Secondary Metabolites as Potential Therapeutic Agents against Parasitic Infections

**Abstract**

Parasitic infections continue to pose a significant threat to global health, particularly in developing countries with limited resources. The emergence of drug-resistant parasites and the side effects associated with conventional therapies have prompted the search for alternative treatment strategies. Secondary metabolites, natural products synthesized by organisms for ecological purposes, have shown great promise as potential therapeutic agents against parasitic infections. This comprehensive review aims to summarize the current state of research on secondary metabolites with anti-parasitic activity, highlighting their mechanisms of action, efficacy, and potential applications.

**Introduction**

1. **Secondary metabolites as therapeutic agents**

The SMBs are produced by plants throughout their life cycle (**Marin et al., 2015).** Primary compounds (carbohydrates, lipids, proteins and nucleic acids) are the precursors of SMBs (**Saltveit 2017)**, which include organic compounds like terpenes, resins, alkaloids, flavonoids and phenolic compounds presented in Figure 1. These SMBs are mostly colour, flavour or fragrant compounds **(Shitan 2016; Bansal et al., 2017; Symeonidou et al., 2018; Chanda and Ramchandra 2019).**The essential oils, polyphenols and glycosidicglucosinolates extracted from various species (e.g. medicinal and aromatic plants) have shown promising anti-microbial activity against several pathogens responsible for diseases.

**Sources of Secondary Metabolites with Anti-Parasitic Activity**

Many secondary metabolites have been harnessed from plants, microorganism and fungi. For example, alkaloids such as morphine and quinine have been derived from plants and used as powerful painkillers and antimalarial drugs, respectively. Other examples include taxol, derived from the Pacific yew tree, which is used in the treatment of various cancers, and artemisinin, obtained from Artemisia annua, which is effective against malaria.Secondary metabolites continue to serve as a rich source of novel drug candidates, and their exploration holds immense promise for the development of new therapeutics to improve human and animal health.

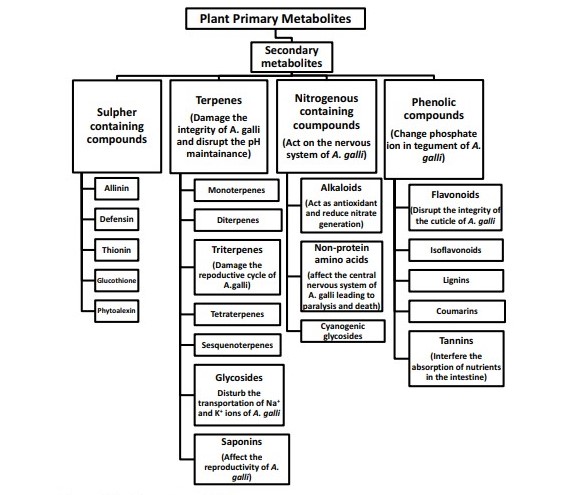
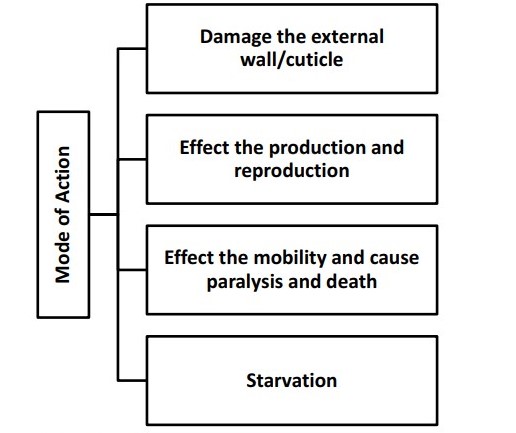
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Figure 1: Classification of secondary metabolites of plants

Several classes of secondary metabolites have demonstrated anti-parasitic potential. Alkaloids, terpenoids, flavonoids, quinones, and polyphenols are discussed in this table

