correspond to 7.23 % yield was obtained, stored in a clean air tight glass bottle and used for the biochemical analysis.

Elemental Analysis

Calcium (Ca), Potassium (K), manganese (Mn), copper (Cu) and zinc (Zn) in the extracts were determined using wet-acid digestion method (AOAC, 2006). About 0.5 g of the pulverized leaves was added into a conical flask, 5 ml of 70% hydrochloric acid was added, mixed and the mixture was heated at 30°C for 15 minutes using a hot plate. A 5 ml of 65% nitric acid was then added and heated again until the sample was digested to a clear solution. A 20ml of distilled H_2O was added mixed thoroughly and heated for 1 minute. The digested sample was allowed to cool, filtered into a 50 ml volumetric flask and made up to the mark with distilled water. This was used for the determination of the element using flame photometer (AA320N, Buck Scientific). The absorbance and concentration of each element were directly indicated on the digital voltmeter attached to the photometer.

Amino Acid Profile

Amino acid content was determined (AOAC, 2006), using phenylthiohydantoin (PTH) amino acid analyzer (120A, Applied Biosystems). A 4g of the dried sample (70˚c) was defatted, hydrolyzed, evaporated and then loaded into the amino acid analyzer. The sample was defatted using chloroform and methanol mixture in a ratio 2:1, put in extraction thimble and extracted for 15 hours using soxhlet apparatus. A 250 mg of the defatted sample was hydrolysed using 7 ml of 6N HCl and heating at 105˚C± 5˚C in oven for 22 hours. The resulting mixture was filtered to remove humins and the filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved in 5ml acetate buffer (pH 2.0) and stored in glass bottle. A 60µL of the dissolved residue was dispensed into the cartridge of the analyzer, which is designed to automatically separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. An integrator attached to the analyzer was used to calculate the peak area proportional to the concentration of each of the amino acids.

Determination of Vitamins A, C and E

Vitamins A, C, and E were evaluated as described by Rutkowski *et al.* (2007).

Vitamin A

The sample solution (1ml) was measured and added in a test tube (Tube I). One milliliter (1 ml) of alcoholic KOH solution was added and the test tube was shaken for 1 minute. The tube was heated in a water bath at 60°C for 20 minutes, 1 ml of xylene was then added and again shaken for 1 minute. The mixture was centrifuged at 1500 rpm for 10 minutes and the supernatant was pipetted into another test tube (Tube II) using pasture pipette. The absorbance of A_1 of the extract was obtained at 335 nm against xylene. Extract in the test tube II was irradiated with UV light for 30 minutes and the absorbance A_2 was also measured. Concentration of vitamin A (µM) in the sample was calculated using;

$$[\text{Vitamin A}] = (A_1 - A_2) \times 22.23 \quad \text{----} \quad 1$$

Where: 22.23 = Multiplier received on the basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm.

Vitamin C

The sample (1ml) was measured into the centrifugal test-tube, 1ml of phosphotungstate reagent (PR) was added, mixed and leave at 30°C for 30 minutes. The tube was centrifuged at 7000 rpm, for 10 minutes and the supernatant was collected using a dropper. The standard was prepared as above using 1 ml of the standard solution instead of the sample, without centrifugation. The absorbance of the test sample (Ax) and of the standard sample (As) were measured at 700 nm using 6705/ UV spectrophotometer, Jenway. The concentration of vitamin C (µM) in the sample was calculated using:

Where: Ax = Absorbance of sample, Cs = Concentration of the standard solution used and As = Absorbance of standard.

$$[\text{Vitamin C}] = \frac{A_x \times C_S}{A_s} \quad \text{-----------} \quad 2$$

Vitamin E

The sample solution (0.5 ml) was measured into the test-tube I, 0.5 ml of anhydrous ethanol was added and shaken for 1 minute. Then, 3 ml of xylene was added, the test tube was Clogged and shaken for another 1 minute and then centrifuged at 1500rpm for 10 minutes. 250µl solution of batophenanthroline was measured into test-tube II, 1.5 ml of the supernatant was transferred to the test-tube II and mixed, 250µl of ferric chloride ($FeCl_3$) solution was added and mixed, 250µl of H_3PO_4 solution was also added to the above and mixed. The standard was prepared by adding 250µl of Trolox and 250µl of deionized water in a test tube, instead of anhydrous ethanol. The absorbance of test sample Ax and standard As were measured at 539 nm against the blank. Concentration of vitamin E (µM) in the sample was determined using the same equation 2 above.

