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Estimation of Total Phenol, Tannin, Alkaloid and Flavonoid in *Hibiscus Tiliaceus* Linn. Wood Extracts.

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Research Article

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ABSTRACT

The present study was performed to evaluate the total phenol, tannin, alkaloid and flavonoid contents in petroleum ether, ethyl acetate and methanol extracts of *Hibiscus tiliaceus* wood. Extraction of powdered wood material was carried out by continuous hot percolation method in soxhlet apparatus using petroleum ether, ethyl acetate and methanol as solvents. Gallic acid was used as standard for the determination of total phenol and tannin by Folin-ciocalteu method. Total alkaloid content was determined by chloride colorimetric method using quercetin as a standard. The results showed that ethyl acetate extract has high concentration of total phenol, tannin, alkaloid and flavonoid contents as compared by bromocresol green solution using atropine as a standard. Total flavonoid content was determined by aluminium to petroleum ether, ethyl acetate and methanol extracts. Ethyl acetate extract contained the total phenol of 30.18 and tannins of 83.03 as mg of gallic acid equivalents (GAE), alkaloids of 66.01 as mg of atropine equivalents (AE) and flavonoids of 91.01 as mg of quercetin equivalents (QE).

INTRODUCTION

Plants have provided mankind with herbal remedies for several diseases for many centuries. In India herbal medicines have been the bases of treatment and cure for various diseases in traditional methods such as Ayurveda, Unani and Sidha. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus the plant species may be consider as a biosynthetic and for the chemical compounds example proteins, carbohydrates, and fats that are utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties.[1]Natural products extracted from plants which belong to the Malvaceae family are used in the treatment of many diseases worldwide. One important genus in this family is Hibiscus spp., with more than 220 species distributed in tropical and subtropical regions.[2] *Hibiscus tiliaceus* L. is a typical plant of tropical climates found in the regions of mangroves in significant quantities.[3] An aqueous extract of wood and fresh flowers is a registered treatment for skin diseases.[4-5] Recently it was shown that methanolic flower extract exerts an antioxidant effect on the yeast *Saccharomyces cerevisiae*, protecting against hydrogen peroxide (H_2O_2) and tert-butylhydroperoxide (t-BHP) cytotoxicities. In addition, the extract was not mutagenic in *Salmonellatyphimurium* or *S. cerevisiae* and showed a significant antimutagenic action against oxidative mutagens in *S. cerevisiae*.[2]It is also reported traditionally, where the leaves are used to treat fevers and soothe coughs, the bark to treat dysentery, and the flowers aid in treating ear infections and abscesses.[6] Previous pharmacological investigations of the genus Hibiscus plants indicated the presence of species with useful biological activities. The studies conducted to date have demonstrated that plants of the

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Hibiscus genus have the potential to provide biologically active compounds that are able to deter the proliferation of malignant cells. Thus, the Hibiscus genus deserves additional evaluation as a provider of chemopreventive agents. Indeed, there is a current need for availability of new plant-derived bioactive molecules; for the development of new drugs and may provide a cost-effective mean of treating cancers and other diseases in the developing world.[7-8]

Present work was undertaken to screen to evaluate the total phenol, tannin, alkaloid and flavonoid contents in petroleum ether, ethyl acetate and methanol extracts of *Hibiscus tiliaceus* wood.

MATERIALS AND METHODS

Plant material

Hibiscus tiliaceus Linn. woodwas collected from Costal side of Ratnagiri district of Maharashtra and authenticated By Dr. J. Jayanthi, Scientist "C" for Director, Botanical Survey of India, Pune, India vide letter No. BSI/WRC/TECH/2010 Voucher No. HIBCA1VIT. The herbarium is kept at BSI, Pune for further reference.

Chemicals and reagents

Aluminium chloride, sodium carbonate, sodium nitrite, sodium hydroxide was obtained from Department of pharmaceutical chemistry, Pravara rural college of pharmacy, Pravaranagar. Folin-Ciocalteu's phenol reagent, aluminium chloride and standard samples of gallic acid, quercetin was obtained from Department of pharmacognosy, Pravara rural college of pharmacy, Pravaranagar.

Instrument used

Ultraviolet (UV) /Visible spectrophotometer

Preparation of extracts

For the present study, the extracts were obtained by continuous hot extraction method using soxhlet apparatus. Freshly collected wood part was cut into pieces to dry in shade and finely powdered. 250 gm of wood powder was passed through sieve no. 60 and packed in soxhlet apparatus and extracted using petroleum ether, ethyl acetate and methanol as solvents. The filtrate was concentrated in rotary evaporator and the extracts were calculated for their extractive value.

