Research Article

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International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Primary Phytochemical analysis and acute toxicity studies of Different Extracts of *Celtis philippensis* Blanco.

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ABSTRACT

*Celtisphilippensis*isalargefugacioustreethatbelongstothefamilyofUlmaceae.Itisdistributedinmost parts of India at an altitude of 1400 m and in indestructible forests. The main aim of the present study is to investigate the phytochemical analysis of leaves of *Celtis philippensis* and to evaluate its acute toxicity study as per OECD guidelines. The phytochemical analysis was performed by following standard procedures. The phytochemical screening results of various extracts showed the presence of alkaloids, sterols, carbohydrates, glucosides, terpenoids and saponins, tannins, gums and mucilage and flavonoids. In the acute toxicity tests, single oral administration of 5, 50, 300 & 2000 mg/kg doses of various extracts of leaves of *Celtis philippensis* did not show any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence, it was concluded from the results that the possible oraltoxicdosesof*Celtisphilippensis*ismorethan2000mg/kg.

Keywords: *Celtis philippensis,* acute toxicity study, phytochemical compounds.

INTRODUCTION

*Celtisphilippensis*isalargefugacioustreethatbelongsto thefamilyofUlmaceaecommonlyknownasVellai Tovarai in Tamil[1,2]. It is distributed in most parts of India at an altitude of 1400 m and in indestructible forests. Traditionally, the roots of *Celtis philippensis* has been used for diarrhoea and as astringent [3,4]. The sap leaves were used for parasites. Although limited pharmacological studies have been carried out with this plant, there is no experimental evidence on its phytochemical and toxicity studies. Hence, in the present study, we planned toevaluatethe phytochemical analysis of leaves of *Celtis philippensis* and to evaluate its acute toxicity study as per OECD guidelines.

MATERIALS AND METHODS

Plant Materials Collection and authentication

The leaves of *Celtis philippensis* Blanco was collected from Thirunelveli District, Tamilnadu in the month of October 2019. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu.

Extraction of the plant

About 500 g of dried leaves were coarsely powdered and subjected to maceration with different solvents of increasing order of polarity such as pet ether, chloroform, ethyl acetate, ethanol, and aqueous [5,6]. The extracts were dried under the rotary evaporator and the crude extract was obtained. The product of the crude extraction fraction was calculated and stored in an airtight container for further use for analysis [7].

Phytochemical screening of the crude drug

Qualitative phytochemical analysis of the Pet. ether, Chloroform, ethyl acetate, ethanol, and aqueous plant

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extracts was carried out to test the presence of phytochemicals such as alkaloid, flavonoids, terpenoids, sterols, tannins, glycosides, etc. The following tests were done for the preliminary phytochemical screening[7].

Test for flavonoids (Shinoda test)

2mL of the *Celtis philippensis* extracts were mixed in the methanol, to this a minor part of magnesium ribbon was added and 1 mL of concentrated hydrochloric acid added from the sides of the test tube. A magenta pink color designates the presence of flavonoids.

Test for saponins

To 5mL of the *Celtis philippensis* extracts, 5mL of distilled water was added, and mix well for the formation of froth which confirms the presence of saponins. Test for steroids: 1mL of plant extracts were taken in test tubes, to which 10mL of chloroform was added. After that 10mL of concentrated sulphuric acid was added along the sides of the test tubes. A color change from violet to blue/green confirms the presence of steroids in the samples.

Test for tannins

0.5Ml of *Celtis philippensis* plant extracts were boiled in10mL of water for 510minutes and filtered. The filtrate was taken and 2mL Ferric chloride (0.1%) was added to the filtrate. The appearance of brownish-green or blue-black coloration formed confirms the presence of tannins.

Test for alkaloids

2mL of the *Celtis philippensis* plant extracts were diluted to10ml with acidified alcohol, boiled, and filtered. To5mL of the filtrate 2mL of dilute ammonia was added. 5mL of chloroform was added and shaken gently to extract the alkaloid base.The chloroform layer was extracted with 10mL of concentrated acetic acid. Few drops of Wagner's solution were added to the chloroform solution and the presence of reddish-brown precipitate indicates the presence of alkaloids.

Test for cardiac glycosides

2mL of plant extracts were treated with 2 mL of glacial acetic acid containing a drop of $FeCl_3$ solution. This was treated with 1 mL of concentrated H_2SO_4. A brown ring obtained at the interface specifies the presence of de-oxy sugar characteristics of cardenolide.

Test for terpenoids

2ml of *Celtis philippensis* extracts were treated with 2mL of chloroform and concentrated H_2SO_4 was sensibly added to form a layer. Areddish-brown color creation at the interface confirms the presence of terpenoids.

Detectionof Carbohydrate
Fehling's test

Plant extract (1mL) mixed with 1mL Fehling solutions A and B and was boiled on a water bath. The colour change was observed. A red precipitate indicated the presence of sugar.