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| 1. **Terpenes** | It is combination of different 5-carbon isoprene units from hydrocarbon are known as terpenes. Glycosides and saponins are categorised in this class, based on their structure. |
| 1. **Glycosides** | Glycosides are acetal alternatives formed by the reaction of monosaccharides and alcohol in the presence of an acid catalyst. |
| 1. **Saponins** | Saponins (SN) contain triterpene or steroidal-aglycone with one or more sugar chains. More than 100 plant families are reported as containing SN and are active ingredients. SN is present mostly in monocotyledons and less frequently in the family of dicotyledons |
| 1. **Phenolic compounds** | Phenolic compounds (PC) have a functional heterogenous group with an aromatic ring. They are metabolites which are formed by acetate unit condensation, and includes flavonoids, isoflavonoids and tannins |
| 1. **Flavonoids** | Flavonoids (FN) are SMBs with low molecular weight and help in the function of defence, UV protection, auxin transport inhibition, allelopathy and flower colouring |
| 1. **Tannins** | Tannins (TN), a water-soluble polyphenolic group, has the ability to precipitate proteins like gelatin. TN is found in number of plants, both woody and herbaceous |



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| **Class** | **Site of infection** | **Host** | **Parasites** | **Consequence of parasitism** |
| Protozoa | Blood | Human | Plasmodium | Anemia |
|  | Blood | Human  Bovine  White footed mouse | Babesia  Ticks (Intermediate host )  *B. divergens*  *B. mircoti*  (Primary host: *Peromyscusleucopus;*secondary host: *Ixodes*) | Fever, chills, haemolytic anemia |
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| Nematode | Abomassum | Sheep, goat | Haemochuscontrotus  Teladorsagiacircumcincta | Anemia  Reduced feed intake |
|  | Small intestine | Sheep, goat | Nematodirusbattus  Trichostrongyluscolubriformis | Reduced feed efficiency  Reduced feed efficiency |
|  | Abomassum | Cattle | Haemochusplacei  Ostertagiaostertagi | Reduced feed intake  Reduced feed intake |
|  | Small intestine | Cattle | Cooperiaoncophora  Cooperiapunctata | Reduced feed efficiency  Reduced feed efficiency |
| Cestode | Small intestine | Human | Taeniasolium  Taeniasaginata | Abdominal distress, anorexia, diarrhea, dyspepsia  Nausea, appetite loss and weight loss |
| Small intestine | Canids | Echinococcusgranulosa | Pulmonary and liver cyst formation |
| Small intestine | Rodents and human (intermediate host) | Hymenolepis nana | Headache, abdominal pain, diarrhoea |

**Secondary metabolites against protozoan**

*Protozoa*

Protozoan disease are associated with the economic health problems across the world. Major human and livestock disease caused by protozoan parasites are malaria, Typanosomiasis, Chagas disease, leshmaniasis, toxoplasmaosis (**Capela et al., 2019)**. The increased emergence of drug resistance and lack of development of new drugs with novel approach reduce the effectiveness and current treatment of these disease. Protozoan are microscopic, unicellular eukaryotic organism found worldwide. There are more than 65,000 species of protozoan parasites are described, most of them are free living parasites. These organisms have complex internal structure along with complicated metabolic activities (**Shanan et al., 2015**). The development stages of protozoan parasites consists of feeding trophozoites either intracellular or extracellular. The transmission mode of parasite involved the fecal oral route, vector borne and predatory-prey interaction **(Imam et al., 2009; Antonovics et al., 2017).** The life cycle include the dormant stage, in this form the parasite are able to survive in the extreme condition without the presence of oxygen, nutrients for a longer period of time.

1. *Giardia*

*Giardia* is a common flagellate of the intestine of mammals (Gardner and Hill, 2001). Giardia is emerging parasite of livestock, wildlife animals, including birds and amphibians across globe. The genus *Giardia* comprises several species which are morphologically similar with multiple host association. The infected animal may act as zoonotic potential of infection of *Giardia*. The transmission may involve from an infected animal to healthy animals or humans. However, the direct transmission of *Giardia* from infected animal to healthy animal or human are still need to be explored. The disease became a global health issue due to development of resistance to commonly used drugs. Since many plant-derived products have been used to treat many parasitic infestations. PipaliRasayana, an ayurvedic herbal medicine is prepared from the *Piper longum*(Pippali) and *B. monosperma* (Palash) in which ash of stem, root, flower and leaves of B. monosperma (Palash) is used and it has shown significant activity against Giardiasis, it produced upto 98% recovery from the infection (Agrawal **et al., 1997).** The most effective herbal medicine against *G. lamblia* in vitro were *Allium satium, Artemisia sieberi* and *Chenopodiumbotrys*which showed the greatest effect at a concentration of 0.1%μg/ml. In vitro studies also demonstrated that the most effective medicinal herbs against animal model giardiasis was *Helianthemumglomeratum*methanolic extract with the ED50 value of 0.125 mg/kg (**Alnomasy et al., 2021).** A dose of 400 mg/ml of hydroalcoholic extract of Lavender is effective against *Giardia lamblia* (**Vanizi et al., 2017**). The alcoholic extract of Ferula assafoetida was 37% at a concentration of 20mg/ml and at the 4th hour it was 100% against *Giardia lamblia* (**Manesh et al., 2012).** In Balb/C mice, the chloroform extract of *Tanacetumparthenium* at a concentration of 100 mg/ml was effective in the treatment of *Giardia lamblia*(**Elmi et al., 2014)*.***The crude extract of leaves of *Agereatumconiyzoides* showed the highest efficacy against *Giardia duodenalis*trophozoites**(Pintong et al., 2020).** The efficacy of ethanolic extract of pomengranate peel were evaluated against *G. lamblia* and has shown significant reduction in the number of cyst and trophozoites in faeces and intestine respectively (**El Kady et al., 2021).**

