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PHYTOCHEMICAL ESTIMATIONS AND ANTIMICROBIAL ACTIVITY OF METHANOLIC AND ACETONE EXTRACTS OF *CORDIA DICHOTOMA* F.

R. M. Mulani[1], P. H. Nandedkar*[1], B. M. Solankar[1]

*[1]DST-FIST Sponsored School of Life Sciences, S. R. T. M. University, Vishnupuri, *Nanded* - 431606, Maharashtra, India.

ABSTRACT

It is well-known that plant produces the chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. Present study deals with the identification of phytochemicals, there quantitative estimation and antimicrobial activity of leaves and fruit of *Cordia dichotoma* F. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, tannins, proteins, terpens and reducing sugars. Quantitative estimation of phytochemicals of methanolic and acetone leaf extract revealed presence of phenols (1.54, 1.93mg/g), flavonoids (1.86, 1.98 mg/g), alkaloids (1.24, 1.48 mg/g), saponins (1.44,1.23 mg/g) respectively.Methanolic and acetone fruit extract revealed higher concentration of bioactive constituents comprising phenols (2.23,1.78 mg/g), flavonoids (2.50,2.09 mg/g),alkaloids (1.98,1.87 mg/g),saponins (1.33,1.14 mg/g) respectively. The presence of these bioactive constituents associated with antimicrobial activity of leaves and fruits. It has been revealed by disc diffusion method and showed moderate activity against pathogenic microorganism such as *Escherichia coli, Staphylococcus aures* and *Candida albicans.* Methanolic leaf extract showed higher antimicrobial activity,as compared to acetone leaf extract.

KEY WORDS

Phytochemicals, Quantitative analysis, Antimicrobial activity, *Escherichia coli*, *Staphylococcus aures* and *Candida albican*s.

Author for correspondence:

P. H. Nandedkar,
DST-FIST Sponsored School of Life Sciences,
S. R. T. M. University, Vishnupuri, *Nanded* - 431606,
Maharashtra, India.

Email: nandedkarph2014@gmail.com.

INTRODUCTION

Phytochemicals are natural bioactive compounds which are present in plants. These natural compounds work with nutrients and dietary fibres to protect animals and man against diseases. Since time immemorial, these plant products which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine, thus indicating that any part of a plant may contain important active compounds[1]. The search for natural products to cure diseases represents an area of great interest in which plants have been

the most important source. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these plants bioactive chemical constituents are alkaloids; tannins, flavonoids, and phenolic compounds[2,3]. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as we use today [4-6].

There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. India is one of the richest countries in the world in regard to genetic resources of medicinal plants. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections[7].

Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds[8] and many much such as flavonoids ,tannins and so on.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant part of cordia *dichotoma was* collected randomly from the area of S. R.T.M .University campus, Nanded , Maharashtra. The selection of plant is on the basis of morphological variation of leaves and fruits. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of Plant Material

The powdered (5g) material was weighed and subjected to extraction using (50 ml) Methanol and Acetone respectively for 48 h. The solvent was then evaporated and the concentrated extract was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation for phytochemical screening, and anti microbial evaluation.

Phytochemical screening

The extracts of the dry powdered leaves and fruit of *Cordia dichotoma* were analyzed for the presence of various phytoconstituents like carbohydrates, reducing sugars, Tannins, Saponnins, Flavonoids (Shinoda's Test), Terpenes/ steroids (Liebermann - Burchard's Test), Alkaloids, proteins (copper sulphate and Folin Ciocalteau solution) and amino acids (Ninhydrin)[9] were identified using standard phytochemical procedures. The results of the phytochemical identification are shown in Table No.1.

Quantitative estimation of phytochemicals

Total phenols determination

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph was shown ,results are shown in Table No. 2[11].

Total flavanoid determination

Total flavonoid content was determined by aluminium chloride method[12] using quercetin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). 5 min after adding 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were

expressed as quercetin equivalents (mg quercetin /g dried extract and the standard graph was shown, results are shown in Table No.2

Alkaloid determination

The sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4 h. Then it was filtered and the extract was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till its settlement. The precipitate was easily collected from the solution and was washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid which was weighed after complete dryness and the percentage was calculated[13].

