International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 14-05-2021, Accepted: 29-05-2021, Published: 12-06-2021
Volume 6, Issue 3, 2021, Page No. 620-626

Screening of nematicidal activity and phytochemical analysis of *Caesalpinia sappan* (Linn).

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Abstract

Introduction: The present investigation has carried out to shed light on the Nematicidal activity and preliminary phytochemical screening of *C. sappan* powder.

Materials and methods: Collection and extraction of *C.sappan*: The Pathimugam powder was collected from the commercial market of Kerala state. The extract was made from the powdered plant material (20g) by using Aqueous, Ethanol and Methanol as solvent with Soxhlet apparatus (500 mL) at 60˚C.

Results: Egg Hatchability Test: The solvents were selected and tested at different concentrations (10, 20, 30 and 40ppm) were tested the egg hatchability of root knot nematode *M. incognita* (Table 1). The results show a decrease in egg hatchability as increasing concentration of the extracts. The increase in exposure period and increasing concentration also decrease in egg hatchability. The *C.sappan 10 ppm* extract has been found to be less toxic when compared to other extracts and the hatchability has been found to be decreasing with increasing concentrations from 10 at 5 ppm to 1 at 25 ppm after 24 hours exposure time. The egg hatchability was observed in the decreasing order *10 ppm > 20 ppm > 30ppm > 40ppm.* Significant results were observed at p>0.0001.

Larval Mortality Test: The present study, bark extract of *C. sappan* was tested nematicidal activity of second stage juveniles of *M. incognita* in the laboratory conditions. The juveniles were exposed for 24, 48 and 72 hours exposure 2 time at different concentrations (5, 10, 15 and 20 ppm) and the percentage of larval mortality was increasing with increasing toxicity of the bark extracts. Toxicity of each extracts LC_{50} and LC_{90}, slope value, chi square value and spontaneous response were calculated (Table 3).

Qualitative Analysis of Phytochemicals from *C. sappan*: Alkaloids, terpenoid, tannin, flavonoids, Steroids and phenolics were present in Ethanolic extract; Alkaloids, terpenoid, tannin and phenols were found in Aqueous extract; all the chemicals except saponins were present in methanolic extract only (Table 4). All the three solvent extracts have shown the negative result of saponin and reducing sugar was absent both in aqueous and ethanol. The solvency of *C.sappan*, methanol exhibit high when compare with two other solvents.

FT-IR Results: Aromatic, aliphatic, ring compounds were present in the experimental samples. The peak values are obtained from FT-IR results shows that 418.55 to 549.71 821.68– Aliphatic Iodo groups, 1035. 77 and 1112.93; 1161.15 – Aliphatic bromo compounds, 1597.06 - Alcohol hydroxyl compound rings, 1737.86 - Hydroxy groups, 2040.69 – dimethyl groups, 2063.83 – Saturated aliphatic groups like methylen C-H bends, 2113.98 – aromatic ring stretch, 2310.72 – Organic nitrates, 2372.44-Methyl groups; 2918.3, 3336.85- Ammonia groups were found in the present experimental samples. **Conclusion**: The present work identify the functional groups these groups do the wonderful role against the pathogens. An attempt has been made in this work to study the functional derivatives of the sample by observing the position and relative intensities of the band in FTIR.

Keywords: *meloidogyne incognita, nematicidal activity, c.sappan*

Introduction

Since, ancient times nature has been an important source of medicinal agents and a large number of natural products have been identified and developed from natural sources based on their use in traditional medicine. Numerous medicinal plants are of global interest today because of their therapeutic and economic significance. According to the World Health Organization, approximately 80% of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk or alcohol [1]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [2]. For a long period of time, plants have been a valuable source of natural therapies. The use of plant compounds for pharmaceutical purpose has gradually increased in the world. A special feature of angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. These so called secondary metabolites have contributed more than 7000 different compounds in use today as cardiac drugs, anticancer agents, hormones, antibiotics, laxatives, diuretics, analgesics, anesthetics, drugs for ulcer treatment and antiparasitic compounds. In USA 74% of drugs are based on plants [3]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that

they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits [4]. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and About 150 phytochemicals have been studied in detail [5].

Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well - known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [6, 7, 8]. Phytochemicals are responsible for medicinal activity of plants these are non-nutritive chemicals that have protected human from various diseases [9]. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. The major constituents are consists of carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs [10]. Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids secreted by the root of their host plant help Rhizobia in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule. In addition, some flavonoids have inhibitory activity against organisms that cause plant diseases [11].

