

Analysis The Extract Of Barks In *Gmelina Arborea* In Different Dilution And Phytochemical Examination In Ethanol Extraction

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Abstract: In this work mainly focused the qualitative estimation phytochemical in the different solvent, Most of the compounded present in the ethanol extract so we selected the ethanol extract for four their investigation like wises in vitro, in vivo and GC-MS analysis examination. Primarymetabolites, which include amino acids, simple sugars, nucleic acids, and lipids are compounds that are necessary for cellular processes. Secondary metabolites are mainly produced by the plants in response to stress.

Key Words: Secondary metabolites, Ethyl acetate, Petroleum ether, *GmelinaArborea*, phytochemical

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I. Introduction

Gmelinaarborea is a fast-growing tree, which grows on different localities and prefers moist fertile valleys with 750–4500 mm rainfall. It does not thrive on ill-drained soils and remains stunted on dry, sandy or poor soils; drought also reduces it to a shrubby form.

The *Gmelinaarborea* tree attains moderate to large height up to 30 m with girth of 1.2 to 4 a chlorophyll layer just under the outer bark, pale yellow white inside.

Gmelinaarborea wood is pale yellow to cream-coloured or pinkish-buff when fresh, turning yellowish brown on exposure and is soft to moderately hard, light to moderately heavy, lustrous when fresh, usually straight to irregular or rarely wavy grained and medium course textured. Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June. The fruit is up to 2.5 cm long, smooth, dark green, turning yellow when ripe and has a fruity smell.

This tree is commonly planted as a garden and an avenue tree; growing in villages along agricultural land and on village community lands and wastelands. It is light demander, tolerant of excessive drought, but moderately frost hardy. It has good capacity to recover from frost injury. Gamhar trees coppices very well with vigorous growth. Saplings and young plants need protection from deer and cattle.

Medicinal plants have been consumed for the health benefits in developed and developing nations. According to the World Health Organization (WHO) many developing nation like India, rely only on medicinal plants and plant based compounds for the treatment of various diseases.

Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of allopathic medicine. Traditional knowledge will serve as a powerful search engine and most importantly, will greatly facilitate intentional, focused and safe natural products research to rediscover the drug discovery process.

People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses.

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell. Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

II. Methods And Materials

2.1. COLLECTION AND IDENTIFICATION OF PLANTS

The bark of GA is collected from south India, Kanyakumari district during the month of January and February. The plant was identified by S. Balasubramaniam, ABS Botanical Garden – Salem.

2.2. PREPARATION OF EXTRACTS

The freshly collected barks were dried in shade, then coarsely powdered. For extraction of crude phytochemical, 25 g of powdered bark material was kept in closed conical flask with 20 mL various solvents like petroleum ether, benzene, chloroform, ethanol, acetone, ethyl acetate and distilled water in a shaker at room temperature for 24 h. After incubation, the extracts were filtered and the extracts were collected and stored in the refrigerator at 4 for further studies. All the extracts were subjected to preliminary phytochemical screening as per the guidelines.

2.3. PRELIMINARY PHYTOCHEMICAL SCREENING OF BARK EXTRACT OF *GREWIA UMBELLIFERA* AND *GMELINA ARBOREA*

The extracts obtained as above were then subjected to qualitative tests for the identification of various plant constituents.

2.3.1. DETECTION OF CARBOHYDRATES

A minimum amount of extracts were suspended in 5 ml of distilled water. The suspension was subjected to General test, Starch test, Barfoed's test, Molisch's test, Fehling's test, Benedict's test, Iodine test as seen below.

a) General Test

The extracts were treated with a few ml of distilled water and sulphuric acid. Formation of dull violet precipitate indicates the presence of reducing sugar.

b) Starch Test

Aqueous extracts were treated with 5ml of 5% potassium hydroxide. Canary coloured solution shows the presence of starch.

