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Phytochemical, Acute Toxicity and Nutrient Composition of *Mallotus oppositifolius*

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Abstract

Background: *Mallotus oppositifolius* is a medicinal herb popular in Nigeria and African countries, consumed as a food thickener known as "Ukpo" among the Igbos. The leaf of this plant is widely used for the treatment of diseases such as infection, ulcer, and wound healing, with a paucity of data on its nutraceutical potentials. This study evaluated the phytochemicals and nutrient composition of *Mallotus oppositifolius* methanol leaf extracts. The fresh *Mallotus oppositifolius* leaves were harvested from their habitat in Amawbia in June 2018, identified, air dried, milled, and extracted with methanol using cold maceration. The extract was concentrated to dryness and kept until required. The dried methanol extract was subjected to phytochemical analysis while the powder of the leaf was evaluated for proximate, minerals, and vitamins profile using a standard Laboratory procedure.

Results: The result showed that *Mallotus oppostifollous* contain alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, steroids, proteins, carbohydrates, anthraquinones, reducing sugars, and anthocyanins while terpenoids were absent. The result further showed that *M. oppostifollous* contained appreciably high amount of essential nutrients: Protein (24.07%), Carbohydrates (54.7%), Fibre (10.59%), Fat (6%), magnesium (7.8 mg), Calcium (79.947 mg), Iron (0.107 mg), Manganese (0.703 mg), Zinc (0.587 mg) and high amount of essential vitamins such as Pyridoxine (8.150 mg), Biotin (4.940 mg), Ascorbic acid (0.920 mg), Retinol (0.837 mg), and Riboflavin (0.580 mg).

Conclusion: These findings showed that the *M. oppostifollous* leaf is rich in phytochemicals, vitamins, protein, carbohydrates, and minerals, which could be exploited as a food source for preventing malnutrition and in food fortification for malnourished children.

Keywords: *Mallotus oppositifolius* • Mineral • Vitamin • Phytochemical • Proximate

Introduction

All over the world, plants serve various functions such as sources of food, phytochemicals, dietary supplements, and crude drugs. Phyto-nutrients including Proteins, Fibre, Vitamins, and various specialized functional additives will remain the top-selling group of nutraceutical ingredients globally due to their health benefits. Proteins, carbohydrates and vitamins remain the most important nutraceutical for food and beverage industries throughout the world due to their leading role in solving problems of malnutrition. Around 80% of rural population in Nigeria depends on plants for their primary health care [1]. High dietary intake of fibres in the form of fruits, vegetables, whole grains is strongly linked to a reduced risk of chronic diseases like cancer and cardiovascular diseases [2]. Pharmacological and epidemiological studies provide convincing evidence that dietary factors may modify carcinogenesis [2] and therefore more effective in the treatment and management of cancer. Herbal formulations with phytochemicals possess an excellent alternative to orthodox drugs in the treatment of cancer and other diseases because of the fact that plant has synthesized in nature more nutrients and therapeutic moiety than man has ever synthesized. *Mallotus oppositifolius* is a popular medicinal herb within its native range in Nigeria and Africa. *Mallotus oppostifollous* belongs to the family Euphorbiaceae and locally known as "Ukpo" which is an edible plant in the South-Eastern part of Nigeria [3]. The seed is a special soup thickener in the region. The leaves have been reported in treatment for dysentery, malaria, and inflammation [4].

Ethnobotanical uses of *Mallotus oppostifollous*

Folklorically, the plant leaf is used for the treatment of eye and kidney infections, painkillers, treatment of paralysis, spasm, headache, inflammation, wounds and antidiabetic [4]. Decoction of the root is used for anaemia, pneumonia, and as an aphrodisiac, and the stem is chewed for oral hygiene [5, 6].

Pharmacological potentials of *Mallotus oppostifollous*

Preliminary phytochemical screening of *Mallotus oppostifollous* revealed the presence of secondary metabolites such as Alkaloids, Phenols, Flavonoids, Anthraquinones and Cardenolides [7, 8].