1. ***Toxoplasma gondii***

*Toxoplasmosis is caused by the obligate parasite Toxoplasma*, is a zoonotic disease and is worldly distributed parasite. T. gondii is considered as single species in the genus toxoplasma, phylum Apicompleaxa The infection is life threatening in immune-compromised patients. Recently, greater prominence has been given towards finding alternative medicine to deal with parasitic diseases and consequently, numbers of studies have been performed **(Nasr et al., 2016).**

Fungi can produce a wide range of secondary metabolites (**Costa et al. 2016**) such as peptides, alkaloids, terpenes, polyketides, quinones, sterols, and coumarins. These secondary metabolites of fungus are effective against the protozoan infections

1. **Malaria**

*Plasmodium* is a protozoan pararsite, a unicellular sporozoa caused malaria that transmitted by the bite of infected Anopheles mosquitoes. The infection of malaria is more common in tropical and sub-tropical countries of the world. *P. falciparum*, *P. vivax, P. malariae* and *P. ovale* are the four common species. Among them, *P. falciparum* and *P. vivax*are the most common. In India, *Plasmodium vivax* is responsible for malarial infection (**Anuvikar et al., 2016).** According to WHO, the global malaria burden reported from 15 countries of sub Saharan Africa and Indian subcontinent. The major 5 countries were responsible for the half of malarial infection including India (WHO, 2018). Many drugs are available in the market for the treatment of malaria like chloroquine, mefloquine, primaquine, arteminsinin. Main constrain for the use of these drug are the development of drug resistance strains of *Plasmodium***(Bahekar et al., 2013).** Over the years *Plasmodium* (especially P. falciparum causing tropical malaria) has become resistant against many of the synthetic drugs. Among the mechanisms of drug resistance an enhanced expression of ABC transporters has been reported which can pump out any drug in an ATP dependent fashion that has entered the parasite **[Wink, 2000**]. A breakthrough for the development of antimalarial drugs was the identification of the sesquiterpeneartemisinin from Artemisia annua (Asteraceae), which can even kill multidrug resistant strains of *P. falciparum* [**Willcox, 2011; Efferth et al., 2011**]. Several semisynthetic derivatives of artemisinin (e.g., the water soluble artesunate) have been developed which are in clinical practice today **[Kuhn and Wang, 2008].** Medicinal plants usually contain complex mixtures consisting of several classes of secondary metabolites. It has been postulated that the combinations found in these extracts exhibit synergistic interaction [**Wink, 2008].** If epigallocatechin 3-gallate (EGCG), a typical polyphenol of green tea, is combined with the saponindigitonin, a synergistic reduction of motility and survival of Plasmodium berghei has been recorded [**Hallmann et al., 2010].**

Large number of plants were identified as anti-malarial medicinal plants. The genus Artemisia is considered for its therapeutic importance. The biomolecule, essential oils were taken into consideration for the treatment of malaria. Artemisinin (ART) is derived from *Artemisia annua*, commonly known as sweet wormwood. The derivatives are sesquiterpene lactone trioxanes. Artemisin, a sesquiterpeneendoperoxide lactone that are responsible for the antimalarial activity **(Loo et al., 2007).***Cinchona officinalis* and related species of *Cinchona* belongs to family Rubiaceae were first used for the development of anti-malarial drug. Various alkaloids present in Cinchona bark such as quinoline alkaloids (quinine) which were used to treat the different development stages of Malaria parasite. It was also suggested that the combination of *Allium sativum* with some other plants such as *Giardiniadiversifolia, Lepidiumsativum, Rutachalepensis, Daturastramonium, Otostegiaintegrifolia, Ocimumbasilicum, Ginger officinale* showed effective cure of *Plasmodium* (**Alebie et al., 2017).**

