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Phytochemical analysis of rare plants from Surgana forest, Nasik district, Maharashtra, India

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Abstract
Phytochemicals are bioactive compounds obtained from the plants and widely applied in the traditional herbal medicines. The Present study was aimed to evaluate the phytochemical constituents of nine rare plants from Surgana forest (Peepalsond) of Nashik division. The phytochemical screening (Qualitative and Quantitative) were carried out of stem-bark of the Plant, by using UV spectrophotometry for Qualitative method and standard procedure method for Quantitative analysis. The plants were *Spondias pinnata, Tecomella undulata, Syzygium cumini, Dyschoriste dalzellii, Sterculia urens, Martynia annua* L., *Costus speciosus, Cassine glauca, Terminalia arjuna*. The qualitative phytochemical test exhibited the presence of common phyto-compounds including Alkaloid, Tannin, Carbohydrates, Proteins, Steroids, Gums. Alkaloids, Steroids, Carbohydrates are the major compounds in the extract of plant extract. Along with it the other compounds like proteins, Tannins, Mucilage were also found in the plant extract. Flavonoids were recorded as least compounds in the plant extact.

Keywords: phytochemical, qualitative, quantitative, *Spondias pinnata, Tecomella undulata, Syzygium cumini, Dyschoriste dalzellii, Sterculia urens, Martynia annua* L., *Costus speciosus, Cassine glauca, Terminalia arjuna*

Introduction
Plants are very important to humans' life as food, shelter and even as medicines. Traditional knowledge of medicinal plants is now considered to play a vital role in addressing the health care needs of developing countries and indigenous people.

The natural vegetation of Nashik District includes a variety of plant species having economic importance. It yields timber, food and fodder plants and plants having medicinal value.

Here the tribal's are largely dependent on forest products for their livelihood. They are knowledgeable about the utility of the majority of these plants. The average rainfall in the district is ca 1,034.5mm. The maximum precipitation recorded at Surgana is 2500mm (1958-59). 'Peepalsond', forest which is 15-20 kms from Surgana, is the next forest area for exploration of Nashik district.

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive compounds. These phytochemicals are the secondary metabolites present in smaller quantities in higher plants and they include the alkaloids, Steroids, flavonoids, Tannin, Carbohydrate, proteins, glycosides and Gums, tannins and many others.

The present investigation is carried out to study the constituents of the plants by phytochemical analysis of the following nine rare plants found rare and endangered-
Spondias pinnata, Tecomella undulata, Syzygium cumini, Dyschoriste dalzellii, Sterculia urens, Martynia annua L., *Costus speciosus, Cassine glauca, Terminalia arjuna*.

Geology and Soil
Forest is enriched with medicinal plants. As it was an onset of rainfall forest department took the efforts of tree plantation and maintained the forest by planting trees like Sissov etc, and in the nursery many plants seedlings are developed Ficus, Fig, Khair). Mixed deciduous forests, occurring in both protected as well as reserved forest. Generally found in black and Grey soil. Here is the floristic data with medicinal plants.

Trees like- *Acacia chundra, Albizia lebbeck, Dalbergia lanceolaria, Terminalia bellerica, T. chebula, T.crenulata, Cassia fistula, Pongamia pinnata, Wrightia tinctoria, Emblica officinalis, Ficus racemosa, Madhuca indica, Syzygium cumini.*

Shrubs were, *Carissa congesta, Carvia callosa, Casearia graveolens, Lantana camara, Meyna laxiflora, Woodfordia fruticosa, Ziziphus glaberrima.*

Climbers were- *Cuscuta reflexa, Acacia pennata, Dioscorea bulbifera*. Some were parasitic like *Cuscuta reflexa*, There were Epiphytic orchids- *Aerides crispum, Eria dalzellii, Nervilia sps.*

Ferns like- *Adiantum philippense, Cheilanthes farinosa* By Flora of Nasik Sharma, B.D and Lakshminarasimhan, P (1991) [2]. Many plants and their parts are used as medicines. Collected plants are verified by using Flora of Nasik Sharma, B.D and Lakshminarasimhan, P (1991) [2].

Nine medicinal important plants were observed and collected for the phytochemical analysis was carried out.

Material and Method
The plants were collected from their natural habitat. Collected plants are verified by using Flora of Nasik Sharma B.D and Lakshminarasimhan, P (1991) [2]. Also collected plant specimen were compared with Traditional Medicines in Satpura – by S.H. Patil.

The collected samples were shade dried and crushed to a coarse powder by grinder. Then the samples were labelled as, A, B, C, D, E, F, G, H, and I, phytochemical analysis was carried out. Phytochemical screening and antioxidant activity of extracts of the leaf and bark of *Albizia lebbeck* (Benth)-

Vasanthi *et al.*

a. *Sterculia urens*
b. *Syzygium cumini*
c. *Spondias pinnata*
d. *Cassine glauca*
e. *Terminalia arjuna*
f. *Tecomella undulata*
g. *Dyschoriste dalzellii*
h. *Costus speciosus*
i. *Martynia annua* L.

