LC-MS analysis of various extracts from different parts of Cassia fistula (Linn.) for identification of Phyto-constituents responsible for anthelmintic activity

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Abstract

Ethnopharmacological relevance:

Medicinal plants have always assisted through the ages in conflict resolution of global health problems of humans and livestock caused by intestinal gastrointestinal parasites. Ethno-medicinal studies associated with ' traditional uses have revealed the application of different parts of *C. fistula* for curing helminth infections.

Aim of the study:

Current research was carried out to screen the anthelmintic properties of *C. fistula* against gastrointestinal parasites of sheep and goats and to carry out a detailed phytochemical profile of different extracts of *C. fistula*.

Materials and methods

The anthelmintic effectiveness of various parts of *C. fistula* (fruits, stem, root, and leaves) was studied using two-fold dilutions of all extracts considering 50 mg/ml as the mother solution. Each Petri dish containing triplicate doses of each plant extract and active mature worms (n = 10) was observed for 12 hours to study the worm's motility. In the egg hatch assay, fully developed female worms were crushed to release eggs, filtered, diluted until 200 eggs/ml in each of 24 multi-well plates, and incubated in a refrigerator for 48 hours. The highly effective fruit ethanol extract and crude fruit powder of *C. fistula* were further processed for i*n vivo* anthelmintic screening in goats (n = 21) and parasitic eggs reduction in fecal samples was observed by MacMaster technique for 9 days at the dose rate of @250, 500 & 1000 mg/Kg body weight. The quantitative analysis of bioactive components of all extracts of *C. fistula* was carried out using the LC-MS method. It was carried out in ESI ionization mode using both positive and negative polarities.

Results

The ethanol fruit and ethyl acetate leave extracts of *C. fistula* revealed a highly potent effect, caused the complete fatality of adult worms (10.00 ± 0.00) within post contact of 4 and 6 hours at the highest dose of @50 mg/ml correspondingly exhibited comparable activity with standard drugs Levamisole. The highest eggs egg inhibition potential was revealed by fruit ethanol extract (90.33%) and the weakest with root methanol extract (60.67%). In the study of *in vivo* trials, the highly potent ethanol fruit extract administered to goats effectively reduced parasite load with the highest efficacy percentages of 79.27% and 90.78% at the dose of 500 and 1000 mg/Kg b.wt. of fruit ethanol extract of *C. fistula*. The phytochemical constituents detected using LC-MS analysis belong to class; flavonoids, phenols,

terpenoids, alkaloids, sterols, glycosides, fatty acids, amino acids, esters, and alkanes, and the maximum number of compounds reported belong to class flavonoids, phenols, and alkaloids exhibiting their maximum role in anthelmintic activities.

Conclusions

The overall findings intensely indicate the anthelmintic potential of all parts of *C. fistula*, specifically of its fruit part. Further, the toxicity study of different extracts of various parts of this plant is desired to assess the optimal non-lethal dose for curing parasitic diseases in livestock as well as in humans.

1. Introduction

Livestock performance and production are inversely affected by the financial costs of gastro-intestinal parasites leading to significant losses in their productivity, longevity, infertility, and survivorship (Oliveira, 2021c). Among the gastro-intestinal helminthiasis, heamonchosis is of high economic worth on the global index that constraint the fertility and continuity of small ruminants (Zajickova et al., 2020), influences the immune system, hematology, and biochemical parameters of host animals, eventually causing inefficient digestion, sustained blood losses, protein depletion and even death of host animals (Charlier et al., 2018). The chief influential causative parasite of heamonchosis, *Heamonchus contortus* (*H. contortus*) leads to a production loss of about ten billion dollars annually to the veterinary market (Bibi et al., 2017). It is considered one of the principle resilient parasite parasites having high pathogenicity, prolific nature, and prompt growth in grazing ranges within a short period (Beleck et al., 2021).

Parasite control was exclusively reliant upon the application of synthetic anthelmintic, dietary, and grazing management. However, the emergence of parasite resistance upon commercial drug acquaintance restricted the use of these standard drugs (Vineer et al., 2020). Besides these, chemical anthelmintics have many toxicity problems, adversative impacts, high economic, costs, and are not easily accessible. The multi-resilient parasite of veterinary concern, *H. contortus* has developed resistance against all broadly used anthelmintics i.e. ivermectin, thiabendazole, benzimidazole, imidazothiazole (Doyle et al., 2020).

Consequently, there is ever present and imperative need to search for alternative consistent ways of parasites (Verma et al., 2018). Nature has provided the plant kingdom with a variety of bioactive substances such as alkaloids, amino acids, poly-acetylenes, terpenoids, sugars, and flavonoids for their defense against pests, viruses, insects, fungi, and bacteria (Rizwan et al., 2019). Further, these plant-based anthelmintics possess fewer or no toxic impacts, are easily accessible, low cast, and have a safe and simple mode of medication, preparation, and application (Ahmed et al., 2023). Besides, there is prolonged affiliation among the concomitance of plant-based medicines, parasites, and humans.

Precise standardizing techniques are in use to search plant's secondary metabolites and to assess their anthelmintic efficacy. The anthelmintic activity of various plants even amongst various parts of the same plants diverges according to their different bioactive compounds, regional, cultivar, or seasonal variation, and various solvents used for their extraction (Ahmad et al., 2020). The peculiarity of in-vitro systems becomes more obvious when revealing plant extract effects upon diverse life phases of parasite; egg, larva, and adult by applying egg hatch assay, larval motility inhibition assay, larval ex-sheathment inhibition assay, and adult-motility assay (Liu et al., 2020).

Liquid chromatography-mass spectrometry (LC-MS) is a key analytical technique used for the quantification, mass analysis, and identification of a wide range of semi-volatile or non-volatile inorganic and organic components within a mixture. It is a particularly effective technique for analyzing complex botanical extracts. It is a highly advanced and powerful tool for detecting both low and high-molecular-weight analytes. One notable advantage of Electrospray Ionization (ESI) in LC-MS is its ability to achieve soft ionization (Kumar et al., 2020).

Cassia fistula (*C. fistula*) of the current study, commonly known as Amaltas is an angiosperm, that belongs to the family Fabaceae. Amaltas is a medium-sized beautiful tree with bright yellow flowers and dark green composite leaves (Ruth et al., 2021). The native residents of the Cholistan desert (Roohelay in the local vernacular) are living nomadic lifestyles and use this plant as a medicament and therapeutic mediator for the prosperity of their livestock. Ethno-veterinary assessment by Siddique et al. (2022) documented the traditional uses of *C. fistula* for the treatment of internal parasites of livestock. The preliminary anthelmintic efficacy of *C. fistula* leaves was revealed using its ethanol and n-hexane extracts against infective larvae (L3) of *H. contortus* by applying a larval migration inhibition assay (Wahyuni et al., 2019). Present research would provoke further toxicity studies of this plant to develop the cheaper, more effective, locally accessible, and optimal non-lethal dose of *C. fistula* for dealing with gastrointestinal nematodes, specifically of the most pathogenic parasite, *H. contortus* of small ruminants.

2. Methodology

2.1. Collection and processing of plant samples

The plant was registered as *Cassia fistula* (voucher # 18-01-025), Department of Botany, Faculty of Sciences, University of Agriculture Faisalabad, Pakistan. Fresh roots and leaves adhering to soft branches of the stem and fruits of *C. fistula* (2 kg) were collected from January to February 2023 from the area of the Cholistan desert and identified by a taxonomist of the Cholistan Institute of Desert Studies. Collected plant parts were separated and washed under fresh water to get rid of all forms of dust and contaminations. Then completely dried under shade places at room temperature (25°C), chopped and crumpled into sufficient coarse particles in a mechanical grinding machine.

2.2. Preparation of plant extracts

The different parts of *C. fistula;* root, stem, leaves, and fruits were extracted successively in methanol, ethanol, ethyl acetate, and aqueous methanol according to the procedure adopted by Rehman et al. (2021) following minor amendments. Plant powder of different parts (each about 100 g) was soaked into 400 ml ethanol in a bucket concealed with a lid and stirred occasionally for 3 days. Then soaked material was filtered through a soft porous piece of cloth and residual plant material was diluted again in 400 ml ethanol above-mentioned procedure was regurgitated for the 2nd and 3rd times. Finally, the filtrates were combined and solvent evaporation from filtrates was carried out in a rotary evaporator at 40°C under reduced pressure. Crude ethanol extract in syrup form appeared, which was preserved in the refrigerator at 4°C until carried out for further analysis. described procedure was reiterated for the preparation of extracts in methanol, ethyl acetate, and 70% aqueous methanol (methanol and water in 3:1 ratio).

2.3. In-vitro anthelmintic assays

2.3.1. Adult motility assay (AMA)

Adult motility assessment was performed according to the procedure described by Rehman et al. (2021) with slight modifications. The mature *H. contortus* worms were immediately collected in phosphate buffer saline (PBS) just after evisceration of a freshly slaughtered sheep. About 10 active mature worms were moved into isolated Petri dishes having different concentrations of each part of the plant extract. Then two more separate petri plates were also encompassed for positive (PBS; 20 ml/petri-dish) and negative (Levamisol @0.55mg/ml) control test. Plant extract doses (50, 25, 12.5, 6.25, 3.125 mg/ml) were deliberated by following a two-fold serial dilution procedure by considering a 50 mg/ml dose as the mother solution. Then three repetitions of each dosage of plant extracts and control groups were conducted at 25–30°C. The worm's paralysis criteria were indiscriminately applied to investigate anthelmintic consequences of various extracts of *C. fistula*, under the inverted microscope at different time intervals of 0, 2, 4, 6, 8, 10, and 12 hours. The immobile parasites revealing no motility in the area of the tail and head were picked out in Luke warm saline for about 5 minutes. Parasites renewing their movement were deliberated as alive, if not, recorded dead.

2.3.2. Egg hatch assays

Egg hatch assessment proceeded according to the guidelines of "World-Association for Advancement of Veterinary-Parasitology" (Rehman et al., 2021) with slight modifications. Fully grown female worms were firstly isolated and washed in saline solution, then crushed to release eggs in a pestle and mortar having 5 ml PBS. The egg-saline solution was filtered by a mesh sieve (80µm). The collected mother fluid was diluted until there were 200 eggs/ml. the three repetitions of each dosage of plant extracts (50, 25, 12.5, 6.25, 3.125 mg/ml) were conducted in 24 multi-well plates by following a two-fold serial dilution procedure considering 50 mg/ml dose as the mother solution.

The labeled plates were incubated in a refrigerator for 48 hours at 27 ^{O}C and relative humidity (70%). Post incubation of 48 hours, un-hatched eggs and hatched larvae (dead/live) were counted under a

compound microscope at a magnification of X40. The number of total eggs-hatch inhibition rates was calculated by following the formula;

$$\%\text{age Egg-hatch inhibition} = \frac{(\text{Total number of eggs}) - (\text{Number of hatched larvae})}{\text{Total number of eggs}} \times 100$$

2.4. In vivo anthelmintic screening of C. fistula fruit ethanol extract

The in vivo anthelmintic assessment of crude fruit powder and the ethanol fruit extracts of *C. fistula* was carried out as per the methodology of Badar et al. (2021) with minor modifications. A total of 21 goats of either sex (male/female) weighting 16–25 kg with aged between 7–10 months previously infested with mixed parasites species of gastro-intestinal nematodes were selected for in vivo anthelmintic screening of fruits of *C. fistula*. The goats were kept on sufficient fodder, feed, and concentrates. The goats (n = 21) were divided randomly into 7 groups (3 goats/group). Group 1 (T1) served as the control group while receiving no treatment. Groups 2,3 and 4 were designated as T2, T3, and T4 treated with 250, 500, and 1000 mg/Kg b.w. of crude fruit powder correspondingly. Groups 5, 6, and 7 were designated as T5, T6, and T7 treated with 250, 500, and 1000 mg/Kg b.w. of ethanol fruit extract respectively.

The collection of fecal samples from each of the animals was carried out directly from the rectum in the morning, starting from pre-treatment (0 day) to post-treatment (days; 0, 5, 7, 9). The nematode eggs per gram (EPG) of fecal samples were counted following the McMaster technique for 9 days. To measure the reduction in fecal eggs, about 2 g of fecal samples were separated in a beaker, stirred in 30 ml of pure tap water to dilute the fecal sample, and then shaken in 30 ml of the saturated salt solution. A small quantity of diluted fecal sample withdraws with a pipette run into the McMaster counting chamber. The McMaster slide was kept to stand for a while countenancing the eggs of the parasite to float under the upper surface of the McMaster chamber and observed under the microscope using low power eyepiece (X6) and objective (X10) and the total number of eggs within each filled area were counted up.

