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Phytochemical, elemental and in-vitro anti-oxidant activity screening of crude methanol *Tapinanthus globiferus* leaf extract

Ajibola M. Umarudeen[1*], Aminu Chika[2]
[1]Department of Pharmacology & Therapeutics, University of Abuja, Abuja, Nigeria
[2]Department of Pharmacology & Therapeutics, Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

Tapinanthus species have been reported with safe toxicity profiles and efficacy in several categories of diseases. Their ethnobotanical/pharmacological activities are viewed to be related to their phytochemical and elemental constituents as well as their anti-oxidant activity. This study carried out phytochemical, elemental and in-vitro anti-oxidant activity screening on crude methanol *Tapinanthus globiferus* leaf extract. Flavonoids, terpenoids, phenols, alkaloids, saponins, tannin, cardiac glycosides and carbohydrates but not anthraquinones were found in the leaf extract. Cadmium, copper, iron, manganese, sodium, potassium, calcium but not lead and cobalt were present in the extract. The extract also demonstrated dose-dependent ferric ion reducing and α, α-diphenyl-β-picrylhydrazyl (DPPH) free radicals scavenging anti-oxidant activities that were inferior to those of the standard anti-oxidant (ascorbic acid). Some of the phytochemicals and the metal elements may be responsible for the anti-oxidant activity of this extract and for the reported ethnobotanical/pharmacological efficacies of *Tapinanthus globiferus* extracts.

Keywords: *Tapinanthus globiferus*, oxidative stress, ferric ion reducing, DPPH radical scavenging

INTRODUCTION

Phytochemical compounds such as phenols, steroids, saponins, carotenoids, tannins, alkaloids, flavonoids, etc., are non-nutrient macromolecular plant-derived bio-active secondary metabolites. These chemical substances have been reported to play preventive roles in several classes of diseases including diabetes mellitus, hypertension, cancers, nervous disorders, atherosclerosis, dementia and microbial infections (Altemimi *et al.*, 2017; Adesina *et al.*, 2013; Noumi and Eloumou, 2011). Ethnobotanical and scientific studies on *Tapinanthus globiferus* and related species from ten different host trees (excluding *Azadirachta indica*) have reported their efficacies in diverse disease spectra and their ubiquitous biological activities have been largely attributed to the presence of arrays of phytochemicals such as chlorogenic acid, caffeic acids, gallic acid, rutin and quarcetin in addition to the above-named compounds present in them (Abedo *et al.*, 2013; Borokini and Omotayo, 2012).

Preliminary activity screening of crude methanol leaf extract of *Tapinanthus globiferus* growing on *Azadirachta indica* showed that the extract possessed significant anxiolytic activity (Umarudeen and Magaji, 2019) but its phytochemical composition is yet unknown. Studies have also shown that the key disease-modulatory effects of medicinal plants largely reflect the levels of anti-oxidant activity of their phytoconstituents (Herrera-Arellano *et al.*, 2007) and that the differences in the anti-oxidant activities of medicinal plants also reflect their differential phytochemical compositions (Belobrajdic and Bird, 2013).

Exogenous anti-oxidant compounds from medicinal plants have been shown to be useful in ameliorating/counteracting the deleterious oxidizing effects of some of the products of cellular respiratory/metabolic activities in scenarios where the endogenous anti-oxidant activity is overwhelmed or defective (Grilo *et al.*, 2014). Anti-oxidant activities have also been reported for extracts of *Tapinanthus bangwensis*, *Piliostigma thonningii*-grown *Tapinanthus globiferus*, *Ficus glumosa* grown-*Tapinanthus globiferus, Persea Americana*-grown *Tapinanthus globiferus and Acacia*-grown *Loranthus*

***Corresponding Author:** Dr. Ajibola M. Umarudeen, Department of Pharmacology & Therapeutics, University of Abuja, Abuja, Nigeria.

E-mail: umarudeen.monisola@uniabuja.edu.ng

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acacia (Abubakar *et al.*, 2018; Emmanuel *et al.*, 2017; Molehin and Adefegha, 2015). But scientific reports on the anti-oxidant activities of *Tapinanthus globiferus* growing on *Azadirachta indica* are sparse in spite of its several reports of diverse ethno-botanical and ethno-pharmacological activities. Inorganic elemental metals have also been viewed to play an important modulatory or adjunctive role not only in the maintenance of brain health but also in the pathogenesis of affective and neurodegenerative diseases and their analysis and quantification has been shown to give insight to their pharmacological and toxicological properties (Piao *et al.*, 2017; Jellinger, 2013).

