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Phytochemical Screening And Gc-Ms Analysis Of Natural Plant Extract Of Dalbergia Sissoo, Hibiscus Rosa And Quisqualis Indica

Kavita Kumari[1], Dr Umesh Kumar Gilhotra[2], Dr Rakesh Chawla[3]

[1] Research Scholar, Rajasthan University of Health Sciences, Jaipur, Rajasthan, India
[2] Professor, Department of Pharmacology, G. D. Memorial College of Pharmacy, Jodhpur Rajasthan, India
[3]Head, Department of Pharmaceutical chemistry, University institute of Pharmaceutical Sciences & Research, Baba Farid University of Health Sciences, Faridkot, Punjab, India

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ABSTRACT

The aim of the study is to elucidate the phytochemical screening and GC-MS analysis of natural plant extract of *Dalbergia sissoo*, *Hibiscus rosa* and *Quisqualis indica***.** These natural plants as *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* has important role in the treatment of various disease. These plants show various pharmacological activity such as *Dalbergia Sissoo* possess antidiabetic, antioxidant, analgesic and antipyretic, ant-termite, anti-spermatogenic, anti-inflammatory, anthelmintic, anti-diarrhoeal. *Hibiscus Rosa* has anti-tumor, antioxidant, anti-ammonemic, anti-diabetic, hypolipidemic, post-coital antifertility, cardio protective, wound healing, pain relieving and *Quisqualis Indica* possess antimicrobial activity, Antiviral activity, Control lungworm infection, Anthelmintic activity, Insecticidal activity, Anti lymphatic filariasis activity, Analgesic activity, Antioxidant activity etc. Ethanolic and hydroalcoholic extract of the plants were prepared and its phytochemical screening and GC-MS analysis were performed and chemical constituent determined as alkaloids, glycoside, carbohydrate, flavonoids, steroids, tannins, and Terpenoids, Saponins, Protein, reducing sugar and amino acids were observed. In extract of *Dalbergia Sissoo* total 20 (R-(2,2,3,3-2H4) Butyrolactone, Formic acid, 1-methylethyl ester, Propene 3,3,3-D3, 2-Propanamine, 2-Amino-1-propanol, Pentanal, Guanosine, Acetaldehyde etc.), in *Hibiscus Rosa* 12 (Propanol,3,3'-dithiobis(2,2-dimethyl-), SS)-or (RR)-2,3-hexanediol, 2-Hydroxy-2-methylbutyric acid, n-Hexadecanoic acid, Heptanoic acid, 2-ethyl-, Trans-(2-Ethylcyclopentyl)methanol, 3-N-Hexylthiolane) and in *Quisqualis Indica* 7 (1-Undccanol, 1-Fluoro-dodecane, Decylundecyl ester carbonic acid , 1-Dodecanol) phytochemical compounds were identified.

Keywords: GC-MS, pharmacological activity, Chemical constituents, flavonoid

INTRODUCTION

In recent years, the use of medicinal plants has gradually increased because they are seen as safe and valuable natural resources.[1] These natural plant *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* has important role in the treatment of various disease. These plant show various pharmacological activity such as *Dalbergia Sissoo* possess antidiabetic, antioxidant, analgesic and

