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Research Article

Potential anthelminthic properties of selected medicinal plants in Brunei Darussalam: a phytochemical review

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Abstract
Parasitic infections caused by nematode parasites are a challenge which causes significant threats to animal health, which results in economic losses. Medicinal plants have been utilised for generations as traditional remedies against various diseases due to the presence of bioactive compounds in the plants. This article reviews four selected plant species in Brunei: *Azadirachta indica*, *Andrographis paniculata*, *Clitoria ternatea*, and *Litsea elliptica* to unravel their potential in combating helminthic infections, considering both their historical significance and known therapeutic values. The phytochemical compounds present in each species that exhibit potential anthelmintic properties are discussed and evaluated. The selected medicinal plants from Brunei have shown their potential as an alternative anthelmintic source, particularly noting their effectiveness against nematode parasites due to the presence of phytochemicals in the plants. *Caenorhabditis elegans* can be used as a model organism to explore the exact active compounds having anthelmintic ability and elucidate its mechanism of action to enhance a better understanding of plant-based anthelmintics and their potential in combating nematode parasitic infections. Hence, the identification and understanding of the phytochemical constituents of these plants can offer promising avenues for the development of novel interventions to combat parasitic infections in livestock, promoting sustainable agricultural practices and securing food production.

Keywords: Medicinal plants, helminth infection, nematode, *Caenorhabditis elegans*

Introduction

Brunei Darussalam, a small equatorial country located in Borneo Island, is widely known for its abundance of natural resources. The economy of Brunei is heavily relied on the income generated from crude oil and natural gas exports, contributing around 50 percent to the country's Gross Domestic Product (Department of Economic Planning and Statistics, 2022). Recognising the importance of sustainable development, the Brunei government has attempted to diversify the economy and reduce the dependence on non-renewable resources since the late 20th century (Hoon *et al.*, 2023). As part of this shift, agriculture and food security have been one of the identified key sectors for investment and innovation (Radzuan *et al.*, 2022).

The progress in agricultural technology has played a pivotal role in enhancing yield and productivity in various agricultural sectors, including the livestock industry. Despite these advancements, challenges persist, especially in the mass production of the livestock. A noteworthy obstacle is the widespread occurrence of parasitic infections in agricultural settings, particularly those caused by nematode parasites. These parasites cause a significant threat to animal health, which result in economic losses (Charlier *et al.*, 2014). Synthetic anthelmintic drugs are usually required to control the parasitic worm infections. However, the usage of these modern drugs is recently restricted due to the high cost and increasing development of helminth resistance (Kaplan, 2004).

An efficient alternative source for anthelmintic drugs is derived botanical, which can provide a simple and sustainable method of controlling the parasitic worms. Therefore, there is an urgent need for novel anthelmintic drugs against intestinal parasitic nematodes derived from herbal sources. In recent years, there has been a growing interest in the bioprospecting of plant species to discover natural compounds with anthelminthic properties (Veerakumari, 2015). The medicinal plants that are often used in the traditional medicine can offer a wide repository of knowledge, which can be leveraged to identify active compounds against parasitic infections.

Brunei Darussalam, renowned for its rich biodiversity and distinctive flora due to the diverse ecosystems, hence, is providing an ideal habitat for the diverse range of plant species with multitude medicinal value. The medicinal plants have been utilised for generations as traditional remedies against various diseases due to the presence of bioactive compounds in the plants. This article reviews four selected plant species with renowned medicinal properties in Brunei: *Azadirachta indica*, *Andrographis paniculata*, *Clitoria ternatea*, and *Litsea elliptica* to unravel their potential in combating helminthic infections, considering both their historical significance, and known therapeutic values.

This review explores the phytochemical profiles of the selected plants to identify active compounds with potential anthelminthic properties. This will in turn, help to uncover novel therapeutic interventions of Brunei Medicinal plants against parasitic nematodes.

***Azadirachta indica* (Sugi India)**

Azadirachta indica is a medium sized tree belonging to Meliaceae family (Table 1). It is commonly known as neem or "Sugi India" in Malay (Table 2), Neem has an average height of 30 meters with exterior bark of dark grey and reddish interior. The leaves are green in colour, alternate, and clustered at the tips of the branches. The fruit of the neem tree is a drupe that undergoes a colour transformation from green to yellow as it ripens (Nicoletti, 2020).

A. indica is originated from India and has been widely distributed and cultivated in tropical and subtropical regions. For thousands of years, neem has been recognized for its multitude beneficial properties, including those in agriculture for pest control and in traditional medicine for various common human ailments. The herb has been used for the treatment of diseases including skin disorders, digestive problems, respiratory issues, infections, wound healing, dental, and other healing and protective properties (Puri, 1999; Subapriya & Nagini, 2005; Kumar & Navaratnam, 2013; Wylie and Merrell, 2022; Haji *et al.*, 2023)

Table 1

Taxonomic classification of A. indica, A. paniculata, L. elliptica and C. ternatea. Data derived from Ngearnsaengsaruay *et al*. 2011; Hossain *et al*. 2014; Sarkar *et al.* 2021 and Suarna and Wijaya, 2021.

