ASSESSMENT OF INORGANIC AND ORGANIC SOLVENTS EXTRACTION YIELDS, PHYTOCHEMICAL, NUTRITIONAL AND MINERAL CONSTITUENTS OF *Ocimum gratissimum* L. AND *Rhoicissus tridentate* L. f ANTIMALARIAL MEDICINAL PLANTS

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ABSTRACT

Malaria is a global health crisis. Drug resistance underscores the urgency of developing new antimalarial treatments. Currently, medicinal plants are being explored for potential solutions, offering hope in the fight against the deadly disease. This study examines the medicinal potential of *Ocimum gratissimum* and *Rhoicissus tridentate* through comprehensive analyses of their phytochemical, nutritional, and mineral profiles, aiming to assess their potential for malaria treatment. The *O. gratissimum and R. tridentate* aqueous and methanol solvents extracts yields were quantified and subjected to qualitative and quantitative phytochemical screening for the determination of their bioactive metabolites. Their nutritional and mineral element profiles and distribution in the plant parts were also assessed. Phytochemical screening identified a range of bioactive compounds, including alkaloids, flavonoids, Quinone's, phenols and carotenoids in both plants. Quantitative analysis revealed that *O. gratissimum* roots contained significantly ($P < 0.05$) highest alkaloid concentration (6.2 ± 0.1 mg/g) compared to its leaves (5.7 ± 0.0 mg/g), while *R. tridentate* leaves contained significantly ($P < 0.05$) higher alkaloid levels (3.9 ± 0.1 mg/g) than its roots (1.3 ± 0.0 mg/g). Carotenoid concentrations were notably higher in *R. tridentate* roots (6.8 ± 0.2 mg/g) compared to *O. gratissimum* roots (0.7 ± 0.1 mg/g). Methanol proved to be the most effective solvent, achieving phytochemical extraction yields of 28.3% from *O. gratissimum* leaves and 26.5% from *R. tridentate* roots, surpassing hexane and dichloromethane in efficacy. Nutritional analysis showed that *O. gratissimum* roots had the highest moisture content (45%), while *R. tridentate* leaves had the highest ash content (25%). Protein content was highest in *O. gratissimum* leaves (35%) and *R. tridentate* roots (30%), whereas carbohydrates were more abundant in *R. tridentate* roots (35%) than in its leaves (25%). Mineral analysis revealed that *O. gratissimum* roots were rich in iron, zinc, and manganese, while its leaves had higher potassium and magnesium. In contrast, *R. tridentate* leaves were higher in sodium, zinc, iron, copper, and manganese, with potassium and magnesium more concentrated in its roots. These results provide validity to the traditional medicinal usage of these plants by herbalists and traditional medicine men. These results equally highlight the significant therapeutic potential of both plants for malaria treatment. There is need for further research to isolate and characterize specific bioactive compounds for enhanced efficacy.

Keywords: Proximate Extract Yield, Phytochemical, Nutritional, Mineral Profiles, *Ocimum gratissimum* L., *Rhoicissus tridentate* L. F., Antiplasmodial

INTRODUCTION

Malaria continues to be a significant global health challenge, especially in tropical and subtropical regions, driven by increasing drug resistance and limited healthcare access (Kolawole *et al*., 2023). This suggests the need for new antimalarial agents, particularly from traditional medicine. Plants like *Ocimum gratissimum* (African basil) and *Rhoicissus tridentate* (three-leafed grape) are gaining attention for their potential therapeutic benefits. *O. gratissimum* has been widely used in traditional medicine for various ailments, including fever and digestive issues (Imosemi, 2020). Its phytochemical profile includes flavonoids, phenols, and essential oils known for their antimicrobial and anti-inflammatory properties (Zareiyan & Khajehsharifi, 2022). This studies have highlighted its significant activity against *Plasmodium falciparum*, the malaria-causing parasite, with compounds like eugenol linked to its antiplasmodial effects (Ain *et al*., 2024). In contrast, *R. tridentate* is traditionally used for treating infections and inflammation, containing phytochemicals such as flavonoids, saponins, and tannins, which may also contribute to its medicinal properties (Mdletshe, 2018; Uche-Okereafor, 2016). Preliminary studies suggest *R*. tridentate exhibits promising antimalarial activity, though it has not been as extensively researched as *O. gratissimum*. Investigating these plants is crucial not only for their potential direct antiplasmodial effects but also for their nutritional benefits, such as essential minerals like iron and zinc, which are vital for managing malaria-associated anemia (Abdulkareem *et al*., 2017). This study aims to comprehensively assess *O. gratissimum* and *R. tridentate* by analyzing their extract yields, phytochemical content, and nutritional profiles. The research seeks to validate their traditional uses and characterize their medicinal properties, exploring their potential as alternative or complementary malaria treatments. By integrating qualitative and quantitative analyses, this study provides valuable data for developing plant-based antimalarial therapies and emphasize their role in enhancing health through therapeutic and nutritional benefits.