Essential minerals like Fe2+, Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, Zn^{2+} and PO_4^{2-} have been previously identified in extracts from citrus-grown *Tapinanthus globiferus*, *Tapinanthus sessilifolius* and *Tapinanthus preusi* but metal elements' analysis of leaf extracts of *Tapinanthus globiferus* growing on *Azadirachta indica* has not been reported (Jeremiah *et al.*, 2018; Tarfa *et al.*, 2018; Tarfa *et al.*, 2002). The aim of the present study, therefore, is to carry out a qualitative phytochemical, elemental and in-vitro anti-oxidant activity screening of the crude methanol leaf extract of *Tapinanthus globiferus* from *Azadirachta indica* tree.

MATERIALS AND METHODS

Collection, identification and extraction of plant materials

Tapinanthus globiferus leaves were collected from an *Azadirachta indica* (Neem) tree at Shuni road, Mabera, Sokoto, Sokoto State, Nigeria. Specimen vouchers were deposited and authenticated at the herbarium of the Botany Department, Faculty of Science, Usmanu Dan Fodiyo University, Sokoto, Nigeria, with the following identification numbers: *Tapinanthus globiferus* (UDUH/ANS/0135). Following collection, the plant materials were briefly washed with clean water in order to remove potential dirt contaminants. They were then air-dried under shade for several days till they attained constant weights. The materials were then ground to a fine powder using a mortar and an electric blender, and stored under room temperature till use. Seventy per cent (70%) crude methanol extract of *Tapinanthus globiferus* (CMTG) leaves was obtained by soaking 250 g of fine powder of the plant material in 1 L of methanol/distilled water (70/30) for 24 hours. The macerate was then filtered using Whatman's paper (150 mm) and evaporated in rotatory water bath at 45-50 degree Celsius. The drying process produced 31.45 g of dry greenish *Tapinanthus globiferus* leaf extract (12.58%).

Phytochemical screening and determination of the elemental constituents of crude methanol *Tapinanthus globiferous* leaf extract

Crude methanol *Tapinanthus globiferus* leaf extract was screened for its phytochemical constituents according to the standard methods described by Sofowora (1993). The elemental composition of CMTG leaf extract was determined by an atomic adsorption spectrophotometric procedure that was previously adopted by Zafar *et al.* (2010). Briefly, a small quantity (0.2 g) of dry powder of CMTG was dissolved in 10 ml of solution of nitric acid (NHO3) and hydrochloric acid (HCL) (1/3: V/V) and the resulting mixture was heated till almost dryness. The almost dry powder was put in a volumetric flask and distilled water was added up to the 100 ml mark. The extracted sample was then filtered with 150 mm Whatman's filter paper and the filtrate was collected and stored in a tightly stoppered labeled plastic bottle. The solution was then analyzed for the elements and the percentages of different elements in the sample were determined by flame atomic absorption spectrophotometer model 6800 Shimadsu, Japan.

Determination of anti-oxidant activity of crude methanol Tapinanthus globiferous leaf extract

The anti-oxidant activity of the crude methanol *Tapinanthus globiferus* leaf extract was evaluated by two different in-*vitro* procedures, i.e. determination of the reducing power of CMTG extract against Fe3+ and determination of the DPPH radical scavenging activity of the extract viz-a-viz a standard anti-oxidant agent. Determination of the reducing power of the crude methanol *Tapinanthus globiferus* leaf extract against Fe3+ was done in accordance with the method of Oyaizu (1986). Briefly, CMTG or vitamin C (0, 1, 2, 3, 4 or 5 mg/kg) was dissolved in 1 ml of distilled water and was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [KFe (CN) e]. The mixture was then incubated at 50 °C for 20 minutes. Then, 2.5 ml of 10% trichloroacetic acid was added to this mixture before centrifuging it at 3000 rpm for 10 minutes. Finally, 2.5 ml of the upper layer of each sample was mixed with an equal volume of distilled water and 0.5 ml of 1 % Fe3+ and the absorbance was determined at 700 mm. Evaluation of the α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity of crude methanol *Tapinanthus globiferus* leaf extract was carried out according to the method of Liyana-Pathiranan and Shahidi (2005). Briefly, 1 ml of a stock solution of 0.135 nM DPPH was mixed with an equal volume of CMTG or vitamin C (25, 50, 125, 250 or 500 μg/ml). The resulting reaction mixture was thoroughly mixed with a vortex mixer and allowed to stay in the dark at room temperature for 30 minutes. Finally, the absorbance of each sample was measured spectrophotometrically at a wavelength of 517 nm. The ferric ion reducing/DPPH scavenging power of each sample was calculated as follows:

DPPH radical scavenging activity = [(Abs control – Abs sample)/Abs control] X 100.