Chemicals

The entire chemicals used in the present study are of analytical grade.

Phytochemical analysis

Experimental Phyto Pharmacognosy –Dr S.S Khadbadi, B.A Baviskar, Dr. S.I Deore

A. Quantitative estimation of Tannin content

Standard: Tannic acid solution Reagent: Folin- Denis Reagent Wavelength: 700 nm

Procedure

- Prepare calibration curve of standard Gallic acid (10-100ug/ml in water).
- Prepare 1mg/ml of extract solutions.
- Mix 1ml of each sampled with 0.25ml of Folin ciocalteu's reagent and 1.25ml of 20% sodium carbonate solution. Allow the mix to react for 40 min. at room temperature.
- After the reaction period, the contents are mixed and measure the blue colour at75 nm in comparison with standards. Calculate the amount of total phenols from calibration curve as Gallic acid equivalent by formula.

B. Quantitative estimation of Flavonoid content

- Prepare the calibration curve of standard Quercetin (10-100ug/ml in methanol).
- Mix 0.5 ml standard solution with 1.5ml of 95% ethanol .0.1ml of 10% aqueous aluminium chloride.0.1 m of 1M potassium acetate and 2.8 ml of D/W.
- Incubate for 30 mins at room temperature. Measure the absorbance of the reaction mixture at 415nm with a UV spectrophotometer.
- To prepare blank solution substitute 10% aluminium chloride with the same amount of D/W.
- Similarly, treat 0.5 ml of plant extract samples with aluminium chloride for determination of flavonoid content from calibration curve.

A. Quantitative estimation of Tannin content

Standard: Tannic acid solution Reagent: Folin- Denis Reagent Wavelength: 700 nm.

Table 2: Absorbance of standard tannic acid

Drug	Concentration (mcg)	Absorbance
Tannic acid	50	0.497
	100	1.059
	150	1.267
	200	1.613
	250	1.813

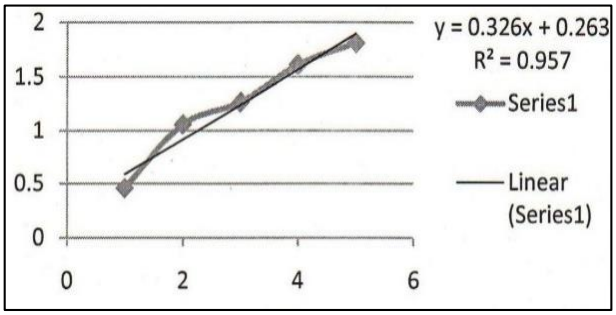

Fig 1: Standard curve of Tannic Acid

Table 3: Total tannin content of plants

Plant Extracts	Total tannin content mcg/gm
Plant A	780
Plant E	3030
Plant F	3670
Plant G	892.8

B. Quantitative estimation of Flavonoid content:

Method: UV spectrophotometry
Standard: Quercetin solution
Reagents: Aluminium chloride, potassium acetate
Wavelength: 415 nm

Table 4: Absorbance of standard Quercetin

Drug	Concentration (mcg)	Absorbance
Quercetin	10	0.384
	20	0.476
	30	0.837
	40	0.956
	50	0.989

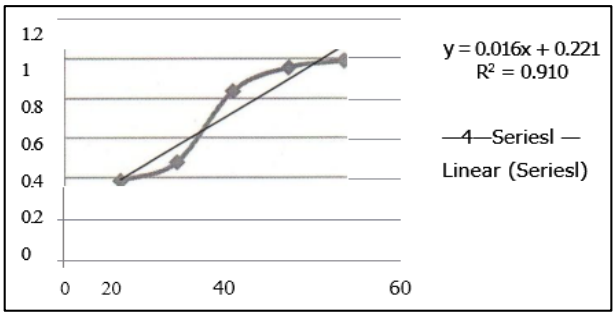

Fig 2: Standard curve of Quercetin

Table 5: Total Flavonoid content of plants

Plant Extracts	Total Flavonoid content mcg/gm
Plant E	660

Qualitative analysis of nine rare plants

Experimental phyto Pharmacognosy –Dr S.S Khadbadi, B.A Baviskar, Dr. S. I Deore

1. Tests for carbohydrates [3, 7-10]

a. Molisch's test: To 2 mL of test solution adds few drops of α-naphthol solution in alcohol and adds 2 mL of concentrated H2SO4 slowly from the sides of the test tube. A purple ring is observed at the junction of two liquids.

b. Fehling's test: Mix 1 mL Fehling's solution A and Fehling's solution B, boil for 1 minute, add equal volume

of test solution, heat in boiling water bath for 10 minutes. First yellow, then brick red precipitate is observed.