In plants, saponins may serve as anti-feedants and to protect the plant against microbes and fungi. Some plant saponins (e.g. from oat and spinach) may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste, and so can reduce plant palatability (e.g., in livestock feeds), or even imbue them with life-threatening animal toxicity. It makes clear that some saponins are toxic to cold-blooded organisms and insects at particular concentrations. Further research is needed to define the roles of these natural products in their host organisms, which have been described as "poorly understood" to date [12]. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body [13]. Pathimugam is widely used since ancient days and is an excellent herbal medicine. Kerala is one state in India which is popular for use of ayurvedic medicines. Herbs and shrubs are said to possess certain medicinal values but less considered that even a tree which does not bear any edible fruits can also possess some medicinal compounds. In order to make a healthy living, one such tree species evolved which is called Pathimugam tree. It is also known by the name sappan wood or East Indian red wood. It is said to be a multipurpose tree and grown in regions where red soil is available in vast [14]. Sappan wood consists of the heartwood of Caesalpinia Sappan, Linn. (Leguminosae family), a tree indigenous to India. The wood occurs in red, hard, heavy pieces, or in orange-red chips. It is whitish when freshly cut but becomes red on exposure to air. A transverse section exhibits well-marked, concentric rings, numerous narrow, modularly rays, and large vessels. The drug has no odour, but an astringent taste. If "Pathimugam" is boiled in water, the water gets purified and thereby drinking this water can prevent epidemic diseases spreading through water. This material is used as an ingredient in almost all Ayurvedic medicines and because of its prophylactic nature; it can be used by itself as a medicine. This way the people in Kerala are trying to make their living hygienic with the use of natural herbals and ayurvedic trees available in that region [15]. The Tibetan medical Encyclopaedia, the Ashtangahridayam, the Sahasrayogam, the Dhan-wanthari Nikhandu and all Pauranic Ayurvedic books carry the benefits of this herbal tree. Experts state that "Pathimugam" can prevent and cure diseases like certain types of cancer, kidney disorder, piles, cholesterol, blood purification, stomach ailments, Skin diseases, diabetics, body heart etc. [16,17,18] Apart from being a valued medicinal herb, this wood is also used for various commercial purposes. The wood when boiled or add to boiling water produces a red colour like ivory young which is generally used for painting, cakes, drinks or as ink and in colouring textiles. Pathimugam is an excellent dye raw material. WTO and WHO has decided to ban the usage of artificially chemical dye for human usage, both internal and external, within a short span of time. By doing so, the experts foreseen that the natural colour dye demand will increase by 1000 times done the current consumption.

Hence, agriculture experts state that Pathimugam has a bright future in the natural colour industry [18, 19, 20].

Nematicidial Activity

Plant parasitic nematodes, are capable of reproducing on over 2,000 species of plants [21] and are responsible for approximately 50% of overall damage [22]. The root knot nematodes (*Meloidogyne incognita*) produce galls on the roots of many vegetable crops, pulses, some of the fruit crops, tobacco, ornamental crops and causes severe losses. This nematode is known to attack more than 3,000 separate host plants. *M. incognita* causes 33 percent loss in vegetables in Bihar and 60 percent loss in New Delhi. The symptoms of nematode infection include formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plants [23]. The root-knot nematode has been variously controlled by nematicides, fungicides or the combination of both with insecticides, although proven to be effective [24], the negative effects on the environment and other ecological consequences cannot be overlooked. Therefore there is an urgent need to shift attention from the conventional use of synthetic hazardous control measures to more environmentally friendly ones such as the use of less toxic methods of pest and disease control such as plant based substances. The use of botanical extracts for controlling *Meloidogyne* is becoming appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides. There has been a de-registration of some hazardous nematicides. Increasing pressure is on farmers to use non-chemical pest control methods that do not pollute the environment. This emphasis the need for new methods of control such as the use of plant extracts [25, 26] studied that the reduction in population densities of plant parasitic nematodes in response to application of inorganic amendments. However, very less research has been done on this bark of the tree and there is a wide scope for ivestigation. In keeping this view in mind, the present investigation has carried out to shed light on the Nematicidal activity and preliminary phytochemical screening of *C. sappan* powder.