antipyretic, ant-termite, anti-spermatogenic, anti-inflammatory, anthelmintic, anti-diarrhoeal, antinociceptive, neuroprotective, antioxidant and osteogenic activities.[2,3] *Hibiscus Rosa* has anti-tumor, antioxidant, anti-ammonemic, anti-diabetic, hypolipidemic, post-coital antifertility, cardio protective, wound healing, pain relieving, antiviral, anti-ovulatory, juvenoid action, hypotensive, antidepressant, mitigating and antiestrogenic movement.[4,5] *Quisqualis Indica* possess antimicrobial activity, Antiviral activity, Control lungworm infection, Anthelmintic activity, Insecticidal activity, Anti lymphatic filariasis activity, Analgesic activity, Antioxidant activity, Anti-inflammatory activity, Antidiarrheal activity, Anticancer activity, Antidiabetic activity.[6] The medicinal value of these plants is identified by the chemical compounds that affect the human body. About 60% of the global population uses medicinal plants for health care.[7] *Dalbergia Sissoo* belongs to the family *Fabaceae* and possess Chemical constituents such as Carbohydrates, proteins, amino acids, flavanoids, alkaloids, phenolic compounds, saponin, phytosterols, steroids and tannins. *Dalbergia sissoo* has 25 meters hight with grey yellow trunk and it is 2-3 meters in diameter. It is found in tropical to subtropical climates in natural and planted forests, it was distributed in Pakistan, India, Cameroon, Afghanistan, Bangladesh, Ethiopia, Persia, Iraq, Sudan Palestine, India, Malaysia, Thailand, Tanzania, Indonesia, Cyprus, Mauritius, Nigeria, Zimbabwe, Kenya, and United States of America.[8] *Hibiscus Rosa* belongs to the family *Malvaceae***,** it contains Anthocyanins & flavonoids; cyanidin-3,5-diglucoside, cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, quercetin3-diglucoside46. 2. Other A cyclopeptide alkaloid47 , cyanidin chloride, quercetin, hentriacontane48 and vitamins : riboflavin, ascorbic acid & thiamine.[9] It is a native of China. It is grown as an ornamental plant in gardens throughout India and often planted as a hedge or fence plant.[10] *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide.[11] *Quisqualis Indica* belongs to the family *Combretaceae* and it's leaves contains trigonelline, L-proline, L-asparagine, quisqualic acid, it contains chemical constituents as rutin and cysteine synthase, isoenzyme A and isoenzyme B. Fruits is sugary such as levulose and an organic same as cathartic acid. Fixed oil present in seeds and it consists of linoleic, oleic, palmitic, stearic and arachidic acids, a sterol, it also has alkaloid and a neuroexcitatory amino acid, quisqualic acid.[12] Its length in cultivation ranges between 2-9 m. It is a large, woody and shrubby climber, trellises. It is distributed in the world mainly in China, Philippines, Bangladesh, Myanmar and Malaysia and now also broadly grown in India as ornamental plant in most of the garden.[13] Gas Chromatography (GC) and Mass Spectroscopy (MS) works a powerful tool to have a deeper insight by identifying the various compounds present in the sample. Present study was focus on identification of compounds by using GC-MS technique and biological activities of identified compounds from plant leaf and flower extract of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica*. [14,15]

MATERIAL AND METHODS

Collection of Plant Material

Selection of material and methods and plant Collection was based on botanical survey, traditional use and literature survey. The leaves of *Dalbergia Sissoo* and mature flowers and leaves of *Quisqualis indica Linn* and leaves and flowers of *Hibiscus rosa* were collected from the nursery and garden from Noida, U.P. in the month of November 2020.

Authentication of plant materials:

The plant was collected and authenticated from Botanical Garden of Indian Republic (BGIR),

Botanical Survey of India, Noida, U.P. for all the plants. *Dalbergia Sissoo* (**Authentication No.:** BGIR 344), *Quisqualis Indica* (**Authentication No:** BGIR 343), *Hibiscus rosa* (**Authentication No.:** BGIR 342)

Extract Preparation

Preparation of powder:

For the preparation of powder shade dried leaves of leaves of *Dalbergia Sissoo*, flower part and leaves of *Quisqualis Indica* and *Hibiscus rosa* were powered separately with a mechanical grinder and it was passed through a 40-mesh sieve.

Powder of the extracts of the *Dalbergia Sissoo (A), Hibiscus rosa(B)* and *Quisqualis indica(C)*.

