INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *BOMBAX CEIBA* BARK

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Article Received on: 16/04/18 Approved for publication: 09/05/18

DOI: 10.7897/2230-8407.09463

ABSTRACT

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently it will lead to drug discovery and development. The present effort has been intended to investigate the phytochemical constituents, quantify the total phenolic and flavonoid contents and to determine the possible bioactive components of methanolic bark extract of *Bombax Ceiba* using GC-MS analysis. Phytochemical screening of bark extract revealed the presence of around 30 compounds such as alkaloid, carbohydrates, tannins, steroids, flavonoids, glycosides and phenolic compounds. Amongst these it was found to contain the highest phenolic content and flavonoids content. The compounds were identified by comparing their retention time, peak area and by interpretation of mass spectra and matched with the National Institute of Standards and Technology (NIST) library. The *Bombax ceiba shows it* is an excellent source of phytocomponents, which are medicinal important to become a potential drug in future perspective. Isolation of individual components would however, help to find new drugs.

KEYWORDS: *Bombax Ceiba*, phytochemical, GC-MS analysis, NIST library.

INTRUDUCTION

Medicinal plants are playing a vital role on the health and healing of several diseases since the down of human civilization[1]. Bombax ceiba is commonly known as silk cotton tree which belongs to the family of Bombacaeae. It is one of the important medicinal plants in tropical and subtropical region in Asia especially in India, Sri Lanka, Pakistan, Malaysia, Myanmar and in Bangladesh. It has number of traditional and medicinal uses in the traditional system of medicine such as Ayurveda, Siddha and Unani[2]. The plant is commonly used for the treatment of diarrhea, fever, chronic inflammation, catarrhal affection and ulceration of the bladder and kidney in traditional systems of medicine[3]. Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design[4]. The herbs are constantly being screened for their biological and pharmacological activities such as anti-diabetic, anti-oxidant, anti-microbial, laxative, and anti-cancer activities[5-9] . Gas-Chromatography-Mass Spectrometry (GC-MS) is a helpful technique for reliable profiling of secondary metabolites[10-11]. So, the present study was aimed to investigate the possible phytochemical components by preparing the methanolic extract and profiling of phytochemicals for subjecting it to GC-MS analysis.

MATERIALS AND METHOD

Chemicals

Rutin and catechol were purchased from Sigma-Aldrich, Bangalore. Folin- Ciocalteu reagent was obtained from Merck, Bangalore. All other chemicals and solvents used in the study were of analytical grade.

Collection of Plant Material

The plant material *Bombax ceiba* bark was collected in June 2016 from Beldal Region of Bidar District Karnataka and authenticated by Dept of botany, Gulbarga University, Kalaburagi. Its voucher specimen number is HGUG-B58. The bark was washed thoroughly with water to remove dust and dried under the shade at room temperature for 20 days. The dried bark was ground using kitchen blender to obtain the course powder and kept in an air tight container till further use.

Preparation of Extracts

The air dried bark powder (100g) was successively extracted by Hot Soxhlet extraction with polar solvent like methanol. The extract was heated at 40ºC till the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for future use.

Phytochemical Screening of Bark Extract

The freshly prepared crude methanolic extract of bark was subjected to qualitative chemical tests to identify various classes of bioactive chemical constituents present in the bark extract using standard procedure[12].

Determination of Total Phenols by Folin-Ciocalteu Reagent Method

Known amount of samples were pipetted out in series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteau reagent (0.5ml) was added to each tube and incubated for 3 min. at room temperature Sodium carbonate (20%; 2ml)

solution was added, mixed thoroughly and the tubes were incubated for 1 min. in boiling water bath.

Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenolic -catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as mg of catechol equivalent[13].

Estimation of Total Flavonoid Content (Tfc) By Aluminium Chloride Colorimetric Method

Known volume of samples were pipetted out in series of test tubes and volume was made up to 0.5 ml with distilled water. Sodium nitrite (5%; 0.03ml) was added to each tube and incubated for 5 min at room temperature. Aluminium chloride solution (10%; 0.06ml) solution was added and incubated for 5 min at room temperature. Sodium Hydroxide solution (1 M; 0.2ml) solution was added and total volume was made up to 1 ml with distilled water.

Absorbance was measured at 510nm against a reagent blank. Standard curve using different concentrations of rutin was prepared. From the standard curve, concentration of flavonoids in the test samples was determined and expressed as mg of rutin equivalent[14].

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

The phytochemical investigation of methanol extract of *Bombax ceiba bark* was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo TSQ 8000 Gas Chromatograph - Mass Spectrometer. The MS part consists of Triple Quadrupole, This mass spectrometer comes paired with the Thermo GC-TRACE 1300. Experimental conditions of GC-MS system are as follows: DB 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40 °C raised to 290 °C at 5 °C/min and injection volume was 1.0 µl. A scan interval of 0.5 seconds with scan range of 40-600 m/z. Total GC running time was 30.09min and the results were compared by using NIST library search programme[15].

