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Phytochemical analysis of stem-bark of the selected trees by biochemical and GC-MS methods

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Abstract

Plant kingdom is known for having various medicinal properties. These medicinal properties of herbs, shrubs and trees are due to presence of various phytochemicals. In the present study, phytochemicals in stem bark of five selected trees (*Holoptelia integrifolia, Syzygium cumini, Pscidium guajava, Pongamia pinnata, and Bombax ceiba*) were analyzed and quantified by using various biochemical methods and Gas chromatography mass spectroscopy (GC-MS) technique. The selected plant part has been found to possess different phytochemicals like carbohydrates, proteins, lipids, phenols, ascorbic acid, saponins, alkaloids, and flavonoids at different level. In GC-MS studies, different flavonoids, alkaloids, steroids were detected in the selected plant part. Further studies of these phytochemicals for their pharmacological activities may have a path for pharmaceutical industries.

Keywords: phytochemicals, biochemical methods, GC-MS technique etc

Introduction

Since ancient time, traditional plant-based medicines are used by tribes, health care workers and ethnic communities for the treatment of various diseases all over the world. Some of them established themselves and gloom worldwide (Patwardhan *et al*, 2005) [20]. World Health Organization (WHO) has started promotion of primary health care (PHC) system development in developing countries. Traditional healthcare systems and medicinal plants have been incorporated in the programme and given equal importance (Sofowora *et al*, 2013) [26].

Plants possess various phytochemicals and these Phytochemicals are naturally occurring compounds in plants. These are produced in plants via primary and secondary metabolic pathways. A special feature of all higher angiosperms is their capacity to produce a large number of organic compounds with high structural diversity called secondary metabolites, which include flavonoids, alkaloids, steroids, tannins, saponins etc. These secondary metabolites can be divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit and Mathur, 1999) [22].

Plants are the oldest source of pharmacologically active compounds and have provided human kind with many medically useful compounds from centuries (Cordell, 1981) [8]. The use of plants and their extracts in treatment of diseases dates back to 460-370 BC when Hippocrates practiced the art of healing by the use of plant-based drugs (Soforowa, 1982). Today, it is estimated that more than two thirds of the world's population relay on plant derived drugs (Anon, 1987) [2]. About 7000 medicinal compounds used in the Pharma industries are derived from plants (Caufield, 1991) [4].

Throughout the world, plant-based medicines are used traditionally to treat many ailments, particularly infectious diseases, such as diarrhoea, fever, cold as well as for the purpose of birth control and dental hygiene (Mitscher *et al*, 1987). In addition, many psychoactive substances used in traditional medicines are of plant origin (Deans and Svodoba, 1990) [9]. Traditionally used medicinal plants produce a variety of known therapeutic properties (Chopra *et al*, 1992 [7]; Harborne and Baxter, 1995 [13]; Ahmad and Beg, 2001) [1].

In India, thousands of species of plants are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient time. Purohit and Vyas reckon that about 70,000 species of the plant kingdom have been used as herbal medicine at one or other time. Ayurveda is the science of life, and the oldest (over 5000 years) and most holistic medical system available today. This system traces its origins to the Vedas, Atharvaveda in particular. Ayurveda or Ayurvedic medicine is a system of traditional medicine, which is native to India (Chopra and Ananda, 2003) [6] and form of an alternative system of medicine.

In the present investigation, five trees were selected for phytochemical evaluation. These are *Holoptelia integrifolia* (Family Ulmaceae), *Bomabax ceiba* (family Malvaceae), *Pongamia pinnaata* (family Fabaceae), *Pscidium guaava* (Myrtaceae), *Syzygium cumini* (Mytaceae). In these selected plants, phytochemical analysis was done in stem bark by different biochemical methods and GC-MS studies. Quantification of different phytochemicals were also done in the study.

Material and Methods

Collection and Processing of Plant Parts

Stem bark of the selected medicinal plants *viz*: *Holoptelia integrifolia, Pscidium guajava, Pongamia pinnata, Syzygium cumini and Bombax cieba* were collected from Jaipur.

All the selected parts of plants were separated and washed thoroughly in running tap water, separately shade dried at room temperature. Dried plant materials were milled to make fine powder in a grinder. Each powdered sample was stored at room temperature in an air-tight polythene bag and were labelled to be used for extraction.

Preliminary Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical analysis was carried out to identify various primary and secondary metabolites present in the stem bark of the selected plants. These were processed further to evaluate the presence of various metabolites using the established protocols (Senguttuvan J., *et al*, 2014) [24].

Extraction and Quantitative Estimation of Phytochemicals

Total soluble sugar (TSS) and starch

Extraction of total soluble sugar and starch: 50 mg of each dried plant material was homogenized in a pestle and mortar with 20 ml of 80% ethanol separately and left overnight. Each of the sample was centrifuged for 15 minutes at 1200 rpm; the supernatants were collected and concentrated on a water bath (Loomish and Shull, 1973) [15]. Later each resultant was made up to final volume of 50 ml with addition of distilled water (extraction A) and processed further for the quantitative analysis of total soluble sugars. The residual pellet obtained after the process of total soluble sugars from each of the test sample was suspended in 5 ml of 52% perchloric acid and 6.5 ml of distilled water was added, shaken vigorously for 5 minutes and then centrifuged at 2500 rpm for 20min (Mc Cready *et al*., 1950) [17]. This step was repeated three times and the supernatants of each sample were pooled together and the final volume was raised up to 100 ml with distilled water (Extraction B), 1 ml aliquot was taken to evaluate the quantity of starch.