2.5. Animal ethical

All the experimental procedures were carried out after approval by the Animal Ethics Committee of Islamia University of Bahawalpur (Approval No. 456/ORIC) and were in accord with the requirements of the Institutional Animal Care and Use Committee (IACUC). The Islamia University of Bahawalpur has recruited the research reviewed all aspects of ethical issues concerning its policy and confirmed that the research procedures were conducted according to established animal welfare guidelines.

2.6. Liquid chromatography-mass spectrometry (LC-MS) analysis

The LC-MS process was conducted following the methodology of Kaur et al., 2022. LC-MS analysis was conducted in a sophisticated systematic instrumentation facility at textile testing international (tti) labs in Lahore, Punjab, Pakistan. The liquid chromatogram system used was the UPLC and acquity H-class

series, with separations carried out on an acquity of BEH-C18 column (2.1 × 100 mm, 1.7 µm). The mobile phases were a mixture of formic acid (0.1%) in water (A) and formic acid (0.1%) in acetonitrile (B), with a flow rate of 0.25 mL/min. The graded elution profile was as follows (Time - A/B): @0 minutes – 90/10, @2 minutes – 90/10, @5 minutes – 80/20, @10 minutes – 70/30, @12 minutes – 50/50, @14 minutes – 90/10, and so on. A 4 µL sample was injected into the column, and then column temperature was maintained at 30°C.

Mass spectrometric examination was carried out using a TOF-MS equipped with an ESI source. The instrument was set at: Cone Gas with a flow rate of 30 L/h, drying or Desolvation gas (Argon and N2) flow rate at 850 L/h; 450°C desolation temperature, 120°C source temperature; 3.22 kV capillary voltage, 50V cone voltage; 4 eV collision energy. Each sample was analyzed in positive ion mode. The secondary metabolites Identification in *C. fistula* extracts was based exclusively on LC retention time and high resolution of mass spectra.

2.7. Statistical analysis

The recorded data obtained from adult motility assays was subjected to One-way ANOVA using SPSS and the percentage (%) of unhatched and hatched eggs was analyzed by applying the formula Ieggs = 100 × (1 – Ptest/Pnon-treated), P-number of eggs hatched (Devrnja et al., 2021). Duncan-multiple-range test (DMRT) was applied to calculate significant differences among different groups. Eggs per gram (EPG) of fecal samples and the effectiveness of various treatments were calculated by the following the formula;

Eggs per gram = Total eggs in 2 chambers * dilution factor/ 0.3

***Dilution factor = Total suspension volume in ml / Total feces volume in g**

Efficacy = EPG before treatment – EPG after treatment /EPG before treatment

The molecular mass and name of phytochemicals and their characterization and identification databases were carried out using various authentic search engines such as MELTIN and HMDB (Human Metabolome Databases).

3. Results

3.1. Percentage yield of extracts

In the current study, the solvent extraction of different parts of *C. fistula* extracted percentage yield as follows; fruit's ethanol extract (12.5%), fruit's methanol extract (13%), fruit's ethyl acetate extract (9.2%), fruit's methanol extract (8%), roots ethanol extract (6.5%), roots methanol extract (7.4%), roots ethyl acetate extract (9%), roots aqueous methanol extract (8.5%), stem ethanol extract (7%), stem methanol extract (9.8%), stem ethyl acetate extract (9.1%), stem aqueous methanol extract (7.5%), leaves ethanol extract (11%), leaves methanol extract (11.5%), leaves ethyl acetate extract (7.5%) and leaves aqueous

methanol extract (7.5%). The highest plant extract yield was recorded by fruit methanol extract and the lowest one by roots ethanol extract. In the study of in vivo screening, the fruit ethanol extract yielded 14.25%. The extract yield percentage was calculated by dividing the weight of plant powder by the weight of plant extract (%w/w *100).

3.2. Adulticidal effects

The adulticidal assessment of various extracts of *C. fistula* in comparison with PBS and Levamisol against the adults *H. contortus* are presented in Figs. 1, 2, 3 & 4. The highest anthelmintic potential was revealed in fruit ethanol and leaves ethyl acetate extracts of *C. fistula* at the highest concentration of @50 mg/ml, causing complete paralysis and fatality of adult worms (10.00 ± 0.00) within post-exposure of 4 and 6 hours correspondingly revealed comparable activity with standard drug Levamisole

The anthelmintic effectiveness of tested *C. fistula* extracts against mature *H. contortus* worms was observed to follow this order: fruit ethanol extract (4 h) > leaves ethyl-acetate extract (6 h) > fruit methanol and ethyl-acetate extract = root ethanol extract (8 h) > fruit aqueous-methanol extract = leaves ethanol extract = root ethyl-acetate, methanol & aqueous-methanol extract (10 h) > stem ethanol-methanol-ethyl acetate extract = leaves methanol extract (12 h). It was observed that the fruit part of *C. fistula* is more potent as compared to the root, stem, and leaves. The effectiveness of all extracts was increased considerably following time and dose-dependent phenomena, while $p < 0.05$. The highest tested dose @50 mg/ml was found to be more potent. Levamisole paralyzed 100% of adult worms (10.00 ± 0.00) within 2 and 4 hours of contact. No dead parasites (0.00 ± 0.00^{a}) were observed in saline solution (PBS) within the 12-hour test period. Additionally, no inhibition of parasite motility (0.00 ± 0.00a) was recorded after a contact period of 2 hours at 3.125 mg/ml.

All extracts of the selected plants exhibited strong homicidal activities against *H. contortus*. However, there was a wide variation in the effectiveness of plants depending on the type of plant, dose, and solvent used in the study, each extract type against *H. contortus*. Across all of these treatments, however, the highest and lowest anthelmintic potency was obtained at a dose of 50 and 3.125 mg/ml, respectively, and at 12 and 2 hours of exposure.

3.3. Ovicidal effects

The results of egg hatch inhibition assessment of various extracts of *C. fistula* in comparison with Oxfendazol and PBS are exhibited in Figs. 5, 6, 7 & 8. All extracts of *C. fistula* significantly inhibited the hatching of *H. contortus* eggs. The stronger inhibitory potential was revealed by fruit ethanol extract (90.33%) and the lowest one with root methanol extract (56.17%) at the highest dose (50 mg/ml) after an exposure period of 48h. The percentage correlation data of egg hatching exhibited dose-dependent response of different parts of *C. fistula* to inhibit development of *H. contortus* eggs into larva and exhibited their effective response against targeted parasites eggs. The highest eggs inhibitory potency was found in the fruit part of *C. fistula* than stem, leaves, and root. The different concentrations of various extracts of different parts of *C. fistula* displayed comparable efficacy with different doses of Oxfandazol.

The highly effective fruit ethanol extract even at a lower dose of 3.125 mg/ml inhibited 68.33% of eggs from developing into larvae. The anthelmintic potency of different extracts of *C. fistula* against the eggs of *H. contortus* at the highest dose of @50 mg/ml was recorded as; fruit-ethanol extract (90.33%), stem-methanol extract (85.83%), fruit methanol extract (85.33%), leaves ethyl acetate extract (83.83%), leaves methanol extract (83.5%), fruit ethyl acetate extract (83.33%), stem aqueous methanol extract (81%), leaves ethanol extract (79.17%), fruit aqueous methanol extract (73.67%), root aqueous methanol extract (69.67%), stem ethanol extract (78.5%), stem ethyl acetate extract (77.33%), root ethyl acetate extract (63.67%), leaves aqueous methanol extract (76.17%) and root methanol extract (56.17%).

3.4. Effect of ethanol fruit extracts on parasite load in goats

The in vitro anthelmintic effectiveness of various extracts of *C. fistula* indicated that the ethanol fruit extracts exhibited excellent worm-killing and egg inhibition effects as exhibited in Table 1. The findings indicated the potential of plants in combating gastrointestinal nematodes in small ruminants. All the animals involved in the study showed a high level of gastrointestinal nematode infection, as determined by the fecal egg count reduction test using the McMaster technique.

Table 1
The in vivo anthelmintic efficacy of ethanol fruit extracts and crude fruit powder of *C. fistula* against gestro-intestinal nematodes in experimental goats using fecael egg count reduction assay.

Treatment groups	Treatments	(Day 0)	(Day 5)	(Day 7)	(Day 9)
		EPG Pre-treatment counting	EPG Post-treatment counting (Percentage reduction in parasitic eggs		
T0 (Control)	No treatment	450	521	685	805
T2	Fruit ethanol extract 250 mg/Kg b.w.	480	390 (18.75%)	261 (45.62%)	122 (74.58%)
T3	Fruit ethanol extract 500 mg/Kg b.w.	796	605 (23.99%)	295 (62.93%)	165 (79.27%)
T4	Fruit ethanol extract 1000 mg/Kg b.w.	510	400 (21.57%)	205 (59.80%)	47 (90.78%)
T5	Crude fruit powder 250 mg/Kg b.w.	630	560 (11.11%)	421 (33.17%)	299 (52.54%)
T6	Crude fruit powder 500 mg/Kg b.w.	650	490 (24.61%)	388 (40.30%)	198 (69.53%)
T7	Crude fruit powder 1000 mg/Kg b.w.	621	406 (34.63%)	303 (51.21%)	185 (70.20%)

In the current study, the goats treated with ethanol extracts of *C. fistula* exhibited the highest efficacy percentages of 79.27% and 90.78% at the concentrations of 500 and 1000 mg per kg b.w. The goats

treated with fruit powder of *C. fistula* exhibited the highest efficacy percentages of 69.53% and 70.20% at the concentrations of 500 and 1000 mg per kg b.w. Furthermore, there was a noticeable increase in the efficacy of ethanol extract from the 5th to the 9th day post-treatment.

Post-treatment of animals with the fruit ethanolic extract derived from *C. fistula*, the primary outcome observed in this study concerning fecal egg excretion depicted a decrease in the level of nematode egg in fecal excretion in studied infested goats. The fruit ethanolic extract and fruit powder significantly increased the percentage of fecal egg excretion reduction. Results indicated that the various treatments administered to animal groups effectively reduced parasite load. There was recorded a significant difference ($p < 0.05$) between animals receiving the ethanolic extract and plant powder of *C. fistula* in comparison with the control group.

3.5. Liquid chromatography-mass spectrometry (LC-MS) analysis of C. fistula

LC-MS analysis of different extracts from various parts of *C. fistula* revealed the presence of various phytochemicals. The maximum number of compounds detected using LC-MS analysis belong to class flavonoids phenols and alkaloids exhibiting their maximum role in anthelmintic activities. Plant secondary metabolites identification was carried out based on LC retention time (RT) relative to compound mass and by comparing the mass peaks from a standard library.

3.5.1. Phytochemical components identified in leaves extracts of C. fistula

A list of identified phytochemical constituents in different leaf extracts of *C. fistula* is presented in Tables 2 & 3. The leaf extracts of *C. fistula* contained several key classes of bioactive compounds, including phenols, flavonoids, alkaloids, terpenoids, and glycosides. Additionally, other classes of compounds such as amino acids, fatty, acids, and lignans also partly contributed to the medicinal value of *C. fistula*. Since some of the compounds were reported to possess pharmacological properties. The bioactive components detected in negligible quantities were excluded from the tables such as Pyroglutamic acid, phytol sodium, myricetin, Adicardin, and lauric acid in leaves ethanol extract, catalpol sodium, Succinylproline, cycloartenol acetate, 3-Indoleacrylic acid and adenosine diphosphate in leaves methanol extract, cycloartenol acetate, Vomifoliol, dihydroquercetin, Arachidonic acid and myricetin in leaves ethyl acetate extract and quercetin 3'-O-glu, levulinic acid, Coniine are present in minor quantities. Apart from compounds present in negligible quantities, a total of 13 phytochemicals in ethanol extract (7 flavonoids, 5 phenolic acids, and 1 lignan), 14 in methanol extract (9 flavonoids, 2 polyphenols, 1 phenolic acid, 1 lignan and1 fatty acid), 11 in ethyl acetate extract (6 flavonoids, 1 phenolic acid, 2 α-amino acids, and 1 alkaloid) and 13 (9 flavonoids, 1 phenolic acid, 1 alkaloid, and 2 glycosides) in aqueous methanol extract of *C. fistula* leaves were detected by LC-MS analysis.

