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PHYTOCHEMICAL AND NEMATICIDAL SURVEYS ON SOME ANTHELMINTIC PLANTS, WITH SPECIAL REFERENCE TO *CASSIA FISTULA* L.

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A B S T R A C T

Preliminary phytochemical screening on boiling water extracts of some known effective parts of three anthelmintic plants; *Commiphora myrrha* (Gum), *Cassia fistula* (Mature Pods) and *Citrullus colocynthis* (Mature Fruits); (1 ml of each extract = 50 mg/g Dry Weight) was done and all the obtained results showed the presence of flavonoids, anthraquinones, steroids and/triterpenoids, tannins, saponins, alkaloids and/or nitrogenous bases, carbohydrates and/or glycosides, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in most samples. *Cassia fistula* boiling water extract' was found to be the richest extract regarding all investigated phytochemicals, so it was selected for further analyses. Preliminary phytochemical screening Results' of different extracts of examined plant parts of *Cassia fistula* indicated the presence of all investigated phytochemicals in most samples; with special reference to boiling water extracts of mature pods, followed by leaf petioles and stems and also in methanol extracts of all investigated parts (leaf petioles, leaf blades, stems and mature pods) in this regard, ethanol extracts were found to contain the lowest amount of all investigated phytochemicals compared to any other extracts. Ethanol and methanol extracts of all investigated parts are devoid of saponins, since saponins were found only in boiling water extracts of all investigated parts; with special reference to fresh samples of leaf petioles, stems and dried samples of mature pods. Regarding total phenolic contents, it was found that, the highest amount was recorded to be in dried samples of mature pods (28.671 ± 0.021) and both fresh and dried samples of leaf blades (28.571 ± 0.020 and 24.927 ± 0.020 mg/g respectively), the least amount was recorded to be in both fresh and dried samples of leaf petioles (19.670 ± 0.011 and 19.686 ± 0.017 mg/g respectively). Meanwhile the differences in all investigated samples are very small regarding their flavonoidal contents, dried samples of mature pods and fresh samples of stems were found to contain the highest amount in this regard (5.963 ± 0.011 and 5.957 ± 0.008 mg/g respectively). Additionally, all extracts of different parts of *Cassia fistula* L. (Golden shower trees) were examined for their nematotoxicity against *Meloidogyne incognita*, results showed that, all investigated extracts were effective against infective juveniles of root knot nematode; the mortality percentages were ranged between 11 to 100%, aqueous extracts of most plant parts achieved the best results in this regard (morality percentages are higher than 50%). Meanwhile, ethanolic extracts of all plant parts caused toxicity percentages' lower than those of water extracts, but higher than methanolic ones with few exceptions; dry leaf blades extracted by ethanol caused mortality to about half of subjected juveniles. On the other hand, methanolic extracts of all plant parts showed lower efficacy than aqueous and methanolic ones, except methanolic extracts of fresh leaf petioles, since it caused complete death of juveniles. It can be concluded that, nematicidal activity of such extracts were positively correlated with their phytochemical contents.

Keywords: Anthelmintic plants, Nematotoxicity, *Cassia fistula* L., Plant extracts, Phytochemical surveys.

INTRODUCTION

The most abundant animals on the world are Nematodes; some of them are plant parasites. A large section of them are in free living forms, those feed on other microorganisms and inhabit both freshwater and marine ecosystem, as well as the soils and sediments of all different types of earth biomes (Bailey, 2021). Plant parasitic nematodes (PPN) are major challenges for growers universally, causing severe crop deficits. PPN are soil borne microorganisms those capable of invade many host plants worldwide. More than 5000 plant pathogenic nematodes are identified, they are causing considerable

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yield losses reached up to $125 billion yearly on the global scales (Hamza *et al*., 2014; Mesa-Valle *et al*., 2020; Pulavarty *et al*., 2021). Root-knot nematode (RKN) is the most damaging phytonematodes. RKN is a sedentary endoparasitic nematode; this parasitic behavior protects all stages except the second juvenile stage or infective stage form unfavorable conditions and enemies. They can attack about five thousand plant species, their effects may be resulted from nematodes parasitizing them only or in combination with other soil pathogens causing severe damage to plants and economic yield losses (Hu *et al*., 2020; El-Nuby and Alam, 2020; Forghani and Hajihassani, 2020).

Nematicides have been used to control nematodes since a long time, either as fumigants or non-fumigants ones, as a simple way to manage PPN. Due to acute and chronic toxicity of chemical nematicides, many of them were restricted, in particularly the soil fumigant methyl bromide which was widely used as a pre-plant for nematode eradication, and it is also banned because of its confirmed effect on the depletion of ozone layer. Accordingly, many researches have been done to find some alternatives for methyl bromide in the last two decades, and the necessity of the usage of non-chemical nematicides (those characterized by being safe and ecofriendly) has been arisen. In the same way, the adverse effects of chemicals prompted many scientists to investigate antinematodal activity of several organisms and plants, in order to find more sustainable and environmentally safe alternatives of these chemical nematicides (Alam and El-Nuby, 2019; El-Nuby *et al*., 2020; 2021; Pulavarty *et al*., 2021; Saad *et al*., 2022).