	A. indica.	*A. paniculata*	*L. elliptica*	*C. ternatea*
Kingdom		Plantae		
Division	Magnoliophyta	Angiosperma	Magnoliaphyta	Magnoliophyta
Order	Rutales	Personales	Magnoliopsida	Fabales
Suborder	Rutinae			
Family	Meliaceae	Acanthaceae	Lauraceae	Fabaceae
Subfamily	Melioideae	Acanthoideae		Faboideae
Tribe	Melieae	Justiciae		Phaseoleae
Genus	Azadirachta	Andrographis	Litsea	Clitoria
Species	Indica	Paniculata	Elliptica	Ternatea

Table 2

Vernacular names for respective species of plants: A. indica, L. elliptica, A. paniculata and C. ternatea.

Country	*A. indica*	*L. elliptica*	*A. paniculata*	*C. ternatea*
Brunei/Malaysia	Sugi India	Pawas	Hempedu Bumi	Telang
Indonesia	Intaran, Mimba	Medang Perawas	Sambiloto	Kembang Telang
Thailand	Saliam, Sadao	Tham-mung	FaThalai Chon	Anchan
Phillipines	Nim, Balunga	Batikuling-surutan	Sinta, Aluy	Pokindong, Kolokanting
India	Neem		Kalmegh	Aparajitha

Phytochemistry of *Azadirachta indica*

Numerous studies have investigated the phytochemical composition of different parts of neem using various extraction methods and solvents (Puri, 1999; Yakubu *et al.*, 2006; Seriana *et al.*, 2021). Phytochemical substances that are present in neem can be grouped into two major sections: isoprenoids and non-isoprenoids (Tiwari *et al.,* 2014). Isoprenoids are usually in a form of aldehyde, alcohol, ester, ether, and ketone (Waterhouse *et al.*, 2016), yet for neem, the active compounds are in the form of diterpenoids and triterpenoids (Sarkar *et al.*, 2021). Non-isoprenoids that have been investigated consist of amino acids, polysaccharides, tannins, coumarin, dihydrochalcone, polyphenolic compounds, sulphurous compounds and aliphatic compounds (Shah *et al*., 2009).

Among the notable isoprenoid constituents, terpenes play a vital role as they have been extensively studied and are known for their pesticidal and medicinal properties (Simmonds *et al*., 2004; Chaudhary *et al*., 2017; Braga *et al*., 2020; Lin *et al*., 2021; Lin *et al*., 2022). Terpenes found in neem include Azadirachtin, Azadirone (from the oil), Gedunin (from the seed oil and bark), Meliacarpin, Nimbin (from the leaves and seed), Salannin (from the leaves and seed), and Vilasinin (from the green leaves). They can be further classified as triterpenoids and, specifically, limonins, which are known for their antifeedant properties against insect pests. From the active compounds being studied, it is believed that these triterpenoids contribute to anthelmintic effects.

Anthelmintic Potential of *Azadirachta indica*

Research on the anthelmintic properties of *A. indica* has explored the various parts of the plant, including leaves, seeds, aerial parts, and flowers (Table 3). However, the leaf part of *A. indica* has been primarily investigated for its anthelmintic ability due to the readily available nature of the leaves throughout its lifecycle. Different forms of *A. indica* extracts have shown promising results in controlling some nematodes in different species of domesticated animals. *Haemonchus contortus*, commonly known as the "barber pole" worm, is one of the major species of parasitic nematodes affecting ruminants, particularly sheep and goats, causing haemonchosis. The methanolic leaf extract of *A. indica* exhibited a dose- and time-dependent response in reducing the egg per gram (EPG) fecal content of infected goats over the course of 3-week treatment (Priscilla *et al.*, 2014). Similarly, the water extract of *A. indica* showed a time- and dose-dependent response, resulting in 89% reduction in EPG fecal content in infected sheep 12 days post-treatment (Nawaz *et al*., 2014). The ethanol extract of the aerial parts of *A. indica* also demonstrated an ovicidal effect on *H. contortus* eggs, inhibiting 97.77% of egg development at a concentration of 3.12 mg/ml (Costa *et al.*, 2008). Additionally, the ethanolic seed extract of *A. indica* produced 93% mortality rate for exsheathed third-stage larvae of *H. contortus*. This effect was observed even at the lowest concentration tested, which is1.3 µg/µl (Hördegen *et al.*, 2006).

