Review Article

Fascioliasis Phytotherapy using Tropical Plant Extracts: A Systematic Review with Meta-Analysis

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Abstract | Fascioliasis is a waterborne and foodborne parasitic disease, involving species *Fasciola hepatica* and *Fasciola gigantica*. This systematic review with meta-analysis (SR-MA) explored the potential of phytotherapy against fascioliasis and as alternative to commercially available anthelmintic drugs. Eligibility criteria for inclusion and protocol was defined for systematic publication database searching. Final reference database consisted of eight (8) published journal articles with a total of 106 *Fasciola* flukes, published between years 2001 to 2021. The mortality time between tropical plant extracts and commercially available drugs posed a significant difference (P < 0.05), while ten (10) among the plant species differed in fasciolocidal activity (P < 0.05). Albendazole (62.5%) was the most used reference drug, along with Piperazine Citrate, and Triclabendazole, wherein ANOVA showed no significant difference in mortality time among them (P > 0.05). Notably, plant parts and extraction techniques used affected the resulting fasciolocidal activity of the plant extracts. Linear regression revealed a low inverse relationship between the plant extract concentration and the mortality time (R^2 = 0.37). Meta-analysis revealed a significant summary effect among included studies, with high statistical heterogeneity (P < 0.05). Moreover, the forest plot showed significant difference (P < 0.05) between the activity of plant extract and comparator drugs. Egger's test revealed that this SR-MA has a low risk of publication bias (P < 0.05). Overall, the results confirm that tropical plant extracts have a potential for phytotherapy against fascioliasis and drug development, and is comparable, in terms of induced mortality time, to commercially available fasciolocidal drugs.

Keywords | Fascioliasis, Fasciola hepatica, Fasciola gigantica, Tropical plant extracts, Mortality time

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INTRODUCTION

Fascioliasis, also known as fasciolosis, is a well-known waterborne and foodborne parasitic disease of ruminants and humans and is recognized as one of the neglected tropical diseases worldwide. The global prevalence of fascioliasis is estimated to be at least 2.4 million in more than 75 countries and is typically under-reported and under-diagnosed (WHO, 2021). It is caused by two species of liver fluke under the class Trematoda and genus *Fasciola*: *F. hepatica* and *F. gigantica*. Both can thrive through its snail intermediate host from the genus *Lymnaea*. For the past 20 years, incident reports of the disease have increased, most especially in countries whose locations are in temperate and tropical regions (Tolan Jr., 2011). In a study by Portugaliza et al. (2019), it was revealed that in Leyte, Philippines, the prevalence of *Fasciola* spp. in animal and herd levels was 63.58% and 86.96%, respectively.

Furthermore, there were two cases of human fascioliasis reported, that were thought to be caused by eating improperly prepared kangkong (*Ipomea aquatica*) (Eduardo, 2001). Harboring liver flukes can lead to a number of significant losses in livestock, including reduced production and quality of wool, poor growth rate of lambs, reduced production and quality of milk in dairy cattle, and lower growth rates and feed conversion rates in fattening cattle (Boray, 2017). In treating the disease, Triclabendazole is the drug of choice (Mas-Coma et al., 2014), however the emergence of fluke resistance due to under- and over-dosing of over-the-counter fasciolocides, as well as cost and inaccessibility instigated a shift in the universal trend of using synthetic drugs to herbal medicine and phytotherapy as an alternative method in controlling fascioliasis (Foran, 2007). Given the great diversity of the plant kingdom, phytotherapy or the use of plants with anthelmintic activity is recommended as the opportunity of finding bioactive compounds, especially anti-fluke properties, increases (Alvarez-Mercado et al., 2015). Moreover, plant-derived compounds may help in the contribution of the development of a novel, more affordable, and attainable drugs and medication. Findings in the study would potentially be substantial for future studies within a similar field. Nevertheless, this review did not cover other phytomedicinal efficacy of tropical plant extracts against other helminths, the characterization of plant extract components into specific compounds, as well as the mechanism of actions of the commercial drug and plant extract. Systematic searching was strictly limited to electronic databases: PubMed, Springer, ScienceDirect, and Google Scholar. In addition, the locale of the eligible study is limited to countries within the tropical climate zone *(23.5 degrees north latitude to 23.5 degrees south latitude)*.

RESULTS AND DISCUSSION

Systematic Search

Summarized in Figure 1 are the individual searches on the four selected databases: PubMed, Springer, ScienceDirect and Google Scholar, that resulted in four hundred thirty-nine (439) articles. After being subjected to cross-checking for duplication, two hundred twenty-six (226) articles advanced to title/abstract screening. One hundred eighty-nine (189) of these were excluded: eighty-five (85) studied a different genus of helminth, fifty-four (54) were outside the geographical scope, twenty-three (23) utilized non-plant extracts, fifteen (15) were paid studies, and twelve (12) were review study designs. Meanwhile, thirty-seven (37) were directed to full-text article eligibility testing. Among them, twenty-seven (29) articles were excluded: fourteen (14) showed different outcomes, five (5) showed abstract only, four (4) showcased different trematodes, two (2) were in vivo studies, and four (4) had no comparator drug. Eight (8) studies were then included in both qualitative and quantitative (meta-analysis) synthesis.

Table 3 below summarizes the eight (8) journal articles that were reviewed — all of which were reliable without restrictions in the bibliographic and scientific data. Three (3) of these were from Nigeria *(9.0820° N, 8.6753° E)*, two (2) were from the Philippines *(12.8797° N, 121.7740° E)*, one (1) was from India *(20.5937° N, 78.9629° E)*, and another one was from Sudan *(12.8628° N, 30.2176° E)*. Five (5) utilized adult *F. gigantica,* one (1) used miracidia of *F. hepatica;* while the remaining two (2) did not specifically characterized the species and used *Fasciola* spp. Four (4) methods of extraction were utilized: methanolic technique, ethanolic technique, aqueous, and hydroethanolic technique. Using one hundred and sixty-three (163) flukes, the fasciolocidal activity of plant extracts were compared to fasciolocidal drugs in terms of mortality time. Among the studies, the evaluated anthelmintic comparator drugs included: albendazole, piperazine citrate, and triclabendazole.