c) Barfoed's test

Aqueous extracts were treated with 1ml of Barfoed's reagent. The solutions were heated in a beaker of boiling water bath gives a red precipitate indicates the presence of reducing sugar.

d) Molisch's Test

The extracts were treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of purple ring between two layers shows the presence of carbohydrates.

e) Fehling's Test

The extracts were treated with Fehling's A and B solution and heated for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

f) Benedict's Test

The extracts were treated with Benedict's reagent and heated for few minutes. Formation of red precipitate shows the presence of reducing sugar.

g) Iodine Test

Add a few drops of iodine solution to 1ml of the extract. Formation of deep blue colour indicates the presence of starch.

2.3.2. DETECTION OF GLYCOSIDES

Minimum quantities of the extracts were hydrolysed with hydrochloric acid for few minutes on a water bath and the hydrolyzate was subjected to Legal's test, Bontrager's test, Ferric Chloride's test as seen below.

a) Legal's Test

To the hydrolyzate 1ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide. The pink colour changes in to red show the presence of glycosides.

b) Bortrager's Test

Hydrozylate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The pink colour changes in to red show the presence of glycosides.

c) Ferric chloride Test

2ml of extracts were treated with 1ml of glacial acetic acid and 1ml of ferric chloride. Also add few drops of concentrated sulphuric acid. Formation of blue colour showed the presence of glycosides.

2.3.3. DETECTION OF PROTEINS AND AMINO ACIDS

A small quantity of extract was dissolved in few ml of water and they were subjected to Million's test, Ninhydrin test, Biuret test as given below.

a) Million's Test

The extracts were treated with Millon's reagent. The precipitate was formed with the extract, which shows the presence of proteins.

b) Ninhydrin test

The extracts were treated with Ninhydrin reagent. The purple colour was formed with extract, which shows the presence of proteins.

c) Biuret Test

To the extracts equal volume of 5% sodium hydroxide solution and 1% copper solutions were added. A violet colour formation indicates the presence of amino acids.

2.3.4. DETECTION OF FIXED OILS AND FATS

A small quantity of extract was subjected to Spot test, Saponification test as follows.

a) Spot Test

Small quantities of extracts were placed between two filter papers. The production of stains with alcoholic extract shows the presence of fats and fixed oils in the extract.

b) Saponification Test

Few drops of 0.5N alcoholic potassium hydroxide was added to the extracts with few drops on phenolphthalein solution. Later the mixture was heated on a water bath for 1-2 hours. The soap formation indicates the presence of fat and fixed oils in the alcoholic extracts.

2.3.5. DETECTION OF ALKALOIDS

A small quantity of the extracts were treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid reagent such as Mayer's reagent, Dragondroff's reagent, Hager's reagent, Wagner's reagent and subjected to potassium dichromate test as given below.

The filtrate was tested with alkaloid reagent such as,

- 1) Mayer's reagent (Cream precipitate)
- 2) Dragondroff's reagent (Reddish brown precipitate)
- 3) Hager's reagent (Yellow precipitate)
- 4) Wagner's reagent (Reddish brown precipitate)
- 5) Potassium dichromate Test

The extracts were treated with concentrated sulphuric acid and add small amount of potassium dichromate. No colour change indicates the presence of indole alkaloid.

2.3.6. DETECTION OF FLAVANOIDS

i) Ferric chloride Test

Aqueous extracts were treated with few drops of 10% ferric chloride. Formation of green precipitate indicates the presence of Flavanoids.

ii) Lead acetate Test

Aqueous extracts were treated with few ml of 10% lead acetate. Buff coloured solution formation indicates the presence of Flavanoids.

iii) Test for Anthraquinone

a) 0.5gm of extract was treated with 10ml of sulphuric acid. This solution was boiled and filtered while hot. From the filtrate add 5ml of chloroform. Pipetted out the formed chloroform layer add 1ml of dilute ammonia. No colour change indicates the presence of Anthraquinone.

b) 0.5gm of Anthroquinone added a drop of benzene and ammonia. Formation of pink colour indicates the presence of Anthraquinone.

iv) Test for Catechins

A drop of Erlich's reagent was added to the 0.5gm of extracts. Formation of pink colour indicates the presence of catechins.

v) Test for Anthocyanin

2ml of plant extracts were treated with 1ml of 2M NaOH and heated for 5 min. Formation of yellow colour indicates the presence of Anthocyanin.