(A) **(B)** **(C)**

Extraction:

***Dalbergia Sissoo*:** The shade-dried and pulverized leaves (1000 g) were defatted with petroleum ether and then extracted with 90% ethanol in a Soxhlet extractor. The ethanolic extract was filtered using Whatman paper and concentrated to dryness under reduced pressure at a controlled temperature of 48°C–50°C with a rotary evaporator. The obtained dried extract (DSLE) was further triturated in ethanol at room temperature, and the alcohol-soluble part was concentrated and dried to obtain the yield value. Then its extract were used for purification and identification of different compounds. The dark brown extract was used for various qualitative phytochemical investigations for the identification of different phytochemical components. [18]

Quisqualis indica: Defatted the 180 g of dried powder with petroleum ether in a closed container, and continued this process for 9-10 days with occasional shaking. After that filtered this extract to get the marc. The marc was removed and it was dried in the shade, after that it was extracted with methanol and water (hydroalcoholic) through the process of cold maceration for another 9-10 days, with occasional shaking, then it was filtered and concentrated under reduced pressure. A semisolid mass was obtained and made it free from the solvent. The extract was weighed properly, and its percentage yield was calculated and stored it in a cool place. [19]

Hibiscus rosa: 500 g fine powder of Hibiscus Rosa was suspended in 1500 ml of ethanol at room temperature for 24 hours. The prepared mixture was filtered by using a fine muslin cloth and it was subsequently filtered through Whatman No. 1 filter paper. Keep the filtrate in a water bath and dried at 40°C then an ethanol-free residue was obtained and it was used for the study.[20]

Percentage yield= weight of dry crude extract obtained (g) x 100

Plant material weight before extraction (g)

Phytochemical screening of plant extracts

Phytochemical screening of plant extracts was performed by following methods:

1) **Detection of Carbohydrates**

Molisch's Test: 2-3 drops of 1 % α-naphthol and 2 ml of concentrated sulphuric acid used carefully added along the sides of test tubes for the treatment of each plant extract and brown ring in the test tube represent the presence of carbohydrates in *Dalbergia sissoo* extracts and not in *Quisqualis Indica* and *Hibiscus rosa.*

2) **Test for Proteins and Amino Acids**

Dissolved Small quantities of alcoholic extract in few ml of distilled water and perform the Ninhydrin test, Xanthoprotein test, test with tannic acid and heavy metals.

Ninhydrin Test The ethanolic extracts of each plants were treated with 0.1% ninhydrin reagent and boiled, then purple colour appeared in *Dalbergia sissoo* and *Quisqualis indica* which show the presence of protein. No any purple colour observed in the extract of *Hibiscus rosa*, which indicating the absence of proteins and amino acids.

3) **Test for triterpenoids and steroids**

In a 5 ml of chloroform, small amount of each plant extracts were dissolved separately then the this chloroform solutions were subjected to the Liberman's, Liberman-Burchard's and Salkowski's test.

Liberman-Burchard's Test: The residue was dissolved in chloroform. To this Liberman-Burchard's reagent was added. Green colour was produced. Green colour was produced in both the extract *Dalbergia sissoo* and *Hibiscus rosa* indicating the presence of steroids and not formed in *Quisqualis Indica* indicates its absence.

4) Test for Tannins

Test for Tannins: Dissolve all three plant extracts in water and filtered it and using various reagent to treat the filtrates as:

Ferric Chloride Test: 5 % ferric chloride solutions used to treat the plant extracts. The presence of bluish black colour in each solution indicates the presence of tannins.

5) Test for Alkaloids

Stirred solvent-free ethanolic and aqueous extracts separately with dilute HCl. Filtered it and used for test of each plant extracts.

For the detection of alkaloid a small amount of the solvent-free ethanolic and aqueous extracts was used and it was separately stirred with a few ml of dilute HCl then it was filtered and various alcoholic reagents were used to test the filtrates.

Dragendorff's Test:

Add Dragendorff's reagent in a small quantity of the extracts. The presence of an orange-brown

precipitate indicates the presence of alkaloids in all plant extracts.

6) **Test for Saponins**

Foam Test: In a 20 ml of distilled water dilute the all extract separately and shake it continue for 15 minutes, the presence of 1 cm foam layer in each extract solution show the presence of saponins.

