

Preliminary phytochemical screening and Antioxidant Property of Leaf and twig Extracts of *Turraea Vogelii* Hook. f. ex. Benth (Meliaceae)

H.Y. Olufadi-Ahmed*¹, P.A. Idowu²

¹Department of Pharmaceutical Microbiology and Biotechnology, University of Ilorin, Kwara State, Nigeria. ²Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria.

*Corresponding author: E-mail address: ah.olufadi@unilorin.edu.ng; Tel: +2348141539861

Abstract

This study investigated the phytochemical constituents by screening the pulverized plant material and the antioxidant potential of the extract of the leaves and twigs of *Turraea vogelii*. The crude powdered leaves and twigs of the plant were subjected to preliminary phytochemical screening using standard procedures. The ethyl acetate extract of the leaves and twigs as well as the methanolic extract of the twigs were subjected to antioxidant activity studies using 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay, catalase activity assay as well as lipid peroxidation activity. The result of phytochemical screening of the crude powdered leaves and twigs of *Turraea vogelii* revealed the presence of tannins, flavonoids, saponins, reducing sugars and alkaloid in the leaves while the twigs contained tannins, flavonoids, saponins, reducing sugars and steroids. The results of the DPPH Radical Scavenging activity studies revealed that ethyl acetate fraction of *Turraea vogelii* twigs has an IC₅₀ of 444µg/ml which was closest to the activity of the reference measured at 409µg/ml. The ethyl acetate fraction of the twigs showed the most promising lipid peroxidation inhibition activity with an MDA concentration of 9.595mMol/g FW when compared with the standard. The catalase enzyme concentration responsible for the mop up of hydrogen peroxide per minute was highest in the methanol extract of twigs at 0.038mMolmin⁻¹g⁻¹. The implication of this study suggests that the twigs of *Turraea vogelii* has note-worthy phytochemical principles and also has a good natural antioxidant source that may be indicated in the treatment and management of diseases associated with oxidative stress.

Keywords: Antioxidant, *Turraea vogelii*, Free radicals

INTRODUCTION

Since the dawn of man, humans have relied on nature for basic needs such as the production of food, shelter, fertilizers, flavours, medicinal plants and phyto-medicine, clothing, means of transportation, fragrances and medicine¹.

Plants are essential crude materials of drugs for treating various human diseases. The universe of science has been keen on medications of natural origin because of their harmonious nature with our biological framework². Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which should therefore be

utilized to combat the disease causing pathogens.³

Turraea vogelii Hook. f.ex. Benth. (Meliaceae) (*T. vogelii*) is a plant with numerous ethno-medicinal uses that is found mostly in Tropical Africa. It is a scandent shrub or woody climber which can grow up to 5m high and is widely distributed in evergreen forests and its edges.⁴ The leaves, roots and bark are used for urogenital infections, intestinal complaints, and as a decoction for wounds, skin disease, rituals and magic.⁵ Plants of the genus *Turraea* (about 60 species) belonging to the Meliaceae family are trees or shrubs distributed mainly in tropical Africa, Asia, Australia, and Madagascar.⁶ Ethno-botanically, it is used to treat stomach pains, eczema and blennorrhoea.⁷

Africa is blessed with enormous biodiversity resources, but plagued with several diseases, including those with ROS (Reactive Oxygen Species) as the etiological factor.⁸ Free radicals are a normal product of human metabolism. They are extremely reactive, highly unstable and potentially damaging transient chemical species.⁹ They are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogeneous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. Halliwell¹⁰ defined an antioxidant as any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. In spite of numerous folkloric claims and uses of *Turraea vogelii*, there are limited scientific reports of the biological activity of the plant. This research therefore seeks to identify the phytochemical principles in the pulverized leaves and twigs and then determine the antioxidant potential of the extracts of *Turraea vogelii*.

MATERIALS AND METHODS

Plant material collection and authentication

The plant sample was collected, identified by Mr. T.K. Odewo and authenticated at the herbarium of Forestry Research Institute of Nigeria Ibadan, Oyo State (FRIN) and assigned the voucher specimen number FHI 110392.

Preparation of the leaves and twigs extract
The leaves and twigs of the medicinal plant were chopped into smaller pieces and shade dried for three weeks and subsequently ground. 400g of each part of the pulverized plant material were successively extracted by soxhlet extraction with hexane, ethyl acetate and

methanol. The obtained extracts were evaporated to dryness and the concentrate used in the antioxidant screening.

