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Detection of Phytochemical Profile of *Cordia Dichotoma* L. Extracts

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ABSTRACT:

Cordiadichotoma is a plant species in the genus *Cordia*. It is called Lashuda, Gunda or Tenti in Hindi and Lasura in Nepali and Bhokar in Marathi. *Cordiadichotoma* (*C. dichotoma*) is one of the traditional medicinally important deciduous plants available all over India. The fruit has been reported to be rich in polysaccharide. Ripe fruit of *C. dichotoma* produces a jelly-like, sticky mass. Unani system of drug medicine uses plant as antibacterial, antiviral and antitussive. Joshandah, polyherbal formulations, are extensively used by the masses in India for the treatment of common cold, catarrh, cough, respiratory distress, fevers of which *C. dichotoma* is chief ingredient. From the ancient time, leaves and stem bark are used in the treatment of dyspepsia, fever, diarrhea, leprosy, gonorrhoea and burning sensation. Leaf of plant traditionally shows the therapeutic uses and actions such as anthelmintic, astringent, diuretic, demulcent, purgative, expectorant, tonic, ulcer and cough. It is used as immunomodulator, antidiabetic, anthelminthic, anti-inflammatory, diuretic and hepatoprotective in folklore medicine. The goal of the present study was to investigate the phytochemical profile of methanolic, ethanolic and Aqueous extracts of *Cordiadichotoma* Fruit, Bark and leaves extracted by Soxhlet and Maceration method.

Keywords: Soxhlet, Maceration, Extract, phytochemicals

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1. Introduction

From time immemorial, the mankind has depended on the nature and herb products for the sustenance and the well-being and the pages of evolution in the lifestyle of mankind has been decorated with the dominant presence of medicinal plants. This medicinal plants have been the most exploited and depended sources for man. Medicinal plants are those plants which exhibit medicinal and therapeutic properties in the form of biologically active compounds and secondary metabolites this compounds are found either incorporated in the plant parts like leaves or flowers, seeds or bark or sometimes found in the form of exo-polysaccharides, resins and gums. This plants are also called medicinal herbs, have been discovered and used in traditional medicine practices since pre-historic time. Plant synthesis hundreds of chemical compounds for function including defence against Insects, Bacterial, Fungal diseases. Numerous phytochemicals with potential or established biological activity have been identified however, since a single plant contains widely diverse Phytochemical, the effects of using a whole plant as medicine are uncertain. In Developing countries, medicinal plants are used in traditional medicine, Research in this field is based on the knowledge of different Scientific disciplines (i.e. Botany. Plant biology. Phytochemistry. Pharmacology. Toxicology. Pharmacokinetics and Clinical trials) with the final goal being the evaluation of the Quality. Efficacy and Safety of Herbal medicines, as requested by many regulatory authorities worldwide (Jitendra M. et.al. 2014). The WHO established definitive guidelines regarding the methodology of clinical research and the effectiveness appraisal of traditional medicines. The use of plants to cure several kinds of Human diseases as long history. Various parts of plants such as Leaf, Stem. Bark. Root etc. are being used to prevent allay symptoms or Revert abnormalities back to normal since the Practice of Herbal remedies does not adhere strictly to facts accrued using scientific approaches, orthodox medicines sees herbal medicines as an alternative medicine. However, most of the pharmaceutical products currently dispensed by Physicians have a long history of use as Herbal remedies including Opium. Aspirin and Quinine. Modern medicine today utilizes active compounds isolated from higher plants and about 80% of this active Ingredient indicate a Positive correlation between their modern Therapeutic uses and Traditional uses. The search for and use of drugs and dietary supplements obtained from plants have increased in recent years (Dinesh K. et al. 2017). *Cordiadichotoma* L. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasaura/Lasura. It is a medium sized tree with short crooked trunk, leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with round and cordate base, flower white, fruit drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part . It grows in sub-Himalayan tract and outer ranges, ascending up to about 1500 m elevation. It is used as immunomodulator, antidiabetic, anthelminthic, diuretic and hepatoprotective in folklore medicine. *Cordiadichotoma* seeds has disclosed the presence of α -amyrins, betulin, octacosanol, lupeol-3- rhamnoside, β -sitosterol, β -sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhamnoside and hesperitin-7-rhamnoside. The seed contain α myrin and toxifolin 3, 5, dirhamnoside, which shows significant anti-inflammatory activity by an oral dose of 1gm/kg in albino rats. The seeds of this plant reported to contain fatty acids and flavonoids.(Reena Singh et.al.2010).The whole plant of *C. dichotoma* is edible and is used as food. Immature fruits are pickled and are also used as vegetable. Mixture of flower and curd applied two times in a day used to protect body against heavy sun heat waves. The rural people of coastal areas of Orissa eat the ripe fruits raw. The seed kernels of *C. dichotoma* contain high quantity of fatty oils and proteins which has potential as cattle feed. The polysaccharide gum (97%) obtained from the plant used for various pharmaceutical purposes. Chromium present in the fruit has therapeutic value in diabetes. A fruit also

