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Phytochemical Screening of Crude Extracts of *Bridelia micrantha*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CM, PGK, CK and ESM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EGM, P. K. Kairigo, P. K. Kimani and DMM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In Kenya, 22 million people are at risk of malaria, 70% of them are in rural areas, and most of these people use traditional plant-based medicines to treat malaria. The use of natural product-derived drugs and drugs from other sources in combating malaria has however been faced with several challenges, including the emergence of drug resistance parasites, thereby making many of the first line drugs such as chloroquine (CQ) not efficient. The aim of this study was to determine the phytochemical properties of *Bridelia micrantha* used to treat malaria among the Digo community in Kenya. The crude extracts were obtained using hexane, ethyl acetate, and methanol. A Shimadzu 8400 FT-IR was used to determine the functional groups present in the crude extracts. From the results obtained, various functional groups characteristic of phenolics, tannins, saponins, alkaloids,

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flavonoids, and glycosides were found to be present. A quantitative assay was performed using Shimadzu 6200 UV-VIS spectrophotometer. The total phenolics and flavonoids were found to be highest in barks with a concentration of 0.84±0.14 mg/g and of 0.86±0.03 mg/g respectively while Saponin content was found to be highest in leaves with a concentration of 12.66±0.23 mg/g. Alkaloid content, on the other hand, was found to be highest in roots with a concentration of 313.44±0.05 mg/g.

Keywords: Resistance; malaria; phytochemical; extracts; isolation.

1. INTRODUCTION

Although there is widespread use of traditional herbal remedies in the management of malaria [1,2], scientific understanding of the plants is, however, largely unexplored [3]. There is a need to collect ethnobotanical information on antimalarial plants which is essential for further evaluation of the efficacy and safety of the plants as antimalarial remedies.

Many tribes in Africa have much-elaborated plant knowledge [3]. Most knowledge on medicinal plants is transferred orally in many communities [4], and there is, therefore, the danger of losing this precious cultural heritage. Given the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, an increasing number of ethnobotanical inventories need to be established [5].

In this study, phytochemical screening of *Bridelia micrantha*, an indigenous plant used as traditional remedy among the Digo community, was undertaken with the aim of documenting its composition. *Bridelia micrantha* is a semi-deciduous to deciduous tree up to 20 m tall with a dense, rounded crown and tall, bare stem. The bark on young branches are grey-brown and smooth, on older branches and stems dark brown and rough, cracking into squares. The branches are often thorny, slash thin, fibrous, and brown to dark red [6].

Phytochemicals play a significant role in plant defense against prey, microorganism, stress as well as interspecies protections; these plant components have been used as drugs for millennia. Thus, phytochemical screening serves as the initial step in predicting the types of potentially active compounds from plants [7]. Phytochemical screening assay is a simple, quick and less costly procedure that gives immediate information of the various types of phytochemicals in a plant extract and a valuable tool in bioactive compound analyses [8]. Knowledge of the chemical constituents of a plant(s) is helpful in the discovery of therapeutic agent(s). The most important of these bioactive plant constituents include saponins, alkaloids, tannins, flavonoids and phenolic compounds [9].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The root, leaves and barks samples from *Bridelia micrantha* were collected from Kwale County, Kenya. The samples were identified with the help of a taxonomist and voucher specimens taken to botany department, J.K.U.A.T. The samples were then dried and phytochemicals extracted using n-hexane, ethyl acetate and methanol solvents sequentially increasing their polarity.

2.2 Fourier-transform Infrared Spectroscopy

The functional groups present were determined using Shimadzu 8400 FT-IR spectrophotometer. The dried extracts were analyzed using the KBr pellet technique.

2.3 Phytochemical Screening

Qualitative phytochemical analysis of the crude extracts was carried out according to a method described elsewhere [10]. These tests are usually based on visual observation of color or precipitate formation after addition of specific reagents [10]. One gram of the crude was re-dissolved in 10mL of distilled water and subjected to qualitative phytochemical tests for saponins, alkaloids, phenolic compounds, tannins, and flavonoids.

