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Phytochemical analysis of successive extracts of the *Cordia macleodii* leaves Hook.: A Folklore medicinal plant

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ABSTRACT

'Preliminary phytochemical analysis of successive extracts, fluorescence analysis and HPTLC of C. macleodiileaves has been planned in the current study.' The extracts of the dried coarse powdered of *C. macleodii* leaves obtained using different polarities solventsin succession were tested for the presence of various active phytoconstituents. Fluorescence analysis was investigated andunsaponifiable fraction of petroleum ether extract was also observed forchromatographic evaluations. The outcome suggest that the existence of various dynamic phytoconstituents having particular solubility in selected solvents of different polarities used in succession. The fluorescent analysis under the visible and UV light by treatment of diverse chemical reagents showed diverse colours. HPTLC profile of unsaponifiable fraction showed 10 and 3 spots at 254 nm and 366 nm radiation respectively. After spraying with diluted H_2SO_4, it showed 5 and 6 spots at 254 nm and 366 nm radiations respectively. The presence of active constituents in different polarities solvent, which were used in progression suggesting the significance of the solvent as a conclusive factor. Further the data suggests that, the successive extractions using Petroleum ether, chloroform, methanol and water solvents of different polarities would maximize the exploitation of the diverse bioactive compounds. Study concluded that it would be help to isolate and characterize the different pharmacologically dynamic principles of the *C. macleodii* such as mouth sores, treating jaundice, hepatoprotective and also as an aphrodisiac. It shows their importance for supporting their claimed uses and biological activities such as wound healing.

Keywords: *Cordia macleodii*, Successive extraction, Phytochemical, Fluorescence Analysis, HPTLC.

INTRODUCTION

Cordia macleodii Hook. Belongs to widely used important family Boraginaceae (Ehretiaceae), a folklore medicinal plant, native to India, is a small-sized tree, commonly known as *Phanki/Shikari*in local language, seldom found in the forests of many state like Orissa, Chhattisgarh and Madhya Pradesh. It is distributed in Deccan and Carnatic region[1]. The plant is used ethnomedicinally for various purposes like healing wounds (leaf, bark), mouth sores (leaf), treating jaundice (bark) and also as an aphrodisiac (seed) by the tribal people of Orissa, Chhattisgarh and Madhya Pradesh [2]. Earlier study of *Cordia macleodii* has been studied included pharmacognostic evaluation of it's leaf [3] and, presence of various phytoconstituents viz; alkaloids, glycosides, phenols, flavonoids, terpenoids, tannin present in extyract of *C. macleodii* leaves using various solvents [4]. The leaf powder of *C. macleodii* has been reported for pharmacological activity like antihypertensive activity [5], antimicrobial and wound healing activities of leaves [6].

However, no study had been conducting regarding the screening of the bioactive principles in successive extracts of the *C. macleodii* leaves using solvents of different polarities in succession.'Preliminary phytochemical analysis of successive extracts, fluorescence analysis and HPTLC of *C. macleodii* leaves has been planned in the current study.'

MATERIALS AND METHODS

Plant materials

For present study, fresh leaves of *C. macleodii* collected from Orissa, India in the month of December 2014 and the collected samples were identified, authenticated by using various floras and texts and also matched with Pharmacognosy Departmental Herbarium, I.P.G.T and R.A. of Gujarat Ayurved University, Jamnagar. The leaves were washed and shade dried. Coarse powder of dried leaves (40#) was prepared and preserved in an air-tight vessel.

Chemicals

Solvents and chemicals of laboratory reagent (L.R) and analytical reagent (A.R) grade were procured from Rankem, Merck and S.D. fine chemicals, Mumbai.

Preparation of successive extracts

500 g of air dried, coarse powdered sample was successively extracted with different solvents in the increasing order of polarity (petroleum ether 60-80 ^{o}C, chloroform, and methanol) using soxhlet apparatus. Each time, before extracting with the next solvent, the powdered material was dried in hot air oven at 40 ^{o}C; finally, the material was macerated using hot water with occasional stirring for 16 h and the water extract filtered. Each extract obtained by successive extraction was concentrated and dried under reduced pressure and weighed. The percentage yield of evaporated extract was calculated. The extract was preserved at 2–4 ^{o}C. This crude extract was used for further investigation.

