"Phytochemical characterizationof extracts obtained from bark of *Spathodeacompanulata(P.Beauv.)*"

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Abstract: Medicinal plants are gift of God having answers for almost all type of diseases. Since ancient time in India many healers used these medicinal plants in treatment of various pathogenesis. Now a days this knowledge is accepted globally. Hurdle in establishing the concept, is proper characterization of these medicinal plants on the basis of physical, chemical and biological properties. Among these medicinal plants, many are reported to have significant effect on various systems and disease. These plants possess great therapeutic effects on human body and can be good cure to many deadly diseases. The plant *spathodeacampanulata* falls under such category. In the present research work the barks have been studied. These are first identified and the bark extracts have been isolated. The extracts were tested for phytochemical constituents such glycosides, alkaloids, terpenoids, steroids, carbohydrates, etc. The TFC and TPC were also calculated.

Keywords- spathodeacampanulata, TFC- total flavonoid content, TPC-total phenolic content.

Introduction

Spathodeacampanulata L. is native of tropical Africa, with orange scarlet bell shaped flowers, three by two and half inch large, that appear in November, the climate of Mumbai seems to suit it and may be seen in full flowering in the month of November. It is generally planted as an ornamental plant along the roadside. Hence, the flowers are easily available and abundant. The flower part is most colourful and consists of maximum amount of chromophores responsible for the activity. Genus name comes from the Greek words*spathe* meaning sheath and *oides* meaning resembling in reference to calyx shape.

Phytochemical studies yield alkaloids, tannin, saponin, steroids, terpenonids, flavonoids. Study to analyze the constituents of the flower yielded four compounds. Butane, 1, 1-diethoxy-3-methyl- (35.11%) and n-Hexadecanoic acid (30.22%) were the major constituents of the ethanolic extract. Stem bark has yielded spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid and pectic substances. Phytochemical screening phytochemical screening yielded carbohydrates, alkaloids,tannins, glycosides in the extracts of flowers and steroids, carbohydrates, alkaloids, tannins and glycosides in the bark.

Traditional uses

The bark is widely used in traditional medicine in Ghana. The bark is used in wound healing and especially burn healing. The bark shows a wide spectrum of antibacterial activity including antimalarial activity. Aqueous alcoholic decoctions of the leaves shows promise to be used for the treatment of malaria. The stem bark decoction has shown hypoglycemic activity in mice. African tulip tree is planted as an ornamental, a wayside tree and shade tree. It is used for soil improvement, reforestation, erosion control and land rehabilitation, and as a live fence. The seeds are edible. The soft white timber used in making paper and a wood is used to make drums. The bark, flowers and leaves are used in traditional medicines in Western Africa. The seeds are eaten in many parts of Africa. The flower buds contain a reddish sap and are used as water pistols by children. In Ethiopia, it is used as firewood and to produce charcoal.

Medicinal uses (huo Yan Shu, 2012)

*Spathodeacampanulata*has many medicinal uses both where it is native and introduced. Extracts of bark, leaves and flowers are used to treat malaria, HIV, diabetes, meliitus, Oedema, dysentery, constipation, gastrointestinal disorders, ulcers, skin diseases, wounds fever, urethral inflammation, liver complaints and as a poison antidote. It may be effective as malaria prophylactic and

in the control of *Aedes* Mosquitoes. The bark has laxative and antiseptic properties and the seeds, flowers and roots are used as medicine. The bark is chewed and sprayed over swollen cheeks. The bark also is boiled in water used for bathing newly born babies to heal body rashes. Bark is commonly used as a dressing for ulcers and skin diseases, applied dried, pulverized, or as fresh inner bark. In Africa, the stem bark is used as a paste for wound healing. In Senegal, bruised leaves and flowers are applied to wounds. In Gabon, flowers are applied to ulcers. In Gold Coast, bark decoction taken for constipation and gastrointestinal problems and dysentery. In Ghana, the stem bark and leaf used for treatment of dyspepsia and peptic ulcer; leaf, root bark and fruit used for arthritis and fractures; the stem bark used for toothaches and stomachaches; root bark seed used for stomach ulcers. In Rwanda decoction of stem bark used for diabetes. In Ayurveda it is used for kidney diseases.

