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Phytochemical Constituents and *in vitro* Antioxidant Properties of the Root of *Mallotus subulatus* Mull. Arg (Euphorbiaceae)

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ABSTRACT

Background and Objective: *Mallotus subulatus* is a tropical plant traditionally used in African medicine to treat various ailments, largely attributed to its phytochemical and antioxidant components. Despite its traditional applications, scientific validation of its phytochemical content and antioxidant potential remains limited. This study aimed to investigate the phytochemical composition and *in vitro* antioxidant activity of the methanolic root extract of *Mallotus subulatus* to support its ethnomedicinal use. **Materials and Methods:** Phytochemical screening was performed on methanolic root extracts to detect the presence of major secondary metabolites. Antioxidant potential was evaluated using the DPPH free radical scavenging assay and the phosphomolybdate method for total antioxidant capacity, with ascorbic acid as the standard. Extract concentrations tested included 62.5, 125.0, 250.0, 500.0, and 1000.0 µg/mL. Percentage inhibition and IC_{50} values were calculated to determine antioxidant efficiency. **Results:** The extract tested positive for alkaloids, flavonoids, tannins, phenolics, anthocyanins, and soluble carbohydrates, but lacked saponins. The DPPH assay results showed concentration-dependent activity, with the highest inhibition (61.11±1.85%) at 1000.0 µg/mL and the lowest (16.67±1.85%) at 62.5 µg/mL. The extract exhibited moderate antioxidant potential compared to the ascorbic acid standard. **Conclusion:** The presence of multiple phytochemicals and notable antioxidant activity suggests that *Mallotus subulatus* root extract may serve as a natural source of antioxidants. Further studies are recommended to isolate active compounds and assess *in vivo* efficacy and toxicity.

KEYWORDS

Mallotus subulatus, phytochemicals, antioxidants, tannins, alkaloids, flavonoids, phenolics, anthocyanins, saponins

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INTRODUCTION

Medicinal plants have been utilized for centuries, long before modern medicine was developed[1]. Herbal remedies can be made from various parts of plants, including leaves, flowers, stems, roots, seeds, fruits, and bark. These plants' phytochemical components, which cause certain physiological effects in the human body, are thought to be responsible for their medicinal qualities[2]. Alkaloids, saponins, tannins, phlobatannins, anthraquinones, glycosides, flavonoids, steroids, and terpenoids are all examples of these phytochemicals[3].

Based on their functions in plant metabolism, phytochemicals are separated into primary and secondary metabolites. Plant viability depends on primary metabolites, which include proteins, lipids, carbohydrates, amino acids, and purines and pyrimidines in nucleic acids. Secondary metabolites, on the other hand, are the extra plant compounds produced by cells via metabolic pathways that originate from primary metabolism[4]. These chemical compounds have been identified as possessing antiviral, antifungal, and antibacterial properties, which play a crucial role in safeguarding plants against pathogens[5].

Phytochemicals are essential for plant survival as they facilitate interactions with competitors, shield plants from diseases, pollution, stress, and UV radiation, and enhance color, aroma, and flavor[6]. The compounds generated by plants to defend against biotic and abiotic challenges have evolved into medicines that humans use for the treatment of various diseases[7].

According to Hernández-Rodríguez *et al*.[8], antioxidants aid in the prevention of cellular damage, which is a common cause of cancer, aging, and a number of other disorders. According to Abraham *et al*.[9], free radicals are produced as a by-product of the body's regular metabolic processes and have two functions in human bodies: They can be harmful and helpful. Tissue damage and many diseases can result from an excess of reactive oxygen species (ROS) and a decline in antioxidant levels[10]. According to recent research, antioxidants derived from plants may have significant therapeutic value in treating free radical-mediated illnesses such as diabetes, cancer, neurodegenerative diseases, cardiovascular diseases, aging, gastrointestinal disorders, arthritis, and the aging process. They may also have the ability to scavenge free radicals[11].