Quantification of total soluble sugar and starch: 1 ml of each extraction (A and B) were used for the quantitative estimation of the total levels of carbohydrates by using Phenol-Sulphur acid reagent (Dubois *et al.,* 1951). A regression curve for the standard glucose was prepared. A stock solution of glucose (100µg/ml) was prepared in distilled water, from which 0.1 to 0.9 ml were pipette out into nine test tubes and the volume of each test tube was raised up to 1 ml with distilled water. These tubes were kept on an ice-chest; 1 ml of 5% phenol solution was added in each and shaken gently. Then 5 ml conc. H_2SO_4 was poured rapidly and tubes were shaken gently during the addition of the acid. These mixtures were allowed to stand in a water bath at 26-30°C for 20 min. Characteristic yellow-orange colour was developed. The optical densities of various samples were taken at 490 nm by a spectrophotometer (Carl Zeiss, Jena DDR, VSU 2 P), after setting for 100% transmission against the blank (distilled water). A standard regression curve was computed according to Lambert Beer's Law between its known concentration of glucose and the respective OD. Optical density of all the samples were analysed in the same above said way and the concentration of total soluble sugar and starch were calculated by computing OD of respective sample with standard curve.

Proteins

Extraction of proteins: 50 mg of each test sample was homogenized in 10 ml of cold 10% TCA for 30 min and kept at 4°C for 24 hours. These all the mixtures were centrifuged at 2000 rpm for 10 minutes separately and supernatants were discarded. Each of the residues was re-suspended in 10 ml of 5% TCA and heated at 80°C on a water bath for 30 minutes. These all the samples were cooled, re-centrifuged (2000 rpm for 10 minutes) and the supernatant obtained from each sample were discarded. The residue was then washed with distilled water and dissolved in 10 ml of 1N NaOH, left overnight at room temperature (Osborne, 1962) [19].

Quantification of proteins: Using 1 ml each of above samples, total protein contents were estimated by following the method of (Lowry *et al*, 1951) [16] and a regression curve was prepared. A stock solution of Bovine serum albumin (BSA; Sigma chem. Co., St. Louis, USA) was prepared in 1N NaOH (1 mg/ml). Out of which nine concentrations ranging from 0.1 to 0.9 mg/ml of the solution was separately measured in the test tubes and the volume of each test tube was made up to 1 ml by adding distilled water. To each, 5 ml of freshly prepared alkaline solution (prepared by mixing 48 ml of 2% Na_2CO_3 in 0.1N NaOH, 1 ml of 1% sodium potassium tartrate in distilled water and 1 ml of 0.5% $CuSO_4.5H_2O$ in distilled water) was added and kept at room temperature for 10 minutes. Later in each of test tube 0.5 ml of Folin-Ciocalteau reagent (diluted with equal volume of distilled water, just before use) was added rapidly with immediate mixing and optical density were measured at 750 nm using a spectrophotometer after 30 min against the suitable blank. All the samples were processed in the above same manner and the concentration of the total protein content individually was calculated by referring the optical density of each test sample with the standard curve. Three replicates of each test sample were examined and their mean value were noted.

Lipids

Extraction and Quantification of lipids: 1gm of each and every dried and crushed test sample was macerated with 10 ml distilled water by using of a mortar and pestle (Jayaraman, 1981) [14]. The resulted paste was mixed thoroughly with 30 ml of chloroform-methanol (2: 1, v/v) mixture in a conical flask. Each and every mixture was left overnight at room temperature and then 20 ml of chloroform and equal volume of distilled water was added and centrifuged. Out of three layers, a clear lower coloured layer of chloroform containing all the lipids. This lower layer was collected in pre-weighted beakers. On the complete evaporation, the weight of beakers was determined again, which was taken as the weight of total lipids/g of the dried plant sample.

Phenols

Extraction of phenols: Each of 200 mg dried test sample was homogenized with 10 ml of 80% ethanol for 2 hours, and left overnight at room temperature. Every mixture was centrifuged and the supernatants were collected individually and then volume of each was raised up to 40 ml with 80% ethanol.

Quantification of phenols: To analysis of total phenol content in each plant sample, the protocol of (Bray and Thorpe, 1954) was followed. A standard curve of caffeic acid (a standard phenol) was prepared. A stock solution of gallic acid was prepared by mixing 100 mg of gallic acid in 10 ml of 80% ethanol. Nine concentrations extending from

0.1 to 0.9 ml (0.1 mg/ml; 100 times dilution) were prepared in the test tube separately and the volume of each test tube was raised to 1ml by adding 80% ethanol. To each of these test tubes, 1ml of Folin-Ciocalteau reagent (commercially accessible reagent which was diluted with distilled water in 1:2 ratio just before use) and 2 ml of 20% Na_2CO_3 solution was added and then mixture was shaken vigorously. Each of test tube was placed on water bath for boiling 1min and then cooled. Each of reaction mixture was diluted with 25 ml of distilled water and then optical density of each mixture was taken at 750 nm against a blank with the help of spectrophotometer. Three similar replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regressive curve. OD were measured for each test sample treated similar of the above method and the total level of phenol was calculated from mean values (With reference to gallic acid) by referring the OD of the test sample with the regression curve of the standard phenol.

Ascorbic acid

Extraction of ascorbic acid: Each of the fresh sample about 400 mg was homogenized with 10 ml of acetate buffer (pH 4.8) and centrifuged for 20 minutes at 1200 rpm. Obtained supernatants were collected separately, out of that 1 ml was reserved to other test tubes in which 4 ml of 4% trichloroacetic acid (TCA) was added and kept overnight and then recentrifuged. Freshly prepared 1 ml of the colour reagent (prepared by mixing 90 ml of 2.2%, 2,4-dinitrophenylhydrazine in 10N H_2SO_4, 5 ml of 5% thiourea and 5 ml of 0.6% CuSO4 solution), was mixed with the supernatant of each sample and which was incubated later at 57^0C for 45 min. After cooling of each sample 7 ml of 65% H_2SO_4 was added to each mixture and cooled it again on ice-chest until used.

