Asian Journal of Chemistry; Vol. 33, No. 11 (2021), 2641-2644

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23363

GC-MS Analysis, Phytochemical Screening and Biological Activity of *Bauhinia tomentosa* (Linn.)

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Received: 10 June 2021; *Accepted*: 30 July 2021; *Published online*: 20 October 2021; AJC-20542

Present study aimed to evaluate the chemical composition and biological activity for methanolic extract of *Bauhinia tomentosa* (Linn.) leaves grown in Western Ghats region of South India. The preliminary phytochemical screening tests revealed the presence of steroids, alkaloids, terpenoids, flavonoids, glycosides and phenolic compounds in the leaf extract. A total of 19 compounds were identified through gas chromatography-mass spectroscopy (GC-MS) analysis of methanolic extract of *B. tomentosa*. The major compounds identified were phytol (23.96%), *n*-hexadecanoic acid (11.62%), squalene (8.85%) and the minor compounds are *trans-bis*(2-methylpropyl)-4,6-dioxane(0.13%), dihydro-*cis*-α-copaene-8-ol (0.14%), tetradecanoic acid (0.81%), respectively. Antibacterial activity of the extract showed the zone of inhibition 18 mm at 200 µg/mL against *S. aureus*, followed by 15 and 16 mm against *S. anginosus*, *K. pneumoniae* at 200 µg/mL, respectively. Antioxidant activity of methanolic extract of *B. tomentosa* leaves showed the maximum IC_{50} value with 75.07 % of scavenging activity at the concentration of 5 µg/mL.

Keywords: *Bauhinia tomentosa*, GC-MS, Antibacterial activity, Antioxidant activity.

INTRODUCTION

Plant based drugs are proven to be less toxic and their side effects are relatively low compared to synthetic drugs. Phytochemicals with antimicrobial properties are medical hubs to identify new medicines [1]. Oxidative stress due to the accumulation of free radicals can be nullified by the antioxidant activity of plant phenolics [2]. *Bauhinia* belongs to the family Fabaceae is distributed throughout Africa, Asia and South America [3]. Special cow's hoof-like lobed leaves and showy flowers are characteristics of the genus [4]. Species of *Bauhinia* has traditional claims for the treatment of various types of ailments, *e.g*. diabetes, liver/abdominal troubles, skin infections, inflammation, wounds and diarrhoea [5]. Species among *Bauhinia* are identified based on the leaf features, number of protruding midribs, trichome style and vein termination arrangements [6].

Bauhinia tomentosa is a multiple stemmed small tree with many slender twigs. It is known as yellow bell orchid and commonly called as Mandaarai in Tamil and Phalgu in Sanskrit. The plant reaches up to 12 feet height with smooth hairy textured barks. The flowers have yellow petals and exhibit a dark maroon marking in the throat. *B. tomentosa* leaves possess antidiabetic, anticancer, antiobesity, antihyperglycemic and antihyperlipidemic activities [7-9]. Flowers and leaf buds were used to treat dysentery and microbial infections [10]. Phytochemicals from the plant were reported to have therapeutic values [11]. Nanoparticles prepared from this plant showed antibacterial activities *in vitro* [12]. Administration of *B. tomentosa* in rats had increased the antioxidant enzymes and potential role in cisplatin induced nephrotoxicity [13]. The plant was also explored for the regulation of antioxidant and inflammatory mediators by Kannan & Guruvayoorappan [14] and stimulated immune response [15]. This study reports the chemical composition, antibacterial and antioxidant activities of methanolic extract of *Bauhinia tomentosa* leaves collected from Western Ghats region of South India

EXPERIMENTAL

Plant material: Fresh leaves of *Bauhinia tomentosa* (1 kg) were collected from the area near Udumalpet, South India (10º35′12.96″ N, longitude: 77º14′37.37″ E) between the periods of September to November 2019. The plant material

was identified and authenticated by a Botanist and the herbarium was deposited in Department of Botany, NGM College, Pollachi, India.

