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Review Article

An overview of some medicinal plants and isolated active compounds with potential antiprotozoal activity

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Abstract

Diarrhoea associated illness presents with mortality and morbidity in rural communities in most low income countries especially in children < 5 years of age. The continuous emergence of several opportunistic infections in immuno-compromised individuals has worsened the burden of diarrhoea in most of these countries. Protozoan infections caused by species of Cryptosporidium, Entamoeba spp. Giardia intestinalis, Blastocystis hominis and Trichomonas vaginalis have received insufficient attention because data on their prevalence and incidence are scanty. The commonly used drugs to treat infections caused by these organisms are becoming less effective due to the development of drug resistance. Evidence from literature has shown that natural products from medicinal plants are likely to be suitable alternatives and complimentary therapeutic drugs to combat most protozoan infections. Natural products and their bioactive compounds could be the solution to treat most protozoan infections that have developed resistance to these drugs. This review provides comprehensive information on the potential and limitations on activity of medicinal plants and their isolated compounds used in the treatment of protozoan diseases. Especially those considered as neglected diseases such as Cryptosporidium and other protozoans that are inadequately funded and possibility of lack of interest in drug developments have made them receive little attention. Isolation and identification of bioactive natural products could be the ultimate panacea to cases of metronidazole resistance and discovery of effective and novel drug for Cryptosporidium infection which is currently suffering inadequate treatment options.

Keywords: *Protozoan parasites, Diarrhoea, Neglected diseases, Medicinal plants, Bioactive compounds*

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INTRODUCTION

The protozoan parasites *Cryptosporidium* species, *Entamoeba* spp. *Giardia intestinalis, Blastocystis hominis* and *Trichomonas vaginalis* are associated with gastroenteritis except *T. vaginalis* that causes sexually transmitted disease. Reports have associated these parasites with morbidity and mortality in developing countries [1-4]. Parasitic infections have continued to be a major challenge to health and well-being of millions of people worldwide and are particularly common in impecunious areas of the tropics and subtropics. These parasites have shown inherent ability to impair

childhood growth, and intellectual development in most people that are infected.

Flooding, famine, migration of huge populations and high prevalence of HIV infection have added to the parasite burden and quite obvious that complex interaction between parasites and other infectious agents contributes to emergence of resistant strains. Moreover, emphasis has been focused on Neglected Tropical Diseases (NTDs) especially soil transmitted helminths and nematodes, but these protozoan diseases are unappreciated and there is insufficient information on their burden and treatment options. Nevertheless, these parasites have shown intrinsic ability to develop resistance to the few effective drugs available.

Currently, there are a number of vaccines trials on parasitic infection in humans but they are ineffective in eradicating most parasitic infections. Furthermore, attempts to control parasitic infection through vector control have yielded emergence of insecticide resistance strains and it is obvious that parasitic infection still presents significant challenges to developing countries.

According to the WHO [5]. Traditional Medicine (TM) is the sum total of knowledge, skill and practises based on theories, beliefs and experiences indigenous to different culture, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

Data were collected using two different approaches. 1). Extensive literature search on the prevalence, epidemiology and available treatment options for the protozoan infections within sub-Sahara Africa. 2). Ethnobotanical/Ethnoveterinary assessment of medicinal plants used in the treatment of diseases related to protozoan infections like diarrhoea. Medicinal plants from indigenous population are accessible, readily available and affordable for the treatment of several ailments in many communities in the developing countries. Plants from time immemorial present abundant source of natural bioactive compounds and certainly have very rich history of use in management of human and animal diseases including parasitic infections.

Enteric parasitic agents such as *Cryptosporidium* and other protozoans reviewed are important cause of diarrhoea in most developing countries. The non-availability of effective drugs to treat cryptosporidiosis prompted the need to search for alternative drug(s) that will be highly effective, with low toxicity and affordable. The selection of most plants by various authors stem from the fact that such plants are conventionally used in some countries for the treatment of gastrointestinal infections and have shown anti-protozoan activities.

