



Phytochemical Analysis of Some Indigenous Plants Potent Against Endoparasite

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Abstract: A study has been done with indigenous plants to explore their phytochemical constituents. About 7 indigenous plants collected from Agra–Mathura Region. The collected plants undergone extraction followed by evaporation. The prepared plant extract goes through phytochemical investigation to explore active constituents which are very significant drug development.

Keywords: *Aegle marmelos*, *Ailanthus excelsa*, *Annona squamosa*, *Bauhinia variegata*, *Butea frondosa*, *Calotropis gigantea*, *Calotropis procera*.

1. Introduction

Since time immemorial plants have been used for the treatment of various ailments. Even today several important drugs used in the modern system of medicine are obtained from plants. The use of medicinal plants has figured in several ancient manuscripts like the Rigveda, The Bible, The Iliad, The Odyssey and the history of Herodotus. As far back as 4000 B.C., the ancient Chinese were using drug plants. The earliest reference to the use of medicinal plants as a cure for diseases is found in the manuscript of 'Eber Papyrus' written in 1600 B.C. with the advancement of our knowledge, such superstitions were gradually lost [1].

In India, the earliest reference to medicinal plants appears in the Rigveda, written between 3500 and 1600 B.C. in Artharveda too, detailed descriptions of several medicinal plants were given. Most of the plants are wild and only a few of them have been cultivated. Studies of medicinal plants based on ancient literature and its investigation in modern light is under process.

The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and Tannins etc. these active principles usually remain concentrated in the storage organs of the plants viz., roots, seeds, bark, leaves etc.

Considering all these facts present investigation is designed to find out phytochemical analysis of some indigenous plants which are potent against endoparasites (ticks and mites).

2. Materials and Methods

2.1 Selection and Authentication of Plants

Plants were selected on the basis of literature and indigenous traditional knowledge. The selected plants were *Aegle marmelos*, *Ailanthus excelsa*, *Annona squamosa*, *Bauhinia variegata*, *Butea frondosa*, *Calotropis gigantea*, *Calotropis procera*, *Chenopodium album*, *Chrysanthemum indicum*, *Cuscuta reflexa*, *Datura stramonium*, *Euphorbia hirta*, *Eucalyptus globulus* and *Ficus religiosa*. All the information regarding taxonomy and action are collected from the book "Encyclopedia of Indian Medicinal Plants" written by Dr. C.P. Khare.

2.2 Collection of Plants

The selected plant materials were collected from the campus of the Central Institute for Research on Goats, Makhdoom, Farah, Mathura and some plant material collected from Botanical garden of School of Life sciences, Khandari, Agra (U.P.) during March-April 2007 and some of them were purchased from

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local market. Plant materials were authenticated by taxonomic characteristics and consultation with experts. The selected plant materials were washed with clean water and allowed to shade dry for about 2-3 weeks. The dried materials were crushed in an electric grinder to coarse powder.

2.3 Preparation of Plant Extracts

The crude plant extract was prepared by the Soxhlet extraction method following Kokate *et al.*, [2]. About 100 gm of powder material was uniformly packed into a thimble and run in Soxhlet extractor. It was exhaustively extracted with methanol for the period of about 48 hours or 22 cycles or till the solvent in the siphon tube of an extractor became colorless. After that extracts were filtered with the help of filter paper and solvent evaporated in Rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove traces of alcohol. Then extract kept in refrigerator at 4°C for detecting antibacterial activity and analysis of their physical and chemical property.

2.4 Physical and Chemical Examination of Crude Extract

Physical properties were noted, mainly color, odour, nature and consistency. Solubility of extracts was checked in commonly used solvent like – Distilled water, ethanol, methanol, petroleum ether, acetone and chloroform.

2.5 Phytochemical analysis of different crude extract

Extracts were tested for the presence of active principles such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins. Following standard procedures [3] were used.

2.6 Test for Protein

Millon's test, Crude extract was mixed with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, which turned red upon gentle heating. Ninhydrin test, Crude extract when boiled with 0.2 % solution of ninhydrin (Indane-1,2,3-trione hydrate), violet color appeared. Suggesting the presence of amino acids and protein.

2.7 Test for Fat

Stain test, the small quantity of crude extract was pressed between two filter papers; the stain on 1st filter paper indicated the presence of fixed oils.

Saponification test, In small quantity of crude extract few drops of 0.5N of alcoholic potassium hydroxide, was added to which a drop of phenolphthalein was added separately and heated in a water bath for 1 hour. The formation of soap indicated the presence of fixed oils and fats.

2.8 Test for Carbohydrate

Benedict's test, Crude extract was mixed with a few drops of Benedict reagent (alkaline solution containing cupric citrate complex) boiled in a water bath; a reddish brown precipitate formed indicating the presence of sugar.

Fehling's test, equal volume of Fehling A (copper sulphate in distilled water) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents were mixed with a few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar is present.

2.9 Test for Tannins

Gelatin test, Crude extract was mixed with 1% gelatin solution containing 10 % sodium chloride. A white precipitate formed, indicated the presence of tannins.