Quantification of ascorbic acid: Various concentration, 0.01 to 0.09 mg/ml were taken in different test tubes from the stock solution (10mg/100ml) which is prepared by mixing of 10 mg ascorbic acid in 100 ml of 4% TCA. Volume of each test tube was raised up to 5 ml by addition of 4% TCA solution and left for 12 hours at room temperature. The colour reagent (1 ml) was added to each, incubated and cooled. Later 7 ml of 65% H_2SO_4 was added to each and brought at the room temperature and then ODs were measured at 540 nm in a spectrophotometer against a blank. A regression curve was figured between the main OD and the concentration of standard ascorbic acid which was followed Beer's Law.

Alkaloids

Alkaloids were extracted from plant part of selected plants by the well-established method (Harborne, 1988) [12]. Finely powdered samples (100 gm) of plants parts were separately extracted with 10% acetic acid in ethanol (500 ml) for 4 hours. Filtered extracts were concentrated and were made alkaline by NH4OH. Thus the obtained Precipitate was collected separately by centrifugation, washed with 1% NH4OH, filtered, dried in *vaccuo* and weighed. Extracts thus obtained were stored in glass vials at 4^0C for further use.

Flavonoids

Different parts of selected plants were subjected to flavonoid extraction by following the method of (Subramanian and Nagarajan, 1969) [28]. Twenty grams of finely powdered plant material was dissolved in 80% methanol (500 ml) by shaking in shaker at 37^0C for 24 hours at 100 rpm and were filtered. Filtrate was re-extracted successively with petroleum ether, diethyl ether and ethyl acetate. Each step was carried out three times to ensure complete extraction. Petroleum ether fraction was discarded due to being rich in fatty substances and diethyl ether fractions (free flavonoids) were collected. Ethyl acetate fractions were analysed for bound flavonoids. Each ethyl acetate fraction was hydrolysed in 7% H2SO4 for 2 hours. Resulting mixture was filtered and filtrate was again extracted with ethyl acetate. The ethyl acetate extract was washed with distilled water to neutrality and collected. The diethyl ether (free flavonoids) and ethyl acetate (bound flavonoids) fractions were dried in *vaccuo*, weighed and stored in glass vials at 4°C.

Preparation of the extract for GC- MS investigation

Shade dried and powdered plant material (about 20gm) were soaked in 100 ml of methanol individually and kept for 24 hours for cold extraction. Dissolved phytoconstituents containing extracts were filtered individually using Whatman No. 1 filter paper. All the extracts were evaluated by GC-MS in Jawahar Lal Nehru University, Advance Instrument Centre and New-Delhi.

Gas Chromatography and Mass Spectrum analysis

The extract and the standard samples were analysed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm ide) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 ml/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library (NIST data bank) or with the published mass spectra.

Results and Discussion

Qualitative estimation

Carbohydrates, Proteins, Tannins, Flavonoids, alkaloids, saponins, and phytosterols were found to be present in the stem bark of all the selected plants.

The results of these studies are shown in Table 1.

Table 1: Qualitative analysis for presence/absence of various metabolites in selected plant parts

S.N.	Plant name	Carbohydrate	Protein	Tannin	Flavonoids	Alkaloids	saponins	Phytosterols
1.	*Holoptelia integrifolia*	+	+	+	+	+	+	+
2.	*Pscidium guajava*	+	+	+	+	+	+	+
3.	*Pongamia pinnata*	+	+	+	+	+	+	+
4.	*Syzygium cumini*	+	+	+	+	+	+	+
5.	*Bombax cieba*	+	+	+	+	+	+	+

Quantitative analysis

Results of the present studies revealed that various important phytochemicals are present in stem bark of all the selected plants in different quantity.

Results of the quantitative studies for total soluble sugar (TSS), starch, proteins, lipids, phenols, and ascorbic acid obtained from each gram of dried weight of the plant material are shown in Table 2 and these results are graphically represented in Figure 1.

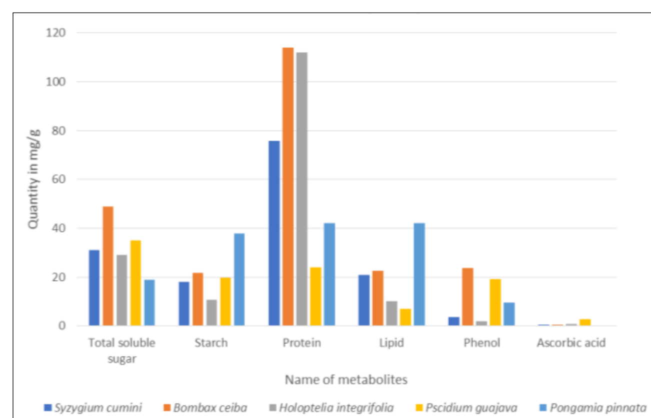

Fig 1: Quantitative estimation of various metabolites in the selected plant parts.

Quantity of flavonoids (total, free and bound) and alkaloids obtained from each gram of dried weight of plant material is shown in Tables 3 and Figure 2.