TREATMENT OF NEGLECTED TROPICAL DISEASES – POTENTIALLY USEFUL NATURAL COMPOUNDS

Efforts have been made globally to verify the efficacy of TM, and key outcomes have led to the isolation, characterisation and production of bioactive compounds from most medicinal plants. This review focuses on human protozoan diseases: cryptosporidiosis, amoebiasis, giardiasis, blastocystosis, and trichomoniasis as well as medicinal activity of isolated bioactive compounds in providing alternative drug(s) to some of the listed diseases.

Cryptopsoridiosis

Cryptosporidium species are associated with diarrhoea in humans and animals worldwide [2-4]. Currently, 27 species have been identified and *Cryptosporidium hominis* and *C. parvum* are the two common species infecting humans [5]. According to authors, other species infect humans include *C. meleagridis, C. felis, C. canis, C. andersoni, C. suis, C. baileyi* and *C. muris* [3,4,6]. The Global Enteric Multicentre Study (GEMS) report and other studies estimated that 30 - 50 % of children < 5 years are infected one of the leading causes of diarrhoea after rota virus [7-9]. The disease results in severe diarrhoea, dehydration and other clinical signs associated with gastroenteritis. The symptoms usually resolve within 2 weeks in immunocompetent individuals, however, in immunocompromised patients, the infection may become persistent and chronic which could be life threatening [10,11].

Several hundred of anti-parasitic drugs have been evaluated for anti-cryptosporidial activity but, the management options are limited to Nitazoxanide (NTZ) which is the current drug of choice. The drug exhibited minimal efficacy in immunocompetent adults, children but ineffective in immunocompromised patients/AIDS [12,13].

Halofuginone used as prophylactic drug in calves suffers from limited safety margin [14]. Reports have documented that over 20 compounds have shown anti-cryptosporidial activity in animal models but the target of most of these compounds require elucidation [15-17].

However, Lendner *et al* [18] demonstrated that BKI 1294 minimally reduced oocysts release in infected calves. This suggests a potential novel lead drug in animals and humans in future for drug discovery [19]. The significance of protein kinase has been fully reviewed and these small molecules have the potential in treating parasitic infections [15,19]. Moreover, halofuginone lactate was reported to reduce oocysts shedding, diarrhoea and mortality in goat kid when given as prophylactic treatment [6].

Recently, the medicinal activity of *Olea europaea* (Linnaeus) against *C. parvum* using water and ethanol extracts showed strong inhibitory activity against the parasite development at MIC 250 µg/mL; IC_{50} 361 (279 - 438) µg/mL; IC_{90} 467 (398 – 615 µg/mL). Four isolated compounds from *O. europaea* (L.) were tyrosol, hydroxytyrosol, trans-coniferyl alcohol and oleuropein. However, they exhibited insignificant inhibitory effects on *C. parvum* [20]. It was suggested that not all chemical compounds from plants are responsible for inhibition of *C. parvum* but rather a synergistic mixture of several substances, which requires standard verification (Table 1).

Amoebiasis

Amoebiasis is of public health significance worldwide, with prevalence in most developing countries (tropics and subtropics). According to the World Health Organisation, the prevalence rate affects over 50-500 million people worldwide and 110,000 deaths annually. Up to 90 % of infected people are asymptomatic carriers, whereas a smaller percentage develops extra intestinal infection such as amoebic liver abscess [21,22].

Documented evidence to metronidazole resistance against protozoans like *Entamoeba* spp., *G. intestinalis* and others abounds in several studies [23-25]. Monitoring such resistance of clinical and reference strains to the commonly used drugs in developing countries where such can be purchased without prescription should be paramount to ending resistance to the drug of choice.

Dysphania ambrosioides (Linnaeus) Mosyakin & Clemants (commonly identified as *Chenopodium ambrosioides*) was reported to have inhibitory activity on a number of parasites in *in vitro* studies [26, 27]. Interestingly, Avila-Blanco *et al* [28] reported amoebicidal effects of *D. ambrosioides* (L.) essential oil during *in vivo* experiment and identified 5 components from the essential oil using GC-MS. The chemical constituents were ascaridole epoxide (45.5 %), cis-ascaridole (34.2 %), 7-oxabyciclo (4.1.0) Heptn 2-one, 3 methyl-6-1 (7-methyl-ethyl (2.5 %), 2-propenoic acid, 2 methyl, dodecyl esther (7.2 %), and methacrylic acid, tetradecyl esther (3.54 %). Oral administration of essential oil to hamster infected with *E. histolytica* showed reversal of infection but the mechanism of how these compounds killed trophozoites requires further elucidation.