Sterosterin A, a terpene compound extracted from the fungi *Stereumostera* have anti-plasmodial effect particularly against *P. falciparum* (**Isaka et al., 2011**). Aurisin A, G, K extracted from *NeonathopanusnambiAnthracophyllum* species against *Plasmodim falciparum*(**Kanokmedhakul et al., 2012; Intaraudon et al., 2013)**. Eurochevailiene, an alkaloid compound extracted from *Eurotiumchevalieri* shown effective results against plasmodium falciparum (**Kanokmedhakul et al., 2011).**Hirsutellone F, alkaloid from *Trichoderma*spp against *Plasmodim falciparum* (**Isaka et al., 2006)**

1. **Coccidiosis**

Coccidiosis is recognized as the major parasitic disease of poultry and is caused by protozoan parasites of genus *Eimeria*. The disease seriously impairs the growth and feed utilization of infected birds resulting in loss of productivity. It is one of a serious poultry parasitic disease that infects the epithelial lining of the intestine of poultry throughout the world (**Conway &Mckenzie, 2007**). It causes massive destruction of the epithelial cells and characterized by droopiness, paleness of the comb, diarrhea and occasional appearance of blood in droppings. The death rate can be quite high, both in chicks and in adults (**Fanatico, 2006).** Current prophylaxis and therapy for coccidiosis comprise anticoccidial chemicals, vaccines, and natural products. Plants are a rich source of phytochemicals against coccidiosis. Anticoccidial chemicals are the chemicals which kill coccidia (coccidiocides) or slow their growth (coccidiostats). Various herbal plants are evaluated for anticoccidial activity against coccidiosis as a safe alternative. Garlic and its sulfur compounds, allicin, alliin, ajoene, diallyl sulfide, dithiin, and allylcysteine, are reported to have broad antimicrobial activities which can eliminate negative factors of microbial infections. An in vitro study has shown that allicin inhibits sporulation of *E. tenella* effectively (**Alnassan et al., 2015; El-khatam et al., 2014; Pourali et al., 2014** ). Green tea extracts have been shown to significantly inhibit the sporulation process of coccidian oocysts [**Jang et al., 2007; Molan and Faraj, 2015**]. Accordingly, the selenium and polyphenolic compounds in green tea are thought to be active compounds to inactivate the enzymes responsible for coccidian sporulation (**Jang et al., 2007; Molan and Faraj, 2015).** The in vivio study evaluated the anticoccidial activities of the extract of Garcinia kola against experimental *Eimeria tenella* infection using broiler chicken (**Shetshak et al., 2020**).Azadirachtaindica commonly known as neem, a traditional medicinal plant which contain limonoids, protolimonoids, tetranortriterpenoids, pentanortriterpenoids, hexanotriterpenoidsnonterpenoids, some of which are thought to have influence on Eimeria life cycle switching **(Biswas et al., 2002; Koul et al., 2006).** The comparsion of anticoccidial efficacy of salinomycin sodium and neem fruit in broilers. It was concluded that the addition of 0.03% ground neem fruit in broiler diet showed efficiency in the repression of coccidiosis as compared to salinomycin sodium (**Tipu et al., 2002**). Various research conducted have already demonstrated the antimicrobial action of several essential oils (EO) of aromatic plants and some of their components on bacteria and fungi in vitro and in vivo (**Rhayour et al., 2003, Chami et al., 2004, 2005 and Bennis et al., 2004).** Recently, **Remmal et al (2011)** have demonstrated that EOs are efficient to destroy Eimeria oocysts in vitro. Since each EO is a mixture of many components such as Carvacrol, carvone, isopulegol, thymol, and eugenol tested for the oocysticidal activity these major components; in order to verify if they are among the active principles that are responsible of the EO’s effect. The treatment of Eimeria oocysts with these components led to their lysis in a dose and time dependant manner as shown by the release of substances absorbing at 273 nm.