Materials and Methods

Description of *C. sappan*

The tree grows upto 8-10 m in height and the trunk reaches upto 15-30 cm in diameter, after a period of 8 years. The heartwood contains the water soluble dye and it has high economic values. It can be cultivated in any type of soil, but only red soil is found to be suitable for maximum growth of the tree. Due to its withstanding power in drought condition, it can be grown in any hot regions of the country, but it can do that for only 10-12 days period. At the month of April, flowering starts and that will continue till November or December. Basically, the golden yellow flowers are cross pollinated by bees, butterflies and insects. The fruits which bear 3-4 seeds will start after 5-15 days of flowering, during the month of September and continues to mature for 3 more months. The seeds are brown/ black in colour with ellipsoid in shape. In order to grow the plant in healthy manner, the side branches of the tree are cut during the flowering period. The tree is cultivated for many purposes such as for horticulture, dye production, fencing and medicinal substances. It is good fencing since it contains spikes all around its branches and trunk. So it is planted at the borders of the paddy or grape field. Tannin is basically used to make leather soft and the pods of the tree contain 40 % of the compound. Hence the fruits can be used to produce tannin and replace it with the sumac (toxic used for tanning purpose).

Collection and extraction of *C.sappan*:

The Pathimugam powder was collected from the commercial market of Kerala state. The extract was made from the powdered plant material (20g) by using Aqueous, Ethanol and Methanol as solvent with Soxhlet apparatus (500 mL) at 60°C. The last trace of solvent was removed under reduced pressure distillation and the crude extract was dried in hot air oven for overnight.

Qualitative estimation of *C.sappan* [27]:

1. Test for alkaloids

The ethanolic extract was warmed with 2% H_2SO_4 for 2 minutes. It is filtered and few drops of Mayer's reagent were added and creamy white coloured precipitation indicates the presence of alkaloids.

2. Test for Flavonoids

A few drops of 1% ammonia solution were added to the extract in a test tube. A yellow coloration was observed for the presences of flavonoids.

3. Test for Terpenoids (Salkowski Test)

The extract was mixed with 2ml of chloroform and concentrated H_2SO_4 is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.

4. Test for Tannins (Ferric chloride)

Small quantity of extract is boiled with 5ml of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. The filtrate was diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black colour indicates the presence of Tannins.

5. Test for Steroids

2ml of acetic anhydride was added to extract and then 2ml of H2SO4was added, the colour changes from violet to blue or green in sample extract indicates the presence of steroids.

6. Test for Saponins

A small quantity of different extract was diluted with 4ml of distilled water. The mixture is shaken vigorously and then observed on standing for stable brake.

7. Test for Glycosides

5ml of diluted H_2SO_4 was added in extract in a test tube and boiled for 15 minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal parts of Fehling's solution was added and boiled for 5minutes. A more dense red precipitate indicates the presence of glycosides.

8. Test for reducing sugar

A small fraction of extract was added vigorously with 5ml of distilled water and filtered to the filtrates while equal volume of Fehling's solution was added and were shaken vigorously. A brick red precipitation indicates the presence of reducing sugar.

9. Test for phenol
The extract was treated with 3-4drops of ferric chloride solution. The formation of bluish black colour indicates the presence of phenol.

Nematicidal Activity
The sappan powder extracts was used for test the nematicidal activity. These plants were collected from Keelamathur area of Madurai. The collected leaves were shade dried and powdered with the help of mixer grinder. The powder was extracted by Soxhlet apparatus with 200ml of methanol as a solvent [28]. The extracted material was then dissolved in methanol (1:10) w/v to prepare stock solution. Different concentrations of plant extracts (5 to 25 ppm) were prepared from the stock solution using distilled water. The egg masses and nematode larvae were collected from the *Acalypha indica* plant near agricultural fields. Effect on hatching was evaluated on five mature uniform size egg masses of *M. incognita* were suspended in the extracts and water (control), replicated three times in cavity blocks. The blocks were kept at room temperature. Observations were recorded on number of hatched larvae, dead and alive after 24, 48 and 72 h. For effect of nematode larval mortality of 30 freshly hatched J_2 of *M. incognita* were kept at room temperature. Mortality of larvae was calculated as a percent of total larvae suspended and LC_{50} and LC_{90} values were determined by using probit analysis [29].

Fourier Transform Infrared Spectrophotometer (FTIR)
Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.
The samples were given for analysis in The Madura College, Tamillnadu, India. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm1 with a resolution of 4 cm1.