Flavonoids and other polyphenolic compounds found in plant extracts and products are potent lipid peroxidation inhibitors and radical scavengers[12]. Numerous researchers are looking for natural antioxidants to replace synthetic antioxidants in food and medicine because of the harmful and carcinogenic effects of many synthetic antioxidant molecules[13]. Approximately 5 meters high, *Mallotus subulatus* is a shrub or tree that grows in the forest zone from Sierra Leone to West Cameroon and beyond to Zaïre. In Nigeria, the fruit, roots, and leaves are all crushed up and used as medicine to treat diarrhea[14]. For stomach-aches, seeds are mashed and eaten by draught[15]. Sap from the bark is applied to scarifications in the location of pain to cure lumbar and side pain in Congo (Brazzaville)[16].

In Nigeria, young boys are administered wet leaves for amudzu convulsions, and the leaves are pulped and put to wounds as a styptic[17]. The Igbo of Southern Nigeria grind the seeds into a powder, which is then used to cover young men's and women's faces. The bark and roots have been shown to contain a small amount of tannin, a lot of saponins, and a trace amount of alkaloid[18]. Recently, a number of artificial antioxidants have been utilized in food manufacturing; they can have a variety of adverse effects. The search for novel natural antioxidants to replace synthetic ones in food and medicine has gotten more attention. This study aims to close the knowledge gap and provide a scientific foundation for the possible health advantages of *Mallotus subulatus* roots by identifying new sources of natural antioxidants that can be utilized to enhance human health.

MATERIALS AND METHODS

Study area and duration of study: This study was carried out at the University of Nigeria, Nsukka, Enugu State, Nigeria, within a period of 6 months, from January, 2023 to July, 2023. The GPS coordinates of Nsukka, Nigeria. Latitude: 6.8578 Longitude: 7.3958. Nsukka is a town and Local Government Area in South-East Nigeria in Enugu State, Nigeria.

Plant collection: Fresh roots of *Mallotus subulatus* were harvested from Obukpa Forest, Nsukka, Enugu State, Nigeria, identified and authenticated in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria.

Sample preparation and extraction: The sample was washed in running tap water and chopped into small pieces, and spread out on newspaper to dry at room temperature. The dried samples were pulverized with a local milling machine and stored in an air-tight container until used. The 2 g of the powdered *Mallotus subulatus* sample were mixed with methanol, and the extraction method of Ifeoluwa *et al*.[19].

Detection of alkaloids: The methanolic plant extract was warmed with 2% H_2SO_4 for 2 min. and filtered; a few drops of Dragendorff reagent were added as described by Kuete[20]. Formation of a reddish-orange precipitate showed the presence of alkaloids.

Detection of flavonoids: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which became colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids[21].

Detection of phenolics: Phenolic detection was carried out by the method described by Pandey and Rajbhandari[22]. The 10 mL of the extracts were treated with a few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenol.

Detection of saponins: Saponin detection was carried out by the method described by Murtiningsih *et al*.[23] where a total of 10 drops of solution, added, 5 drops of hot water were added, then cooled, shaken vigorously for 10 sec. If a lot of foam is formed for 10 min, as high as 1 to 10 cm, and does not disappear with the addition of a drop of 2N hydrochloric acid, it indicates the presence of saponin content.

Detection of tannins: A total of 10 drops of solution, added to distilled water until the color fades, and then 1-2 drops of iron (III) chloride reagent. When formed blue-black or green-black color indicates the presence of tannin compounds in the sample[24].

Detection of carbohydrates: A 2 mL of Fehling's solutions A and B were added to 2 mL of the extract, and the test tube containing the mixture was placed in a boiling water bath for ten minutes. The presence of carbohydrates was confirmed by the formation of a red precipitate[25].

Detection of anthocyanins: The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract to 2 mL of 2N HCl. The appearance of a pink-red colour that turns purplish blue after the addition of ammonia indicates the presence of anthocyanins[26].