2. Test for gums

Hydrolyse test solution using dilute HCl. Perform Fehling's or Benedict's test. Red color is developed.

3. Test for mucilage:

a) Powdered drug material shows red color with Ruthenium red.
b) Powdered drug swells in water or aqueous KOH.

4. Tests for proteins

a. Biuret test (General Test): Test solution treated with 4% sodium hydroxide and dilute copper sulphate (1%) solution gives violet or pink color.
b. Millon's test: Mix 3ml of test solution with 5 mL Million's reagent. White precipitate is obtained which turns into brick red or red colored solution on warming. Millon's reagent- Dissolve 3 mL of mercury in 27 mL of fuming nitric acid, keep the mixture to cool. Dilute the solution with an equal quantity of distilled water.

5. Tests for amino acids

a) Ninhydrin test (General test): Heat 3 mL test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 minutes. Purple or bluish color appears.

6. Test for fats and oils

a) Solubility Test: Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water. (Exception is castor oil, soluble in alcohol).
b) Saponification Test: Evaporate extract to get 10 mL oil. To oil add 25 mL 10% NaOH. Boil in water bath for 30 minutes. Cool. Add excess Na2SO4 solution. Soap forms and rise to the top. Filter, to filtrate add H2SO4.Evaporate, collect residue, dissolve in ethanol. With ethanolic solution, perform following tests:
1. To ethanolic solution, add few crystals of KHSO4.Heat vigorously. Pungent odour of acrylic aldehyde is produced.
2. To ethanolic solution, add few drops of CuSO4 and NaOH solution. Clear blue solution is formed.

7. Test for sterols and triterpenoids

a) Salkowaski test: When a few drops of concentrated H_2SO_4 is added to the mixture of chloroform and test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols and formation of yellow colour in the lower layer indicates the presence of Triterpenoids.

8. Tests for alkaloids

a) Mayer's test: Test solution with Mayer's reagent (potassium mercuric iodide) gives cream-colored precipitate.
b) Wagner's test: The acidic solution with Wagner's' reagent (iodine in potassium iodide) gives brown precipitate.
c) Hager's test: The acidic solution with Hager's reagent (saturated picric acid solution) gives yellow precipitate.
d) Dragendorff's test: The acidic solution with Dragendorff's reagent (potassium bismuth iodide) shows orange brown precipitate.

9. Tests for saponins

a) Foam test: Saponins when mixed with water and shaken, shows the formation of foam which is stable at least for 15 minutes.
b) Haemolysis test: 2 mL of 18% sodium chloride solution in two test tubes is taken. To one test tube added distilled water and to the other 2 mL of filtrate. Few drops of blood are added to both the test tubes. Mixed, observed for haemolysis under microscope.

10. Tests for flavonoids

a) Shinoda test: Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid, shows pink to magenta red colour.

11. Test for tannins and phenolic compounds

a) Ferric-chloride test: Test solution treated with few drops of ferric chloride solution gives dark color.
b) Lead Acetate Test: Add 10% w/v solution of lead acetate in distilled water to the test filtrate. Precipitate indicates presence of tannins.

Table 1

S.N.	Chemical Test For Plant	A	B	C	D	E	F	G	H	I
1	Carbohydrates (Molisch Test)	-	-	-	+	+	+	+	+	+
2	Proteins (Biuret Test)	-	+	+	-	++	-	-	+	-
3	Tannins and Phenolic compounds (Ferric chloride Test)	++	-	-	+	++	++	++	-	-
4	Mucilage (Ruthenium Red Test)	-	+	+	+	-	-	-	+	-
5	Flavonoids (Shinoda Test)	-	-	-	-	+	-	-	-	-
6	Gums (DilHCl and Fehling's Test)	+	-	-	+	+	+	+	-	-
7	Steroids (Salkowski)	+	-	+	+	++	+	+	-	-
8	Alkaloids (Dragendorff's Test)	+	+	-	-	+	+	+	+	+

Result

The qualitative phytochemical test exhibited the presence of common phyto-compounds including Carbohydrates, proteins, Alkaloids, Steroids, Tannin, Mucilage, Flavonoids are the major compounds in the plant-extract. (+represent presence and ++ represent high concentration). Proteins, Tannins, Mucilage were also found in the plant extract.

Flavonoids were recorded as least compounds in the plant extract.

The result of phytochemical screening revealed the presence of Flavonoids and Tannin in the stem-bark of nine rare plants found in Kalwan forest region. The stem bark of the plants investigated for phytochemical constituents seems to have the potential to act as a source of medicines and also to enhance the health status of the consumers due to the presence of various compounds they play a vital role for good health.

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