Results and Discussion
Egg Hatchability Test
The solvents were selected and tested at different concentrations (10, 20, 30 and 40ppm) were tested the egg hatchability of root knot nematode *M. incognita* (Table 1). The different concentrations (10, 20, 30 and 40ppm) were tested among the three solvents. The results show a decrease in egg hatchability as increasing concentration of the extracts. The increase in exposure period and increasing concentration also decrease in egg hatchability. The *C.sappan 10 ppm* extract has been found to be less toxic when compared to other extracts and the hatchability has been found to be decreasing with increasing concentrations from 10 at 5 ppm to 1 at 25 ppm after 24 hours exposure time. Similarly all the bark extracts were increasing egg hatchability at low concentration and decreasing at high concentration. The egg hatchability was observed in the decreasing order *10 ppm > 20 ppm > 30ppm > 40ppm.* Significant results were observed at p>0.0001. Decrease larval hatchability in the high concentration of the bark extract and the increase in number of mortality were observed. Similarly Oka *et al. (*2013) [19] reported that stem, bark, root and flower of *Ochradenus baccatus* aqueous extract against the root-knot nematode *M.javanica*. Hundred percent of second-stage juveniles were immobilized after exposure to 4% root extract.

Larval Mortality Test
The present study, bark extract of *C. sappan* was tested nematicidal activity of second stage juveniles of *M. incognita* in the laboratory conditions. The juveniles were exposed for 24, 48 and 72 hours exposure 2 time at different concentrations (5, 10, 15 and 20 ppm) and the percentage of larval mortality was increasing with increasing toxicity of the bark extracts (*Aqueous extract* (56%), *Ethanolic extract* (66%), *Methanolic extract* (70%) (Table2). Toxicity of each bark extracts LC_{50} and LC_{90}, slope value, chi square value and spontaneous response were calculated (Table 3). Similarly Dongre and Simon (2013), reported that nematicidal activity of different plant extracts namely Neem (*Azadirachta indica*), Bael (*Aegle marmelos*), Jatropha (*Jatropha curcas*), Eucalyptus (*Eucalyptus globus*), Sahjan (*Moringa oleifera*), Ber (*Ziziphus mauritiana*), Sarifa (*Annona reticulate*), Congress grass (*Parthenium argentatum*) against *Meloidogyne graminicola*. *In vitro* studies 25%, 50% and 100% concentrations of bark extracts significantly reduced second stage juvenile of mortality after 24 and 48 h. The plant leaf extracts of Bael and Neem` exhibited highly promising mortality range 80-88% after 48 h exposures. Ardakani *et al*. (2013) also reported that essential oil from dried leaves of true myrtle (*Myrtus communis* L.) at the rates of 8000, 4000, 2000, 1000, 500, 250, 125, 62.5 and 0 mg/l were tested for its nematicidal activity against the second stage juvenile (J2) of root knot nematode, *M. incognita*. Essential oils of 8000 and 4000 mg/l were showed that 100% nematode mortality. No nematode mortality was seen in treatments of 250, 125 and 62.5 mg/l of the oil.

Table 1: Effect of different concentrations of bark extract on egg hatchability of root knot nematode *Meloidogyne incognita*.

		Egg hatchability at different concentrations (ppm)				
Plants	**Exposure time (Hours)**	**Control**	**10ppm**	**20ppm**	**30ppm**	**40ppm**
Aqueous extract	24	10	8	7	5	3
	48	15	12	10	7	4
	72	18	15	11	8	3
Ethanolic extract	24	14	10	7	5	3
	48	18	12	8	3	2
	72	22	18	15	6	3
Methanolic extract	24	14	11	9	7	6
	48	19	15	10	6	4
	72	22	17	13	10	6

Table 2: Effect of different concentrations of bark extracts on larval mortality of root knot nematode *Meloidogyne incognita*

Plants	Exposure time (Hours)	Control	10ppm	20ppm	30ppm	40ppm
Aqueous extract	24	0	7.66	10.0	14.0	18.0
	48	0	10.0	13.0	17.33	21.0
	72	0	13.33	15.0	19.83	21.66
Ethanolic extract	24	0	10.66	13.33	18.66	19.66
	48	0	13.33	18.66	21.0	21.66
	72	0	20.66	21.66	25.0	25.0
Methanolic extract	24	0	5.33	7.0	11.66	18.0
	48	0	10.33	11.66	15.0	20.0
	72	0	14.0	16.0	19.33	21.66

Table 3: Toxic effect of different plant extracts against *Meloidogyne incognita*