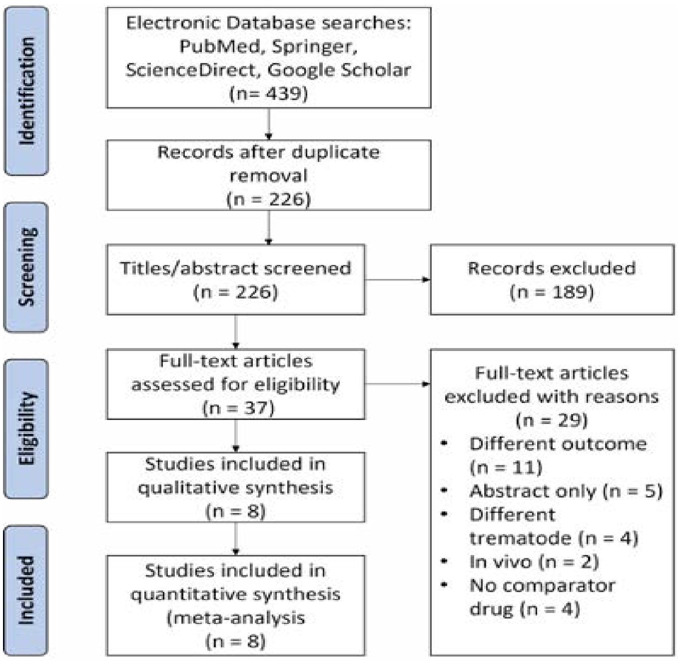


Figure 1: PRISMA flowchart of screening and selection

The prevalence of fascioliasis has been reviewed in several parts of the world, most especially in Africa, South America, Western Europe and Asia. In this systematic review, African countries Nigeria and Sudan, as well as Asian countries the Islamic Republic of Iran, India, Thailand, and the Philippines, were highlighted as those with the highest endemicity of fascioliasis in humans, as well as high parasitic infections in livestock and in other domestic animals. *F. hepatica* is found all across the world, however it is most common in temperate zones, whereas *F. gigantica* is primarily found in tropical areas (Mas-Coma et al., 1999). In addition to this, a report by the World Health Organization revealed that there is an overlapping distribution of

Table 1: Population, intervention, comparison, and outcome keywords and search strategy for anthelmintic activity and fascioliasis phytotherapy in different target databases

Database	**Search String**
PubMed	(*Fasciola*[tw] OR fascioliasis[tw] OR "*F. hepatica*"[tw] OR "*F. gigantica*"[tw]) AND ("plant extract*"[tw] OR phytochemical*[tw] OR "secondary metabolite*"[tw]) AND ("control drug*"[tw] OR triclabendazole[tw] OR mebendazole[tw] OR "anthelmintic drugs"[tw]) AND ("paralysis time"[tw] OR toxicity[tw] OR "mortality time"[tw] OR mortality[tw])
Springer	TITLE-ABS-KEY ((*Fasciola* OR fascioliasis OR "*F. hepatica*" OR "*F. gigantica*") AND ("plant extract" OR phytochemical OR "secondary metabolite") AND ("control drug" OR triclabendazole OR mebendazole OR "anthelmintic drugs") AND "paralysis time" OR toxicity OR "mortality time" OR mortality))
ScienceDirect (Elsevier)	(*Fasciola* OR fascioliasis OR "*F. hepatica*" OR "*F. gigantica*") AND ("plant extract" OR phytochemical OR "secondary metabolite") AND ("control drug" OR triclabendazole OR mebendazole OR "anthelmintic drugs") AND ("paralysis time" OR toxicity OR "mortality time" OR mortality)
Google Scholar	Where my words occur: anywhere in the article 1. With all of the words: *Fasciola*, plant extract, mortality time, control drug

Table 2: Cohen's d result interpretation according to Cohen (1992) reported by Cadena et al. (2015)

Cohen's d effect size	**Interpretation**	**Differences in SD**
d= .0-.19	Trivial effect	< 1/5 from a SD
d=.20	Small effect	1/5 from a SD
d= .50	Medium effect	1/2 from a SD
d= .80 or higher	Large effect	8/10 from a SD

Table 3: Reference database summary

Country	**Coordinates**	**Fluke Species & Developmental Stage**	**No. of Flukes**	**Extraction Technique**	**Outcome**	**Comparator Drug**	**Quality Assessment**	**Risk Of Publication Bias**	**Authors**
Nigeria	9.0820° N, 8.6753° E	adult *F. gigantica*	10	Methanol	Mortality Time	Albendazole	Reliable without Restriction	Low to None	Adeniran, A.A., & Sonibare, M.A. (2013)
Nigeria	9.0820° N, 8.6753° E	adult *F. gigantica*	8	Methanol	Mortality Time	Piperazine Citrate	Reliable without Restriction	Low to None	Ajaiyeoba, E.O. et al. (2001)
Sudan	12.8628° N, 30.2176° E	adult *F. gigantica*	18	Methanol & Aqueous	Mortality Time	Albendazole	Reliable without Restriction	Low to None	Ali, S.A. et al. (2012)
Iran	32.4279° N, 53.6880° E	miracidia *F. hepatica*	60	Hydroethanolic	Mortality Time	Triclabendazole	Reliable without Restriction	Low to None	Ghafari, A. et al. (2021)
India	20.5937° N, 78.9629° E	adult *F. gigantica*	6	Methanol	Mortality Time	Albendazole	Reliable without Restriction	Low to None	Hossain, E. et al.(2013)
Nigeria	9.0820° N, 8.6753° E	adult *F. gigantica*	20	Methanol	Mortality Time	Piperazine Citrate	Reliable without Restriction	Low to None	Lasisi, A. & Idowu, O. (2011)

Philippines	12.8797° N, 121.7740° E	*Fasciola* spp.	25	Ethanol	Mortality Time	Albendazole	Reliable without Restriction	Low to None	Luis, H.C. et al. (2021)
Philippines	12.8797° N, 121.7740° E	*Fasciola* spp.	16	Ethanol	Mortality Time	Albendazole	Reliable without Restriction	Low to None	Yamson, E. C. et al. (2019)

Table 4: Categorization of tropical plant species, part, extract type, concentration, and mortality time used in the different fascioliasis phytochemical studies.

Study	Tropical Plant Species	Common Name	Plant Part	Extract Type	Concentration	M (Mortality Time in min)
Adeniran, A.A., & Sonibare, M.A. (2013)	*Dioscorea bulbifera* Russ. ex Wall.	Air Yam	Flesh Peel	Methanol	100 mg/mL 20 mg/mL	15.81 ± 2.13
Ajaiyeoba, E.O., Onocha, P.A., & Olarenwaju, O.T. (2001)	*Buchholzia coriaceae* Engl.	Musk Tree	Leaves	Methanol	100 mg/mL	6 ± 0.9 5 ± 0.2
	Gynandropsis gynandra (L.) Briq.	Spiderwisp				
Ali, S.A., Mohammed. G. E., & Gameel, A.A. (2012)	*Capparis decidua* (Forssk.) Edgew.	Hedge Caper	Stem	Methanol Aqueous	1200 ug/mL 300 ug/mL	816 ± 1.25 779 ± 3.57
	Moringa oleifera Lam.	Horseradish	Leaves	Methanol	1200 ug/mL	
Ghafari, A., Arbabi, M., Mosayebi, M., Hooshyar, H., Nickfarjam, A.M. (2021)	*Zingiber officinale* Roscoe	Garden Ginger	Not indicated	Hydroethanolic	10 ug/mL	1.75 ± 0.05
Hossain, E., Chandra, G., Nandy, A.P., Gupta, J.K., Mandal, S.C. (2013)	*Dregea volubilis* (L.fil.) Benth.	Cotton Milk	Leaves	Methanol	100 mg/mL	38.83 ± 3.41
Lasisi, A. & Idowu, O. (2011)	*Berlinia confusa* Hoyle	Ebbiara	Stem bark	Methanol	100 mg/mL	40 ± 0.5
Luis, H.C., Matias, F.B.R., Tubalinal, G.A.S.P., Mingala, C.N. (2021)	*Cleome rutidosperma* DC.	Spiderflower	Leaf	Ethanol	100 mg/mL	55 ± 5
Yamson, E. C., Tubalinal, G. A. S. P., Viloria, V. V., Mingala, C. N. (2019)	*Areca* catechu L.	Betel-nut Palm	Leaf Nut	Ethanol	100 mg/mL	0.22 ± 0.0997 220 ± 40.3556

both species *F. hepatica* and *F. gigantica* across countries in only African and Asian continents (WHO, 2007).