Saponin determination

The method used was that of[14], 20 g of samples powder was put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue reextracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated; 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Tannin Determination

The level of tannin in the plants was determined using the method[15]. 500mg of the sample was weighed into a 50mL plastic bottle. 50mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50mL volumetric flask and made up to the mark. Then 5ml of the filtered was pipette out into a test tube and then mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min using spectrophotometer.

Bacterial Strains

Microorganisms were provided by the school of life sciences, S.R.T.M. university. Three microorganisms were used in investigation named as, *Escherichia coli, Staphylococcus aures*. and *candida albicas*. All the Microorganisms were maintained at 32°C and 40°C on nutrient agar and YPD slants.

Anti-bacterial activity assay

The antimicrobial activity of plant extract was determined on *Escherichia coli, Staphylococcus aures*. and *candida albican* using agar disc diffusion method, paper discs of 5mm diameter were prepared by keeping dipped for overnight in three different concentrations i.e. 50 mg/L, 100 mg/L, 200 mg/L of the test compound. Sterile paper discs of 5mm diameter [containing 500 ppm drug] along with one standard antibiotic containing disc were placed in each plate[16] after spreading of microbial strain. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. The experiment was done three times and the mean values are presented by figures

RESULTS

The two solvents, Methanol and Acetone extraction of *Cordia dichotoma* show positive tests for few phyto chemicals , while some of secondary metabolites of plant extract may not get extracted by using acetone or methanol, the results are shown in Table No.1. Qualitative estimation of phyto chemicals was indicated in Table No.2. Moderate antibacterial activity were shown by extract of leaves and fruit and result were indicated in Table No.3.

DISCUSSION

The present investigation was carried out to study the presence of medicinally active phytochemicals in the leaves and fruit of *Cordia dichotoma* plants from Nanded District (MS) India. The results are

summarized in table 1 and 2. Alkaloids, flavonoids, tannins and saponins are present in both parts of plants investigated. Methanolic extracts of the plant show positive results and deep coloration for number of tests, indicates that methanol is used as a best solvent for extraction of phytochemicals. Some chemical components can not show +ve results for solvent like acetone. The quantitative phytochemical estimation in *Cordia dichotoma* study showed that the leaves are very rich in saponins, and flavonoids (Table 1). The presence and large amount of tannins also confirms its astrigent property[17]. The saponin content makes the leaves an important source of detergents, surface active agents used in industrial applications and also possesses beneficial health effects[18]. Quantitative estimation of crude phytochemicals from these plant parts is given in Table No.2. The methanol extract of fruit and acetone extract of fruit contained highest percentage of flavonoid and alkaloids (2.50±0.22, 2.09 ±025 and 1.98 ±012, 1.87± 0.12 respectively. The solvent extract of leaves and fruits show antimicrobial growth inhibition in disc diffusion method, indicates that the solvent extracts show resistant against the growth of microorganisms. Methanolic extracts shows best results against the growth of Staphylococcus *aures* and the minimum inhibition in acetone extract for *Candida albican* . Comparatively between leaves and fruit, leaves extract shows good antibacterial activity.

Table No.1: Results of the phytochemical screening of *Cordia dichotoma*

S.No	Secondary metabolites	Test Name	Methanolic extract		Acetone extract	
			Leaves	Fruit	Leaves	Fruit
1.	Carbohydrates	Molisch's test	-	+ +	-	++
2.	Reducing sugars	Fehling test	-	+	-	+
3.	Tannins	.Ferric chloride test	+	+	+	-
4.	Saponnins	Foam test	++	+	+	-
5.	Flavonoids	Alkaline Reagent test	+++	+	++	+
6.	Alkaloids	Mayer's test	+	+	+	-
7.	Proteins	copper sulphate and Folin Ciocalteau solution	+ +	+ +	+ +	+ +
8.	amino acids	Ninhydrin test	+	+	-	+

- =Not detetcted; + = Slight coloration; ++ = Deep coloration; +++ = Very deep coloration

Table No.2: Phytochemicals in selected medicinal plant C*ordia dichotoma* (mg/g)