MATERIAL AND METHODS

Spathodeacompanulata was collected and authenticated by Dr. Zia-Ul-Hasan,HOD, Department of Botany. The parts where extraction was to be performed were washed under running tap water and kept in shade for drying. They were later pulverized using mechanical grinder. They were then packed in labelled containers. Then they were subjected to extraction using hot percolation 'Soxhlation'. Pulverised dried material of *Spathodeacompanulata*were placed in thimble of Soxhlet apparatus. Extraction of *Spathodeacomanulata* was done with percolation method. Extraction was performed at 60°C using petroleum ether as non-polar solvent at first. Exhausted plant material (mark) was dried and afterward extracted with ethyl acetate, chloroform and methanol. Obtained extracts were evaporated and using rotary vacuum evaporator (Buchi type) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

Phytochemical characterization

1. **Qualitative phytochemical screening**

Preliminary phytochemical screening of extracts were subjected to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins.

2. **Quantitative phytochemical testing**

a. **Total Phenolic Content Estimation**

Reagents and Chemicals: Gallic acid, Methanol, FolinCiocalteu Reagent, Sodium carbonate solution (7.5 % w/v sodium carbonate solution)

Methodology: Gallic acid and test sample solutions (10-100 µg/ml) were prepared using methanol as solvent. Gallic acid (0.5 ml) or test sample (0.5 ml) dilutions were added to Folin-Ciocalteu Reagent (2 ml). Sodium carbonate solution (4 ml) was added to the above mixture and incubated for 30 minutes at room temperature with intermittent shaking. Absorbance was noted at the wavelength of 765 nm utilizing methanol as blank. Standard curve for Gallic acid was prepared. Total phenolic content in the test sample was estimated as µg/mg galic acid equivalent using Gallic acid standard curve (Ainsworth et al., **2007**; Alhakmani, **2013**).

b. **Total Flavonoid Content Estimation**

Chemicals: Rutin, methanol, etc.

Methodology: Different concentrations of Rutin and test sample were prepared in methanol (10-100 µg/ml). Sample solution (0.5 ml) was mixed with distilled water (2 ml), followed by 5% $NaNO_2$ solution (0.15 mL). $AlCl_3$ solution (10%) was added after 6 minutes and allowed to stand for 6 min followed by 4% NaOH solution (2 ml). Mixture was further diluted to 5 ml using water and allowed to stand for 15 minute. Absorbance was noted at 510 nm utilizing water as blank. Standard curve for Rutin was prepared. Total flavonoid content µg/mg Rutin equivalent was estimated using the absorbance value of sample (Zhishen et al., **1999)**.

Thin Layer Chromatography

TLC is a method for analyzing mixtures by separating the compounds in the mixture. TLC is used to determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analyzed by TLC - and it takes little time for an analysis (about 5-10 minutes). TLC consists of three steps - spotting, development, and visualization.

Results and discussion

Phytochemical Identification:

In order to determine the presence of chemical constituents, phytochemicals test were performed, which revealed the presence of phytoconstituents in Petroleum ether, Ethyl Acetate, chloroform and methanol extracts of bark. Following results were obtained:-

BARK :