Table 2
Phytochemical constituents identified by LC-MS analysis in leaves ethanol and methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* leaves ethanol extract					
Apigenin-6,8-di-C- glycoside	Flavonoid	0.69	2.13	594.5	2.11
Apigenin-6-C- pentoside-8-Chexoside (Isomer 1)	Flavonoid	1.81	1.54	564.5	5.30
Protocatechuic acid-4-Oglucoside	Phenolic acid	0.82	1.31	316.3	1.73
Gallic acid	Phenolic acid	1.59	1.23	170.12	1.06
Quercetin	Flavonoid	2.75	1.89	302.2	3.33
Coumaric acid derivative	Phenolic acid	2.91	5.75	308.5	1.67
Kaempferol rhamnosyl xyloside	Flavonoid	3.34	4.24	564.5	1.73
3,4-di-O-caffeoylquinic acid	Phenolic acid	3.48	2.68	516.5	1.06
Myricetin hexoside	Flavonoid	1.79	7.46	480.4	3.33
Proanthocyanidin B dimer	Flavonoid	5.28	4.14	578.5	1.14
Ferulic acid	Phenolic acid	6.77	7.93	194.2	3.04
Syringaresinol	Lignan	1.52	5.31	418.4	2.97
Astragalin kaempferol O-glucoside	Flavonoid	1.09	1.17	448.4	1.96
Phyto-constituents identified in C. fistula leaves methanol extract					
Vitexin	Flavonoid	1.52	3.44	432..4	1.60
Rutin	Flavonoid	1.52	6.40	610.5	1.25
Apigenin-C-hexosideO-pentoside	Flavonoid	0.81	1.30	270.2	4.29
Apigenin-6-C- pentoside-8-Chexoside (Isomer 2)	Flavonoid	0.79	5.85	564.5	2.84
Apigenin-6-C- pentoside-8-Chexoside (Isomer 3)	Flavonoid	2.67	9.39	564.5	2.68
p-Coumaric acid	Phenolic acid	3.49	2.56	164	2.90
Quercetin-acetyl-glycoside	Flavonoid	2.85	6.65	506.4	7.19

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* leaves ethanol extract					
Neochlorogenic acid	Polyphenol	5.29	4.88	354.3	1.46
Kaempferol-acetyl-glycoside	Flavonoid	6.20	1.36	490.4	1.96
Isorhamnetin-3-O-glucoside	Polyphenol	6.01	5.53	478.4	1.86
Quercetin-O-hexoside	Flavonoid	7.69	6.04	463.4	2.99
Syringaresinol	Lignan	6.57	6.64	418.4	3.91
Isorhamnetin	Flavonoid	8.37	7.42	316.2	6.27
α-Linolenic acid	Fatty acid	2.25	3.49	278.4	4.04

Table 3
Phytochemical constituents identified by LC-MS analysis in leaves ethyl acetate and aqueous methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (mint.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* leaves ethyl acetate extract					
Apigenin-6,8-di-C- glycoside	Flavonoid	2.25	7.47	564.5	4.04
Trigonelline	Alkaloid	5.53	8.17	137.1	2.14
L-Valine	α-amino acid	6.04	4.14	117.1	4.54
Apigenin-6-C- pentoside-8-Chexoside (Isomer 2)	Flavonoid	6.47	1.36	564.5	4.52
L-Leucine	α-amino acid	5.21	4.89	131.1	4.10
Coumaric acid derivative	Phenolic acid	3.78	2.29	308.5	2.10
Kaempferol rhamnosyl xyloside	Flavonoid	4.79	3.33	564.5	3.07
3,4-di-O-caffeoylquinic acid	Phenolic acid	1.91	1.67	516.5	6.21
Myricetin hexoside	Flavonoid	1.09	1.73	480.4	3.65
Proanthocyanidin B dimer	Flavonoid	2.48	1.14	578.5	2.63
Quercetin-O-hexoside	Flavonoid	4.67	3.04	463.4	5.00
Phyto-constituents identified in C. fistula leaves aqueous methanol extract					
Apigenin-6,8-di-C- glycoside	Flavonoid	5.51	3.06	564.5	3.11
Apigenin-6-C- pentoside-8-Chexoside (Isomer 1)	Flavonoid	1.75	1.26	564.5	4.48
Apigenin-C-hexosideO-pentoside	Flavonoid	2.05	1.48	270.2	1.88
Apigenin-6-C- pentoside-8-Chexoside (Isomer 2)	Flavonoid	1.24	2.33	564.5	1.65
Apigenin-6-C- pentoside-8-Chexoside (Isomer 3)	Flavonoid	1.51	6.54	564.5	2.32
Trigonelline	Alkaloid	1.70	1.61	137.1	2.67
Vicenin-2	Glycosides	2.11	1.22	594.5	8.53
3,4-di-O-caffeoylquinic acid	Phenolic acid	1.91	1.40	516.5	2.68
Kaempferol-3-O-rutinoside	Glycosides	1.58	1.82	594.5	2.02
Proanthocyanidin B dimer	Flavonoid	2.52	3.81	578.5	3.49

Compounds name	Phytochemical class	RT (mint.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* leaves ethyl acetate extract					
Quercetin-O-hexoside	Flavonoid	2.59	1.23	463.4	1.44
Isoquercitrin	Flavonoid	1.65	4.09	464.4	4.90
Kaempferol	Flavonoid	1.77	1.57	286.2	2.30

3.5.2. Phytochemical components identified in stem extracts of C. fistula

Tables 4 & 5 show percentage abundance as well as retention time and compound mass for identified phytochemicals in different stem extracts of *C. fistula*. The stem extracts of *C. fistula* contained several key classes of bioactive compounds, including phenols, flavonoids, alkaloids, terpenoids, and glycosides. Additionally, other classes of compounds such as amino acids, fatty acids, amino acids, and lignans also partly contributed to the medicinal value of C. fistula, since some of the compounds were reported to possess pharmacological properties. The bioactive components in stem ethanol extract; are propyl gallate, renylated Chalcone and Campesterol, stem methanol extract; vanillic acid 4-sulphate, myrin succinyl, rutin acetate and myricetin-3-O-xyloside, stem ethyl acetate extract; asparagine, leucyl alanine and phtytol, stem aqueous methanol extract; pterostilbene caffeine and pallidol malonyl as detected in negligible quantities were excluded from the tables. Apart from compounds present in negligible quantities, a total of 14 phytochemicals in ethanol extract (3 flavonoids, 3 fatty acids, 2 terpenoids, 1 phenolic acid, 1 phenol, 1 phenyl propanoic, 1 amino acid, and 1 alkaloid), 12 in methanol extract (3 flavonoids, 5 fatty acids, 3 terpenoids, and 1 phenolic acid,), 13 in ethyl acetate extract (2 flavonoids, 2 fatty acids, 3 terpenoids, 4 phenols, 1 amino acid, and 1 alkaloid) and 12 (3 flavonoids, 3 fatty acids, 1 terpenoids, 3 phenols, and 1 alkaloid) in aqueous methanol extract of *C. fistula* stem were detected by LC-MS analysis.

Table 4
Phytochemical constituents identified by LC-MS analysis in stem ethanol and methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* stem ethanol extract					
Aloin A	Phenol	1.52	13.44	418.4	1.26
Adipic acid	Fatty acid	1.80	3.61	146.1	6.83
Betaine	Amino acid	2.18	3.57	117.1	1.73
β-Asarone	Phenyl propanoid	4.87	2.79	208.3	3.68
Luteolin	Flavonoid	5.38	2.67	286.2	1.70
Quercetin	Flavonoid	5.78	4.41	302.2	1.66
Erucamide	Fatty acid	5.47	4.99	337.6	2.89
(22E)-Stigmasta-5,22-dien3-ol	Terpenoid	5.50	6.04	412.7	1.16
Lupa-12,20(29)-dien-3-one	Terpenoid	3.98	7.64	422.7	2.89
4-Methoxycinnamic acid	Phenolic acid	2.71	7.79	178.2	1.71
Kaempferol	Flavonoid	1.39	12.64	286.2	1.64
n-Hexadecanoic acid	Fatty acid	1.87	3.98	256.4	4.57
(E)-parinaric acid	Fatty acid	6.71	10.90	276.4	2.69
Quinine	Alkaloids	6.79	12.57	324.4	6.22
Phyto-constituents identified in C. fistula stem methanol extract					
Aloe-emodin	Fatty acid	3.19	10.74	270.2	2.01
Apigenin	Flavonoid	3.22	6.03	270.2	1.05
Abietic acid	Terpenoid	1.01	1.08	302.4	1.06
Quercetin	Flavonoid	1.30	1.46	302.2	1.05
Lup-20(29)-en-28-al, 3betahydroxy	Terpenoid	1.77	6.55	641	1.23
Erucamide	Fatty acid	6.13	1.36	337.3	3.94
Betulin	Terpenoid	7.13	3.22	442.7	6.77
Kaempferol	Flavonoid	4.30	1.16	286.2	1.79
Nervonic acid	Fatty acid	1.58	2.87	366.6	5.28

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* stem ethanol extract					
n-Hexadecanoic acid	Fatty acid	1.79	4.05	256.4	5.09
3-hydroxy-3,7- dimethyl-6-octenedioic acid	Fatty acid	2.90	6.81	186.3	2.52
4-Methoxycinnamic acid	Phenolic acid	1.19	2.11	178.1	2.15

Table 5
Phytochemical constituents identified by LC-MS analysis in stem ethyl acetate and aqueous methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* stem ethyl acetate extract					
alpha,omega-dicarboxylic acid	Fatty acid	2.82	1.04	286.4	4.62
Epicatechin	Flavonoid	1.07	1.83	290.2	1.11
4-Hydroxycoumarin	Phenol	4.83	2.38	162.1	1.35
8-Hydroxyquinoline	Phenol	2.49	3.07	145.2	2.24
Quinine	Alkaloid	1.30	3.01	324.4	6.75
Caffeic acid	Phenol	3.01	1.60	180.2	2.97
Aloin A	Phenol	1.39	2.94	418.4	1.03
Valine	Amino acid	3.36	1.35	117.1	1.36
Aloe-emodin	Fatty acid	1.24	1.27	270.2	7.33
Betuline	Terpenoid	1.30	1.30	442.7	1.77
Rhamnetin	Flavonoid	1.32	2.39	316.2	1.35
Oleanolic acid	Terpenoid	8.56	1.16	456.7	5.91
Lupa-12,20(29)-dien-3-one	Terpenoid	1.10	1.05	422.7	3.18
Phyto-constituents identified in C. fistula stem aqueous methanol extract					
alpha,omega-dicarboxylic acid	Fatty acid	1.77	6.76	146.1	2.17
(-)-Epigallocatechin	Flavonoid	1.63	6.74	458.4	5.99
3-Hydroxypyridine	Phenol	1.11	5.18	95.1	1.01
Quinine	Alkaloid	2.87	3.76	324.4	1.18
Valine		1.76	1.42	117.2	1.30
3,7-Dimethyl-2- octaen-1,8-dioic acid	Fatty acid	1.90	2.81	200	2.03
Aloe-emodin	Fatty acid	1.06	3.07	270.2	6.68
Oleanolic acid	Terpenoid	4.38	8.34	456.7	3.40
(E)-Ferulic acid	Phenol	1.48	4.44	194.2	1.05

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* stem ethyl acetate extract					
7-Ethoxycoumarin	Phenol	2.70	1.10	190.2	1.17
Apigenin	Flavonoid	1.52	1.61	270.2	1.98
2’,3’,6-Trimethoxyflavon	Flavonoid	243	1.81	313.1	1.98

3.5.3. Phytochemical components identified in root extracts of C. fistula

The roots of *C. fistula* were subjected to LC-MS analysis to identify bioactive compounds that may contribute to the plant's pharmacological functions. The identified compounds are shown in Tables 6 & 7. These bioactive compounds belong to various classes, including flavonoids, phenolic acids, and fatty acids, all of which are known for their pharmacological activities. These compounds may have synergistic effects in reducing oxidative stress and immunosuppressive properties. A few phyto-compounds were detected in minor quantities and were excluded from the table. Apart from compounds present in negligible quantities, a total of 14 phytochemicals in ethanol extract (2 flavonoids, 4 terpenoids, 3 phenolic acids, 1 phenol, 2 fatty acids, 1 amino acid, and 1 stilbene), 13 in methanol extract (4 flavonoids, 2 terpenoids, 2 polyphenols, 1 phenolic acid, 1 glycoside, 1 steroid, 1 amino acid, and 1 alkaloid), 14 in ethyl acetate extract (2 flavonoids, 2 fatty acids, 3 terpenoids, 4 phenols, 1 amino acid, and 1 alkaloid) and 14 (3 flavonoids, 3 fatty acids, 1 terpenoid, 3 phenols and 1 alkaloid) in aqueous methanol extract of *C. fistula* leaves were detected by LC-MS analysis.

