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Preliminary Phytochemical Screening of Five Plants as Possible Antileishmaniasis Control Agent

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Leishmaniasis is a major public health problem globally and manifests in three clinical forms including visceral cutaneous and mucocutaneous. Visceral leishmaniasis is fatal if left untreated for a period of 2 years, while cutaneous leishmaniasis cause crusted papules or ulcers on exposed skin. Plant families containing active compounds against other protozoan diseases may be suitable against leishmania parasites. This study report the compounds extracted from five plants (*Olea europaea, Kigelia Africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha* extracts). The plants were collected from Baringo County in Kenya and authenticated at the National Museums of Kenya (Department of Botany). The plant samples were dried, pulverized into fine powders and extracted using methanol at the Center for Traditional Medicine and Drugs Research, KEMRI. The plant extracts contained varying amounts of phytochemical compounds such as tannins, phenols, flavonoids, steroids, alkaloids, saponins, anthraquinone, cardiac glycoside, polyphenols, cumarins, anthocyanins, trepenoids, glycosides and triterpenoids. The presence of tannins, flavanoids, alkaloids and saponins with known biological activities offer opportunity to test these compounds against leishmania parasites.

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Keywords: Leishmaniasis; leishmania parasites; phytochemical screening; active compounds; plant extracts.

1. INTRODUCTION

Leishmaniasis is a major public health problem, which cause significant morbidity and mortality in tropics and subtropical regions mainly in Africa, Asia and Latin America [1,2]. The disease affect 340 million people in 88 countries around the World, with approximately 2 millions being infected annually [3]. The disease is caused by more than 20 leishmania species transmitted to humans by 30 different species of phlebotomine sandflies [4,5]. The Eurocentric world view groups the *Leishmania* parasites into Old World species: *L. major*, *L. aethiopica*, *L. infantum*, and *L. tropica* (prevalent in the Mediterranean basin, the Middle East, the horn of Africa, and the India), and New World species, such as *L. amazonensis*, *L. infantum, L. mexicana*, *L. naiffi*, *L. braziliensis*,and *L guyanensis* (endemic in Middle and South America). Among these, cutaneous leishmaniasis is rarely fatal apart from disfiguring scars but visceral leishmaniasis if left untreated for a period of two years causes fatality [6]. Death can result directly from the disease through organ failure or wasting syndromes or as a result of a secondary bacterial infection such as pneumonia [7].

Despite the existence of antiparasitic agents, conventional antileishmanial drugs are expensive and out of reach for most rural communities [8]. Moreover, there is increasing evidence of parasite resistance to conventional drug therapies [9]. The treatment cost is also high and takes minimum of 17 days inpatient treatment [10] thus, necessitate for alternatives therapeutics. Traditional medicine form a cornerstone for the treatment of various diseases of parasite and non-parasite origin in many rural settings [11,12]. Recently, as a consequence of the constraints to chemical use and the encouraging results obtained from plant extracts, interest in plants with antiparasite properties have increased [13-15]. Numerous plants have been screened for antiparasite activities using a standard WHO procedure [16-19]. However, there is still lack of vital information on the chemical constituents possessing anti-leishmania activities. Furthermore, plant extracts are known to be affected by among other things such as location, amount of active compounds in the plants, extraction procedure and species of organism under study [20], which makes it very difficult to generalize the chemical compounds of many plant species.

