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Phytochemical Screening and Anthelmintic Activity of Methanolic Extracts of Herbal Plants Schinus limonia L

Omkar Devade[a]*,Shinde rucha[b], Vijapure Gautami[c], Sokate Nisarga[d],Ranpise pallavi[e], Randave utkarsha[f]

[a]Assistant Professor, Department of pharmacology, AISSMS College Of Pharmacy,Pune-India

[bcdef]Shinde rucha, Vijapure Gautami, Sokate Nisarga,Ranpise pallavi, Randave utkarsha.AISSMS College Of Pharmacy,Pune-India

A B S T R A C T

In Indian medicinal plant literature, plant of Schinus limonia L have been traditionally reported medicinal value as astringent, anticancer activity. Diuretic activity, Hepatoprotective activity, Antispermatogenic activity, Antioxidant activity, Antidiabetic activity, Nootropic activity. The methanolic extract of Whole plant of Schinus limonia L were screened for anthelmintic activity on Indian earth worm in comparison to standard drug Albendazole. The concentrations Schinus limonia L extracts and Albendazole were kept same for comparative activity. Saline water was kept as control. Determination of anthelmintic activity was done by recording the paralysis time and death time. Phytochemical test on plant extracts were carried out. The result showed that the anthelmintic activity of plant extracts were comparable to that of the reference drug Albendazole.

Keywords: Helminthiasis, Anthelmintic activity, Methanolic extracts, Albendazole.

1. INTRODUCTION

Helminth is Greek word meaning is worm .Helminth infections among the most common infections in men. Helminthiasis is macroparasitic diseases and the most common infectious agents of humans in developing countries1, 2. Helminthiasis, or worm infestation, is one of the most prevalent disease and one of the most serious public health problems in the world3. Helminthes infections are, affecting a large proportion of the world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, etc. Human beings have depended on nature for their simple requirements as being the sources for medicines, plants have been playing an essential role in the human culture4-6 .For much of our past history for ages, medicinal plant parts or entire plant extracts have been used to cure Helminth infections.7-8 According to the WHO current estimate, roundworms alone infect 1.5 billion people worldwide 9.

2. MATERIAL AND METHODS

2.1Collection, identification and processing of plant samples

Plant material was collected from Satara, identified, and authenticated by the Department of Botany, Yashwantrao Chavan Institute of Science, Satara, and Maharashtra, India. A voucher specimen (no: 57) the fruit, leaves, flowers of Schinus limonia L was collected in the month of December. Collected plant material was initially rinsed with water to remove soil and other contaminants. The fruits was cracked with hammer a separated seed and pulp, collected part was kept for shade dried for two weeks at room temperature. By the help of grinder the dried leaves, pulp, seed was powered to get coarse powder and stored in air tight container.

2.2Preparation of Extract:

The powdered plant of Schinus limonia L parts ratio1:1:1:1 (seed, pulp, flowers and leaves). (40 gm.) were successively extracted with methanol in a soxhlet extractor at elevated temperature(30-50°C). The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath10.

2.3Animals

Indian adult earthworms collected form moist soil and washed with normal saline to remove all fecal matter was used for the anthelmintic study.

* *Corresponding author.* Tel.:7775912234;
E-mail address: om.devade@gmail.com

The earthworms of 4-6 cm in length and 0.3-0.4 cm in width were used for all experimental protocol.

2.4Drugs and Chemicals:

The following drugs and chemicals were used.

Drugs: Albendazole **Chemicals:** Methanol and saline water

3.Preliminary Phytochemical Screening11-15

Chemical tests for the screening and identification of bioactive chemical constituents in Schinus limonia L were carried with extracts prepared using the standard procedures.

3.1Detection of alkaloids

Mayer's Test-Schinus limonia L filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test-Schinus limonia L filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formed of brown precipitate indicates the presence of alkaloids.