Table 6
Phytochemical constituents identified by LC-MS analysis in the roots ethanol and methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* roots ethanol extract					
Phytol	Terpenoid	6.47	4.21	296.5	3.66
Vitispirane	Terpenoid	2.36	2.48	192.2	2.96
Catechin	Flavonoid	7.95	5.25	290.3	3.23
Protecatechuic acid	Phenolic acid	2.65	3.70	154	2.15
p_coumaric acid	Phenolic acid	1.49	1.59	164	2.67
Resveratrol	Stilbenes	2.82	3.85	228.2	2.59
4-O-Methylmelleolide	Sesquiterpenoid	4.66	3.98	414.5	1.76
Levulinic acid	Fatty acid	2.68	1.64	116	2.52
Traumatin	Fatty acid	5.16	21.17	212.3	21.50
Tyramine	Monoamine	2.08	1.42	137.2	1.56
Lansioside A	Terpenoid	4.09	11.07	659.9	17.22
Catechin hydrate	Flavonoid	1.93	1.63	290.3	2.48
Mahuannin D	Phenol	3.75	1.89	528.5	2.29
Gallic acid	Phenolic acid	1.69	2.05	170.1	1.54
Phyto-constituents identified in C. fistula roots methanol extract					
Quercetin-3β-D-glucoside	Flavonoid	3.48	1.86	464.5	1.84
Caffeic acid	Polyphenol	1.82	5.39	180.2	8.95
Gallic acid	Phenolic acid	1.46	3.64	170.1	3.05
Demethoxycurcumin	Polyphenol	1.66	3.54	368.4	3.20
3',4',5,7,8-Pentamethoxyflavanone	Flavonoid	3.82	2.33	372.4	2.68
6-beta-D-Glucopyranosyl-4',5-dihydroxy3',7-dimethoxyflavone	Flavonoid c-glycosides	1.33	4.43	4773.2	8.66
Isoacteoside	Glycosides	1.78	2.06	664.6	1.74
Pilosanol A	Flavonoid	6.60	8.02	540.6	8.32

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* roots ethanol extract					
6'-O-E-Caffeoyl-mussaenosidic acid	Terpenoid	1.79	2.87	376.4	3.18
Tyramine	Monoamine	2.14	14.58	137.2	7.21
Armillarivin	Terpenoid	2.87	3.18	384.5	2.14
6alpha-Fluoroprednisolone	Steroids	3.29	1.44	378.4	1.50
Senampeline A	Alkaloids	1.48	1.70	473.5	2.23

Table 7
Phytochemical constituents identified by LC-MS analysis in the roots ethyl acetate and aqueous methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* roots ethyl acetate extract					
Protecatechuic acid	Phenolic acid	1.50	8.38	154.1	6.72
Salicylic acid	Phenolic acid	2.23	1.89	138.1	1.68
Gallic acid	Phenolic acid	2.72	3.85	170.1	5.26
Quercetin 3-O-rhamnoside-7-O-glucoside	Glycosides	3.07	3.14	610.5	2.17
(3R)-Hydroxy-beta-ionone	Sesquiterpenoid	1.75	7.69	208.3	4.03
Armillarivin	Terpenoide	1.72	2.28	384.5	3.11
Epicatechin	Flavonoid	2.11	8.55	290.2	5.03
rosmarinic acid	Phenolic acid	3.77	6.26	360.3	7.54
Betasitosterol	Phytosterol	1.82	3.77	414.7	4.30
Luteolin	Flavonoid	3.49	9.76	286.2	8.62
Catechin	Flavonoid	1.81	1.84	290.3	1.51
Myricetin	Flavonoid	1.54	2.25	318.2	2.92
Silymarin	Flavonoid	1.94	3.55	482.4	4.32
Phyto-constituents identified in C. fistula roots aqueous methanol extract					
Perillic acid	Terpenoid	2.27	5.65	166.2	3.70
Pantothenic acid	Vitamin	1.31	4.82	219.2	8.38
13-Hydroxy-9,11-octadecadienoic acid	Fatty acid	1.64	2.60	296.4	1.77
Dodecanedioic acid	Fatty acid	1.82	2.13	200.3	3.15
Astragalin	Flavonoid	1.69	2.72	448.4	1.57
Gallic acid	Phenolic acid	2.85	1.32	170.1	1.39
Rutin	Flavonoid	1.83	2.01	610.5	2.57
6-beta-D-Glucopyranosyl-4',5-dihydroxy3',7-dimethoxyflavone	Flavonoids c-glycosides	2.61	1.68	4773.2	2.75
2",6"-Di-O-acetylononin	Phenol	2.53	7.64	514.5	2.76

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* roots ethyl acetate extract					
Myricetin	Flavonoid	1.43	1.50	318.2	1.96
3-hydroxy-4- methoxymandelate	Phenol	1.73	1.44	166.1	2.32
Silidianin	Flavonoid	1.27	1.33	482.4	1.39
Catechin	Flavonoid	1.25	4.41	290.3	4.04
4-O-Methylmelleolide	Sesquiterpenoid	1.65	3.44	414.5	5.85

3.5.4. Phytochemical components identified in fruit extracts of C. fistula

The roots of *C. fistula* were subjected to LC-MS analysis to identify bioactive compounds that may contribute to the plant's pharmacological functions. The identified compounds are shown in Tables 8 & 9. These bioactive compounds belong to various classes, including flavonoids, phenolic acids, and fatty acids, all of which are known for their pharmacological activities. These compounds may have synergistic effects in reducing oxidative stress and immunosuppressive properties. A few phyto-compounds were detected in minor quantities and were excluded from the table. Apart from compounds present in negligible quantities, a total of 14 phytochemicals in ethanol extract (2 flavonoids, 4 terpenoids, 3 phenolic acids, 1 phenol, 2 fatty acids, 1 amino acid, and 1 stilbene), 13 in methanol extract (4 flavonoids, 2 terpenoids, 2 polyphenols, 1 phenolic acid, 1 glycoside, 1 steroid, 1 amino acid, and 1 alkaloid), 14 in ethyl acetate extract (2 flavonoids, 2 fatty acids, 3 terpenoids, 4 phenols, 1 amino acid, and 1 alkaloid) and 14 (3 flavonoids, 3 fatty acids, 1 terpenoid, 3 phenols and 1 alkaloid) in aqueous methanol extract of *C. fistula* leaves were detected by LC-MS analysis.

Table 8
Phytochemical constituents identified by LC-MS analysis in the fruits ethanol and aqueous methanol extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* fruits ethanol extract					
Propane, 1,1,3-triethoxy	Ester	2.69	1.18	176.3	5.63
Caprylic acid	Fatty acid	4.16	0.55	144.2	8.71
Methyl salicylate	Ester	5.20	1.44	152.1	4.92
Decanoic acid	Fatty acid	6.74	0.74	200	6.23
8-Methylnonanoic acid	Fatty acid	9.82	5.28	171.2	9.14
Pentacosane	Alkanes	17.2	0.92	352.7	9.83
Griseophenone, trimethyl ether	Benzophenone	17.60	0.02	387.2	5.41
Morphinan-4-ol-6,7-dione, 3-methoxy-N-methyl	Alkaloid	17.60	0.02	315.4	32.32
Pancracine, O,O-dimethyl-	Alkaloid	17.60	0.02	287.3	39.91
6-Chloro-4-phenyl-2-propylquinoline	Alkaloid	19.98	23.74	256.7	38.61
4-epi-Dehydroabietinol acetate	Terpenoid	19.52	0.84	328.5	42.53
2-(Ethyl)oxybenzylidene acetophenone	Flavonoid	19.41	7.29	294.4	78.72
2 2-(2-Methylpropyl)oxybenzylidene...	Ester	19.18	6.35	162.2	35.64
Terephthalic acid, di(4-octyl) ester	Ester	18.85	5.71	376.5	46.74
3-Hydroxy-4'-methoxy-6-methylflavone	Flavonoid	18.79	3.94	282	56.61
Phenol, 2,2'-methylenebis[6-(1,1..	Phenol	18.1	1.78	340.5	53.42
2-Aminofluorene, N-heptafluorobu.	Aryl amine	17.89	0.61	181.2	37.73
6-Chloro-2,4-diphenylquinoline	Alkaloid	17.89	0.61	315.8	31.54
Phyto-constituents identified in C. fistula fruits aqueous methanol extract					
Corchorifatty acid F	Fatty acid	17.51	21.6	328.22	13.31
6-Chloro-4-phenyl-2-propylquinoline	Alkaloid	19.98	23.74	256.7	14.43

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* fruits ethanol extract					
Trifolin	Flavonoid	18.62	7.83	448.10	21.52
4-epi-Dehydroabietinol acetate	Terpenoid	19.52	0.84	328.5	34.74
ß-Sitosterol acetate	Terpenoid	14.53	15.2	456.39	31.31
2-(Ethyl)oxybenzylidene acetophenone	Flavonoid	19.41	7.29	294.4	38.23
Stigmasterol	Terpenoid	12.51	9.81	412.37	26.72
2 2-(2-Methylpropyl)oxybenzylidene...	Ester	19.18	6.35	162.2	23.31
Propane, 2,2-diethoxy	Aceton	17.74	16.81	132.20	20.81
n-Hexadecanoic acid	Fatty acid	15.63	7.81	256.42	25.52
Gallic acid	Phenolic acid	8.72	9.81	170.1	27.73
p_coumaric acid	Phenolic acid	13.71	11.51	164	19.94
1-Caffeoyl-β-D-glucose	Phenolic	11.71	13.5	342.09	17.1
Myricetin-3-o-Galactoside	Flavonoid	23.511	21.6	480.09	26.73
3,4-O-Dimethylgallic acid	Benzoic acid	32.32	25.5	198.05	23.72
Dihydroquercetin-glucosyl-rhamnosy	Flavonoid	29.93	27.3	613.11	22.74

Table 9
Phytochemical constituents identified by LC-MS analysis in the fruits methanol and ethyl acetate extracts of *C. fistula*.

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* fruit methanol extract					
Propane, 1,1,3-triethoxy	Ester	2.69	1.18	176.3	4.3
Sinapinic acid	Phenolic acid	3.67	3.2	224.2	3.71
2-Ketoepimanoo	Terpenoid	6.43	2.65	304.2	6.93
Isoquercitrin	Flavonoid	6.43	7.86	464.1	9.87
Caprylic acid	Fatty acid	4.16	0.55	144.2	3.7
4-O-sinapoyl-5-O-caffeoylquinic acid	Phenolic acid	9.71	11.73	560.2	13.78
Decanoic acid	Fatty acid	6.74	0.74	200	7.98
Gallic acid	Phenolic acid	7.91	8.73	170.1	7.89
p_coumaric acid	Phenolic acid	17.71	13.73	164	9.83
Phenol, 4-propyl-	Phenylpropane	8.74	1.72	136.1	7.34
Syringic acid	Phenolic acid	1.08	3.54	194.4	7.71
phenol 2 2'-methylenebis 6-(1 1-dimethylethyl)-4-methyl-	Phenolic acid	12.79	10.93	340.5	9.78
Corchorifatty acid F	Fatty acyl	11.89	19.91	328.2	9.72
Dodecanedioic acid	Fatty acyl	7.87	16.71	230.2	15.57
N-Acetyl-Lphenylalanine	Aromatic	13.79	14.17	207.08	13.78
Pyridoxine	Vitamin	18.98	19.37	169.07	21.71
Pyridoxine	Terpenoid	27.71	24.51	262.2	26.76
Hexadecanoic acid, ethyl ester	Fatty acid	23.45	24.52	284.5	26.76
Phyto-constituents identified in C. fistula fruit ethyl acetate extract					
Prenylated Chalcone	Flavonoid	21.21	23.71	324.3	27.71
Propane,1,1,3-triethoxy	Ester	19.98	20.36	182.12	17.17
Chloroacetic acid, 2,2-dimethylphenyl ester	Ester	9.82	5.28	198.6	7.89
Perillic acid	Terpenoid	7.83	9.81	166.09	10.73

Compounds name	Phytochemical class	RT (min.)	% Area	MW (g/mol)	% Abundance
Phyto-constituents identified in *C. fistula* fruit methanol extract					
8-Methylnonanoic acid	Fatty acid	9.82	5.28	171.2	8.76
Pentacosane	Alkanes	17.21	0.92	352.7	5.67
Melilotoside	Aromatic	13.76	13.71	326.10	15.6
Octadecane	Alkanes	17.2	7.92	254.5	13.46
16-Hydroxyhexadecanoic acid	Fatty aacyl	13.76	15.67	272.2	3.71
Griseophenone, trimethyl ether	Benzophenone	17.60	9.02	387.2	13.07
n-Propyl acetate	Ester	17.71	13.78	102.13	9.08
Myricetin-3-o-Galactoside	Flavonoid	5.06	7.13	480.09	9.08
Dihydroquercetin-glucosyl-rhamnoside	Flavonoid	11.09	13.17	613.11	9.18
Amyrone succinyl	Terpenoid	13.73	9.17	524.4	10.08
Astragalin	Flavonoid	1.73	3.45	448.10	5.67
Gallic acid	Phenolic acid	5.06	3.18	170.1	9.81
p_coumaric acid	Phenolic acid	2.08	3.18	164	9.19

4. Discussion

The anthelmintic activity of various plants even amongst various parts of the same plants diverges according to their different bioactive compounds, regional, cultivar or seasonal variation, and various solvents used for their extraction (Ahmad et al., 2020). The highest percentage yield in the current study was recorded in fruit methanol (13%) and fruit ethanol (12.5%) extracts of *C. fistula*. The difference in percentage yield of plant extract might be due to variations in the biochemical composition of each plant because some bio-chemicals are more soluble in one solvent and less soluble in others (Ahmed et al., 2023). The variation in plant extract yield might be due to the difference in the polarity of different solvents used and the variance in their biological activity might indicate the difference in active components proportion responsible for that biological activity (Abubakar and Haque, 2020).