S.No	Phytochemicals	Methanolic Extract		Acetone Extract	
		Leaves	Fruit	Leaves	Fruit
1	Phenols	1.54 ± 0.22	2.23±0.24	1.93 ± 0.2	1.78±032
2	Flavonoids	1.86 ± 0.20	2.50±0.22	1.98 ± 0.11	2.09 ±025
3	Alkaloids	1.24 ± 0.11	1.98 ±012	1.48 ± 0.02	1.87± 0.12
4	Saponins	1.44± 0.23	1.33 ±0.13	1.23 ±003	1.14 ±0.02
5	Tanins	0.06 ± 0.10	1.20 0.19	0.51 ± 0.20	0.90±0.12

Results are mean of triplicate determinations on a dry weight basis ± standard error

Table No.3: Anti-bacterial activity assay

S.No	Microorganism	Concentration of test compound	Methanolic Extract		Acetone Extract		Standard Antibiotic
			Zone of inhibition (mm)		Zone of inhibition (mm)		Zone of inhibition (mm)
			Leaves	Fruit	Leaves	Fruit	
1	*Escherichia coli*	50mg/L	7.2	6.0	6.4	5.9	10.6 (Gentamycin)
2	*Staphylococcus aures*.	100mg/L	10 .2	6.5	9.2	7.2	11.5(Gentamycin)
3	*Candida albicans*	200mg/L	8.0	5.8	8.6	8.7	11.2 (Fluconazole)

Figure No.1: Fruits and Leaves of *Cordia dichotoma*

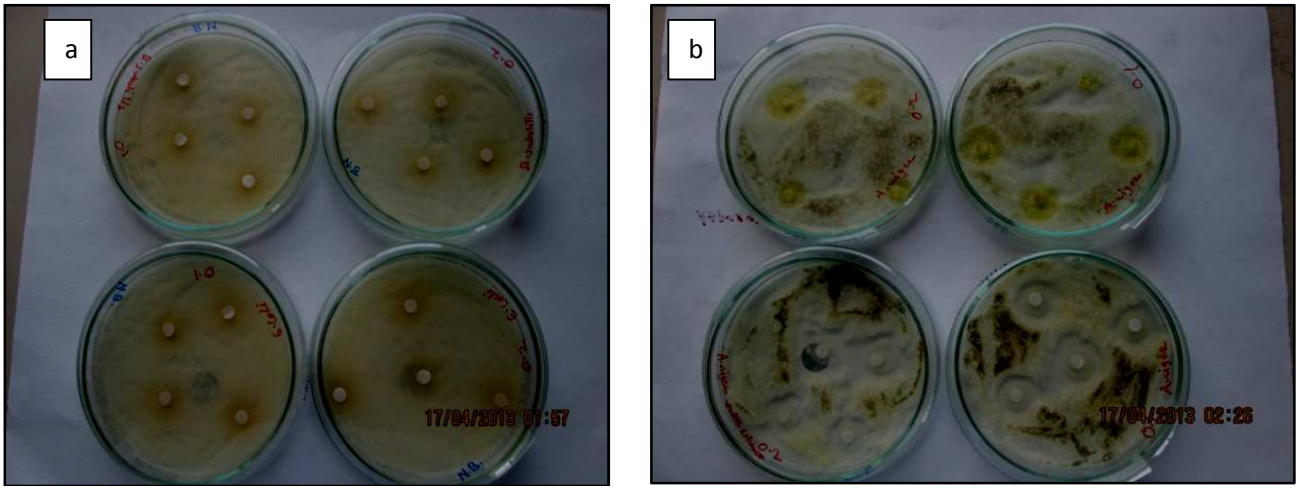

Figure No.2: a. Antimicrobial activity of extract of *Cordia dichotoma* against bacteria **b.** Antimicrobial activity of extract of *Cordia dichotoma* against fungi

CONCLUSION

Presence of medicinally active constituents in the two plant parts of the plant, studied paves a new way in the study of these compounds for their activity .The phytochemical compound identified in this studied have earlier been proved to be bioactive . The plant extract could therefore be seen as a potential source for useful drugs. By the use of these chemicals can cure the diseases without any side effects, unlike the modern drugs that cause many side effects.

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