

Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India.

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ABSTRACT: Phytochemicals are secondary metabolites produced by all plants in which some has medicinal uses. The phytochemical analysis of leaf extracts in aqueous, methanol, acetone, petroleum ether and chloroform extracts of indigenous medicinally important plants of *Holoptelea integrifolia* and *Celestrus emarginata* were investigated. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, Xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations. This research supports the local use of the leaf extract of the plant *Holoptelea integrifolia* for pediculoses and *Celestrus emarginata* for increasing male sex vigour. This plant belongs to family **Euphorbiaceae** and **Celestraceae** respectively. The present study provides evidence that solvent extract of *Holoptelea integrifolia* and *Celestrus emarginata* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

KEY WORDS: *Celestrus emarginata*, *Holoptelea integrifolia*, indigenous, leaf extracts, phytochemical analysis

I. INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [1]. Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections [2]. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed)[3]. (Amrit pal Singh, 2005). India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants.

According to an estimate, 120 or so plant based drugs prescribed for use through the world come from just 95 plant species [4]. Natural antimicrobials can be derived from plants, animal tissues and microorganisms[5]. The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents from medicinal plant research[6]. The amount of phytochemical substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors[7]. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives[8]. Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defense mechanisms against predation by microorganisms, insects and herbivores [9]. Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings.

II. MATERIALS AND METHODS

2.1 Collection and authentication of plant materials

The leaves of the plant species were collected wildy from the forest areas of Seethagondi Grampanchayath, Adilabad District with the help of local tribal people. The collected plants were identified using available published literature [10, 11, 12, 13] at the Department of Botany, Osmania University, Hyderabad, India

2.2 Preparation of extracts

To prepare the Methanolic, Acetonic, Chloroformic and Petroleum ether extracts, 150 g of each of the ten plant material was collected, dried in the oven at 70°C for 4 h and reduced to powder. It was separately macerated with the above solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use. For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Erlen-Mayer flask and to this was added 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whatman filter paper (W and R Balson Ltd, England). The filtrate was then filtered sterilized using a membrane filter of pore size 0.45 µm diameter (millipores corp, England). The extracts were concentrated in a hot water bath at 80°C for 5 h during which 0.5 g charcoal was added to decolorize it. Sterile decolorized filtered extract was then refrigerated at 5°C until required for use.

2.3 Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described by [14, 15, 16, 17].

2.4 Qualitative analysis

Preparation of reagents: Preparation of Maeyer's reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Preparation of Dragendorff's reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

2.5. Phytochemical screening for different compounds

2.5.1. Test for Flavonoids:

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: (a) 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones. (b) 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of Flavonoids. (c) 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H₂SO₄. The appearance of the yellow colouration indicated the presence of flavonoids.

2.5.2. Test for alkaloids:

0.5 to 0.6 g of various extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

2.5.3. Test for Glycosides:

Five ml each of various extract were hydrolysed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

2.5.4. Test for steroids:

0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

2.5.5. Test for Phenols:

To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

2.5.6. Test for Terpenoids (Salkowski test):

5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

2.5.7. Test for Saponins:

0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

2.5.8. Test for Resins:

One ml of various solvent extract were treated with few drops of acetic anhydride solution followed by one ml of conc. H_2SO_4 . Resins give colouration ranging from orange to yellow.

2.5.9. Test for Tannins

0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

2.5.10. Test for Cardiac glycosides (Keller-Killani test)

5 ml of various solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

2.5.11. Test for Carboxylic acid:

One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

2.5.12. Test for Coumarins:

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

2.5.13. Test for Quinones

One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinones give coloration ranging from red to blue.

2.5.14. Test for Xanthoproteins

One ml each of the various extracts were treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

III. RESULTS AND DISCUSSIONS

The results of the phytochemical analysis of the leaf extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of phytochemicals in various solvents are shown in TABLE 1 and 2. The results obtained are also shown in Fig. 1 and 2. The study revealed that the leaf extracts of *Holoptelea integrifolia* are showing maximum presence of alkaloids in aqueous, methanolic and acetonic solvent extracts but adequately present in petroleum ether and Chloroformic extracts, whereas in petroleum ether and chloroform the cardiac glycosides are maximum, but adequately present in aqueous, methanolic and acetonic extracts, coumarins and quinines are maximum in acetonic extracts, but are completely absent in petroleum ether and Chloroformic extracts. Glycosides are completely absent in aqueous, methanolic and Chloroformic extracts. Steroids on other hand in methanolic, petroleum ether and Chloroformic extracts are completely absent but are adequately present in acetonic extract. In contrast to all this Xanthoproteins are maximum in all the solvent extract including aqueous one. In aqueous extracts flavonoids, phenols, tannins, cardiac glycosides are adequately present, whereas terpenoides, saponins and carboxylic group members are in minimum presence.

In *Celestrus emarginata*, alkaloids, glycosides, resins and quinones are showing maximum presence in acetonic extracts, phenols are maximum in methanolic extracts and quinones are maximum in aqueous, methanolic and acetonic extracts. Saponins are absent in methanolic and acetonic extracts, but are adequately present in petroleum ether and Chloroformic extracts. Tannins are absent in acetonic, petroleum ether and Chloroformic extracts but are adequately present in methanolic and aqueous extracts. Coumarins are adequately present in aqueous, methanolic and acetonic extracts but completely absent in petroleum ether and Chloroformic extracts. The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous findings of the various researchers.