In vitro antioxidant screening

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay: The DPPH radical scavenging assay was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Polile *et al*.[27] with slight modifications. Briefly, five different concentrations of the studied plant extracts (62.5, 125, 250, 500, and 1000 mg/mL) were prepared in methanol (analytical grade). The same

concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. A 1 mL of each studied extract was transferred into a clean test tube, into which 0.5 mL of 0.3 mM DPPH in methanol was added. The mixture was shaken and left to stand in the dark at room temperature for 30 min. Blank solutions comprising of the studied extract solutions (2.5 mL) and 1 mL of methanol were used as baseline. The negative control comprised 2.5 mL of DPPH solution and 1 mL of methanol, while L-ascorbic acid at the same concentrations as the studied extracts was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a UV-Vis spectrophotometer UV-6300PC (VWR International, ThermoFisher Scientific, China).

Total antioxidant capacity (TAC): The total antioxidant capacity of the methanol extract was determined by the phosphomolybdate method using ascorbic acid as a standard, as described by Pisoschi and Pop[28]. The stock solution (1 mg/mL) of plant extract was diluted to lower concentrations: 20, 40, 60, 80, 100 µg/mL. An aliquot of 0.1 mL of sample solution was mixed with 1 mL of reagent solution (0.6 M sul40phuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Sample tubes were 60 capped and incubated in a water bath at 95°C for 90 min. Once the sample had cooled down to room temperature, the absorbance of the mixture was measured at 695 nm against a blank on a UV-visible spectrophotometer. A typical blank contained 1 mL of the reagent solution along with an appropriate volume of the solvent and was incubated under similar conditions.

Test for reducing power: The crude extract was subjected to reducing power assay following the method of Alam *et al.*[29]. A 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of K_3Fe (CN) 6 (1% w/v) were added to 1.0 mL of the sample dissolved in distilled water. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 mL of Trichloroacetic acid (10% w/v). The 2.5 mL of the sample was obtained from the mixture and mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance was then measured at 700 nm against a blank.

RESULTS

The presence and absence of these phytochemicals were verified by the appearance of various color changes that were seen upon the addition of particular reagents, as indicated in Table 1. The presence of tannins was verified by the formation of a dark green precipitate upon the addition of ferric chloride; the presence of total anthocyanin content was verified by the appearance of pink red upon the addition of HCl, which turned purplish blue upon the addition of ammonia; and the presence of total flavonoids was verified by the formation of an intense yellow precipitate that vanished upon the addition of diluted hydrochloric acid. The presence of carbohydrate was verified by the appearance of red precipitate upon the addition of Fehling's solution, the presence of alkaloids by the appearance of reddish orange precipitate upon the addition of Dragendorff reagent, and the presence of total phenolics by the formation of bluish black precipitate upon the addition of ferric chloride solution. The extracts did, however, show that the plant sample lacked saponins.

Quantitative test: The soluble carbohydrate content was 45.77±0.55, while that of alkaloids was 9.36±0.30, tannin 1.68±0.20, total phenolics 0.69±0.02, flavonoids 0.33±0.03, and anthocyanins 0.02±0.00, while saponin was absent. This shows that there were more soluble carbohydrates in the extract, followed by alkaloids, phenolics, flavonoids, and anthocyanins, respectively shown in Table 2.

Reducing power assay: The reducing power antioxidant capacity was determined by comparing the sample with ascorbic acid. As the ascorbic acid increased, the reducing power of the root also increased, but the standard (ascorbic acid) had a higher absorbance value than the absorbance of the extract. The power of the extract increased with the concentration of the sample in Table 3.

