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Mayuri Chelkar
MVSc., Department of Veterinary Pathology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Mahesh Harke
MVSc., Department of Veterinary Pharmacology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Arul Pandiyan
MVSc., Department of Veterinary Pathology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Corresponding Author:
Mayuri Chelkar
MVSc., Department of Veterinary Pathology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Identification of chemical compounds from the ethanolic extract of *Bauhinia racemosa* Lam. Bark by GC-MS analysis

Mayuri Chelkar, Mahesh Harke and Arul Pandiyan

Abstract
Bauhinia Racemosa Lam. belonging to the family Cesalpiniaceae is a small deciduous tree found throughout India. Its traditionally used in the indigenous system of medicine Ayurveda, Unani and Siddha for the treatment of several ailments like fever, skin and blood diseases, diarrhea, malaria, tumors and cancer. In order to review full pharmacological and therapeutic potential, the detailed pharmacognostic study and screening of bark was carried out. GC-MS analysis revealed the presence of twenty phytochemical constituents, which were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The major chemical constituents are octacosane, Heptadecane, 9-hexyl and Heptadecane, 9-octyl, hentriacontane, Di-n-octyl phthalate, hexacosane, nonacosane and pntacosane, ascorbic acid 2, 6 dihexadecanoate, Tridcanoic acid 12-methyl-, methyl ester, Vitamin E, heptadeconic acid, octadeicadinoic acid, hexadecanoic acid which possess many biological activities. Hence these studies help for the screening of varied bioactive components.

Keywords: *Bauhinia racemosa*, phytochemistry, GC-MS, Soxhlet's extractor

1. Introduction
Bauhinia is a well known genus for its therapeutic potential. One of the most important species of this genus *Bauhinia racemosa* L. is commonly known as Kachnal in Hindi and Sonpatta or Apta in Marathi (Garodia *et al.* 2007) [5]. *Bauhina racemosa* L. is distrbuted throughout southern India, Assam and Bihar. Its dried leaves, buds and flowers are used for anti-hypergycaemic and anti- lipedemic activity (Gopalkrishnan *et al.* 2011) [2, 4]. The dried bark is applied externally to tumors and wounds (Gopalkrishnan *et al.* 2012) [12]. An infusion of the bark is used as astringent gargle.
The medicinal plants are having varied bioactive components which are identified in minute concentrations by GC-MS analysis (Sahaya *et al.* 2012) [7]. Gas Chromatography-Mass Spectrometry is the most commonly used technique for the identification and quantification of bioactive components. For the analysis of medicinal plants, GC-MS has proved to be widely used technique for analysis of non-polar components, volatile essential oils, fatty acids, lipids and alkaloids.
The aim of the present study is to identify the phytoconstituents of this plant and subjecting the ethanolic extract of the bark to Gas Chromatography- Mass Spectrometry analysis. This work will help to identify the compounds, which may be used in therapeutic value.

2. Materials and Methods
2.1 Plant Materials
The plant bark was collected in the month of September from National Bureau of Soil Survey and Land Utilization Planning, ICAR Research Institute in Amravati Road, Nagpur. The plant was identified by Department of Botany, Rashtrasant Tukdoji Maharaj University, Nagpur. A voucher specimen was preserved in the herbarium of the Department of Veterinary Pathology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur.

2.2 Extraction of plant Material
The fresh bark was cleaned with distilled water to remove extraneous matter, shade-dried until to get constant weight and then powdered. Thimbles made up of whatman filter paper no.1 enclosing the required powder was used. The dried powder of the bark of the plant (500 g) was successively defattened using petroleum ether (40-60 °C), and then subjected to 70% ethanolic

extraction by Soxhlet's extractor. The last trace of solvent was removed under reduced pressure distillation and then the recovered solvent was kept for drying at water bath (40-60 °C) in clean sterilized petri plates. The collected extract was then stored at 4 °C. The dried crude ethanolic extract was used for the organoleptic, preliminary phytochemical screening and GC-MS analysis.

2.3 GC-MS analysis
05 grams of ethanolic extract of *Bauhinia racemosa L*. bark was diluted in distilled water till it formed a uniform solution and was packed in sterile, bottles to be employed for GC-MS analysis.

2.4 Instruments and chromatographic conditions
Gas Chromatograph with a mass Spectrometry was performed with an instrument Make- Bruker Scion, Model TQ-MS System for the analysis of plant extract sample under following chromatographic conditions. The machine was equipped with the column DB-5MS Agilent (30m x 0.25 mm 1D) composed of 100% dimethyl polysiloxane. Electron ionization system with ionization energy of 70 eV was used. Helium gas was used as carrier gas at constant flow rate 1.0 ml/ min with a split ratio of 10:1. The oven temperature was operated in following manner: 40 °C (isothermal for 2 min), with an increase of 20°C/min, to 150 °C/min, then upto 300 °C with 10 min held, injector temperature and volume 250 c and 2 µl..