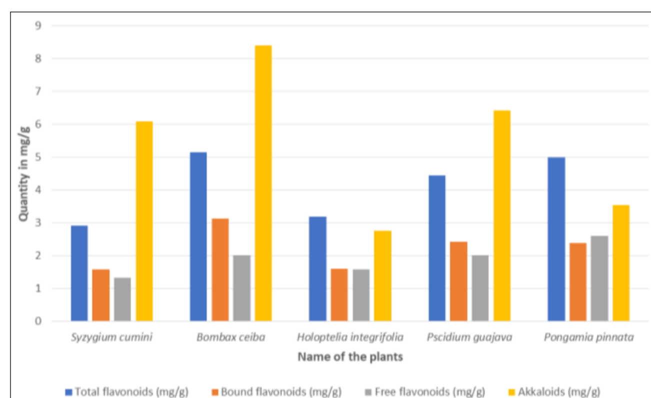

Fig 2: Quantitative estimation of extraced secondary metabolites from the selected plant parts

Total soluble sugar was found to be 31±2.02 mg/gdw, 49±1.20 mg/gdw, 29±1.52 mg/gdw, 35±0.57 mg/gdw and 19±1.85 mg/gdw in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Starch was found to be 18±2.33 mg/gdw, 21.6±1.33 mg/gdw, 10.8±0.88 mg/gdw, 19.8±2.08 mg/gdw, and 37.8±1.45 mg/gdw in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Protein was found to be 75.87±3.84 mg/gdw, 114±4.84 mg/gdw, 112±3.28 mg/gdw, 24 ±1.15 mg/gdw, and 42±1.73 mg/gdw in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Lipid was found to be present as 21±2.88 mg/gdw, 22.5±2.18 mg/gdw, 10±2.30 mg/gdw, 7±3.46 mg/gdw, and 10±2.51 mg/gdw in *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Phenol was found to be present as 3.5±0.33 mg/gdw, 23.75±1.00 mg/gdw, 2±1.66 mg/gdw, 19.125±2.00 mg/gdw, and 9.62±3.21 mg/gdw in *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Ascorbic acid was found to be present as 0.35±0.12 mg/gdw, 0.59±0.27 mg/gdw, 0.66±0.32 mg/gdw, 2.65±0.22 mg/gdw, and 0.29±0.20 mg/gdw in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Total flavonoids were recorded to be 2.915±0.18 mg/gdw (1.59 mg/gdw bound flavonoids & 1.325 mg/gdw free flavonoids), 5.15±0.08 mg/gdw (3.13 mg/gdw bound flavonoids & 2.02 mg/gdw free flavonoids), 3.2±0.04 mg/gdw (1.61 mg/gdw bound flavonoids & 1.59 mg/gdw free flavonoids), 4.45±0.09 mg/gdw (2.43 mg/gdw bound flavonoids & 2.02 mg/gdw free flavonoids), and 4.99±0.11 mg/gdw (2.38 mg/gdw bound flavonoids & 2.61 mg/gdw free flavonoids) in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Alkaloids were recorded to be 6.1±1.1 mg/gdw, 8.4±0.11 mg/gdw, 2.76±0.07 mg/gdw, 6.42±0.09 mg/gdw, and 3.55±0.02 mg/gdw in stem bark of *Syzygium cumini, Bombax ceiba, Holoptelia integrifolia, Pscidium guajava, and Pongamia pinnata* respectively.

Maximum total flavonoids were found to be in stem bark of *Bombax ceiba*, bound flavonoids were found to be maximum in stem bark of *Bombax ceiba* while free flavonoids were found to be maximum in stem bark of *Pongamia pinnata.*

Maximum alkaloids were found to be in stem bark of *Bombax ceiba*.

Table 2: Quantitative estimation of various metabolites in the selected plant parts.

S.N.	Name of plant	Total soluble sugar	Starch	Protein	Lipid	Phenol	Ascorbic acid
1.	*Syzygium cumini*	31±2.02	18±2.33	75.87±3.84	21±2.88	3.5±0.33	0.35±0.12
2.	*Bombax ceiba*	49±1.20	21.6±1.33	114±4.84	22.5±2.18	23.75±1.00	0.59±0.27
3.	*Holoptelia integrifolia*	29±1.52	10.8±0.88	112±3.28	10±2.30	2±1.66	0.66±0.32
4.	*Pscidium guajava*	35±0.57	19.8±2.08	24 ±1.15	7±3.46	19.125±2.00	2.65±0.22
5.	*Pongamia pinnata*	19±1.85	37.8±1.45	42±1.73	10±2.51	9.62±3.21	0.29±0.20

Note*: All values are in mg/gdw (milligram per gram of dry weight of plant

Table 3: Quantitative estimation of extracted secondary metabolites from the selected plant parts.

S.N.	Name of plant	Total flavonoids (mg/gdw)	Bound flavonoids (mg/gdw)	Free flavonoids (mg/gdw)	Alkaloids (mg/gdw)
1.	*Syzygium cumini*	2.915±0.18	1.59	1.32	6.10±1.1
2.	*Bombax ceiba*	5.15±0.08	3.13	2.02	8.40±0.11
3.	*Holoptelia integrifolia*	3.2±0.04	1.61	1.59	2.76±0.07
4.	*Pscidium guajava*	4.45±0.09	2.43	2.02	6.42±0.09
5.	*Pongamia pinnata*	4.99±0.11	2.38	2.61	3.55±0.02

Note*: all values are in mg/gdw.

Results of GC-MS analysis

Result of GC-MS studies of methanolic extracts of stem bark of *Syzygium cumini, Holoptelia integrifolia, Pongamia pinnata, Pscidium guajava,* and *Bombax ceiba* are shown in Table 4-8 respectively and Chromatographs for these plants are shown in Figure 3-7 respectively.