METHODOLOGY

Study Area, Plants Collections and Identifications

In February 2021, plant materials (roots and leaves) of *O. gratissimum* and *R. tridentate* were collected from Tingwo Forest, Maoi, Elgeyo Marakwet County (latitude 0.24°N, longitude 35.65°E, 1990 m altitude). The specimens were preserved and deposited at the University of Eldoret Herbarium (voucher numbers UOE/OCGR/JCK/07/17 and UOE/RHTR/JCK/03/17) before being transported to KEMRI for bioassay analysis.

Processing Plant Materials for Solvent Extraction

The collected plant materials were washed, chopped into small pieces, air-dried for a period of three weeks in a well-aerated room at room temperature. The grinding of plant materials into a coarse-fine powder was done using electric grinder in the school of agriculture at the university farm facility. The respective powders were packaged in well-labelled 1kg bags each and transported to KEMRI where extractions and bioassays experiments were conducted.

Aqueous Extraction

Briefly, 100 grams of the respective powdered plant materials was transferred into clean labelled 1000 ml capacity beakers and then 600 ml of distilled water with polarity of 1.00 was added. The respective concoctions in beakers were covered with aluminum foil papers and placed in a water bath (80°C) for 1.5 hours to facilitate extraction. The mixtures were then filtered through Whatman's number 1 filter papers before subjecting them to freeze drying for 48 hrs. The dry and lyophilized samples were transferred into clean, dry, pre-weighed universal bottles, and their respective percentage yields were determined and stored at -20°C in a freezer until use.

Organic Solvent Extraction

Sequential extraction using Hexane, Ethyl acetate, Dichloromethane and Methanol with polarity of 0.009, 0.228, 0.309, and 0.762 respectably was adopted. Briefly, 250 grams of the respective plant materials were soaked in 600 ml in 1000 ml capacity beakers at room temperature (25°C) for 48 hours. The mixtures were then filtered through double-layer Whatman's number one filter papers after which the filtrates were reduced *in vacuo* at solvent temperature set using a rotary evaporator. The respective extracts were soaked daily for three days in 600 ml volumes of Methanol in 1000 ml capacity beakers in the same way as for dichloromethane and other solvent extraction standards. The extracts were obtained by filtration and concentrated *in vacuo* using a rotary evaporator set at respective temperatures. The resultant extracts were transferred into clean pre-weighed glass bottles and their yields were determined and stored at -20°C in a freezer until use. Percentage yield of extract was calculated by dividing the weight of a dry extract by the weight of the dry plant biomass and multiplying by 100.

Phytochemical Screening Methods

The *O. gratissimum and R. tridentate* extracts were subjected to qualitative and quantitative phytochemical screening for the identification of metabolites, including Flavonoids, terpenoids, Alkaloids, tannins, anthraquinones, cardiac glycosides, Saponins, Phytosterols, Saponins, coumarins, Quinone s, Phenols, and carotenoids, according to standard methods with some modifications. Shinoda's test for the detections of Flavonoid, Salkowski's test for Terpenoids, Dragendorff's test for Alkaloids, the Ferric Chloride test for Tannins, the Froth test for Saponins, Keller Killiani's test for Cardiac Glycosides, Modified Borntrager's test for Anthraquinone and the NaOH paper test for coumarins.

Preparation of Stock Solutions of Plant Extracts

Stock solutions of the studied total extracts (200μg/ml) were prepared in sterile deionized water and filtered through 0.22μm membrane filters under aseptic conditions in a laminar flow hood. The water insoluble extracts were first dissolved in 0.02% dimethyl sulphoxide (DMSO) before diluting them to the required concentrations using sterile deionized water. All the prepared stock solutions of plant extracts and standard drugs were stored at -20°C and retrieved only during use.

Nutritional Analysis in Plants

Ash contents, moisture contents, crude protein by Macrokjeldahl method, and carbohydrates were determined by standard methods following AOAC (AOAC International, 2000).