contains some anti-nutritional factors such as phytic acid (355 mg), phytate phosphorus (100 mg) and oxalic acid (250 mg) per 100 g. New natural cellulose fabrics were identified from the branches of the *C. dichotoma*. (Prasad G. Jamkhande et.al.2013).

Phytochemical are chemical compounds produced by Plants, generally to- help them to resist Fungi, Bacteria and Plant viral infection, and also consumption by Insects and other Animal. Therefore in the present investigation efforts were made to detect the phytochemicals from the different plant part's like Leaves, Bark and fruit of *Cordia dichotoma*L

2. Materials and Methods

Cordia dichotoma Leaves, Bark and fruit samples were collected from near Canteen of Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar. Collected samples were washed under tap water and then shade dried for 10-15 days, then dried sample were grinded using mortar and pestle.

Extraction of Plant part

Extraction of plant part extracts were carried out by

1. Hot Continuous extraction method(Soxhlet apparatus)
2. Maceration Technique.

Hot Continuous Extraction Method (Soxhlet Apparatus) :(Sukhdev swami handa. et.al., 2008) (James Redfern et.al.2014)10 gram of grinded powder of samples were kept in “thimble”made up of Whatmann filter paper no. 1.Then thimble were inserted into the thimble chamber or the Soxhlet extractor of the Soxhlet apparatus.Extraction were carried out in different solvents like Ethanol , Methanol and Water etc. 100 ml of which were filled in the round bottom flask.The upper part were fitted with a condenser by introducing water inflow & outflow.The solvent were heated at its boiling point using the isomantle, the solvent vaporizes and then condenses. The condensate then drips into the reservoir containing thimble.When the liquid extract reaches the siphon arm and hence the capacity it pours back into the flask and the cycle begins again.The process were continued until solvent drop cannot leave residue when evaporated.Extracts were collected in vials and stored at 4°C

Maceration Technique

10 gram grinded powder of samples were kept in air tight flasks and 100 ml of solvent were added. The flasks were kept on shaker at 120 rpm for 3 days. Extracts were collected in vials and stored at 4°C.



Different Extracts of *C. dichotoma* extracted by Soxhlet Method.

Phytochemical Detection: The extracts were subjected to analysis of different phytochemicals.

