Original Research Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND INHIBITORY POTENTIAL OF FIVE PLANTS FOR POSSIBLE ANTILEISHMANIAL ACTIVITY

Abstract

Leishmaniasis is a major public health problem globally and manifests in three clinical forms namely; Cutaneous, mucocutaneous and Visceral leishmaniasis (kala-azar). Visceral leishmaniasis is known to fetal 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.

Keywords: Leishmaniasis, Leishmania parasites, Phytochemical screening, Active compounds, Plan 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. chagasi, 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 by visceral leishmaniasis if left untreated for a period of two years causes fatality.

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 [6].

Despite the existence of antiparasitic agents, conventional antileishmanial drugs are expensive and out of reach for most rural communities [7]. Moreover, there is increasing evidence of parasite resistance to conventional drug therapies [8]. Also, the cost of the treatment regimes is high and takes minimum of 17 days inpatient treatment [9] thus, there is need for alternatives. Traditional medicine form a cornerstone for the treatment of various diseases of parasite and non-parasite origin in many rural settings [10, 11]. 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 [12-14]. Numerous plants have been screened for antiparasite activities using a standard WHO procedure. 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, 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 [15]. Subsequently, the extractions of purified forms of complex molecules with various functional structures such as polyphenols, flavonoids, terpenoids and coumarins have been accomplished [13, 14, 16]. 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 [17]. 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 specimen 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 [18]. 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 (Harborne, 1973; Evans, 2002; Godghate *et al*., 2012; Jaradat *et al*., 2015).

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 [19, 20]. 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 (TFC)

Total flavonoid content (TFC) in plant species were determined by colorimetric method [21]. 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% $AlCl_3$ 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 [22]. 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 and Discussion

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, Figure 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 [23, 24]. These phytochemicals in these plants contain biological activities of the plant extracts against a range of parasites.