Another plant *Persea americana* Mill. (Seed) traditionally used in treating skin rashes, diarrhoea and dysentery caused by both protozoans and soil transmitted helminths in some countries, are used alone and/or in combination with species of *Psidium guajava, Mentha piperita or Ocimum basilicum* for the treatment of diarrhoea [29]. The seed showed antiprotozoal activity against protozoan parasites in an *in vitro* study and phytochemical analysis using Thin Layer Chromatography (TLC) of the chloroformic extract ($CHCl_3$) identified β-sitosterol, phytol and palmitic acid while the ethanol extract (EtoH) identified catechin and epicatechin as active compounds [30]. Both ethanol and choloroform extracts displayed substantial antiprotozoal activity possibly due to epicatechin (Table 1).

These compounds could be potential sources of bioactive lead molecules for the development of unique antiprotozoal agents to emerging metronidazole resistance. *Thymus vulgaris* (Linnaeus) (garden thyme) was reported to have various chemical constituents such as borneol, carvacrol, cymol, linalool in the essential oil and the oil has been used in food, pharmaceutical and cosmetics industries for different purposes [31,32]. Interestingly, Behnia *et al* [31] evaluated anti-amoebic effect of *T. vulgaris* (L.) essential oil against *E. histolytica* and the report showed significant activity on the trophozoites. Results of MIC after 24 h was 0.7 mg/mL, whereas the MIC for metronidazole was 2 µg/mL and 1.5 µg/mL after 24h and 48 h respectively. Other studies reported antibacterial activity of the oil on *Staphylococcus aurerous* 33] and antiprotozoal activity against *Trypanosoma brucei* and *T. cruzi* [34] signifying that the oil could be an important anti-protozoan agent. Moreover, methanol extract of *Thymus vulgaris* showed moderate activity on *E. histolytica* and *G. intestinalis* [35]. It is important to verify the activity of each isolated compounds from this plant on *Entamoeba* spp. to ascertain whether they acted alone or in synergy.

Interestingly, Calzada *et al* [36] isolated epicatechin from *Germanium mexicanum* (HBK) and the result showed strong antiprotozoan

activity *against E. histolytica* and *G. intestinalis*. Similarly, Jimenez-Arellanes *et al* [37] isolated bioactive compounds ((-) - epicatechin and (+) - catechin, while β-sitosterol, squalene, nigaichigoside F1 and 3.4 - hydroxybenzoic acid) from *Rubus liebmannii* plant traditionally used for dysentery and cough in North America. These compounds showed moderate anti-protozoan activity against *E. histolytica* and *G. intestinalis*. Deducing from the report, *R. liebmannii* (Focke) was found to be non-toxic however, it is important to ascertain if the isolated compound(s) could be an alternative therapeutic agent for future use. Additionally, Tona *et al* [38] investigated antiamoebic activity of some congolese medicinal plants, where metronidazole showed more pronounced action when compared with 45 plant extracts tested. This pointed to the fact that not all traditionally used medicinal plants are effective antiamoebic agents, but confirmation of their potency is still important.

Nevertheless, some active plant extracts used traditionally in most developing countries for the treatment of intestinal amoebiasis includes *Euphobia hirta, Mangifera indica, Carica papaya*, *Psidium guajava* and *Morinda morinoides* [39]. It will be of immense benefit if the bioactive compounds from these plants are tested alone or synergistically for their activity on *Entamoeba* and other protozoans. The isolation of novel Galacto-glycerolipid (GGL) from *Oxalis corniculata* to cure dysentery and diarrhoea associated with *E. histolytica* and *G. intestinalis* supported the importance of medicinal plants in treating protozoan infections. Analysis of phytochemical components of *O. corniculata* recognised several compounds that displayed antiamoebic activity in axenic cultures of *E. histolytica*. Bioactive compounds were characterised by Nuclear Magnetic Resonance (NMR), Infra-red and Mass spectrometry which confirmed the presence of (i) Oc-1, a mixture of saturated fatty acids C24 to C38 (ii) Oc-2, a mixture of long chain alcohols C18 to C28 and (iii) Oc-3, a single compound that was galacto-glycerolipid (GGL) [21] . It is conceivable that other compounds isolated could have strong activity against other parasitic organisms alone or in combinations when tested. In future, GGL need to be tested on clinical isolates from humans to verify their potency as alternative drug to metronidazole.