Biocides are any formulations, those used to control various pests, they are mainly derived from either microorganisms or plant extracts. Bio-nematicides are promised substitutes to combat PPN, they can eliminate or minimize the hazardous effects of chemicals on various elements of the ecosystem, also they are cost effective approaches in managing nematodes (Danahap and Wonang, 2016). Higher plants are major sources of many important bioactive compounds; including antinematodal compounds those involved in plant defense mechanisms against various stresses either biotic or abiotic. These small organic molecules those synthesized and produced by various plants are called secondary metabolites. Since few decades ago, the exploration of antinematodal potential of botanicals and their applications is of increasing manner. Phyto-nematicides could be important in the integrated pest management and in also the sustainability of our natural resources. Using botanical extracts and bioagents can change the indigenous microbial communities of the rhizosphere in a great approach that leads to enhance the soil suppressive efficacies to nematodes and other plant pathogens in the soil, also the application of these phyto-nematicides are generally safe and ecofriendly (Shaukat and Siddiqui, 2001; Chitwood, 2002; D'Addabbo *et al*., 2014). Plants-derived natural products (botanical nematicides), can represent helpful tools in suppressing populations of tolerant plant parasitic nematodes; those infecting many economic crops and tolerate classical che mical nematicides. Researchers investigated many wild and cultivated plants for their nematicidal properties against several phytonematodes and their obtained results confirmed variations between these plants in this regard (Vázquez-Sánchez *et al*., 2018; El-Nuby *et al*., 2020; El-Nuby and Alam, 2020).

Cassia spp. included approximately five hundred species; it is considered to be the largest genus of the family Fabaceae (Leguminosae); the third largest family of flowering plants on the earth (Lodha *et al*., 2010). *Cassia* species are used enormously for the treatment of many diseases in the traditional medicine. In the folk medicinal history, these plants are used as laxative and purgative agents. The phytochemical screening of different extracts of genus *Cassia* plants' indicated the presence of some groups of secondary metabolites including: anthraquinones, flavonoids, triterpenoids, pentacyclic sterols, alkaloids, phenylpropanoids and γ-naphthopyrones. Among these phytochemicals, flavonoids and anthraquinones are the major groups of bioactive constituents found in genus *Cassia* (Khurm *et al*., 2020). It had been stated that, *C. fistula* leaves' extract contains phenol hydroquinone, flavonoids, steroids, triterpenoids, tannins, and saponins, but this extract is devoid of alkaloids (Wahyuni *et al*., 2019). On the other hand, tannins, flavonoids, alkaloids, and phenolic compound, glycosides, saponins, triterpenoids, amino acids and proteins were detected in *C. fistula* extracts (Panda *et al*., 2016). Danish *et al*. (2011) found that *C. fistula* leaves mainly contain anthraquinone derivatives, oxy-anthraquinones, oxalic acid and tannins. Agarwal and Paridhavi (2005) found that the stem bark, and leaves of this plant contain a variety of biologically active compounds such as; anthraquinones, flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, steroids and reducing sugars, those have various medicinal properties. Leaves and stem bark extracts were found to have various activities including antipyretic, anthelmintic, antidiabetic, protective, antioxidant, antiulcer, antitumor and antimicrobial activities etc., Extracts of the plant showed its effect against constipation, piles and also it is considered to be a detoxifier. Anthelminthic potential of *C. fstula* was previously reported also, in this regard, Silva *et al*. (2018) assessed the larvicidal and ovicidal activity of protein preparations obtained from *Cassia fstula* leaves on the gastrointestinal parasites of goats. *Cassia fistula* pod extract' showed anthelmintic potential, eggs hatching inhibition, and larval mortality were observed also on gastrointestinal nematodes of sheeps (Zaragoza-Bastida *et al*., 2019). Thirumal *et al.* (2012) reported that *Cassia fistula* possessing a wide range of phytochemicals almost in every plant part of it and has been reported to possess various activities like anthelmintic, larvicidal and antiparasitic. Antinematodal activity of *C. fistula* against plant parasitic nematode, however these activities were investigated only in a few studies; Leaf aqueous extract of *Cassia fistula* exhibited highly mortality rates of *Meloidogyne incognita* juveniles after two days of exposure (Asif *et al*., 2013). Another study using aqueous extract of *C. fistula* seeds, high mortality percentage of root knot nematode larva was recorded in this study (khurma and Mangotra, 2004).

This study was carried out as a survey using phytochemical screening to detect the richest plant among these investigated anthelmintic plants regarding some investigated phytochemicals. Moreover, to detect the richest extract belonging to the best plant part regarding these investigated phytochemicals. Additionally, this current research was aimed to assess the nematicidal potential of *C. fistula* L. ' aqueous, methanolic and ethanolic extracts of fresh and dry plant parts against *M. incognita* juveniles.