Barfoed's test

To 1 mL of extract, 1 mL of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. The colour change was noted and recorded. A red precipitate indicated the presence of sugar.

Benedict's test

To 0.5 mL of extract, 0.5 mL of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes and the result was observed. A red precipitate indicated the presence of sugar.

Detection of Proteins

The plant extracts were dissolved in 10 mL of distilled water and filtered through Whatman No.1 filter paper and the filtrate is exposed to many tests for proteins. Millon's test: To 2 ml of the plant filtrate, few drops of Millon's reagent are added. The result was detected and the form of white precipitate specified the presence of proteins.

Biuret test

To 2 mL of filtrate, a drop of 2% copper sulfate solution was added. To this, 1 mL of 95% ethanol was added, followed by an excess of potassium hydroxide solution (60%). The appearance of a pink colour in the ethanol layer designates the existence of proteins.

Total phenol content determination

The total phenol content was determined by Folin-Ciocalteu's assay using gallic acid as standard[8]. In this method,0.5 ml of plant extracts were mixed with 1.5 ml Folin- Ciocalteu's reagent. After 5 minutes, 1.5 ml of 7% sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 minutes at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a Shimadzu 1601 UV spectrophotometer. All the readings were repeated three times for precision and

values were expressed in mean ± standard deviation in terms of phenol content (Gallic acid equivalent, GAE) per g of dryweight.

Total flavonoid content determination

Total flavonoid content was determined by the Aluminium chloride method using quercetin as a standard[9]. 1 ml of thetestsampleand4ml ofwaterwereaddedtoa10mlvolumetricflask.Tothis0.3 mlof5%Sodiumnitriteand0.3 ml of 10% Aluminium chloride were added after 5 minutes. The mixture was incubated for 6 min at room temperature, then 1 ml of 1 M Sodium hydroxide was added and the final volume was made up to 10 ml with distilled water. The absorbance of the sample was measured against the blank at 510 nm using a Shimadzu 1601UVspectrophotometer. All the readings were repeated three times for precision and values were expressed in mean ± standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight.

Animals

Female Swiss albino mice weighing about 30-35g were used for the study. They were housed in polypropylene cages and fed with a standard chow diet and water ad libitum. The animals were exposed to an alternative cycle of 12 h of darkness and light each. Before each test, the animals have fasted for atleast 12h. The experimental protocols were subjected to the scrutinization of the institutional animal ethics committee and were cleared by the same.

Acute Oral Toxicity Study

The acute toxicity studies were performed as per OECD guidelines 423[10,11]. A total of 48 mice weighing between 30-35g were randomly divided into twelve groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld over-night). Following the period of fasting, the bodyweight of the animals was measured and the chloroform, ethyl acetate, ethanol, and aqueous extracts of leaves of *Celtis philippensis* was administered to each group at single doses of 5, 50,300, and 2000 mg/kg, respectively, by oral gavage. The control groups were treated with the same volume of distilled water, ceaselessly for cyanogenetic symptoms throughout the primary half-hour once dosing and discovered sporadically (with special attention given throughout the primary four hours) for consecutive twenty-four hours and then daily after that, for 14 days. Acute oral toxicity study of various extracts of *Celtis philippensis* leaves in mice was determined by observing the changes in skin and fur, eyes and mucous membranes, and behavioral pattern. Attention was given to observations of tremors, convulsions, salivation, lethargy, sleep, Diarrhoea, Respiratory, Circulatory, Autonomic, and Central nervous system, Somatomotor activity, changes in body weight, andmortality.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaves of *Celtis philippensis*

The present study was carried out to analyze the extractive value, percentage yield, and the presence of bioactive compounds in the various extracts of leaves of *Celtis philippensis*. The colors of the extracts were green color particularly ethyl acetate extract was greenish-brown. The percentage yield of these extracts was also measured, and it was the ethyl acetate extract 15.43% showed maximum yield in comparison with other solvent extracts. Chloroform and ethyl acetate extracts were sticky semisolid, ethanol, and aqueous extracts were powder in their consistency. The extractive values and percentage yield of leaves of *Celtis philippensis* were shown in Table.No.1. The qualitative phytochemical screening of Pet ether, chloroform, ethyl acetate, ethanol, and aqueous extracts of leaves of *Celtis philippensis* and its secondary metabolites were shown in Table No.2. The results showed the presence of phytochemical constituents, namely alkaloids, sterols, carbohydrates, glycosides, fixed oils and fats, phenolic compounds, proteins and aminoacids, terpenoids and saponins, tannins, Gums and mucilage and flavonoids. Among the four solvents used, ethyl acetate extracts yielded maximum bioactive compounds followed by ethanol extract, aqueous extract, and a minimum amount of compounds present in chloroform extract. Alkaloids, sterols and carbohydrates were present in all extracts in varying concentrations. Ethyl acetate and ethanol extracts were yielded similar bioactive compounds such as alkaloids, carbohydrates, sterols and flavonoids. Ethyl acetate extract yields the compounds are alkaloids, sterols, carbohydrates, flavonoids, tannins, phenols, protein, and amino acids. Ethanol and aqueous extracts showed the presence of all compounds of ethyl acetate extract. Glycosides, fixed oils and fats, phenolic compounds, proteins, and amino acids, terpenoids, saponins, tannins, gums, and mucilage and were absent in chloroform extracts. Further, ethyl acetate, ethanol, aqueous extracts were showed the absence of Glycosides, Fixed oils & Fats, Terpenoids, Saponins, Gums and mucilage.