The native residents of the Cholistan desert (Roohelay in the local vernacular) are living nomadic lifestyles and use this plant as a medicament and therapeutic mediator for the prosperity of their livestock. Ethno-veterinary assessment by Siddique et al. (2022) documented the traditional uses of *C. fistula* for the treatment of internal parasites of livestock. Wahyuni et al. (2019) revealed the anthelmintic efficacy of four Cassia species (*C. fistula, C. siamea, C. spectabilis*, and *C. surattensis*) using its ethanol and n-hexane extracts (200, 100, 50, 25 mg/ml) against infective larvae (L3) of *H. contortus* by applying

larval migration inhibition assay. *C. surattensis* explored the highest efficacy at the maximum dose of 200 mg/ml. All species of Cassia significantly inhibited larval motility. The larvicidal and ovicidal effects from protein fractions of *Combretum leprosum* and *C. fistula* against the various nematodes of goats were demonstrated by Silva et al. (2018). *Combretum leprosum* didn't cause a significant effect on egg hatching and larval development. *C. fistula* significantly inhibited egg hatching (38%) and larval development (61–69%) of endo-parasites of naturally infected goats.

In the current study, the highest anthelmintic potential was revealed in fruit ethanol and leaves ethyl acetate extracts of *C. fistula* at the highest concentration of @50 mg/ml, causing complete paralysis and fatality of adult worms (10.00 ± 0.00) within post-exposure of 4 and 6 hours correspondingly. The fruit ethanol extract also showed comparable activity with the standard drug Levamisol. All extracts of *C. fistula* even at lower doses of 6.25 and 3.125 mg/ml exhibited maximum worms paralyzing potential within a tested 12 hours.

Plants' bio-active ingredients pierce through the parasite eggshell and inhibit to hatching first stage larva (L1) hence reducing the chances of parasite load in host animals and averting the contamination of grazing areas with parasitic eggs (Sambodo et al., 2018). Different plants have been studied for their anthelmintic effects against multiple phases of multi-resilient parasites *H. contortus*. Complete inhibition of egg hatching of *H. contortus* (100%) upon exposure to aqueous extract of *Aesculus hippocastanum, Laurus nobilis*, *Chelidonium majus*, was reported by Esteban-Ballesteros et al. (2019), 98% on interaction with n-butanol leaf extracts of *Icacina trichantha* at 12.5mg/ml (Olayemi et al., 2022). The methanol leaves extract of *C. macrostachyus*, *Z. officinale*, and *N. tabacum* significantly produced 100% fatality and mortality of adult worms of *H. contortus* correspondingly at the concentration of 125, 62.5, and 250 mg/ml in comparison with albendazole after an exposure period of 4 h (Mumed et al., 2022). All extracts of *C. fistula* in the current study represent a close correlation with the anthelmintic effects of other plants.

Most in vivo studies of anthelmintic medicinal plants have demonstrated lower efficacy compared to synthetic anthelmintic drugs. Moreover, challenges such as poor absorption in the gastrointestinal tract, as well as issues with compound stability and solubility after oral administration, are major obstacles in developing herbal formulations with good bioavailability and effective anthelmintic properties. Further, the pH levels in the gastrointestinal tract may reduce the bioactivity of plant extracts (Francois Ngnodandi Belga et al., 2024). Those plant products are considered effective in the anthelmintic sense if they cause a reduction of more than 90% in both adult parasites as well as eggs per gram (EPGs). Reinecke (1980) classifies an extract as highly effective if it achieves a fecal egg count reduction (FECR) of over 90%, moderately effective for 80% FECR, and poorly effective for 60% FECR. If FECR is below 60%, the product is considered ineffective. In the current study, the FECR value at the dose of 1000 mg/Kg b.w. of *C. fistula* fruit ethanol extract was recorded at 90.78% reflecting its highest efficacy as compared to other extracts.

In the current study, the phytochemical constituents detected using LC-MS analysis belong to the class; flavonoids, phenols, terpenoids, alkaloids, sterols, glycosides, fatty acids, amino acids, esters and alkanes that might be responsible for their anthelmintic potential. Flavonoids, phenols, and alkaloids are the phytochemicals in *C. fistula* that are present in maximum numbers *and* seem to be more responsible for its anthelmintic activity. The results of the present research show agreement with Abdullah et al., 2024 who also reported the maximum number of alkaloids and flavonoids in the ethanol leaves extract of *C. fistula*. The bioactive compounds present in various extracts of *C. fistula* exhibited strong binding affinity to the target protein, suggesting they could effectively replace synthetic drugs for treating Haemonchosis.

Those plant extracts having potent anthelmintic potential against all life phases of the parasite (egg-larvae-adult) would have greater chances of reducing the parasite's capability to develop resistance against anthelmintic extracts (Mrifag et al., 2021). Thus, fruits of *Cassia fistula* may serve as a valuable source of secondary metabolites with notable anthelmintic properties, offering a promising natural alternative to synthetic drugs for managing gastrointestinal diseases.

Conclusion

The overall verdicts of the current study intensely advocate that all parts of *C. fistula* specifically fruit contain such bioactive ingredients that possess strong anti-parasitic inhibitory potential against the egg and adults of gastro-intestinal parasites in general, specifically *H. contortus.* All extracts of *C. fistula* revealed significant anthelmintic potential, however, fruit ethanol extract of *C. fistula* exhibited comparable efficacy with standard therapy. Further *in-vivo* and toxicity study of different extracts of various parts of this plant of this plant is desired to assess the optimal non-lethal dose for curing parasitic disease in livestock as well as in humans.

Abbreviations

LC-MS: Liquid chromatography-mass spectrometry

ESI: Electrospray Ionization

AMA: Adult motility assay

PBS: Phosphate buffer saline

b.w. Body weight

EPG: eggs per gram

H. contortus: Heamonchus contortus

C. fistula: Cassia fistula

IACUC: Institutional Animal Care and Use Committee

tti labs: textile testing international labs

UPLC: Ultra-performance liquid chromatography

DMRT: Duncan-multiple-range test

Lev. Levamisol

Oxf. Oxfandadazole

RT: retention time

C.: Cassia species

FECR: fecal egg count reduction

Declarations

Credit authorship and contribution statement

Azra Anwer: were responsible for conception and design the study, were involved in writing and editing the manuscript. Khalid J. Iqbal: supervised the study, were involved in project administration. Khalid Mehmood: were responsible for conception and design the study, edit and revised the manuscript. Muhammad Abdullah: helped in plant collection, were involved in conception and design the study. Nawal Al-Hoshani: were involved in conception and design the study. Khalid M. D. Alsyaad: were involved in conception and design the study.

Declaration of competing interest

All authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Azra Anwer: were responsible for conception and design the study, were involved in writing and editing the manuscript. Khalid J. Iqbal: supervised the study, were involved in project administration. Khalid Mehmood: were responsible for conception and design the study, edit and revised the manuscript. Muhammad Abdullah: helped in plant collection, were involved in conception and design the study. Nawal Al-Hoshani: were involved in conception and design the study. Khalid M. D. Alsyaad: were involved in conception and design the study.

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References

1. Abdullah, R., Younas, Q., Kaleem, A., Iqtedar, M., Aftab, M., Saleem, F., 2024. Phytochemical and antimicrobial properties of different plants and in silico investigation of their bioactive compounds in wound healing and rheumatism. Saudi. J. Biol. Sci. 31, 1–8.
2. Abubakar, R.A., Haque, M., 2020. Preparation of medicinal plants. Basic extraction and fractionation procedures for experimental purposes. J. Pharm. Bioallied Sci. 12, 1–10.
3. Ahmad, S., Sajid, M.S., Khan, M.K., Nadeem, R., Rizwan, H.M., Malik, M.A., Yosuf, F., 2020. Effect of trace element supplementation on the gastrointestinal parasites of grazing sheep of Multan district, Pakistan. J. Anim. Plant Sci. 30, 72–80.
4. Ahmed, H., Kilinc, S.G., Celik, F., Kesik, H.K., Simsek, S., Ahmad, K.S., Afzal, M.S., Farrakh, S., Safdar, W., Pervaiz, F., Liaqat, S., Zhang, J., Cao, J., 2023. An Inventory of Anthelmintic Plants across the Globe. Pathog. 12, 131.
5. Badar, S.N., Iqbal, Z., Sajid, M.S., Rizwan, H.M., Shareef, M., Malik, M.A., Khan, M.N. 2021. Comparative anthelmintic efficacy of *Arundo donax*, *Areca catechu* and *Ferula assa-foetida* against *Haemonchus contortus*. *Rev. Bras. Parasitol. Vet*. 30, e001221.
6. Belecke, A., Kupcinskas, T., Stadaliene, I., Höglund, J., Thamsborg, M.S., Stuen, S., Petkevicius, S., 2021. Anthelmintic resistance in small ruminants in the Nordic-Baltic region. Acta Vet. Scand. 63, 18.
7. Bibi, R., Afshan, K., Khan, I.A., Iqbal, Z., Kayani, A.R., Mushtaq, M., Irfan, M., Qayyum, M., Hassan, M.F., 2017. Phenotyping and prevalence of *Haemonchus contortus* (Nematoda: *Trichostongylidae*) in ruminants from endemic areas of Pakistan: influence of host species and geographical area on phenotypic traits of worms. Pak. Vet. J. 37, 170 –174.
8. Charlier, J., Thamsborg, S. M., Bartley, D. J., Skuce, P. J., Kenyon, F., Geurden, T., Hoste, H, Williams, A, R., Sotiraki, S., Hoglund, J, Chartier, C, Geldhof, P., Van Dijk, J., Rinaldi, L., Morgan, E.R., Von Samson-Himmelstjerna, G., Vercruysse, J., Claerebout, E., 2018. Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs. Transbound. Emerg. Dis. 65, 217–234.
9. Delfin, E., Cabardo, Jr., Harvie, P., Portugaliza., 2017. Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae. Int. J. Vet. Sci. Medicne. 5, 30–34.
10. Devrnja, N., Krstic-Milosevic, D., Janosevic, D., Tesevic, V., Vinterhalter, B., Savic, J., Calic, D., 2021. In vitro cultivation of tansy (*Tanacetum vulgare* L.): A tool for the production of potent pharmaceutical

agents. Protoplasma, 258, 587–599.