MATERIAL AND METHODS

Sample collection: Selected parts of different plants' known with their anthelmintic efficiency; *Commiphora myrrha* (Gum) and *Citrullus colocynthis* (Mature Fruits), were obtained from the herbal market, Cairo, Egypt. Meanwhile, different plant parts (stems, leaf petioles, leaf blades and mature pods) of wild *Cassia fistula* L. were collected from Desert Research Center (DRC), Cairo, Egypt. All samples were identified in the Taxonomy Unit, Plant Ecology and Ranges Department, voucher herbaria' specimens were deposited in DRC herbarium.

Preparation of different extracts: Selected parts of different known plants with anthelmintic efficiency; *Commiphora myrrha* (Gum), *Cassia fistula* (Mature Pods) and *Citrullus colocynthis* (Mature Fruits), were extracted by boiling water. Plant materials (Stems, leaf petioles, leaf blades and mature pods) of *Cassia fistula* L. tree were cleaned, air dried, extracted by using different solvents in a descending successive extraction manner according to their polarity gradients (boiling water, followed by methanol then ethanol) then filtered; these extracts (1 ml of each extract = 50 mg/g Dry Weight or Fresh Weight) were screened phytochemically and both total phenolic and flavonoidal contents were estimated also, this is done after choosing *Cassia fistula* as a model plant regarding results of preliminary phytochemical screening on boiling water extracts of known effective parts of different anthelmintic plants; *Commiphora myrrha* (Gum), *Cassia fistula* (Mature Pods) and *Citrullus colocynthis* (Mature Fruits); (1 ml of each water extract = 50 mg/g Dry Weight) (Alam, 2019).

Preliminary Phytochemical Screening: Flavonoids were screened by following the method of Mabry *et al*. (1970).

Anthraquinones screening was done according to Farnsworth *et al.* (1969) method.

Unsaturated sterols and/or Triterpenes were screened according to a-Liebermann- Burchardt's test (Claus, 1967) and b-Salkowiskit's test (Schmidt, 1964).

Tannins were screened according to the method of Trease and Evans (1978).

Saponins were determined according to the methods adopted by Hungund and Pathak (1971) via a-Forth test and b-Blood hemolysis test.

Alkaloids and/or Nitrogenous bases were checked according to the method of Shellard, (1957).

Carbohydrates and/ or Glycosides were detected according to the method of Stank *et al.* (1963).

Cardiac glycosides screening was carried out according to Balbaa *et al*. (1981). a-Killer –Kiliani test. b-Kedde's reaction. c- Libermann's reaction.

Chlorides and Sulphates were determined according to the method adopted by Islam *et al*. (1993).

Irodoids were checked according to the method obtained by Weiffering (1966).

Sublimation: The presence and accumulation of sublimable substances in different investigated extracts were detected using the method of Afifi (1972).

Assay for total phenolics: Total phenolics were estimated in all extracts by following the method of Gursoy *et al.* (2009).

Assay for total flavonoids: Total flavonoids were determined in all extracts also using the method of Gursoy *et al.* (2009).

Source of Nematodes: Culture of root knot nematode, *Meloidogyne incognita,* was prepared to be used in the current study, this culture was reared on eggplants grown in plastic posts and maintained inside glasshouse. To prepare freshly hatched second stage juveniles' suspension; the infected plants were carefully uprooted then washed under stream of water to remove the soil particles. Using sterilized forceps, from heavily infected roots egg masses were picked by hand. Collected egg masses were washed with sterile distilled water and then filtered by using small sieve having tissue paper above its net, then it is poured in Petri plates having water enough to touch the egg masses to allow releasing of hatching juveniles to the bottom of plates passing through the sieve, the count of hatched juveniles were observed each six hours and the collected nematode suspension was adjusted to about five hundreds juveniles per 1ml then preserved in refrigerator, it will be ready to be used within few hours of preservation in the refrigerator (El-Nuby and Alam, 2020).

Determination of Nematotoxicity of different extracts: The toxicity of *C. fistula* parts' extracts was tested in terms of nematode' immobility leading to confirmed death. Five milliliters of water suspension containing ≃100 second stage juveniles (J_2S) of *M. incognita* were poured in petri dishes containing different extracts (boiling water, methanol, ethanol extracts) of various plant parts; leaf blades, leaf petioles and stems (El-Nuby and Alam, 2020). These Petri plates were placed at 28°C, there were four replicates / treatment. The immobilized J_2S were counted after 24 hours of the exposure period by the aid of microscope (Meiji 100X). Recovery test was done by empting the dishes and replacing the extracts with distilled water, the immobile juveniles were considered dead (permanent death) if they remain immovable after touching them with a fine needle (Cayrol *et al*., 1989). The mean percentage of mortality was calculated. Mortality percentage (M%) was calculated by the ratio of the dead nematodes/number of total nematodes multiplied by 100 i.e. (M %) = [(No. of dead J_2S)/ (No. of total J_2S)] x 100. The experiment was conducted twice typically to previous protocols.

Statistical analysis:

Nematicidal Activity Studies: In current study experiments completely randomized design was used. All results were undergone to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at 5% significance level (Duncan's, 1955) was used for comparing between treatments means. This analysis was done using statistical program of CoStat version 6.303, copyright (1998-2004), CoHort software statistical packages.

