INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL STUDY AND ANTIMICROBIAL ACTIVITIES OF *CORDIA DICHOTOMA*

Rawat Suman[1]*, Saini Ruchi[2] and Sharma Ankita[1]
[1]Department of Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India
[2]Department of Pharmaceutical Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India
*Corresponding Author Email: sumanman.2008@rediffmail.com

Article Received on: 10/09/13 Revised on: 01/11/13 Approved for publication: 26/12/13

DOI: 10.7897/2230-8407.041212

ABSTRACT
The present study was aimed at detecting the phytochemicals and evaluating antimicrobial activities of *Cordia dichotoma* known for their medicinal properties in folk medicine. Phytochemical screening was carried out on the leaves of *Cordia dichotoma*; the results latifoliate the presence of alkaloids, proteins, carbohydrates. The assessment of antifungal activity was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against *Aspergillus flavus* and *Penicillium expansum*. The antibacterial effect was studied by the agar direct contact method using *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia colistrains*. The phytochemical estimation revealed the presence of alkaloids, flavonoids and saponosides. These phytochemicals were isolated from the plant with yields of 5.709 % of Petroleum ether extract, 6.69 % of Chloroform extract, and 9.45 % of methanol extract. Finally, the results of antimicrobial activity of the aqueous extract showed a pronounced antifungal activity against the tested strains. The percentage inhibition values were found to be in the range of 12-24 mm against *Candida albicans* and 11 to 19 mm against *Aspergilus niger*. The results revealed that the methanolic extract exhibited significant antimicrobial activity of concentration of 100-500 µ/ml respectively against tested organisms, particularly more effective against *Bacilus cereus* and *Escherichia coli* than the other extract when compared to the standard drug (chloramphenicol).
Keywords: *Cordia dichotoma,* Antimicrobial activity, Phytochemical Studies.

INTRODUCTION

Cordia dichotoma Forst. f. A plant belonging to family Boraginaceae[1]. It is a tree of about 15 meters high, found spanning from north India and south China to Australia and Polynesia. It grows wild in the northern part of Peninsular Malaysia but is planted in the south[2]. Leaves are Simple, alternate, entire to slightly lobed, it occurs wildly all over Bangladesh (Moist sites, along watercourses), India (Several states), China, Taiwan, Australia, and North America. The fruits of the plant are used as cooling, astringent, emollient, expectorant, anthelmintic, purgative and diuretic[3]. A number of pharmacological properties such as analgesic, anti-inflammatory and hepato-protective have been reported. *Cordia dichotoma* reduce the blood glucose level when compared to diabetic control group and exert a significant hypoglycemic and antidiabetic activity[4]. Leaves used in Ulcers and in headache[5].

MATERIALS AND METHODS

Collection of leaves of *Cordia dichotoma*

Leaves of *Cordia dichotoma* were collected from locality of Kachchi Garhi, Distt. Shamli (U.P.), India. Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun, India (BSI). Authenticated specimen no is- A/C no.113678.

Extraction of leaves of *Cordia dichotoma* in different solvents (Non-polar to Polar)

The collected plant Material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (200 g) of *Cordia dichotoma* were crushed. The crushed leaves extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, methanol by hot percolation method using Soxhlet Apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

Phytochemical Analysis of different extracts

Phytochemical Tests: The different extracts of leaves of *Cordia dichotoma* were tested for various components as follows:

Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dil HCl and filtered. The filtrate was then tested for following color test:

Mayer's test

(a) 1.36 g of mercuric chloride was dissolved in 60 ml distilled water. (b) 5 g of potassium iodide was dissolved in 20 ml of distilled water. (a) And (b) was mixed and the volume was adjusted to 100 ml with distilled water. Appearance of cream color precipitate with Mayer's reagents showed the presence of alkaloids.

Wagner's Test

1.27 g of iodine and 2 g of potassium iodide was dissolved in 5 ml of water and make up the volume to 100 ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloids.

Hager's test

Take 20 ml of saturated solution of picric acid and add few drops of it to 2-3 ml of extract. A yellow color was observed.