Phytochemical screening of *Epinestrum vilosum* revealed the presence of alkaloid, sterols and/or triterpenes, saponins and reducing sugars. The antiprotozoal effects of *E. vilosum* extract against *E. histolytica* exhibited strong activity but the active compound(s) from the extract needs to be isolated and tested. Other studies have ascribed the strong activity of *Quassia africana* against *E. histolytica* to the presence of alkaloids and quassinoids [40,41]. Moreover, McGaw *et al* [42] reported strong antiamoebic activity of *Acorus calamus* (L.) and the study credited that to presence of the toxic phenyl propanoid β-asarone.

Another plant *Aristolochia elegans* Mast. (Aristolochiaceae) commonly known as duck fever, usually cultured as ornamental plant has been employed as expectorant, an antitussive, anti-asthmastic, analgesic, antihistamine, anti-diarrhoea, and antidote for snake bites and toothache [43,44]. Its phytochemical constituents revealed the presence of alkaloids, lignin, neolignans, monoterpenoids, diterpenoids, sesquiterpenoids, tetralones, isoquinolines, porphyrins, biphenyl ethers, aristolactoleans and aristolochic acid dimers from leaves, stems and roots.

To support previous observations, Jimenez-Arellanes *et al* [45] purified bioactive compounds such as eupomatenoid-1, fargesin and 8R, 8' R, 9R-cubebin from hexane extract of *A. elegans* rhizomes and the effects of these purified compounds against *E. histolytica* and *G. intestinalis* showed eupomatenoid-1 was the most active compound against *E. histolytica* and *G. intestinalis* with IC_{50} of 0.624 and 0.545 µg/mL respectively. Both fargesin and 8R, 8' R, 9R-cubebin demonstrated moderate antiprotozoal activity with IC_{50} < 275.00 µg/mL against both parasites. This may provide invaluable resource for antiprotozoal activity of eupomatenoid-1 in *in vivo* studies and clinical trials to be used for drug new drug discovery and understanding the mechanism of action is important. Additionally, eupomatenoid-1, fargesin and 8R, 8'R, 9R-cubebin has been isolated from other medical plants such as *Eupomatia laurina*, *A. taliscana* and *Caryodaphnosis baviensis* [46-49]. Taken together, these compounds should be tested *in vivo* on other protozoan parasites to confirm their potency and possibility of developing prototype drugs as alternatives to metronidazole as shown in Table 1.

Recently, Njoya *et al* [50] isolated and identified Ceramide a bioactive lipid from *Codiaeum variegatum* (L.) leaves which showed anti-amoebic activity against *E. histolytica* through disruption of cell membrane including differentiation, proliferation and inhibition of cell growth.

Table 1: Some medicinal plants and isolated active compounds with potential antiprotozoal activity

