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ARTICLE

Phytochemicals, Nutritional and Antibacterial Evaluation of a Domestic Plant – *Tetradenia riparia*

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Abstract

Medicinal plants have been established to be endowed by nature as sources of phytochemicals employed in the treatment and management of various health disorders. Preliminary phytochemical composition and mineral content of *Tetradenia riparia* leaf was analyzed, using standard procedures. The radical scavenging ability of the methanol extract was determined using the DPPH assay. Flavonoids, phenols and tannins were detected in abundance in the phytochemical analysis. At 500 µg/mL, it had percentage inhibition of 68% when compared with ascorbic acid, the standard reference drug which was 85%. Antibacterial investigation of both the dry and wet leaf extracts in ethanol and water was determined against four pathogenic organisms; *Bacillus substilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and Streptococcus pneumonia. Amongst the tested extract, wet leaf methanol extract was the most active with inhibitory activity of 20 mm and 22 mm against *P. aeruginosa* and *E. coli*, respectively. The results from the study, showed that *Tetradenia riparia* plant could be useful as a food supplements and as alternative antibacterial remedy.

Keywords: Antibacterial, Phytochemical, Anti-oxidants, Mineral element, *Tetradenia riparia*

1. Introduction

Medicinal plants are well known natural sources bioactive agents that are used for the treatment and management of various health disorders and challenges. Several studies have recorded over 20,000 species of plant that have been discovered to serve as food and medicine according to World Health Organization [1–4]. Natural products (plants secondary metabolites) affords unparalleled phytochemicals which can be used both as pure compounds or as standardized plant extracts and has played a crucial role in drug development and lead to discovery of new efficient drugs [4–8]. The upsurge demand in medicinal plants for management of diseases can be attributed to cultural belief, affordability, and usage over a long time and unlimited access of traditional medicine [9].

Herbal medicine in the management, control and cure diseases is as old as man. Man has been dependent on plant for some simple requirement as being the source of food and shelter. Some plants provides man with fragrances, flavours, animal feeds, fertilizers and medicine [10]. Many domestic herbs and spices used by man as sources of food, shelter, decorations and ornamental purposes yield useful phytochemical and medicinal compounds. Activities demonstrated by these naturally occurring phytochemicals as an effective agents in combating and preventing diseases have been attributed to their antioxidant effects [11–13]. Naturally occurring compounds generally called phytochemicals are bioactive compounds that occur in medicinal plants as non-nutritive and non-essential plant chemicals which are largely responsible for the protective or disease preventive properties of the plants. These phytochemicals are

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responsible for the colour, flavour, and odour of plant foods, for example blueberrie is dark hue, broccoli got the bitter taste, and the pungent odour of garlic. They play a major role in plant growth and also used as defensive agents against predators. Over tens of thousands of phytochemicals are known and it is assumed that many are yet to be discovered in the plants [2–4,8–13].

Phytochemicals are grouped into various classes based on their biosynthetic origin, the common structural characteristics and the solubility properties. Major classes of these phytochemicals are saponins, terpenoids, tannins and phenols. Isolation and characterization has led to identification of several compounds [14]. Natural products are significant for the cure and management of many of the diseases that currently pose challenge to man today. Examples, Indoles, which are found in cabbages, could reduce the risk for breast cancer. Protease inhibitors from soy and beans, slows down the progression of HIV, terpenes (citrus fruits and cherries) are antimicrobial, antifungal and antiparasitic. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry and also reduces the risk of urinary tract infections and improves dental health [15,16].

Tetradenia riparia is a traditional plant found within the southern coast of Africa, which span across South Africa, Namibia, Angola and Botswana [17]. This plant is commonly known as misty plume or bush ginger. The common indigenous name of *T. riparia* is 'Ibosa' which is a Zulu word meaning aromatic leaves which is frequently prescribed to provide relieves from many ailments. Traditional uses of *T. riparia* include as an antiseptic, and for the treatment for coughs, respiratory problems, stomach ache, diarrhoea, dropsy, fever, malaria, headache, yaws and toothache [18]. Many medicinally active compounds have been identified in the plant including diterpenes and high valued essential oils [19].

2. Materials and methods

The reagents used in this research are standard analytical reagents and were carried out based on specified standards.

2.1. Sample collection and preparation of plant material

The fresh leaves of *T. riparia* were collected within the premises of Federal University Otuoke, Ogbia Local Government Area of Bayelsa State, Nigeria. The plant was identified and authenticated in Botany Department of University of Benin, Benin City, Edo State, Nigeria. The collected plant samples were washed with distilled water and then air dried for about 14 days. The dried leaf samples were pulverized and stored in air tight container for further analysis. Twenty grams of both the wet and dry powdered leaf samples were packed into the extractor and extracted into 200 mL of distilled water and ethanol respectively. The crude extracts were obtained by concentrating them via rotary evaporator to give for distinct extracts namely, Aqueous extract dry leaf (AEDL), Aqueous extract wet leaf (AQWL), Ethanol extract dry leaf (EEDL), Ethanol extract wet leaf (EEWL) extracts. The filtrates were stored in sterilized bottles and properly labelled for further analysis.

