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Screening of various extracts of *Grewia hirsuta* leaves and fruits for secondary metabolites

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Abstract
Secondary metabolites present in plants are responsible for medicinal activity of plants. The present investigation is designed in screening of secondary metabolites present in the leaves and fruits of *Grewia hirsuta* an ethnomedicinaly important plant. *Grewia* belonging to the family Tiliaceae is reported to have high medicinal value. The qualitative analysis for secondary metabolites was performed with different solvents such as ethanol, ethyl acetate, acetone, chloroform and distilled water. Phytochemical analysis revealed the presence of various chemical compounds like alkaloids, flavonoids, terpenoids, glycosides, steroids and phenols in various extracts.

Keywords: *Grewia hirsuta*, Secondary metabolites, Medicinal plants, Phytochemical screening

Introduction
Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical semi-synthesis. When a plant is designated as a "medicinal" it is implied that the said plant is useful as an active ingredient of medicinal preparations [12].
Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments [13]. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic importance. Different phytochemicals have been found to have broad range of activities which may help protection against chronic diseases [8]. In India 95% of prescriptions were plant based in the traditional system of Unani, Ayurveda, Homeopathy and Siddha [11].
Grewia hirsuta belong to family Tiliaceae also known as kukuebicha, Govli. It is shrub with 30-90 cm high branches slender, leaves 5-11, oblong linear- lanceolate, stellate, small hairy above, flowers white turning yellow, fruit 2-4 lobed, shining brown, hairy. *G. hirsut*a has high medicinal value; leaves are useful in nose and eye diseases, anthelmintic [3]. The root is astringent to the bowel; useful in cholera, hydrophobia, kidney pain, piles, anthelmintic [2]. Leaves and fruits are purgative, expectorants, carminative, abortifaciant, galactagogue; useful in splenic enlargement, eye troubles, piles, rheumatism pain in joints and in breasts [6]. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals in the five different extracts of the leaves and fruits of *G. hirsuta*.

Material method
Collection of plant material
The fresh sample of leaves and fruits of *G. hirsuta* were collected from Brahminvada region, Undri, Dist-Buldana, Maharashtra and identified with the help of floras. Collected materials were washed under running tap water to remove the surface pollutants and air dried under shade. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

Preparation of the plant extracts
Crude plant extracts were prepared by Soxhlet extraction method. About 50 gm of powdered plant material was uniformly packed into a thimble and extracted with 500 ml of different solvents separately. Solvents used were ethanol, ethyl acetate, acetone, chloroform and distilled water. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless.

After that the extracts were taken in a beakers and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis.

Qualitative Phytochemical test

The solvent free extract obtain as above was then subjected to qualitative preliminary phytochemical screening for identification of various plants constituents following the standard methods [5, 7].

Test for Alkaloids

Solvent free extracts, 50 mg was stirred with few ml of dilute HCL and filtered. The filtrate was tested with various alkaloidal reagents as follows:

Mayer's test

Few ml of filtrate and a drop or two of Mayers reagent were added by the side of the test tube. A white or creamy ppt indicates the presence of alkaloids.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown ppt confirms the presence of alkaloids.

Hager's test

To a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow ppt indicates the presence of alkaloids.

Test for phenolic compound

Lead acetate test

The extract (50mg) was dissolved in distilled water and to this; 3ml of 10% lead acetate solution was added. A bulky white ppt indicates the presence of phenolic compounds.

Test for Tannins

About (0.5g) of the plant extract was added in 10 ml of water in test tube and filtered. A few drops of 0.l% ferric chloride was added and observed for brownish green or blue- black coloration.

Test for proteins

To 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for amino acids

To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acid in the sample.

Test for reducing sugars

To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Test for glycoside

Each extract was hydrolyzed with HCL and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red ppt indicates the presence of glycosides.

Test for Flavonoids

(a) 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCL were added. A yellow solution that turn colorless indicates the presence of flavonoids

(b) To 2 ml of test solution, 0.5ml alcohol was mixed. Then a bit of magnesium and 1 or 2 drops of con. HCL were added and heated. The mixture was analyzed for reaction.

Test for Phenols

To 2 ml of test solution, alcohol and then few drops of neutral ferric chloride solution was added. A dark green clour indicated the presence of phenolic compound.

Test for Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow color indicates the presence of coumarins.

Test for Resins

To the 0.2 g of each extract, 10 ml of glacial acetic acid was added then heated and cooled. A drop of conc. H_2SO_4 was added. Purplish red color shows the presence of resins.

Test for Steroids/ Terpenoids

1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of con. H_2SO_4 was added by the side of the test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence indicated the presence of steroids.

Observations and Results

Table 1: Nature and color of various extracts of *Grewia hirsuta* (Leaf & Fruit)

Extracts	Texture		Color	
	Leaf	Fruit	Leaf	Fruit
Ethyl alcohol	Sticky	Sticky	Dark green	Light brown
Ethyl acetate	Sticky	Sticky	Dark green	Light brown
Acetone	Sticky	Sticky	Dark green	Light brown
Chloroform	Sticky	Sticky	Dark green	Light brown
Distilled water	Sticky	Sticky	Brown	Light brown

Table 2: Phytochemical analysis of various extracts from leaves and fruits of *Grewia hirsuta*

Phytochemical components	Leaf Extract					Fruit Extract				
	Ethyl alcohol	Ethyl acetate	Acetone	Chloroform	Aqueous	Ethyl alcohol	Ethyl acetate	Acetone	Chloroform	Aqueous
Alkaloids Mayer's Hager's Wagner's	- + +	- + +	- + +	- + +	+ + +	- + -	+ + +	- + +	- + +	_ + +
Phenolic compounds Lead acetate	+	+	+	+	+	+	+	+	+	+
Tannin Ferric chloride	+	-	-	-	+	-	-	-	-	-
Protein	-	-	+	+	-	+	+	+	+	-
Amino acids	-	-	-	-	-					
Reducing sugar	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	+	-	-	-	-	-
Flavonoides	-	-	+	+	-	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
coumarins	+	+	+	+	+	+	+	+	+	+
Reasins	-	-	-	-	-	-	-	-	-	-
Steroids/ Terpenoids	-	-	-	-	+	+	+	+	+	+

Key: (+) = indicate present, (-) = indicate absent

Table 1 indicated nature and color of leaf and fruit extracts of *G. hirsuta*. In all five solvents both leaf and fruit extracts showed sticky nature of extract. All extracts of fruits exhibited dark brown color and dark green color for leaf extract except distilled water (Brown).

The phytochemical characteristics of leaves and fruits of *G. hirsuta* were summarized in the table-2. The results revealed the presence of medically active compounds in *G. hirsuta*. From the table it could be seen that alkaloids, phenolic compounds, phenols, caumarins were present in leaf extract of the solvents such as ethanol, ethyl acetate, acetone, chloroform and distilled water. However proteins, flavononoids, tannins were found in some extracts like acetone, chloroform, distilled water. Reducing sugar, glycosides and resins were completely absent in leaf of *G. hirsuta*. In all the extracts fruit alkaloids, phenolic compounds, flavonoides, proteins, caumarin, steroids/ terpenoids were present while tannin, reducing sugar, glycosides and resin were absent.

According to previous study secondary metabolites saponin, tannin, flavonoids and phenols [9]; alkaloids, flavonoids, phenol and ternenoids [1]; terpenoid, fatty compound, flavonoids, steroids, saponins and tannins [4]; tannins, saponins, steroids, terpenoids, phenols [10] were present *G. hirsuta*. Present results also in support of them.

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