Fasciolosis is a widespread parasitic disease occurring in livestock, mainly caused by *Fasciola hepatica* and *Fasciola gigantica*. Different parts of *A. indica* have been explored for anthelmintic ability against these parasitic worms (Table 3). 10 mg/ml aqueous extract of *A. indica* leaves has been shown to eradicate *F. hepatica* over a 3-hour period *in vitro* (Ibekwe, 2019). In contrast, the ethanol extract was shown to be ineffective according to motility score interpretation criteria when tested against *Fasciola sp.* in infected buffalo liver (Yamson *et al*., 2019). The oil extract from dried neem seeds also did not cause significant motility against *F. gigantica in vitro* (Jeyathilakan *et al.*, 2010).

A. indica has also been tested against the roundworm species *Ascaridia galli*, a parasitic roundworm causing helminth infection in chickens. The aqueous extract of *A. indica* at 40 mg/ml significantly demonstrated maximum efficacy in the mortality of *A. galli* (Rabiu & Subhasish, 2011). Moreover, n-haxane seed extract of *A. indica* also exhibited the ability to eliminate and inhibit the development of eggs to larvae at 20 mg/ml (Hellawi & Ibrahim, 2020). The trematode parasite, *Gastrothylax indicus*, was exposed to ethanolic and aqueous extracts of *A. indica*, and both extracts at 50 mg/ml caused significant anthelmintic effects at 4 hours post exposure (Aggarwal *et al.,* 2016). Both extracts were also shown to produce worm motility inhibition of 89.6 % (Aggarwal *et al.*, 2016). It is believed that the extract might alter the enzymes responsible for the normal metabolism of the absorptive surface on the treated worm. Furthermore, extracts from different parts of neem have been tested for their antiparasitic activities against species other than helminths. Maran *et al*. (2021) demonstrated that the leaf aqueous extract of *A. indica* exhibited effectiveness against the marine parasitic leech *Zeylanicobdella arugamensis*. The total mortality of leeches was noticed with exposure to the *A. indica* aqueous extract in a dose-dependent manner. All leeches were eliminated in an average period of 6.45 ± 0.45 min (100 mg/mL), 11.69 ± 1.11 min (50 mg/mL), and 42.65 ± 9.20 min (25 mg/mL). Additionally, petroleum ether flower extract of *A. indica* was shown to cause paralysis in adult earthworms at a concentration of 40 mg/ml (Salma *et al*., 2021).

Andrographis paniculata (HEMPEDU BUMI)

Andrographis paniculata (family: Acanthaceae) (Table 1) is an annual, branching, herbaceous plant that grows to a height of 30 to 110 cm. It is characterised by its slender green stems, vivid green, lance-shaped leaves measuring approximately 8 cm in length and 2 cm in width. *A. paniculata* is commonly known as Hempedu Bumi in Malaysia/Brunei and Kalmegha in India (Table 2).

A. paniculata is native to Taiwan, Mainland China, and India. It is also commonly found in tropical Asian countries including Malaysia, Indonesia, and Brunei. Traditional medicinal practices have long utilised *A. paniculata* to treat various ailments such as bronchitis, worm infestation, influenza, dyspepsia, flatulence, and diarrhea (Maiti *et al*., 2006; Okhuarobo *et al*., 2014; Sharma and Sanadhya, 2017). This herb is popularly known as the "King of bitters" due to its distinctive bitter taste.

Phytochemistry of *Andrographis paniculata*

The bioactive components of *A. paniculata* generally consist of lactones, diterpenoids, flavonoids, quinic acid, xanthones, and noriridoids (Rao *et al*., 2004; Xu *et al*., 2010; Hossain *et al.*, 2021). Andrographolide emerges as the main constituent of diterpenoids in *A. paniculata* and is found abundant constituting 15% of the extract (Dwivedi *et al.*, 2021). The derivatives of the Andrographolide were also detected in this plant which are deoxyandrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographide and isoandrographolide (Chao *et al.*, 2010). Andrographolide exhibits multiple pharmacological properties and is a potential chemotherapeutic agent.

Andrographolide and its derivatives have been recognised as effective natural compounds for use as pesticides. The compounds exhibit various modes of actions and interactions against different pest species. For instance, andrographolide acts as an antifeedant against *Plutella xylostella* and *Helicoverpa armigera,* similar to the limonins found in *A. indica* (Hermawan *et al*., 1993; Ramya *et al*., 2008). Edwin *et al*. (2021) also demonstrated the antifeedant effect of andrographolide, resulting in a change in the enzymatic profile of the insect pests, *Spodoptera litura* (Edwin *et al.,* 2021). Different fractions of *A. paniculata* extracts have also been found to possess insecticidal and repellent properties against *Tribolium castaneum* and *Callosobruchus maculatus* (Adekunle and Ayodele, 2014; Baliyarsingh *et al*., 2021).