2.2. Preliminary phytochemical screening

The two extracts of *T. riparia* leaves were screened respectively to identify the phytochemicals present [20]. 1 g of the crude extracts obtained was dissolved in 100 mL of each solvents used to obtain the stock solution and was subjected to phytochemical screening as describe below.

2.2.1. Flavonoids test

Dilute ammonia solution was added dropwise to 2 mL of the extract. A yellow colour appears and the solution becomes colourless with the addition of few drops of dilute H_2SO_4. This indicates the presence of flavonoids.

2.2.2. Alkaloid test

Wagner reagents (2 mL) was added of to 2 mL extract, formation of reddish precipitates indicates the presence of Alkaloid.

2.2.3. Steroids test

2 mL of the extract was added 2 mL of acetic anhydride and 1 mL of dilute H_2SO_4. A colour changed from violet to green is indicative of the presence of Steroids.

2.2.4. Anthraquinone test

To 2 mL of the extract was added 20 mL of benzene, the mixture was shaken was filtered. 5 mL of 10% ammonia solution was added to the filtrate. Free Anthraquinones is detected when pink, red or violet colour appears in the ammonical phase.

2.2.5. Saponins test

5 mL of the filtrate was added 2 mL of water. It was shaken vigorously and an observed persistent frothing indicates the presence of Saponins.

2.2.6. Anthocyanoside test

2 mL of the extract was measured and mixed with 2 mL of dilute HCl. The presence of pale pink colour indicates the presence of Anthocyanoside.

2.2.7. Tannins test

2 mL of ferric chloride was added to 2 mL extract, brownish green colouration shows the presence of Tannins.

2.2.8. Terpenoids (Salkowski test)

To 5 mL extract was added 2 mL of chloroform and 3 mL conc. H_2SO_4. Terpenoids is confirmed if a reddish brown colouration of the inter face was formed.

2.3. Determination of mineral elements content

Determination of mineral elements content was determined following standard procedures [21]. Mineral elements assessment is a determinant of the amount of inorganic elements present in the sample. Briefly, the sample was first ashed and dissolved in a solvent, and the resultant solution aspirated into air-acetylene flame. The amount of magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), calcium (Ca), iron (Fe), and phosphorous (P) was performed by atomic absorption spectrophotometric methods. Sodium (Na) and potassium (K) was determined by flame emission spectrophotometer.

2.4. Antimicrobial assay

Four different organisms were employed for the screening tests in line with previous reported method [12,13]. The organisms were used for the assays includes: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *and Bacillus subtilis*. The agar well diffusion method was employed to determine the antimicrobial properties of the test extracts with 24 h broth culture. 200 μL of the bacteria culture was inoculated into 20 mL of molten and cooled nutrient agar [1–4,12,13]. The agar was mixed for homogeneity and poured into 8.5 cm petri dishes and was allowed to set. Equidistant wells were bored into the agar using sterile cork borer (8 mm diameter). The extract (5 mg/mL) was dissolved in methanol and added into the wells. All plates were incubated at 37 °C for 24 h. Gentamicin (0.5 mg/mL) was included as positive control and methanol was used as negative control. The experiments were carried out in duplicates and diameter of zones of inhibition measured.

2.5. DPPH-radical scavenging activity

The antioxidant ability of *T. riparia* leaf extracts was carried out by the DPPH radical scavenging assay method. In this study, 3 mL 2,2-diphenyl-2-picrylhydrazyl (DPPH) solution in methanol (2.0 mg/mL) was added to 1 mL of the extracts at five different concentrations ranging from 0.1 to 0.5 mg/mL [1–4],. The mixtures were incubated at room temperature for 30 min in the dark environment and absorbance read sing a spectrophotometer at 517 nm. The experiment was carried out in triplicate. The % inhibition was calculated and compared with the standard reference, ascorbic acid.

3. Results and discussion

3.1. Phytochemical analysis of extracts

Plants generally contain chemical compounds called secondary metabolites, which are biologically active and may be applied in nutrition and pharmacologically active agent [3,4,13,22]. Table 1 showed that flavonoids, tannin, terpenoids, phenol, steroids, alkaloids and saponins were present in ethanol extracts of the leaves of *T. riparia*. Anthraquinones and anthocyanosides were notably absent. Phytochemicals are found in various parts of plant with numerous functions which include strength to the plant, colours to the plant while some act as defence. Alkaloids, flavonoids, Saponins, and tannins have shown medicinal activities as well as physiological activities [2,3]. Herbal preparations of the leaves of *T. riparia* leaves which are traditionally used for various medicinal purposes (disinfectant, coughs, fever, malaria, toothache, stomach ache, back pain, diarrhoea, dropsy and respiratory problems) was found to contain tannins, phenol, saponins, steroids, flavonoids, terpenoids, and alkaloids. Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre [3,12,23,24]. Komape et al. [25] reported