TESTS	**PET ETHER**	**ETHYLACETATE**	**CHLOROFORM**	**METHANOL**
GLYCOSIDES				
Borntrager's test	- Ve	- Ve	- Ve	- Ve
Legal's test	- Ve	+ Ve	+ Ve	+Ve
Killer killiani test	- Ve	+ Ve	+ Ve	+ Ve
ALKALOIDS				
Mayer's test	- Ve	- Ve	- Ve	+ Ve
Hager's test	- Ve	- Ve	- Ve	+ Ve
Wager's test	- Ve	- Ve	- Ve	+ Ve
CARBOHYDRATES				
Molish test	+ Ve	+ Ve	+ Ve	+ Ve
Fehling's test	- Ve	+ Ve	+ Ve	+Ve
Benedict's test	+ Ve	+ Ve	- Ve	+Ve
PROTEINS AND AMINO ACIDS				
Biuret's test	+ Ve	+ Ve	- Ve	- Ve
Ninhydrin test	+ Ve	+Ve	- Ve	- Ve
SAPONINS				
Froth's test	- Ve	- Ve	- Ve	+ Ve
FLAVONOIDS				
Lead acetate test	- Ve	- Ve	+ Ve	- Ve
Alkaline reagent test	- Ve	+ Ve	+ Ve	+ Ve
Shinoda test	- Ve	+ Ve	+ Ve	+ Ve
TRETERPENOIDS AND STEROIDS				
Salkowski's test	+ Ve	+ Ve	+ Ve	+ Ve
Libbermann's test	+ Ve	+ Ve	+ Ve	+ Ve
TANNIN AND PHENOLIC COMPOUNDS				
Ferric chhloride test	- Ve	+ Ve	+Ve	+ Ve
Lead acetate test	- Ve	-Ve	- Ve	- Ve
Dilute iodine solution test	-Ve	- Ve	+ Ve	+ Ve
Gelatin test	- Ve	+ Ve	+ Ve	+ Ve

RESULTS AND DISCUSSION

The extracts of bark of spathodeacampanulatawere tested for the presence of phytochemical constitiuents. The extracts were obtained after soxhlation of pulverized bark in Petroleum ether, ethyl acetate, chloroform and methanol. The extracts in various solvents were then tested for phytochemical constituents such as glycosides, alkaloids, proteins, steroids, carbohydtrates, etc. amongst the glycosides the extracts were tested for borntrager's test, legal's test and kellerkilliani test. The bark extracts of methanol, chloroform and ethyl acetate tested positive for legals test and kellerkilliani test indicating presence of glycosides.

Then the samples of extract of bark were tested for alkaloids. The tests they underwent were mayers test,hagers test, wagers test. The extracts of bark in petroleum ether and chloroform tested positive for all the three tests while the alcoholic extract tested negative for mayers test signifying absence of alkaloids.

The extract of bark in ethyl acetate and methanol tested positive for all the tests clearly symbolising presence of carbohydrates while the petroleum ether extract tested negative for fehlings test while chloroform extract tested negative for benedicts test indicating insolubility of carbohydrates in these solvents.

The extract of bark was then tested for proteins and amino acids. The tests applied were biurets test, ninhydrins test. The chloroform and methanol extract tested negative for all the tests while petroleum ether and ethyl acetate tested positive for all the tests.