11. Doyle, S.R., Tracey, A., Laing, R., Holroyd, N., Bartley, D., Bazant, W., Beasley, H., Beech, R., Britton, C., Brooks, K., Chaudhry, U., Maitland, K., Martinelli, A., Noonan, J.D., Paulini, M., Quail, M.A., Redman, E., Rodgers, F.H., Salle, G., Shabbir, M.Z., Sankaranarayanan, G., Wit, J., Howe, K.L., Sargison, N., Devaney, E., Berriman, M., Gilleard, J.S., Cotton, J.A., 2020. Genomic and transcriptomic variation defines the chromosome-scale assembly of *Haemonchus contortus* a model gastrointestinal worm. Commun. Biol. 3, 1–16.
12. Esteban-Ballesteros, M., Sanchis, J., Gutierrez-Corbo, C., Balana-Fouce, R., Francisco, A. Rojo-Vazquez, Gonzalez-Lanza, C., Martinez-Valladares, M., 2019. In vitro anthelmintic activity and safety of different plant species against the ovine gastrointestinal nematode *Teladorsagia circumcincta*. Res. Vet. Sci. 123, 153–158.
13. Francois Ngnodandi Belga F.N., Raulf, M.K., Spiegler, V., Liebau, E., Hensel, A., Strube, C. and Ndjonka, D. In vivo anthelmintic activity of *Combretum mucronatum schumach* & *Thonn* leaf extract against *Haemonchus contortus* in goats. Vet. Parasitol. 331, 1–8.
14. Hardianto, M.A., Widiyant, S., 2022. In Vitro Anthelmintic Activity of *Limonia acidissima*, L. Leaves Aqueous Extract on *Haemonchus contortus* (Rudolphi, 1803). Adv. Biol. Sci. Res. 22, 73–77.
15. Higuera-Piedrahita RI, Dolores-Hernandez, M., Jimenez-Perez, L.G., Camacho-Enríquez, B.C., Zamilpa, A., Lopez-Arellano, R., Mendoza-de-Gives, P., Cuellar-Ordaz, J.A., Lopez-Arellano, M.E., 2021. In vitro nematocidal effect and anthelmintic activity of Artemisia cina against *Haemonchus contortus* in gerbils and relative expression of Hc29 gene in transitional larvae (L3–L4). Acta Parasitol. 66, 938–946.
16. Higuera-Piedrahita, R.I., Dolores-Hernandez, M., de la-Cruz-Cruz, H.A., Andrade-Montemayor, H.M., Zamilpa, A., Lopez-Arellano, R., Gonzalez-Garduno, R., Cuellar-Ordaz, J.A., Mendoza-de-Gives, P., Lopez-Arellano, M.E., 2022. An Artemisia cina n-hexane extract reduces the *Haemonchus contortus* and *Teladorsagia circumcincta* fecal egg count in naturally infected periparturient goats. Trop. Anim. Health Prod. 54, 1–10.
17. Jelalu, K. Tesfaheywet, Z. Sisay A., Sali,K., Nasir, M., Abraha, A., Feyera, T., 2020. “In vitro acaricidal activity of selected medicinal plants traditionally used against ticks in eastern Ethiopia”. J. Parasitol. Res. Pages 10. https://doi.org/10.1155/2020/7834026
18. Karim, M.A., Islam, M.R., Lovelu, M.A., Nahar, S.F., Dutta, P.K., Md Talukder, H., 2019. In vitro evaluation of anthelmintic activity of tannin-containing plant Artemisia extracts against *Haemonchus contortus* from goat: Anthelmintic activity of tannin-containing plants Artemisia. J. Bangladesh Agric. Univ. 17, 363–8.
19. Kaur, Dhiman, V., Bhadada, S., Katare, O.P., Ghoshal, G., 2022. LC/MS guided identification of metabolites of different extracts of *Cissus quadrangularis* Jaspreet. Food Chem. Adv. 1, 1-11.
20. Kaur, J., Sharma, G., Mahajan, A., Katare, O. P., Bhadada, S. K., Ghoshal, G., 2021. Role of *Cissus quadrangularis* in the management of osteoporosis: An overview. Crit. Rev. Ther. Drug. 38, 27–51.

21. Klavin, A., Keidane, D., Sukele, R., Bandere, D., Kovacuka, L., 2021. Traditional Latvian herbal medicinal plants used to treat parasite infections of small ruminants: A review. Vet. World, 14, 1548–1558.
22. Kumar, T., Khan, Z., Oulkar, D., Singh, B. K., Maurya, A., Singh, B., Banerjee, K., 2020. High resolution LC-MS characterization of phenolic compounds and the evaluation ofantioxidant properties of a tropical purple radish genotype. Arab. J. Chem. 13, 1355–1366.
23. Liu, M., Panda, S. K., Luyten, W., 2020. Plant-based natural products for the discovery and development of novel anthelmintics against nematodes. Biomol. 10, 426.
24. Matsabisa, M.G., Campbell, W.E., Folb P.I., Smith, P.J., 2013. Treatment of Parasitic Infections in Humans and Animals. U.S. Patent. 8, 586–112.
25. Mrifag, R., Lemrabott, M. A., El Kharrim, K., Belghyti, D., Basco, L. K., 2021. S*etaria labiatopapillosa* (Filarioidea, Nematoda) in Moroccan cattle: atypical localization and morphological characterization of females and microfilariae by light and scanning electron microscopy. Parasitol. Res. 120, 911–918.
26. Mumed, H.S., Nigussie, D.R., Musa, K.S., Demissi, A.A., 2022. In Vitro Anthelmintic Activity and Phytochemical Screening of Crude Extracts of Three Medicinal Plants against *Haemonchus Contortus* in Sheep at Haramaya Municipal Abattoir, Eastern Hararghe. J. Parasitol. Res. 8, 6331740.
27. Olayemi, O., Onakpa, M. and Jegede, O., 2022. In vitro Anthelmintic Activity of *Icacina trichantha* Leaf Crude Extract and Fractions on *Haemonchus contortus*. Vet. Arhiv. 92, 331–337.
28. Oliveira, M., Hoste, H., Custodio, L., 2021c. A systematic review on the ethnoveterinary uses of mediterranean salt-tolerant plants: exploring its potential use as fodder, nutraceuticals or phytotherapeutics in ruminant production. J. Ethnopharmacol. 1, 113464. doi: 10.1016/j.jep.2020.113464.
29. Ramdani, D., Yuniarti, E., Jayanegara, A., Chaudhry, A.S., 2023. Roles of essential oils, polyphenols, and saponins of medicinal plants as natural additives and anthelmintics in ruminant diets: A Systematic Review. Anim. 13, 767.
30. Rehman, T., Iqbal., K.J., Anwar, A., Abbas, R.Z., 2021. In vitro anthelmintic efficacy of *Citrullus colocynths* against *Haemonchus contortus*. Vet. Arch. 91, 309–318.
31. Reinecke, R.K., 1980. Chemotherapy in the control of helminthiasis. Vet. Parasitol. 6, 255–292.
32. Rizwan, H.M., Sajid, M.S., Iqbal, Z., Saqib, M., 2019. Association of phytomineral with gastrointestinal parasites of grazing sheep in Sialkot district, Punjab, Pakistan. Pak. J. Agric. Sci. 6, 459–468.
33. Ruth, W., Mwangia, John, M., Machariab, Isabel, N., Wagaraa, Raposa and L. Bence., 2021. The medicinal properties of *Cassia fistula* L: A review. Biomed. Pharmacother. 144, 1–9.
34. Sambodo, P., Prastowo, J., Kurniasih, K., Indarjulianto, S., 2018. In vitro potential anthelmintic activity of *Biophytum petersianum* on *Haemonchus contortus*. Vet. World, 11, 1–4.
35. Siddique, Z., Ahmed, H.M., Hussein, K.N., Hassan, K.I., Meena, B.I., 2022. Herbal medicinal uses and their practice in human health care and livestock from district Haripur, Khyber Pkhtunkhwa, Pakistan. Vet. Med. Sci. 8, 2683–2695.

36. Silva, M.T.D.O., Maia, M.A.C., Silva, M.D.C.D., Torres, T.M., Pereira, J.C.D.S., Araujo, J.R.C., Silva, R.E.D., Souza, A.L.D.C., Barbosa, T.N., Bezerra, A.C.D.S., 2018. Anthelmintic effect of *Cassia fistula* and *Combretum leprosum* protein fractions against goat gastrointestinal nematodes. Braz. J. Vet. Parasitol. Jaboticabal. 27, 237–241.
37. Verma, R., Lata, K., Das, G., 2018. An overview of anthelmintic resistance in gastrointestinal nematodes of livestock and its management: India perspectives. Int. J. Chem. Stud. 6, 1755–1762
38. Vineer, H.R., Morgan, H., Hertzberg, E. R., Bartley, H., Bosco, D. J., A., Charlier, J., Chartier, C., Claerebout, E., Waal, T,de, Martinez-Valladares, M., Mateus, T.L., Mclntyre, J., Mickiewicz, M., Munoz, A.M., Phythian, C.J., Ploeger, H.W., Rataj, A.V., Skuce, P.J., Simin, S., Sotiraki, S., Spinu, M., Stuen, S., Thamsborg, S.M., Vadlejch, J., Vrady, M., Samson-Himmelstjerna, G.V., Rinaldi, L., 2020. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite. 27, 69.
39. Wahyuni, S., Sunarso, S., Prasetiyono, B.W.H.E., Satrija, F., 2019. Exploration of anthelmintic activity of Cassia spp. extracts on gastrointestinal nematodes of sheep. J. adv. Vet. Anim. Res. 6, 236–24 0.
40. Zajickova, M., Nguyen, L.T., Skalova, L., Stucklikova, L.S., Matouskova, P., 2020. Anthelmintics in the future: current trends in the discovery and development of new drugs against gastrointestinal nematodes. Drug Discov. Today. 25, 430–437.

Figures

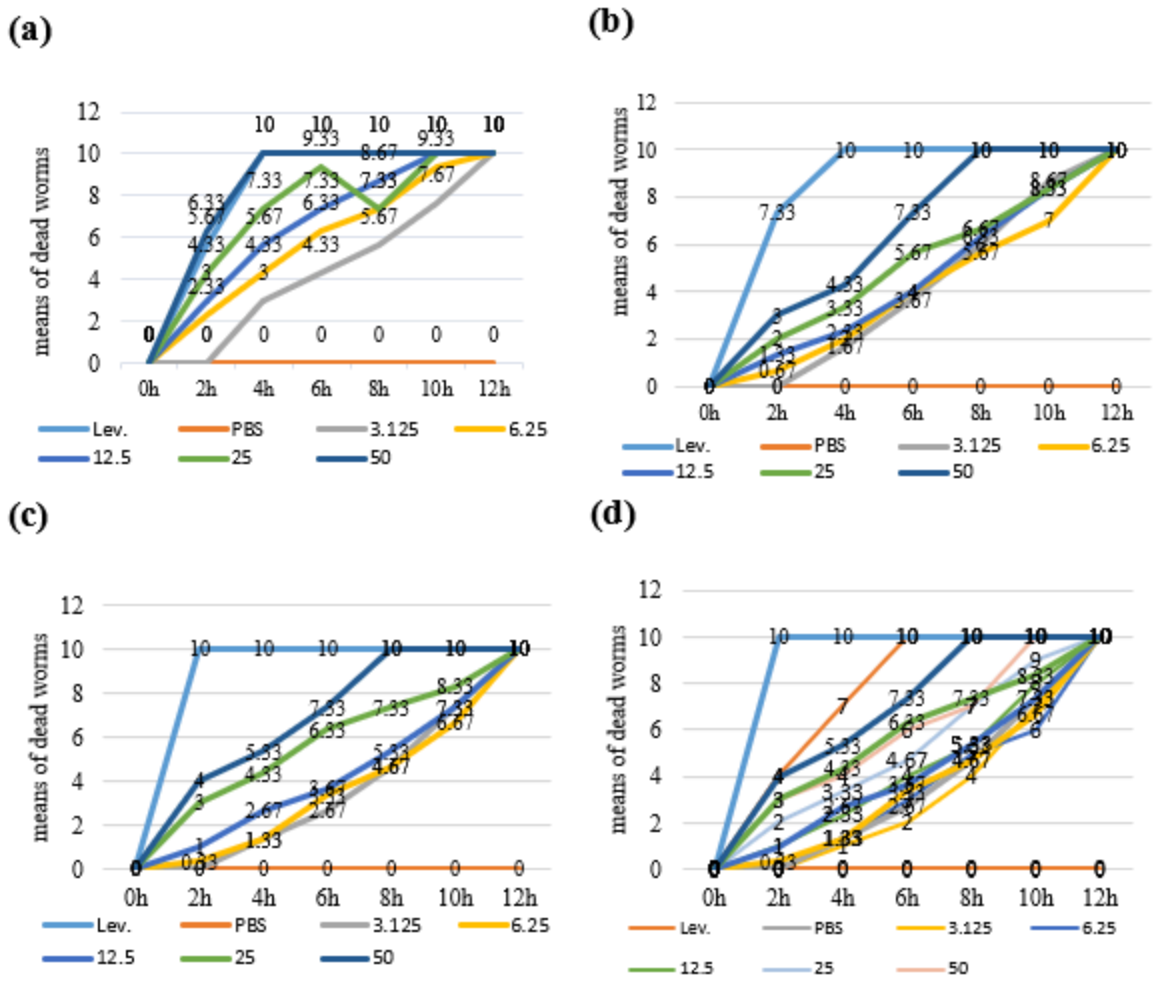

Figure 1

Photomicrographs of in vitro adulticidal assessment of various fruit extracts of C. fistula in comparison to PBS and Levamisol against the worms's motility of H. contortus. Anthelmintic efficacy of (a) fruits ethanol extract (b) fruits methanol extract (c) fruits ethyl acetate extract (d) fruits aqueous methanol extract.