2.3.7. DETECTION OF PHYTOSTEROLS

Small quantities of extracts were suspended in 5ml of chloroform separately. The above obtained chloroform solution was subjected to LibermannBurchard test, Salkowski test as given below.

a) LibermannBurchard Test

The above prepared chloroform solutions were treated with few drops of concentrated sulphuric acid. A bluish green solution indicates the presence of phytosterols.

b) Salkowski Test

To the above prepared chloroform solutions, a few drops of concentrated sulphuric acid were added. Formation of brown ring with chloroform extract indicates the presence of phytosterols.

2.3.8. DETECTION OF TANNINS- PHENOLIC COMPOUNDS

All the extracts were dissolved or suspended separately in minimum amount of water and filtered. The filtrate was subjected to General test, Ferric Chloride test, Lead acetate test and Phlonatannins as given below.

a) General Test

Plant extracts were treated with a few drops of sulphuric acid and 1 drop of 5% HCl. Formation of green colour indicates the presence of tannins.

b) Ferric chloride Test

To the filtrates few drops of ferric chloride was added. Violet colour precipitate indicates the presence of tannins.

d) Test for Phlonatannins

The ethanol extract of plant material was treated with 5ml of 1% hydrochloric acid. Formation of red precipitate indicates the presence of phlonatannins.

2.3.9. DETECTION OF SAPONINS

The extracts were subjected to Foam test, Haemolysis test, as seen below.

a) Foam Test

The extract was diluted with 20ml of distilled water and then agitated in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of Saponins.

b) Haemolysis Test

About 2ml of blood was taken two test tubes separately. To one of the test tubes, equal quantity of water was added. To the other test tube, an equal quantity of ethanolic extract dissolved in water was added. A clear red liquid was formed in the first test tube, which indicates the red blood corpuscles were haemolysed. The extract in the second tube also haemolysed. It indicates the presence of Saponins.

2.3.10.DETECTION OF STEROIDS, VITAMINS AND TERPENOIDS

The extracts were subjected to various tests, as follows.

a)Detection of Steroids

To the ethanolic extract add few drops of acetic anhydride and a drop of concentrated sulphuric acid. Appearance of green or brown colour was the end point.

b)Detection of Vitamins

One or two drops of plant extracts were treated with 1ml of chloroform and a drop of concentrated sulphuric acid. A colour changes from violet to brown colour indicates the presence of vitamins.

c)Detection of Terpenoids

Aqueous extract of plant materials were treated with 2ml of chloroform and few drops of concentrated sulphuric acid. Formation of reddish brown colour at interphase indicates the presence of terpenoids.

Name of the compound	<i>Gmelinaarboreabark</i> extracts						
	Pet.ether	Benzene	Chloroform	Ethanol	Acetone	Ethyl acetate	Dist. Water
CARBOHYDRATES	Absent	Absent	Present	Present	Present	Present	Absent
GLYCOSIDES	Absent	Absent	Present	Present	Present	Present	Absent
PROTEINS	Absent	Present	Present	Present	Present	Absent	Absent
AMINO ACIDS	Absent	Present	Present	Present	Present	Absent	Absent
OILS	Present	Present	Absent	Present	Present	Absent	Absent
FATS	Present	Present	Absent	Present	Present	Absent	Absent
ALKALOIDS	Absent	Present	Absent	Present	Present	Present	Absent
FLAVANOIDS	Absent	Absent	Present	Present	Present	Absent	Absent
PHYTOSTEROLS	Present	Absent	Absent	Present	Present	Absent	Absent
TANNINS	Absent	Absent	Present	Present	Present	Absent	Absent