Anthelmintic Potential of *Andrographis paniculata*

The leaves and roots of *A. paniculata* extracts have shown to have anthelmintic activity against different parasites (Table 3). Banerjee *et al*. (2019) investigated the anthelmintic properties of *A. paniculata* extracts by focusing on the ovicidal and larvicidal activities against *Ancyclostoma duodenale*. Both ethanol and methanol extracts of the *A. paniculata* leaves exhibited significant inhibition of egg hatching in *A. duodenale* (Human hookworm) with median effective dose (ED_{50}) of 0.017 mg/ml and 0.02 mg/ml, respectively. Meanwhile, ethyl acetate and ethanol extracts demonstrated the highest larvicidal activity with half-maximal inhibitory concentration (IC_{50}) values of 0.001 mg/ml and 0.0019 mg/ml, respectively. The andrographolide component in the extracts is one of the main phytochemicals responsible for significant inhibitory effects both on ovicidal and larvicidal activities against field isolates of *A. duodenale* (Banerjee *et al.,* 2019).

Another study by Kamaraj *et al*. (2011) showed that the methanolic leaf extract of *A. paniculata* was as effective as Albendazole and Ivermectin in preventing the hatching of eggs and inhibiting larval development of *H. contortus* at a concentration of 25 mg/ml. Similarly, Singh *et al*. (2011) demonstrated that treatment of *H. contortus* eggs with*A. paniculata* aqueous leaf extract significantly inhibited egg hatching at a concentration of 1.25 mg/ml. However, moderate larvicidal activity (43.58%) was observed at a concentration of 20 mg/ml post 72 hours of treatment with the extract.

When tested against parasitic worms *Ascaris lumbricoide,* the ethanolic plant extract of *A. paniculata* was significantly paralysed and killed the parasitic worms (Raj, 1975). Similarly, the ethanolic leaf extract of *A. paniculata* exhibited a time- and dose- dependent response in the mortality of nematode from the same genus of different species, *Ascaris suum,* achieving complete eradication at 80% concentration within 5.89 ± 5.84 hours (Chastity *et al.,* 2015).

Both aqueous and ethanolic extracts of *A. paniculata* demonstrated significant paralysis and mortality against the earthworm *Pheretima prosthuma*, with a maximum effective inhibitory concentration of 50 mg/ml for both extracts (Murali *et al.,* 2014). The finding also showed that *A. paniculata* exhibits potent anthelmintic activity when compared to the standard drug, albendazole (Murali *et al*., 2014). Dutta and Sukul (1982) studied the efficacy of the aqueous extract of *A. paniculata* leaves against the microfilariae, *Dipetalonema reconditum* in dogs, and it was found that the extract was able to reduce more than 85% of microfilariae at a dosage of 0.06 ml per kg of body weight. Additionally, the ethyl acetate *A. paniculata* leaf extract showed a toxic effect on *Paramphistomum cervi* (sheep fluke), with lethal concentration 50 (LC50) value of 0.45 mg/ml (Elango and Rahuman, 2011).

Clitoria ternatea (TELANG)

Clitoria ternatea, commonly known as Butterfly pea or Telang in Malay is a perennial herbaceous plant from the Fabaceae family. (Table 1 and Table 2). It usually grows as a vine or creeper that thrives in moist and neutral soil conditions. One of its distinctive features is the striking vivid deep blue flowers which resemble butterflies, where the outer petals display a predominant blue hue that gradually transforms into whitish or yellowish tones towards the center. The plant has compound odd-pinnate leaves that are alternately arranged with 5-7 elliptic or ovate leaflets. *C. ternatea* produces fruit pods which resembles peas, that undergo a transformation into a pale brown shade upon reaching maturity.

Clitoria ternatea is native to tropical Asia and widely distributed in India, South and CentralAmerica, the Caribbean, and Madagascar (Lakshan *et al*., 2019). The genus Clitoria encompasses several species, with many known for their traditional medicinal properties, specifically in the realm of reproductive health, libido enhancement, and the treatment of gonorrhea (Mukherjee *et al*., 2008). *C. ternatea* has long been widely used as a brain tonic and is believed to promote memory and intelligence (Talpate *et al.*, 2013).

In Malay culture, the flower of *C. ternatea* holds cultural significance and practical applications. The juice extracted from its vibrant flowers serves as a natural dye, imparting a distinctive blue colour to a signature rice dish, adding visual appeal to culinary traditions. Additionally, the flower juice can be used as a treatment for snake bites (Sahu *et al.*, 2023). Furthermore, the roots of Butterfly pea are believed to exhibit valuable medicinal properties, offering potential relief for conditions such as ascites, abdominal viscera enlargement, skin diseases, and sore throat (Al-Snafi, 2016).