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric method. [9]Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.[10-12]

Determination of tannin Content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.[13-16]

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Determination of Alkaloid

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.[17-18]

Determination of Total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.[19-22]

RESULTS AND DISCUSSION

Petroleum ether, ethyl acetate and methanol extracts were prepared to examine thetotal phenolic content, tannin, alkaloidand flavonoid. The results for extractive valueand for total phenol, tannin, alkaloid, flavonoid contents are tabulated in Table 1 and 2, 3, 4, 5 respectively. Calibrationgraphs for total phenol, tannin, alkaloidand flavonoid contents were shown in figure 1, 2, 3 and 4respectively.

Table 1: Extractive values for different solvents used

Extract	Percentage yield
Petroleum ether	9.3
Ethyl acetate	3.5
Methanol	6.8

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.006x - 0.002$, $R^2 = 0.997$). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 2).The total phenolic contents in the examined extracts ranged from 11.90 to 30.18 mg GA/g. The highest concentration of phenols was measured in ethyl acetateextracts. Petroleum ether and methanol extracts contains smaller concentration of phenols. The total phenolic contents in plant extracts of the species *Hibiscus tiliaceus* depend on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.[23-24]

Table 2: Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GAE/g of extract)

Extract	mg of GAE/g of extract
Petroleum ether	11.90 ± 0.301[1]
Ethyl acetate	30.18±0.321
Methanol	23.09±0.095

[1]Each value is the average of three analyses ± standard deviation; Where GAE is gallic acid equivalent.

The tannins contents was examined in plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.057x - 0.071$, $R^2 = 0.997$). The values obtained for the concentration of tannin contents are expressed as mg of GA/g of extract.The highest concentration of tannins was measured 83.03in ethyl acetate extracts(Table 3).

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Table 3: Tannin contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GAE/g of extract)

Extract	mg of GAE/g of extract
Petroleum ether	44.26 ± 0.062[1]
Ethyl acetate	83.03±0.029
Methanol	55.04±0.019

[1]Each value is the average of three analyses ± standard deviation;Where GAE is gallic acid equivalent.

The alkaloid contents was examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/g of extract (the standard curve equation: y = 0.006x – 0.003, R^2 = 0.997). The highest concentration of alkaloid was measured 66.01in ethyl acetate extracts(Table 4).

Table 4: Alkaloid contents in the plant extractsexpressed in terms of atropine equivalent (mg of AE/g of extract)

Extract	mg of AE/g of extract
Petroleum ether	19.10 ± 0.013[1]
Ethyl acetate	66.01±0.049
Methanol	41.00±0.031

[1]Each value is the average of three analyses ± standard deviation, Where AEis atropine equivalent.

The concentration of flavonoids in various plant extracts of *Hibiscus tiliaceus*L. woodwas determined using spectrophotometric method with aluminum chloride. The content of flavonoidswas expressed in terms of quercetin equivalent (the standard curve equation: y = 0.009x– 0.006, R^2 = 0.999), mg of QE/g of extract (Table 5). Ethyl acetate extracts of wood contains the highest flavonoid concentration as 91.01 mg QE/g. The concentration offlavonoids in methanol extract was 66.08 mg QE/g. The lowest flavonoid concentration was measured in petroleumether extract. The concentration of flavonoids in plant extracts depends on thepolarity of solvents used in the extract preparation.[25]

Table 5: Concentrations of flavonoids in the plant extractsexpressed in terms of quercetin equivalent (mg of QE/g of extract)

Extract	mg of QE/g of extract
Petroleum ether	60.41 ± 0.012[1]
Ethyl acetate	91.01±0.046
Methanol	66.08±0.033

[1]Each value is the average of three analyses ± standard deviation; Where QE is quercetin equivalent respectively.

Figure 1: Calibration graph for Total Phenolic Content

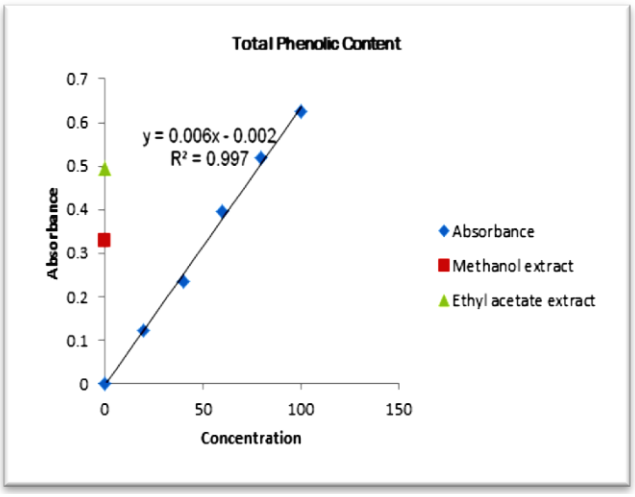

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Figure 2: Calibration graph for Tannin Content