Table 1: Qualitative phytochemical results

Phytochemical	Qualitative test
Total phenolics (mg/g)	+
Tannins (mg/g)	+
Flavonoids (mg/g)	+
Soluble carbohydrates (mg/g)	+
Saponins (mg/g)	-
Alkaloids (mg/g)	+
Anthocyanins (mg/100 g)	+

+: Indicates presence and -: Indicates absence

Table 2: Quantitative estimation of phytochemicals present

Phytochemical	Quantitative test
Total phenolics (mg/g)	0.69±0.02
Tannins (mg/g)	1.68±0.20
Flavonoids (mg/g)	0.33±0.03
Soluble carbohydrates (mg/g)	45.77±0.55
Alkaloids (mg/g)	9.36±0.30
Anthocyanins (mg/100 g)	0.02±0.00
Saponins (mg/g)	0.00±0.00

Values expressed as Mean±Standard Error of duplicate analysis

Table 3: Reducing power activity of *Mallotus subulatus* root compared with ascorbic acid

Concentration (ug/m)	Sample (Abs)	Ascorbic acid (Abs)
62.5	0.07±0.00	0.433
125.0	0.09±0.00	0.526
250.0	0.10±0.00	0.684
500.0	0.13±0.00	0.866
1000.0	0.18±0.00	1.025

Values expressed as Mean±Standard error of duplicate analysis

Table 4: Percentage inhibition of DPPH antioxidant capacity of *Mallotus subulatus* aqueous root extract

Conc. (ug/m)	Percentage inhibition 1	Percentage inhibition 2	Mean±SEM	Ascorbic acid (%)
62.5	18.52	14.81	16.67±1.85	47
125.0	29.63	25.93	27.78±1.85	64
250.0	37.04	33.33	35.19±1.85	74
500.0	48.15	44.44	46.30±1.85	82
1000.0	62.96	59.26	61.11±1.85	92

Table 5: Percentage inhibition of total antioxidant capacity of *Mallotus subulatus* aqueous root extract

Conc. (ug/m)	Percentage inhibition 1	Percentage inhibition 2	Mean±SEM	Ascorbic acid %
62.5	37.97	38.16	38.06±0.10	47
125.0	40.23	40.41	40.32±0.09	64
250.0	45.86	45.68	45.77±0.18	74
500.0	49.44	49.62	49.62±0.19	82
1000.0	57.33	57.42	57.42±0.10	92

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay: Percentage inhibition was determined at different concentrations as shown in (Table 4) When the concentration was at 62.5, 125, 250, 500 and 1000, the percentage inhibition of the root extract was at 16.67, 27.78, 35.19, 46.30 and 61.11% and the ascorbic acid was at 47, 64, 74, 82 and 92%, respectively. The IC_{50} value for *Mallotus subulatus* was 674.2 mg/mL and the IC_{50} value of the standard (L-ascorbic acid) was 171.0 mg/mL.

Total antioxidant activity: The total antioxidant capacity was determined, and the samples were compared with ascorbic acid, as seen in Table 5. As the ascorbic acid increased, the percentage inhibition also increased. When the concentration is at 62.5, 125, 250, 500, and 1000, the percentage inhibition is at 38.06, 40.32, 45.77, 49.62 and 57.42% at IC_{50} of 602.7 mg/mL for extract and 145.7 mg/mL for ascorbic acid.

DISCUSSION

Table 1 lists the tannins, soluble carbohydrates, alkaloids, anthocyanins, phenols, and flavonoids found in *Mallotus subulatus* roots. Since these substances are said to be antioxidants or free radical scavengers, they may be the cause of the roots' antioxidant activity[30].

As indicated in Table 1 and 2, phenolic compounds found in *Mallotus subulatus* roots are known to be potent antioxidants and significant plant elements. Research has demonstrated the antibacterial, anti-inflammatory, antiallergic, and antioxidant properties of flavonoids[31]. According to Ramesh *et al.*[32] flavonoids are polyphenolic chemicals that are present in a variety of fruits, vegetables, barks, tea plants, and stems. Their anti-inflammatory, anti-oxidative, and anti-carcinogenic qualities give them a wide range of uses.