Synthesis & Statistical Report of Selected Studies

The selected studies have examined ten (10) species of tropical plants: *Dioscorea bulbifera* Russ. ex Wall., *Buchholzia coriaceae* Engl., *Gynandropsis gynandra* (L.) Briq., *Capparis decidua* (Forssk.) Edgew., *Moringa oleifera* Lam., *Zingiber officinale* Roscoe, *Dregea volubilis* (L.fil.) Benth., *Berlinia confusa* Hoyle, *Cleome rutidosperma* DC., and *Areca* catechu L.

Fasciolocidal Activity of Extracts from Tropical Plant Species

As shown in Table 4, extracts from evaluated tropical plant species differ in fasciolocidal efficacy, in terms of mortality time ($P < 0.05$). Data evaluation of the selected studies revealed that extracts derived from these tropical plant species recorded mortality time ranging from 0.22 minutes to 779 minutes with extract concentrations between 1.0 mg/mL and 100 mg/mL. Particularly, the tropical plants *Zingiber officinale* (M = 1.75±0.05 mins), *Buchholzia coriaceae* (M = 6±0.9 mins), and *Gynandropsis gynandra* (M = 5±0.2 mins) exhibited remarkably low mortality time, indicating potential toxicological effect on *Fasciola* spp. flukes.

In a study by Soren and Yadav (2020), they explored the in vitro and in vivo anthelmintic concentration-dependent efficacy of traditionally used anthelmintic plants in parasite-animal models. Results showed that the highest concentration (30 mg/mL) of *Cyperus compressus* L. methanolic root extract yielded the fastest mortality rate against cestode *Hymenolepis diminuta*. With the same highest concentration, efficacy was observed against nematode *Syphacia obvelata,* although with a much lower mortality rate. In another in vitro study by Bazh et al. (2013), *Zingiber* and *Curcuma* species exhibited more anthelmintic activity on *A. galli* in plant extract concentrations with higher dosage and increased exposure time. Similarly, a study on the anthelmintic effects of plant extracts from *Melia azedarach* L. and *Trichilia claussenii* C.DC. against egg and larval nematodes produced results of increased inhibition of egg and larval formation and development, as concentrations for the plant extracts increased, exhibiting a dose-dependent response (Cala et al., 2012).

Country of Origin

As seen in Figure 2A, fifty percent (50%) of plants showing high fasciolocidal activity were from Asia, particularly in the Philippines (25%), India (12.5%), and Iran (12.5%). The remaining 50% were from Africa, majority of which from Nigeria (37.5%). In sub-Saharan Africa, the country of Nigeria is one of the four leading livestock producers of the world, wherein the country's cattle and other small ruminants contribute up to 12.7% of the total Nigerian agricultural gross domestic products.

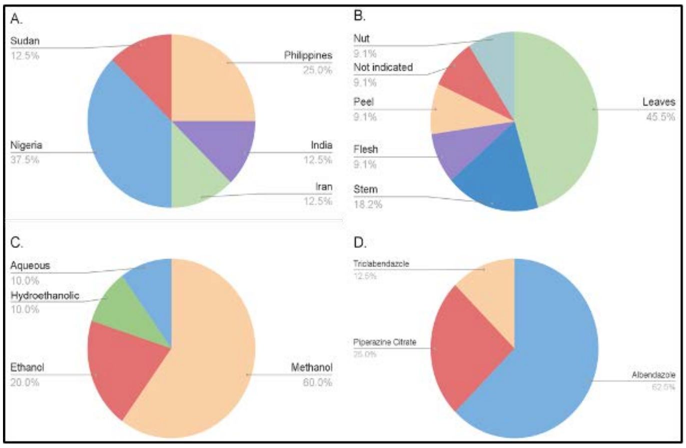


Figure 2: (A) Distribution of studies according to the country of origin (B) Distribution of plants used according to the plant parts. (C) Distribution of solvent used for plant extraction techniques. (D) Distribution of control drugs

Prevalence studies of liver flukes in the country's livestock reached up to 48%, resulting in 32.34% economic loss from 47, 931 infected livers of cattle (Isah, 2019). These economic losses are associated with anthelmintics for treatment, liver condemnation, metabolic diseases and infertility, among others (Ibrahim et al., 2014). In northeast Africa, the country of Sudan showed evidence of endemic bovine fascioliasis in the swampy areas of Northern Bahr el- Ghazal, Jonglei, lakes and central equatorial states. Moreover, the overall prevalence of fascioliasis in the area was 790 of 4,642 which is 17% (Mousa et al., 2013).

Covering the Asian continent and beginning with the middle east, the Islamic Republic of Iran showed evidence that high infection of cattle, sheep, buffalo, and goat with fascioliasis is prevalent in several parts of the country. The first outbreak of human fascioliasis occurred in 1989 in the province of Guilan, wherein 10,000 people were infected. A second outbreak occurred in the same province 10 years later, wherein 5,000 people were infected. Both events revealed a Caspian pattern that describes the specific large-scale transmission of the disease among its residents (Ashrafi, 2015). More recent studies conducted in the province of Guilan revealed that 71.4% of cows were infected with *F. gigantica* and 28.5% with *F. hepatica*, giving a total of 32.1% infected of 445 cows, and an infection rate of 55.2%. Aside from cows, coprological studies conducted in the province of Mazandaran also showed 25.4% of infected cattle and 7.3% of infected sheep (Moghaddam et al., 2004). Contributing factors include the higher amount of rainfall, usage of infested water, and consumption of wild aquatic plants (Salahi-Moghaddam & Arfaa, 2013). In south Asia, the country of India is characterized by having agro-climatic conditions, which differ significantly and as a result, infection with *F. gigantica* varies according to the geographic region of the country. Several studies conducted in north India showed how larger ruminants such as cattle and buffalos have higher prevalence during the winter months, while small- to medium-sized ruminants such as sheep and goats have higher prevalence during the rainy season (Garg et al., 2009). Another study that was conducted in Rajasthan showed seasonal prevalence of the fascioliasis disease, wherein it was at a maximum during monsoon and at a minimum during winter in small ruminants (Swarknar et al., 2021). Consequently, the prevalence rate of infection of *F. gigantica* in domestic ruminants vary significantly from 30% to 80%, depending on the different regions and parts of the country (Lalrinkima et al., 2021). Finally, the Philippines in southeast Asia is reported to have *F. gigantica* as the common species infecting bovines and other ruminants. Investigations using qPCR and FEA-SD revealed high infestation in six different barangays in the province of Northern Samar, wherein 93-97% of 45 cattle and 95-96% of 105 carabaos had fascioliasis (Gordon et al., 2015).