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

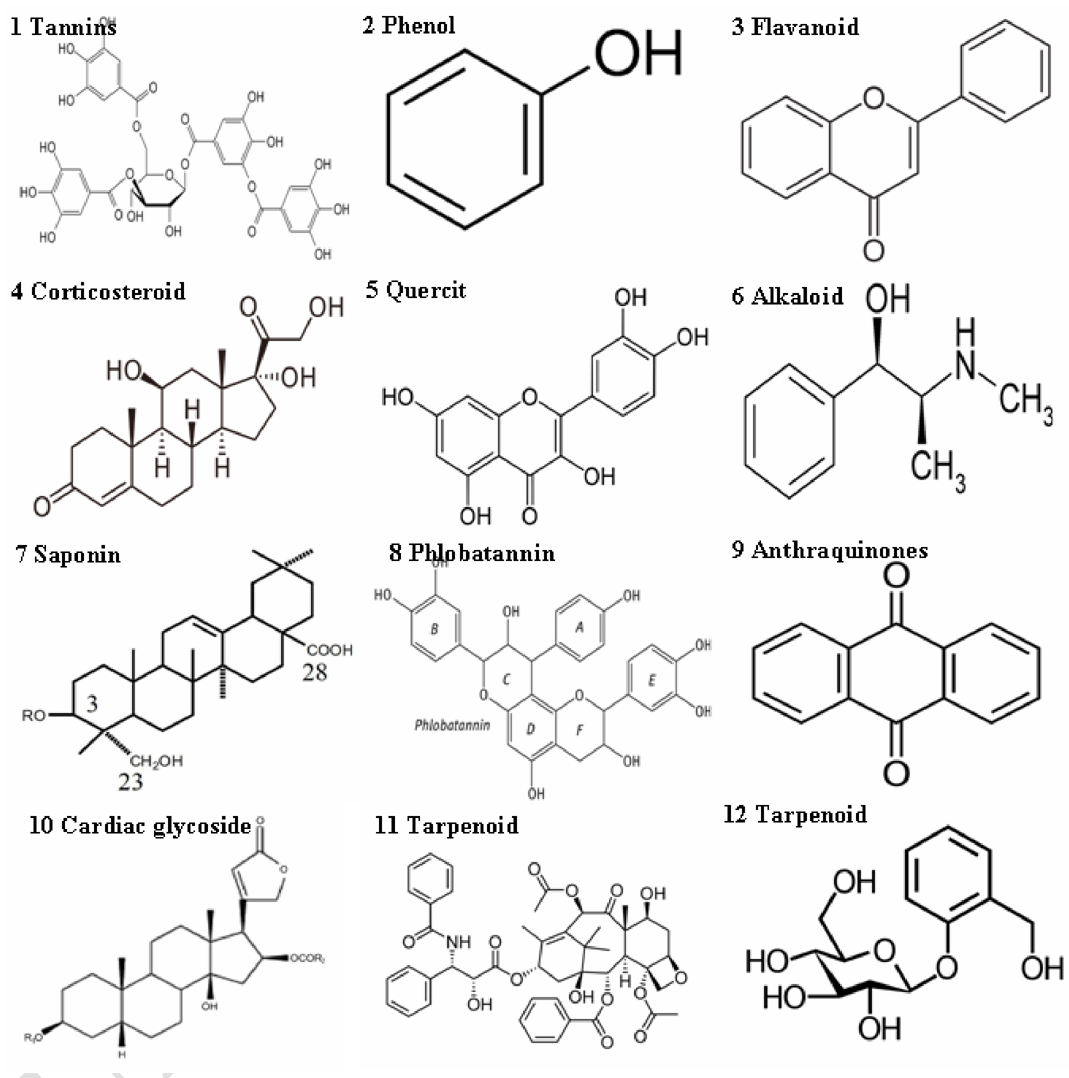

Figure 1. Structure of compounds isolated from the bark of the plants

3.2 Total phenolics contents (TPC) measurement

This study determined TPC value as Equivalent Gallic Acid using an equation obtained from a standard gallic acid graph (R^2 = 0.992). As shown in Figure 2, the concentration of TPC was significantly (P < 0.05) the highest in *Olea europaea* bark extracts (124.5 ± 15.4 µg GAE/mg of dry extract) followed by *K. africana* (92.5 ± 9.5 µg GAE/mg of dry extract.

Theses measured concentration of tested plants is higher than that of control test (0. 57 ± 0, 14 µg GAE/mg of dry extract). 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).