Phytochemical analysis

For analysis different stock solution in concentration of 1% (W/V) of each successive extract obtained using petroleum ether, chloroform, methanol and water was prepared using the respective solvent. These extracts were tested for the presence of active phytochemicals viz; tannins, alkaloids, phytosterols, triterpenoids, falvonoids, glycosides, saponins, carbohydrates, amino acids and fixed oils & fats by the following standard methods [7, 8, 9] as briefed below:

Tests for Alkaloids

1. **Mayer's reagent:** The substance to be tested was treated with few drops of dilute 2N HCl and 0.5 ml Mayer's reagent, alkaloids give a white precipitate.
2. **Wagner's reagent:** The substance was treated with few drops of dilute 2N HCl and 0.5 ml Wagner's reagent, brown flocculent precipitate with alkaloids.
3. **Draggen Dorff's reagent:** The substance was treated with few drops of dilute 2N HCl and 0.5 ml Draggen Dorff's reagent. Brown precipitate is obtained.

Test for Amino acids

Alcohol and water extracts were tested for presence of amino acids.

Ninhydrin Test: Heat 3 ml test solution, add 3 drops of 5% Ninhydrin solution in boiling water-bath for 10 minutes. Purple or bluish colour appears.

Tests for Carbohydrates

Alcohol and water extracts were tested for presence of carbohydrate.

1. **Molish Test:** To 2 ml extract add 2 drops of fresh 10% α-Naphthol reagent and mix. Pour 2 ml of conc. H_2SO_4, it form layer below the mixture. A red-violet ring indicates the presence of carbohydrates.
2. **Fehling's test:** Mix each 1 ml Fehling's A & B solutions; boil for 1 min, add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then brick red precipitate is observed.

Test for Fats and fixed oils

Place 0.05 ml of the oil on a filter paper. The volatile oil evaporates completely within 24 hrs. without leaving a translucent or greasy mark (volatile oil). If greasy mark appears that indicates the occurrence of fixed oil.

Test for Flavonoid

1. **Shinoda's test:** Dissolve sample in 10% HCL and add Zinc (dust). Observe effervesces with pink color indication of flavonones, flavonol and the glycosides.
2. **Lead acetate test:** When treated with 10% lead acetate gives yellow, orange, red or brick color precipitation.
3. **Alkaline reagent:** Flavonoid dissolved in 5% NaOH with the formation of yellow solution which undergoes rapid color changes on standing for sometime on addition of few drops of HCL the color disappears.

Tests for Glycosides

Saponin Glycosides

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

Coumarin Glycoside

Alkaline reagent: Alcoholic extract when made alkaline shows blue or green fluorescence.

Flavonoid Glycoside

Lead acetate test: To small quantity of residue, add lead acetate solution. Yellow colored ppt was formed.

Test for Resin

Shake a little powdered drug extract with light petroleum and filter. Shake the solution with about twice of its volume of dilute solution of copper acetate. The petroleum layer becomes emerald-green in colour.

Tests for Tannins & phenolic compounds

1. **$FeCl_3$ test:** To aqueous extract of sample, very dilute solution of ferric chloride was added, blue colour was obtained, which changes to olive green by the addition of more amount of ferric chloride.
2. **Iodine test:** With iodine solution extract gives red coloration due to the presence of tannins.
3. **Gelatin test:** To solution of tannin containing sample add 1% solution of gelatin in 10% NaCl precipitation of gelatin occurs.

Tests for Steroids and Terpenoids

1. **Salkowski reaction:** To 2 ml of extract, add 2 ml chloroform and 2 ml concentrate H_2SO_4, shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Liebermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of concentrate H_2SO_4 from the side of test tube. First red then blue and finally green color appears.