Plant Part Used for Extraction

Figure 2B presents the part of the plants sampled in the selected studies. The most utilized plant part for extraction were the leaves at 45.5%, followed by parts of the stem at 18.2%, and flesh, peel and nut used by only one study

(9.1%). Ghafari et al. (2021) did not indicate the specific part of the plant used for extraction. Analysis of variance revealed that there is a significant difference among the mortality means of different plant part extracts at 100 mg/mL concentration ($Q_c(4,14) = 6.295$, $P < 0.05$). A Tukey post hoc test showed that mortality time was statistically significantly lower in plant leaf extract ($P < 0.05$) and in plant stem extract ($P < 0.05$) compared to plant nut extract. No significant difference was found between flesh and peel plant extract ($P > 0.05$).

A study conducted by Salmeron-Manzano and colleagues revealed that the parts of plants most studied for modern medicinal research, in order of importance, are the leaves, roots, seed, stem, fruit, bark and flower (Salmeron-Manzano et al., 2020). This is because biological activities initiated by various secondary metabolites are distributed in most, if not all, plant organs. Specifically, while polyphenols are distributed in all parts, phenolic acids are found in seeds, leaves, roots, and stems. Furthermore, flavonoids are found in aerial parts, while tannins are found in roots, barks, and seeds (Chiocchio et al., 2021). Among the plant parts in this SR-MA, leaves were the most utilized for extraction and this is because they can be harvested easily without harming the whole plant (Bhat et al., 2013). Leaves are also preferred to be used because they supply a variety of raw materials and are considered to contain bioactive compounds such as tannins and alkaloids (Moshi et al., 2012; Mbuni et al., 2020).

Extraction Techniques

Further, among the solvents used for extraction techniques, the majority of the studies utilized methanol (60%), followed by ethanol (20%), and aqueous and hydroethanolic, each used in one study (10%) as demonstrated by Figure 2C. Three studies (Lasisi & Idowu, 2011; Hossain et al., 2013; Adeniran & Sonibare, 2013) that posed statistically significant mortality time favoring plant extracts (Figure 5) utilized methanol as the main solvent in their extraction method, while one study (Luis et al., 2021) used ethanolic extraction.

Zhang et al. (2018) reported that the efficiency of extraction is enhanced by the particle size of solutes, temperature, and extraction duration. The small particle size of solute increases the penetration of solvent, but too small particles may lead to filtration difficulty due to excessive absorption. Further, the extraction duration is found to be directly proportional to extraction efficiency until it reaches equilibrium. These are supported by a study by Che Sulaiman et al. (2017) where they noted that prolonged exposure at a minimum temperature for a longer period increased the yield while exposure at a maximum temperature caused degradation of compounds.

Moreover, solvents used for extraction techniques of these plant extracts are usually chosen based on the polarity of the solute. It is necessary to choose a solvent of similar polarity to make sure that the solute will be dissolved properly. The polarity from least to most polar of some common solvents is as follows: Hexane < Chloroform < Ethyl Acetate < Acetone < Methanol < Water (Altemimi et al., 2017). Apart from polarity, other factors such as type of plant, part of the plant to be extracted, selectivity, safety, recovery, viscosity, boiling temperature, and availability of solvent affect the type of solvent to be utilized in different studies (Abubakar & Haque, 2020; Pandey & Triphani, 2014). Alcohols such as methanol and ethanol are commonly used as solvents because they are nontoxic at low concentrations, and require small amounts of heat for concentrating the extract (Das et al., 2010). In particular, methanol was noted to be more efficient in extracting lower molecular weight polyphenols from plants due to its polar nature, while ethanol is known as a good solvent for polyphenol extraction and is safe for human consumption.

Comparator Drugs

In Figure 2D, among the three commercially available drugs, the majority of the studies utilized albendazole (62.5%), followed by piperazine citrate (25%) and triclabendazole (12.5%). Further, it was found that there is no statistically significant difference, in terms of mortality time, among these trematocidal drugs ($Q_c(2,6) = 1.244$, $P > 0.05$).

Triclabendazole is the medicine of choice for treating fascioliasis (CDC, 2020). Treatment of human fascioliasis with triclabendazole has been examined for approximately 30 years in various dosages and regimens in a number of geographic regions. Clinical investigations have demonstrated that triclabendazole is beneficial in the treatment of both chronic and acute fascioliasis, as well as in *F. hepatica* and *F. gigantica* infections (Keiser et al., 2005). Since the first human trials, several case reports effectively utilizing triclabendazole in the treatment of human fascioliasis have been documented in a variety of countries such as Bolivia (Villegas et al., 2012), Egypt (El Morshedy et al., 1999), Iran (Talaie et al., 2004), Peru (Maco et al., 2015), and Vietnam (Hien et al., 2008). These include data on adult, child, and adolescent treatment (Gandhi et al., 2019). However, resistance to triclabendazole has been commonly reported in fascioliasis in livestock (Fairweather, 2009). A tiny number of cases of patients failing to react to numerous treatment courses with approved doses of triclabendazole have been recorded in human illnesses (Cabada et al., 2016).

Moreover, the most used comparator drug of choice in the studies was albendazole. Albendazole is an alternative for fascioliasis treatment, however it has poor anthelmintic efficacy against adult flukes 12 weeks and older (McKellar & Scott, 1990; Fairweather & Boray, 1999). A previous report on the efficacy and safety of albendazole against *F. hepatica* infections in goats by Foreyt (1988) showed 95.9% efficacy rate and no signs of toxicity was observed. In recent studies however, albendazole resistance in *F. hepatica* infections in cattle has been published in Egypt (Shokier et al., 2013), Peru (Chavez et al., 2012), Tanzania (Nzalawahe et al., 2018), and Turkey (Elitok et al., 2006). Additionally, the efficacy of the said drug was reassessed by Babják et al. (2021) and the study confirmed that albendazole reduced in its efficacy with a percentage of 77.0-81.8%. Lastly, piperazine is an anthelmintic treatment that is generally deemed safe for usage, despite the fact that it has been phased out in several developed countries due to concerns about probable carcinogenicity and electroencephalographic alterations (Aronson, 2015).

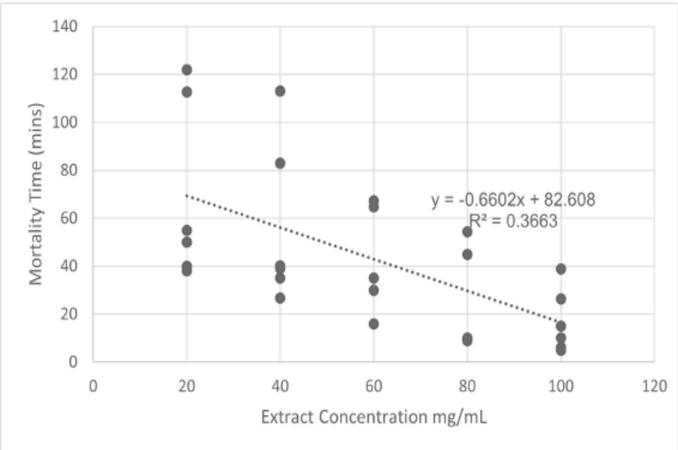


Figure 3: Tropical plant extract concentration (mg/mL) versus mortality time versus mortality time (min) linear regression

Regression analysis results are demonstrated in Figure 3, showing a relationship between the plant extract concentration and the mortality time (R^2 = 0.3663). The trend of the line exhibits that, as the plant extract concentration increases, the mortality time decreases, proving an inversely proportional relationship between the two variables. In consideration of the R^2 value, 37% of the data points pass through the regression model; however, the model cannot be used as a probability for likelihood to predict other outcomes using other extract concentration due to the low R^2 value. The same relationship is exhibited by various extracts of *Corallocarpus epigaeus* (Rottler) C.B. Clarke (methane, hexane, chloroform, and ethyl acetate) against *Pheretima posthuma* (earthworms) in a study by Ishnava and Konar (2020). Another study by Mathias et al. (2021) that explored the anthelmintic activity of *Celosia laxa* extracts against the similar organism *P. posthuma,* showed similar results for the aqueous and ethanolic extracts however the hexanic extract showed no activity on all test concentrations employed.