Ferric chloride test, Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.

2.10 Test for Saponins

Froth test, Crude extract was mixed in 1 ml water in a semi-micro tube, shaken well and noted the S Table froth. S Table froth indicated the presence of saponins.

Haemolysis test, 0.2 ml of crude extract was mixed with 0.2 ml of blood (containing normal saline) and centrifuged. A red supernatant thus resulted which was then matched with colourless control, suggesting the presence of saponins.

2.11 Test for Alkaloids

Mayer's test, Crude extract was mixed with Mayer's reagent (potassium mercuric iodide solution). The cream color precipitate was formed, indicating the presence of alkaloids.

Dragendorff's test, Crude extract was mixed with Dragendorff's reagent (potassium bismuth iodide solution). The reddish brown precipitate was formed which suggested the presence of alkaloids.

2.12 Test for Flavonoids

Shinoda test, Crude extract was mixed with a few fragments of magnesium ribbon and concentrated hydrochloric acid was added dropwise. The Pink scarlet color appeared after a few minutes, indicated the presence of flavonoids.

Alkaline reagent test, Crude extract was mixed with a few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of a few drops of diluted acid, marked the presence of flavonoids.

2.13 Test for Glycosides

Borntrager's test, 200 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5 % aqueous ferric chloride solution, boiled for 5 minutes

which lead to oxidation to anthraquinones, indicating the presence of glycosides.

Kedde's test, Crude extract was mixed with chloroform, one drop of 90% alcohol and 2 drops of 2% 3,5-dinitrobenzoic acid in 90% alcohol and made alkaline with 20% sodium hydroxide. A purple colour so produced, suggested the presence of glycosides.

2.14 Test for Steroids and Triterpenoids

Liebermann-Burchard test, Crude extract was mixed with a few drops of acetic anhydride, boiled and cooled, concentrated H₂SO₄ was then added from the sides of the test tube. A brown ring at the junction of two layers was formed. The upper layer turned green which showed the presence of steroids and the formation of deep red colour indicated the presence of triterpenoids.

Salkowski test, Crude extract was mixed with chloroform and a few drops of concentrated H₂SO₄, shaken well and allowed to stand for some time. Red color appeared at the lower layer indicated the presence of steroids and formation of a yellow colored layer indicated the presence of triterpenoids.

3. Results

In present study total of fifteen crude methanolic extracts was prepared by using different plant parts. *Eucalyptus globulus* (leaves) yielded highest (28.42%), while *Bauhinia variegata* (leaves) yield was low (18.67%). In case of fruit extract highest yield (33.40%) was in *Annona squamosa* followed by *Aegle marmelos* (32.23%) and in the case of the seed extract, *Butea frondosa* yielded highest (30.67%) while *Chenopodium album* yielded lowest (18.45%). The details of all extract about percent yield and physical characteristic are given in Table 1.

Phytochemical analysis of different extracts was conducted by different tests to know the presence of

(active constituents) alkaloids, flavonoids, saponins, steroids, carbohydrates, glycosides, tannins, phenolic compounds, protein, amino acids and triterpenoids.

Phytochemical analysis of methanolic extract of *Ailanthus excelsa* (leaves) showed the presence of alkaloids, flavonoids, tannins and phenolic compounds and triterpenoids while in case of methanolic extract of *Annona squamosa* (leaves) flavonoids, tannins and triterpenoids were present, methanolic extract of *Annona squamosa* (seeds) showed alkaloids, carbohydrates, tannins and fixed oils.

Methanolic extract of *Aegle marmelos* (leaves) showed the presence of alkaloids, flavonoids and triterpenoids were present during analysis while methanolic extract of *Butea frondosa* (seeds) alkaloids, tannins and fixed oils were present, *Bauhinia variegata* (leaves) showed the presence of alkaloids, flavonoids and tannins.

In case of methanolic extract of *Calotropis procera* and *Calotropis gigantea* (leaves) alkaloids, flavonoids and tannins were present in both the extracts while in methanolic extract *Cuscuta reflexa* (whole plant) only alkaloids and triterpenoids were present. In the extract of *Chrysanthemum indicum* (leaves) three compounds alkaloids, flavonoids and triterpenoids were present while in seeds of *Chenopodium album* alkaloids, Saponins, glycosides, fixed oils and tannins were present, methanolic extract of *Datura stramonium* (leaves) showed the presence of alkaloids, flavonoids and tannins.

Methanolic extract of *Euphorbia hirta* (leaves) contains alkaloids, flavonoids and tannins whereas methanolic extract *Eucalyptus globulus* (leaves) alkaloids, flavonoids, tannins and triterpenoids were present. *Ficus religiosa* (bark) contains alkaloids, carbohydrates and tannins. Results are portrayed in Table 2.

Table 1. Physical characteristic and percent yield of crude methanolic extract of various plants.