Chemical Studies: Fisher analyses of variance methodology was used for statistical analyses. A least significant difference test was applied at 5 and 1% probability level in order to determine the differences among treatment means (Steel and Torrie, 1984). The CO-STAT computerized package program was undergone to the regular statistical analyses of variance (Nissen *et al.,* 1985), using 2 designs -1- Anova-1 completely randomized design (CRD) -2- Factorial implemented in completely randomized design. Each reading = mean of four replicates ± SE for all experiments.

RESULTS

Chemical Studies: Preliminary phytochemical screening on boiling water extracts of some known effective parts of three anthelmintic plants; *Commiphora myrrha* (Gum), *Cassia fistula* (Mature Pods) and *Citrullus colocynthis* (Mature Fruits); (1 ml of each extract = 50 mg/g Dry Weight) was done and all the obtained results showed the presence of steroids and/triterpenoids, anthraquinones, flavonoids tannins, alkaloids, nitrogenous bases, and/or saponins, carbohydrates and/or glycosides, cardiac glycosides, iridoids, chlorides, sulphates and sublimable substances in most samples. *Cassia fistula* boiling water extract' was found to be the richest extract regarding all investigated phytochemicals, so it was selected for further analyses. (Table.1).

Preliminary phytochemical screening Results' of different extracts of examined plant parts of *Cassia fistula* indicated the presence of all investigated phytochemicals in most samples; with special reference to boiling water extracts of mature pods, followed by leaf petioles and stems and also in methanol extracts of all investigated parts (leaf petioles, leaf blades, stems and mature pods); in this regard, ethanol extracts were found to contain the lowest amount of all investigated phytochemicals compared to any other extracts. Ethanol and methanol extracts of all investigated parts are devoid of saponins, since saponins were found only in boiling water extracts of all investigated parts; fresh samples of leaf petioles having specific reference, dried samples of mature pods and stems (Tables. 2-4).

Data in Table. 5 showed that, regarding total phenolic contents, it was found that, the highest amount was recorded to be in dried samples of mature pods (28.671 ± 0.021) and both fresh and dried samples of leaf blades (28.571 ± 0.020 and 24.927 ± 0.020 mg/g, respectively), the least amount was recorded to be in both fresh and dried samples of leaf petioles (19.670 ± 0.011 and 19.686 ± 0.017 mg/g, respectively). Meanwhile the differences in all investigated samples are very small regarding their flavonoidal contents, fresh samples of stems and dried samples of mature pods were consisted of the highest amount in this regard (5.963 ± 0.011 and 5.957 ± 0.008 mg/g, respectively).

Table 1. Preliminary phytochemical screening on boiling water extracts of *Commiphora myrrha* (Gum), *Cassia fistula* (Mature Pods) and *Citrullus colocynthis* (Mature Fruits)

Experiment	*Commiphora myrrha* (Gum)	*Cassia fistula* (Mature Pods)	*Citrullus colocynthis* (Mature Fruits)
1-Flavonoids	+	+++	+
2-Anthraquinones	+	+++	+
3-Unsaturated sterols and/or Triterpenoids	+	+++	+
4-Tannins	+	+++	+
5- Saponins	+	+++	+
6-Alkaloids	+	+++	+
7-Carbohydrates and/or Glycosides	+	+++	+
8-Cardiac Glycosides	+	+++	+
9-Chlorides	+	+++	+
10-Sulphates	+	+++	+
11-Iridoids	+	+++	+
12-Sublimable substances	+	+++	+

Table 2. Preliminary Phytochemical Screening on boiling water extracts of different plant parts of *Cassia fistula*:

Experiment	Fresh Samples			Dry Samples			
	Leaf blades	Leaf Petioles	Stems	Leaf blades	Leaf Petioles	Stems	Mature Pods
1-Flavonoids	++	++	++	+++	+++	+++	+++
2-Anthraquinones	+	++	++	++	+++	+++	+++
3-Unsaturated sterols and/or Triterpenoids	+	++	+	++	++	++	+++
4-Tannins	++	+++	+++	++	+++	+++	+++
5- Saponins	+	++	++	+	+	+	+++
6-Alkaloids	+	++	++	+++	+++	+++	+++
7-Carbohydrates and/or Glycosides	+	++	++	++	++	++	+++
8-Cardiac Glycosides	++	++	++	+++	+++	+++	+++
9-Chlorides	+	+	+	++	++	++	+++
10-Sulphates	+	+	+	++	+++	++	+++
11-Iridoids	+	+	+	+	++	++	+++
12-Sublimable Substances	+	++	++	++	+++	+++	+++

Table 3. Preliminary Phytochemical Screening on methanol extracts of different plant parts of *Cassia fistula:*

Experiment Stems	Fresh Samples			Dry Samples			
	Mature Pods	Leaf Petioles	Stems	Mature Pods	Leaf Petioles	Stems	Mature Pods
1-Flavonoids	+++	+++	+++	+++	+++	+++	+++
2-Anthraquinones	+++	+++	+++	+++	+++	+++	+++
3-Unsaturated sterols and/or Triterpenoids	+++	+++	+++	+++	+++	+++	+++
4-Tannins	+++	+++	+++	+++	+++	+++	+++
5- Saponins	-	-	-	-	-	-	-
6-Alkaloids	++	++	++	+++	+++	+++	+++
7-Carbohydrates and/or Glycosides	+++	+++	+++	+++	+++	+++	+++
8-Cardiac Glycosides	++	++	++	+++	+++	+++	+++
9-Chlorides	++	+	++	+++	+++	+++	+++
10-Sulphates	++	+	+	+++	+++	+++	+++
11-Iridoids	++	++	++	+++	+++	+++	+++
12-Sublimable substances	+++	+++	+++	+++	+++	+++	+++