Relevance of Anthelmintic Plant Extract Studies

Recent surveys of plants used in ethnoveterinary medicine, particularly one in which information was provided by 20 participants that includes including scientists, field staff members from non-governmental organizations, and farmers from Asian countries—Cambodia, India, Indonesia, Laos, Philippines, Sri Lanka, and Thailand—identified 230 beneficial plants, with 23 of them being utilized as anthelmintics for intestinal helminths: 6 for poultry, 10 for pigs, and 11 for ruminants (Allon, 1994). Though these surveys convey relevance to anti-intestinal helminths, none are recorded for liver helminths, specifically for *F. gigantica* and *F. hepatica*. This is indicative of the lack of plant studies against *Fasciola* flukes, which helps conclude that the fasciolocidal activities of such tropical plants provide a step forward for the formulation of a good alternative. Furthermore, albendazole is known to have failed to treat trematode infections such as *F. hepatica* because of the differing kinetics and active metabolite albendazole sulphoxide in humans (Horton, 2000). It is also noted that when administered in high doses for a long period of time, it may cause hepatic injury, congenital abnormalities and eventually damage the fetus (Fahlevi et al., 2021). In addition, inappropriate administration may result in anthelmintic resistance, and has been reported to leave drug residues in meat as well as the environment. In some countries like the Philippines, it has been proven, in water buffaloes, that *Fasciola* spp. has developed resistance to albendazole, triclabendazole, and bromofoenosfos which are all known anthelmintic drugs that are effective against trematodes (Venturina et al., 2015).

Apparently, attempts have been made to test plant extracts against different stages of a parasite life cycle. Even though plant extracts are validated using in vitro anthelmintic activity, the best way to determine the anthelmintic activity of plant extracts and natural compounds is to test them on naturally infected hosts (Jayawardene et al., 2021). Several methodologies have been proposed. One of which is the suggested fecal egg counts by Taylor et al. (2009) that can be conducted weekly or monthly during a grazing experiment. It is worth noting that five plants utilized in the meta-analysis can be found in the Philippines and two of them, namely *Zingiber* officinale and *Areca* catechu as shown in Figure 4 show great potential in its application against *Fasciola* spp.. Apart from its capability to be cultivated with high chances of survival in the country, the following plants are already used for their medicinal properties for other diseases.

As an example, thirteen species of *Zingiber* are endemic in the Philippines, and an additional two were discovered in the province of Sorsogon (Doctot et al., 2019). Two of its several uses include its beneficial effects in reducing pro-inflammatory cytokines of patients suffering from osteoarthritis, as well as reducing cardiovascular diseases and diabetes by controlling body weight, and reducing serum levels of glucose and lipids (Sharifi-rad et al., 2017). Another example is *Areca* which consists of fifty-one species in the Philippines and is highly prevalent in several parts of the country. Over the years, it has shown high significance for its economic contributions to the country such as being a component in the Agroforestry (AF) Systems in Ifugao, and a natural resource for the production of materials, among others (Latap, 2013). For its medicinal value, studies show the effectiveness of root decoction or sap as an alternative treatment for kidney stones, itchiness, and sprains (Tantengco et al., 2018).

Figure 4: *Zingiber officinale* (left; Gupta et al., 2016) and *Areca catechu L.* (right; Peng et al., 2015)

Many plants are used as anthelmintic alternatives in Africa, according to Bizimana (1994), the combination of different plant extracts is perhaps considered one of the most effective fasciolocidal preparations in traditional veterinary medicine. Therefore, it is highly beneficial for these countries that use ethnomedicinal plants to expand the variety of plants available for them to make a highly effective alternative treatment for fascioliasis.

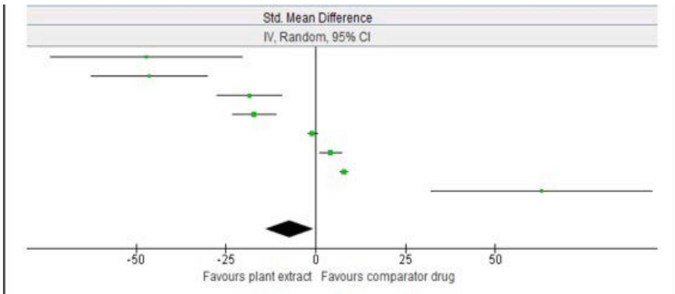
Quality Assessment

All journal articles were assessed using the Toxicological Data Reliability Assessment Tool (TOXRTool). Questions under different criteria: Group I: Test substance identification (4), Group II: Test system characterization (3), Group III: Study design description (6),Group IV: Study results documentation (3), and Group V: Plausibility of study design and data (2) are answered by giving points (1) if the condition is met.

The TOXRTool quality assessment of all the studies across different criterias (see *Appendix B*) as well as overall Reliability Categorization (see *Appendix C*) was tabulated . All articles' scored within the range of 16-18 which falls under the category "Reliable Without Restrictions". Studies classified with these scores are considered as useful and with high relevance for the purpose of systematic review. Included studies are all presented with clearly identified test substance, test, species, administration method, outcomes, and study design.

Meta-analyses

The forest plot in Figure 5 shows the meta-analyzed results of the selected studies. Five studies (Luis et al., 2021; Lasisi & Idowu, 2011; Hossain et al., 2013; Adeniran & Sonibare, 2013; Yamson et al., 2019) showed significant fasciolocidal activity, in terms of mean mortality time, for plant extracts (P < 0.05). The effect size plot of these studies are seen on the left side of the line of no effect (LONE) in the forest plot.