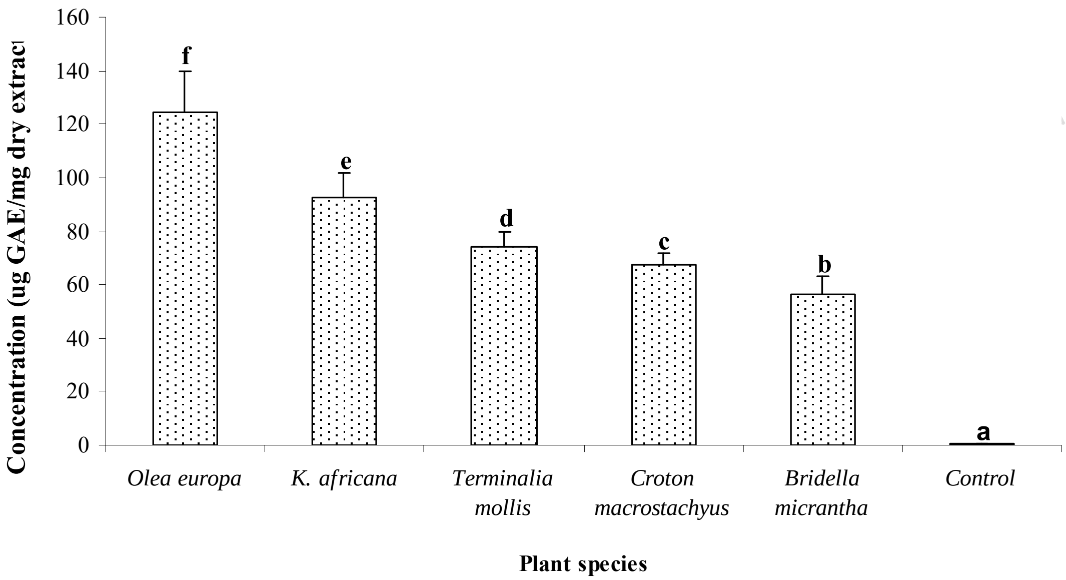

Figure 2: Total flavonoid contents analyzed in the plant extracts

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

3. Discussion

Leshmaniasis has been implicated in leishmaniasis diseases which causes considerable mortality and morbility in Sub Saharan African which is regarded as resource poor. 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 [25]. To avoid this problem, scientific researchers have returned to folk medicine to investigate and find certain bioactive molecules, which may offer resistance against Leishmania parasite by scavenging free radicals and inhibiting lipid peroxidation [26]. In this study, we aim to determine the phytochemical compounds in *Olea europaea*, *Kigelia africana, Terminial mollis, Croton macrostachyus* and *Bridella micrantha* and determine the 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. Some screening compounds of our preliminary phytochemical analyses have been reported previously [27]. The concentration of these most active compound, 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 [28]. Moreover, the TPC in all the tested plants were higher than the control suggesting that they may be active against Leishmania parasites

4. 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. The presence of compounds such as polyphenols, alkaloids, flavonoids, cumarins, anthocyanins, trepenoids, saponins and tannins suggest that these plants contain anti-leishmanial properties. The knowledge of traditional medicine practices can be a source of useful information for the isolation of natural extracts to develop new products for natural health care. Further investigations for potential applications of these plants for antileishmanial activities require anyway, in vivo studies in order to better establish the functionality of the examined plant species for control of Leishmania.

ETHICAL APPROVAL

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

References

1. Cunze S, Kochmann J, Koch LK, Hasselmann KJ, Klimpel S. Leishmaniasis in Eurasia and Africa: geographical distribution of vector species and pathogens. Royal Society open science 2019; 6(5): 190334.
2. Tabbabi A. Review of Leishmaniasis in the Middle East and North Africa. African health sciences 2019; 19(1): 1329-37.

3. Berger S. Cutaneous and Mucosal Leishmaniasis: Global Status: GIDEON Informatics Inc; 2018.
4. Hashiguchi Y, Gomez EA. Importance of Leishmania species and vector sand fly (Diptera: Psychodidae) identification. Journal of medical entomology 2018.
5. Lopes JV, Michalsky EM, Pereira NC, et al. Entomological Studies in Itaúna, Brazil, an Area With Visceral Leishmaniasis Transmission: Fauna Survey, Natural Leishmania Infection, and Molecular Characterization of the Species Circulating in Phlebotomine Sand Flies (Diptera: Psychodidae). Journal of medical entomology 2019; 56(5): 1368-76.
6. Taxy JB, Goldin HM, Dickie S, Cibull T. Cutaneous Leishmaniasis. The American journal of surgical pathology 2019; 43(2): 195-200.
7. Croft SL, Chatelain E, Barrett MP. Antileishmanial and antitrypanosomal drug identification. Emerging Topics in Life Sciences 2017; 1(6): 613-20.
8. Sundar S, Chakravarty J, Meena LP. Leishmaniasis: treatment, drug resistance and emerging therapies. Expert Opinion on Orphan Drugs 2019; 7(1): 1-10.
9. Hendrickx S, Caljon G, Maes L. Need for sustainable approaches in antileishmanial drug discovery. Parasitology research 2019; 118(10): 2743-52.
10. Parsaei P, Karimi M, Mardani M. A review of treatments for leishmaniasis wound using the prescriptions of traditional medicine. INTERNATIONAL JOURNAL OF ADVANCED BIOTECHNOLOGY AND RESEARCH 2017; 8: 2050-8.
11. de Oliveira RM, de Araújo Melo S, da Penha-Silva TA, Almeida-Souza F, Abreu-Silva AL. Alternative Treatment for Leishmaniasis. Leishmaniases as Re-emerging Diseases 2018: 145.
12. Al-Hajj MMA, Al-Shamahy HA, Alkhatib BY, Moharram BA. In vitro anti-leishmanial activity against cutaneous leishmania parasites and preliminary phytochemical analysis of four Yemeni medicinal plants. Universal J Pharm Res 2018; 3: 48-54.
13. Zeouk I, Et-Touys A, Balouiri M, Fellah H, Lalami AEO, Bekhti K. Leishmanicidal activity of plant extracts from Sefrou, a Moroccan focus of Leishmaniasis, against various Leishmania parasites in the promastigote stage. Phytothérapie 2019; 17(2): 83-9.
14. Shamsi M, Abbasi N, Mohajer A, Hoseini M, Rafieian-Kopaei M. The most important native medicinal plants effective against cutaneous leishmaniasis in mouse. International Journal of Life Science and Pharma Research 2018; 8(2): P1-P7.