2.5 The total GC running time was 36 min.
The mass spectrometer operating conditions were: ionization voltage 70eV, source temperature of 250 °C inlet line temperature of 280 °C MASS SCAN (M/Z)-30-500, solvent delay: 3 min with total MS running time for 34 min. The mass spectra of compounds were identified by comparing the mass spectra obtained from related chromatographic peaks with NIST mass spectral libraries. Further the identified compounds were searched over online literature for detailed information.

3. Results and Discussion
Gas Chromatography-Mass Spectrometry (GC-MS) chromatogram of the ethanolic extract of the bark of *Bauhinia racemosa Lam*. plant showed 20 peaks indicating the presence of twenty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the phytoconstituents were characterized and identified. The chemical name, retention time (RT), molecular formula, molecular weight (MW), peak area (%) and biological activity of various are presented in Table 1. They were identified as octacosane, heptadecane, 9-hexyl heptadecane, 9-octyl, hentriacontane, Di-n-octyl phthalate, hexacosane, nonacosane, pentacosane, ascorbic acid 2, 6 dihexadecanoate, tridcanoic acid 12-methyl-, methyl ester, vitamin E, heptadeconic acid, octadeicadinoic acid and hexadecanoic acid respectively.
Presence of antitumor agent, octacosane may play a prominent role in curing cancer which is in agreement with the previous reports (Panda *et al.* 2015) [9]. Heptadecane, 9-hexyl and Heptadecane, 9-octyl component is useful as an antifungal agent according to reports (Abubacker *et al.* 2014). Presence of anti-tubercular constituent hentriacontane may be responsible for its protective activity against Tubercular cervical lymphadenitis. The presence of Di-n-octyl phthalate inferred anti-inflammatory properties to the plant (Gunalan *et al.* 2014) [6]. The presence of hexacosane, nonacosane and pentacosane phytoconstituent provides insecticidal properties to the plant. Ascorbic acid 2, 6 dihexadecanoate, tridcanoic acid 12-methyl-, methyl ester, vitamin E, heptadeconic acid infers antioxidant property as reported previously (Murugan *et al*. 2012) [8]. Presence of octadeicadinoic acid, hexadecanoic acid confers antimicrobial activities.

Table 1: GC-MS Chromatogram of Bauhinia racemosa Lam. Bark Ethanolic extract

Sr. No.	RT	Name of Compound	Peak Area	%
1	19.756	Octasane	58925734	89.33
2	24.545	Tetratetracontane	65964680	100
3	33.31	Octadecane,3-ethyl-5-(2-ethylbutyl)	4217462	6.39
4	37.68	Vitamin E	33802848	51.24
5	38.38	Tocopherol	18285629	27.72
6	39.03	Heptadecane,9-hexyl	4210090	6.38
7	39.72	Heptadecane,9-octyl	2604040	3.95
8	42.911	Hentriacontane	2492489	3.78
9	44.79	Di-n-octyl-phthalate	4937382	7.48
10	45.38	Hexacosane	2428852	3.68
11	47.17	Nonacosane	7590088	11
12	50.21	Pentacosane	6357620	9.6
13	53.2	9-Octadecnoic acid ethyl ester	5430416	8.23
14	58.42	Methyl-17-methyl-octadecanoate	5758432	8.73
15	24.55	Hexadecanoic acid, ethyl ester	65964660	99
16	37.6	9-octadecenoic acid-methyl ester	7725766	12.1
17	30.29	Heptadecanoic acid 16 methyl-methyl ester	4120945	63
18	18.72	Methyl-10-methyl-undecanoate	56432161	88.0
19	42.91	Tridecanoic acid, 12-methyl, methyl-ester	2492489	3.78
20	32.42	Ascorbic acid 2,6- dihexadecanoate	18285629	27.7

4. Conclusion
In the present study, 13 bioactive phytoconstituents have been extracted and identified from bark of *Bauhinia Racemosa L.* have been identified. The presence of various bioactive phytoconstituents justifies its role as a indigenous medicinal plant The GC-MS analysis of the 13 bioactive phytoconstituents that have been extracted and identified from bark of *Bauhinia Racemosa* L. will help in understanding the nature of active principles which will be further useful for detailed phytochemical studies. However isolation of

individual phytochemical constituents and subjecting it to biological activity will definitely give beneficial results. It could be concluded that the ethanolic extract of the bark of *Bauhinia racemosa* L. contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance.

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