Determination of Mineral Elements

Plants parts of *O. gratissimum* and *R. tridentate* were sampled according to the general procedure used for plant diagnosis. Collected plant parts were washed and dried for one week at 23ºC and ground to coarse fine powder. Nutrient elements such as Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu) were determined by AAS (Atomic Absorption Spectrophotometer) Potassium (K) by AES (Atomic Emission Spectrophotometer) and Phosphorus (P) was analyzed by calorimetry. The methods used to digest the samples were by an improved wet digestion method (7-8 min) based on the addition of hydrogen peroxide (H_2O_2) to the sample previously introduced in concentrated Nitric acid ($HN0_3$) for 24hours.

Statistical Analyses

Statistical analysis was conducted using Gen-Stat Software Version 14.1. Descriptive statistics were expressed as mean ± SEM. One-way ANOVA assessed treatment outcomes and mean separations. Student’s t-test compared longevity between treated and untreated mice, and differences in activity, IC50, MIC, and LC/LD50 values. Significance was set at $P \leq 0.05$.

Ethical Clearance

Ethical clearance was granted by the University of Eastern Africa Baraton (ISERC/01/05/2022) and NACOSTI (NACOSTI/23/P/23/23966). The study adhered to SERU, KEMRI guidelines, the Ethical Review Committee, and the Animal Care and Use Committee (ACUC).

RESULTS

Determination of Phytochemical

The methanol (MeOH) extracts of *O. gratissimum* and *R. tridentate* exhibit distinct compositions of phytochemicals across different solvents, highlighting their potential pharmacological and nutritional benefits.
O. gratissimum Methanol extract contains Alkaloids, Flavonoids, Phytosterols, Saponins (in water), and Coumarins as in table 1. In contrast, *R. tridentate* MeOH extract shows Alkaloids, Flavonoids, Phytosterols (in Dichloromethene and Methanol), and additional coumarins and Quinones (in Ethyl acetate and Methanol). Tannins are found in *O. gratissimum* and *R. tridentate* Methanol extracts, Phenols and carotenoids, present in all

solvents for both plants. The choice of solvent influences the phytochemical profile, with *O. gratissimum* showing a broader range of compounds, particularly Saponins, while *R. tridentate* exhibits specific compounds like Quinone s. These findings underscore the potential of both plants for pharmaceutical and nutritional applications, tailored to exploit their diverse bioactive properties based on solvent-specific extraction methods.

Table 1: Qualitative Phytochemical Screening of *O. gratissimum* and *R. tridentate* Extracts

	O. gratissium Solvent Extracts					*R. tridentate* Solvent Extracts				
Phytochemicals	Hex	DCM	EA	MeoH	H_2O	Hex	DCM	EA	MeoH	H_2O
Alkaloids	+	+	-	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+
Phytosterols	-	+	-	+	-	-	+	-	+	-
Saponins	-	-	-	-	+	-	-	-	-	+
Coumarines	-	-	-	+	+	-		+	+	+
Quinones	-	+	-	+	+	-	-	-	+	+
Tannins	-	-	-	+	-	-	-	-	+	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Phynols	+	+	+	+	+	+	+	+	+	+
Caratinoids	-	-	-	-	-	+	+	+	+	+

Key: + = present; - absent; Hex= Hexane; DCM= Dichloromethane; EA= Ethyl acetate; MeoH= Methanol; H_2O= Water; Mean ± Standard Error; n =3

Quantitative Phytochemical Screening of *O. gratissimum* and *R. tridentate.*

The quantitative screening of Methanol extracts from *O. gratissimum* and *R. tridentate* in Table 2 reveals varying concentrations of phytochemicals across various parts of the plants. In *O. gratissimum*, alkaloid content is highest in roots (6.2 ± 0.1), followed by leaves (5.7 ± 0.0), while *R. tridentate* shows highest alkaloid levels in leaves (3.9 ± 0.1), with significantly lower amounts in roots (1.3 ± 0.0). Carotenoid levels are notably higher in *R. tridentate* roots (6.8 ± 0.2) compared to *O. gratissimum* roots (0.7 ± 0.1), showing a stark contrast in carotenoid accumulation. Flavonoids show consistent levels across *O. gratissimum* leaves (1.3 ± 0.2) and roots (1.8 ± 0.2), whereas *R. tridentate* displays slightly lower levels in leaves (1.3 ± 0.0) and the lowest in roots (0.8 ± 0.1). Glycosides are more prominent in *O. gratissimum* compared to *R. tridentate*, while Phenols and Saponins show similar trends across both species but vary in concentration among plant parts, showing differential distribution and accumulation. These results highlight the importance of plant part selection for targeted phytochemical extraction and suggest potential differences in bioactive compound content that may influence the medicinal and nutritional value of *O. gratissimum* and *R. tridentate* in pharmaceutical and dietary applications.