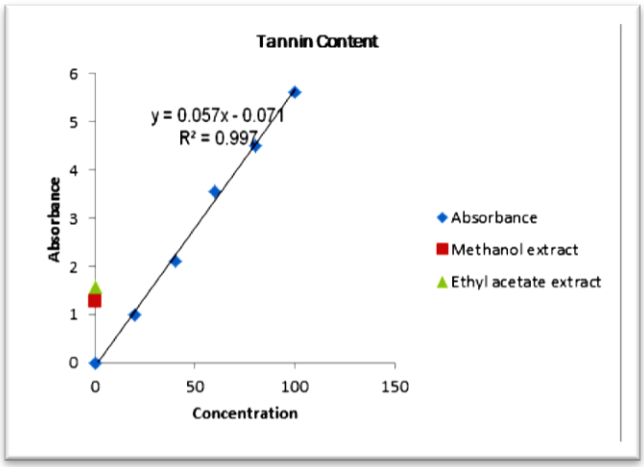


Figure 3: Calibration graph for total alkaloid content

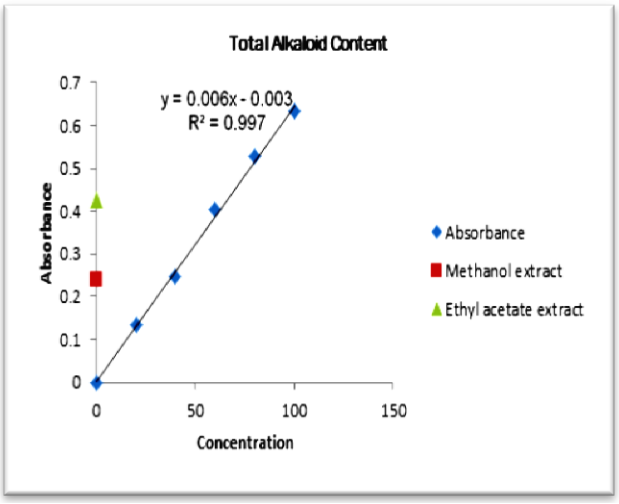
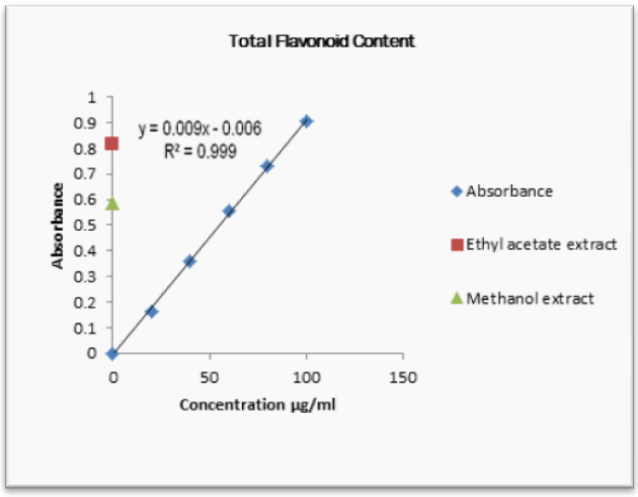


Figure 4: Calibration graph for Total flavonoid content



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All the calibration graphs showed that strong positive linear correlation (r) which is close to +1. These graphs indicate that as the value of concentration increases, values for absorbance also increase. Total phenol, tannin, alkaloid and flavonoid contents in ethyl acetate extract were found to be 30.18 mg of GAE/gm, 83.03 mg of GAE /gm,66.01 mg of AE/gm,and 91.01 mg of QE/gm of extract respectively. Petroleum ether and methanolic extracts were made known less content of total phenol, tannins, alkaloid and flavonoid.

CONCLUSION

In this study, total phenol, tannin, alkaloid and flavonoid contents from petroleum ether, ethyl acetate and methanol extracts of *Hibiscus tiliaceus* L. woodwere investigated. Ethyl acetate extract showed high contents of phenol, alkaloid, flavonoid and tannin as compared to petroleum ether and methanolic extract. The pharmacological action of ethyl acetate extract of *Hibiscus tiliaceus* L. woodwill be determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects.

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