	Plant Extract-Induced Mortality Time (min)			Comparator Drug-Induced Mortality Time (min)					
Study	Mean	SD	Total	Mean	SD	Total	Weight	SMD	95%-CI
Luis et al. 2021	55	0.5	5	240	0.01	5	4.8%	-47.26	[-73.90, -20.63]
Lasisi et al. 2011	40	0.5	10	60	0.3	10	9.0%	-46.46	[-62.55, -30.37]
Hossain et al. 2013	38.83	3.41	6	222.67	12.59	6	13.6%	-18.40	[-27.45, -9.35]
Adeniran et al. 2013	15.81	2.13	10	50.6	1.72	10	15.8%	-17.21	[-23.23, -11.20]
Yamson et al. 2019	0.22	0.0997	5	0.38	0.2003	5	17.8%	-0.91	[-2.26, 0.43]
Ajaiyeoba et al. 2001	6	0.9	4	3	0.05	4	17.3%	4.09	[0.96, 7.23]
Ghafari et al. 2021	1.75	0.05	60	1.001	0.125167	60	17.9%	7.81	[6.74, 8.88]
Ali et al. 2012	779	3.57	6	576	2.22	6	3.8%	63.04	[32.24, 93.83]
Random effects model			**106**			**106**	**100%**	**-7.29**	**[-14.10, -0.47]**

Heterogeneity: $Tau^2 = 67.27$ $Chi^2 = 237.43$, df = 7 (P < 0.00001); $I^2 = 97\%$

Test for overall effect: Z = 2.10 (P = 0.04)

Figure 5: Random effects model forest plot and summary effect

Oppositely, three studies (Ajaiyeoba et al., 2001; Ghafari et al., 2021; Ali et al., 2012) posed significant fasciolocidal activity favoring the comparator drugs (P < 0.05) whose effect size are plotted on the right side of the LONE. All these effect size plots have a corresponding 95% confidence interval (CI). By convention, wider CIs are indicative of less precised results while narrower CIs entail more precision. CIs that do not pass the line of no effect, indicates that the result for that study is significant in the meta-analysis. Furthermore, the diamond plot represents the pooled/summary effect size and CIs (SMD = -7.29 [-14.10, -0.47]). As graphically shown, the pooled estimate plot and its CI (represented by the lateral points of the diamond) did not cross the LONE which is indicative of a significant pooled effect estimate favoring the intervention (P < 0.05). Further, the pooled random-effect estimates of mortality time revealed that the mean mortality time of plant extracts is 7.29 standard deviations lower (-14.10 to -0.47) than in the comparator drugs' mortality time.This lower SD entails that the obtained data, from the eight selected studies with 106 sample flukes, is close to the mean of the entire case population. In addition, the standard deviation differences reflect the different scales of measurement utilized among the meta-analyzed studies and variability among the study populations.

The summary effect is further supported by summary statistics. Specifically, test for overall effect validates the significance of the pooled effect size and CIs ($P < 0.05$). Summary statistics of mortality time data, as also depicted in Table 5, showed a lower mean mortality time in the tested plant extracts (M = 104.6233 ±165.729, 95% CI) as compared to the comparator anthelmintic drugs (M = 128.5168 ±125.775, 95% CI). However, Cohen d guidelines for effect sizes suggest that there is a trivial effect size between the mean mortality time of plant extract intervention group and the comparator group, as shown in the interpretative ranges in Table 2.

Table 5A: Summary of extraction technique used in different fascioliasis phytochemical studies

Extraction Technique	M (Mortality Time in min)	P-value
Methanolic	176.32	0.0857
Ethanolic	91.74	
Hydroethanolic	1.75	
Aqueous	857	

Table 5B: Summary of Comparator Drugs used in the different fascioliasis in vitro phytochemical studies

Comparator Drug	M (Mortality Time in min)	P-value
Albendazole	537.34	0.125
Piperazine Citrate	23	
Triclabendazole	28.38	

Focusing on fasciolocidal activity, the synthesized scientific information of this review paper showed that there is a significant difference ($P < 0.05$) favoring the plant extracts supports the great potential of plants as alternative anthelmintics. This is in congruence with a study by Anuracpreeda et al. (2017) which demonstrated that minimal concentration of *Terminalia catappa* L. extract caused equal or more damage characterized by swelling, blebbing, loss of spines, and disruption of total tegument in *F. gigantica* compared to triclabendazole. Several researches within the past ten years suggest that plants with anthelmintic properties affect multiple morphological, physiological, and metabolic targets. The chemical compounds found in plant sources are classified as primary and secondary metabolites on the basis of their chemical structure and metabolic derivation (Selvaraju & Dhanraj, 2019).

Secondary metabolites are the most important substances as novel alternatives for parasite management and those with anthelmintic properties are proven to reduce motility, as well as create epidermal lesions, degrade esophagus and gut tissues, decrease egg production in females, inhibit eggs from transforming into larvae, and cause death within 24–72 hours (French, 2017). Because of this, the chances of finding bioactive chemicals with anti-fluke capabilities increase. These metabolites such as alkaloids, saponins, skimmiarins A and C, tannins, flavonoids, sterols, and terpenes have been demonstrated to have antiparasitic activity against a variety of parasites (Alvarez-Mercado et al., 2015).

Specifically, Fahlevi et al. (2021) reported that alkaloids are proven to break down cell walls, proteins, and lipid content of the worms, which leads to death; flavonoids inhibit the NAD+ catabolizing enzyme that regulates calcium content, which interferes with the motility of the worms; saponin increases calcium activity, triggering an increase in muscle activity that eventually causes paralysis to the worms; tannins cause lysis of cell membranes due to destruction of membranes and proteins; and triterpenoid compounds inhibit thioredoxin glutathione reductase, an enzyme which plays a role in cellular processes such as DNA synthesis, ceasing the reproduction of worms. In addition, condensed tannins may function against parasites in a variety of ways, including impairing parasite feeding and reproduction activities as well as disrupting its cuticle (Abbas et al., 2020). Numerous studies also demonstrate that saponins have a cytolytic effect on parasites via affecting membrane-associated sterols and increasing cell permeability (Geidam et al., 2007).

In the Zingiberaceae family, *Zingiber officinale* Roscoe is one of the most extensively utilized herbs. This plant's therapeutic potential has been documented in recent years. Ginger has long been used to treat throat infections, and it has been shown to inhibit a wide spectrum of harmful germs. In previous investigations, the phytochemical content of *Z. officinale* has been extensively evaluated (Ali et al., 2008). The principal phytochemical categories found in *Z. officinale* are essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids, and tannin, which play significant roles in its medicinal properties. Methanol extracts of *Z. officinale* were tested for *in vitro* anthelmintic action in ruminant nematode research. The results showed that *Z. officinale* was 100% effective in killing all of the test worms within two hours of exposure, which may indicate the potential of the plant for anti-fascioliasis activity (Kumar et al., 2011).

Areca (*Areca catechu* L.) phytochemical compounds include 20% polyphenol, 0.5% alkaloids, 15% fat, 20% starch and mineral content (Amudhan et al., 2012). On flukes, Areca had more severe effects, including abrupt paralysis. Areca extract caused mortality (scoring 0) at 5% concentration in a span of 30 seconds, showing potential fasciolocidal activity. Moreover, according to Yamson (2019), after exposure to *Fasciola* spp., betel nut extract had stronger fasciolocidal activity and no movement upon contact than albendazole at 10%, 20%, and 40% extract concentrations.