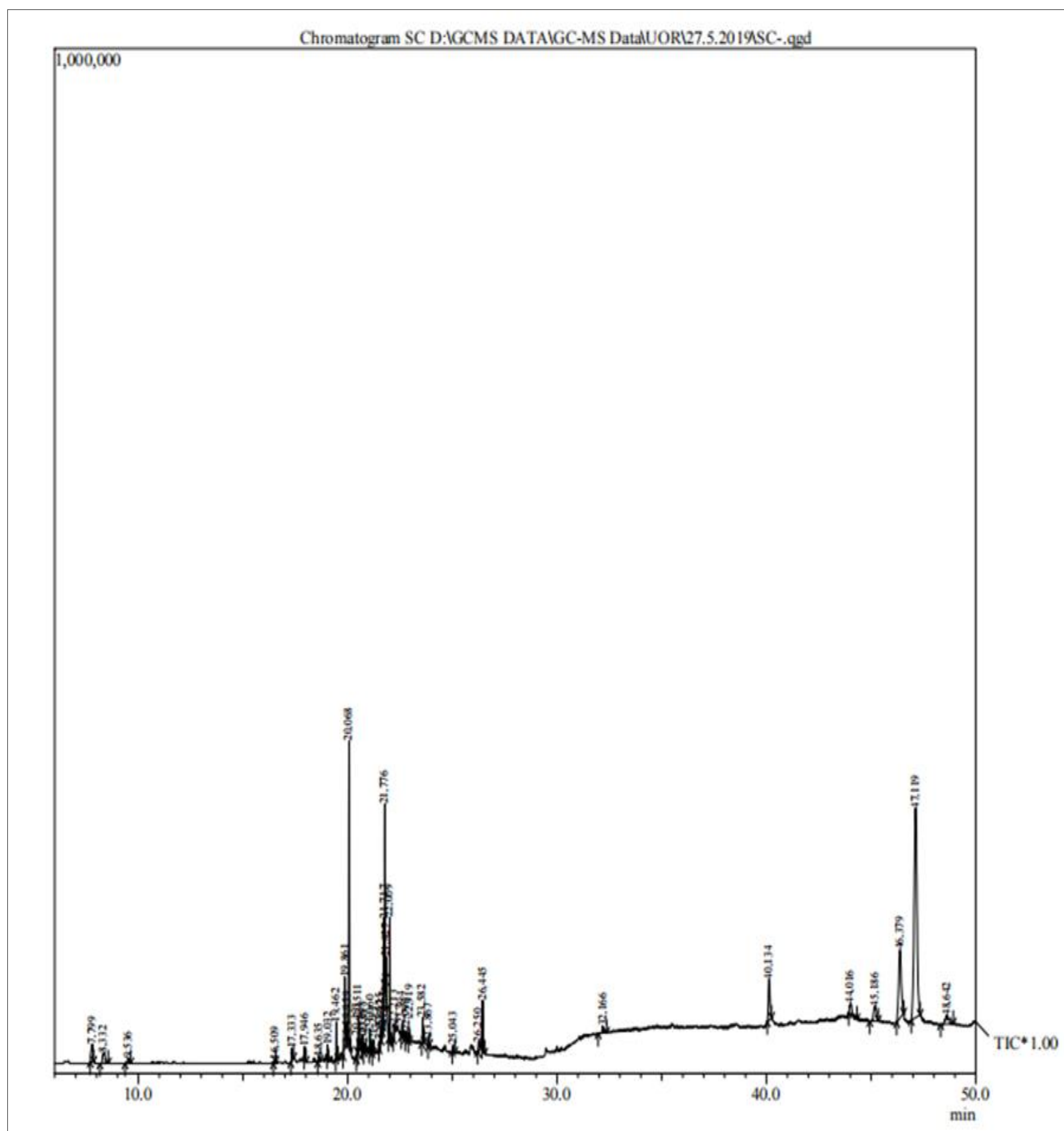

Fig 3: Chromatogram of methanolic extract of *Syzygium cumini* by GC-MS study

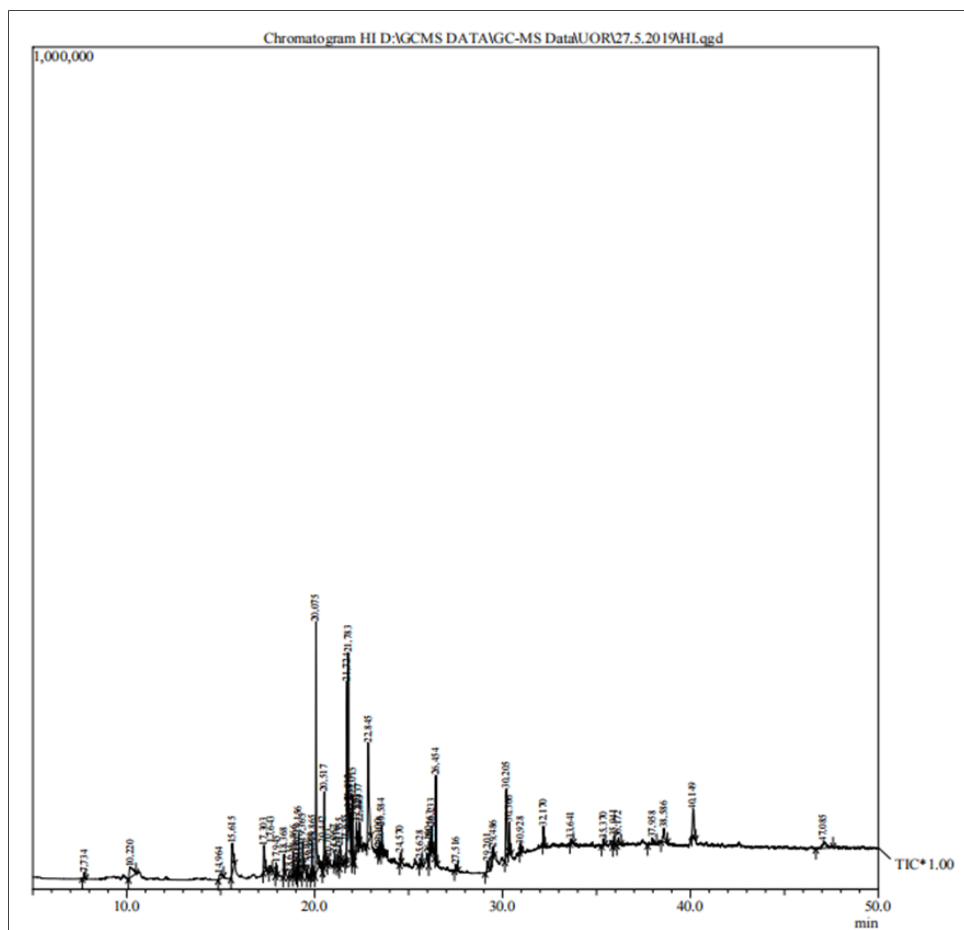

Fig 4: Chromatogram of methanolic extract of *Holoptelia integrifolia* by GC-MS study