2.4 Quantification of Total Phenol

The total phenolic content was determined using folin-ciocalteau reagent and tannic acid method [11].

2.5 Quantification of Total Flavonoid

The total flavonoid was measured by the aluminum chloride colorimetric assay [12]. The solution was mixed well and the absorbance measured at 510 nm against a blank. The flavonoid content was determined using quercetin standard.

2.6 Quantification of Saponins

Saponins were quantified by extracting 1.0 g of the sample in a soxhlet using methanol. The methanolic extracts were evaporated under reduced pressure to afford crude methanolic extracts which were partitioned between hexane and water in a separating funnel. The aqueous layer were then extracted with diethyl layer. The aqueous layer were then further partitioned with n-butanol and then washed with 15 ml of 5% sodium chloride and then evaporated to yield crude saponins [13].

2.7 Quantification of Alkaloids

The quantity of alkaloids in the plant extract were determined gravimetrically [14,15].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

The phytochemicals tested include saponins, alkaloids, tannins, glycosides, phytosterols and flavonoids and the results are presented in Table 1.

Table 1. Qualitative phytochemical analysis results for *Bridelia micrantha* grinded powder samples

Phytochemical	Leaves	Barks	Roots
Saponins	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Phytosterols	+	+	+
Glycosides	+	+	+
Flavonoids	-	+	-

Key: '+' Present '-' Not present

Important phytochemicals such as saponins, steroids, flavonoids, phenolic compounds, and tannins were found to be present in all the *Bridelia micrantha* samples (leaves, barks and roots). Alkaloids saponins, tannins, and glycosides were found to be present in all the three samples (leaves, barks, and roots) but their presence varied according to the solvents used. Flavonoids were present in methanol and ethyl acetate bark extracts only while phytosterols were not present in leaf extracts. Phytochemicals play a significant role in plant defense against prey, microorganism, stress as well as interspecies protections; these plant components have been used as drugs for millennia. Thus, phytochemical screening serves as the initial step in predicting the types of potentially active compounds from plants [16].

3.2 FT-IR Results for Crude Extracts

Fig. 2 below represents FT-IR spectra for hexane, ethyl acetate and methanol leaf extracts.

The spectra obtained showed presence of various functional groups that are characteristic to phytochemicals. O-H broad peaks at around 3300 shows the presence of phenolics, strong C=O absorption band at 1720 and C-H stretch at around 2850 shows presence of saponins. The C=C aromatic bonds at 1600 and 1400 shows the presence of flavonoids, C-O-C glycosidic linkage between 1100-1000 shows presence of glycosides and a strong absorption peak at around 2900 also shows presence of saponins.

The OH, CH, C=C, C=O and C-O-C infrared absorptions observed in the various fractions of the herbal drug are suggestive of oleanane triterpenoid saponins [13,16] and are characterized by the C=O infrared absorbance due to oleanolic acid/ester. Such triterpenoid saponins are also likely to be bidesmosides since they have two attachments of glycones (i.e. glycosidic and ester groups) to the sapogenin due to the presence of a C=O functional group [13,16].

3.3 Quantitative Analysis Results

3.3.1 Estimation of total phenolic content

The phenolic content in the samples was expressed in µg/ml and were represented in a bar graph as shown in Fig. 2 below.

Phenolics were found to be highest in barks with a concentration of 0.84±0.14 mg/g followed by leaf extracts with 0.52±0.02 mg/g, and the root extracts had the lowest concentration of phenolics with 0.29±0.04 mg/g. Statistical analysis of the mean concentration revealed that total phenolic content in leaves, bark and root sample are not significantly different, $F(2, 6) = 4.409$ $P = 0.06$.