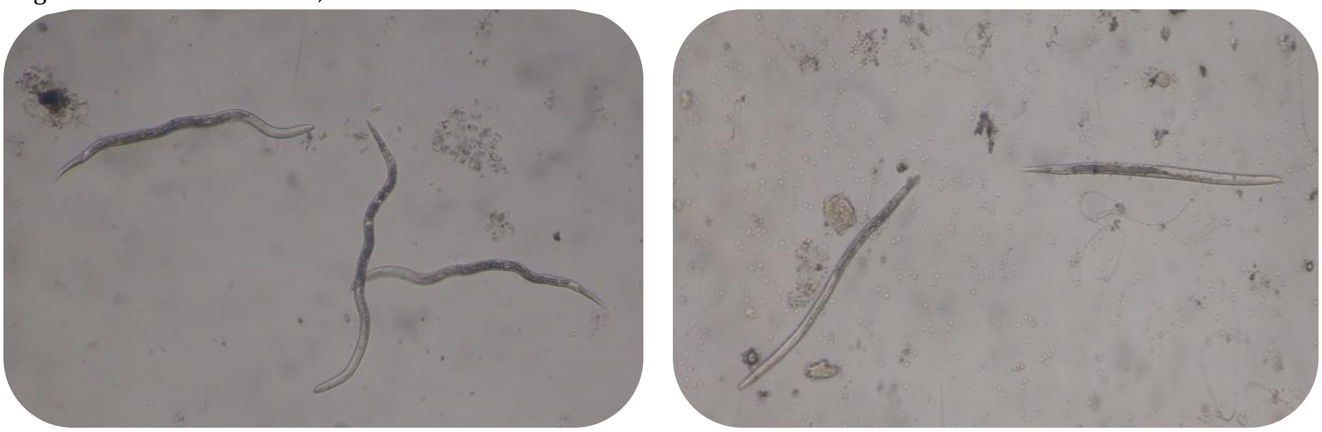
Table 4. Preliminary Phytochemical Screening on ethanol extracts of different plant parts of *Cassia fistula:*

Experiment Stems	Fresh Samples			Dry Samples			
	Mature Pods	Leaf Petioles	Stems	Mature Pods	Leaf Petioles	Stems	Mature Pods
1-Flavonoids	+	+	+	++	+	+	++
2-Anthraquinones	+	+	+	++	+	+	++
3-Unsaturated sterols and/or Triterpenoids	+	+	+	++	+	+	++
4-Tannins	+	+	+	++	+	+	++
5- Saponins	-	-	-	-	-	-	-
6-Alkaloids	+	+	+	++	+	+	++
7-Carbohydrates and/or Glycosides	+	+	+	++	+	+	++
8-Cardiac Glycosides	+	+	+	++	+	+	++
9-Chlorides	+	+	+	++	+	+	++
10-Sulphates	+	+	+	++	+	+	++
11-Iridoids	+	+	+	++	+	+	++
12-Sublimable Substances	+	+	+	++	+	+	++

Table 5. Total phenolic and flavonoidal contents (mg/g) in different plant parts of *Cassia fistula*:

Experiment	Fresh Samples			Dry Samples			
	Leaf blades	Leaf Petioles	Stems	Leaf blades	Leaf Petioles	Stems	Mature Pods
Total phenolic contents	28.571 ± 0.020	19.670 ± 0.011	22.752 ± 0.019	24.927 ± 0.020	19.686 ± 0.017	24.927 ± 0.020	28.671 ± 0.021
Total flavonoidal contents	5.952 ± 0.011	5.951 ± 0.010	5.957 ± 0.008	5.954 ± 0.008	5.940 ± 0.007	5.887 ± 0.006	5.963 ± 0.011

Nematicidal potential of aqueous, ethanol and methanol extracts of various portions of *C. fistula* on *M. incognita* juvenile mortality: Various extracts viz., ethanolic, methanolic and water of *C. fistula'* different parts (either dried or fresh parts of leaf petioles, stems, leaf blades, seeds and mature pods) showed varied nematotoxical efficiencies. Obtained results showed that all tested extracts having nematotoxic effects as they achieved mortality percentages varied between 12 to 100%. The most effective extracts were boiling water extracts, these extracts showed percentages reached to 80%, 66.7% and 58.7 % in extracts of dry leaf petioles, dry Stems and dry leaf blades, respectively. All methanolic extracts recorded mortality percentages lower than 35% with one exception of fresh leaf petioles which achieved 100% mortality. On the other hand, ethanolic educes of dried and fresh parts of the plant recorded the lowest toxicity toward nematode' juveniles. Only dry leaf blades ethanolic extract' gave mortality percentage around 50%, while the rest ethanolic extracts achieved mortality around 30% and less. In general, water was the most appropriate solvent that release many antinematodal compounds from all *C. fistula* parts and this was observed by giving the higher mortality percentages compared with other solvents. Methanolic extracts were categorized in the second rank, as its extracts' showed lower efficacy in killing nematode juveniles' except one extract. Ethanol extract was the less effective one since it possessed the lowest toxicity effect on root-knot nematode juveniles (Fig. 2). It was noticed that, juveniles in the control replicates which received distilled water only instead of extract were mobile and live till the end of bioassay, while in treated dishes the dead juveniles were static and have attained the shape of straight or semi-straight bodies (Figure 1)