Previous studies have reported the following activities of *Mallotus oppostifollous*: Antifungal and Antibacterial activities of the plant [9, 10], Antitrypanosomal and Anti-Helmintic activity *in vitro*, Anti-Inflammatory activity [4], and Antioxidant activity [11].

The demand for nutraceutical products and their derivatives is projected to increase by 6.8 percent annually to $28.8 billion between 2017 to 2022 [12].

Herbal formulations should be properly labeled for the nutraceutical database and safety profile. Nutrients such as Ca, I, Zn, Fe, Mn, Mg, and other mineral elements are essential components for human health, and deficiency of any one of these minerals may cause serious health problems [13]. Phytochemicals on the other hand possess potency for therapy and serve as Modulators, Ligands, Cofactors, Scavengers, Growth Inhibitors and Drug enhancers. Combination of the nutrients and therapeutic activity in one is of great potential in both the pharmaceutical and nutraceutical industries which remains largely untapped in Nigeria. The present study evaluated the Proximate, Phytochemicals, Minerals, and Vitamin profile of *M. oppositifolius*.

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Methods

Plant collection

Fresh leaves of *Mallotus oppostifollous* were collected in June 2019 from their habitat Amawbia, Awka Local government Area, Anambra State. The plant was identified by a plant taxonomist a voucher number PCG/UNN/0337/ Euphorbiaceae was obtained and deposited in the herbarium. The plant leaves were air dried at room temperature for 14 days.

Animals

Thirteen healthy adult mice (males) weighing between 25 g to 30 g were used in this work. The animals were maintained at room temperature and humidity (25˚C, 70% relative humidity) and allowed to acclimatize for 2 weeks at the animal housed using a standard cage on arrival. The mice used in this work were purchased from a private farm and the written consent to use them is not applicable to this research which required only 13 mice for acute toxicity studies. All the animals were fed with a standard pelleted diet and water ad libitum. The study followed ethical guidelines for investigations using experimental animals established by [14].

Extraction

The air-dried plants were ground and extracted using methanol by cold maceration. 500 g was extracted using 5 litres of methanol. The extract was concentrated to dryness using a rotary evaporator at 30˚C. The concentrated extract was scrapped into the bottle and kept in the refrigerator until required for use.

Phytochemical screening

The Phytochemical analysis was conducted using the method of [14, 15] to determine the presence of secondary metabolites in *Mallotus oppostifollous*.

Estimation of the amount of phytochemicals present was assayed using the standard procedures described by [16-19]. The values of the amount of phytochemicals present were read at absorbance of 470 nm for Alkaloids; Glycosides at 530 nm; Steroids at 540 nm; Terpenoids at 538 nm; Saponins at 527 nm; Flavonoids at 510 nm; Tannins at 640 nm and reducing Sugar at 510 nm.

Acute toxicity studies

The acute toxicity test to determine the LD50 was done as described by [20] modified for *Mallotus oppostifollous*. A total of 13 mice were used for the Acute Toxicity Studies. Briefly, nine mice (male) were randomly allocated into 3 groups of 3 mice each. Animals in groups 1, 2 and 3 were given 10, 100, and 1000 mg/kg respectively of the extract through the oral route. Animals were monitored for signs of mortality and adverse effect for two days (48 hours). All the animals survived, and the extract was further subjected to the second phase of acute toxicity test with higher doses in the second trial. In the second trial, 4 mice (male) were randomly allocated to 4 groups of one animal each. In the second trial the group 1 received 1200 while groups 2 to 4 received 1600, 2900, and 5000 mg/kg of the extract respectively. The study followed ethical guidelines for investigations using experimental animals established by [21]. After the experiment, the mice used in the study were deep anesthetized by administration of ketamine 60 mg/kg and euthanized. The liver and kidney of the mice were harvested for Histopathological studies.

Proximate analysis

The Proximate analysis was done by the standard method of [22].

Determination of minerals

The Mineral analysis was done by the method of [22] as follows

The sample was first ashed in the oven at 600˚C and 2 g of the samples were analyzed for the selected nutrients (Manganese, Sodium, Iron Phosphate, Sulphate, and Calcium) using the standard protocol according to [22].