Phytochemistry of *Clitoria ternatea*

The potential of *C. ternatea* as an anthelmintic candidate is supported by phytochemical studies that found several active ingredients such as tannins, plobatins, saponins, triterpenoids, phenols, flavonoids, alkaloids, anthraquinones, anthocyanins, flavonols, resins, glycosides, steroids, essential oils, and cholestenones (Manjula *et al*., 2013; Jeyaraj *et al.*, 2021; Siddham *et al.*, 2023). GC-MS analysis identified 30 compounds in *C. ternatea*, with the main predicted compounds being Butyl-2-ethyl-hexyl-phthalate (30.19%), Butyl-2-methyl-propylphtalate (20.11%), Butyl-2-methylpropyphthalate (10.39%), and Butyloctyl-phtalate (11.29%) (Thakur *et al*., 2018).

Out of the several bioactive compounds of *C. ternatea* being mentioned, the peptide-based compounds exhibit significant importance in pest control and as potential anthelmintics. Finotin, a protein extracted from the seeds was found to cause 100% larvae mortality in bruchids *Zabrotes subfasciatus* and *Acanthosceldius obtectus* at 5% and 1% dosage, respectively (Kelemu *et al*., 2004). Another plant-derived peptide compound with insecticidal properties is cyclotides, which are often utilised by plants for defence mechanisms. The cyclotide Cter M, exhibited mortality in L3 larvae of *Helicoverpa armigera* at a concentration of 1 μmol peptide/g diet (Poth *et al.*, 2011). This finding correlates with other studies on cyclotides, demonstrating their insecticidal properties in various plant families (Jennings *et al*., 2001; Barbeta *et al*., 2008; Colgrave *et al*., 2008).

Anthelmintic Potential of *Clitoria ternatea*

Few studies have been conducted on the anthelmintic potential of *C. ternatea* extracts in combating parasitic nematodes and other similar organisms (Table 3). Khadatkar *et al*. (2008) demonstrated that the crude ethanolic extract, petroleum ether fraction, ethyl acetate fraction and methanol fraction of *C. ternatea* exhibited significant motility and mortality against non-nematodes, the annelid *P. posthuma* at a concentration of 50 mg/ml. Similarly, Nirmal *et al.* (2008) conducted a study using different parts of the plant, including flowers, leaves, stem, and roots, and found that the methanol root extract of *C. ternatea* demonstrated the highest potency as compared to other plant parts at a concentration of 20 mg/ml when tested against *P. posthuma*. The study is also in concordance with Shekhawat and Vijayvergia (2011), which demonstrated a time- and dose-dependent efficacy in the motility and mortality of the earthworm, with the maximum dose of 100 mg/ml for complete mortality post treatment with the plant's ethanolic extract. Another study focused on the different species of earthworm, *Eisenia fetida*, and reported the significant effects on the motility and mortality of *E. fetida* at a concentration of 100 mg/ml for both aqueous and ethanolic extracts of *C. ternatea* (Salhan *et al.*, 2011).

Litsea elliptica (PAWAS)

Litsea elliptica, of the Lauraceae family (Table 1), is a plant that holds significance both as a culinary ingredient and in traditional medicine. Locally known as Pawas or Madang Pawas (Table 2) in Brunei, the plant is characterised as a tall tree, that can reach a height of up to 30 meters. The bark of the tree displays a smooth texture and is grey brown in colour, while the inner bark exhibits a pinkish hue. The leaves are green, elliptic in shape, and are arranged alternately, with a leathery texture. Young shoots of *L. elliptica* exhibit a striking reddish-maroon colour, and the leaves will emit an aromatic fragrance when crushed. The leaves of *L. elliptica* are often susceptible to gall attacks in the wild (Ngearnsaengsaruay *et al*., 2011; de Kok, 2021).

L. elliptica, particularly the young shoots are commonly consumed as salads (ulam) by the local community, in addition to its utilisation for therapeutic purposes. Traditional applications include the treatment of headaches, fever, and stomach ulcers (Grosvenor *et al*., 1995; Taib *et al.*, 2009; Goh *et al.*, 2022). In addition, *L. elliptica* has been reported to reduce the incidence of gastric cancer (Bhamarapravati *et al*., 2003; Taib *et al.*, 2009). Modern pharmacological studies of *Litsea elliptica* have demonstrated its antimicrobial and antioxidant properties (Wong *et al.*, 2014).