(A) (B)

Figure 1. Survive juviniles in distilled water (A), dead juviniles in *Cassia fistula* extract (B)

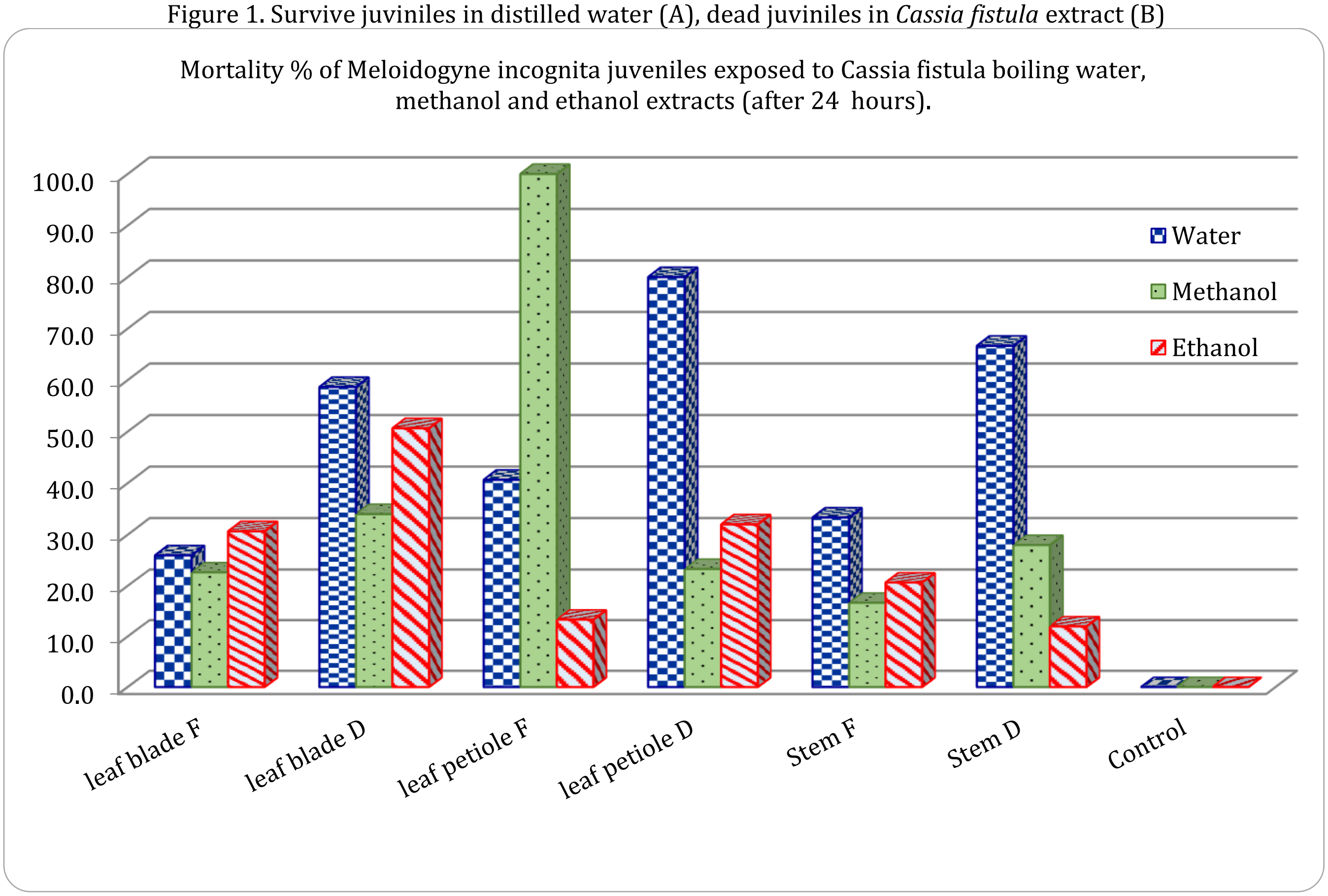


Figure 2. Effect of diffenrt extacts of *cassia fistula* on *M. incognita* juveniles' mortality F= fresh samples, D= dried samples