Name	Protozoan parasite	Part used	Solvent used for extraction	Active Compounds	Plant Origin	Activities	Ref.
Olea europaea L. (Oleaceae)	*C. parvum*	Olive oil	Water, ethanol and heptane	Tyrosol, hydroxytyrosol, trans-coniferyl alcohol, oleuropein	Italy	Insignificant inhibitory activity on *E. histolytica*	[20]
Dysphania ambrosoides (L.) (Chenopodiaceae)	*E. histolytica*	Essential oil	Whole plant	Ascaridole epoxide	Mexico	Inhibit trophozoite growth	[28]
Persea americana Mill. (Lauraceae)	*E. histolytica, G. intestinalis, T. vaginalis*	Seed	Chloroform, ethanol	β-sisterol, phytol, palmitic acid, catechin and epicatechin	Mexico	Antiprotozoal activity	[30]
Thymus vulgaris (L.) (Lamiaceae)	*E. histolytica*	Essential oil	Hexane, aqueous Ethanol	Thymol, carvacol, borneol and linalool	Iran	Inhibitory activity on *E. histolytica*	[31]
Rubus liebamannii (Rosaceae)	*E. histolytica* and *G .lamblia*	Whole plant	Methanol	Epicatechin, catechin, β-sisterol squalene, ngaichigoside F1, 3,4-hydroxybenzoic	Mexico	Inhibitory activity on *E. histolytica* and *G. intestinalis*	[36,37]
Oxalis corniculata Linn. (Oxalidaceae)	*E. histolytica* and *G. intestinalis*	Whole plant (weed)	Methanol, Aqueous Methanol, water	Saturated fatty acid (Oc-1, Oc-2, Oc-3), Galacto-glycerolipid (GGL)	India	GGL showed strong activity antiprotozoan activity	[21]
Acorus calamus L. (Acoraceae)	*E. histolytica*	Rhizome and roots	Hexane, Ethanol and water	Phenyl propanoid, β-asarone	South Africa	Inhibited trophozoite growth	[42]
Aristolachia elegans Mast (Aristolochiaceae) *Eupomatia laurina, R.Br.* (Eupomatiaceae) *Aristolochia taliscana* (Aristolochiaceae) *Caryodaphaosis baviensis* (Lauraceae)	*E. histolytica* and *G. intestinalis*	Rhizome	Hexane extract	Eupomatenoid-1, fargesin and 8R, 8' R, 9R-cubebin	Mexico, Brazil	Fargesin and 8R, 8' R, 9R-cubebin showed moderate antiprotozoal activity	[45,48]

Table 2: Some medicinal plants and isolated active compounds with potential antiprotozoal activity

Name	Protozoan parasite	Part used	Solvent used for extraction	Active Compounds	Plant Origin	Activities	Ref.
Codiaeum variegatum (L.) Rumph. ex A. Juss. (Euphorbiaceae)	*E. histolytica*	Leaves	Water, Methanol and Ethyl acetate	Ceramide	Cameroon	Disrupts cell membrane, inhibit cell growth and apoptosis	[50]
Fragaria x ananassa (Lag. ex Dunal) (Rosaceae) *Rubus chamaemorus* L. (Rosaceae)	*G. intestinalis*	Fruits	Polyphenol extracts	Ellagitannins (Ellagic acid)	United Kingdom	Inhibitory activity on trophozoites	[58]
Pulsatilla chinensis Bunge (Finet & Gagnap) (Ranunculaceae)	*G. intestinalis*	Roots	Aqueous and Ethyl acetate	β-daucasterol, anenoside A3, ferulic acid and caffeic acid	China	Inhibitory activity on growth and adherence	[64]
Artemisia absinthium L. (Asteraceae)	*T. vaginalis*	Seeds, Essential oil	Methanol, Ethanol, Hexane , Dichloromethane	cis-epoxyocimene , (-)-cis chrysanthenol , dihydrochamzulene and chrysanthenyl acetate , camphor, trans-caryophyllene and chamzulene, Linalool, (-)-(5Z)-2, 6-dimethyllocta-5, 7-diene-2, 3-diol, germacrene-D, β-selinene and (E)-3-hexenyl butyrate	Spain	Trans-caryophyllene and (-)-cis chrysanthenol showed activity on *T. vaginalis* trophozoites	[67]
Eurycoma longifolia Jack. (Simaroubaceae)	*Blastocystis hominis*	Roots	Aqueous and Ethyl acetate	Quassinoids, β-carboline alkaloids and Canthin-6-one alkaloids	Malaysia	Antiprotozoal activity on *B. hominis*	[88]
Geranium mexicanum Kunth (Geraniaceae) *Cuphea pinetorum,* (Benth (Litraceae) *Helianthemum glomeratum* (Lag.) Lag. ex Dunal (Cistaceae) *Rubus pavifolius* (Rosaceae)	*G. intestinalis*	Whole plant	Methanol	Flavonoids-Kaempferol,tiliroside and epicatechin ,(+)-catechin, tyramine and β-sitosterol 3-O-β-D-glucopyranoside,	Mexico	Epicatechin showed remarkable inhibitory activity	[36, 54-56]

However, the mechanism of action requires elucidation and its non-toxicity to human requires verification. In view of the several evidences that numerous natural products that displayed anti-amoebic properties have been recognised, it is important that such compounds be tested further to validate their activity *in vivo* and in human studies. The isolation and identification of most bioactive compounds from different medicinal plants with promising potential as anti-protozoan are as shown in Table 2.