.7) **Tests for Glycosides**

In 5 ml of distilled water dissolve each plant extracts separately then filter it and another portion of the extracts was hydrolyzed with hydrochloric acid for one hour in a water bath and subjected to different test as Legal's, Baljet's, Borntrager's, Keller-Killiani's and cyanogenetic glycosides.

Legal's Test:

Hydrolyzed the plant extracts by adding 1 ml of pyridine and a few drops of sodium nitroprusside solution and followed by making the solution alkaline with sodium hydroxide. Then a pink colour was observed in *Dalbergia sissoo* and *Quisqualis indica* extracts, but not in the *Hibiscus rosa* extract.

8) **Test for Flavonoids**:

All plant extracts were dissolved in ethanol and tested with different reagent as:

Ferric chloride Tests: Add few drops of neutral ferric chloride solutions in a small quantity of the ethanolic extract. Then a blackish-red colour in all plant extracts indicate the presence of flavonoids.

9) **Test for Fixed Oils and Fats**

Spot Test: For the test of fixed oil and fats a small amount of extract was pressed between two filter papers, and oil stains appeared which showed the presence of fixed oils and fats.

Table: Phytochemical study of the extracts of leaves of *Dalbergia sissoo, Quisqualis indica, Hibiscus rosa*

+ =Presence; - = Absence, *Dalbergia sissoo* = A, *Quisqualis Indica* = B, *Hibiscus Rosa* = C

Phytoconstituents/ Extracts	***Dalbergia Sissoo* (A)**	***Quisqualis Indica* (B)**	***Hibiscus Rosa* (C)**
Alkaloids	+	+	+
Glycosides	+	+	-
Flavonoids	+	+	+
Steroids	+	-	+

Tannins	+	+	+
Carbohydrates	+	-	-
Saponins	+	+	+
Terpenoids	+	-	+
Protein	+	+	-
Reducing sugar	+	+	-
Amino acids	+	+	-

RESULT

Phytochemical study and its results was performed on the ethanol extracts of the leaves of *Dalbergia Sissoo*, Hydro-alcoholic extract of *Quisqualis Indica* and ethanolic extract *Hibiscus Rosa* of the presence of various phytoconstituents such as alkaloids, glycoside, carbohydrate, flavonoids, steroids, tannins, and Terpenoids, Saponins, Protein, reducing sugar and amino acids were observed. Phytochemical present in *Dalbergia sissoo* as alkaloids, glycoside, flavonoids, Steroids, tannins, Carbohydrates, Saponins, Terpenoids, Protein, reducing sugar and amino acids.

Preliminary Phytochemical studies of the hydroalcoholic extract of *Quisqualis indica* was performed for major classes of constituents like as alkaloids, glycoside, flavonoids, tannins, Saponins, Protein, reducing sugar and amino acids were observed.

Phytochemical studies of the ethanolic extract of *Hibiscus Rosa* was performed for major classes of constituents like as alkaloids, flavonoids, tannins, Saponins, Terpenoids were observed.

Determination of Ash value: Ash value determined as per formula for all the plant extracts.

Total ash : Weighed empty silica crucible (W1). Added about 3 g (W2) of the air-dried PHP in the previously weighed crucible. Gradually the sample was ignited in an electrical muffle furnace and increasing the heat to 500- 600° to get it white which indicate the absence of carbon. Then cooled it in a desiccator and reweighed (W3).

Total ash content = ((W3- W1)/(W2-W1))×100.

Analysis of powder of plant extracts *Dalbergia sissoo, Hibiscus rosa, Quisqualis indica*

Few common test conducted for the standardization of powder of plant extracts (*Dalbergia sissoo, Hibiscus rosa, Quisqualis indica*) among the various pharmacopeial analysis of herbal origin. Following results were obtained in the physical standardization of powder.