Fig 5: Chromatogram of methanolic extract of *Pongamia pinnata* by GC-MS study

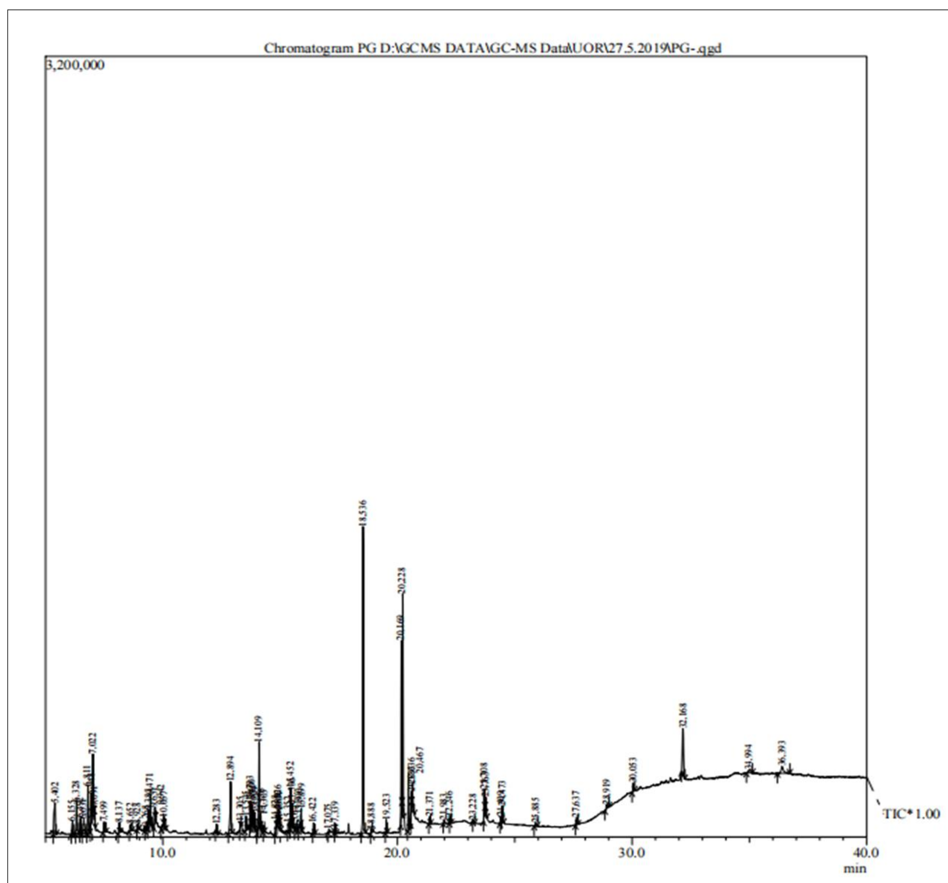

Fig 6: Chromatogram of methanolic extract of *Pscidium guajava* by GC-MS study

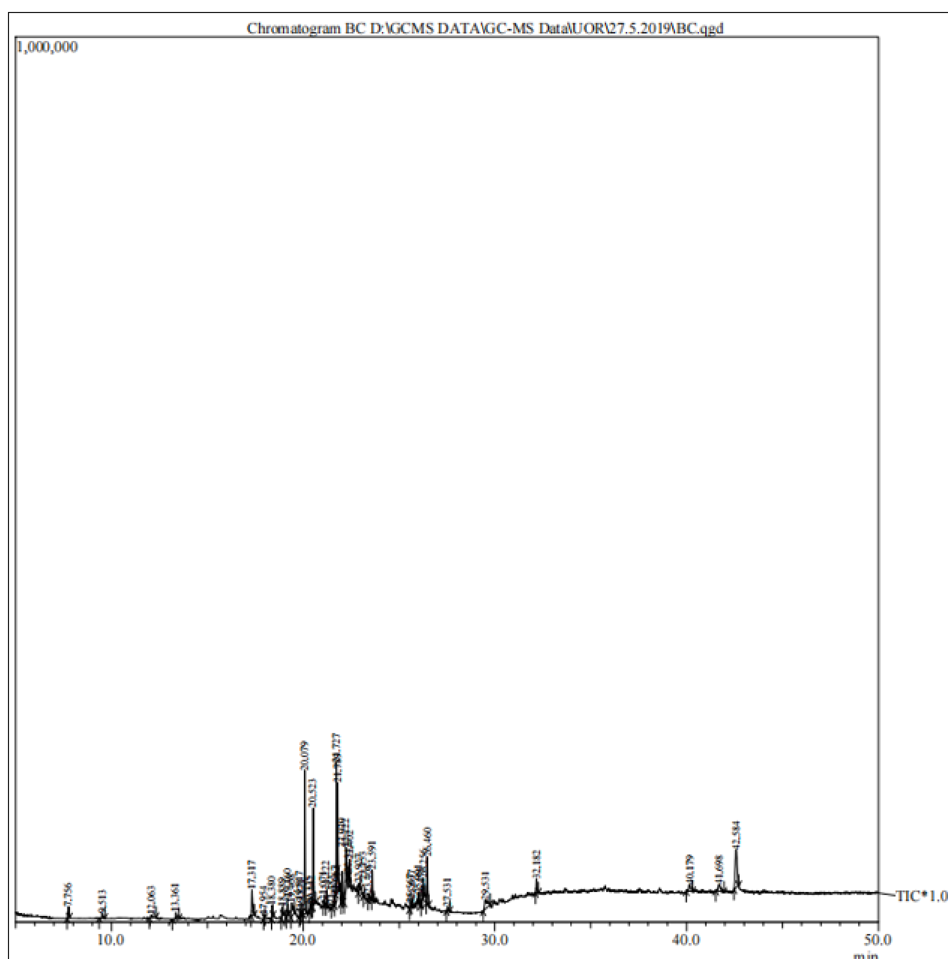

Fig 7: Chromatogram of methanolic extract of *Bombax ceiba* by GC-MS study

In *Syzygium cumini*, about 15 compounds were found mainly out of which Friedelan-3-one (34.58 %), Hexadecanoic acid, methyl ester (11.88 %), and (2, 2, 6-Trimethyl-bicyclo [4.1.0] hept-1-yl)-methanol (10.15 %) were present abundantly.

In *Holoptelia integrifolia*, about 23 compounds were found to be present significantly out of which Hexadecanoic acid, methyl ester (11.95 %), and Phenol, 4, 4'-(1-methylethylidene) bis- (10 %) were found abundantly.

In *Pongamia pinnata*, about 23 compounds were found to be present significantly out of which Zingiberene (21.37%), beta.-Sesquiphellandrene (12.39 %), and Ar-Curcumene (10.78 %) were found abundantly.

In *Bombax ceiba*, 15 compounds were found mainly out of which Pentadecanoic acid, 14- methyl-, methyl ester (12.60 %), 9,12-Octadecadienoic acid, methyl ester (7.67 %), Z-10-Pentadecen-1-ol (7.47 %), and 8,11,14-Docosatrienoic acid, methyl ester (7.30 %) were present significantly.

In *Pscidium guajava*, about 25 compounds were found to be present mainly out of which Hexadecanoic acid, methyl ester (15.87 %), 11-Octadecenoic acid, methyl ester (9.93 %), and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (7.89 %) were present significantly.

In the present investigation, it was found that the stem bark of the selected plants, possess various phytochemicals. As the selected plants have been identified previously to possess various pharmacological uses (Santosh Kumar, 2018; Prashanth G.K., *et al*., 2014; Shaukat Khalid *et al*., 2013; Ekeleme Kenneth *et al*., 2017; Chopade V.V., *et al*., 2012) and these effects might be due to presence of these phytochemicals.

Table 4: Name of compounds found in methanolic extract of *Syzygium cumini* by GC-MS studies.

Peak #	R.Time	Area	Area %	Name
1	7.799	98935	1.73	DODECANE, 1,1-DIFLUORO-
2	8.332	73706	1.29	CYCLOHEXENE, 1,4-DIMETHYL-4-VINYL-