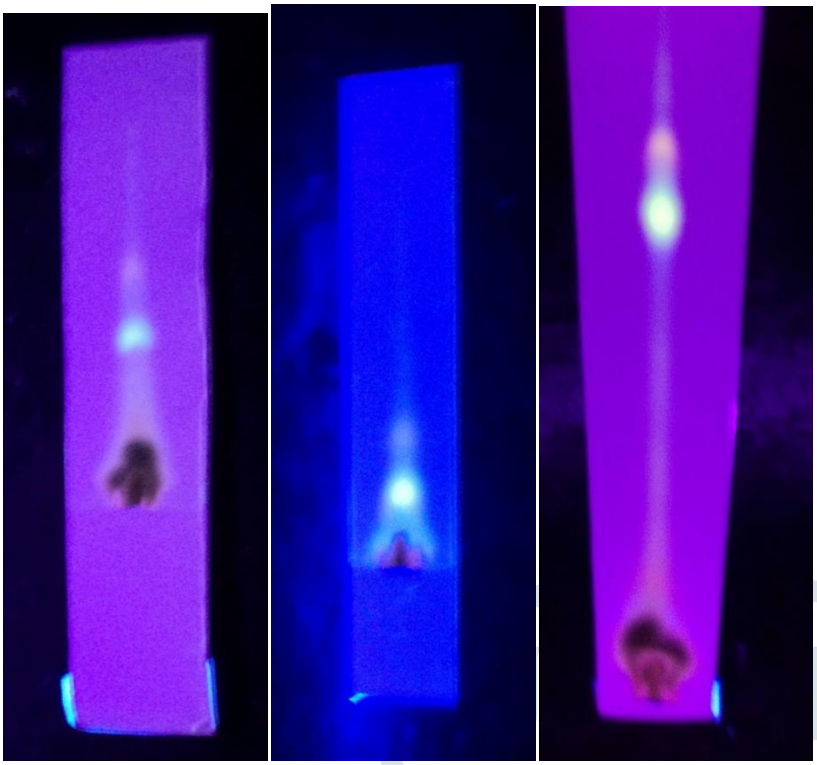
Then the bark extracts were tested for froths test (saponins). All the extracts tested negative while methanol extract tested positive only. All the extracts tested negative while only chloroform extract tested positive indicating the presence of saponins.

The bark extracts were tested for flavonoids. The tests applied to check presence of flavonoids were lead acetate test, alkaline reagent test, shinoda test. Petroleum ether extract tested negative for all the tests while ethyl acetate and chloroform extract tested negative for lead acetate test and positive for the remaining. The methanol extract tested negative for shinoda test indicating absence of flavonoids.

The barks were tested for Triterpenoids and steroids. The tests applied were salwoski's test and libberman's test. The extracts in all the four solvents tested positive for all the tests visualising presence of triterpenoids and steroids.

The bark extracts were tested for tannin and phenolic compounds. The tests applied were ferric chloride test, lead acetate test, dilute iodine solution test, gelatin test. All the solvent extracts tested negative for dilute iodine solution test while all tested positive for lead acetate test. The extracts of petroleum ether and ethyl acetate tested negative for ferric chloride test while positive for gelatin. The extracts of chloroform tested positive for ferric chloride test while tested negative for gelatin test. The test results were similar for dilute iodine solution test and lead acetate test. However for ferric chloride test only petroleum ether and ethyl acetate extract tested negative and positive for gelatin test indicating presence of tannins.