Phytochemistry of *Litsea elliptica*

A comprehensive investigation conducted by Goh *et al*. (2022) explored the phytochemical composition of methanolic extracts derived from young leaves and mixed leaves of *L. elliptica*, where the identified secondary metabolite groups consist of various compounds, including aliphatic alcohol, aromatic alcohols, coumaran, cyclic alcohol, fatty acid ester, diterpene, phthalic acid monoester, flavonoid, phenolics, furaldehyde, furanone, terpene, triterpene, and sesquiterpenoids. Notably, four groups among the identified compounds have been demonstrated to possess pesticidal or nematicidal properties, namely aliphatic alcohol, aromatic alcohols, fatty acids, and triterpenes. Aromatic alcohols such as catechol and pyrogallol are commercially utilised as precursors for pesticides, highlighting their potential pesticidal activity (Lim *et al*., 2009). In the aliphatic alcohol group, heptacosan-1-ol exhibited promising efficacy, surpassing the nematicide *A. indica,* when the ethanolic extract of the aerial parts of another plant, *Buddleja crispa* having this compound was tested against the nematode *Meloidogyne incognita* (Sultana *et al*., 2010).

Among the fatty acid group, hexadecanoic acid, a soap concentrate insecticide and acaricide, has been employed to control soft-bodied insects such as aphids, mealybugs, and thrips (Lewis *et al*., 2016). Additionally, the triterpene squalene demonstrated significant mortality against the adult *Melanaphis sacchari* at a concentration of 2.5 mg/ml (Sotelo-Leyva *et al.*, 2023). The presence of phytochemical compounds in *L. elliptica*, particularly those exhibiting the potential as pesticidal and nematicidal properties, requires further study, as the potential bioactive compounds derived from the plant to combat pests and parasitic worms are still lacking.

Anthelmintic Potential of *Litsea elliptica*

Although the anthelmintic properties based on the active constituents of *L. elliptica* are still lacking, insights into its potential anthelmintic activity can be extrapolated from the active constituents reported from previous studies, as well as anthelmintic investigations conducted on other species within the same genus, Litsea. *In vitro* studies revealed that the methanolic leaf extract of *Litsea monopetala* was effective in causing a reduction in the motility and mortality of adult earthworms, specifically *P. posthuma*, with the most effective inhibitory concentration observed at 60 mg/ml (Mohammad, 2021; Dewanjee *et al*., 2022).

Essential oils extracted from the fruit of *Litsea cubeba* also exhibited significant mortality at an LC_{50} value of 0.504 mg/ml when tested against the Pine Wood nematode, *Bursaphelenchus xylophilus* (Park *et al*., 2007). The major constituents of the essential oil contributing to such effects were identified as Limonene (14.64%), Neral (30.27%), and Geranial (39.23%) (Park *et al*., 2007).

In the case of *Litsea elliptica*, it was reported to have potential insecticidal activities against three mosquito species: *Anopheles maculatus*, *Aedes aegypti*, and *Culex quinquefasciatus* (Jantan *et al*., 1996). The essential oil extracted from the leaves exhibited an impact on the larval survival of all three mosquito species, with LC_{50} values of 13.61 μg/ml, 16.01 μg/ml, and 14.63 μg/ml, respectively (Jantan *et al*., 1996). An aqueous cream with 1/3 essential oils from *L. elliptica* has a protective role up to 96.6% against mosquito bites (Ibrahim and Zaridah, 1998). Additionally, the methanol fraction of *L. elliptica* leaves has been shown to control the vector of dengue fever (Hidayatulfathi *et al.*, 2004). Although direct anthelmintic studies focusing specifically on *L. elliptica* are still limited, the available evidence from previous studies on other Litsea species related to their anthelmintic and insecticidal properties suggests that *Litsea elliptica* can be a potential candidate to fight against helminth parasites. Furthermore, the presence of the active compounds of *Litsea elliptica* needs to be further investigated to understand the potential active constituents contributing to such effects.

Table 3

Summary of the studies done from the medicinal plants: A. indica, A. paniculata, C. ternatea and Litsea sp. *on nematodes and other organisms*.

