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Research Article

Comparative Phytochemical Screening and Estimation of Bioactive Constituents of Leaves of *Lagerstroemia parviflora*, *Gardenia latifolia* and *Terminalia tomentosa*

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, saponin, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. *Gardenia latifolia* (*G. latifolia* Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of various ailments such as snake bite, skin diseases, stomach pains, caries in humans and ephemerical fever in live stocks. *Terminalia tomentosa* (*T. tomentosa* Combretaceae) occurs frequently in Indonesia, Malaysia, China and India as wasteland weed and also found in most parts of the world with a warm climate in dry, sandy and alkaline soils. The powdered leaves are useful for fast healing of wounds, as purgative, to treat liver problems, to promote sexual health, to relieve stomach ache, headache, also applied in sprain to ease swelling and pain. In Indian Ayurvedic system, *Lagerstroemia parviflora* (*L. parviflora*, Lythraceae) are well-known plants used for major and minor ailments. The aim of the present study is to examine leaf of *G. latifolia*, *T. tomentosa* and *L. parviflora* for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folin's Ciocalteu reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The present study concluded that the crude extract of *G. latifolia*, *T. tomentosa* and *L. parviflora* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Gardenia latifolia*, *Terminalia tomentosa*, *Lagerstroemia parviflora*, Phytochemical, Folin's ciocalteu reagent**Article Info:** Received 21 June 2019; Review Completed 17 Aug 2019; Accepted 28 Aug 2019; Available online 30 Aug 2019

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INTRODUCTION

Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects¹. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. Large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care². Medicinal plants are plants in which one or more of their organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. WHO consultative group that formulated this definition stated also that, such a description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to a

thorough scientific study³. Such plants should be investigated to better understand their properties, safety and efficacy. The medicinal properties of plants are due to some chemical constituents that produce certain pharmacological action on the humans. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques⁴. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available⁵. *G. latifolia* (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree or large shrub that occurs throughout the greater parts of Indian common in deciduous forests along the streams. Root used as a remedy for indigestion in children. Fruits used in affections of the mammary glands. Pounded pulp is

applied to forehead in fever. Stem and fruit used for stomach pain. Fruit extract is used in treating snake bite, sores of hand and feet, stomach ache and wounds. To treat caries, stem bark crushed and boiled in water is applied to affected areas. Bark is used in skin diseases. The bark and wood gave beta sesterol, hederegenin, Me-esters of oleanic and gypsogenic acids. Root gave gardenins. Saponins from bark decreased formation of histamine and may find use in asthma (market drug is expectorant and weak spasmolytic, but was not found effective in asthma). The stem bark contains hederagenin, D-mannitol, sitosterol and siarresinolic, episarresinolic, oleanolic and spinosic acid^{6,7}. *L. lanceolata* Wall. (Lythraceae) is a moderate to large deciduous tree, sometimes attaining 30 metres in height and 2.4 to 3.0 metres in girth with a clean cylindrical bole of 12 to 15 metres. It is found from Bombay to Kerala and in the hills of Deccan Peninsula upto an altitude of 1,200 metres. Bark is smooth, greenish or yellowish white, exfoliating in papery strips; leaves elliptic – lanceolate or broadly ovate, 6.2 to 10.0 cm x 1.8 to 5.0 cm, coriaceous, glabrous, shining above, usually white or grayish blue; flowers small, white, in large panicles; capsules ellipsoid; seeds winged⁸. *L. lanceolata* has been used in the treatment of asthma, diabetes mellitus, chronic bronchitis, cold and cough. Seeds have been documented for its multiple pharmacological activities including narcotic principle. Steroid, terpenoids, phenols, flavonoids, alkaloids, ellagic acid & tannins are the major components present in the plant⁹. Mazumder et al. (2003)¹⁰ reported the antibacterial activities of the leaves of the plant and Bhakuni et al. (1969)¹¹ reported the antiasthmatic activity of the flowers of *L. parviflora*. The leaf juice of this plant is used in traditional medicine to treat fever in Jharkhand, India¹². *T. tomentosa* (Combretaceae) is a large tree found in deciduous forests and extensively disseminated in south East Asian countries including India and Burma. Many plants of the genus *Terminalia* have been reported to possess medicinal values such as antidiabetic, cardioprotective, anti-inflammatory and antioxidant¹³⁻¹⁶. The common polyphenolic compounds reported in many *Terminalia* species are: Ellagic acid, Dimethyl ellagic acid, Pentamethyl flavellagic acid, Trimethyl flavellagic acid and β -sitosterol¹⁵. Although, *T. tomentosa* bark is used in Indian traditional and folklore medicine for wound healing, GI disorders and anti-inflammatory purposes¹⁷, it lacks adequate scientific evidences. Ramachandra Row and Subbarao (1962) reported the chemistry and constituents of *T. tomentosa*¹⁸. They isolated β -sitosterol, oleanolic acid, arjunolic acid, barringtonenol and tomentosic acid. Anjum Ghalaut et al. (2013)¹⁹ developed and optimized a convenient, high throughput, and reliable UPLC-QTOFMS method to analyze crude water extract from *T. tomentosa*. They identified the presence of 5-Aminovaleric acid, Zeatin riboside, Thymine, 4-Methoxy cinnamic acid, Niacinamide, (-) Epigallocatechin, Indole-3-aldehyde, Resveratrol, Chlorogenic acid, (+)-Epicatechin, Quercetin-3-O-rhamnopyranoside, Quercetin and Kynurenic acid. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds of leaf of *G. latifolia*, *T. tomentosa* and *L. parviflora*.