Plants	Exposure time (Hours)	LC_{50}	LC_{90}	Slope ± S.E	Chi Square (χ^2)
Aqueous extract	24	20.41	275.38	1.95±0.45	0.533
	48	14.39	433.47	1.61±0.43	0.829
	72	11.37	297.19	1.59±0.43	1.248
Ethanolic extract	24	14.03	477.19	1.58±0.43	0.537
	48	8.34	845.84	1.30±0.43	0.215
	72	5.69	42.31	1.75±0.49	1.016
Methanolic extract	24	30.72	512.39	1.94±0.48	2.047
	48	17.22	1339.85	1.46±0.43	1.429
	72	10.33	907.54	1.36±0.43	1.896

Fig 1: Show the chips of C.sappan

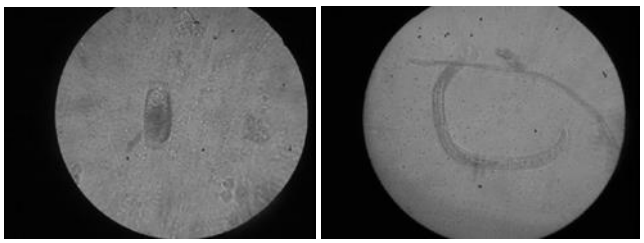

Fig 2: Shows that egg and larval stages of *M. incognita.*

Qualitative Analysis of Phytochemicals from *C. sappan*: The phytochemical analysis of nine different chemical compounds (Alkaloids Terpenoids, Saponins, Flavonoids, Tannin, Glycosides, Phenols, Steroids and Reducing sugars) were tested in three different extracts Methanol, aqueous and Ethanol, respectively. However, all these chemicals were not extractable in one solvent. Alkaloids, terpenoid, tannin, flavonoids, Steroids and phenolics were present in Ethanolic extract; Alkaloids, terpenoid, tannin and phenols were found in Aqueous extract; all the chemicals except saponins were present in methanolic extract only (Table 4). All the three solvent extracts have shown the negative result of saponin and reducing sugar was absent both in aqueous and ethanol. The solvency of *C.sappan*, methanol exhibit high when compare with two other solvents.

Table 4: The qualitative test for phytochemical analysis of various extracts of *C.sappan*

	Solvents		
Compounds	**Methanol**	**Aqueous**	**Ethanol**
Alkaloids	+	-	+
Terpenoids	++	++	+
Saponins	-	-	-
Flavonoids	+	-	+
Tannin	++	+	+
Glycosides	-	-	-
Phenols	+	+	+
Steroids	+	-	+
Reducing sugars	+	-	-
Carbohydrate	+	-	+