RESULTS

Preliminary phytochemical analysis was showed the presence of major classes of secondary metabolites such as Alkaloids, Carbohydrates, Tannins, Steroids, Saponins, Flavonoids, Glycosides, Phenols, Amino acids and proteins present in the extract as shown in Table-1

Total Phenolic Content

Based on the result obtained from phytochemical screening, the methanol extract is further subjected to quantitative estimation of phenols and flavonoids. phenolic –catechol was used as a standard compound and the total phenols were expressed as mg of catechol equivalent using the standard curve equation: y = 0.16x -0.155, R2= 0.999, Where y is absorbance at 650 nm and x is total phenolic content in the methanol and expressed in mg/ml as shown in Figure-1.The maximum phenolic content was found to be 243.75 µg/ml in the methanol extract.

Total Flavonoid Content

The TFCs of the methanol crude extracts is expressed in terms of rutin and is presented in Figure-2. The TFCs were calculated using the following linear regression equation obtained from the standard plot of rutin: y= 0.009x+0.003, $r2$= 0.998 Where y is absorbance and x is the amount of rutin in µg. maximum amount of flavonoid content was found in methanol extract is 2204.4 µg/ml. From the standard curve, concentration of flavonoids in the test samples were determined and expressed as mg of rutin equivalent.

Identification of Phytochemical Components Present in the Methanolic Extract of *Bombax ceiba* Bark

GC-MS of the Methanolic extract of *Bombax ceiba bark* is presented in table. 2. Mass spectra of the Methanolic extracts of the bark are depicted in Fig. 3. The fragmentation patterns of the mass spectra was compared with those of the known compounds stored in the National Institute of Standards and Technology (NIST) research library. In the GC-MS analysis, Total 30 active components were detected. The identification of phytochemical compounds was based on peak area, molecular weight and molecular formula. The compounds detected were 1H-Cyclopropa[3,4]benz[1,2e]azulene5, Olean12-ene3, hexamethyl Olean12ene3, Morphinan, Astaxanthin, Digoxigenin, Dodecanoic acid, Butanoic acid, Glycine etc.

DISCUSSION

The results of preliminary phytochemical testing confirmed the presence of various classes of bioactive chemical constituents in the methanolic extract of *Bombax ceiba* bark including polyphenols (tannins and flavonoids), steroids, alkaloid, carbohydrate and glycosides. many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities[16]. Numerous reports available on phenolic compounds have demonstrated their usefulness in exhibiting potential biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer[17] *etc*. Flavonoids and tannins are considered to be the most promising polyphenolic compounds among plant secondary metabolites[18]. Therefore, based on the phytochemical screening results, the total phenolic and flavonoid contents of methanolic extract of *Bombax ceiba bark* was estimated. Methanol, the most polar extract, was found to contain the highest content of total phenol (243.75 µg/ml) and flavonoid (2204.4 µg/ml). Based on the results of total phenol and flavonoid content in the bark of *Bombax ceiba*, it can be proposed that biological activity of this species could be due to the presence of flavonoids and other phenolics in it. A total of 30 phyto chemical compounds, belonging to hydrocarbons, esters, alcohols, fatty acids, ketones, *etc*. were identified and characterized from methanol extracts through GC-MS analysis. These compounds have previously been isolated from other medicinal plant species and were believed to play an important role in plant defence system[19].

Table-1. Phytochemical screening of Methanolic Extract of *Bombax ceiba*

Phytochemicals	Methanolic Extract
Alkaloids	Present
Carbohydrates	Present
Tannins	Present
Steroids	Present
Saponins	Present
Flavonoids	Present
Glycosides	Present
Phenols	Present
Amino acids and Proteins	Present

Table.2. Phytochemical components present in the methanolic extract of *Bombax ceiba* bark