Table: Analysis of powder of plant

S. No.	Parameters	Value obtained (%W/W)		
		A	B	C
1	Ash content	7.0	6.3	7.1
2	Acid-insoluble ash	1.5	1.8	1.2
3	Water-soluble ash	0.8	1.1	0.6
4	Ethanol soluble extractive	47	32	38
5	Water soluble extractive	51	47	42

A= *Dalbergia sissoo*, B= *Hibiscus rosa*, C= *Quisqualis indica*

Identification of components -*Hibiscus Rosa*

GC-MS interpretation was conducted by using the database of National Analytical Services (NAS) having more than 40,000 patterns. Unknown component spectrum was compared with the spectrum of known components in the NAS library. Then determine the all information as name, molecular weight and structure of the components of the test materials.

GC-MS analysis of plant extract:

Interpretation on mass spectrum GC-MS of ethanolic extract of Dalbergia Sissoo, Quisqualis Indica and Hydroalcoholic extract of Hibiscus Rosa were conducted using the database of **National Analytical Services,** Jaipur, Rajasthan with sample record no.: **NAS/AD/601,** Reg. No.: **AD/601 and** Ref: **033/05-2024.** Compare the spectrum of the unknown component was with known components which was stored in the library. The name, peak value and retention time of the components of the test materials were ascertained

Dalbergia Sissoo

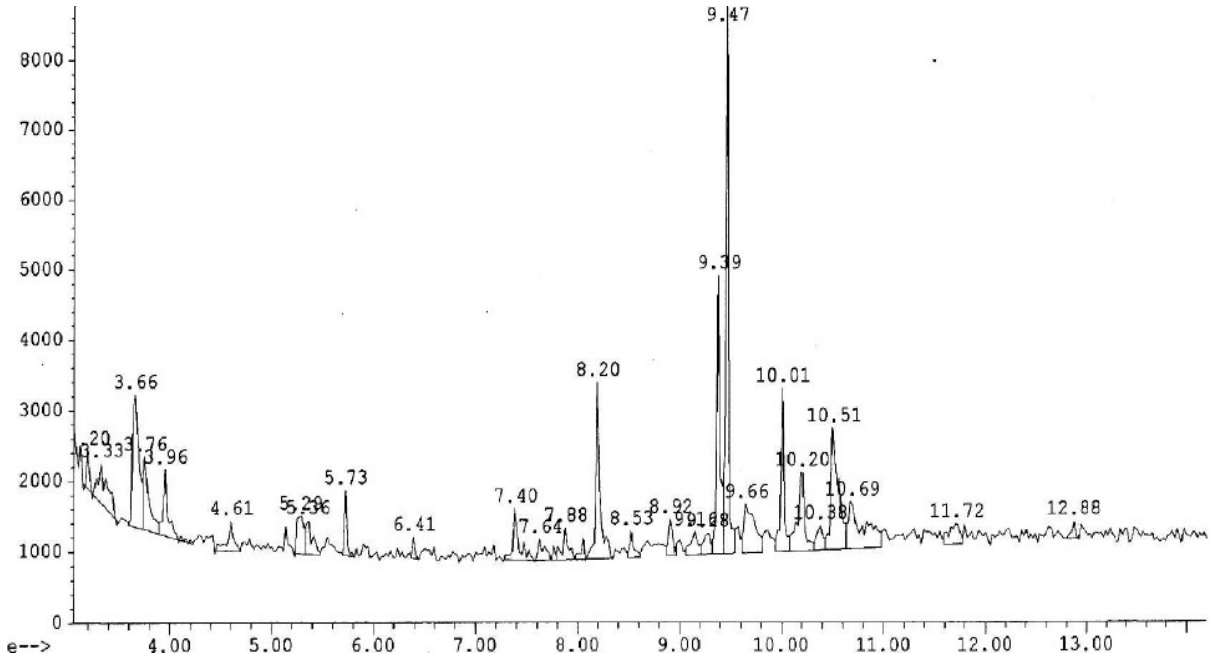

Figure: Chromatogram of *Dalbergia Sissoo* by GC-MS

Table: Phytocompounds identified in the ethanolic extract of *Dalbergia Sissoo* by GC-MS