15. Bouyahya A, Assemian ICC, Mouzount H, et al. Could volatile compounds from leaves and fruits of Pistacia lentiscus constitute a novel source of anticancer, antioxidant, antiparasitic and antibacterial drugs? Industrial crops and products 2019; 128: 62-9.
16. Barone CD, Zajac AM, Ferguson SM, et al. In vitro screening of 51 birdsfoot trefoil (Lotus corniculatus L.; Fabaceae) strains for anti-parasitic effects against Haemonchus contortus. Parasitology 2019; 146(6): 828-36.
17. Et-Touys A, Fellah H, Mniouil M, et al. Screening of antioxidant, antibacterial and antileishmanial activities of Salvia officinalis L. extracts from Morocco. Microbiology Research Journal International 2016: 1-10.
18. Azmir J, Zaidul I, Rahman M, et al. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of food engineering 2013; 117(4): 426-36.
19. Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of phytology 2011.
20. Angelova N, Kong HW, Van Der Heijden R, et al. Recent methodology in the phytochemical analysis of ginseng. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 2008; 19(1): 2-16.
21. Tristantini D, Jessica A. Determination of flavonoid content of mixed herbs extract using colorimetric method and thin layer chromatography (TLC). AIP Conference Proceedings; 2019: AIP Publishing LLC; 2019. p. 030004.
22. Box J. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. Water research 1983; 17(5): 511-25.
23. Hajimehdipoor H, Shahrestani R, Shekarchi M. Investigating the synergistic antioxidant effects of some flavonoid and phenolic compounds. Research Journal of Pharmacognosy 2014; 1(3): 35-40.
24. Hajimehdipoor H, Esmaeili S, Shekarchi M, Emrarian T, Naghibi F. Investigation of some biologic activities of Swertia longifolia Boiss. Research in Pharmaceutical Sciences 2013; 84(4): 253.
25. Magalhães LG, Souza JM, Candido ACB, et al. In vitro evaluation of the leishmanicidal potential of selected plant derived extracts against Leishmania Leishmania amazonensis. International Journal of Complementary & Alternative Medicine 2019; 12(12).

26. Cortes S, Bruno de Sousa C, Morais T, Lago J, Campino L. Potential of the natural products against leishmaniasis in Old World-a review of in-vitro studies. Pathogens and Global Health 2020: 1-13.

27. Gutiérrez-Rebolledo GA, Drier-Jonas S, Jiménez-Arellanes MA. Natural compounds and extracts from Mexican medicinal plants with anti-leishmaniasis activity: An update. Asian Pacific journal of tropical medicine 2017; 10(12): 1105-10.

28. Zeouk I, Et-Touys, A.,, Balouiri M, Fellah H, Lalami AEO, Bekhti K. Leishmanicidal activity of plant extracts from Sefrou, a Moroccan focus of Leishmaniasis, against various Leishmania parasites in the promastigote stage. . Phytothérapie 2019; 17(2): 83-9.