S.N	RT	Compound Name	Molecular Formula	Area %
1	8.25	3[(acetyloxy)methyl]1a,1b,4,4a,5,7a,8,9octahydro 1,1,6,8tetramethyl,5,9,9atriacetate	C28H38O9	3.25
2	8.76	hexamethyl Olean12ene3,16,21,22,23,28hexol,	C18H30O2	4.94
3	8.86	Olean12ene3,16,21,22,23,28hexol,	C30H50O6	2.22
4	9.04	1H-Cyclopropa[3,4]benz[1,2e]azulene5,7b,9,9a-tetrol	C24H34O7	7.31
5	9.25	4'Apoá,.psi.carotenoic acid	C35H46O2	2.33
6	9.47	Olean12ene3,16,21,22,28pentol,21(2methyl2butenoate),	C35H56O6	6.20
7	9.98	Oleic acid, eicosyl ester	C38H74O2	1.85
8	12.56	8,8abis(acetyloxy)2a[(acetyloxy)methyl] 6btrihydroxy1,1,5,7tetramethyl	C26H34O11	1.52
9	13.95	12-olide 2-[d-Lyxo-d-manno-octahydroxyoctyl] Benzimidazole	C15H22N2O8	0.35
10	14.63	Glycine	C36H69NO6Si3	0.86
11	18.65	9,12,15Octadecatrienoicacid	C27H52O4Si2	1.46
12	18.80	Oleic acid, eicosyl ester	C38H74O2	0.54
13	18.96	Olean12ene3,16,21,22,28pentol,	C35H56O6	0.65
14	19.71	3[(acetyloxy)methyl]1a,1b,4,4a,5,7a,8,9octahydro 1,1,6,8tetramethyl, 5,9,9atriacetate	C28H38O9	0.41
15	21.43	13,14boctadecahydro2Hpicene4acarboxylicacid,	C33H52O5	0.53
16	21.76	1,1,6,8tetramethyl,9,9a-diacetate,	C24H34O7	0.46
17	22.02	8,9octahydro3(hydroxymethyl)1,1,6,8tetramethyl	C24H34O7	1.28
18	23.69	Oleic acid, eicosyl ester	C38H74O2	0.69
19	23.97	Morphinan	C22H33NO3Si2	2.55
20	25.90	Glycine,	C26H43NO5	1.77
21	28.03	Butanoic acid,	C24H32O6	0.95
22	28.16	Astaxanthin	C40H52O4	1.63
23	28.37	Digoxigenin	C23H34O5	1.03
24	28.84	9Octadecenoicacid, 2(octadecyloxy)ethyl ester	C38H74O3	0.77
25	28.94	Dodecanoic acid	C32H48O6	1.25
26	29.52	4Hexyl1(7methoxycarbonylheptyl)bicyclo	C25H40O2	0.43
27	29.77	1,4,10,13Tetraoxa7,16diazacyclooctadecane,	C32H62N2O6	1.02
28	32.60	L-Lysine,	C53H72N8O9	0.67
29	32.82	Astaxanthin	C40H52O4	1.96
30	33.04	3,11dihydroxy17,21bis[(trimethylsilyl)oxy],Omethyloxime	C28H53NO5Si2	0.48

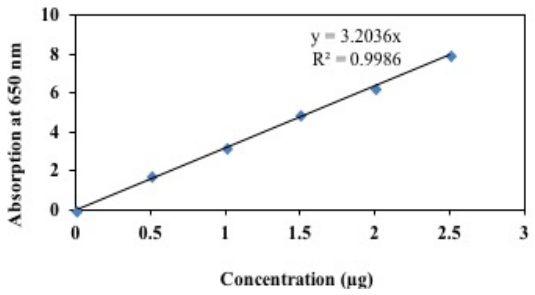

Figure-1. Standard calibration curve of catechol for the determination of total phenolic content

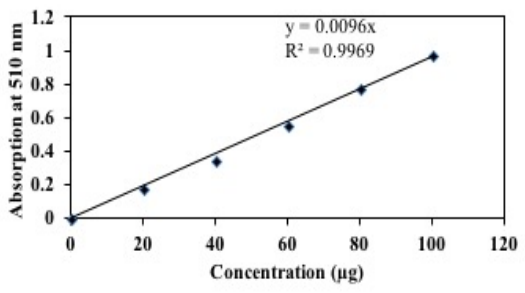

Figure 2. Standard calibration curve of rutin for the determination of total flavonoid content.

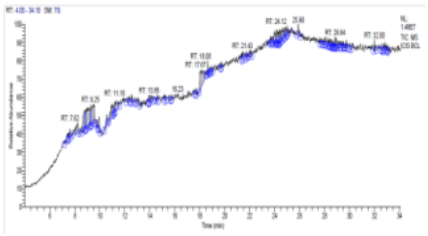

Figure 3. GC-MS chromatogram of Methanol extract of *Bombax ceiba* bark

CONCLUSION

Results of this study show that the bark of *Bombax ceiba* are the rich source of phenolic and flavonoid compounds that can play an important role in preventing the progression of many Diseases. Phytochemical screening has showed that maximum presence of phytoconstituents in methanolic extracts. The association among the phytochemical constituents with their biological activities is pioneering. *Bombax ceiba is* a plant, commonly used for the treatment of diarrhea, fever, chronic inflammation, catarrhal affection and ulceration of the bladder and kidney in traditional systems of medicine. But till date, there are few or no reports on GC-MS analysis of methanolic extract of the plant. Hence the presence of some important compounds in this plant isolated by GC-MS analysis is undertaken in this study. This type of study may give information on nature of active Phytoconstituents present in the medicinal plants. These identified phytoconstituents presumed to be responsible for proving the medicinal value of this plant *Bombax ceiba.*

ACKNOWLEDGMENT

The authors are sincerely thankful to SAIF Panjab University, Chandigarh for providing the GC-MS facility. We also thank DST for financial support in the form of Inspire fellowship.

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Cite this article as:

Meenakshi S C *et al*. Phytochemical screening and GC-MS analysis of methanolic extract of *Bombax ceiba* bark. Int. Res. J. Pharm. 2018;9(4):67-70 http://dx.doi.org/10.7897/2230-8407.09463

Source of support: DST Inspire fellowship, Conflict of interest: None Declared