RT	Name of the compound	Peak area (%)
3.33	R-(2,2,3,3-2H4) Butyrolactone	3.58
3.66	Formic acid, 1-methylethyl ester	7.38
3.76	Propene 3,3,3-D3	4.22
3.96	2-Propanamine	3.03
4.61	2-Amino-1-propanol	1.71
5.29	Pentanal	2.29
5.36	Guanosine	2.02
5.73	Acetaldehyde	1.47

6.41	Cyclobutanol	0.47
7.40	3-Amino-2-ethylbutanoic acid	2.63
7.64	2-Oxo-Butanoic acid	1.49
8.20	Benzenemethanol, 2-2-aminopropxy	7.29
8.53	2-Fluro-betahydroxy benzeneethanamine	1.00
9.16	L-Alanine, methyl ester	1.49
9.40	3-Hydroxycarbonyl-2,5-diethylpyrrolidine	7.83
9.47	1,2-Bnezenedicarboxylic acid dibutyl ester	13.68
10.20	2,2-Dimethyl-4-methylaminobutanone	5.27
10.51	5-Nitro,2,4-Pyrimidinedione	7.94
10.69	2-Isocyanato-Propane	6.56
11.721	Oxirane	.98

Hibiscus Rosa

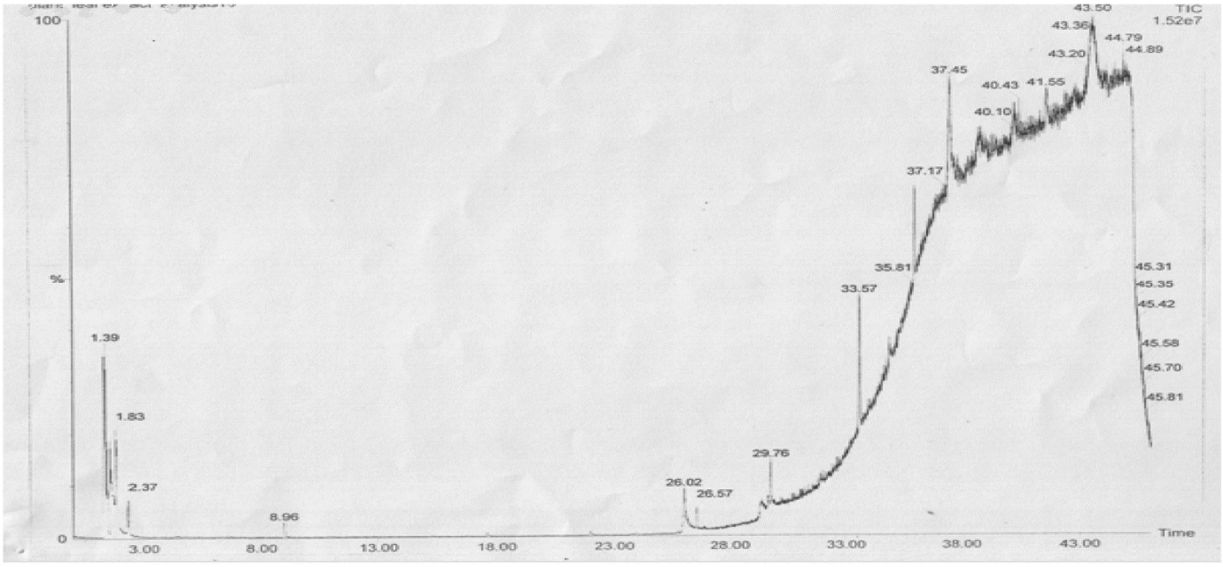

Figure: Chromatogram of *Hibiscus Rosa* by GC-MS

Table: Phytocompounds identified in the ethanolic extract of *Hibiscus rosa* by GC-MS