Determination of vitamins

The Vitamin profile was done by the method of [22].

The sample was first ashed in the oven at 600˚C, and then dissolved with 5 ml of 30% HCl and 2 ml of the samples were analysed for the selected vitamins (Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B 7, Vitamin B9, Vitamin K and others Vitamins) using standard protocol [22] and the vitamin content was determined at 510, 560, 540, 460, 420, 520, 379 and 635 nm respectively using UV-Visible Absorption Spectrometer.

Statistical analysis

The data were analyzed using statistical package for social science version 20.0. One-way ANOVA was used to analyze the variation between means of each group while Duncan multiple range tests was used to separate the means. The data were expressed as mean ± SEM while ulcer inhibition was expressed as index number and percentage. $P<0.05$ were considered significant.

Results

Phytochemicals

The result of the phytochemical screening of *Mallotus oppostifollous* is presented in Tables 1 and 2.

Qualitative phytochemicals analysis

The result of the qualitative phytochemical analysis is presented in Table 1. The result showed that *Mallotus oppostifollous* contains Alkaloids, Glycosides, Flavonoids, Tannins, Phlobatanins, Saponins, Steroids, Proteins, Carbohydrates, Anthraquinones, Reducing Sugars and Anthocyanins while Terpenoids were absent (Table 1).

Quantitative phytochemical analysis

The result of the quantitative phytochemical analysis is presented in Table 2. It showed that *Mallotus oppostifollous* contained an abundant amount of Flavonoids (28.2 mg/g), Tannins (17.7 mg/g), Alkaloids (7.8 mg/g), and Saponin (0.2 mg/g). It further showed that *Mallotus Oppostifollous* contained a high amount of carbohydrates (54.7%), and Reducing Sugar (2.0%) which is important nutrients (Table 2).

Acute toxicity test of *Mallotus oppostifollous* leaf extract

The result of the acute toxicity studies is presented in Table 3. It showed that the LD50 of *Mallotus oppostifollous* is above 5000 mg/kg of crude extract because no mortality was recorded at 5000 mg/kg in the mice, therefore, this implied that the crude extract of *Mallotus oppostifollous* is safe for consumption at dosage less than or equal to 5000 mg/kg at acute administration (Table 3).

Table 1. The qualitative phytochemical constituents of *M.oppositifolius*.

S/N	Bioactive compound	Qualitative
S/N	Alkaloids	Present
S/N	Glycosides	Present
S/N	Flavonoids	Present
S/N	Tannins	Present
S/N	Phlobatanins	Present
S/N	Saponins	Present
S/N	Steroids	Present
S/N	Proteins	Present
S/N	Terpenoids	Absent
S/N	Carbohydrates	Present
S/N	Anthraquinones	Present
S/N	Anthocyanins	Present
S/N	Reducing Sugar	Present

Proximate profile of *Mallotus oppostifollous*

The result of the proximate analysis is presented in Table 4. The result showed that *Mallotus oppostifollous* leaf is rich in carbohydrates (54.7%), protein (24.1%), ash (13.6%), fiber (10.6%), and fat (6.0%) which are of great importance to nutraceutical industries. The dried leaves also had a moisture content of 4.5% which is far below the tolerable limit (Table 4).

Mineral content of *Mallotus oppostifollous*

The result of the mineral profiling of *Mallotus oppostifollous* is presented in Table 5. The result showed that *Mallotus oppostifollous* leaf richly contained Chlorine (159.7 mg), Calcium (80 mg), Phosphorus (59.7 mg), Magnesium (7.8 mg), Manganese (0.7 mg), Zinc (0.5 mg), Sulphate (0.2 mg) and Iron (0.1 mg) while Potassium (0.09 mg), Sodium (0.07 mg) and Cadmium (0.01 mg) were in traces. This implied that the leaf of *Mallotus oppostifollous* is rich in mineral elements which are of great nutraceutical importance (Table 5).