Plant species	Plant parts	Extraction Solvent	Assay	Species Tested	MEIC	Remarks	Reference
A. indica	L	Methanol	EPG	*Haemonchus contortus*	0.5ml/kg	*In vivo* test in goats	Priscilla *et al.*, 2014
A. indica	L	Water	EPG	*Haemonchus contortus*	2ml/kg	*In vivo* test in sheep	Nawaz *et al.*, 2014
A. indica	AeP	Ethanol	ES	*Haemonchus contortus*	3.12mg/ml	*In vitro* study	Costa *et al*., 2008
A. indica	SD	Ethanol	LS	*Haemonchus contortus*	1.3µg/µl	*In vitro* studies	Hördegen *et al.*, 2006
A. indica	L	Water	M/M	*Fasciola hepatica*	10mg/ml	*In vivo* test in cattle	Ibekwe, 2019
A. indica	L	Ethanol	M/M	*Fasciola sp.*	Unspecified (-)	Worm paralysed but not dead	Yamson *et al.*, 2019
A. indica	SD	Essential oil	M/M	*Fasciola gigantica*	Unspecified (-)	*In vitro* No significance in motility	Jeyathilakan *et al.*, 2010
A. indica	SD	Isolate	M/M	*Rotylenchulus reniformis*	0.096mg/ml	*In vitro study*	Sharma *et al.*, 2003
A. indica	L	Water	M/M	*Ascaridia galli*	40mg/ml	*In vitro* study	Rabiu and Subhasish, 2011
A. indica	SD	n-hexane	ES	*Ascaridia galli*	20mg/ml	*In vitro* study	Hellawi & Ibrahim., 2020
A. indica	L	Ethanol and water	M/M	*Gastrothylax indicus*	50mg/ml	*In vitro* study	Aggarwal *et al.*, 2016
A. indica	L	Water	M/M	*Zeylanicobdella arugamensis*			Maran *et al.*, 2021
A. indica	Fl	Petroleum Ether	M/M	*Pheretima posthuma*	40mg/ml	*In vitro* study	Salma *et al.*, 2021
A. paniculata	L	Ethanol and methanol	ES	*Ancyclostoma duodenale*	0.017mg/ml, 0.02mg/ml	*In vitro* study	Banerjee *et al.*, 2019

A. paniculata	L	Ethyl acetate and Ethanol	M/M	*Ancyclostoma duodenale*	0.001mg/ml, 0.0019mg/ml	*In vitro* study	Banerjee *et al.*, 2019
A. paniculata	L	Methanol	ES and LS	*Haemonchus contortus*	25mg/ml	*In vitro* study	Kamaraj *et al.*, 2011
A. paniculata	L	Water	ES and LS	*Haemonchus contortus*	1.25mg/ml, 20mg/ml	Moderate larvicidal activity at 20mg/ml	Singh *et al*., 2011
A. paniculata	AeP + Rt	Ethanol	M/M	*Ascaris lumbricoides*	Unspecified (-)	*In vitro* study	Raj, 1975
A. paniculata	L	Ethanol	M/M	*Ascaris suum*	80% concentrate from ethanol extract	*In vitro* study	Chastity *et al.*, 2015
A. paniculata	L	Water and ethanol	M/M	*Pheretima posthuma*	50mg/ml	*In vitro* study	Murali et al., 2014
A. paniculata	L	Water	M/M	*Dipetalonima reconditum*	0.06ml/kg	*In vivo* study	Dutta & Sukul, 1982
A. paniculata	L	Ethyl acetate	M/M	*Paramphistomum cervi*	LC_{50} 0.45mg/ml	*In vitro* study	Elango and Rahuman, 2011
C. ternatea	Rt	Ethanol, ethyl acetate and methanol	M/M	*Pheretima posthuma*	50mg/ml	*In vitro* study	Khadatkar *et al.*, 2008
C. ternatea	Rt	Methanol	M/M	*Pheretima posthuma*	20mg/ml	*In vitro* study	Nirmal *et al.*, 2008
C. ternatea	AeP + Rt	Ethanolic	M/M	*Pheretima posthuma*	100mg/ml	*In vitro* study	Shekhawat and Vijayvergia, 2011
C. ternatea	L	Water and ethanol	M/M	*Eisenia fetida*	100mg/ml	*In vitro* study	Salhan *et al.*, 2011
L. monopetala	L	Methanol	M/M	*Pheretima posthuma*	60mg/ml	*In vitro* study	Mohammad, 2021
L. cubeba	Fr	Essential oil	M/M	*Bursaphelenchus xylophilus*	LC_{50} 0.504mg/ml	*In vitro* study	Park *et al*., 2007

AeP: Aerial parts, L: Leaves, SD: Seed, Fl: Flower, Rt: root, Fr: fruit, EPG: Egg Per gram, ES: Egg survival/ovicidal, LS: larval survival, M/M: Motility and mortality, MEIC: Most Effective Inhibitory concentration

Future directions

Plants may serve as an alternative source to combat helminth parasites due to their richness in bioactive chemicals, effective against a number of species, including specific target-parasitic nematodes, and are biodegradable. This review aims to summarise the anthelmintic properties of selected Brunei medicinal plants: *A. indica*, *A. paniculata*, *C. ternatea* and *L. elliptica* with the association of active chemical compounds contributing to the detrimental effects against helminth parasites. The review identifies several future directions for enhancing our understanding and utilization of plant based-anthelmintics.