The presence of technological techniques available for purification of the active compounds could render the purified forms of the plant extracts more efficient in management of the leishmaniasis [21]. Subsequently, the extractions of purified forms of complex molecules with various functional structures such as polyphenols, flavonoids, terpenoids and coumarins have been accomplished [14,15,22]. Recent studies on antileishmanial activities of medicinal plant products demonstrated the success of such purified extracts in inhibiting growth of several *Leishmania* species compared to the crude form [23]. Therefore, continuous screening of the medicinal plants against leishmaniasis is expected to top research priorities. In light of the scanty data on herbal medicine especially in the tropical regions where there are large forested land under these plants, the aim of this study is to evaluate the phytochemical compounds in *Olea europaea*, *Kigelia africana, Terminial mollis, Croton macrostachyus* and *Bridella micrantha* and determine the possibility of anti-leishmanial therapy

2. MATERIALS AND METHODS

2.1 Sources of Plant Extracts

Five plants species: *Olea europaea*, *Kigelia africana, Terminalia mollis, Croton macrostachyus* and *Bridella micrantha* were collected from Baringo County in Kenya and preserved in cool boxes to maintain the integrity of the sample. The voucher specimens were taken to the herbarium of the Museums of Kenya for authentication. The plant extracts were then taken to the CTMDR, KEMRI Nairobi for methanolic extraction.

2.2 Sample Preparation and Extraction of Compounds of Plant Species

The stem barks were cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shred using an electrical mill in readiness for extraction. Cold sequential extraction were carried out on plant material with analar grade organic solvents of increasing polarity, which includes hexane, dichloromethane, ethyl acetate, methanol and aqueous. Six hundred milliliters of *n*-hexane were added to 300 g of the shred specimen and flasks

placed on a shaker and soaked for 48 h. The residue were filtered using a Buchner funnel under vacuum until the sample dry. The sample were soaked further with 600 ml of hexane for 24 h until the filtrate remain clear. The filtrate will then be concentrated under vacuum by rotary evaporation at 30 - 35°C [24]. The concentrate were transferred to a sample bottle and dried under vacuum; the weight of the dry extract were recorded and stored at -20°C until required for bioassay. The process were repeated sequentially for dichloromethane, ethyl acetate, methanol and aqueous. All the extracts (0.05 g/ml) were subjected to preliminary phytochemical screening following standard methods [25-27].

2.3 Phytochemical Analysis

All the extracts (0.05 g/ml) were submitted to phytochemical analysis for secondary metabolites identification using the phytochemical methods, which were previously described [28,29]. In general, tests for the presence or absence of phytochemical compounds involved the addition of an appropriate chemical agent to the preparation in a test tube. The mixture was then vortexed. The presence or absence of compounds were subsequently detected.

2.4 Determination of Total Flavonoid Content (TPC) and Total Phenolic Content (TPC)

Total flavonoid content (TFC) in plant species were determined by colorimetric method [30]. Briefly, 0.075 mL of 5% $NaNO_2$ was mixed with 0.5 mL of the sample (1 mg/mL). After 6 min, 0.15 mL of a 10% AlCl3 solution was added and the mixture was putted at ambient temperature for 5 min. Then, 0.5 mL of NaOH (1 M) was added, and the volume was made up to 2.5 mL with distilled water. The absorbance was measured at 510 nm using a spectrophotometer (UNICO, USA), against the blank containing the extraction solvent instead of the sample. The concentration of total phenolics (TPC) was determined using spectrophotometric analysis with Folin Ciocalteau's phenolic reagent [31]. The TFC was calculated using a standard calibration of Catechin solution and expressed as micrograms of Catechin Equivalent (CE) per gram of dry extract. All tests were achieved in triplicate.

3. RESULTS

3.1 Phytochemical Screening of Plant Extracts

The phytochemical analysis conducted on the extracts revealed the presence of tannins, flavonoids, steroids phlobatannins, cardiac glycoside, terpenoids and saponins (Table 1). The *K. africana* and *Olea europa* had the largest number of phytochemical compounds. herbal medicines (especially from large families, Asteraceae, Rosaceae and Lamiaceae) have been used from ancient times as remedies for the treatment of diseases because they contain pharmacological and biological active ingredients [32, 33]. These phytochemicals in these plants contain biological activities of the plant extracts against a range of parasites.