Phytochemical Analysis

The extracts were screened for some secondary metabolites and the amount of saponin, phenolics, flavonoid and alkaloids present in the extracts were determined using standard laboratory procedures (El- olemy *et al.*, 1994; Obadoni and Ochuko 2001).

The 1-Diphenyl-2-picrylhydrazyl Radical Scavenging Assay.

The 1-Diphenyl-2-picrylhydrazyl (DPPH) assay was carried out using the method of Dias *et al.* (2007). This is based on reduction of the violet DPPH radical to a colourless non-radical DPPH-H in presence of a hydrogen atom donor and absorbance of disappearance of the purple colour read at 517nm. Exactly 100µl of the methanol extract in varying concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.6 and 7.8µg/ml) were added to 200µl of DPPH radical solution in methanol. The mixture was shaken and allowed to stand at room temperature in dark for 30 minutes. A 200µl of methanol and 100µl plant extract solutions were used as the blank. A 200µl of DPPH and 100µl methanol solutions were used as the control. Decrease in absorbance of the resulting mixtures were measured at 517nm using micro plate reader spectrophotometer multi scan go FI-01620 (Vantaa, finland) and the radical scavenged potential was calculated using equation below (Dias *et al.* 2007).

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}} \right) \times 100 \quad \text{-----} \quad 3$$

Total Phenolic Assay

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method described by Dias *et al.* (2007). The method is based on reducing Folin-Ciocalteu reagent (phosphotungstic acid) with phenolic compound to blue chromophore, with maximum absorbance at 725 nm**.** To the reaction mixture (50µl of extract, 500µl of distilled water, and 50µl of the Folin-Ciocalteu reagent), after a period of 3 minutes, 100µl of saturated sodium carbonate solution (Na_2CO_3) was added. The mixture was shaken, allowed to stand for 1 hour and the absorbance was measured at 725 nm using micro plate reader spectrophotometer multi scan go FI-01620 (Vantaa, Finland). Concentrations of the TPC in the sample was determined by interpolation from absorbance of the standard (Galic acid) calibration curve at varying concentrations plotted against it absorbance and the results were expressed as mg GAE (Gallic acid equivalents)/g of dry extract.

Data Analysis

Results were presented as mean ± standard deviation (SD). The significance of difference among groups was determined by the independent sample-t test, one-way analysis of variance and Pearson correlation using statistical package for social science (SPSS-21) and $p < 0.05$ as significant.

RESULTS AND DISCUSSION

Table 1: Selected Essential Elements in Methanolic Leaf Extract of *Tapinanthus globiferus* from Two Hosts

Elements (mg/L)	***Tapinanthus globiferus* host trees**		Difference (%)
	Azadirachta indica	***Albizia lebbeck***	
Potassium	514.41 ± 0.96[a]	716.25 ± 0.96[b]	32.80*
Calcium	106.41 ± 0.97[a]	36.93 ± 0.95[b]	96.95#
Manganese	58.30 ± 0.51[a]	158.29 ± 0.82[b]	92.33*
Copper	85.14 ± 0.81[a]	177.69 ± 0.79[b]	70.43*
Zinc	211.33 ± 0.53[a]	288.62 ± 1.91[b]	30.92*

Values are mean ± SD of four determinations. Values across the row with different superscript letter for each parameter are significantly different ($p < 0.05$).≠ Percentage increase, ∗percentage decrease.

Table 2: Amino Acid Composition of Methanolic Extract of *Tapinathus globiferus* Leaves from Two Hosts