MATERIALS AND METHODS

Plant material

The leaves of *G. latifolia*, *T. tomentosa* and *L. parviflora* were collected from ruler area of Bhopal (M.P.) in the month of Feb, 2018. The leaves plant sample were separated and

washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form. The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction

Dried powdered leaf of *G. latifolia*, *T. tomentosa* and *L. parviflora* were successive extracted with various solvent (chloroform, ethyl acetate, methanol and aqueous) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts²⁰.

Qualitative phytochemical analysis of plant extract

The *G. latifolia*, *T. tomentosa* and *L. parviflora* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate^{21,22}. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al²³. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al²³. 1ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

Table 1 Results of percentage yield of leaf extracts

Plant Name	Percentage yield (%)			
	Chloroform	Ethyl acetate	Methanol	Water
<i>G. latifolia</i>	12.4	10.5	10.87	14
<i>T. tomentosa</i>	0.9	1.1	8.71	7.3
<i>L. parviflora</i>	1.8	3.5	8.21	9.6

The results of qualitative phytochemical analysis of the crude powder of leaf of *G. latifolia*, *T. tomentosa* and *L. parviflora* were shown in Table 2-4. Methanolic extracts of *G.*

latifolia, *T. tomentosa* and *L. parviflora* showed the presence of glycosides, flavonoids, phenols, saponins, tannin and carbohydrate.

Table 2 Result of phytochemical screening of extracts of *L. parviflora*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	-Ve -Ve	-Ve -Ve	-Ve +Ve	-Ve -Ve
2.	Glycosides A) Legal's Test:	-Ve	-Ve	+Ve	+Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	-Ve +Ve	+Ve +Ve	+Ve +Ve	+Ve +Ve
4.	Saponins A) Froth Test:	-Ve	-Ve	+Ve	-Ve
5.	Phenolics A) Ferric Chloride Test:	-Ve	-Ve	+Ve	+Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	-Ve	-Ve	-Ve	-Ve
7.	Carbohydrate A) Fehling's Test:	-Ve	-Ve	+Ve	-Ve
8.	Diterpenes A) Copper acetate Test:	-Ve	-Ve	-Ve	-Ve
9.	Tannin Gelatin test:	-Ve	-Ve	+Ve	+Ve

Table 3 Result of phytochemical screening of extracts of *G. latifolia*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	-Ve +Ve	-Ve -Ve	-Ve -Ve	-Ve -Ve
2.	Glycosides A) Legal's Test:	-Ve	-Ve	+Ve	-Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	+Ve -Ve	+Ve +Ve	+Ve +Ve	+Ve +Ve
4.	Saponins A) Froth Test:	-Ve	-Ve	+Ve	+Ve
5.	Phenolics A) Ferric Chloride Test:	-Ve	-Ve	+Ve	-Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	+Ve	-Ve	+Ve	-Ve
7.	Carbohydrate A) Fehling's Test:	-Ve	-Ve	+Ve	-Ve
8.	Diterpenes A) Copper acetate Test:	-Ve	+Ve	+Ve	+Ve
9.	Tannin Gelatin test:	-Ve	-Ve	-Ve	-Ve