Table 2: Quantitative Screening of Alkaloids, Carotenoids, Flavonoids, Glycosides, Phenols and Saponins in *O. gratissimum* and *R. tridentate* Methanol extracts

Phytochemicals	***O. gratissimum***	**Extracts**	***R. tridentate***	**Extracts**
	Leaves	**Roots**	**Leaves**	**Roots**
Alkaloids	5.7 ± 0.0^{c}	6.2 ± 0.1^{c}	3.9 ± 0.1^{c}	1.3 ± 0.0^{b}
Carotenoid	0.1 ± 0.0^{a}	0.7 ± 0.1^{a}	1.8 ± 0.0^{b}	6.8 ± 0.2^{c}
Flavonoids	1.3 ± 0.2^{b}	1.8 ± 0.2^{b}	1.3 ± 0.0^{b}	0.8 ± 0.1^{a}

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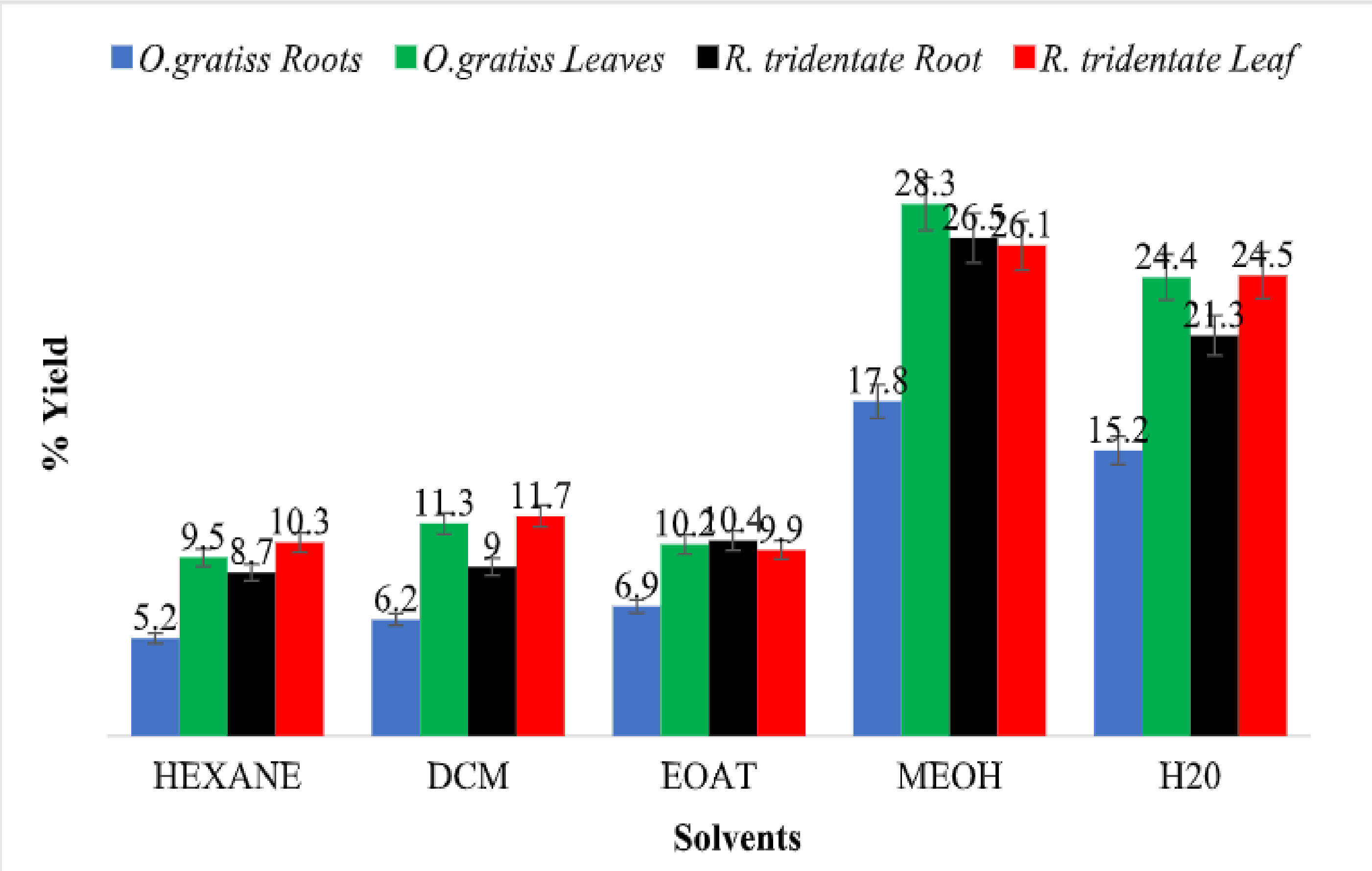
Glycosides	0.3 ± 0.1[a]	0.8 + 0.1[a]	0.2 ± 0.0[a]	0.7 ± 0.0[a]
Phenols	0.6 ± 0.2[a]	0.6 ± 0.1[a]	0.6 ± 0.0[a]	0.4 ± 0.0[a]
Saponins	0.5 ± 0.2[a]	0.7 ± 0.1[a]	0.4 ± 0.0[a]	0.2 ± 0.0[a]