Extraction process: The collected fresh leaves of *Bauhinia tomentosa* was washed with tap water to remove the external contaminants from the plant. Leaves of the plant were shade dried and then coarsely powdered. The powered material was extracted with 80% methanol in Soxhlet apparatus for three day and the methanol extract was collected and filtered, evaporated to dryness for further studies.

Preliminary phytochemical analysis: The methanol extracts of *Bauhinia tomentosa* was qualitatively analyzed to identify the nature of phytochemical constituents present in the leaves [16,17].

GC-MS analysis: GC-MS analysis of leaves of *Bauhinia tomentosa* methanolic extract was carried out using Thermo GC-Trace ultra-version: 5.0 coupled with thermo MS DSQ II INSTRUMENT. Compounds were separated on DB-35, MS capillary standard non-polar column (30 × 0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the initial oven temperature was set at 70 ºC and held for 2 min. Later the oven temperature was raised to 260 ºC for 10 min, which was further raised 6 ºC per min with a final temperature of 300 ºC for 10 min. The sample of 100 µL was dissolved in 1 mL of methanol and injected with split less mode. Mass spectra were recorded with electron impact energy 70 eV and the temperature was set at 230 and 280 ºC, respectively.

Identification of chemical constituents: From GC-MS analysis, the obtained peaks were identified by comparing their mass spectra with those of National Institute of Science and Technology (NIST) mass spectral library. Comparison was done further by matching the retention time with those of authentic compounds.

Antibacterial activity: Whatman No: 1 filter paper was taken and punched in to small circular discs (6 mm dia) and then wrapped in aluminium foil and sterilized using an autoclave. Muller Hinton agar (MHA) was prepared, autoclaved, poured into the sterile petri dishes. After solidification, test organisms were subjected to preliminary screening in order to determine their antimicrobial activity against the extracts using disc-diffusion method. The sterilized filter paper discs were treated with the extracts under laminar air flowchamber. The indicator strains (50-200 µL) of human pathogens was spread plated over the petri plate to form a lawn on the agar. The extract treated discs was placed in their respective place in the petri plates using sterile forceps under aseptic conditions and the plates were incubated for 24-48 h at 37 ºC. After 24 h of incubation the plates were observed for activity against the bacterial pathogens. The zone of growth inhibition was measured in millimeter for those extract which showed activity.

***in vitro* antioxidant activity:** Free radical scavenging activity of different extracts was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 mL) was added to 3 mL of different extracts at different concentration (5, 10 and 15 µg/mL, respectively). The mixture was shaken vigorously and allowed to stand at room temp for 0.5 h. Finally, the absorbance was measured at 517 nm by using UV-vis spectrophotometer (Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of control reaction and A_1 is the absorbance in presence of test or standard sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Janaki *et al* [18] reported the preliminary phytochemical screening of *Bauhinia tomentosa* leaves of methanolic extract showed the presence of saponin, alkaloids, flavonoids, steroids, phenols, coumarins and acids. In present study, the qualitative phytochemical screening of *B. tomentosa* leaves methanolic extract revealed the presence of alkaloids, phenols, flavonoids, tannins, steroids, terpenoids, carbohydrates, acids and saponins. Preliminary Phytochemicals present in methanolic extract of *B. tomentosa* leaves extract is given in Table-1.

TABLE-1
PRELIMINARY PHYTOCHEMICALS PRESENT IN THE METHANOLIC EXTRACT OF *Bauhinia tomentosa* LEAVES

Compounds	Methanolic extract of *Bauhinia tomentosa*
Alkaloids	+++
Phenols	+++
Flavonoids	++
Tannins	++
Steroids	+++
Terpenoids	+++
Carbohydrates	+++
Acids	+++
Saponins	++

+++ indicates higher quantity of compounds, ++ indicates medium quantity of compounds.