According to Ayo *et al.*[13], flavonoids are known to have antioxidant activity and to have scavenging or chelating effects on human nutrition. The work of Valko *et al.*[33] shows that saponins, which are lacking from *Mallotus subulatus* roots, are present in trace amounts in the fruit.

According to Table 2, the high concentration of tannins (1.68 mg/g) in *Mallotus subulatus* root can be linked to its hepatoprotective, antibacterial, antiviral, anti-mutagenic, anti-carcinogenic, anthelmintic, antioxidant, and free radical scavenging properties[34].

A significant class of polyphenols, anthocyanins, have anti-atherosclerotic, antihypertensive, antithrombotic, anti-inflammatory, and anticancer properties in addition to positive effects on oxidative stress and endothelial function. *Mallotus subulatus*' therapeutic properties are due to the presence of alkaloids in its roots[20].

As compared to ascorbic acid, a known reducing agent (Table 3), which has been demonstrated to exhibit antioxidant action by breaking the free radical chain by donating a hydrogen atom, the reducing power of the plant root extract is found to increase with increasing concentration[35].

Mallotus subulatus has less of an antioxidant effect on DPPH than ascorbic acid (Table 4). The study also found that the extracts can donate protons and may operate as scavengers or inhibitors of free radicals, potentially functioning as main antioxidants. The antioxidants' percentage inhibition (Table 5) is comparatively modest.

The present study, while insightful, has several limitations. It evaluates antioxidant activity solely through *in vitro* assays, which may not accurately reflect *in vivo* efficacy or safety. Additionally, although phytochemical screenings were likely performed, advanced analytical techniques such as HPLC, LC-MS/MS, or NMR were not employed, limiting the identification of specific bioactive compounds. The study's focus on only the root of *Mallotus subulatus* may have overlooked antioxidant-rich components in other plant parts like leaves or bark. Moreover, the extract's antioxidant potential was not compared against standard references (e.g., ascorbic acid), making it difficult to contextualize its potency. The lack of toxicity data further restricts any therapeutic recommendations. Future research should validate antioxidant activity *in vivo* using animal models or clinical trials, and apply advanced chromatographic and spectrometric tools to isolate and characterize active compounds. Investigations should also encompass other plant parts to discover additional antioxidant sources and explore underlying mechanisms such as free radical scavenging or enzymatic modulation. Toxicity profiling of both crude extracts and isolated compounds is essential to ensure safety. Ultimately, efforts should be made toward developing stable, bioavailable formulations for use as antioxidant supplements or therapeutic agents.

CONCLUSION

The root of *Mallotus subulatus* is rich in bioactive phytochemicals such as anthraquinones, soluble carbohydrates, flavonoids, tannins, phenolics, and alkaloids, which contribute to its notable antioxidant properties. These findings highlight its potential as a natural source of antioxidants, offering promising applications in pharmaceuticals and the food industry as a substitute for synthetic additives. The presence of these compounds also suggests possible antimicrobial activity, supporting its traditional medicinal use. Further research is recommended to explore its nutritional profile, detailed biochemical composition, and potential for drug discovery.

SIGNIFICANCE STATEMENT

This study discovered the presence of important phytochemical constituents such as alkaloids, flavonoids, phenolics, tannins, anthraquinones, and soluble carbohydrates in the root extract of *Mallotus subulatus* that can be beneficial for developing natural antioxidant and antimicrobial agents. The antioxidant potential demonstrated by the root extract suggests its possible application in pharmaceutical formulations and food preservation, offering an alternative to synthetic additives. Additionally, the study reinforces the ethnomedicinal relevance of *M. subulatus* and supports its potential in drug discovery. This study will help the researchers to uncover the critical areas of plant-based bioactive compound research that many researchers were not able to explore. Thus, a new theory on natural antioxidant drug development may be arrived at.

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