Amino acids (g/100g protein)	***Tapinanthus globiferus* host trees**		Difference (%)
	Azadirachta indica	***Albizia lebbeck***	
Leucine	7.47 ± 0.04[a]	6.79 ± 0.01[b]	9.54#
Lysine	3.51 ± 0.08[a]	5.09 ± 0.08[b]	36.74*
Isoleucine	3.54 ± 0.05[a]	4.07 ± 0.09[b]	13.93*
Phenylalanine	4.60 ± 0.02[a]	3.83 ± 0.09[b]	18.27#
Tryptophan	1.14 ± 0.05[a]	1.19 ± 0.01[a]	4.29*
Methionine	1.40 ± 0.03[a]	1.54 ± 0.05[b]	9.52*
Histidine	2.22 ± 0.01[a]	2.31 ± 0.02[b]	3.97*
Threonine	4.12 ± 0.05[a]	4.35 ± 0.02[b]	5.43*
Valine	3.92 ± 0.10[a]	3.84 ± 0.08[a]	2.06#
Glycine	4.49 ± 0.09[a]	3.90± 0.12[b]	14.04#
Alanine	4.19 ± 0.24[a]	4.44± 0.04[a]	5.79#
Arginine	5.10 ± 0.07[a]	6.09± 0.08[b]	17.69*
Cysteine	1.06 ± 0.03[a]	1.19± 0.03[b]	11.56*
Proline	3.85 ± 0.02[a]	4.17 ± 0.13[b]	7.98*
Serine	4.27 ± 0.70[a]	4.75 ± 0.12[b]	10.64*
Aspartate	7.37 ± 0.06[a]	8.08 ± 0.10[b]	9.19*
Glutamate	11.14 ± 0.11[a]	12.37 ± 0.04[b]	10.46*

Values are mean ± SD of four determinations. Values across the row with different superscript letter for each parameter are significantly different ($p < 0.05$).≠ Percentage increase, ∗percentage decrease.

Table 3: Concentrations of Vitamins A, C and E in Methanolic Leaf Extract of *Tapinanthus globiferus* from Two Hosts

	***Tapinanthus globiferus* host trees**		
Vitamins(µM)	***Azadirachta indica***	***Albizia lebbeck***	**Difference (%)**
Vitamin A	7.84 ± 0.23 [a]	2.94± 0.03 [b]	90.91[#]
Vitamin C	30.01± 0.38 [a]	19.78 ± 0.17 [b]	41.09[#]
Vitamin E	7.40 ± 0.17 [a]	4.58± 0.03 [b]	47.07[#]

Values are mean ± SD of four determinations. Values across the row with different superscript letter for each parameter are significantly different ($p < 0.05$).[≠] Percentage increase, *percentage decrease.

Table 4: Concentrations of some Secondary Metabolites and the Total Phenolic Content in Methanolic Leaf Extract of *Tapinanthus globiferus* from Two Hosts

	***Tapinanthus globiferus* host trees**		
Parameters	***Azadirachta indica***	***Albizia lebbeck***	**Differences (%)**
Secondary metabolites (mg/L)			
Flavonoids	31.05 ± 0.57[a]	36.15± 1.48[b]	15.18[*]
Phenols	3.90 ± 0.48[a]	3.28 ± 0.80[a]	17.27[#]
Tannins	95.40 ± 0.94[a]	94.01 ± 0.99[a]	1.47[#]
Alkanoids	2.78 ± 0.47[a]	19.81 ± 0.51[b]	150.77[*]
TPC **(mgGAE/g)**	99.30± 0.02 [a]	106.20± 0.01[b]	6.72[*]

Values are mean ± SD of four determinations. Values across the row with different superscript letter for each parameter are significantly different ($p < 0.05$). [≠] Percentage increase, *percentage decrease. GAE: Gallic acid equivalent

Table 5: The 2,2-Diphenyl-1-picryl-Hydrazyl Radical Scavenging Activity of the Methanolic Extract of *Tapinanthus globiferus* Leaves from Two Hosts

	Inhibition (%) for the host trees		
Concentration (µg/ml)	***Azadirachta indica***	***Albizia lebbeck***	**Vitamin C**
1000	83.61 ± 0.61	86.65 ± 2.28	96.10 ± 0.83
500	96.10 ± 0.67	87.93 ± 0.89	95.63 ± 0.29
250	83.71 ± 0.35	87.77 ± 0.11	95.34 ± 0.40
125	77.92 ± 1.29	89.11 ± 0.73	95.31 ± 0.66
62.5	60.87 ± 0.54	88.53 + 0.55	94.82 ± 0.70
31.3	46.24 ± 0.54	87.93 ± 0.83	92.86 ± 2.11
15.6	37.39 ± 0.70	85.22 ± 0.77	90.84 ± 3.67
7.8	30.18 ± 1.50	68.32 ± 4.05	81.90 ± 13.22
IC_{50} (µg/ml)	28.73[a]	0.02[b]	0.01[b]

Values are mean ± SD of four determinations.