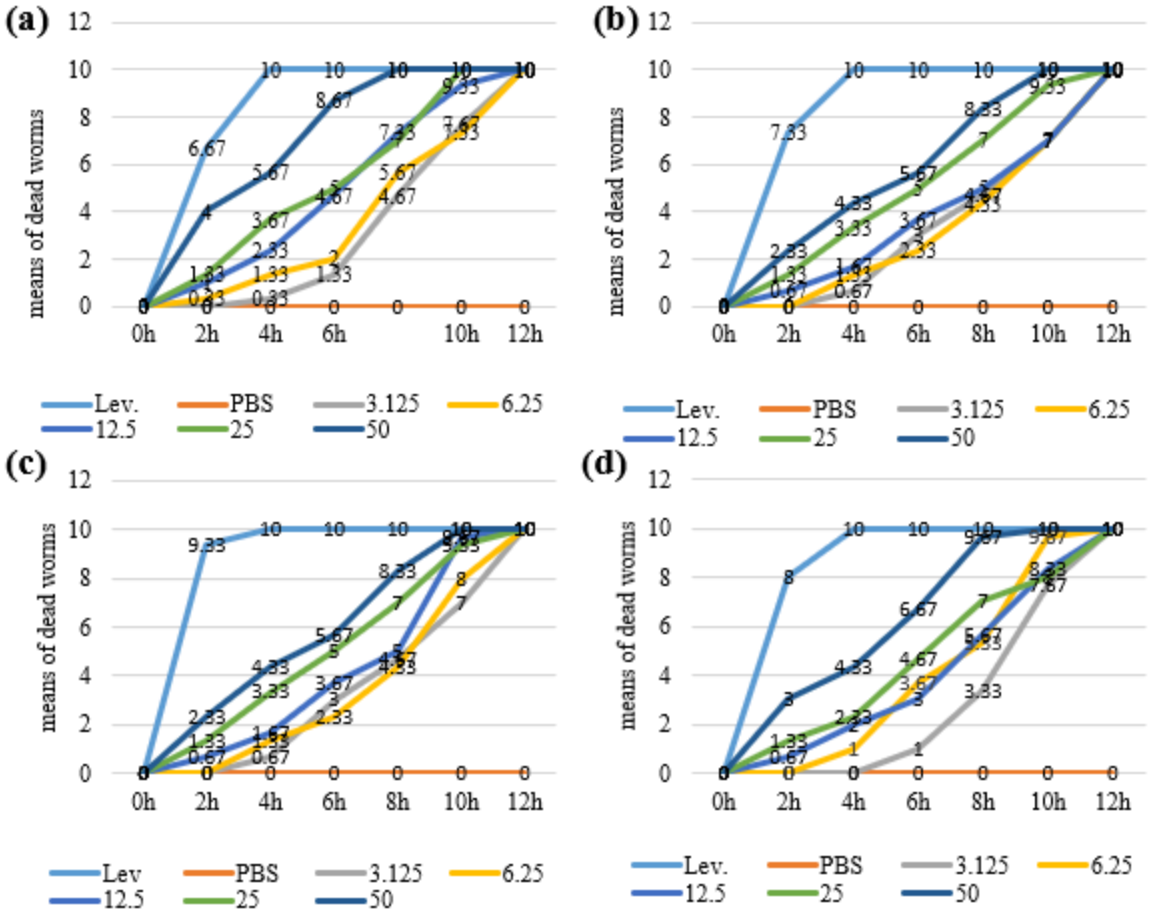

Figure 2

Photomicrographs of in vitro adulticidal assessment of various root extracts of C. fistula in comparison to PBS and Levamisol against the worms's motility of H. contortus. Anthelmintic efficacy of (a) roots ethanol extract (b) roots methanol extract (c) roots ethyl acetate extract (d) roots aqueous methanol extract.

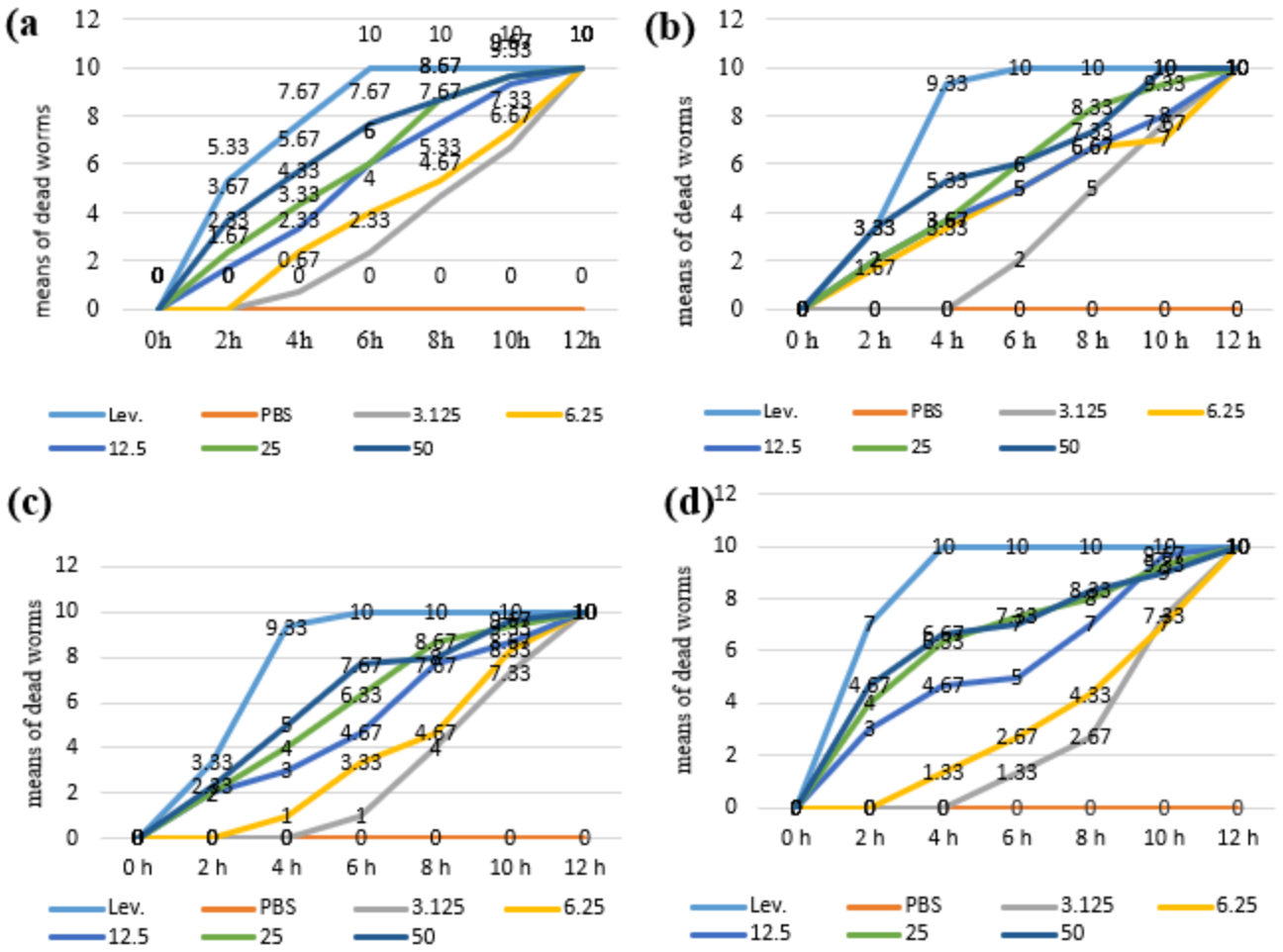

Figure 3

Photomicrographs of in vitro adulticidal assessment of various stem extracts of C. fistula in comparison to PBS and Levamisol against the worms's motility of H. contortus. Anthelmintic efficacy of (a) stem ethanol extract (b) stem methanol extract (c) stem ethyl acetate extract (d) stem aqueous methanol extract.

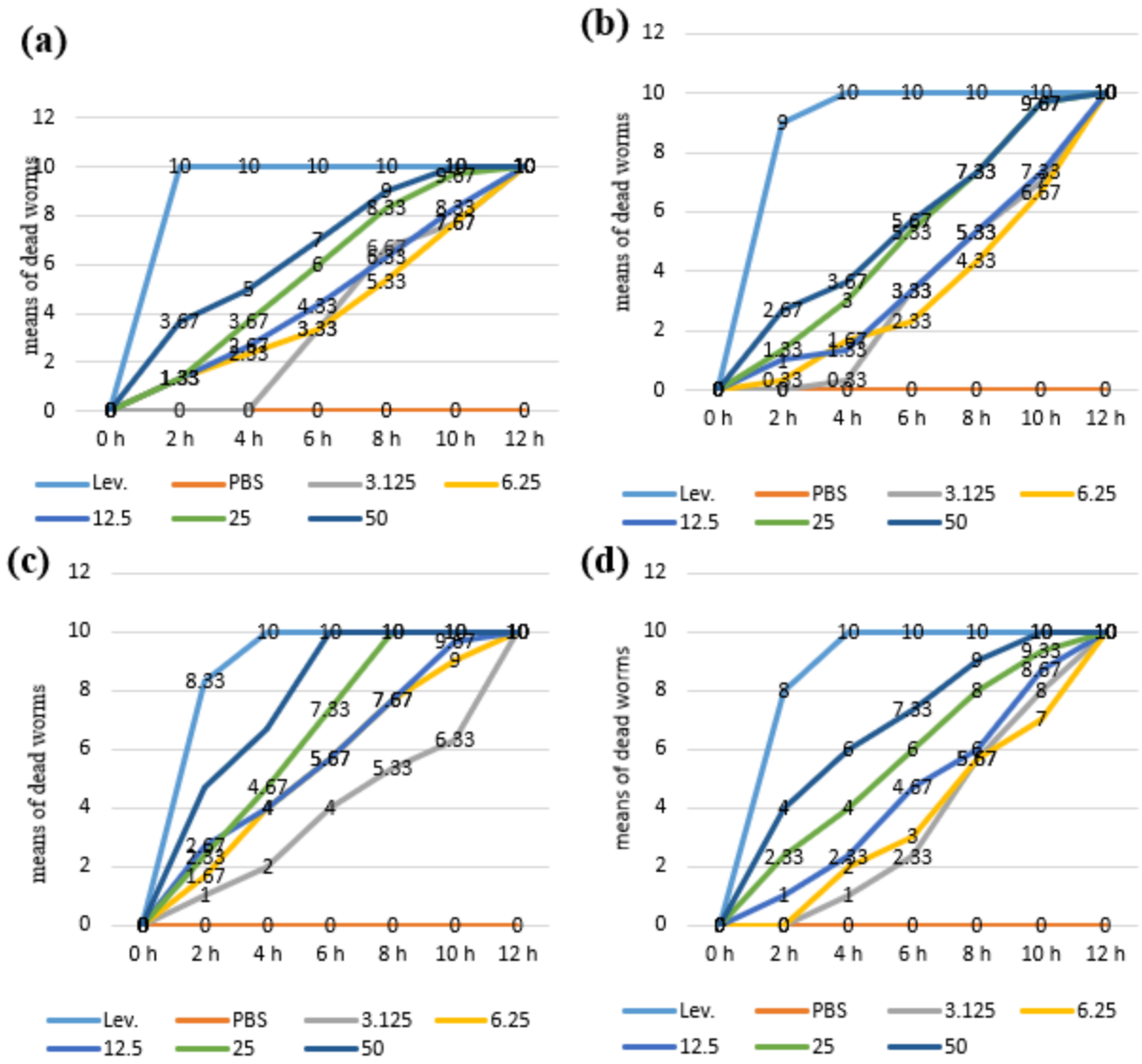

Figure 4

Photomicrographs of in vitro adulticidal assessment of various leaves extracts of *C. fistula* in comparison to PBS and Levamisol against the worms's motility of *H. contortus*. Anthelmintic efficacy of (a) leaves ethanol extract (b) leaves methanol extract (c) leaves ethyl acetate extract (d) leaves aqueous methanol extract.