DISCUSSION

Plant parasitic nematodes are serious pests facing the agricultural production as well as impairing food security universally. Root knot nematodes (RKNs) represent a great challenge and the most destructive pest in agronomic crops, as they occurring in all over globe, and particularly their damage more obvious in warm countries. RKNs can attack thousands of plants causing severe losses especially in favorable conditions and with interaction with other plant pathogens. Because of obvious pesticides' hazards, the other ecofriendly control approaches become necessary. On this concern biological control of nematodes and plant based nematicides are the proper alternatives for synesthetic chemicals. These manners not only beneficial for the growers via reducing the production costs and improving their livelihood but also to reduce the ecosystem pollution, improving human health and mitigating the climatic changes impacts as well as combating desertification. Consequently, many researches seeking some alternatives; such as soil amendment using organic matters which achieved suppressing of nematodes with varying degrees (El-Nuby, 2002). The focus was increased on developing Botanical-based products to manage phytonematodes as well as to conserve the sustainability of natural resources. Various plants have been evaluated for their nematicidal potential, utilizing plant extracts for suppressing nematodes was previously adopted (Alam and El-Nuby, 2019; El-Nuby *et al*., 2020).

To preserve the various elements of the environment, this research was conducted to assess the nematicidal potential of *C. fistulas* various extracts towards root knot nematode.

Results showed that extracts of various parts of *C. fistulas* possessed various degrees of nematotoxicity. Also extracts' efficacy was affected with the used part of the tree, drying and the used solvent. The aqueous extracts achieved nematicidal activity slightly higher than methanolic and far from ethanolic ones. Previous investigation of Cassia spp*.* showed toxicity against parasitic nematodes; Silva *et al*. (2018) investigated the ovicidal and larvicidal activity of protein preparations achieved by *Cassia fistula* leaves on the gastrointestinal parasites of goats. Their results revealed tha hatching of eggs inhibition is done by of *C. fistula protein fraction*, in addition, additionally, these fractions of *C. fistula* caused a significant inhibition in the larval development also. Similarly, Zaragoza-Bastida *et al*. (2019) noticed also the anthelmintic potential, eggs hatching inhibition and larval mortality of pod extract of *Cassia fistula* on sheep nematodes. Phytoparasitic nematodes have also adversely affected by various *Cassia* extracts; aqueous extract of *C. fistula* seeds killed high percentage of root knot nematode larvae (khurma and Mangotra, 2004). Also, leaf aqueous extract of *C. fistula* exhibited high mortality rates of *Meloidogyne incognita* juveniles after two days of exposure (Asif *et al*., 2013), these outcomes match with our results. The antinematodal activities of four *Cassia* species; *Cassia surattensis, C. siamea*, *C. fistula*, and *C. spectabilis* were evaluated, in this regard the significant and highest suppression of juveniles was achieved by *C. surattensis* extract (Wahyuni *et al*., 2019). Phytochemical Screening was carried out on *Cassia spp*. And its results revealed the presence of tannins, triterpenoids, alkaloids, flavonoids, saponins, phenol hydroquinone and steroid (Dehuri *et al*., 2021), these findings are in harmony with our results. Flavonoids and anthraquinones are the main groups of bioactive constituents present in *Cassia spp* among various chemical compounds (Khurm *et al*., 2020).

The Anthelmintic properties maybe related to tannin contents in the plant; some studies have demonstrated the role of tannins to block through uncoupling the oxidative phosphorylation causing death of nematodes. Also due to the binding of tannins to glycoprotein on the nematode cuticle which lead to paralysis and death of parasites. Tannins also can damage both cuticle and digestive tissues of nematode juveniles (Tiwari *et al*., 2011). Anthelmintic activities of *C. fistula* (ovicidal, larvicidal, reduction of deposited eggs) may be related to the presence of condensed tannins (Brigida *et al*., 2015). Tannins may affect via two mechanisms; the first is direct, which may be done by altering biological processes inside nematode (Hoste *et al*., 2006), the second effective mechanism may be done indirectly through the inhibition of egg hatching (Butter *et al*., 2000). The larvicidal action observed by *C. fistula* protein preparations may be related to the presence of *lectins*, the larvicidal action is genuine to this class of proteins (Silva *et al*., 2018). It was suggested that the adverse effect of *Cassia alata* against nematodes may linked to the presence of saponins, which in the extract, and it may also be related to the ability of saponins to disrupt eggs membranes and to increase their permeability, thus countering egg maturation (Mansfield *et al*., 1992). Some findings augmented our findings and interpreted the nematicidal efficacy of *C. fistula* extracts also. They suggested that flavonoid, saponins, alkaloids and terpenoids may exert an anthelmintic action (Patel *et al*., 2010; Botura *et al*., 2013; Jain *et al*., 2013). Similarly, another interpretation was introduced by Zaragoza-Bastida *et al*. (2019), they attributing the antinematodal action of *C. fistula* to the presence of phenolic compounds. However, further studies are needed to elucidate the active component(s) in the ovicidal and larvicidal activity of *C. fistula* and its probable mechanism of action.