*Same Superscript Letters Indicate there is No Significant Difference in Mean Results across the Rows and Columns; Mean ± Standard Error; n =3

Percentage Yields of *O. gratissimum* and *R. tridentate* Extracts

Figure 1 shows the percentage (%) yield from *O. gratissimum* and *R. tridentate* solvent extraction of various plant parts using five different solvents: hexane, Dichloromethane, Ethyl acetate, Methanol and water. In *O. gratissimum*, the highest yield was seen in the leaves extract with Methanol (28.3%), followed by the roots Methanol extract (14.3%). The roots extract had the lowest yield, with hexane and Dichloromethane yielding 5.2% and 6.2%, respectively. In *R. tridentate*, the highest yield was seen in the root extract with Methanol (26.5%), followed by the leave's extracts with Methanol (24.5%).



DCM= Dichloromethane; EOAT= Ethyl acetate; MEOH= Methanol; H_2O= Water; Mean ± SEM (n=3)

Figure 1: Percentage (%) yield extracted from 100g *O. gratissimum* and *R. tridentate* Solvent Extraction**.**

Determination of Nutrients Composition in *O. gratissimum* and *R. tridentate* Extracts.

Figure 2 illustrates the nutrient composition of *O. gratissimum* and *R. tridentate* extracts in terms of moisture, ash, protein, and carbohydrate percentages. The roots of both plants generally exhibit higher moisture content compared to their respective leaves. Specifically, *O. gratissimum* roots stand out with the highest moisture content at around 45%, while *R. tridentate roots* and leaves show similar moisture levels ranging from 35% to 40%. In terms of ash content, the leaves of both species have higher percentages compared to their roots. Notably, *R. tridentate* leaves have the highest ash content at approximately 25%, while both plants' roots have ash levels averaging between 15% and 20%. Protein content varies significantly between plant parts: *O. gratissimum* leaves record the highest protein content at about 35%, contrasting with around 25% in its roots, while *R. tridentate* roots exhibit higher protein levels around 30% compared to 20% in its leaves. Carbohydrate percentages show an inverse pattern, favoring the roots of both plants. Specifically, *R. tridentate* roots prove the highest carbohydrate content at approximately 35%, while its leaves contain around 25%. In contrast, *O. gratissimum* roots and leaves show similar carbohydrate levels, each around 30%.