Where: Abs control = Absorbance of Fe^{3+} ion/DPPH free radical and methanol; and Abs sample = absorbance of Fe^{3+}/DPPH free radical and CMTG or vitamin C (standard). All data were expressed as means of triplicate values.

RESULTS

Phytochemical constituents and elemental metals detected in crude methanol *T. globiferus* leaf extract

The phytochemical constituents detected in crude methanol T. globiferus leaf extract are shown in Table 1. Cadmium, calcium, sodium, magnesium, copper, iron, zinc and manganese were the elemental metals detected, but lead and cobalt were not detected (Table 2).

Table 1: Phytochemicals detected in crude methanol *T. globiferus leaf extract*

Constituents	Inference
Tannin	+
Saponin	+
Terpenoids	+
Cardiac glycosides	+
Alkaloids	+
Flavonoids	+
Anthraquinone	-
Carbohydrates	+
Phenols	+

+ present; - not detected

Table 2: Elemental metals and their concentration in crude methanol *T. globiferus leaf extract*

Element	Concentration (mg/l)
Cadmium	0.0037
Calcium	0.5699
Sodium	1.8285
Magnesium	0.7292
Cobalt	0.0000
Copper	0.0108
Lead	0.0000
Iron	1.3869
Zinc	0.0807
Potassium	0.0000
Manganese	0.2252

Anti-oxidant activities of crude methanol *T. globiferus* leaf extract

The Fe3+ reducing activity of both the crude methanol *T. globiferus* leaf extract and vitamin C were dose-dependent. The ferric ion reducing potential of the crude methanol *T. globiferus* leaf extract was lower than that of vitamin C. However, the IC50 of the extract (3.0 mg/kg) was lower than that of vitamin C of 3.6 mg/kg (Figure 1). Both the crude methanol *T. globiferus* leaf extract and vitamin C dose-dependently increased the percentage inhibition of DPPH free radicals. However, the extract's scavenging activity against DPPH free radicals with an IC50 of 180μg/ml was significantly lower than that of the standard drug (vitamin C) with an IC50 of 20 μg/ml (Figure 2).

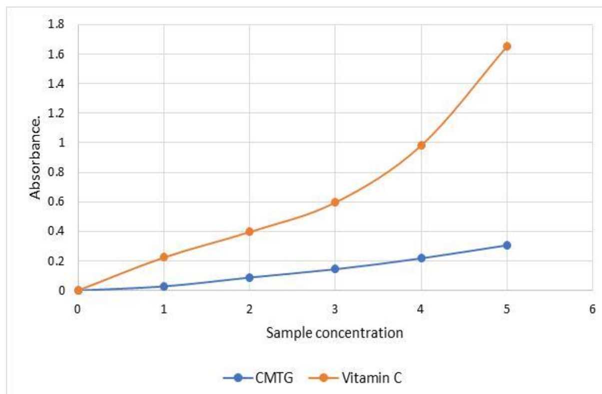

Figure 1: Ferric ion reducing power of crude methanol *T. globiferus* leaf extract

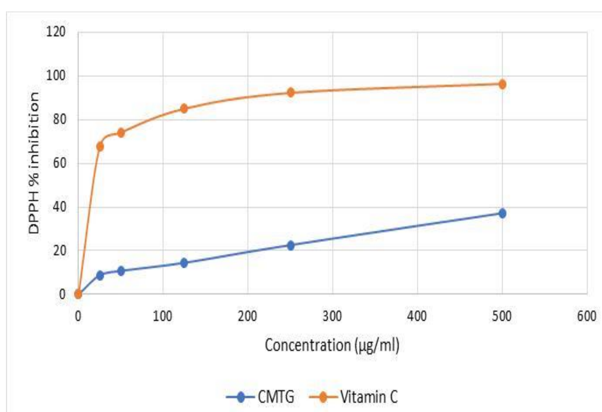

Figure 2: DPPH radical scavenging activity of crude methanol *T. globiferus* leaf extract