Dragendroff's Test-Schinus limonia L filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test-Schinus limonia L filtrate was treated with hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

3.2Detection of flavonoids

Alkaline Reagent Test-Schinus limonia L extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test-Schinus limonia L extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of carbohydrates-Schinus limonia L extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test-Schinus limonia L filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test-Schinus limonia L filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of glycosides-Schinus limonia L extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test-Schinus limonia L extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

3.3Detection of saponins

Froth Test-Schinus limonia L extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test-0.5 gm of Schinus limonia L extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

3.4Detection of proteins

Biuret's Test-To 3 ml of Schinus limonia L extract 1 ml of 4 % w/v sodium hydroxide and 1 ml of 1% w/v copper sulphate was added. The change in colour of the solution to violet or pink indicates the presence of proteins

Million's Test-To 3 ml of Schinus limonia L extract 5 ml of million's reagent was added and heated the appearance of white precipitate which changed to brick red on heating indicates the presence of proteins.

Xanthoprotein Test-To 3 ml of Schinus limonia L extract 1ml of concentrated sulphuric acid was added. The appearance of white precipitate which turned to yellow on boiling and orange on addition of ammonium hydroxide (1ml) indicated that presence of proteins containing tyrosine tryptophan.

Ninhydrin Test-To 3 ml of Schinus limonia L extract 3 drops of 5% v/w lead acetate solution was added and boiled on water bath for 10 min. The changed in colour of solution to purple or blue indicates the presence of amino acids.

Detection of steroid-Two ml of acetic anhydride was added to 0.5 gm of Schinus limonia L extract with two ml of sulphuric acid. The changed in colour from violet to blue or green in sample indicates presence of steroid.

Detection of phenols Ferric Chloride Test-Schinus limonia L extracts were treated with 3-4 drops of ferric chloride solution. It Forms of bluish black colour indicates the presence of phenols.

4. Anthelmintic activity:

The entire plant extract of Schinus limonia L were evaluated for anthelmintic activity in Pheretimaposthuma (earth worm).Indian earthworm is used due to its physiological and anatomical resemblance with the intestinal roundworm parasite of human begins, Because of easy availability of earthworms, they have been used widely for the initial evaluation of the anthelmintic compounds16-18.The worms were acclimatized to the laboratory condition before

experimentation. The earthworms were divided into five groups of six earth worms in each and placed in eight Petri dishes containing the extract solutions.

Group I- Normal saline solution which served as the control

Group II -Standard (Albendazole 10mg/ml)

Group III -Methanolic extract 100 mg/ml

Group IV -Methanolic extract 200 mg/ml

Group V- Methanolic extract 300mg/ml

5. RESULTS AND DISSCUSION

5.1Preliminary Phytochemical Analysis

2. Phytochemical screening From the phytochemical studies, it has evaluated in all extracts remarkable presence of flavonoids carbohydrates Glycosides and alkaloids, Others metabolites and bioactive compounds were identified such as saponosides.. They are present in methanolic extracts. protein, and hydrolysable tannin and phenols are absent in the extracts (Table 1). The results of the preliminary phytochemical screening of the methanolic extract of Schinus limonia L was shown below.

Table 1: Phytochemical screening of Schinus limonia L extracts

Sr.No	PhytochemicalTests	Results
1.	TestforAlkaloids	+Ve
2.	Testflavonoids	+Ve
3.	Testforcarbohydrates	+Ve
4.	TestforGlycosides	+Ve
5.	TestforSaponin	+Ve
6.	Testforproteins	-Ve
7.	Testforsteroids	-Ve
8.	TestforPhenols	-Ve