- 1. Tannin (FeCl₃ test):** Ferric test is specific for phenolic compounds only for Tannin. It is based on principle that phenolic reacts with iron salt forming bluish green colour. 2 ml of 1% aqueous FeCl₃ was added in 2 ml of extract, then formation of Bluish green colour indicates presence of Tannin. (Prajwala B. et.al. 2018)
- 2. Alkaloid (Wagner's test):** Alkaloid are basic in nature when acids added they form precipitate after boiling it gives reddish brown colour. 1% HCl was added in 2ml extract, then solution was steamed for 2-3 minutes, formation of reddish brown colour indicates presence of Alkaloid (Prajwala B. et.al.2018)
- 3. Saponin (Frothing test) :** In aqueous solution saponin align themselves vertically on the surface with their hydrophobic ends oriented away from the water. These has the effect of reducing the surface tension of the water causing it to a foam. 5ml of Distilled water was added in 0.5 ml of extract solution was shook for 2-3 minutes, frothing persistence indicates presence of Saponins. (Prajwala B.et.al. 2018)
- 4. Cardiac glycoside (Keller-Kiliani test):** The test is based on specificity of action of the acid hydrolysis of deoxy-sugar like digitoxin (Glycoside) that is transform to digitoxigenin (Aglycon) and 3-digitox (Sugar residue) and eventually cymarose. Digitoxin is obtained first by alkaline hydrolysis from acetyl digitoxin and reddish ring forms at the inter phase the upper acetic acid layer soon turns bluish green. 1 ml of Glacial acetate. 1% aqueous FeCl₃ and 2-3 drops of concentrated H₂SO₄, were added in 2 ml of extract, formation of Blue green colour indicates presence of Cardiac glycoside (Prajwala B. et.al., 2018).
- 5. Flavonoids (Shinoda test):** Shinoda test was detected the presence of Flaven-3, 4-diol group flavonoids or iso flavonoids. In the Shinoda test strong acid was hydrolysed the Glycoside- flavonoid to aglycon-flavonoid, then form tomato red complex with magnesium. 1ml concentrated HCl was added in 2ml of extract, magnesium ring was put in the solution, appearance of Tomato pink colour indicates presence of Flavonoids (Prajwala B. et al., 2018).
- 6. Phenol:** The principle of Folin Ciocalteu assay is the reduction of the Folin Ciocalteu reagent in the presence of phenolic resulting in the production of molybdenum-tungsten blue colour in alkali sodium carbon component. 0.75ml of Folin Ciocalteu reagent (1:10 dilution with water) was added in 1ml of extract, then it was incubated at room temperature for 10 min. in that 0.75ml of 6% sodium carbonate was added, again it was incubated at room temperature for 90 min, formation of blue colour indicates positive test. (Prajwala B. et.al. 2018).
- 7. Steroids:** When chloroform solution of steroid (if steroid present in extract) is treated with concentrated H₂SO₄ red colour is formed. 10 ml of Chloroform was added in 1ml of plant extract, then equal volume of concentrated H₂SO₄, was added, the upper layer in the test tube was turned into red and sulphuric acid layer showed yellow colour with green fluorescence. (Prajwala B. et al. 2018).

8. Glycosides: Aqueous NaOH was added in 0.5ml of extract, formation of yellow colour indicates presence of glycoside. (Prajwala B. et.al, 2018).

1. For Carbohydrates

1. Fehling's test: (Khandelwal, 2011): 1 ml of Fehling's A and 1 ml of Fehling's B Solution were mixed. Then boiling for 1 minute. Equal volume extract were added. Heating in boiling water bath for 5-10 minutes. First yellow then brick red precipitate Indicates a positive result and presence of reducing sugars.

2. Benedict's test (Khandelwal, 2011): Test - Equal volume of Benedict's reagent and Extract were mixed in test tube. Heated in boiling water bath for 5 minutes Observation – Green, yellow or red coloured solution, Inference - Presence of reducing sugars.

J.For Starch-1.Barfoed's test- (Khandelwal, 2011): Equal volume of Barfoed's reagent And extract were mixed. Heated for 1-2 minutes in boiling water bath and cooled Observation – Formation of red precipitate, Inference - Presence of monosaccharide

K.For Proteins:Biuret test-(Khandelwal, 2011) :Extract + 4 % NaOH + few drops of 1% CuSO₄ solution, Observation – Violet or pink colour, Inference - Presence of proteins.

L.For Amino-acids-Ninhydrin test: (Khandelwal, 2011): 3ml of extract and 3 drops of 5% Ninhydrin solution was heated in boiling water bath for 10 minutes, **Observation** – Purple or bluish colour, Inference - Presence of amino Acid.

M. For Anthraquinone glycosides-Borntrager's test- 3ml of Extract + dilute H₂SO₄, Boiled and filtered. To cold filtrate, equal volume of benzene or chloroform was added, shaken well. Lower organic layer was separated and to this ammonia was added slowly. Observation - Pinkish red color to ammonical layer. Inference - Anthraquinone glycosides present.

N. Test for Tannins and Phenolic Compounds-(Khandelwal, 2011)

1. KmNo₄test: (Potassium Permagnate)-Extract + dilute Potassium Permagnate Solution. Indicator- Decolourization, Inference- Presence of Tannin.