Fig 3: Different concentrations of solutions

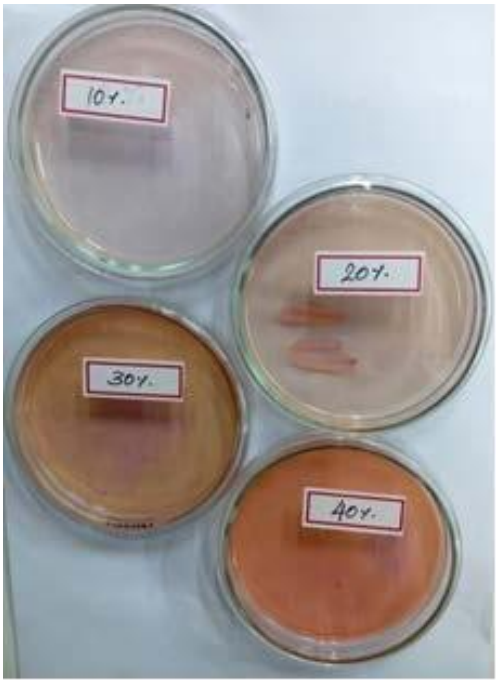

Fig 4: egg hatchability test

Fig 5: Larval mortality test

Phytochemical analysis of 13 medicinally important plants of Margalla hills and surroundings and investigated the qualitative and quantitative analysis of the major bioactive constituents [30]. Alkaloids, saponins, tannins, anthraquinones, flavonoids, flavons, flavonols and chalcones, terpenoids, phlobatanins, coumarins, steroids and cardiac glycosoides were analyzed qualitatively whereas alkaloids, flavonoids, tannins, phenols and saponins were analysed quantitatively too. Quantitative analysis of total polyphenols, tannins, proanthocyanidins and flavonoids in 20 Serbian and Chinese cultivars of *Soybean* (*Glycine max* L.) was performed [31]. In the present study Successive extractive values revealed the solubility and polarity particulars of the metabolites in the plant. Methanolic extract showed high extractive yield 8.8 %w/w when compared to other extracts. Phytochemical and nutrient evaluation of *Spondias mombin* leaves was performed and reported the qualitative and quantitative analysis of various groups of chemical constituents, minerals and vitamins [32]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Quantitative phytochemical estimation and antioxidant studies on aerial parts of *Naravelia zeylanica* was done [33]. It was a woody climber belonging to Ranunculaceae family and whole plant is used as medicine for different problems. Powdered plant material was found to have alkaloid 0.86%w/w, total phenol 0.72 %w/w, tannin 8.72 %w/w, flavonoids 0.56 %w/w and saponin 2.86 %w/w were present in the aerial parts. High concentration of phenols and tannins in this plant cause greater reducing powered which in turn responsible due to the presence of these constituents. Quantitative estimation of methanolic extract of various phytoconstituents *viz* total tannins (156.5 mg/g), total phenolics (146.40 mg/g), total flavonoids (30 mg/g) and total flavonols (3.6 mg/g) content on *Cinnamomum wightii* Meissn flowers of family Lauraceae which may serve as diagnostic tools for identification of crude drug [33]. In vitro in the form of aqueous extract and in vivo as different dry bark powder against nematode *M. incognita*. The inhibition of egg hatching of *M. incognita* by different plant bark extracts in decreasing order was as follows: *Aegle marmelos* >*Prosopis cineraria* >*Nerium oleander* > *Clerodendron aculeatum* > *Bougainvillea spectabilis*> *Lantana camara* > *Withania somnifera* >*Thevetia peruviana* > *Cassia fistula* > Nemacon > Vircon > Control at both lower and higher concentrations and was nemostatic in nature. Minimum gall formation was observed in *Aegle marmelos* and *Prosopis cinerarea* treated plants. Use of *Aegle marmelos* and *Prosopis cinerarea* were recommended for the management of root knot disease both *in vitro* and *in vivo*.[34]

FT-IR Results

Aromatic, aliphatic, ring compounds were present in the experimental samples. The peak values are obtained from FT-IR results shows that 418.55 to 549.71 821.68–
Aliphatic Iodo groups, 1035.77 and 1112.93; 1161.15 – Aliphatic bromo compounds, 1597.06 - Alcohol hydroxyl compound rings, 1737.86 - Hydroxy groups, 2040.69 – dimethyl groups, 2063.83 – Saturated aliphatic groups like methylen C-H bends, 2113.98 – aromatic ring stretch, 2310.72 – Organic nitrates, 2372.44-Methyl groups; 2918.3, 3336.85- Ammonia groups were found in the present experimental samples (Plate:5). In the earlier FTIR study revealed that *L.leucocephala* and *G.sepium* leaf extracts resulted in reduced nematode population in okra plant due the presence of phenolic compound, aromatic amide and carboxylic acid [35]. Another researcher identified the analgesic, anti - inflammatory and anxiolytic activity of *C. sappan* [36]. *C. sappan* has been used as a folkloric medicine to treat amentia, anorexia, fever, swelling and rheumatism [37, 38] antileishmanial activity of acetone and methanol extract. *C. sappan* also exhibited significant antinociceptive activity, this is due to the presence of novel pregnane glycoside named Carumbelloside II and V, and anti - inflammatory activity due to the presence of pregnane glycoside Carumbelloside II and III. The present work identify the functional groups these groups do the wonderful role against the pathogens. An attempt has been made in this work to study the functional derivatives of the sample by observing the position and relative intensities of the band in FTIR. The spectral analysis indicated that the specific functional groups. FTIR spectroscopy technique showed that the presence of functional groups which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of compound by use of different analytical method in future.

Conclusion

C. sappan is widely used in ayurvedic medicine for the treatment of various ailments. It is reported that extract of *C.sappans* has good immunomodulating effect. It also has the ability to scavenge free radicals and to block free radicals and to inhibit radical induced membrane damage. It also has the antibacterial activity and antifungal activity. It also has ability to protect the liver from various diseases. It is found that it is non-toxic in acute toxicity studies. Various types of studies, which have been done on *C. sappan*, reveal that it is an excellent drug, which could be a good remedy for various ailments of animals as well as human beings yet the safety and the potential indications in human beings and animals have to be established using modern techniques. The experimental plant *C. sappan* was exhibited the plenty of phytochemical compounds. From my investigation, it was estimated by both qualitatively as well as quantitatively. The plant chemical like a secondary metabolites will use in discovery of new drugs for benefit of human life. In future the work will be carried out and established with Gas

chromatography - Mass spectrometry analysis for the structure elucidation and identification of the phytochemical compounds which are present in the experimental plant and useful for the human welfare.

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