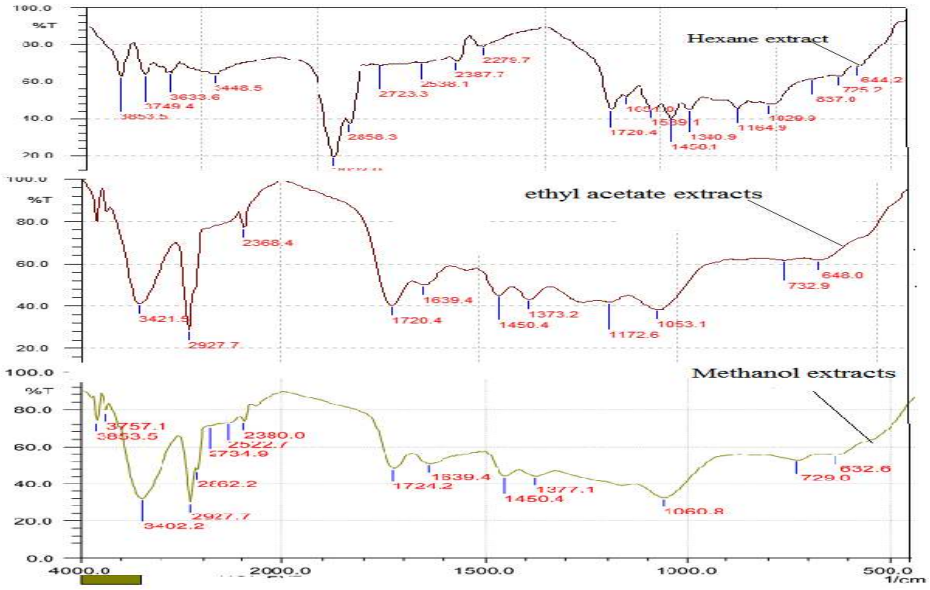

Fig. 1. FT-IR spectra of hexane ethyl acetate and methanol leaves extracts of *Bridelia micrantha*

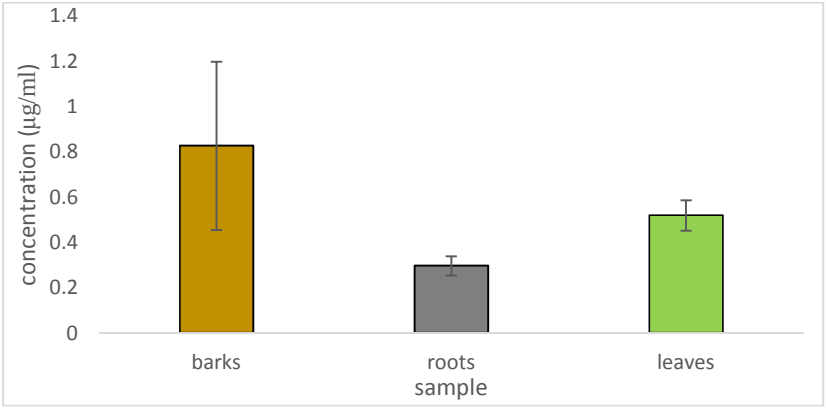

Fig. 2. Bar graphs showing the total phenolic content in *B. micrantha* samples

At 95% confidence level, margin of error is 5% = 0.05. The p-value obtained was 0.066372 which is greater than 0.05, therefore the null hypothesis was accepted. That the phenolic content in *Bridelia micrantha* root, bark and leaf samples is not significantly different at 95% confidence level.

3.3.2 Total flavonoid assay

The flavonoid content in the samples was expressed in µg/ml [17] and are represented in a bar graph as shown in Fig. 3.

Flavonoids were found to be highest in barks with a concentration of 0.85±0.03 mg/g followed by leaf extracts with 0.76±0.01 mg/g, and the root extracts had the lowest concentration of flavonoids with 0.47±0.06 mg/g. Flavonoids were found to be absent in leaves and roots from the phytochemical screening results. However, they were found present in the standard quantitative results. At 95% confidence level, margin of error is 5%=0.05. The p-value is 5.48E-14 which is less than 0.05, therefore the null hypothesis was rejected. That the total flavonoid content in *Bridelia micrantha* root, bark and leaf samples are significantly different at 95% confidence level.

3.3.3 Quantification of saponins

The saponin content was calculated and expressed in mg/g [17].