3	19.462	75790	1.32	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER
4	19.861	212700	3.71	METHYL 9-OCTADECENOATE
5	20.068	680825	11.88	Hexadecanoic acid, methyl ester
6	20.511	73143	1.28	HEXADECANOIC ACID
7	21.717	136316	2.38	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER
8	21.776	295448	5.16	9-OCTADECENOIC ACID, METHYL ESTER, (E)-
9	22.009	236728	4.13	OCTADECANOIC ACID, METHYL ESTER
10	23.582	66168	1.16	CHLOROMETHYL 2-CHLORODODECANOATE
11	26.445	141128	2.46	1,2-BENZENEDICARBOXYLIC ACID
12	40.134	221587	3.87	Ethyl iso-allocholate
13	45.186	132674	2.32	8-NITRO-12-TRIDECANOLIDE
14	46.379	581714	10.15	(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol
15	47.119	1980735	34.58	Friedelan-3-one

Table 5: Name of compounds found in methanolic extract of *Holoptelia integrifolia* by GC-MS studies.

Peak #	R.Time	Area	Area %	Name
1	10.220	172130	2.98	1-Butanol, 3-methyl-, formate
2	15.615	208021	3.60	.beta.-D-Glucopyranose, 1,6-anhydro-
3	17.303	135769	2.35	BENZENE, 1,1'-[OXYBIS(METHYLENE)]BIS-
4	18.866	91902	1.59	BENZENEMETHANOL, 2-(PHENYLMETHYL)-
5	19.156	62684	1.08	Neophytadiene
6	19.365	140321	2.43	Benzenemethanol, 4-(phenylmethyl)-
7	19.865	75883	1.31	7-Hexadecenoic acid, methyl ester, (Z)-
8	20.075	690944	11.95	Hexadecanoic acid, methyl ester
9	20.517	217431	3.76	n-Hexadecanoic acid
10	21.355	65333	1.13	2-PHENYL-3-BENZOYLMETHYL-3,4-DIHYDRO-QUINOXALINE
11	21.724	298479	5.16	9,12-Octadecadienoic acid, methyl ester
12	21.783	304684	5.27	8,11,14-Docosatrienoic acid, methyl ester
13	22.015	153410	2.65	OCTADECANOIC ACID, METHYL ESTER
14	22.220	163630	2.83	17-Octadecynoic acid
15	22.845	578058	10.00	Phenol, 4,4'-(1-methylethylidene)bis-
16	23.584	98482	1.70	Nonanoyl chloride
17	26.233	79517	1.38	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
18	26.454	288640	4.99	Di-n-octyl phthalate
19	29.201	61381	1.06	4-tert-pentylphenol, trifluoroacetate ester
20	30.366	124824	2.16	4-(N-METHYLAMINO)-2-PHENYL-6-N-PROPYLPHENOL
21	32.170	78283	1.35	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
U	38.586	97726	1.69	2-Nonyn-1-ol
23	40.149	194899	3.37	Ethyl iso-allocholate

Table 6: Name of compounds found in methanolic extract of *Pongamia pinnata* by GC-MS studies.