Table 1. Phytochemical constituents of crude extract of T. riparia *dry leaf.*

Test	Aqueous	Ethanol
Flavonoids	+	+
Phenolic	+	+
Alkaloids	+	+
Steroids	+	−
Anthraquinones	−	−
Saponins	+	+
Anthocyanosides	−	−
Tannins	+	+
Terpenoids	+	+

Key of distribution: + = present − = absent.

that these leaves are commonly valued as antiseptic, and are used for the treatment for coughs, respiratory problems, stomach ache, diarrhoea, dropsy, fever, malaria, headache and yaws. These phytochemical are responsible for the therapeutic activities of the plant [2–4,8,13,25].

3.2. Mineral elements determination

Results from the elemental analysis (Table 2) indicated that *T. riparia* leaves contain relatively higher quantities of sodium, potassium, calcium and magnesium. Iron, zinc, copper and manganese were present in minute quantities. Calcium, magnesium, sodium and potassium which are present in high concentrations are essential minerals for life; they are important in the formation of bones, teeth and as a cofactor for enzymes and a component of ATP, DNA, RNA and cell membranes respectively. Iron, zinc, copper and manganese, though occur in low quantity in the plant is important in the formation of haemoglobin, growth and sexual maturation, facilitating iron intake, as cofactor for enzymes [13,22,26].

3.3. Antioxidant activity of the T. riparia leaf extract

The antioxidant capacity of the leaves of *T. riparia* was used to determine the active extracts as measured by the DPPH method. Results from Table 3 showed the inhibitory properties of *T. riparia* leaves ethanol extract being the most active extract in the antioxidant activity. It was observed that the radical scavenging activity was not concentration dependent. The inhibitory property of *T. riparia* leaves fall within the range of 49–68% at 518 nm with a higher percentage inhibitory property in ascorbic acid with value ranging between 81 and 86%. The therapeutic benefit of medicinal plants is mostly ascribed to their antioxidant property. Antioxidants play very important roles in coetaneous tissue repair as they significantly prevent tissue damage that excites wound healing process [2–4,7,8,13,27].

Table 2. Elemental analysis of T. riparia *for ethanol leaf extract.*

Sample	Na (mg/ml)	K (mg/ml)	Ca (mg/ml)	Mg (mg/ml)
Tetradenia riparia crude extract.	6.20	5.54	75.34	18.30

Table 3. Antioxidant activity of ethanol leaf extract of T. riparia.

Samples	Conc. (mg/mL)	Abs @ 518 nm	% inhibition
Tetradenia riparia extract	0.1	0.621	59.14
	0.2	0.650	57.23
	0.3	0.780	48.68
	0.4	0.752	50.52
	0.5	0.486	68.02
Ascorbic acid (Standard)	0.1	0.284	81.31
	0.2	0.321	78.88
	0.3	0.350	76.97
	0.4	0.293	80.72
	0.5	0.220	85.52

Table 4. Antimicrobial analysis of T. riparia *extracts showing zone of inhibition (mm).*

Organisms	Strain type (+/−)	EEDL	EEWL	AEDL	AEWL	GEN
Staphylococcus aureus	+	10	14	16	8	29
Bacillus subtilis	+	10	13	0	0	25
Pseudomonas aeruginosa	−	12	20	0	0	28
Escherichia coli	−	18	22	0	0	30

Note: DL = Dry Leaf, WL = Wet leaf, GEN = Gentamicin.

3.4. Antimicrobial activities of the T. riparia leaf extract

The result of the antibacterial activities of ethanoic and aqueous extracts of *T. riparia* leaf are shown in Table 4. The extracts showed varying degree of activities on the test organisms. The most susceptible organism was *Staphylococcus aureus* which growth was inhibited by the two extracts. The ethanoic extracts of wet leaves was the most active extract. It significantly inhibited the growth of all the tested organisms [2,4,12,13,25]. The aqueous extracts have no inhibitory activity on any of the bacteria screened. This is in accordance with past studies [12–14,25], this plant contains large number of medicinally active compounds including diterpenes which combat different ailments and has high valued essential oils for man's consumptions. The leaves, roots and young stems of *T. riparia* are commonly used in traditional medicine. Flavonoids are hydroxylated phenolic constituents that are manufactured by plants in response to microbial contamination and have been found to demonstrate antimicrobial activities against a wide range of pathogens [4,7,8,13,27].

4. Conclusions

The high composition of macro nutrients, calcium, potassium, and iron in *T. riparia* suggests the plant as a prospective nutritious food supplement to

enhance the health grade of its consumers. Phenols are good antimicrobial and antioxidant agents and also serve as nutritional supplement; this indicate possible synergistic antioxidant activities between various constituents of the crude extracts. Also, the presence of phytochemicals such as flavonoids, tannins in the plants coupled with the antibacterial activities demonstrated by the extracts confirmed its high medicinal properties.

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