Giardiasis

The flagellate, *G. intestinalis*, has worldwide distribution and apparently more prevalent in children than in adults. It is one of the most commonly identified protozoan parasite in intestinal tract and probably the most diagnosed protozoan in some areas of the world [51,52]. WHO estimated that over 280 million people are infected yearly [52]. Transmission is by ingestion of viable cysts through water or food borne while foodborne outbreaks are common in developing countries [53].

In traditional medicine, various parts of the plants are used for treatment of gastrointestinal illnesses such as diarrhoea and dysentery. Barbosa and co-workers [54] assessed the chemical constituents of three flavonoids (Kaempferol, tiliroside and (-) epicatechin isolated from *Geranium mexicanium* (HBK) *Cuphea pinetorum, Helianthemum glomeratum* (Lag & Dunal) and *Rubus corifolius* (Focke) and all demonstrated *in vivo* antiprotozoal activity. The most effective flavonoid was (-) - epicatechin which showed higher activity than metronidazole and emetine commonly used as antiprotozoan medications against diarrhoea. Suggesting that kaempferol, tiliroside and epicatechin could be leading therapeutic compounds for the treatment of gastroenteritis.

Other studies have confirmed that epicatechin as active flavonoid in the treatment of diarrhoea [39,55-56]. In another study, leaves of *Achyrocline satureioides* (Asteraceae), barks of *Eugenia uniflora* (Myrtaceae), aerial parts of *Foeniculum vulgaris* (Apiaceae) extracts revealed cytotoxic effect on *Giardia trophozoites* [57]. Interestingly, the exposure of *G. intestinalis* trophozoites to wide variety of polyphenols-rich extracts from berries and other fruits showed inhibitory activity at 166 µg gallic acid equivalent (GAE)/mL. Extracts from strawberry and cloudberry were effective as metronidazole. A similar study [58] proposed that the presence of ellagitannins from cloudberry extract which could be an important compound in treating giardiasis. The compound contains substantial quantity of unconjugated P-coumaric and benzoic acid which possibly contributed to their inhibitory activity. Several studies have reported the effects of ellagitannins on human *Trypanosoma* [53], *Plasmodium* species [59] and *Leishmania* spp [60]. Ellagic acids formed by ellagitannin degradation are active against malaria parasites *in vivo* and *in vitro* [61] and was confirmed as anti-*Giardia* agent in *Rubus coriifolius* [56]. Previously, Yamasaki *et al* [62] revealed the toxic effect of ellagitannin against *C. elegans* and therefore it is necessary to verify the effectiveness of these compounds in *in vivo* and clinical trials in patients with gastroenteritis.

Another study [63] investigated *in vitro* anti-giardial effects of some plants extracts such as *Alpinia galanga, Boesenbergia pandurata, Eclipta prostrata*, etc. and the outcome showed strong anti-*Giardia* activity, but the isolation of the bioactive components is still required. The need to search for bioactive compounds/products to treat giardiasis has increased tremendously to ameliorate the side effects and resistance to metronidazole. The antiprotozoal activity of *Pulsatilla chinensis* Bunge (Finet & Gagnap) extracts and fractions against *G. intestinalis* trophozoites and its effect on the parasite physiology showed that aqueous and ethyl acetate extracts exhibited significant inhibitory activity on growth and adherence [64]. Assessment of various compounds in *P. chinensis* revealed that ethyl acetate extract contain β-daucasterol, anenoside A3, ferulic acid and caffeic acid among others. Translation of these compounds to new drugs will be meaningful in the search for antiprotozoans. Moreover, Vidal *et al* [65] assessed the antigiardial activity of *Menthax piperita* on trophozoites of *Giardia* and the report showed remarkable anti-giardial activity. Further studies are mandatory to identify major bioactive compound(s) and their toxic effects on *G. intestinalis* trophozoites and humans.