DISCUSSION

The detection of bio-active macromolecules such as phenols, flavonoids, tannin, saponin, terpenoids, cardiac glycosides, alkaloids and carbohydrates in the crude methanol *Tapinanthus globiferus* leaf extract is an indication that the extract is rich in phytochemicals. Phytochemical compounds are secondary metabolites that have been shown to play a vital role in chronic disease prevention (Molehin and Adefegha, 2015). The rich phytochemical profile of the extract being investigated in this study implies that *Tapinanthus globiferus* leaf extracts have the potential to be effective in disease prevention across a broad spectrum, thus justifying the traditional use of this plant for diverse therapeutic indications (Adesina *et al*., 2013). Additionally, the presence of these compounds and the absence of anthraquinones in the methanol *Tapinanthus globiferus* leaf extract in this study are in agreement with the qualitative phytochemical findings in previous studies on the methanol extracts of *Tapinanthus globiferus* from different host trees (Abedo *et al*., 2013; Jeremiah *et al*., 2018), and of *Tapinanthus dodoneifolius* growing on locust beans tree (Ndamitso *et al*., 2013). Terpenes (Wang and Heinbockel, 2018), saponins (Gujar and Kasture, 2005), flavonoids (Zhang *et al*., 2012), alkaloids (Flausino *et al*., 2007) and carbohydrates extracted from plants have been shown to exhibit significant anxiolytic activities in animal studies. These bio-active compounds present in the crude methanol extracts of the *Tapinanthus globiferus* leaf extract in the present and previous studies must have been responsible for their reported anxiolytic and other biologic activities (Umarudeen and Magaji, 2019; Jeremiah *et al*., 2018; Adesina et al., 2013; Ndamitso *et al*., 2013; Noumi and Eloumou, 2011).

The elemental analysis revealed the presence of magnesium and zinc among other elements in the extract of this study. This finding may explain in part, the multiple efficacies of various *Tapinanthus globiferus* leaf extracts that were reported in previous studies (Umarudeen and Magaji, 2019; Szklany *et al*., 2016; Adesina *et al*., 2013). Emerging evidence indicates that magnesium and zinc have not only been shown to play important roles in cellular metabolism, neurotransmission and neuromuscular conduction, but are also thought to have a critical adjunctive role in the anxiolytic mechanism (s) of some known and putative agents (Singewald *et al*., 2014). Magnesium has been shown to be vital for optimal nerve transmission and neuromuscular conduction, for increasing brain GABA levels and neural plasticity, for reducing brain cellular inflammation and stress hormone level, and for detoxifying the brain cells by binding and removing heavy metals (Kirkland *et al*., 2018). Magnesium's neuroprotective activity has been shown to be connected to its interaction with the N-methyl-D-aspartate (NMDA) receptor where it plays a gating role on calcium ion influx into neuronal receptors, thereby limiting neuronal excitatory signals (Stroebel *et al*., 2018). Zinc is an essential nutritional trace element that has been shown to play a critical role in neurogenesis, cognition and affective disorders deriving from its demonstrated roles in several important protein-linked cellular biochemical processes such as DNA replication and transcription, enzyme catalysis and cell signaling (Molina-Navarro and Garcia-Verdugo, 2016). Studies have further indicated that the putative role of zinc in anxiolytic mechanism may be related to its reported modulatory attenuation on the excitatory glutamatergic neurotransmission by allosterically interacting with the NMDA glutamate receptor (Mlyiec *et al*., 2017).

The elemental analysis of the methanol *Tapinanthus globiferus* leaf extract also shows that the extract is devoid of detectable levels of toxic metals such as lead and cobalt. This may partly explain the relative safety profiles reported for extracts from *Tapinanthus globiferus* (Shehu *et al*., 2017; Abdullahi *et al*., 2016). This study has also demonstrated a dose-dependent in-vitro anti-oxidant activity of the methanol *Tapinanthus globiferus* leaf extract in the ferric ion reducing and DPPH free radicals scavenging assays. This finding is in consonance with the finding-of significant anti-oxidant activities of extracts of *T. globiferus f*rom different host trees (Jeremiah *et al*., 2018; Adekunle *et al*., 2012; Ogunbolude *et al*., 2014).

Anti-oxidant activity has been shown to be partly responsible for the disease-preventing property of many medicinal plants such as *Tapinanthus* species against degenerative diseases such as cancers, atherosclerosis, Parkinson's disease and Alzheimer's disease (Zhang *et al*., 2015). The anti-oxidant property of these plants is believed to derive from their innate ability to synthesise natural secondary metabolites and other exogenous anti-oxidants (some of which have been confirmed to be present in the methanol *Tapinanthus globiferus* leaf extract in this study) (Kasote *et al*., 2015). The essential elements zinc and magnesium have been shown to exhibit significant anti-oxidant activity and to be effective in countering cellular oxidative stress by enhancing endogenous anti-oxidant defence mechanisms (Salmonowiccz et al., 2014; Valco et al., 2005). The presence of these metal elements may contribute to the observed anti-oxidant activity of the extract *Tapinanthus globiferus* in this study.

CONCLUSION

This study has shown that crude methanol leaf extract of *Azadirachta indica*-grown *T. globiferus* possesses rich phytochemical and elemental compositions and dose-dependent in-vitro anti-oxidant activity. Some of the phytochemicals and metal elements may be responsible for the anti-oxidant activity of the leaf extract. These findings may be a justification for the traditional uses of extracts of *Tapinanthus globiferus* in the management of diseases.

Source of support

Nil.

Conflict of interest

None declared.

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