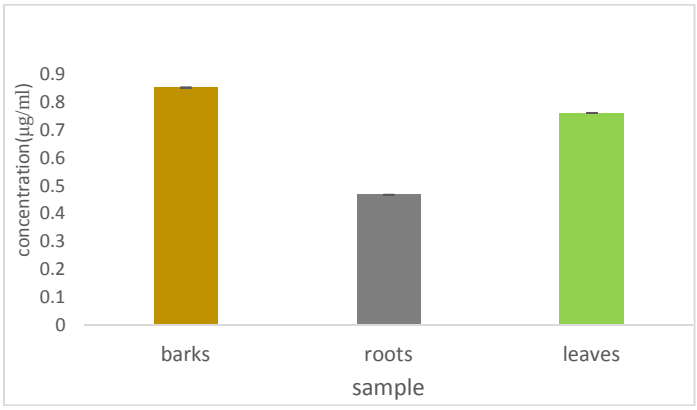

Fig. 3. Bar graphs are showing total flavonoid content in *B. micrantha* samples

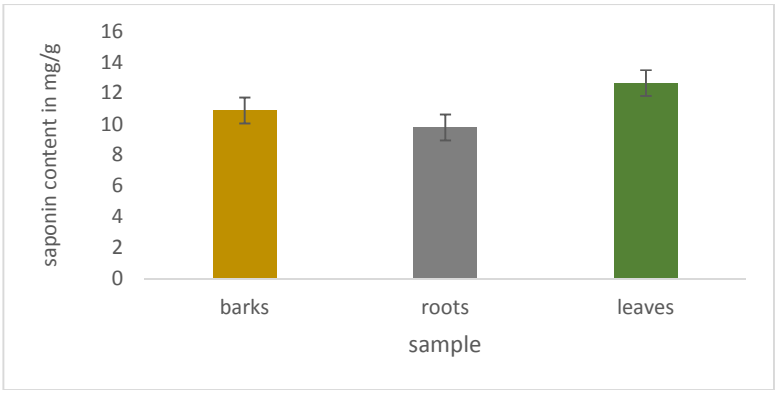

Fig. 4. Bar graphs showing total saponin content in *B. micrantha* samples

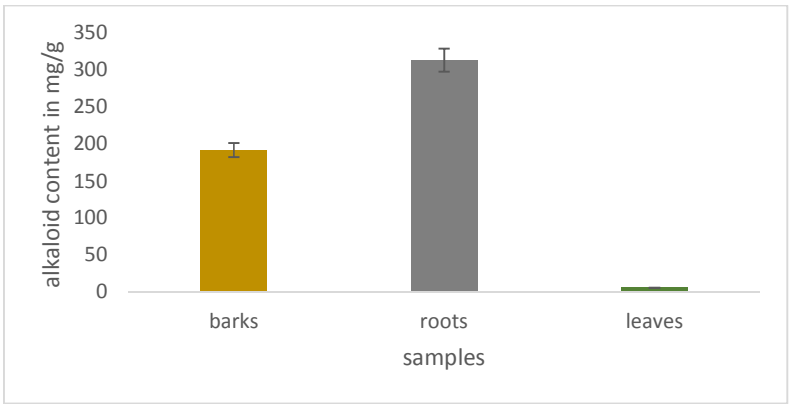

Fig. 5. Bar graphs showing total alkaloid content in *B. micrantha* samples

Saponin content was found to be highest in leaves with a concentration of 12.66±0.05 mg/g followed by barks with a concentration of 10.89±0.08 mg/g, and the roots had the lowest saponin content with a concentration of 9.78±0.02 mg/g.

3.3.4 Quantification of alkaloids

The alkaloid content was calculated and expressed in mg/g [17].

The alkaloid content was found to be highest in roots with a concentration of 313.44±0.09 mg/g

followed by barks with a concentration of 192.02±0.06 mg/g and the leaves had the lowest alkaloid content with a concentration of 5.72±0.04 mg/g.

4. CONCLUSIONS

Phytochemicals in all the extracts were found to vary in concentration with bark extracts having the highest quantity of phytochemicals. A variety of phytochemicals such as alkaloids, saponins, flavonoids, phenolic compounds, and tannins were found to be present in the extracts, and could be responsible for the anti-malarial activity exhibited by the plant, *Bridelia micrantha.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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