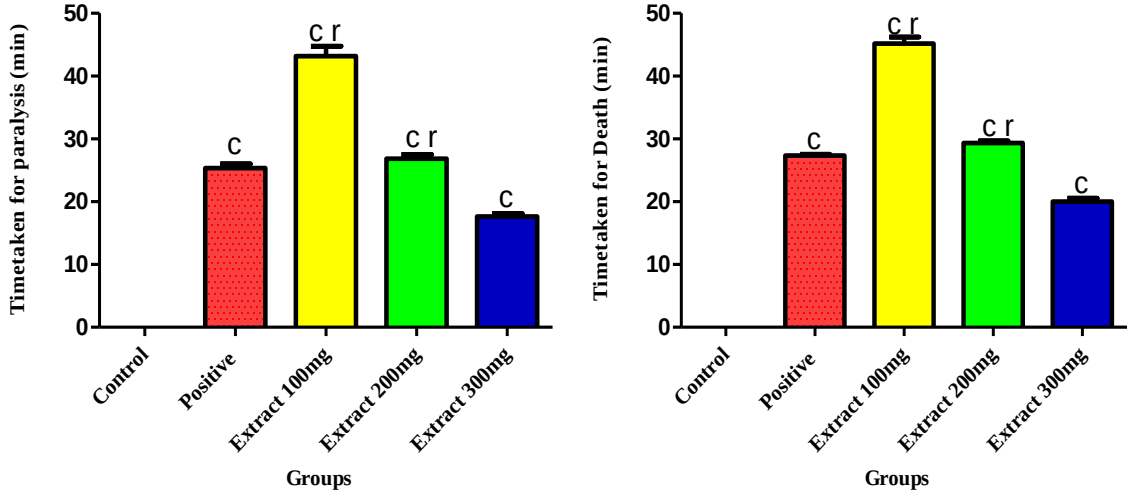
+Ve indicates the presence of compounds, -Ve indicates the absence of compounds

5.2Anthelmintic activity of aqueous extract of Schinus limonia L analysis

Aqueous extract of Schinus limonia L at 100 mg/ml, 200 mg/ml, 300 mg/ml was given, which shows significant activity on earthworm. It was seen that when control group was compare with that of positive treatment and extract group (100mg/ml, 200mg/ml, 300mg/ml) both positive and extract group showed highly significant. Positive group was compared with group 100mg/ml, 200mg/ml group of extract group it showed significant ($p<0.001$) that is paralytic condition and death.

Table 2: Effect of Methanolic extract of Schinus limonia L on Anthelmintic activity

Treatment	Concentrationused (mg/ml)	Time taken for paralysis(min)	Timetaken fordeath (min)
Control	-	Noparalysis	No death
Positive (Albendazole)	10mg/ml	25.33± 1.63	27.33± 0.51
Methanolic extractof *Schinus limonia L*	100mg/ml	43.17± 3.97	45.17± 2.16
	200mg/ml	26.83± 1.72	29.33± 0.81
	300mg/ml	17.67± 1.03	20.67± 1.4



values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA Followed by Tukey's multiple comparison tests. A p value less than 0.05 was considering as statistically significant. p value: a<0.05, b< 0.01, c< 0.001 when compared with control. p< 0.05, q<0.01, r<0.001 when compared with positive control. x< 0.05, y< 0.01, z< 0.001 when compared with standard group.

Significance:

1. When control was compared with standard and 100mg/ml, 200mg/ml, 300mg/ml of extract group it showed significant (p<0.001) that is paralytic condition and death.

2. When positive group was compared with group 100mg/ml, 200mg/ml group of extract group it showed significant (p<0.001) that is paralytic condition and death.

6. CONCLUSION

Phytochemical analysis of the extacted revealed presence of phytoconstituents such as alkaloid, favonoids, carbohydrates, glycoside, and protein.The present data indicate that Methanolic extract of Schinus limonia L is to be a safe anthelmic effect and could be used as a part of therapy to treat parasitic infections of humans. Based on the findings of the present study it is concluded that, the Methanolic extracts of Schinus limonia L found to have confirm their anthelmintic activity. We can conclude that Schinus limonia L exihibited most significant anthelmintic activity among the other Group. During study this plant showed very significant anthelmintic activity at 100 mg/ml Conc. measured by time taken for paralyse / death of the earth worms. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.**7.**

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