S. No.	Extract	Botanical Name	Nature	Color	Odour	Yield %
1.	Ardu leaves	<i>Ailanthus excelsa</i>	Nonsticky	Dark green	Aromatic	24.42
2.	Sharifa leaves	<i>Annona squamosa</i>	Oily	Solid Green	Aromatic	21.50
3.	Sharifa fruit	<i>Annona squamosa</i>	Sticky	Yellowish brown	Peculiar	33.40
4.	Bel fruit	<i>Aegle marmelos</i>	sticky	Brownish	Peculiar	32.23
5.	Palas seeds	<i>Butea frondosa</i>	sticky	Yellowish brown	Aromatic	30.67
6.	Kachnar leaves	<i>Bauhinia variegata</i>	Nonsticky	Blackish green	Aromatic	18.67
7.	Aak leaves	<i>Calotropis procera</i>	Nonsticky	Dark green	Aromatic	21.50
8.	Aak leaves	<i>Calotropis gigantea</i>	Nonsticky	Blackish green	Aromatic	19.10
9.	Amar bel whole plant	<i>Cuscuta reflexa</i>	Sticky	Brownish	Peculiar	22.38
10.	Guldaudi leaves	<i>Chrysanthemum indicum</i>	Sticky	Blackish green	Aromatic	24.32
11.	Bathua seeds	<i>Chenopodium album</i>	Sticky	Brownish	Aromatic	18.45
12.	Dhatura	<i>Datura stramonium</i>	Nonsticky	Blackish green	Aromatic	22.87
13.	Dudhi leaves	<i>Euphorbia hirta</i>	Nonsticky	Brownish	Peculiar	24.72
14.	Peepal leaves	<i>Ficus religiosa</i>	Nonsticky	Black	Aromatic	27.23
15.	Eucalyptus leaves	<i>Eucalyptus globulus</i>	Nonsticky	Yellowish brown	Peculiar	28.42

Table 2. Chemical Constituents in different crude plants methanolic extracts.

S. No.	Plant extract	Plant constituents									
		alkaloids	flavonoids	saponins	carbohydrates	steroids	glycosides	fixed oils	tannins & phenolic compounds	Protein & amino acids	triterpenoids
1.	<i>Ailanthus excelsa</i> (Leaves)	+	+	-	-	-	-	+	-	+	
2.	<i>Annona squamosa</i> (Leaves)	-	+	-	-	-	-	+	-	+	
3.	<i>Annona squamosa</i> (seeds)	+	-	-	+	-	-	+	-	-	
4.	<i>Aegle marmelos</i> (Leaves)	+	+	-	-	-	-	+	-	-	
5.	<i>Butea frondosa</i> (seeds)	-	+	-	-	-	-	+	-	-	
6.	<i>Bauhinia variegata</i> (Leaves)	+	+	-	-	-	-	+	-	-	
7.	<i>Calotropis procera</i> (Leaves)	+	+	-	-	-	-	+	-	+	
8.	<i>Calotropis gigantea</i> (Leaves)	+	+	-	-	-	-	+	-	+	
9.	<i>Cuscuta reflexa</i> (whole plant)	+	-	-	+	-	-	-	-	+	
10.	<i>Chrysanthemum indicum</i> (Leaves)	+	+	-	-	-	-	-	-	+	
11.	<i>Chenopodium album</i> (seeds)	+	-	+	-	-	+	+	-	-	
12.	<i>Datura stramonium</i> (Leaves)	+	+	-	-	-	-	+	-	-	
13.	<i>Euphorbia hirta</i> (Leaves)	+	+	-	-	-	-	+	-	-	
14.	<i>Ficus religiosa</i> (Leaves)	+	+	-	-	-	-	+	-	+	
15.	<i>Eucalyptus globulus</i> (Leaves)	+	-	-	+	-	-	+	-	+	

4. Discussion

In the present study, a total of fifteen crude methanolic extracts was prepared by using different plant parts. *Eucalyptus globulus* (leaves) yielded highest (28.42%) while *Bauhinia variegata* (leaves) yield was low (18.67%). In case of fruit extract *Annona squamosa* yielded highest (33.40%) followed by *Aegle marmelos* (32.23%) and in the case of the seed extract, *Butea frondosa* yielded highest (30.67%) while *Chenopodium album* yielded lowest (18.45%). Difference of percent yield of extraction product among different extractions might be due to difference in solvents used and the solubility of various ingredients and method and type of extraction used [4].

Chemical evaluation of different extracts of all four plants was conducted by various chemical tests to know the active principle present in different extracts [3].

Methanolic extract of *Ailanthus excelsa* (leaves) extract was positive for alkaloids, flavonoids, tannins and phenolic compounds and triterpenoids during analysis. In *Calotropis procera* (leaves) methanolic extract alkaloids, flavonoids and tannins were identified in the methanolic extract while in case of methanolic extract of *Chenopodium album* (seeds) alkaloids, Saponins, glycosides, fixed oils and tannins were present [5].

It was observed from the above observations that the presence of the alkaloids, flavonoids and tannins

were necessary for anthelmintic activity. It was also interesting to know the absence of amines in all the preparation of all the four plants which indicates towards the safety of feeding because amines which are usually toxic substances and this observation was further supported by our observations on the safety and toxicity trials of the plants.

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