1. **Trichomonas**

Trichonomiasis is caused by *Trichomonasvaginalis* and constitutes a common sexually-transmitted protozoan infection (Table 1). It mostly affects women and to minor degree men; about 170 million people are infected worldwide. It is often associated with HIV and cervical cancer (Gehrig et al., 2009). Because some of the Trichomonas strains have become resistant to the standard drugs, new chemicals are needed. Natural products have been analysed as an alternative and several alkaloids (e.g., berberine), dibenzofurans, anthraquinones, polyacetylenes, saponins, and diterpenes [Gehrig et al., 2009].

**Secondary metabolites against nematode**

**Lymphatic filariasis**

LF is one of the major health problem affecting 120 million people in 72 countries. Lf is caused by nematode parasite *Wuchereriabancrofti, Brugiamalayi*and*Brugiatimori.* It is vector borne disease that transmitted through bite of infected mosquoites species such as culex, Anopheles, Aedes and Mansonia (Lourens et al., 2019). LF is related Lymphodema (or elephantiasis), in which the swelling of limbs, breast, genitials. The the aim of WHO is to stop the spread of infection and organized Global program to eliminate Lymphatic filariasis (GPELF) for the elimination of LF and also alleviate suffering of infected persons. The available drugs such as Albendazole, Diethylcarbamazine (DEC) and Ivermectin under the mass drugs administration for the treatment of LF (Lourens et al., 2019, WHO). The recommended drugs were effective against the microfilariae stages but insufficient in the killing of the adult worms. Various herbal plants were studied for the antifilarial activity. The Oldenlandiaherbaecea has been reported for anti filaricial activity (Bahera et al., 2018). Another studies reported that *Buteamonsperma* have significant properties against anti-filarial effect (Radhika et al., 2014). An experiment was conducted on Brugiamalayi infected BALBC/C mice using different solvents (methalonic leaf extracts, n hexane, chloroform, n-butanol fraction of female P. betel) for observing anti filarial effects (Singh et al., 2009). Streblusasper (Moraceae) has proven anti-filarial activity both in vitro and in vivo and has been tested clinically in India (Singh and Singh, 1987). The cardiac glycosides asperoside and strebloside appear to be the active components [Chatterjee et al., 1992]. Tannins and flavonoids are the major polyphenols present in the bark of the trunks of P. biglobosa. These constituents have shown to be effective in the treatment of inflammatory diseases (Ouedrago et al., 2020). Fruit pulp extract of *Adansoniadigitata* (baobab) possesses anti‐ inflammatory, hepatoprotective, anticlastogenic, and other medicinal properties (Adegoke et al., 2021). Baobab affords anti‐inflammatory compounds (campesterol, cholesterol, isofucosterol, β‐sitosterol, stigmasterol, and tocopherol) that can aid in several conditions, from injuries, aches, and pains to stomach upset and respiratory conditions. Polysaccharides from Adansoniadigitata have proven to be a potential antioxidant and anti‐inflammatory food supplement **(Ibrahim et al., 2014)** It is paramount to reduce or regulate inflammation to levels that is tolerable for the body, therefore this plant and its components could help reduce elevated inflammation as seen in individuals with LF pathologies (Figure 3)

**Ascardiagalli (poultry)**

Ascaridia (A.) galli (Family: Ascaridiae) is an economically important and common nematode in poultry all over the world (**Kaufmann et al., 2011).** For the control of A. galli infection, various anthelmintics like piperazine, albendazole, levamisole, ivermectin, benzimidazole and fenbendazole have been used for times with good success. Fenbendazole has been reported most effective, due to its unique mode of action **(Bazh and El-Bahy 2013; Yazwinski et al., 2013; Umar et al., 2018).**Certain plants exhibit anthelmintic effects through secondary metabolites (SMBs), such as terpenes (glycosides and saponins), phenolic compounds (flavonoids and tannins) and nitrogen-containing compounds (alkaloids, cyanogenic glycosides and non-protein amino acids). Generally, SMBs exhibit control of nematodes by causing starvation, damaging the external membrane, impairing fertility and growth rate and damaging musculature (**Zaman et al., 2020).** I*n vitro* and *in vivo* anthelmintic activity of citrus peels against *Ascaridia galli* was investigated. Ethanolic extracts of three citrus peels species containing of Limonene (96%), β-Pinene (1.5%), α-Pinene (0.5%), and Sabinene (0.3%) were concluded that citrus peels extracts have potential anthelmintic properties against *A. galli* (**Abdelqader et al., 2012).**

***Taeniasonium*** (Tapeworm): Cysticercosis is emerging as a serious public health and agricultural problem in lesser developed countries of Latin America, Africa, and Asia where pigs are raised for consumption under traditional pig husbandry practices. Caused by *Taeniasolium*, the pork tapeworm, this zoonotic disease forms cysts in people and pigs that can lead to epilepsy and death in humans, reduces the market value of pigs and makes pork unsafe to eat.*T. solium* is a tapeworm (cestode) transmitted among humans and between humans and pigs or sometimes among humans to cause cysticercosis (Willingham and Engels, 2006).