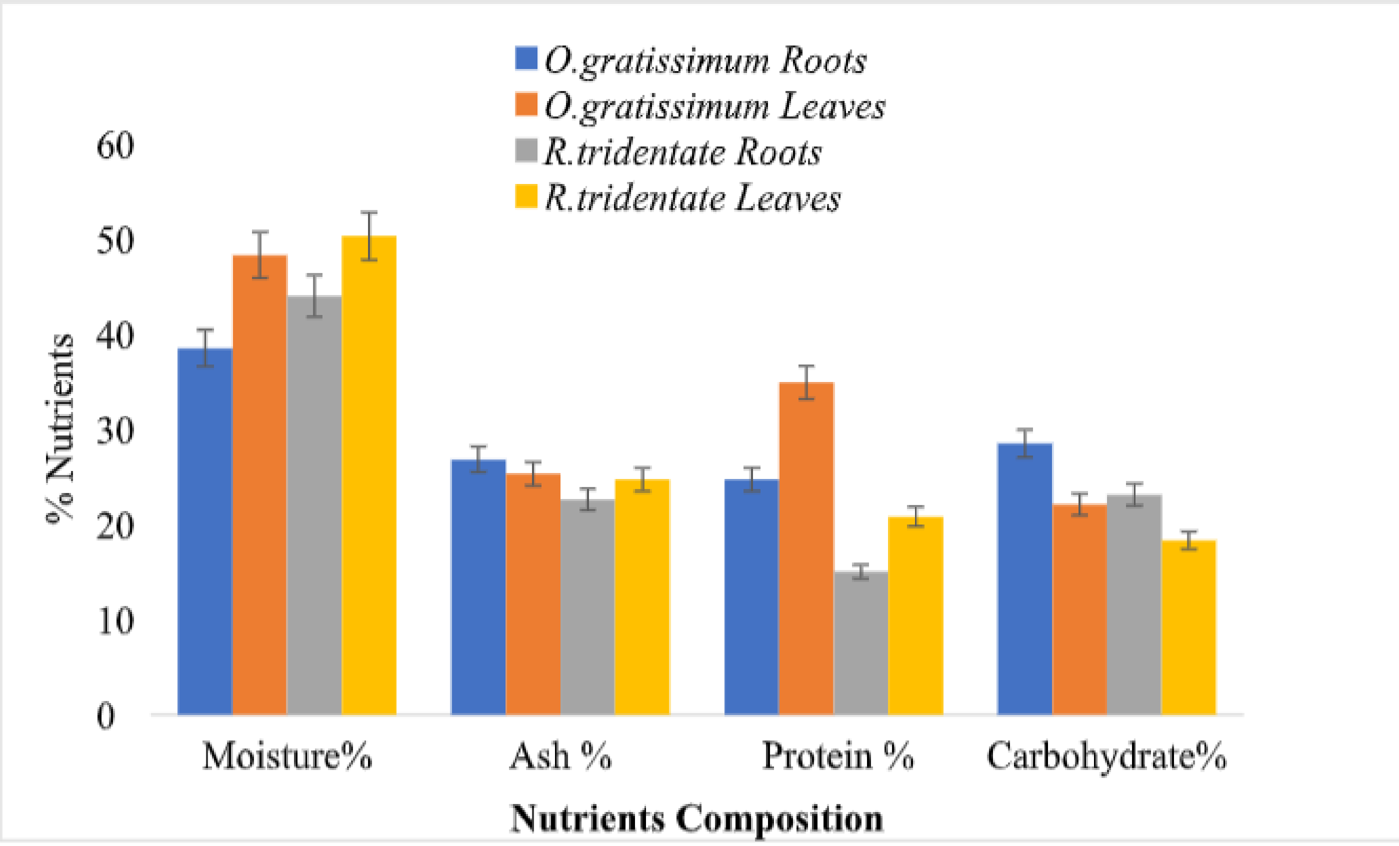
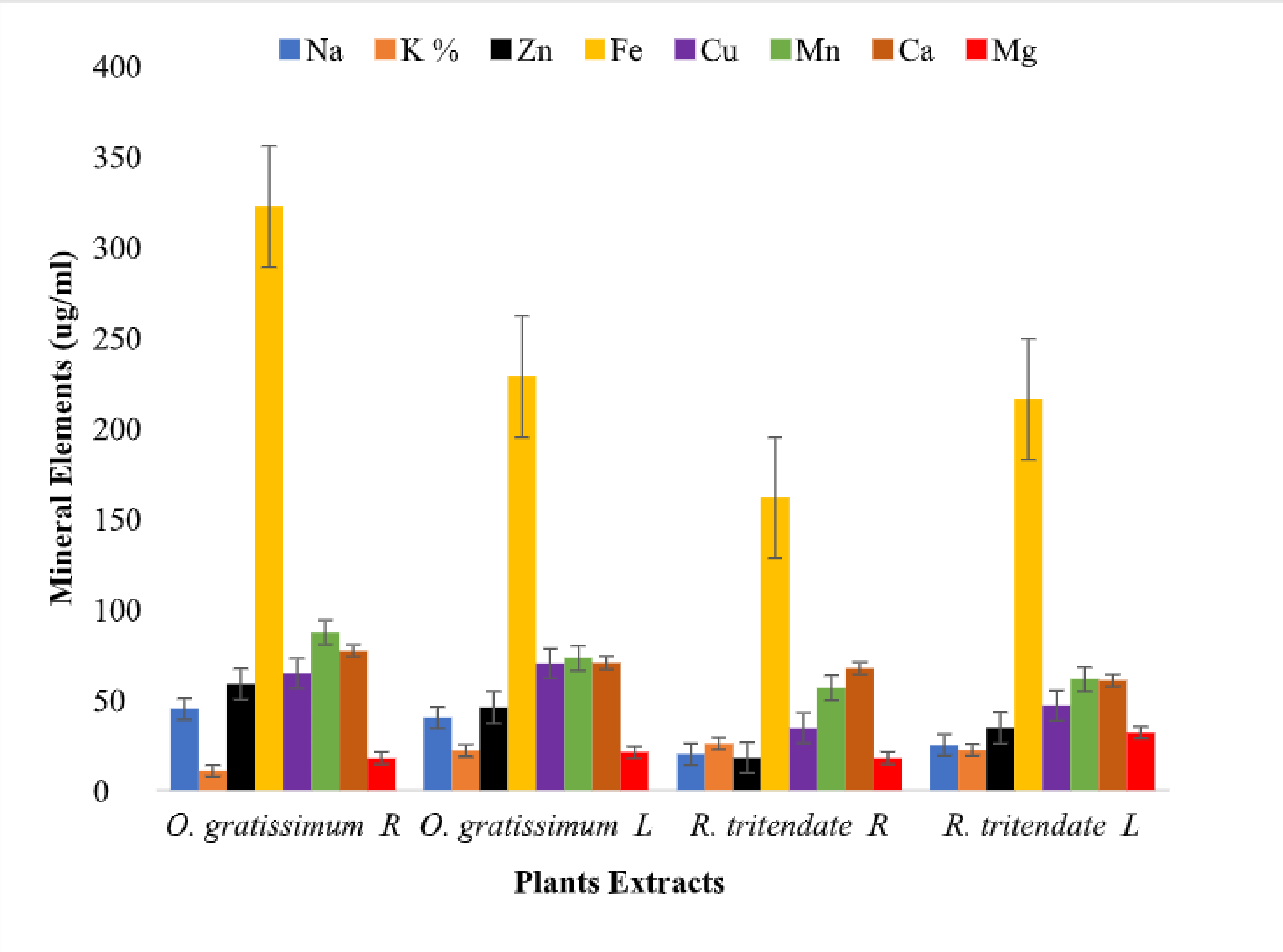