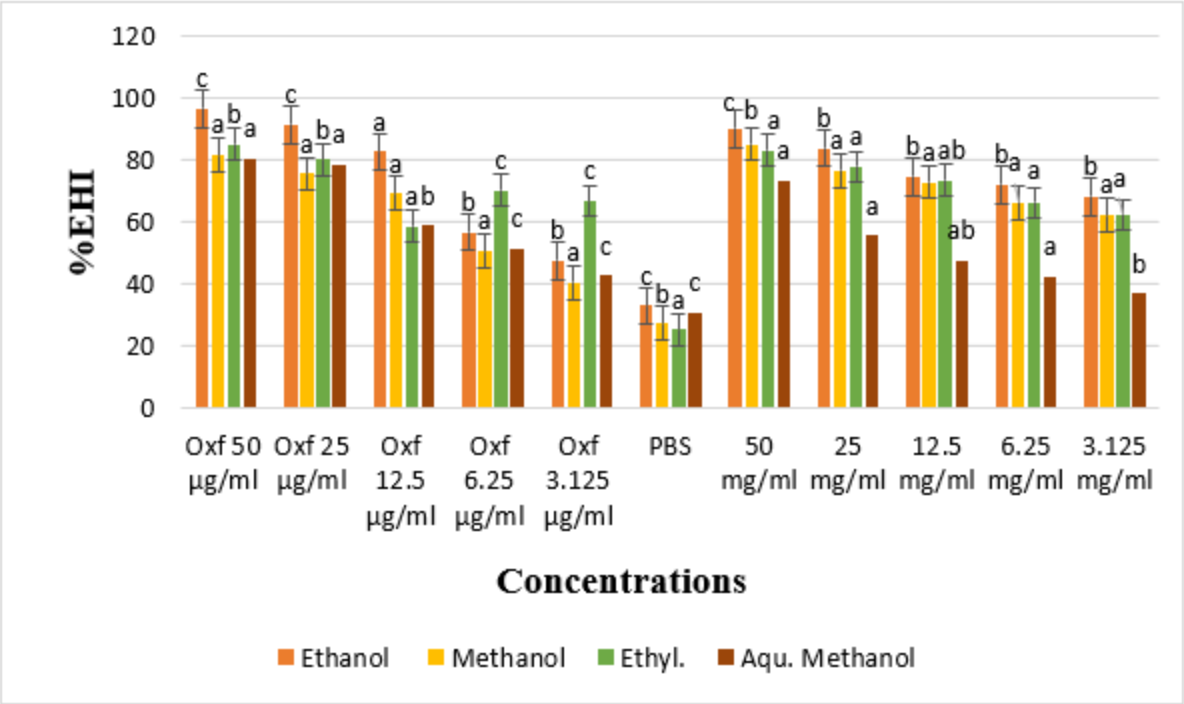

Figure 5

The percentage egg hatch inhibition effects of C. fistula fruit extracts upon H. contortus eggs in comparison across different doses of oxfendazole. The distinct letters within the column are denoting the statistical variances between groups.

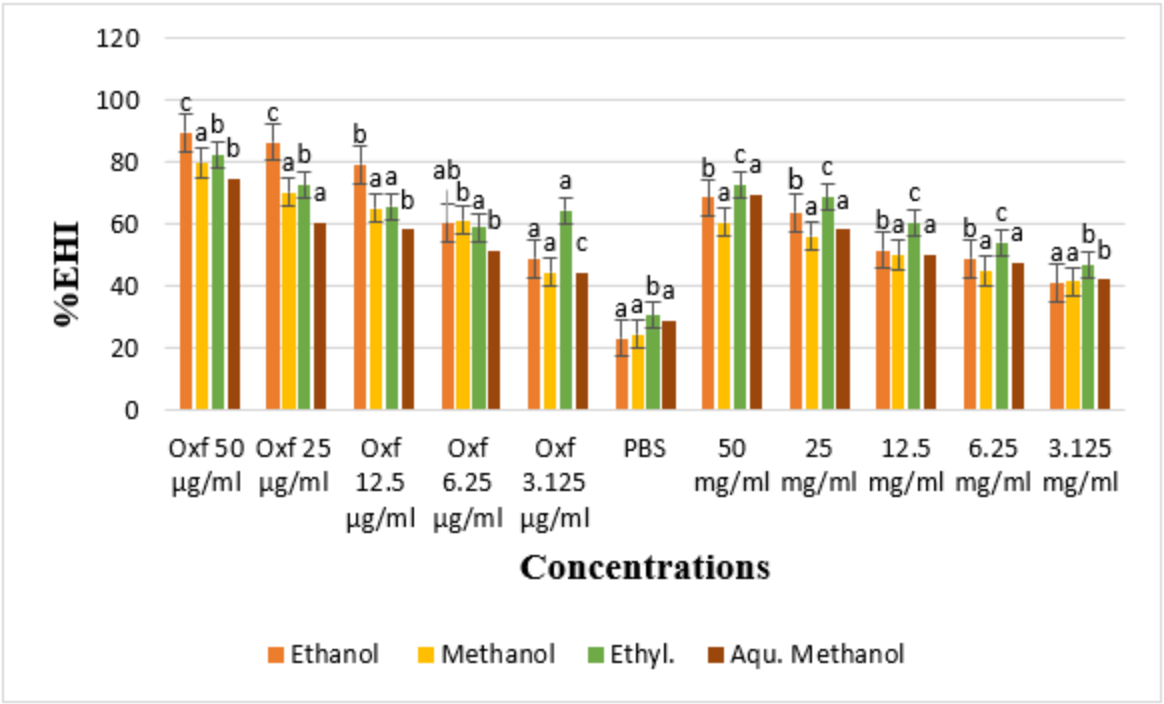

Figure 6

The percentage egg hatch inhibition effects of C. fistula root extracts against H. contortus eggs in comparison across different doses of oxfendazole. The distinct letters within the column are denoting the statistical variances between groups.

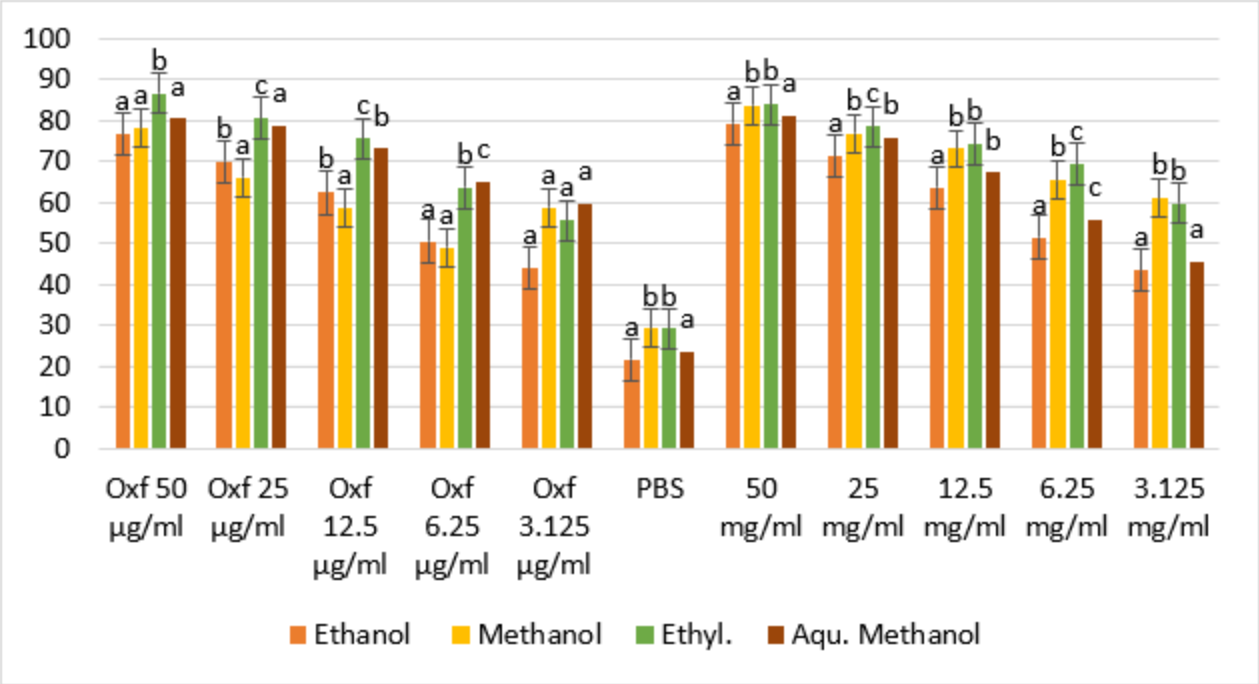

Figure 7

The percentage egg hatch inhibition effects of C. fistula leave's extracts against H. contortus eggs in comparison across different doses of oxfendazole. The distinct letters within the column are denoting the statistical variances between groups.

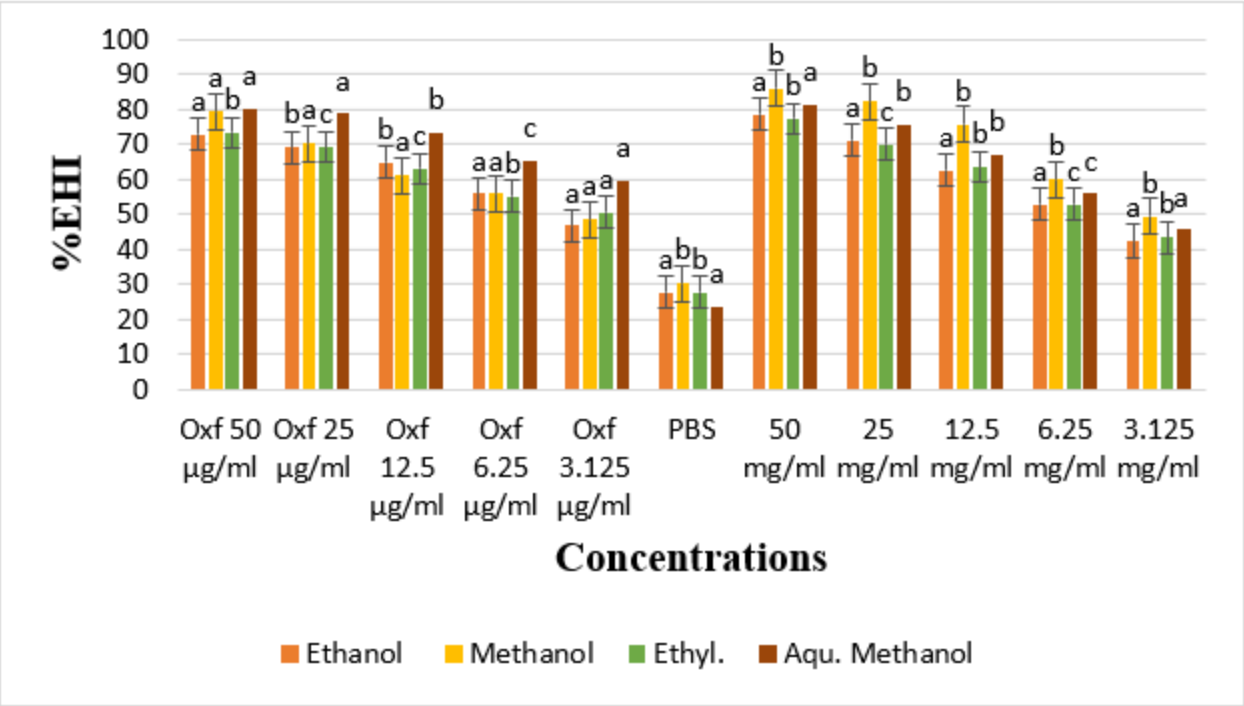

Figure 8

The percentage egg hatch inhibition effects of C. fistula stem extracts against H. contortus eggs in comparison across different doses of oxfendazole. The distinct letters within the column are denoting the statistical variances between groups.