**Secondary metabolites against trematode**

**Fasciola (liver fluke)**

**Schistosomamansoni**

Schistosomiasis is treated with praziquantel alone or in combination with albendazole or ivermectin. Also oxamniquine, and antimalarial drugs, such as quinoline alkaloids and artemisinin and its derivatives have been employed [**Mullner et al., 2011**]. Anthraquinones in *Rheum palmatum* and *Rumexdentatus* (Polygonaceae), phorbolesters from *Jatrophacurcas* (Euphorbiaceae), and saponins in general exhibited molluscicidal activity against schistosomias vector snails *Oncomelania*, *Biomphalaria* and *Bulinus* [**liu et al., 1997**]. Curcumin and derivatives from Curcuma plants (Zingiberaceae) are parasiticidal in *Schistosoma* [**Haddad et al., 2011**].

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| Family | PLANT NAME | ACTIVE COMPOUNDS | PARASITE TYPE | REFERNCE |
| Malvaceace | Adansoniadigitata | Polysaccharide | LF | Ouedrago et al., 2020 |
| Fabaceace | P. biglobosa | Flavonoids, saponins, tanins and triterpenes | LF | Saleh et al., 2021 |
|  | Azadirachtisindica | Tetranotriterpenoids | LF | Mukherjee et al., 2019 |
| Lamiaceace | Ocimumtenuiflorum | Essential oil | LF | Malebo et al., 2012 |
| Lamiaceace | Ocimumgratissimum | Essential oil | LF | Malebo et al., 2012 |
| Lamiaceace | Hyptissuavedens | Essential oil | LF | Malebo et al., 2012 |

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| --- | --- | --- | --- | --- | --- |
| Family | Plant | Active compound | Parasitic form | Concentration | Reference |
| Laminaceae | Lavandulastoechas L | Flavonoids, phenolic acids, dipropenes, triplepenes, tannins, materials as bitter, resins, saponin | G. lamblia | 400 mg/ml | 22 Vazini et al., 2017 |
| Apicaceae | Ferula assafoetida | α-Pinene, β-pinene, sabinene, eremophilene, β-caryophyllene and himachalen-7-ol |  | 20 mg/ml | 23 Rezaee-Manesh and Shirbazou, 2012 |
| Asteracecae | Tanacetumparathenium | Parthenolide |  | 100 mg/ml | 24 Elmi et al., 2014 |
| Asteraceace | Allium paradoxum | Limonen, Spathulenol, alpha-Bisabolol, Z-Nerolidol, n-Tricosane, n-Docosane |  | 100 mg/ml | 25 Elmi et al., 2014 |
| Amaranthaceae | Chenopodiumbotrys L | Linoleic acid, ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine) |  | 20 mg/ml | 26 Shirbazoo et al., 2013 |
| Apiaceae | Carumcopticum | Thymol, Trinin, Pinen and Myrcens |  | 8 mg/ml | 27 Shahabi et al., 2008 |
| Amaryllidaceae | Allium sativum | Allicin | Giardia cyst | 80 mg/ml | 28 Harandi et al., 2006 |
| Asteraceae | Artemisia annua | Artemisinin | Cyst | 100 mg/ml | 29 Esboei et al., 2012 |
| Amaryllidaceae | Allium ascalonicum | Thymol and carvacrol | Giardia | 0.2 mg/ml | 30 Azadbakht et al., 2003 |
|  | Ziziphoraclinopodioide | thymol and phenols | Giardia | 0.015 mg/ml | 31 Najafi et al., 2003 |
|  | Zatariamultiflorahad | thymol and carvacrol |  | 0.01 mg/ml | 32 Farsangi et al., 2001 |
|  | Eucalyptus globulus | cineol |  | 0.022 mg/ml | 33 Saiedi, 1999 |
|  | Lippiaberiandievi | tannins and verinalin |  | 0.85 mg/ml | 34 Ponce et al., 1994 |
|  | Punicagranatum |  |  | 17 mg/ml | 35 Berrington et al., 2012 |
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