2. Lead acetate Test: Extract + lead acetate solution. Indicator- White Precipitate, Inference- Presence of Tannin.

O. Coumarin Glycosides:Extract + 1N NaOH, Indicator- Blue or Green Fluorescence Inference- Coumarin Glycosides present.

Phytochemical activity:

Result summarized in table no.1 revealed that when *Cordia dichotoma* Leaves, Bark and fruit samples were processed for phytochemical tests by Soxhlet Method. Methanol & Ehtanol extract showed variation in presence and absence of biochemical. In Methanol & Ehtanol extract of leaves, bark & fruit showed Tannin, Glycosides, Carbohydrates, Anthraquinone glycosides and Phenols were present. Whereas Flavonoids, Alkaloid, starch and Amino acids were absent.

Table No.1: Phytochemical tests results of the plant extracts extracted by Soxhlet Method

Sr.No	Test	Leaves		Fruit		Bark	
		Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
1.	Tannin(FeCl_3 test)	+	+	-	-	-	-
2.	Saponin(Frothing test)	-	-	-	-	-	-
3.	C. Glycosides(Keller-Kiliani)	+	+	+	+	+	+
4.	Glycosides	+	+	+	+	+	+
5.	Steroid	-	-	+	+	+	+
6.	Fehling's test(For Carbohydrates)	-	-	+	+	-	+
7.	Benedict's test(For Carbohydrates)	+	+	+	-	+	+
8.	Barfoed's test (For Starch)	-	-	-	-	-	-
9.	Biuret test (For Proteins)	-	-	-	-	-	-
10.	Ninhydrin test (For amino acids)	-	-	-	-	-	-
11.	Borntrager's test(For Anthraquinone glycosides)	+	+	-	-	-	-
12.	KMnO_4 test (For tannin)	-	-	+	+	+	+
13.	Lead acetate test(For tannin)	-	-	+	+	+	+
14.	Test for Coumarin glycosides	-	-	+	+	+	+
15.	Alkaloid test	-	-	-	-	-	-
16.	Flavonoids test	-	-	-	-	+	-
17.	Phenols	+	+	+	+	+	+

+ = Present

- = Absent

Table No.2: Phytochemical tests results of the plant extracts extracted by Maceration

Sr.No.	Test	Leaves	Fruit	Bark
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		Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
1.	Tannin(FeCl_3 test)	+	-	-	-	-	-
2.	Saponin(Frothing test)	-	-	-	-	-	-
3.	C. Glycosides(Keller-Kiliani)	+	+	+	+	+	+
4.	Glycosides	+	+	-	-	+	+
5.	Steroid	-	-	+	+	-	-
6.	Fehling's test(For Carbohydrates)	-	-	+	+	-	+
7.	Benedict's test(For Carbohydrates)	+	+	+	-	+	+
8.	Barfoed's test (For Starch)	-	-	-	-	-	-
9.	Biuret test (For Proteins)	-	-	-	-	-	-
10.	Ninhydrin test (For amino acids)	-	-	-	-	-	-
11.	Borntrager's test(For Anthraquinone glycosides)	+	+	-	-	-	-
12.	KMnO_4 test (For tannin)	-	-	+	+	+	+
13.	Lead acetate test(For tannin)	-	-	+	+	+	+
14.	Test for Coumarin glycosides	+	+	-	-	+	+
15.	Alkaloid test	-	-	-	-	-	-
16.	Flavonoids test	-	-	+	+	-	-
17.	Phenols	+	+	+	+	+	+

Result summarized in table no.2 revealed that when *Cordia dichotoma* Leaves, Bark and fruit samples were processed for Phytochemical tests by Maceration Method. Methanol & Ehtanol extract showed variation in presence and absence of biochemical. In Methanol & Ehtanol extract of leaves showed presence of CGlycosides, glycosides, Carbohydrates Anthraquinone glycosides, Coumarin glycosides and Phenols.In Methanol and Ehtanol extract of fruit showed presence of Steroids, C Glycosides, glycosides, Carbohydrates ,tannins, flavonoids,

phenols, whereas in bark extract presence of Steroids, C Glycosides, glycosides, Carbohydrates ,tannins, Coumarin glycosides and phenols,

3. Conclusion

The plant extracts shows presence of various biologically active phytochemicals like Tannin, Cardiac Glycosides, Glycosides, Steroids, Carbohydrates, Anthraquinone Glycosides, Flavonoids, Alkaloids, Coumarin Glycosides and Phenols. Therefore there is a urgent need to do further research on the same topic.

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