Peak #	R.Time	Area	Area %	Name
1	7.023	930990	1.01	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHENYL)-, (S)-
2	13.622	9896286	10.78	Ar-Curcumene
3	13.791	19607668	21.37	Zingiberene

4	13.869	4203234	4.58	alpha.-(Z,E)-Farnesene
5	13.948	4755255	5.18	.beta.-Bisabolene
6	14.162	11368994	12.39	beta.-Sesquiphellandrene
7	14.510	1867831	2.04	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R (1.alp
8	14.588	2025012	2.21	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-
9	15.264	1286434	1.40	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol
10	15.539	953757	1.04	Viridifloral
11	15.827	1391156	1.52	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R (2.alpha.,4
12	16.102	1224100	1.33	7-epi-trans-sesquisabinene hydrate
13	16.217	1852137	2.02	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-
15	20.648	3608018	3.93	cis-9-Hexadecenal
16	24.476	952605	1.04	1,2-BENZENEDICARBOXYLIC ACID
17	25.970	2084521	2.27	2-[5-(2-METHYL-1,3-BENZOXAZOL-7-YL)-1H-PYRAZOL-3-YL]PHENOL
18	29.344	1412092	1.54	3H-Pyrazol-3-one, 4-[[4-(diethylamino)phenyl]imino]-2,4-dihydro-5-methyl-2-phenyl-
19	31.055	2680175	2.92	5,7-Dimethoxy-3-(3,4-methylenedioxyphenyl)-4H-chromen-4-one
20	31.925	1553199	1.69	Cholest-5-en-3-ol, (3.alpha.
21	34.775	2316856	2.52	N,N'-Bis(fluoren-9-ylidene) hydrazine
22	36.438	2100585	2.29	5-Methoxy-2,3-dimethyl-7,9-diphenyl-1H-pyrrolo[2,3-f]quinoline
23	38.443	1763553	1.92	Lupeol

Table 7: Name of compounds found in methanolic extract of *Bombax ceiba* by GC-MS studies

Peak #	R.Time	Area	Area %	Name
1	7.756	43611	1.42	BUTYL 2,4-DIMETHYL-2-NITRO-4-PENTENOATE
2	17.317	116960	3.80	BENZENE, 1,1'-[OXYBIS(METHYLENE)]BIS-
3	18.380	32307	1.05	PHENOL, 2,4,5-TRIMETHYL-
4	20.079	387823	12.60	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER
5	20.523	281778	9.15	n-Hexadecanoic acid
6	21.222	37120	1.21	HEXADECANOIC ACID, TRIMETHYLSILYL ESTER
7	21.727	236241	7.67	9,12-Octadecadienoic acid, methyl ester
8	21.789	224768	7.30	8,11,14-Docosatrienoic acid, methyl ester
9	22.020	72889	2.37	OCTADECANOIC ACID, METHYL ESTER
10	22.222	229870	7.47	Z-10-Pentadecen-1-ol
11	22.402	68520	2.23	2-AMINOETHANETHIOL HYDROGEN SULFATE (ESTER)
12	23.591	89135	2.90	1-TRIDECANOL
13	26.256	85346	2.77	CHLOROMETHYL 2-CHLORODODECANOATE
14	26.460	153215	4.98	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
15	42.584	301028	9.78	Thunbergol

Table 8: Name of compounds found in methanolic extract of *Pscidium guajjava* by GC-MS studies

Peak #	R.Time	Area	Area %	Name
1	5.402	500887	3.24	BICYCLO[3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL-
2	6.328	333366	2.16	1,6-OCTADIENE, 7-METHYL-3-METHYLENE-
3	6.631	235885	1.53	1,3-CYCLOHEXADIENE, 2-METHYL-5-(1-METHYLETHYL)-
5	6.811	520880	3.37	1,3-CYCLOHEXADIENE, 1-METHYL-4-(1-METHYLETHYL)-
6	6.948	315671	2.04	BENZENE, METHYL(1-METHYLETHYL)-
7	7.022	660530	4.28	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHENYL)-, (S)-
8	9.383	154757	1.00	CYCLOHEXANOL, 5-METHYL-2-(1-METHYLETHYL)-, [1R-(1.ALPHA.,2.BETA.,5.AL
9	9.471	255854	1.66	CYCLOHEXANOL, 5-METHYL-2-(1-METHYLETHYL)-, [1R-(1.ALPHA.,2.BETA.,5.AL
11	12.894	573408	3.71	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9
12	13.545	187296	1.21	1H-CYCLOPROP[E]AZULENE, DECAHYDRO-1,1,7-TRIMETHYL-4-METHYLENE-, [1
13	13.733	161498	1.05	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta
14	14.109	860132	5.57	NAPHTHALENE, 1,2,3,5,6,8A-HEXAHYDRO-4,7-DIMETHYL-1-(1-METHYLETHYL)-,
15	15.452	322624	2.09	Junenol
16	15.536	161312	1.04	(-)-GUAIOL
17	18.536	2450980	15.87	Hexadecanoic acid, methyl ester
18	20.169	1217721	7.89	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
19	20.228	1532918	9.93	11-Octadecenoic acid, methyl ester
20	20.467	429154	2.78	Methyl stearate
21	20.587	161498	1.05	9,12-Octadecadienoic acid (Z,Z)-
22	20.636	169822	1.10	HEPTADECENE-(8)-CARBONIC ACID-(1)
23	23.708	203324	1.32	1,8,11-Heptadecatriene, (Z,Z)-
24	32.168	673385	4.36	STIGMAST-5-EN-3-OL, OLEAT
25	36.393	183592	1.19	Cholesterol, chloroformate

Conclusion

By using results of the present study, specific compound/compounds can be identified for the specific effect by isolating and evaluating them. So, this study may pave a path in pharmacological industry to use

phytochemicals isolated from stem bark of these tree against various illness alone or in combinatorial therapy.

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