Phytochemical Screening

The pulverized plant materials were subjected to preliminary phytochemical screening for the identification of secondary plant materials using standard procedures described by Harbone.¹¹

DPPH radical scavenging activity

Zero point one (0.1 ml) of the extract (100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml) was added to 3.9 ml of DPPH solution (0.025 g/L) and the reactants were incubated at 25 °C for 30 minutes. In place of an extract, a positive control – ascorbic acid – was used. The mixture was shaken and allowed to stand in the dark at room temperature for 35 minutes. Free radical scavenging activity was calculated from absorbance values at 517 nm.

Percentage reduction of DPPH was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_1) \div A_0] \times 100$$

Where A_0 = Absorbance of positive control

A_1 = absorbance of different concentration of extracts

The IC_{50} of each extract (concentrations of the extracts that inhibit 50 % of the free radicals) was calculated from the graph of scavenging activity plotted against sample concentrations using Microsoft excel software.¹²

Catalase enzyme assay

Preparation of sample for enzyme assay

One gram of leaf sample was ground in 10ml solution containing 0.1M phosphate buffer (pH 7.5) and containing 0.5mM Ethylene diaminetetracetic acid (EDTA). The mixture was centrifuged for 20 minutes at 15,000rpm at 4°C and the supernatant was collected for the enzyme assay.

Enzyme assay

The lamda 25uv/visible spectrum (Parlan Eber) was adjusted to 240nm. The assay mixture contained 1.9ml of phosphate (PO₄) buffer, 1.0ml of H₂O₂ and 0.1ml of extract. The decomposition of H₂O₂ was recorded as the decline in absorbance at 240nm for 3minutes then change in ΔA 240/min was calculated from the initial (4 seconds) linear portion of the curve.

Determination of Lipid peroxidation rate
Oxidative damage of leaf lipids, resulting from salt stress, was estimated by the content of total 2-thiobarbituric acid reactive substances (TBRAS) expressed as equivalent of malondialdehyde (MDA). The TBRAS content was estimated by the method of Cakmak and Horst ^[13] with some modifications. Extract sample 0.2g were homogenized in 5ml of 0.1%w/v

TBA in 20%w/v TCA. Samples were heated at 90°C for 30 minutes. Thereafter, the reaction was stopped in ice bath. Centrifugation was performed at 1000rpm for 5 minutes and absorbance of the supernatant was recorded at 532nm on a spectrophotometer.

RESULTS

The result of Phytochemical screening of the Crude powdered leaves and twigs of *Turraea vogelii* revealed the presence of tannins, flavonoids, saponins, reducing sugars and alkaloid in the leaves while the twigs contained tannins, flavonoids, saponins, reducing sugars and steroids (Table I).

Table 1: Phytochemical analysis of powdered leaves and twigs of *Turraea vogelii*

Phytochemical components	Powdered Leaves	Powdered twigs
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Phlobatannins	-	-
Steroids	-	+
Cardic glycosides	-	-
Reducing sugars	+	+
Anthraquinones	-	-
Phenols	-	-
Alkaloids	+	-
Glycosides	-	-

+ / - indicates presence or absence of phytochemical component

Antioxidant Activity Study

The results of *in vitro* DPPH radical scavenging activity of each extract of the

plant are illustrated in Table 2 and 3 below while the lipid peroxidation inhibition activity of the plant extracts is represented in Table 4. Figure 1 shows the catalase enzyme activity of the plant extracts.

Table 2: % of Inhibition at different concentrations ($\mu\text{g/ml}$) compared with Vitamin C.

Concentration ($\mu\text{g/mL}$)	Ethyl acetate fraction of leaves	Ethyl acetate fraction of twigs	Methanol fraction of twigs	Vitamin C
100	41.96	43.89	33.91	16.2
200	38.34	75.41	68.9	24.6
400	42.87	83.81	86.81	36.1
600	48	39.41	26.22	96.5
800	50.81	13.44	24.58	96.8
1000	55.6	18.13	79.68	97.1

Table 3: IC_{50} values of the ethyl acetate fractions of leaves and twigs as well as methanolic fraction of twigs

SAMPLE	IC_{50} ($\mu\text{g/ml}$)
Ethyl acetate fraction of leaves	730
Ethyl acetate fraction of twigs	444
Methanol fraction of twigs	770
Vitamin C	409