Detection for carbohydrates and glycosides

Molisch's test

10 g of alpha naphthol was dissolved in 100 ml of 95 % alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet color appeared at the junction.

Benedicts test

The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present.

Fehling's Test

6.932 g of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 g of potassium sodium tartarate and 10 g of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drop of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

Test for sterols and triterpenoids

Salkowski test

Extract was treated with few drops of conc. Sulfuric acid, shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Sulphur powder test

Small amount of sulphur powder was added to the test solution, it sinks at the bottom.

Test for proteins and amino acids

Million test

1 g of mercury was dissolved in 9 ml of fuming nitric acid, keeping the mixture well cooled during the reaction. When the reaction was completed, equal volume of distilled water was added. 2 ml Million reagent was added to the extract gave white precipitate which turns red upon gentle heating.

Ninhydrin test

1 g of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and make the volume to 100 ml. Extract treated with this solution gave violet color on boiling.

Biuret test

To 3 ml test solution 4 % w/v NaOH and few drops of 1 % w/v copper sulphate solution were added. A blue color was observed.

Test for sponins

Foam test

1 ml of extract was diluted with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicated the presence of sponins.

Test for tannins and phenolic compounds

Ferric chloride test

Extract was treated with ferric chloride solution, blue color was appeared if hydrolysable tannin was present and green color was appeared if condensed tannins was present.

Vanillin hydrochloride test

1 g Vanillin was dissolved in 10 ml alcohol and 10 ml concentrated hydrochloride solution. Extract was treated with this solution gave pink or red color due to the presence of tannins and phenolic compounds.

Anti-microbial activity of different extracts

The anti-microbial activity of the leaves of *Cordia dichotoma* was carried out. The leaves extract were screened for anti bacterial and anti fungal activities.

Anti bacterial activity of leaves extract

In this study, the anti bacterial activity was studied against the micro organism and the bacterial cultures used in the study were:

- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Bacillus cereus*

These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37^0c for about 18-24 hours and then stored at 4^0c as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37^0c overnight. To test anti bacterial activity, the well diffusion method used.

Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-Media Laboratories, Mumbai, India. The media used for anti-bacterial activity Muller- Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121^0C at 15 psi for 15-30 minutes autoclave.

Plate preparations

25 ml of pre autoclaved Muller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri-plates. These petri-plates were allowed to solidify at room temperature.

Well diffusion method

After the plated solidified the freshly prepared microbial growth culture suspension (about 20 µl) was spread over the Muller – Hinton agar (MHA) media using L shaped sterilized glass spreader separately under the aseptic condition using laminar air flow. Then well were made in each plate with the help of borer of 8 mm diameter .In these well, about 100 µl of each leaves extracts individually was loaded. This method depend upon the diffusion of leaves extracts from hole through the solidified agar layer of petri-dish to such an extent that the growth of added micro organism is prevented entirely in a circular area or Zone around the hole containing leaf extract.

Incubation

Petri plates were incubated for overnight at 37^0C ± 0.5^0C in the incubator.

Inhibition Measurement of zone of inhibition

After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with standard drug.

RESULTS

Table 1: Percentage yield of different extracts *Cordia dichotoma* leaves

S. No.	Solvent system	% age yield
1.	Petroleum Ether	5.70 %
2.	Chloroform	6.69 %
3.	Methanol	9.45 %

Table 2: Qualitative Phytochemical Chemical Analysis of Extract of *Cordia dichotoma* leaves

Test performed	Pet. Ether Extract	Chloroform Extract	Methanol Extract
Test for Alkaloids			
Mayer's test	_	_	_
Hager's test			
Wagner's test	_	_	_
Test for Carbohydrates			
Fehling test	_	+	+
Molish test	_	_	+
Benedict test		+	+
Barfoed's Test			+
Test for phenolic compounds and Tannins			
Vanillin HCL acid test	_	+	+
Dil. $Fecl_3$.test	_	_	+
Lead Acetate Test	_	_	+
Test for Sterols / Triterpenoids			
Salkowaski test	_	_	+
Test for Saponins			
Saponins Test	_	_	_
Test for Proteins and acids			
Ninhydrin test	_	_	+
Biuret test	_	_	_
Test for Terpenoids			
Sulpher powder test	+	+	+