GC-MS analysis: Panda *et al*. [19] reported that *Bauhinia tomentosa* leaves contains the major compound was 3-*o*-methyl-D-glucose (91.89%) and the minor compound was phthalic acid, ethyl pentyl ester, 2-butanone, 3-methoxy-3-methyl,2,2-dimethylpropionic acid, cyclopentyl ester, 2-hexen-1-ol, 2-ethyl, 5-hydroxy-2,2-dimethylhexan-3-one, pentanoic acid, 2-methyl and butane, 1-bromo-2-methyl were present in trace amount with lower peak areas less than 1%. Janaki *et al*. [18] reported the major compounds *viz*. isopropyl stearate (30.02%), 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosa-pentaene (24.77%), 1,3-pentanedione (10.91%) and the minor compounds are *Z*,*E*-2-methyl-3,13-octadecadien-1-ol (2.98%), methyl abietate (3.61%), respectively. In present GC-MS analysis of *B. tomentosa* leaves of methanolic extract showed the presence of 19 chemical constituents is shown in Table-2 and the GC-MS Chromatogram is shown in Fig. 1. In current result the presence of major compounds was phytol (23.96%), *n*-hexadecanoic acid (19.93%), squalene (11.62%), methyl

TABLE-2
CHEMICAL CONSTITUENTS PRESENT IN THE METHANOLIC EXTRACT OF *Bauhinia tomentosa* LEAVES

Compound name	Retention time (min)	Compound total (%)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	21.330	1.79
6,10,14-Trimethyl-2-pentadecanone	24.726	0.54
Methyl ester-hexadecanoic acid	25.883	8.85
Dihydro-*cis*-α-copaene-8-ol	26.822	0.14
trans-bis(2-Methylpropyl)-4,6-dioxane	27.548	0.13
Methyl stearate	29.146	1.51
Methyl ester-13-octadecenoicacid	29.385	1.56
(Z,Z)-Methyl ester-9,12-octadecadienoic acid	30.106	2.30
(Z,Z,Z)-Methyl ester-9,12,15-octadecatrienoic acid	31.015	2.96
Phytol	31.018	23.96
Tetradecanoic acid	32.412	0.81
7,9-Di-*tert*-butyl-1-oxaspiro-(4,5)-deca-6,9-diene-2,8-dione	33.504	0.88
n-Hexadecanoic acid	34.258	19.93
Squalene	35.450	11.62
Octadecanoic acid	35.902	7.56
2,2'-Bi[1,4,7,10,13-pentaoxacyclopentadecane	36.034	0.45
6-Octadecenoic acid	36.228	4.52
Isopropyl linoleate	36.852	5.20
15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	39.214	1.97

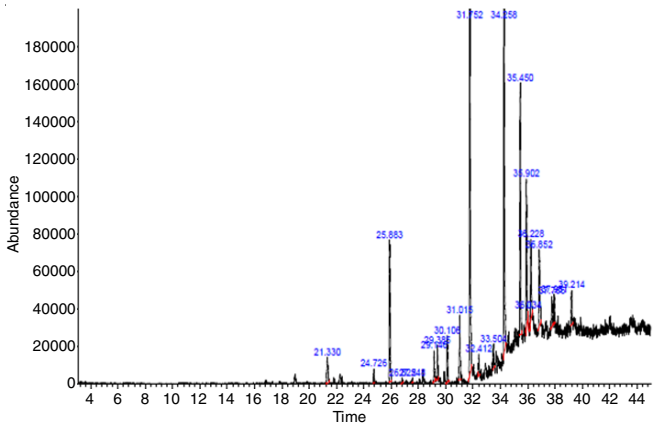

Fig. 1. GC-MS chromatogram of methanolic extract of *B. tomentosa* leaves

ester-hexadecanoic acid (8.85%), octadecanoic acid (7.56%), isopropyl linoleate (5.20%), 6-octadecenoic acid (4.52%) and the minor compounds are *trans-bis*(2-methylpropyl)-4,6-dioxane (0.13%), dihydro-*cis*-α-copaene-8-ol (0.14%), tetradecanoic acid (0.81%), 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.88%), methyl stearate (1.51%), methyl ester-13-octadecenoic acid (1.56%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.79%), 15,15′-bi-1,4,7,10,13-pentaoxacyclohexadecane (1.97%), (*Z,Z*)-methyl ester-9,12-octadecadienoic acid (2.30%), (*Z,Z,Z*)-methyl ester-9,12,15-octadecatrienoic acid (2.96%), respectively. This GC-MS spectrum entirely different from the previously reported GC-MS. Since, first time we reported the major compound phytol (23.96%) and minor compound *trans-bis*(2-methylpropyl)-4,6-dioxane (0.13%) along with squalene (11.62%), isopropyl linoleate (5.20%) and methyl stearate (1.51%) in the methanol extract of *B. tomentosa* leaves.