Thin layer chromatography

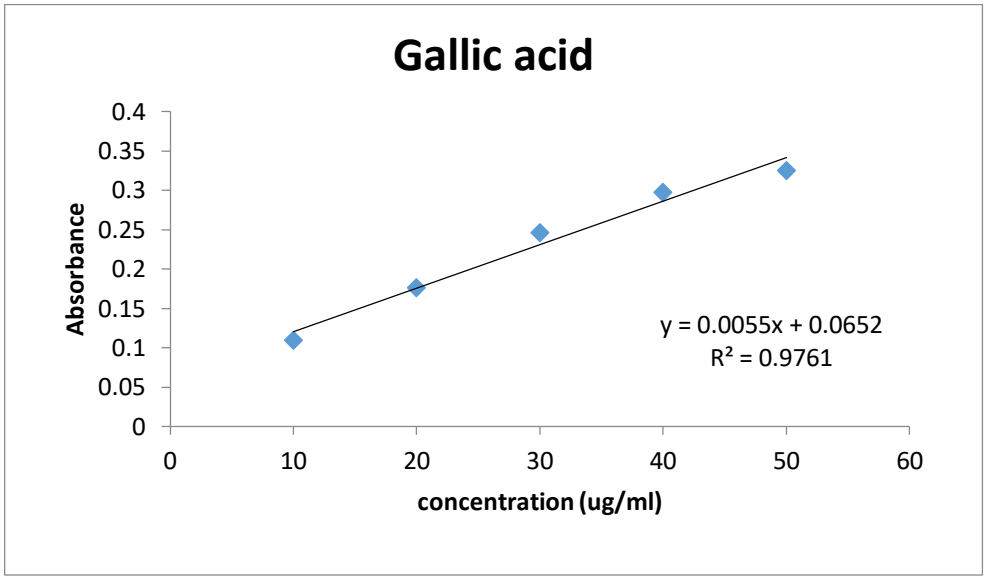


Ethyl acetate **chloroform** **methanol**

Total phenolic content

Standard curve of Gallic acid –

S No	Conc (µg/ml)	Absorbance
1	10	0.1098
2	20	0.1763
3	30	0.2468
4	40	0.2981
5	50	0.3258



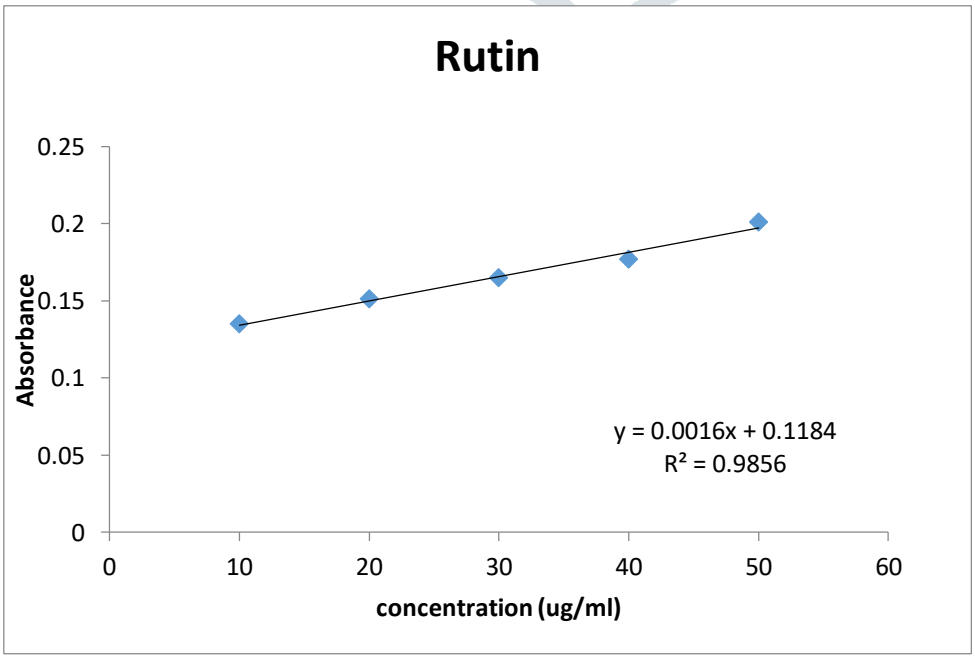
S. No.	Solvent used for extraction	Total phenolic content
1.	Ethyl acetate	177.00±0.529
2.	Chloroform	160.00±0.529
3.	Methanol	184.07±0.306

The total phenolic content was calculated in all the three extracts. The maximum content of phenol was found in the extract of methanol (184.07±0.306 mg/g) followed by ethyl acetate (177.00±0.529 mg/g) and chloroform (160.00±0.529 mg/g).

Total flavonoid content

Standard curve of Rutin –

S No	Conc (µg/ml)	Absorbance
1	10	0.135
2	20	0.151
3	30	0.165
4	40	0.177
5	50	0.201



S. No.	Solvent used for extraction	Total phenolic content (mg/g)
1.	Ethyl acetate	97.00±2.646
2.	Chloroform	45.67±2.082
3.	Methanol	156.67±4.041

The total flavonoid content was calculated by taking rutin as its reference. The maximum flavonoidal content was found in methanol (156.67±4.041 mg/g) and followed by ethyl acetate (97.00±2.646 mg/g) and chloroform (45.67±2.082mg/g).

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