Figure 2: Determination of Nutrients composition in *O. gratissimum* and *R. tridentate* Extracts.

Determination of Mineral Elements in Digested 100g of *O. gratissimum* and *R. tridentate* Extracts.

Figure 3 provides a detailed comparison of mineral element concentrations between the roots and leaves of *O. gratissimum* and *R. tridentate*. The results reveal distinct patterns in the accumulation of various minerals, showing potential differences in their nutritional and pharmacological characteristics. In *O. gratissimum*, sodium levels are higher in roots (45µg/ml) than in leaves (40 µg/ml), whereas *R. tridentate* exhibits higher sodium content in leaves (25 µg/ml) compared to roots (20 µg/ml). Potassium concentrations are significantly elevated in *O. gratissimum* leaves (22.03%) compared to roots (10.87%), whereas *R. tridentate* shows higher potassium in roots (25.87%) than in leaves (22.32%). Zinc content is higher in *O. gratissimum* roots (58.71 µg/ml) than in leaves (45.78 µg/ml), while *R. tridentate* leaves (34.56 µg/ml) have higher zinc levels compared to roots (18.03 µg/ml). Iron concentrations are notably higher in *O. gratissimum* roots (322.4 µg/ml) than in leaves (228.5 µg/ml), whereas *R. tridentate* leaves (215.9 µg/ml) have higher iron content than roots (161.71 µg/ml). Copper concentrations are slightly higher in *O. gratissimum* leaves (69.9 µg/ml) than in roots (64.67 µg/ml), whereas *R. tridentate* leaves (46.76 µg/ml) contain more copper than roots (34.3 µg/ml). Manganese levels are generally higher in *O. gratissimum* roots (87.08 µg/ml) than in leaves (72.98 µg/ml), while *R. tridentate* shows comparable manganese levels between roots (56.55 µg/ml) and leaves (61.34 µg/ml). Calcium concentrations vary with no consistent trend between *O. gratissimum* roots (77.05 µg/ml) and leaves (70.314 µg/ml), as well as *R. tridentate* roots (67.4 µg/ml) and leaves (60.5 µg/ml). Magnesium concentrations are higher in *O. gratissimum* leaves (20.9 µg/ml) than in roots (17.8 µg/ml), whereas *R. tridentate* leaves (31.8 µg/ml) have higher magnesium levels than roots (17.9 µg/ml).



Legend: Na = Sodium; K= Potassium; Zn= Zinc; Fe= Iron; Cu= Copper; Mn= Manganese; Ca= Calcium; Mg= Magnesium; Mean ± Standard error; n=3

Figure 3: Determination of Mineral Elements in *O. gratissimum* and *R. tridentate* Extracts.