Antibacterial activity: The antibacterial assay was performed by agar disc diffusion method. The nutrient agar plates were inoculated with the bacterial strains *viz*. *S. aureus*, *S. anginosus* and *K. pneumonia*. The extract was dissolved in solvents at a concentration of 200, 150, 100 and 50 µg/mL. Solvents used for extraction served as control. Amoxicillin 10 µg/mL was used as standard for bacteria. All the petriplates with sampled disc for bacteria were incubated at 37 ºC for 24 h. The assessment of antibacterial activity was based on the diameter of inhibition zone formed and the zone was measured.

Earlier study [20] reported *in vitro* antibacterial activity of *B. tomentosa* aqueous extract showed the zone of inhibition of 14 mm, 13 mm against *S. aureus* and *K. pneumonia*, respectively. In present experimental work, the extract showed zone of inhibition 18 mm at 200 µg/mL against *S. aureus* bacteria strain. The antibacterial activity of *B. tomentosa* leaves of methanolic extract showed the zone of inhibition 15 mm, 16 mm at 200 µg/mL against *S. anginosus* and *K. pneumonia*. The antibacterial activity results of *B. tomentosa* leaves methanol extract is shown in Table-3.

TABLE-3
ANTIBACTERIAL ACTIVITY DATA OF METHANOLIC EXTRACT OF *B. tomentosa* LEAVES

Name of the organisms	Zone of inhibition (mm)			
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL
Staphylococcus aureus	10	11	13	18
Streptococcus anginosus	8	9	12	15
Klebsiella pneumonia	7	10	14	16

Antioxidant activity: The antioxidant activity of *B. tomentosa* leaves methanol extract exhibited 74.42% at 50 µg/mL concentration by using DPPH radical scavenging activity is reported by Rhama & Madhavan [20]. In present study, *in vitro* antioxidant activity of *B. tomentosa* leaves methanol extract was measured on the basis of DPPH radical scavenging activity. The methanolic extract of *B. tomentosa* leaves showed most potent antioxidant activity for the concentration of 5, 10 and 15 µg/mL was found to be 75.07%, 56.04% and 38.08%, respectively (Fig. 2).

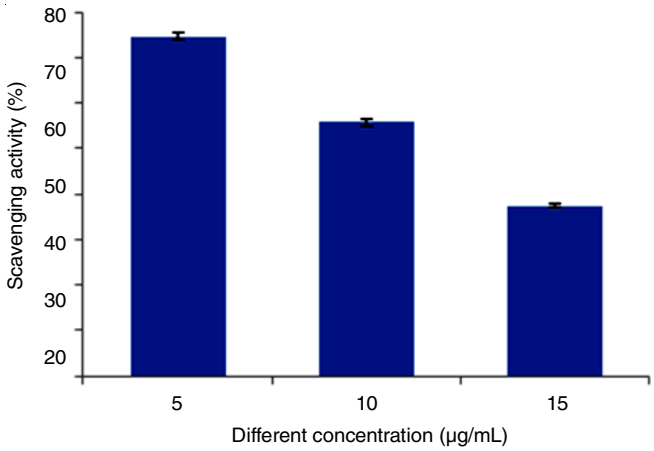

Fig. 2. Antioxidant activity of the *B. tomentosa* methanolic leaves extract

Conclusion

The present study revealed the methanolic extract of *Bauhinia tomentosa* leaves showed the presence of three chemical compounds along with major and minor compounds which is not reported previously. The reason is attributed due to the climatic conditions and soil nature of the area. This plant exhibits some moderate antibacterial and antioxidant activity is due to the presence of phytol as a major compound along with some other chemical constituents.

ACKNOWLEDGEMENTS

The authors are thankful to SITRA, Coimbatore and NGM College, Pollachi for providing the useful information during our study like GC-MS analysis and biological studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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