Buchhlozia coriacea Engl., also known as the "Musk Tree", and *Gynandropsis gynandra* (L.) Briq., also known as spider flower or cat whiskers, are both part of the Capparacaea family. Both plants have been utilized in African traditional medicine for many years, with various elements such as the bark, leaves, and even the leaves being employed. The seed of the plant tree possesses anti-pathogenic properties, according to different studies. Constipation, hypertension, poor sperm count, malaria, and typhoid are just a few of the problems they can help with. The chemical constituents of *B. coriacea* include alkaloids, saponins, resin, flavonoids, cyanogenic glycosides, cardiac glycosides, tannins, acidic compounds, steroids, and terpenoids (Duru et al., 2012). Most of which are compounds known to elicit anthelmintic properties. A study by Nwankwo et al. (2019) states that *B. coriacea* had a complete anthelmintic action. *B coriacea* was found to entirely eradicate *Ascaris lumbricoides* and *Trichuris trichiura* in all concentrations evaluated. For *G. gynandra*, its phytochemical properties included the presence of tannins, alkaloids, flavones, sugar, phenolic compounds, saponin, amino acid, and essential oils. All similar to that of its family member, *B. coriacea*, *G. gynandra* exhibits anthelmintic compounds (Borgio et al., 2008).

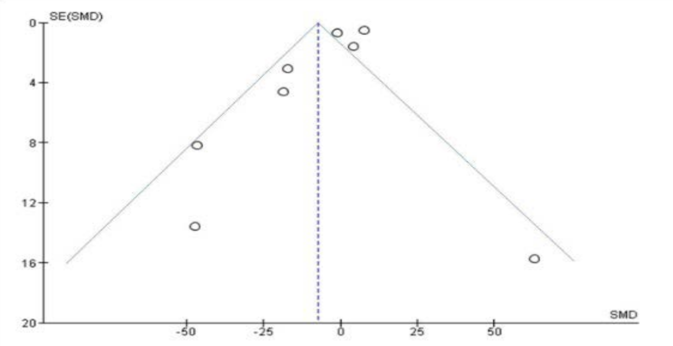
Determination of Studies' Weight & Heterogeneity

Weights of the study are also reported in Figure 5. Ghafari et al. (2021), Yamson et al. (2019) and Ajaiyeoba et al. (2001) exhibited the greatest percentage (>14%) weight among the selected studies. This suggests that these three studies have the greatest influence on the overall/summary effect, and possess the greatest precision. Test for heterogeneity (I^2) suggests that there is 97% statistical heterogeneity, which means that the effect sizes vary significantly among each other ($P < 0.05$). In consideration of the scope of the study, the potential evidence and likely causes for the high heterogeneity include differences in experiment design, administration methods, measurement instruments, concentration of interventions, timing of outcome measurements, and analytical methods. Meta-analyses addressing broad scope of research questions, such as the evaluation of plant species within a vast geographical confinement and diverse environments may assemble highly heterogeneous studies, similar to the meta-analysis conducted by Sabitova et al. (2020). Further, Alba and colleagues (2016) reported that meta-analysis of continuous outcomes (e.g. intervention dosage, drug response in terms of time) tend to have high statistical heterogeneity.

High degree of heterogeneity leads to the possibility of interpretative challenges (Higgins et al., 2002); however, the use of the RE model addresses these statistical challenges (Serghiou & Goodman, 2019; Schroll et al., 2011). Hence, in such cases, a random-effects model is favored over a fixed-effects model (Barili et al., 2018). Meta-analyses have revealed that the outcomes of the research included are frequently heterogeneous (Field, 2005; Higgins et al., 2003). To account for the heterogeneity in studies, one approach is to assume that it is completely random (Viechtbauer, 2007).

Assessment of Bias Risk and Sensitivity Analysis

Egger's test revealed a statistically significant symmetry in the effect estimate plots which is indicative of low risk of publication bias ($P > 0.05$). The produced funnel plot can be visually confirmed through the comparison of the locations of the studies within the scatter plot.



Egger's test

	P-value	Intercept	95% CI	Comment
Plant Extract vs. Control Drug	0.6956	1.6207	-8.0352 to 11.2765	No publication bias

Figure 6: Egger's test results and funnel plot

As shown in Figure 6, effect estimate plots of the majority of the included studies are seen localized and scattered inside the pyramid due to sample variation which suggests that studies with smaller study size dominate bigger studies, in terms of producing statistically significant results. Further, the positive result of the Egger's test of asymmetry between the effect estimates of the intervention group and the control group ($P > 0.05$) from the selected studies does not further necessitate the need of sensitivity analysis. As the name suggests, publication bias is caused by selective publication of studies with statistically significant results and positive direction, as compared to weak and insignificant studies (Ayorinde et al., 2020). So to speak, negative results are less likely to be published, hence available studies in bibliographic databases tend to be a biased sample which can lead to inflation of effect sizes (Ropovik et al., 2021). Joober et al. (2012) identified that highly competitive environment for career promotion and grants, and citation index incite research to mainly release positive results for publication. This results in publication bias which has a detrimental effect on the integrity of knowledge, particularly in conjecturing relationships between variables, as well as the purpose of hypothesis testing (Joober et al., 2012). Nair (2019) reported that there is clinical importance to studies with negative results in such that there is the knowledge of having insufficient evidence to accept the formulated hypothesis which means it is not failed research.

CONCLUSION AND RECOMMENDATIONS

This SR-MA confirmed the fasciolocidal activity of tropical plant extracts against different developmental forms of *Fasciola* spp. In *in vitro* setup, extracts of interest have lower standardized mean mortality time compared to commercially accessible anthelmintic drugs, such as Albendazole. This finding supports the possible use of tropical plant extracts as alternative phytotherapy against fascioliasis, the disease caused by *Fasciola* species. Specifically, these include the tropical plants *Zingiber officinale*, *Buchholzia coriaceae*, and *Gynandropsis gynandra* which induced remarkably low mortality time. Potential factors affecting the mortality time of intervention-exposed liver flukes and overall toxicological effect of these extracts include plant parts used in extraction, plants' geographical origin, extract concentration, and extraction technique. Sixty percent of plants showing high fasciolocidal activity are from Asia, particularly in the Philippines, India, Iran and Thailand.

With that, the authors suggest exploring other outcomes of interest could be covered such as motility index, LC50, and LC99, alongside mortality. Also, studies examining plants of different origin other than the tropics could also be synthesized. To further expand the research, the addition of more bibliographic databases and more inclusive study selection criteria are recommended to increase the number of studies included in the meta-analysis. Also, aside from the use of commercial drugs as positive control, a negative control may be added to further strengthen and establish the experimental variable as cause of results. Lastly, the authors view that more toxicological studies involving efficacy of plant extracts tested on different helminths, as well as the characterization of phytochemicals, should be done.

Supplementary Data – Methodology Search Strategy

Information Sources & Eligibility Criteria for Inclusion: Population-Intervention-Comparison-Outcome (PICO) framework was utilized in framing the main research question of the present study, as suggested by Munn et al. (2018). The main field of study that was considered in this review paper included controlled experimental studies of fasciolocidal activity of the crude extracts of tropical plants against *Fasciola* species. Specifically, selected studies possess the following attributes: i) original and freely available articles with full manuscript published up to November 2021, ii) presented in the English language, and iii) conducted in a tropical country/locale in congruence with the geographical scope of the study. Further, articles of interest were retrieved from the following electronic databases: PubMed, Springer, Science Direct, and Google Scholar. In terms of study coverage, studies eligible for inclusion involved the following: i) extracts derived from tropical plants, ii) *Fasciola* species and fascioliasis, iii) at least one comparator treatment as a control group, iv) anthelmintic assay result of mortality.