Isolation and Characterization of Active compounds

Most conducted research has utilised various parts of the plants with different solvent extracts to evaluate their anthelmintic properties. Yet, there has been a scarcity of studies specifically on investigating the exact phytochemical compounds of the extracts having anthelmintic effects. Previous studies primarily examined the phytochemical composition, which are often encompassing a wide range of secondary metabolites, with only rare instances involving the isolation and characterisation of specific active constituents to test their effectiveness against parasitic nematodes. Therefore, a detailed exploration into the chemical constituents of the plants is crucial to enhance our understanding of their anthelmintic potential by isolating the necessary compound. Each species may harbour unique analogues and derivatives of bioactive compounds that have yet to be isolated and evaluated for their anthelmintic properties. By exploring these untapped sources, potential anthelmintic agents can be discovered, contributing to the development of novel treatments against nematode parasitic infections.

Metabolic Pathway Exploration

The underlying mechanisms of action for the anthelmintic of the plants remain largely speculative although efforts have been made to identify the specific stages of the parasite's lifecycle targeted by the plant extracts (Kumarasingha *et al.*, 2016). However, establishing the metabolic responses induced by the extracts in the target organisms has proven challenging due to inherent limitations in the experimental protocols. Studies often focus on toxicity, motility, and reduction in number of eggs laid by the nematodes, neglecting metabolomics for identifying disruptions caused in the metabolic pathways (Kumarasingha *et al.*, 2016). Utilising parasitic nematodes as test subjects for anthelmintics poses several challenges that complicate experimental design and data interpretation. The availability and maintenance of parasitic nematodes in laboratory settings are complicated, which requires specialised facilities, host animals, and ethical considerations (Gilleard *et al*., 2021). The complex life cycles of many parasitic nematodes also involve various developmental stages, making it challenging to synchronize experimental conditions (Perry & Clarke, 1981) and the genetic variability among individual parasitic nematodes within a population may impact the reproducibility of the results (Haag *et al.*, 2018). The intricate interactions between parasitic nematode and its host organism further complicate the isolation of intrinsic nematode responses to anthelmintic treatments. Moreover, the risk of developing anthelmintic resistance in parasitic nematode populations poses a significant concern, potentially limiting the effectiveness of these secondary metabolites over time (Peña-Espinoza *et al.*, 2016).

Alternative Model Organism

These challenges highlight the importance of considering alternative model organisms, such as the non-parasitic nematode *Caenorhabditis elegans*, which offers experimental advantages and contributes valuable insights to anthelmintic research without the complexities associated with the parasitic species. *C. elegans* is a free-living nematode that is easy to culture in the laboratory and has a short life cycle of about three days allowing for rapid experimentation and high throughput screening. The transparency of *C. elegans* allows for the easy visualisation of internal organs and processes, facilitating the observation of the effects of secondary metabolites on the nematode's anatomy and physiological changes. This transparency provides valuable insights into the mode of action of anthelmintic compounds (Izquierdo *et al.*, 2021).

Genetic Manipulation Studies

C. elegans is amenable to genetic manipulation, enabling researchers to knock down or overexpress specific genes associated with drug sensitivity or resistance. This genetic tractability allows for the study of molecular mechanisms underlying anthelmintic responses (Sugi, 2016). Notably, many biological processes and pathways are conserved between *C. elegans* and parasitic nematodes, offering relevant information about potential drug targets in parasitic nematodes (International Helminth Genomes Consortium, 2019). The use of *C. elegans* helps circumvent the ethical concerns associated with working directly with parasitic nematodes, especially those infecting humans or animals. This model organism allows researchers to study anthelmintics without causing harm to the hosts or using live animals (Ha *et al.,* 2022). The complete and well-annotated genome of *C. elegans* further facilitates the identification of potential drug targets and enhances our understanding of the molecular basis of anthelmintic action (Sugi, 2016). Various research has been done to investigate the anthelmintic effect on *C. elegans* of the various plants including *Guiera senegalensis* (Gagman *et al.,* 2022), *Leucena leucocephala* (Widaad *et al.,* 2022) and *Rumex crispus* (Idris *et al.,* 2022). Understanding the metabolic pathways that the novel anthelmintic compounds act using

C. elegans can help to combat helminth parasites effectively and overcome modern drug resistance.

Conclusion

In conclusion, the review highlights the potential of selected Brunei medicinal plants as alternative anthelmintic sources, particularly noting their effectiveness against nematode parasites due to the presence of the phytochemicals in the plants that contributing to such effect. The review also highlights existing research limitations in identifying specific phytochemical compounds and understanding the underlying mechanisms. The proposed use of *Caenorhabditis elegans* as a model organism to explore the exact active compounds having anthelmintic ability and elucidating the mechanism of action suggest promising avenues for future research to enhance better understanding of plant-based anthelmintics and their potential in combating nematode parasitic infections.

Conflict of interest

We declare that we have no conflict of interest.

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