DISCUSSION

The analysis of extraction yields, phytochemical content, and nutrient composition of *O. gratissimum* and *R. tridentate* underscores their significant medicinal and nutritional potentials. Methanol extraction proved to be the most effective for both plants, yielding higher concentrations of bioactive compounds. *O. gratissimum* showed extraction yields of 28.3% from leaves and 14.3% from roots, outperforming hexane and dichloromethane, while *R. tridentate* yielded 26.5% from roots and 24.5% from leaves. This highlights methanol's efficacy in extracting a diverse range of phytochemicals, which is essential as higher yields correlate with elevated concentrations of beneficial compounds, particularly flavonoids and alkaloids significant for their therapeutic properties (Ashley & Poespoprodjo, 2020). Qualitative and quantitative phytochemical analyses revealed a range of bioactive compounds in both species. *O. gratissimum* exhibited higher concentrations of alkaloids (roots: 6.2 ± 0.1; leaves: 5.7 ± 0.0), indicating stronger therapeutic potential in its roots. In contrast, *R. tridentate* had greater alkaloid levels in its leaves (3.9 ± 0.1) compared to its roots (1.3 ± 0.0), suggesting varied medicinal uses among different plant parts. Flavonoids were notably abundant in *O. gratissimum*, with levels in leaves (1.3 ± 0.2) and roots (1.8 ± 0.2) exceeding those of *R. tridentate* (leaves: 1.3 ± 0.0; roots: 0.8 ± 0.1), emphasizing its potential for antioxidant benefits crucial for managing oxidative stress and inflammation associated with malaria (Wessels *et al*., 2017). Conversely, *R. tridentate* roots contained higher carotenoid levels (6.8 ± 0.2), suggesting superior antioxidant properties, highlighting its use in nutritional supplements aimed at oxidative stress defense. Nutrient and mineral composition analyses revealed distinct differences between the plant parts. *O. gratissimum* roots had a high moisture content (45%), beneficial for herbal extract stability, while its leaves had a higher protein content (35%) compared to roots (25%). In contrast, *R. tridentate* roots exhibited more protein (30%) than leaves (20%), reflecting their different metabolic roles. Carbohydrate levels were highest in *R. tridentate* roots (35%), supporting energy provision. The mineral profile indicated that *O. gratissimum* roots are rich in iron, zinc, and manganese, critical for addressing anemia in malaria patients (Ashley & Poespoprodjo, 2020). The diverse mineral profiles of both plants suggest their potential to support overall health and aid malaria recovery, highlighting the importance of balanced intake to avoid toxicity (Kotepui *et al*., 2023). This study positions *O. gratissimum* and *R. tridentate* as valuable sources of bioactive compounds and essential nutrients, reinforcing their traditional applications in herbal medicine and potential roles in addressing health issues like malaria.

CONCLUSIONS

The study on *O. gratissimum* and *R. tridentata* reveals their substantial therapeutic potential. Methanol extraction yielded significant quantities of bioactive compounds, demonstrating its effectiveness for isolating beneficial phytochemicals. Both plants contain a rich array of bioactive compounds, such as alkaloids, flavonoids, phytosterols, quinones, and phenols, which are recognized for their anti-inflammatory, antioxidant, and antimicrobial properties, thereby contributing to their observed antimalarial activity. Nutritionally, *O. gratissimum* leaves are notably high in protein (35%), while *R. tridentata* roots also exhibit significant protein levels (30%), with *R. tridentata* roots having higher carbohydrate content (35%) compared to its leaves (25%). Mineral analysis showed that *O. gratissimum* roots are rich in iron, zinc, and manganese, whereas its leaves have elevated potassium and magnesium levels; *R. tridentata* leaves are abundant in sodium, zinc, iron, copper, and manganese, with its roots displaying higher potassium and magnesium content. This rich mineral profile underscores the plants' potential to support various physiological processes, enhance overall health, and aid in malaria recovery. *O. gratissimum* and *R. tridentate* present considerable antimalarial and nutritional benefits, emphasizing their role as promising alternative or complementary treatments for malaria. The study recommends continued use of *O. gratissimum* and *R. tridentate* extracts for malaria treatment, emphasizing the need for purification analysis of responsible biomolecules in bioactive compounds that are responsible for antimalarial and mineral content. Expanding research to include various *Plasmodium* species and careful consideration of dosage, solvent selection, and safety will enhance therapeutic efficacy and health benefits.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research paper. We affirm that this study was conducted independently and without any external influences.

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