Fluorescence analysis

Fluorescence study of leaf powder was performed as per reported standard procedures [10]. A small quantity of the leaf powder was placed in clean petridish and 1-2 drops of freshly prepared reagent solution was added, gently mixed by tilting and kept as such for few minutes. It was then placed inside the UV chamber and observed in visible light, short (254nm) and long (365nm) UV radiations. The colour

observed by application of different reagents in different radiations was recorded.

HPTLC analysis of unsaponifiable fraction

Chromatograpic technique was also done for separation and confirmation of chemical compound by using various solvents and spraying reagents by following standard procedures [11].

Sample preparation

Unsaponifiable fraction was used which was obtained from alkaline hydrolysis of petroleum ether extract and sample was isolated compound from *C. macleodii* leaves.

Mobile phase : Petroleum ether: Diethyl ether: GAA (90:10:1)

Stationary phase : Precoated Silica gel G_{254} (Merck)

Detection : (1) Short U.V. (254 nm)

(2) Long U.V. (366 nm)

(3) Spraying with diluted H_2SO_4

Track : *C. macleodii* leaves (unsap)

RESULTS AND DISCUSSION

Fundamental phytochemical investigations of the extracts for their most important phytoconstituents are very important as the active principles of many drugs. And these essential secondary metabolites found in plants which is responsible for particular action of drug. Previous information about study on preliminary phytochemical analysis have documented the presence of various phytoconstituents in the *C. macleodii* leaves using a single solvent but in the present study, the successive extracts in varying polarity solvents were obtained using petroleum ether, chloroform, methanol and water and further used for analysis. The yield obtained for each successive extracts of the *C. macleodii* leaves in present study using different solvents like petroleum ether, chloroform methanol and water (aqueous) is recorded to be different yield according to their polarities and highest in the case of methanol followed by water, petroleum ether and chloroform used in succession (Table No.1). The phytochemical tests shows presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides and tannins, phenols, steroids, terpenoids, resin (Table No.2). The fluorescence characteristics of powdered drug plays essential role in resolving the quality of and transparency of the drug material. The fluorescence characteristics of leaf powder of *C.macleodii* is given in the Tables No.3. Fluorescence study of the leaf powder helps in a quick method for resolution of doubtful specimen[12]. The fluorescent analysis under the visible light and UV light by treatment of different chemical reagents showed different colours. Thus fluorescence is used for qualitative assessment of crude drug [13].

HPTLC profile of unsaponifiable fraction showed 10 and 3 spots at 254 nm and 366 nm radiation respectively. After spraying with diluted H_2SO_4 it showed 5 and 6 spots at 254 nm and 366 nm radiation respectively.This spray reagent is used for Steroids type compound which means Steroids type compounds are present in *C.macleodii* (unsap) (Table No.4).

the existence of varied active principles having selective solubility in successive solvents of different polarities, which were used in succession suggesting the importance of the solvent as a considerable factor. Further in the study the data suggests that, the successive extractions by different solvents in increasing order of polarities would maximize the exploitation of the diverse bioactive compounds as shown in results.

Table 1: Successive Solvent Extraction of *Cordia macleodii* leaves Hook.

Solvents Used	Color of extract	Yield(% w/w)
Petroleum ether	Greenish brown	2.60
Chloroform	Greenish brown	1.37
Methanol	Yellowish brown	9.95
Water	Brown	9.85

Table 2: Phytochemical analysis of Successive Extracts of *C. macleodii* leaves.

Phytoconstituents	Name of test	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	Mayer's	--	--	++	++
	Wagner's	--	--	++	++
	Dragen dorff's	--	--	++	++
Amino acids	Ninhydrin's	--	--	++	--
Carbohydrates	Molish	--	--	--	++
	Fehling's	--	--	--	--
Fats and fixed oils	Filter paper	++	--	--	--
Flavonoids	Shinoda's	--	--	--	--
	Lead acetate	--	--	++	--
	Alkaline reagent	--	--	++	--
Glycosides					
Saponin	Froth Foam	--	--	--	--
Coumarin	Alkaline	--	--	--	--
Flavonoid	Lead acetate	--	--	++	--
Resin	Copper acetate	++	++	--	--
Tannin & phenolic compounds	$Fecl_3$	--	--	++	++
	Iodine	--	--	++	++
	Gelatin	--	--	++	++
Terpenoids& steroids	Salkowski	++	--	--	--
	Liberman- burchard	++	--	--	--