Trichomoniasis

Trichomonas vaginalis is a cosmopolitan protozoa commonly found in reproductive area of both men and women. Commonly found in urogenital tract of females and in urethra, seminal vesicle and prostate of men and transmitted sexually [66]. For several decades, metronidazole has been the drug for the treatment for trichomoniasis.

Recently, Martinez-Diaz *et al* [67] evaluated the trypanocidal, trichomocidal and cytotoxic components of cultivated *Artemisia absinthium* (L.) essential oil. Composition of the oil consist of cis-epoxyocimene (40 %), (-) -cis chrysanthenol (12 %), dihydrochamzulene (6%) and chrysanthenyl acetate (5.3 %), camphor (4.5%), trans-caryophyllene (4%) and chamzulene (3%). Linalool, (-) - (5Z)-2, 6-dimethyllocta-5, 7-diene-2, 3-diol, germacrene-D, β-selinene, (E)-3-hexenyl butyrate were detected in very low concentration. It is noteworthy that fraction VLC1 trans-caryophyllene (29.5 %), germacrene D (15.5 %) and β-seliene (8.8 %) and VLC2 contain dihydrochamazulene (42.5 %) and chamazulene (41.4 %). Trans-caryophyllene exhibited strong activity against *T. cruzi* and *Trichomonas* and the anti-parasitic effects of both fraction (VLC 1and 2) against *T. cruzi* and *T. vaginalis* were attributed to the presence of trans-caryophyllene. Significant activity of (-) - cis-chrysanthenol against *T. vaginalis* was noted. Surprisingly, fraction VLC1 and 2 were not cytotoxic against non-tumor cell line HS5, suggesting selective antiparasitic activity. A promising result was the testing of essential oils rich in trans-caryophyllene and caryophyllene oxide from plant species on *T. cruzi*, and *T. vaginalis* and they were found to be effective as anti-protozoan [26,68-69]. There is possibility that this compound could have significant activity against a number of protozoan parasites that have not been considered in either *in vitro* or *in vivo* studies. Considering the high prevalence of trichomoniasis and metronidazole-resistant cases, Brandelli *et al* [70] evaluated the activity of 10 medicinal plants against seven *T. vaginalis* isolates. The aqueous extract of *Verbena* spp. and *Campomanesia xanthocerpa* showed highest activity against *T. vaginalis* with MIC value of 4.0 mg/mL exhibited 100% efficacy against the parasite. The extract did not support any substantial haemolytic activity against human erythrocytes and it is necessary to test and characterise the active compounds in these extracts responsible for such activity.

The medicinal plant *Polygala decumbens* aqueous extract was reported to be effective in reducing significantly trophozoites viability [71]. The extract displayed strong capacity in removing *T. vaginalis* trophozoites at small concentrations (1.56 mg/mL) against all ATCC and fresh clinical isolates. Could such activity make it a target for future novel drug to treat trichomoniasis? *In vitro* evaluation of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil on *T. vaginalis* showed 100% inhibition at 15 mg/mL and 30 µg/mL after 24 h respectively. Confirming that both *P. lentiscus* mastic and *O. basilicum* oil displayed harmful effect on trophozoites growth with ultrastructural changes on the membrane of the parasite [72]. Other studies suggested that anti *T. vaginalis* effect of *P. lentiscus* was attributed to the presence of mastic which induced apoptosis and the presence of other phytochemical constituents such as α-pinene, β-pinene, β-myrcene, limonene, trans-caryophyllene, camphene, and phenolic compounds are medically important [64,73]. Certainly, it should be of interest to ascertain the activity of *P. lentiscus* constituents only or in synergy in *in vivo* or *in vitro* studies. Most of these substances have demonstrated activity against different flagellates for instance *T. vaginalis*, *G. intestinalis* and *Trichomonas gallinae* [54,74-75].