Table 2. The quantitative phytochemical constituents of *M.oppositifolius*.

S/N	Bioactive compound	Quantitative (mg/g) (Mean ± SE)
1	Alkaloids	7.797 ± 0.02
2	Flavonoids	28.213 ± 0.03
3	Tannins	17.723 ± 0.03
4	Saponins	0.239 ± 0.00
5	Steroid	0.077 ± 0.000
6	Terpenoids	-
7	Carbohydrate%	54.737 ± 0.02%
8	Reducing sugar%	2.013 ± 0.029%

Table 3. Acute toxicity test result of *M.oppositifolius* leaf extract.

Phase	Dose (Mg/kg)	No of animals	Death ratio
Phase 1	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase 2	1200	1	0/1
	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

Table 4. Proximate analysis of *M.oppositifolius*.

S/N	Parameter	Value (%) (Mean ± SE)
1	Protein	24.07 ± 0.01
2	Ash value	13.63 ± 0.06
3	Fiber content	10.59 ± 0.01
4	Fat	6.00 ± 0.00
5	Moisture	4.60 ± 0.01
6	Carbohydrate	54.74 ± 0.02
7	Energy (kcal)	411.61 ± 0.16

Table 5. The mineral components of *M.oppositifolius*.

S/N	Parameter	Values (mg/100g) (Mean ± SE)
1	Magnesium	7.820 ± 0.17
2	Calcium	79.947 ± 0.10
3	Chlorine	159.730 ± 0.02
4	Sodium	0.067 ± 0.02
5	Sulphate	0.223 ± 0.01
6	Iron	0.107 ± 0.01
7	Phosphorous	59.703 ± 0.14
8	Manganese	0.703 ± 0.00
9	Zinc	0.587 ± 0.01
10	Potassium	0.087 ± 0.03
11	Cadmium	00.157 ± 0.00

Table 6. The Vitamins profile of *M.oppositifolius* leaf extract in 100 g dry weight base.

S/N	Vitamin	M. oppositifolius (mg/100 g) (Mean ± SE)
1	Vit A/Retinol	0.837 ± 0.01
2	Vit B2/Riboflavin	0.580 ± 0.01
3	Vit B3/Niacin	0.303 ± 0.01
4	Vit B6/Pyridoxine	8.150 ± 0.09
5	Vit B7/Biotin	4.940 ± 0.12
6	Vit B9/Folate	0.533 ± 0.01
7	Vit C/Ascorbic acid	0.920 ± 0.01
8	Vit K/Tocopherol	0.053 ± 0.01

Vitamin profile of *Mallotus oppostifollous*

The result of the vitamin profiling of *Mallotus oppostifollous* leaf is presented in Table 6. The result showed that *M. oppositifolius* leaf contained vitamin B complex of Pyridoxine (8.2 mg), Biotin (5 mg), Riboflavin (0.6 mg), Folate (0.5 mg) and Niacin (0.3 mg). Also, the result further showed that M. oppositifolius leaf is rich with Ascorbic acid (0.9 mg), Retinol (0.8 mg) while Tocopherol (0.05) is in traces. This implied that pyridoxine and biotin was the highest vitamin content of *M. oppositifolius* leaf and the plant could be used as supplements for vitamin deficiency ailments (Table 6).

Discussion

The Phytochemical analysis revealed the presence of Tannins, Saponins, Glycosides and Flavonoids and this is similar to the findings of [11]. The abundant presence of secondary metabolites such as Flavonoids, Alkaloids, and Saponins showed that the plant could have great potential as an antioxidant. Phytochemical compounds, such as Flavonoids, Phenols, Saponins, and Tannins, are considered major secondary metabolites in plants with nutraceutical potentials. These natural antioxidants could be obtained through diet, dietary supplements, and herbs in the form of Fruits, Spices, Vegetables, or processed foods or diets. It is established in works of literatures that plant-based antioxidants such as Flavonoids, Phenolic Acids, and Tannins exert numerous therapeutic effects such as Anti-Inflammatory, Anti-Carcinogenic and Antiantherosclerotic agents as a result of their antioxidant activity [23]. Plants possessing natural antioxidants are widely distributed in food and medicinal plants as polyphenols and carotenoids [24].