++ Present & -- Absent

Table 3: Fluorescence analysis of leaf powder of *C. macleodii*

Light →	Visible Light	UV Light	
Plant + Reagent ↓		Short UV (254 nm)	Long UV (366 nm)
Leaf Powder	Olive Green	Green	Brown
Leaf Powder + 1N HCl	Creamish yellow	Yellow	Brown
Leaf Powder +NaOH+ Methanol	Yellowish brown	Brown	Reddish orange
Leaf Powder + NaOH	Yellowish brown	Brown	Reddish brown
Leaf Powder + Methanol	Parrot green	Green	Reddish brown

Table 4: HPTLC Analysis of Unsaponifiable fraction in Petroleum ether: Diethyl ether: GAA (90:10:1) solvent system

Sample	Short U.V. (254 nm)		Long U.V. (366 nm)		After spray Short U.V. (254 nm)		After spray Long U.V. (366 nm)	
	No. of Spot	Rf Value	No. of Spot	Rf Value	No. of Spot	Rf Value	No. of Spot	Rf Value
C.macleodii (unsap)	10	0.03, 0.07, 0.15, 0.21, 0.24, 0.30, 0.61, 0.68, 0.78, 0.95	3	0.03, 0.07, 0.21	5	0.02, 0.09, 0.15, 0.18, 0.22	6	0.02, 0.06, 0.09, 0.15, 0.18, 0.22

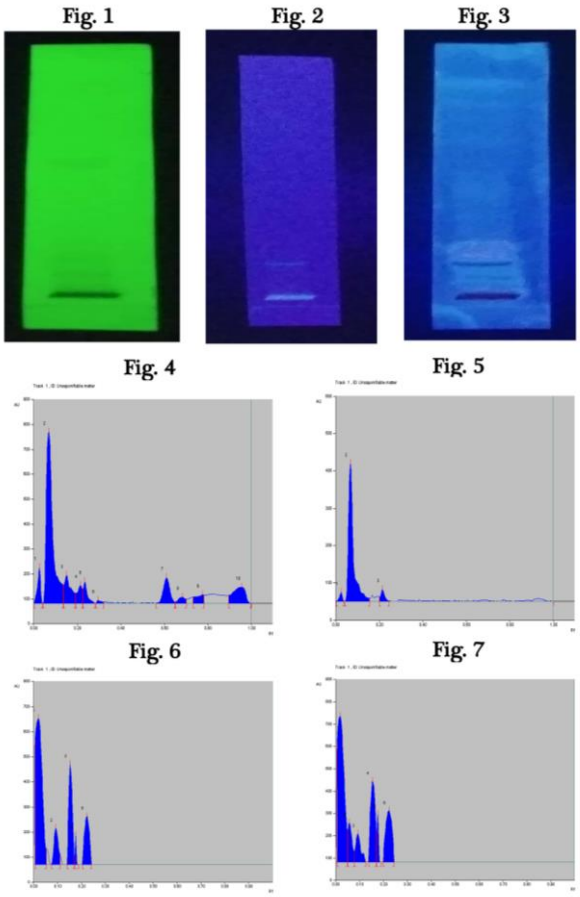

Fig.1. 254 nm, Fig.2. 366 nm, Fig.3. after spray 366 nm, Fig.4. Densitogram at 254nm, Fig.5. Densitogram at 366 nm, Fig.6. Densitogram at 254 nm after spray, Fig.7. Denstigram at 366nm after spray

CONCLUSION

From the present study, it is concluded that study in successive extraction would be help to isolate and characterize the diverse pharmacologically active principles of the *C. macleodii* such as mouth sores, treating jaundice, hepatoprotective and also as an aphrodisiac. It shows their importance for supporting their claimed uses and biological activities such as wound healing.

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