Key: (-) Absence, (+) Presence

Table 3: Antibacterial activity of different extracts of *Cordia dichotoma* and standard drug chloramphenicol, Streptomycin, Ampicillin

S. No.	Test organism	Inhibition zone in mm					
		Pet. Ether	Chloroform	Methanol	Standard drug		
					Ampicilline	Streptomycin	Chloram-phenicol
1	*E. coli*	_	1 mm	8.6 mm	20 mm	17 mm	20 mm
2	*Bacillus cereus*	_	2 mm	24 mm	15 mm	16 mm	18 mm
3	*Pseudomonas*	_	11 mm	5 mm	_	16 mm	17 mm

Table 4: Antifungal activity of different extract *Cordia dichotoma* and standard drug Amphotericin-B and Clotrimazole

S. No.	Test Organism	Inhibition zone in mm				
		Pet. Ether	Chloroform	Methanol	Standard drug	
					Amphotericin-B	Clotrimazole
1	*Aspergillus niger*	_	_	19 mm	_	11 mm
2	*Sclorotium*		4 mm	6 mm		
3	*Candida- albicans*			24 mm		12 mm
4	*Rhizopus*	_	_	7 mm	_	_

DISSCUSSION

Phytochemical studies reveal that methanol extract was the richest extract for phytoconstituents, except alkaloids and saponin. It contains all tested phytoconstituents viz. carbohydrate, glycosides, phenolic compound, tannins, triterpenoids of sterols, fats and fixed oil, protein and amino acid. The chloroform extract contains carbohydrate, Phenolic compound (except $Fecl_3$ test), terpenoids; while Petrolium ether extract contains only terpenoids. The antimicrobial activity of leaves extracts of *Cordia dichotoma* was found active against *E. coli*, *Bacillus cereus*, *Pseudomonas* in chloroform and methanol extracts whereas leaves extracts of Pt. ether was found inactive against *E. coli*, *B. cereus*, *Pseudomonas.* The leaves extracts of methanol was found highly active against *Candida albicans* and *Aspergillus niger* while less active against Rhizopus and Sclorotium. The leaves extracts of Pt. ether did not found any activity. The results revealed that the methanolic extract has shown more degree of anti microbial activity than other extract when compared to the standard drug (ampicillin). It shows anti microbial activity when compared to standard drug. It is due to presence of chemical constituents like carbohydrates, phenolic compounds, tannins, triterpenoids, saponins, terpenoids, proteins and amino acids which was confirmed by phytochemical studies. From the above study it is concluded that is the Methanol extract showed the maximum Anti-microbial activity in comparison to other extracts (Chloroform and Pt. ether).

ACKNOWLEDGEMENT

We are thankful to S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun, India (BSI) for authentication of plant material.

REFERENCES

1. Sharma A, Bhardwaj S, Mann AS, Jain A, Kharya MD. Screening Methods of Antoxidant Activity: An Overview, Phcog Rev 2007; 16(2): 232-238.
2. Yoganarsimhan SN. Medicinal Plants of India, Interline publishing Pvt. Ltd. Ban galore, Karnataka; 2000. p. 38.

3. Evans WC, Trease GE. Text book of Pharmacognosy, 13th edition, ELBS, London; 1984.
4. Mishra Anurag, Garg Ganesh Prasad. Antidiabetic Activity Fruit Pulp of *Cordia Dichotoma* in Alloxan induced diabetic Rats, IJPSR 2011; 2(9): 2314-2319.
5. Chopra RN, Chopra CC, Handa KL, Kapur LD. Indigenous Drugs of India 2nd ed. Academic Publishers, New Delhi; 1982. p. 607-610.

Cite this article as:
Rawat Suman, Saini Ruchi and Sharma Ankita. Phytochemical study and antimicrobial activities of Cordia dichotoma. Int. Res. J. Pharm. 2013; 4(12):53-56 http://dx.doi.org/10.7897/2230-8407.041212

Source of support: Nil, Conflict of interest: None Declared