Table 1: Colour, Extractive values and Percentage yield of various extracts of leaves of *Celtis philippensis*

Plant name	Part used	Methodof extraction	Solvent	Colour of extract	Nature of extract	% yield of extract
Celtis philippensis	Leaves	Maceration	Chloroform	Green	Semisolid	5.56
			Ethyl acetate	Greenish brown	Greasy solid	15.43
			Ethanol	Green	powder	11.86
			Aqueous	Green	powder	12.45

Table 2: Preliminary phytochemical screening of the different extracts of leaves of *Celtis philippensis*

S.No	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	Mayer'stest	+	+	+	+
		Dragondraff's test	+	+	+	+
		Hager'stest	+	+	+	+
		Wagner's test	+	+	+	+
2	Sterols	Burchard test	+	+	-	-
		Salkowski's	+	-	+	-
3	Carbohydrates	Molisch's test	+	+	+	+
		Fehling's test	-	+	+	+
		Benedict'stest	+	+	+	+
		Bontrager's test	+	+	+	+
4	Glycosides	Legal test	-	-	-	-
		Kellerkiallani test	-	-	-	-
5	Fixed oils & Fats	Spot test	-	-	-	-
		Saponification test	-	-	-	-
6	Phenolic Compounds	Ferric chloride	-	+	+	+
7	Proteins & amino acids	Biuret test	-	+	+	+
		Ninhydrin test	-	+	+	+
		Millon's test	-	+	+	+
8	Terpenoids& Saponins	Foam test	-	-	-	-
		Haemolysis test	-	-	-	-
9	Tannins	Gelatin test	-	+	+	+
		$Fecl_3$ test	-	+	+	+
10	Gums & mucilage	Precipitation to 90%alcohol	-	-	-	-
11	Flavonoids	Shinoda test Lead	-	+	+	+
		acetate test	-	+	+	+
		Ferric chloride test	-	+	+	+
		Zinc HCL test	-	+	-	-

+Presence, -Absent

Acute oral toxicity study

To determine the safety of plant products and drugs for human use, toxicological evaluation is carried out in experimental animals to predict the toxicity and to provide for selecting 'safe' doses. In the acute toxicity tests, administration of various leaf extracts at different doses of 5, 50, 300 & 2000 mg/kg of *Celtis philippensis* did not show any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence the effective dose was found to be 200mg/kg.

Total phenol and flavonoid content

The main active compounds present in the extracts were found to be phenolic and flavonoids. In the present study, the total phenolic content of different extracts of leaves of *Celtis philippensis* Blanco was determined by the Folin– Ciocalteu reagent method and expressed as GAE/g of plant extracts. Ethanol extract exhibited the maximum amount of phenolic content among the extracts, i.e., (55.48±0.35) mg/g GAE followed by (41.37±0.21) mg/g GAE in the aqueous extract. Similarly, the total flavonoid content for all the extracts was measured with the aluminium chloride colorimetric assay using quercetin as standard. Itshowed 18.51±0.23 and 14.39±0.27mg of quercetin equivalent/g for EECPB and AECPB respectively. Aluminium chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols.

Besides, it also forms liable complexes with orthodihydroxide groups in A/Brings of flavonoids.

The results are shown in TableNo.3.

Table 3: Estimation of total phenolic and flavonoids from the various extracts of Leaves of *Celtis philippensis*.

S.No	Plant part used	Extracts	Total phenoliccontent (mgof gallic acid equivalent/g dry material)	Total flavonoid content(mgof quercetin equivalent/g dry material)
1	Leaves	Pet ether	11.43±0.27	2.30±0.20
2		Chloroform	20.53±0.25	5.36±0.19
3		Ethyl acetate	32.23±0.14	8.55±0.29
4		Ethanol	55.48±0.35	18.51±0.23
5		Aqueous	41.37±0.21	14.39±0.27

Values are expressed as mean ± SD, n=3

CONCLUSION

Inconclusion, it was observed that the plant *Celtis philippensis Blanco* contains several bioactive components and a high level of total phenolics and flavonoids content. Hence, the plant was considered as an enriched source of different phytocompounds. Acute toxicity studies also revealed the safe level of the plant extracts. This forms the basis for scientific evidence to conduct further studies and to investigate the lead compounds present in the plant, and to evaluate its various pharmacological activities.

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