According to Mandloi *et al*. (2018), nature of active principles may be helped by the periliminary phytochemical screening of plant parts and further it can cause to the discovery and development of new important compounds. The presence of secondary metabolites like flavonoids, tannins, carbohydrates, saponins, glycosides and phenols are proved by the conduction of phytochemical analysis on *cassia fistula* in the leaf extracts while flavonoids, carbohydrates, tannins, saponins, glycosides, phenols and terpenoids in the bark of stem. The ethanolic extract of *Cassia fistula* contains significant amounts of phenolics and flavonoids. Phenolics and flavonoids are ubiquitously seen in most of the plant species and reported to possess a broad spectrum of biological properties. *Cassia fistula* having many pharmacological spectrum is an important medicinal plant showed by extensive literature survey. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. This plant is widely used by tribal people to treat various ailments including ringworm, in addition to other fungal skin infections. Mwangi *et al*. (2021) mentioned that, in traditional medicine, this plant has been used in the treatment of diabetes, hematemesis, leucoderma, pruritis, intestinal disorder and as antipyretics, analgesic and laxative agent. The fruits of this plant was used to cure chest problems, inflammation, liver complications, throat disorders, rheumatism and asthma, this may expressed on the basis of the obtained results in this study, those indicated that, all investigated parts of this plant are rich sources of phenols.

Preliminary phytochemical screening on all tested extracts of *C. fistula* showed the presence of tannins, flavonoids, alkaloids and/or nitrogenous bases, anthraquinones, cardiac glycosides, carbohydrates and/or glycosides, chlorides, steroids and/triterpenoids, sublimable substances, saponins, iridoids and sulphates in most extracts of various plant parts. In this concern, results of preliminary phytochemical investigation on all studied extracts of all plant parts showed that, the richest extracts in these studied phytochemicals are belonging to water and methanol extracts of all plant parts samples. On the other hand, the ethanolic extracts were found to be the poorest extract regarding the screened phytochemicals. These results supported the relation between the phytochemical composition and nematicidal potential of these tested extracts. Results of the phytochemical screening of different extracts of *Cassia spp.* leads to the isolation and identification of some groups of secondary metabolites including alkaloids, anthraquinones, flavonoids, pentacyclic triterpenoids, sterols, phenylpropanoids, and γ-naphthopyrones. Among these chemical compounds, anthraquinones and flavonoids are the major groups of bioactive constituents found in genus *Cassia* (Khurm *et al*., 2020). *Cassia fistula* extracts have some chemicals like; carbohydrate lipid and free amino acid and secondary metabolites, notably phenolic compounds, oxyanthraquinone, dihydroxyanthraquinone, glucoside, proanthocyanidins, flavonoids (Khatri *et al*., 2021). Similarly, Thirumal *et al*. (2012) reported many phytochemicals in leaves of *Cassia sp.*; free rhein, and its glycosides- sennosides; anthraquinone, tannin, oxyanthraquinone and volatile oils, epiafzelechin, epiafzelechin-3-Oglucoside, epicatechin, procyanidin B2, flavonoids. In general, the constitution and concentration of secondary metabolites in specific plant is affected by many factors e.g., its genotype, soil properties, climate elements, collection time and solar radiation (Gobbo-Neto and Lopes, 2007). Although, obtained nematotoxic compounds may be directly used as crud plant extracts, phytochemical formulations, soil amendments and used as model compounds for the development of chemically synthesized derivatives. Our findings introduced a non-traditional tool of managing nematodes via *C. fistula extracts.* Further works for the determination of the best extract including the most potent compound (s) responsible for nematotoxicity but less or nontoxic to plant and human are needed.

CONCLUSION

Aqueous, ethanolic and methanolic extracts of both fresh and dried parts (leaf petioles, stems, seeds, mature pods and leaf blades) of *C. fistula* contained antinematodal compounds against infective stage juveniles of root knot nematode and there is a positive relation between the nematicidal action and phytochemical composition of each examined extract. Results showed that aqueous extracts of *C. fistula* possessed the highest toxic effects against nematodes' juveniles, especially extracts of dried plant parts. Methanol extract of some plant parts showed high nematotoxicity. The evaluated extracts offer a safe alternative to chemical nematicides. Future researches must be focused on the identification of nematotoxic molecules, also the more suitable extraction approaches and then assessment the isolated compounds under lab and greenhouse conditions. According to the current study, it is advisable the use of boiling water extract of *C. fistula* to control nematodes, in integration with regular combating measures, or soil amendments for plant leaves and other plant residues. Results of this study are considered to be an initial point to produce plant based-nematicide or bio-nematicide. Additionally, it is recommended to introduce these preparation or materials in an affordable way and cost effective product to expand their availability for low income and stallholder farmers especially in desert lands.

Based on the previously mentioned results, it could be concluded that, different extracts of all investigated plant parts of *Cassia fistula* are rich sources of all examined phytochemicals of interest. These extracts are rich in their phenolic and flavonoidal contents. Accordingly, more studies for *in vitro* production of these secondary metabolites are highly required to improve this phytochemical productivity in this important anthelmintic plant to combat nematodes.

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Eman A. A. H. Alam	:	All preparations of extracts and all phytochemical examinations of these prepared extracts, participation in the writing and editing processes before and after the submission of the manuscript till publication as a corresponding author, general supervision on the study
Ahmed S. M. El-Nuby	:	*In vitro* nematicidal studies of the prepared extracts, participation in the writing process