3.2 Total Flavonoid and Phenolics Contents in the Plant Extracts

This study determined total flavonoid contents (QE/mg of extract) and TPC (mg g^{-1} DW) (Fig. 1). The concentration of flavonoid content was significantly (P < 0.05) the highest in *Olea europaea* bark extracts (12.4 ± 1.54 mg QE/mg of dry weight extract) followed by *K. africana* (9.2 ± 0.95 mg QE/mg of dry weight extract). The measured concentration of tested plants is higher than that of control test (0. 57 ± 0, 14 µg mg QE/mg of dry weight extract). The total phenolic content in the plant extracts was highest for *K. africana* (9.4 ± 0.46 mg/mg DW), followed by *T. mollis* (7.4 ± 0.53 mg/mg DW) while the range of TPC in the remaining plant extracts were similar (0.6-1.8 mg/mg DW) but higher than control (0.23 ± 0.02 mg/mg DW). Polyphenols and flavonoids are the common antioxidant natural products found in medicinal plants. The results are statistically significant in comparison with the control (0.59 ± 0.04 µgCE/mg of dry extract).

4. DISCUSSION

Leshmania has been implicated in leishmaniasis diseases which cause considerable mortality and morbility in Sub Saharan African which is regarded as resource poor [34]. In the face of no commercial interest for new and the current therapeutics, herbal products could be an inspiration of new prototype for the drug development against leishmaniasis [35]. To avoid this problem, scientific researchers have returned

to folk medicine for bioactive molecules, which may offer resistance against Leishmania parasite by scavenging free radicals and inhibiting lipid peroxidation [36]. In this study, we aim to determine the phytochemical compounds in *Olea europaea*, *Kigelia africana, Terminial mollis, Croton macrostachyus* and *Bridella micrantha* for a possibility of anti-leishmanial therapy.

The qualitative phytochemical analyses of these extracts showed the presence of major known family compounds like polyphenols, alkaloids, flavonoids, cumarins, anthocyanins, trepenoids, saponins and tannis which are known compounds against Leishmania parasites [37-39]. Some screening compounds of our preliminary phytochemical analyses have been reported previously [40]. The concentration of these most active compounds, the total phenolic contents in some of the plants such as *Olea europaea* and *Kigelia africana* were in large enough quantity and may be suitable for control of *Leishmania* parasites. These results obtained suggest that concentration of TPC is in values that may provide active bioactive ingredients for a range of parasites including leishmaniacidal properties [41]. Moreover, the TPC in all the tested plants were higher than the control suggesting that they may be active against Leishmania parasites.