CONCLUSION

Thus, from the present study the plant leaf extracts of *Holoptelea integrifolia* and *Celestrus emarginata* showed an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug against pediculosis and increasing male sex vigour. The studies result of the above two plants gives a basis of its use in traditional medicine to manage ailments and disorders. It also contains some biologically active constituents worthy of further investigations.

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TABLE-1

S.No	PHYTOCHEMICAL CONSTITUENTS	DIST. WAT	METH	ACE	P.ETH	CHL
1	Flavonoids	++	+	+	-	+
2	Alkaloids	+++	+++	+++	++	++
3	Glycosides	-	-	++	+	-
4	Steroids	+++	-	++	-	-
5	Phenols	++	++	++	+	+
6	Terpenoids	+	-	-	+	+
7	Saponins	+	++	++	+	+
8	Resins	-	+	-	-	-
9	Tannins	++	+	+	-	+
10	Cardiac Glycosides	++	++	++	+++	+++
11	Carboxylic acid	+	+	-	++	+
12	Coumarins	-	-	+++	+	-
13	Quionones	-	++	+++	+	-
14	Xanthopretins	+++	+++	+++	+++	+++

Phytochemical studies in leaf powder extracts of *Holoptelea integrifolia*

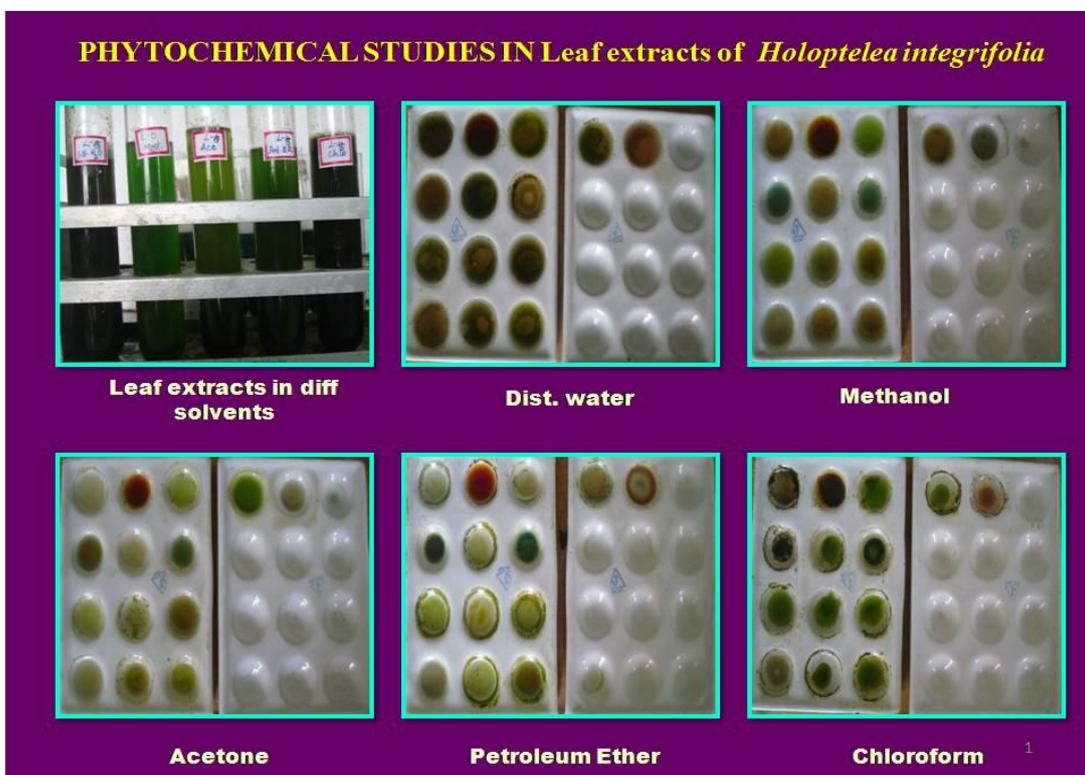


TABLE-2

S.No	PHYTOCHEMICAL CONSTITUENTS	DIST. WAT	METH	ACE	P.ETH	CHL
1	Flavonoids	++	+	-	-	-
2	Alkaloids	++	++	+++	++	++
3	Glycosides	+	+	+++	-	+
4	Steroids	++	+	++	+	-
5	Phenols	++	+++	+	-	-
6	Terpenoids	+	+	-	++	+
7	Saponins	+	-	-	++	++
8	Resins	+	+	+++	+	-
9	Tannins	+	++	-	-	-
10	Cardiac Glycosides	++	+	+	+	++
11	Carboxylic acid	-	-	-	+	-
12	Coumarins	+	++	++	-	-
13	Quinones	+++	+++	+++	-	-
14	Xanthoproteins	+	-	-	++	+

Phytochemical studies in leaf powder extracts of *Celestrus emarginata*