Table 4 Result of phytochemical screening of extracts of *T. tomentosa*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	-Ve -Ve	-Ve -Ve	-Ve -Ve	-Ve -Ve
2.	Glycosides A) Legal's Test:	-Ve	-Ve	+Ve	-Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	-Ve -Ve	-Ve -Ve	+Ve +Ve	+Ve +Ve
4.	Saponins A) Froth Test:	-Ve	-Ve	+Ve	-Ve
5.	Phenolics A) Ferric Chloride Test:	-Ve	-Ve	+Ve	-Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	-Ve	-Ve	-Ve	-Ve
7.	Carbohydrate A) Fehling's Test:	-Ve	-Ve	+Ve	-Ve
8.	Diterpenes A) Copper acetate Test:	-Ve	-Ve	-Ve	-Ve
9.	Tannin Gelatin test:	-Ve	+Ve	+Ve	+Ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC of methanolic extract of *G. latifolia*, *T. tomentosa* and *L. parviflora* showed the content

values of 3.150, 3.860 and 4.900 respectively. The total flavonoid content of *G. latifolia*, *T. tomentosa* and *L. parviflora* methanolic extract showed the content values of 2.492, 3.928 and 3.685 respectively. Results are provided in (Table 5-7 and Fig. 1, 2).

Table 5 Estimation of total phenolic and flavonoids content of *L. parviflora*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Chloroform	-	4.428
2.	Ethyl Acetate	-	4.342
3.	Methanol	4.900	3.685
4.	Aqueous	5.010	4.257

Table 6 Estimation of total phenolic and flavonoids content of *G. latifolia*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Chloroform	-	4.350
2.	Ethyl Acetate	-	4.428
3.	Methanol	3.150	2.492
4.	Aqueous	-	2.542

Table 7 Estimation of total phenolic and flavonoids content of *T. tomentosa*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Methanol	3.860	3.928
2.	Aqueous	-	1.521

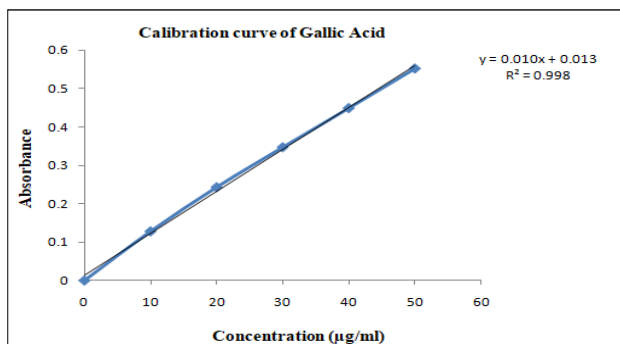


Figure 1 Graph of estimation of total phenolic content

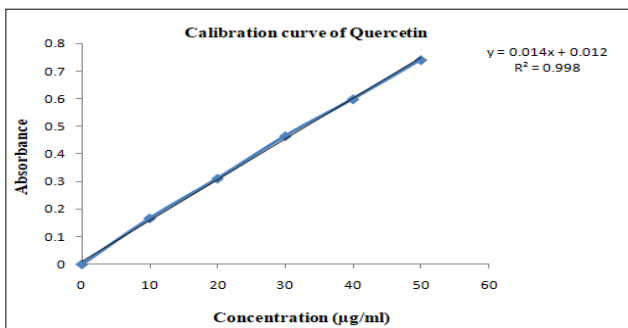


Figure 2 Graph of estimation of total flavonoid content

CONCLUSION

Qualitative and quantitative analysis of phenolics and flavonoids from leaves extract of *G. latifolia*, *T. tomentosa* and *L. parviflora* was achieved first time in this work. The observed level of phytoconstituents revealed that *G. latifolia*, *T. tomentosa* and *L. parviflora* is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

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