Blastocystosis

The parasite *Blastocystis hominis* an inhabitant of gastrointestinal tract first described in 1912. There is still insufficient information about the diversity, pathogenicity and most importantly treatment options of the parasite. When *B. hominis* is diagnosed in the absence of other pathogenic parasites, it may probable be the cause of diarrhoea and this may require treatment. The parasite has been found in wide range of animals, birds and amphibians and over 17 subtypes has been described with subtype (ST) 1-9 found in humans [76-79]. As a result of inadequate information about this parasite, there is still much controversy as to whether it is pathogenic or non-pathogenic. It is seemingly clear that there are conflicting opinions about the efficacy of treatments and this aspect requires more attention from researchers.

Metronidazole is the frequently prescribed drug for *B. hominis* infection or used in combination with other drugs such as trimethropin-sulfamethoxazole (TMP-SMX) and paromomycin [80,81]. There are reports of *B. hominis* metronidazole resistance in some studies [82,83] and others have reported the use of nitazoxanide for the treatment of a wide variety of protozoan infections [84,85]. Treatment failure was reported in some studies to metronidazole, iodoquinol and others for the treatment of *Blastocystis* [76-77,86] and extensive variations in drug sensitivities among subtypes of *Blastocystis* to metronidazole resistance was investigated [87]. Taking into account these observations, it is important to find alternative natural drugs to treat *Blastocystis* through *in vivo* and in vitro studies from medicinal plants that may have bioactive potent compounds to cure the infection.

Recently, Girish *et al* [88] described inhibitory activity of water and ethyl acetate fraction of *Eurycoma longifolia* (Tongkat Ali) on *Blastocystis* isolates (ST1, ST2 and ST5). The extract of *E. longifolia* exhibited very high percentage of antiprotozoal activity at 1.0 mg/mL and which is comparable with the reference drug, metronidazole. Further analysis of both extracts using LCMS/MS showed several types of quassinoids, β-carboline alkaloids and Canthin-6-one alkaloids. Ten compounds were identified from water fraction, six quassinoids, three β-carboline alkaloids and one canthin-6-one alkaloid; for ethyl acetate fraction, 9 compounds were identified, seven quassinoids, one β-carboline alkaloids and onecanthin-6-one alkaloid. With such remarkable outcome, it is necessary to recognise the active compounds in *E. longifolia* extract that could be responsible for anti-protozoal activity observed in the study and this could likely produce a novel drug for the future (Table 2).

CONCLUSION AND PERSPECTIVES

In this review, medicinal plants were characterised for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. It is interesting to note that quite a number of bioactive compounds were isolated using standard laboratory procedures but, understanding their mode of actions are needed to ascertain their real potential. Moreover, it is important to conduct *in vivo* studies using animal models to determine their beneficial effects through additive or synergistic action of the several compounds. Those bioactive compounds with various potency may reduce the chances of these pathogens developing resistance to the new drugs.

Parasitic pathogens associated with diarrhoea remains a challenge in most developing countries where the burden of infection is enormous and requires urgent consideration and remedies. Clearly, special consideration should be given to studies on antiprotozoal activity of medicinal plant extracts, essential oil and many compounds that have been isolated and identified from them. It is evident that several isolated compounds showed remarkable activities against protozoan infections either alone or in synergy with other compounds. *In vivo* studies are required on isolated compounds that exhibited anti-protozoan activity to confirm their toxicity to cells in animals and humans. The mechanism of toxicity of these compounds need adequate elucidation. In respect of *Cryptosporidium* and other protozoans, new effective drug must be a priority. It is imperative to prevent new forms of drugs resistance through testing of isolated natural compounds from medicinal plants that have been confirmed to possess anti-protozoan properties. This could be promising for development of novel drugs with very low toxicity to humans and possibly animals. For instance, the isolation of novel galacto-glycerolipid (GGL) and the amoebicidal concentration of GGL had no effect on intestinal microbial flora and mammalian cell line HEK-293. This in our view will provide the much-needed information on whether those compounds could be novel drugs to treat most parasitic infections.

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Conflict of interest

The authors declared that there is no conflict of interest associated with this work.

Authors' contributions

OTO participated in the design and helped to draft the manuscript and AOA conceived the study, coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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