RT	Name of the compound	Peak area (%)
8.96	Propanol,3,3'-dithiobis(2,2-dimethyl-)	1.39
17.59	SS)-or (RR)-2,3-hexanediol	0.452
21.98	2-Hydroxy-2-methylbutyric acid	0.32
26.02	n-Hexadecanoic acid	8.78
26.57	Heptanoic acid, 2-ethyl-	1.42
29.65	Trans-(2-Ethylcyclopentyl)methanol	1.83
29.76	3-N-Hexylthiolane, SS-dioxide	7.24
33.57	Hexanedioic acid, bis(2-ethylexyl) ester	12.88
35.91	1,2-Benzenedicarboxylic acid, diisoocytl ester	7.66
37.45	1,3-Benzodioxole, 5.5'-(tetrahydro-1H,3H-furo{3,4-c)furan-1,4-diyl)bis-,(1S-(1α,3a α,4β, 6a α))-	23.49
41.55	Squalene	5.25
43.50	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyk-2-buten-1-yl)-1c-cyclohexanol	29.28

GC – MS analysis- *Hibiscus rosa*

Preparation of the extract

The *Hibiscus rosa* extract contained both polar and non-polar phytocomponents of the plant material which was used. 2µl of these extract solutions was employed for GC/MS analysis [17]. GC-MS analysis performed on a GC clarus 500 Perlin Elmer system comprising a AOC-20i autosampler and interfaced gas chromatograph to a mass spectrophotometer (GC – MS) instrument employing the conditions as: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; carrier gas is helium (99.999%) and it was used as at a constant flow of 1 ml/min and injection volume was 0.5 EI (split ratio of 10:1 injector temperature 250 C; ion-source temperature 280 C. The temperature of oven kept 110 C (isothermal for 2 min) and increase of 10 C/min to 200 C then 5 C/min to 280 C, ending with a 9 min isothermal at 280 C. 70 eV is used to take mass spectra; a scan interval of 0.5s and fragments from 40 to 550 Da.

***Quisqualis Indica*: GCMS**

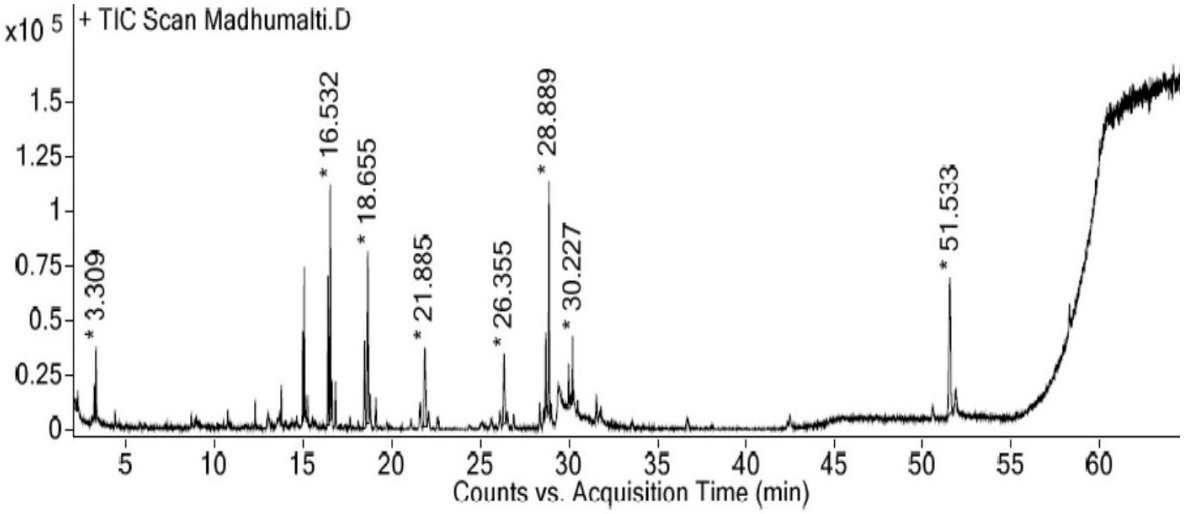

Figure: Chromatogram of *Quisqualis Indica* by GC-MS

Table: Phytochemicals identified from ethanolic extract of *Quisqualis Indica*.