Search strings, as shown in Table 1, were utilized with Boolean operators, truncations, and field tags (i.e. [tw], [tiab], & [all]). In addition to the search strings, subject heading indexing (i.e. MeSH in PubMed) was also utilized to find eligible studies. Advanced search options (e.g. search builders) of each database were fully utilized in order to have higher capture and inclusion of relevant studies. In the case of a wide array of available studies, outcome terms were included to increase the specificity of the inclusion criteria. Conversely, limited sources from the initial screening meant that outcome terms will not be included. Studies within the criteria of inclusion were inspected with keywords in abstracts and study titles to screen eligibility for full-publication review.

Selection Process: The inclusion criteria of articles in the meta-analysis included in vitro experimental studies involving plant extracts, anthelmintic bioassay, anthelmintic comparator drugs, and *Fasciola* flukes. Relevant studies involved the key terms in their abstract or title for the initial screening. Specifically, studies that mention plant extracts, with or without phytochemical screening and characterization, and fascioliasis or *Fasciola* flukes, regardless of species, fluke size, and fluke developmental stage, were retrieved. Studies encompassing other helminths or trematodes were excluded. Furthermore, outcomes of interest were limited to time of death or mortality time. Also, control treatment characteristics (i.e. type of fasciolocidal drug & dosage) were obtained. Studies incorporating other assay or outcomes were set outside inclusion. In similar terms, parallel publications with abstracts or titles that did not indicate these key terms were excluded. In terms of article availability, studies were included if full manuscripts were freely available and not limited to the articles' abstract. The present study was not encompassed by books, book chapters, reviews, editorials, news, response of authors, conference papers, and letters.

Data Extraction: Five researchers independently extracted and gathered data from studies encompassed under the criteria of inclusion. After the initial abstract and title screening, full-text publications were downloaded. Articles meeting the eligibility criteria after full-publication review were compiled and included in a reference database using Microsoft Excel 2020. Outcomes of interest, specifically *Fasciola* mortality time, were the main targets for extraction. Other information that was extracted included the following: name of authors, publication year, study locale/country, study design, *Fasciola* flukes' characteristics, control treatment characteristics, and plants' description. In such cases where unclear or missing information was encountered in a study, the researchers inquired the study author, through email, regarding the concern. After a minimum of two attempts of email inquiry, uncleared information, leading to incomplete necessary data extraction, were marked as "no data available". Further, counterchecking, and round table discussion was employed to promote accurate and consistent data collection in instances of disagreement among researchers.

META-ANALYSIS

Tabulation and Data Synthesis: In Microsoft Excel 2020, the database spreadsheet was categorized as follows: (1) each row represented a study while each column will delineated study parameters, (2) columns were subdivided by bibliographic data (authors, publication year, country, database), *Fasciola* characteristics (species and developmental stage), plant extract characteristics (concentrations, type of extract based on method, and plant source), outcome (mortality time), control treatment characteristics (i.e. type of fasciolocidal drug & dosage), and quality assessment. In anticipation of possible human error, data counterchecking was observed. Further, extracted data in the extraction sheet were cleaned in such a format that it could be read by RevMan 5.4.1 (The Cochrane Collaboration, 2020) and R software (R Core Team, 2020).

Effect Measures: Fasciolocidal activity measures have continuous outcomes; specifically, the results of mortality time of *Fasciola* flukes after exposure to test extracts that lead to the mortality of the flukes' total population. As suggested in Cochrane Handbook for Systematic Reviews of Intervention (2021), continuous outcomes were measured as standardized mean difference (SMD) and 95% confidence interval (CI) providing those selected studies involved different scales (e.g. mortality time). SMD was advantageous in this meta-analysis as this tool standardizes mean differences into a common scale (e.g. different extract and drug concentration). Interpretative ranges of values for standardized mean difference based on Cohen's d guidelines (Cohen, 1988) are elucidated in Table 2.

Meta-analysis Model: To assess the summary effect, the random-effects (RE) model was used in meta-analyzing extracted results. This was in consideration of the possible variation of effect size due to inter- and intra-study differences (e.g. sampling frame, experimental conditions, human error, utilization of techniques) (Cochrane Handbook, 2021). In ecological statistics, random effects models are particularly suitable in such cases of: 1) multiple levels (e.g. various species or units); 2) minimal data is presented about the species being examined; 3) uneven sampling across species (Fox et al. 2015).

Inverse variances of effect estimates procedure was performed using the software RevMan 5.4.1 in order to obtain the weight of studies and evaluate their contribution to the total meta-analysis information. Also, RevMan 5.4.1 was utilized in the calculation of SMD, and the visual representation of results in the form of *forest plots*. *Forest plot* represented all included studies with a confidence interval of effect size at 95%. Such plots were interpreted as reported by Lester (2014).

Subgroup Analysis: Analysis of variance (ANOVA) was utilized in analyzing the difference of means, as per the guideline of Borenstein et al. (2009), among the following subgroups: 1) plant parts used for extraction, 2) different anthelmintic comparator drugs, and 3) solvents used in extraction techniques. Tukey's post hoc test was used in assessing the significance within these subgroups and to determine where the significant mean differences existed. Test of association using logistic regression between concentration and mortality time was also conducted. PAST4.03 was the software of choice in running analysis of variance.

Test of Heterogeneity: I^2 statistic was used to evaluate the statistical heterogeneity of the extracted outcome data. This considered the total variation proportion among studies as inter-study variation (heterogeneity) and not as intra-study variation (chance). Interpretation of I^2 statistic based on magnitude were as follows: low (I^2 <25%), medium (I^2 = 25 to 50%), or high heterogeneity (I^2 >50%) which will be considered as substantial (Higgins et al., 2003). RevMan 5.4.1 was used in computing for I^2 statistics.

Quality & Risk of Bias Assessment: Toxicological data reliability assessment tool (ToxRTool) was utilized in conducting quality assessment of the in vitro experimental studies. Guidelines of ToxRTool were followed as suggested by Schneider et al. (2009) and were performed independently by all of the researchers. Further, reporting bias was assessed and graphically represented using Egger's funnel plot asymmetry test. In the funnel plots, scattered or distributed plots (symmetrical) were interpreted as low risk of bias while heavily skewed or clustered (asymmetrical) plots were considered as high risk of bias (Sterne et al., 2011).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

NOVELTY STATEMENT

The novelty of our review paper consolidates and meta-analyzes findings from existing literature regarding the overall phytotherapy potential of tropical plant extracts against fascioliasis. Many researchers have explored anthelmintic properties of different plants using various bioassays against other helminth genera. Progressively, this systematic review focuses on Fasciola flukes— a well-known cause of fascioliasis among ruminants and humans in tropical countries. In these regions, tropical plants are traditionally used as herbal medicine and treatment alternatives for different local diseases. In line, the considerable ranges of mortality time and strong in vitro fasciolocidal properties of tropical plants, obtained from systematic review with meta-analyses, poses as literature to support medicinal plant alternatives and advances for possible drug discovery.

AUTHORS CONTRIBUTION

All authors designed the study, gathered the materials and wrote the manuscript. ACC and CLT reviewed the manuscript. All authors read and approved the final manuscript.

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