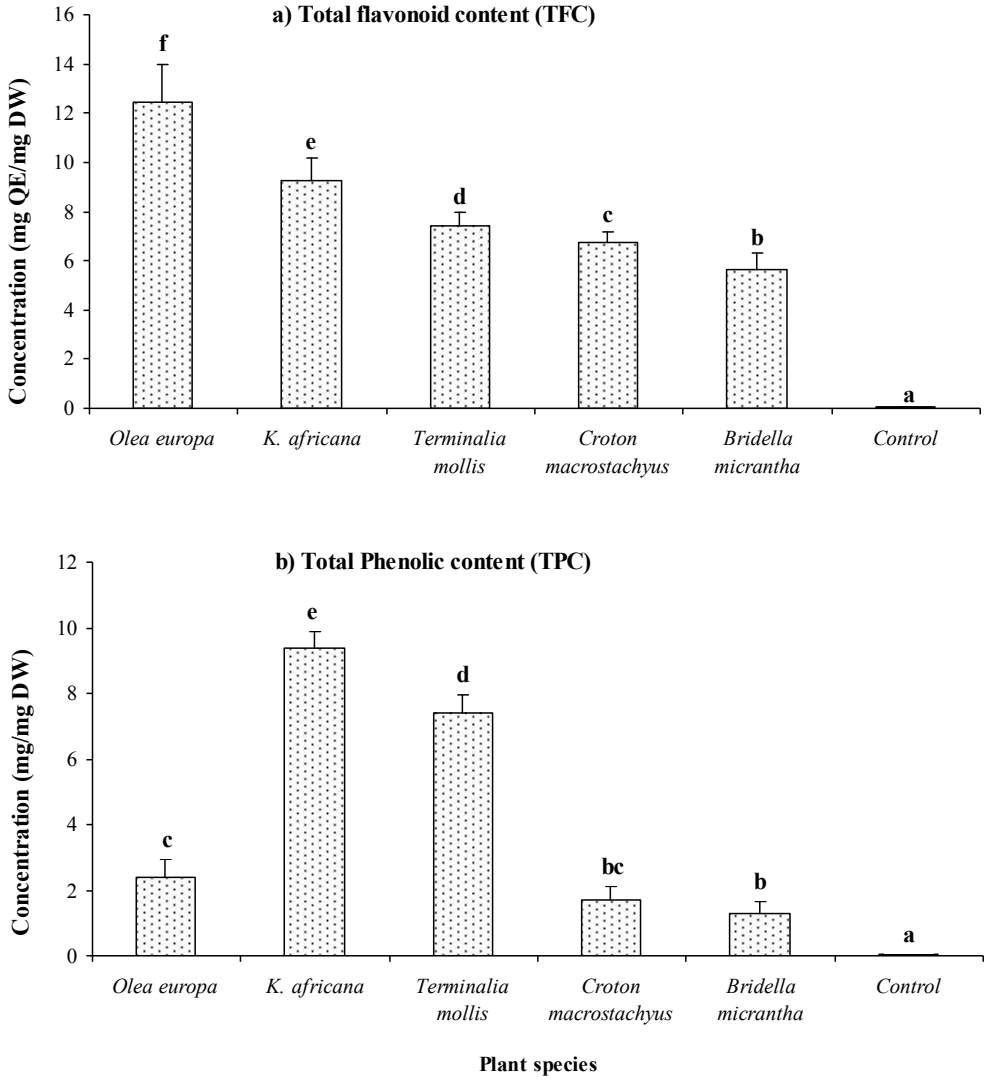

Fig. 1. Total flavonoid and phenolic contents analyzed in the plant extracts

*Data are presented as mean ± SD, n=3 experiments, p values; *: p < 0.05, **: p < 0.01, ***: p < 0.001*

Table 1. The phytochemical components of the plant extracts based on the preliminary extract screening

Phytochemical compound	*Olea europa*	*K. africana*	*Terminalia mollis*	*Croton macrostachyus*	*Bridella micrantha*
Tannins	++	++	+++	++	++
Phenols	-	-	+++	+	+++
Flavonoids	+	++	++	++	++
Steroids	-	+	+	+	+++
Alkaloids	+	++	-	+++	-
Saponins	+	++	+++	++	-
Alkaloid	++	-	++	-	+
Phlobatannin	-	+	+	-	-
Anthraquinone	+	++	+++	-	+
Cardiac glycoside	++	++	++	-	+
Terpenoids	-	+++	+	-	++
Polyphenols	++	-	-	-	+++
Cumarins	+	++	-	-	+++
Anthocyanins	+	-	++	++	-
Glycosides	-	+++	-	+	-
Triterpenoids	-	+	+	++	

+++ = high amount; ++ = moderate amount; + = trace amount; - = Not detected

5. CONCLUSIONS

The aim of this study was to test whether bark extracts of five plants used for traditional medicine practices could be promising sources of natural antioxidants. There were presence of compounds such as polyphenols, alkaloids, flavonoids, cumarins, anthocyanins, trepenoids and saponins. Presence of these compounds predicts that the screened plants may have some degree of anti-leishmanial compounds. Further investigations for potential applications of these plants for antileishmanial activities require anyway, *in vivo* and *in vitro* studies in order to better establish the functionality of the examined plant species for control of Leishmania.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was done in accordance with ethical guidelines of Maseno University.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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