PHENOLIC COMPOUNDS	Absent	Absent	Present	Present	Present	Absent	Absent
SAPONINS	Absent	Absent	Absent	Present	Present	Absent	Absent

Table 1 *Gmelinaarborea* bark extracts. The table show present and absent compound of different dilution of GA

Table 2 Preliminary phytochemical screening of bark extract of *Gmelinaarborea*

S. No	Name of the test	<i>Gmelinaarborea</i> bark extracts						
		Pet. ether	Benzen e	Chloroform	Ethanol	Acetone	Ethyl acetate	Dist. water
1	CARBOHYDRATES							
	a) General test	+	+	+	+++	+	+	+
	b) Starch test	+	+	+	+++	+	+	+
	c) Barfoed's test	+	+	+	+++	+	+	+
	d) Molisch's test	+	+	+	+++	+	+	+
	e) Fehling's test	+	+	+	+++	+	+	+
	f) Benedict's test	+	+	+	+++	+	+	+
	g) Iodine test	+	+	+	+++	+	+	+
2	DETECTION OF GLYCOSIDES							
	a) Legal' test	+	+	+	++	+	+	+
	b) Borntrager's test	+	+	+	++	+	+	+
3	PROTEINS AND AMINO ACIDS							
	a) Million's test	-	+	+	++	+	-	-
	b) Ninhydrin test	-	+	+	++	+	-	-
4	FIXED OILS AND FATS							
	a) Spot test	+	+	-	++	+	+	+
	b) Saponificati-on test	+	+	-	++	+	+	+
5	DETECTION OF ALKALOIDS							
	a) Mayer's test	-	+	-	+++	+	+	-
	b) Dragondroff-'s test	-	+	-	+++	+	+	-
	c) Hager's test	-	+	-	+++	+	+	-
	d) Wagner's test	-	+	-	+++	+	+	-
	e) Potassium dichromate test	-	+	-	+++	+	+	-
6	DETECTION OF FLAVANOIDS							
	a) Ferric chloride test	+	+	+	++	+	+	+
	b) Lead acetate test	+	+	+	++	+	+	+

	c) Test for Anthraquinone	+	+	+	++	+	+	+
	d) Test for Catechins	+	+	+	++	+	+	+
	e) Test for Anthocyanin	+	+	+	++	+	+	+
7	DETECTION OF PHYTOSTEROLS							
	a) LibermannBurchard test	+	-	-	+	+	+	-
	b) Salkowski test	+	-	-	+	+	+	-
8	TANNINS AND PHENOLIC COMPOUNDS							
	a) General test	-	+	+	+	+	+	+
	b) Ferric chloride test	-	+	+	+	+	+	+
	c) Test for Phloratannins	-	+	+	+	+	+	+
9	DETECTION OF SAPONINS							
	a) Foam test	-	-	-	+	+	-	-
	b) Haemolysis test	-	-	-	+	+	-	-
10	DETECTION OF STEROIDS	-	-	-	+	+	-	-
11	DETECTION OF VITAMINS	-	-	-	+	+	-	-
12	DETECTION OF TERPENOIDS	-	-	-	+	+	-	-

III. Discussion

Plants produce primary and secondary metabolites which encompass a wide array of functions. Primary metabolites, which include amino acids, simple sugars, nucleic acids, and lipids are compounds that are necessary for cellular processes. Secondary metabolites are mainly produced by the plants in response to stress. In the present study both the plants showed the presence of alkaloids, flavonoids, tannins and saponins etc., (Table . Among all the extracts the ethanol extract of the plants were found to contain many components. So the ethanol extract of the plants were taken for further studies.

IV. Conclusion

Most of the compounded present in the ethanol extract so we selected the ethanol extract for four their investigation like wises in vitro, in vivo and GC-MS analysis examination.

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