The findings from these studies showed that the LD50 of the plant is above 5000 mg/kg and this implies that the plant is safe for consumption at a dosage less than or equal to 5000 mg/kg at an acute administration as no death was recorded. This plant could be exploited as an active ingredient for the production of feeds used in animal husbandry.

Furthermore, the proximate and mineral analysis showed that the leaf of *Mallotus oppostifollous* is rich in Vitamins, Minerals, Fiber and Proteins, which implies that the leaf is a nutraceutical condiment and could be useful as a food source for the prevention of malnutrition. Vitamins and minerals are important in maintenance of the body equilibrium thereby reducing the risk associated with nutrient deficiency in humans. The study also showed high proteins and carbohydrates content in *M. oppostifollous* and this makes it a good source of supplement for functional nutrients. Micronutrients have numerous health benefits in public health due to their significance in maintaining the physiological well-being of humans and animals. They play a vital role in combating cases of malnutrition and are major components of biomolecules. Among the nutrients of great importance found in *M. oppostifollous* are: Calcium, Zinc, Iron, Magnesium, Phosphorous, Manganese, and Cadmium; they all play essential roles in stability of the human body's immune system. Iron is very essential for blood plasma ferritin, which fluctuates not only in response to iron supply but serves as a very important indicator in blood plasma deficiency leading to acute infections or chronic inflammatory processes. *M. oppostifollous* is rich in iron and this could serve as a supplement of iron in food. Iron is essential in the maintenance of body physiological pathways. Iron essentially affects almost all living organisms as it is involved in a wide variety of metabolic processes such as Oxygen transport, Deoxyribo Nucleic Acid (DNA) synthesis, and Electron

transport [24]. The result also showed that *M. oppostifollous* is essentially rich in Retinol, Riboflavin, Niacin, Pyridoxine, Biotin, Folate, Ascorbic acid and Tocopherol and this could be harnessed as an alternative source of vitamins for lactating Mothers, Adults, and Children. *M. oppostifollous* is rich in Vitamin A (0.8 mg) which is essential for the visual system, growth, and development, maintenance of epithelial cellular integrity, immune function, and reproduction. The retinol content of *M. oppostifollous* is slightly above the WHO recommended for adult which is 270-300 µRE/day estimated mean requirement and 500 to 600 µRE/day recommended safe intake for [25]. Also, the Riboflavin (Vitamin B2) content of *M. oppostifollous* leaf is 0.58 mg/100 g and this is higher than the WHO recommended daily requirement for children less than 6 years (0.5 mg/day) and this, therefore, implies that the plant is rich in vitamin B2 content and could be a possible source of supplements for children less than 5 years [25]. Vitamin B2 is essential for breakdown of proteins, fats, and carbohydrates, and also plays a vital role in maintaining the body's energy supply hence the reason it is called, "energy vitamin". It helps to convert carbohydrates into Adenosine Tri-Phosphate (ATP) [26]. Vitamin B2 is essential for the maintenance of the immune system during pregnancy [26]. The Riboflavin daily requirement for adults is 1.1 to 1.6 mg/day slightly double the *M. oppostifollous* riboflavin content (0.58 mg). The pyridoxine content is 8.1 mg and this is slightly above the daily requirement for lactating mothers which is 2.0 mg/day while adults are 1.4 mg/kg [27]. This study has proven that *M. oppostifollous* could be harnessed as a powerful nutraceutical condiment and therefore should be exploited by nutraceutical and livestock feed processing industries.

Conclusion

The result of the findings suggests that the *M. oppostifollous* leaf contains a high amount of phytochemicals and nutrients such as Proximate, Vitamins and Minerals. This makes *M. oppostifollous* leaf a good supplement for daily human nutrient requirements needed to combat diseases associated with nutrient deficiencies. We, therefore, conclude that this plant leaf could be harnessed for supplementation and fortification of diet both for human and animal consumption.

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