RT	Name of compound	Peak area
14.971	1-Undccanol	3.2
15.069	1-Fluoro-dodecane	4.52
16.532	Decylundecyl ester carbonic acid	11.48
18.477	1-Dodecanol	6.24
21.885	Decylheptyl ether	8.83
28.889	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	13.12
51.533	3,5-Dedihydro-stigmastan-6,22-diene	17

Plant's extract of *Quisqualis indica* were used for the estimation of GC-MS analysis. A regular Perkin Elmer Auto System XL GC-MS analyzer used for the GC-MS analysis of plant extracts. For the detection of GC-MS, 70eV ionization energy was used in an electron ionization energy system. As a carrier gas, helium gas (99.999%) at a constant flow rate of 1.51 ml/min and injection volume kept 2µl. 22 min was total GC running. To handle mass spectra adopted the software and chromatograms were Turbo Mass. On the basis of molecular structure, molecular mass compounds was identification. Calculation for the relative percentage amount of each component was done by comparing its average peak area to the total areas. [14,15]

RESULT AND DISCUSSION

GC-MS analysis of *Dalbergia Sissoo, Hibiscus Rosa* and *Quisqualis Indica* indicate the presence of various phytochemical compounds in plant. In ethanolic extract of *Dalbergia Sissoo* total 20 phytochemical compounds were identified such as R-(2,2,3,3-2H4) Butyrolactone, Formic acid, 1-methylethyl ester, Propene 3,3,3-D3, 2-Propanamine, 2-Amino-1-propanol, Pentanal, Guanosine, Acetaldehyde, Cyclobutanol, 3-Amino-2-ethylbutanoic acid, 2-Oxo-Butanoic acid, Benzenemethanol, 2-2-aminopropxy, 2-Fluro-betahydroxy benzeneethanamine, L-Alanine, methyl ester, 3-Hydroxycarbonyl-2,5-diethylpyrrolidine, 1,2-Bnezenedicarboxylic acid dibutyl ester, 2,2-Dimethyl-4-methylaminobutanone, 5-Nitro,2,4-Pyrimidinedione, 2-Isocyanato-Propane, Oxirane. The extract of *Hibiscus Rosa* contains Propanol,3,3'-dithiobis(2,2-dimethyl-) (1.39%) retention time 8.96, SS)-or (RR)-2,3-hexanediol (0.452) retention time 17.59, 2-Hydroxy-2-methylbutyric acid (0.32) retention time 21.98, n-Hexadecanoic acid (8.78) retention time 26.02, Heptanoic acid, 2-ethyl- (1.42) retention time 26.57, Trans-(2-Ethylcyclopentyl)methanol (1.83) retention time29.65, 3-N-Hexylthiolane, SS-dioxide (7.24) retention time 29.76, Hexanedioic acid, bis(2-ethylexyl) ester (12.88) retention time 33.57, 1,2-Benzenedicarboxylic acid, diisoocytl ester (7.66) retention time 35.91, 1,3-Benzodioxole, 5.5'-(tetrahydro-1H,3H-furo{3,4-c)furan-1,4-diyl)bis-,(1S-(1α,3a α,4β, 6a α))- (23.49) retention time 37.45, Squalene (5.25) retention time 41.55, 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyk-2-buten-1-yl)-1c-cyclohexanol (29.28) retention time 43.50 The Extract of *Quisqualis Indica* contains 1-Undccanol (3.2) retention time 14.971, 1-Fluoro-dodecane (4.52) retention time 15.069, Decylundecyl ester carbonic acid (11.48) retention time 16.532, 1-Dodecanol (6.24) retention time 18.477, Decylheptyl ether (8.83) retention time 21.885, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (13.12) retention time 28.889, 3,5-Dedihydro-stigmastan-6,22-diene (17) retention time 51.533.

CONCLUSION

An insight into the active components of plants was getting from the generated results. Also the several phytochemical compounds were being identified which were present in the plant leaf extract of *Dalbergia sissoo, Hibiscus Rosa, Quisqualis Indica* with several biological properties revealing immense medicinal potential of the plant.

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