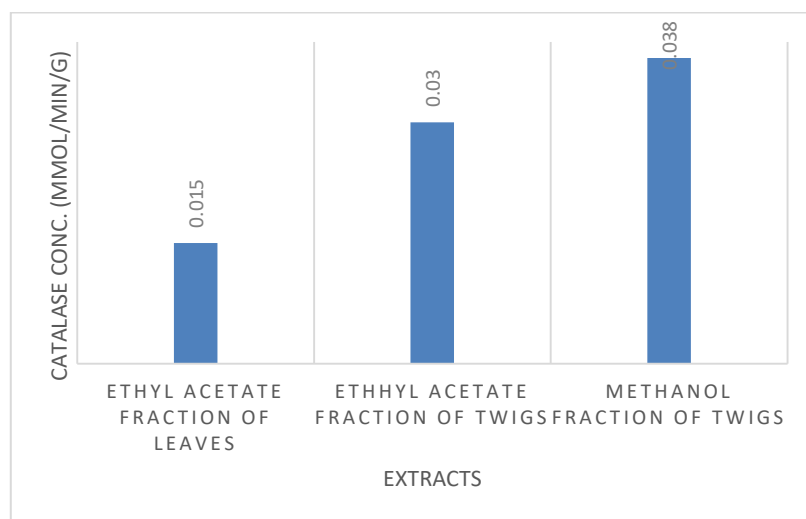


Figure 1: Catalase concentration in mMol/min/g responsible for the decomposition of hydrogen peroxide per minute

Table 4: Concentration of Malondialdehyde (MDA) formed per gram of sample

SAMPLE	MDA Concentration (mmol ⁻¹ of sample)
Ethyl acetate fraction of leaves	10.225
Ethyl acetate fraction of twigs	9.595
Methanol fraction of twigs	9.64
Vitamin C	8.572

DISCUSSION

Phytochemical screening of the crude powdered leaves and twigs of *Turraea vogelii* revealed the presence of tannins, flavonoids, saponins, reducing sugars and alkaloid in the leaves while the twigs contained tannins, flavonoids, saponins, reducing sugars and steroids. Tannins play important role in promoting wound healing, this could be the reason why the *T. vogelii* is applied on wound portion of the body to give relief from pain. In Democratic republic of Congo, the leaves and bark of the plant are powdered and applied for wound healing.¹⁴ Flavonoids are most commonly known for their antioxidant activity; they are the most common group of polyphenolic compounds in the human diet and are distributed ubiquitously in plants.¹⁵ Flavonoids have been reported to have anti-viral, anti-inflammatory, antitumor and antioxidant activities while tannins have been reported to be active against ulcerated and inflamed tissue as well as possessing antioxidant activity.¹⁶ The plant was also found to be rich in alkaloids which is in agreement with Adegoke *et al.*¹⁷ who observed that the leaves of *T. vogelii* growing in Nigeria gave moderate precipitate in only one (phosphotungstic acid) of five alkaloid tests. The leaves of this plant are ethno-botanically known to be used as poisons.⁵ The poisonous nature of the plant may be due to the presence of saponins which are bitter and known to be poisonous.¹⁸ The genus *Turraea* is rich in

limonoids and other triterpenoids known to possess various biological activities¹⁹ and *Turraea vogelii* is not an exception with the presence of saponins which may be triterpenoidal.

This research measures the activity of catalase enzyme present in plant extract and was documented as concentration of catalase enzyme responsible for the decomposition hydrogen peroxide per minute for every gram of the plant extract. The catalase enzyme concentration responsible for the mop up of hydrogen peroxide per minute was highest in the methanol extract of twigs at 0.038 mMolmin⁻¹g⁻¹. This means that for every gram of the plant extract, 0.038 mMol of catalase will mop up 1.0ml of hydrogen peroxide present (even in-vivo) per minute.

DPPH is an established radical and a trap (Scavenger) for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction.²⁰ Percentage DPPH scavenging activity as well as the IC₅₀ of the plant extracts was measured and it was found that the ethyl acetate fraction of the twigs possessed a value closest to ascorbic acid (which was used as standard).

Lipid peroxidation can generally be described as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond(s) especially polyunsaturated fatty acids.²¹ An increased concentration of end

products of lipid peroxidation (Malondialdehyde and 4-hydroxy-2-Nonenal) is a function of how much lipid peroxidation has occurred. Lipid peroxidation activity is measured by the concentration of MDA formed. The lower the concentration of MDA formed, the higher the lipid peroxidation inhibition activity of the extract. The ethyl acetate fraction of the twigs showed the most promising lipid peroxidation inhibition activity with an MDA concentration of 9.595 mMol/g FW when compared with the standard used which displayed an MDA concentration of 8.572 mMol/g FW. From the results above, it may be inferred that the twigs of *Turraea vogelii* seems to possess better antioxidant activity than the leaves. It can also be concluded that the bioactive fractions of the twigs of the plant has an appreciable amount of antioxidant

activity which justifies its use as a decoction applied for wounds and skin diseases.

CONCLUSION

This study has shown that the leaves and twigs of *T. vogelii* contain major secondary plant metabolites such as alkaloids, tannins, saponins, sterols, flavonoids and reducing sugars. The twigs of the plant can be considered a good source of natural antioxidant since it demonstrated potent antioxidant activity *in-vitro*.

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CONFLICT OF INTEREST

The authors declare that there are no conflicting interests.

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