Extract of *Tapinanthus globiferus* leaves from both host trees (*Azatharica indica* and *Albizia lebbeck)* analysed contained K, Ca, Mn, Cu and Zn (Table 1), amino acids (Table 2)as well as vitamins A, C and E (Table 3). The two hosts revealed varying concentrations of tannins, alkaloids, phenols, flavonoids, total phenolic content "TPC" (Table 4) and also scavenge DPPH radicals (Table 5). Manganese, zinc, lysine, isoleucine, tryptophan, and methionine had positive significant correlations ($r = 0.70 - 0.80$, $p<0.05$) with TPC. The K and Ca could contribute to relaxing walls of blood vessels and then lowering blood pressure (Alicia and Mien 2012). Therefore, this may confer on the plant antihypertensive potential.

The Mn, Cu and Zn are positive activators of super oxide dismutase and glutathione peroxidase making them essential in oxidation-reduction reactions and in the scavenging of superoxide or hydroxide radicals. The amino acids, each with its distinctive side chain (R groups) could endow antioxidant abilities in the *T. globiferus*. These may include the alkaline amino acids like histidine, lysine and arginine with amino (NH) group, serine and threonine with alcohol (OH) R-group, the aromatic tyrosine with its phenolic group and the indole in tryptophan as well as the low molecular weight and hydrophobic alanine and threonine with methyl (CH_3) group, all of which can act as proton donor making them strong antioxidants (Farvin*et al.,* 2016; Dash and Ghos, 2017). These could also justify the antioxidant potential of *T. globiferus*. However, *A. lebbeck* may exhibits stronger antioxidant potential than the *A. indica,* due to significantly increased concentrations in most of the amino acids.

Vitamins C and E are potent dietary antioxidants, vitamin C being water soluble could scavenge aqueous peroxyl radicals and the Vitamin E (α-tocopherol) a lipid-soluble vitamin can acts as a lipid peroxyl radical scavenger, preventing lipid peroxidation chain reactions in cell membranes (Birben *et al.,* 2012). These antioxidant molecules may contribute to and promote antioxidant potential of the plant, which can protect the cell membrane from free radicals damaging effect and oxidative stress implicated in the pathogenesis of numerous ailments. This consistently affirm antioxidant potential of the leaf earlier reported by Achi, (2017). Vitamins A, C and E which were significantly higher in *A. indica* may endowed it a better source of vitamin supplement.

Furthermore, phenolic compounds that are contained in plants, have redox properties (Pietrzak *et al.,* 2017). The TPC in the extract may implies that *T. globiferus* exhibited potential of acting as free radical scavenger and efficient antioxidant due to phenolic acids and flavonoids. Inhibition of DPPH radical by the extract at the highest concentration was similar to the minimum for ascorbic acid, the standard control. In addition, the half maximal inhibitory concentration value (IC_{50}) for the extracts were also within the acceptable concentration limit of <50µg/ml as reported for potent antioxidant activity (Irda *et al*., 2015). These implies that, the extract could be a strong free radical scavenger. The finding agrees with that reported by Umarudden, (2019). Moreover, alkaloids are analgesic and anti-inflammatory agents which may relief of pains or fever associated with several illness. Phenols may act as antiseptic and disinfectant against microorganisms (Lin *et al.,* 2018; Heinnch *et al*., 2021) and therefore, could inhibit growth of pathogens causing morbidity and mortality in infectious diseases including wound healing. Higher concentrations of alkaloids and the phenols in the lebbeck could make it a more potent pain reliever, anti-inflammatory and antimicrobial agent.

The percentages differences which imply unequal distribution in concentrations of each chemical constituents indicated that extract from *A. lebbek* has significantly higher concentrations in 21 (67.74%) of the 31 parameters analysed than the 9 (29.03%) for the *A. indica*.

CONCLUSION

Base on the results of this study, it could be concluded that Mn, Cu and Zn, amino acids, vitamins C and E as well as flavonoids and phenols could reduce disease risk due to their antioxidant properties and the protection against free radicals' damaging effects to cellular components. The possible antimicrobial potential of the phenols as well as the analgesic and anti-inflammatory effects of alkaloids in diseases could justify the medicinal effect of *Tapinanthus globiferous in* which these bioactive constituents were also found. The *Albizia lebbeck* may produce more efficacious and diverse targeted pharmacological actions than *Azadirachta indica* due to the higher concentrations of these phytochemicals in it. Higher concentrations of vitamins C and E in *A. indica* could make it a better source of antioxidant vitamin supplement.

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