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Chemical characterization of *Caesalpinia sappan* L. for natural dye

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Abstract
Caesalpinia sappan L. is found to be the one of widely used plant dye for its red color. The Gas Chromatography and Mass spectroscopy (GC-MS) analysis of Brazil wood is found to contain two major dyestuff components namely Brazilin and Brazilein. The major dyestuff components of *Caesalpinia sappan are* Brazilin and Brazilein. The major coloring component of the *Caesalpinia sappan L.* is Brazilin but Brazilein which is the oxidized product of Brazilin. The molecular weight of Brazilin is found to be 286 has been found to be the major dyestuff component of the extract, however this component can be easily oxidized by air and light to produce Brazilein with a molecular weight of 284 (Rosenburg, 2008). The sample undertaken for this study are found to contain Brazilin and Brazilein which confirms that *Caesalpinia sappan* is a potential source of dye which can replace the synthetic dyes in textile industry. *Caesalpinia sappan* L. is a potential source of dye with good dye stuff components so it can be used in textile industry as a natural dye. The extraction of dye from *Caesalpinia sappan* can be done easily and a wide spectrum color range is also available which will be helpful to replace the synthetic dyes. This study may give information to the possible production of natural dyestuff component called Brazilin from *Caesalpinia sappan* in a large scale as a source of natural dye.

Keywords: *Caesalpinia sappan* L, natural dye, brazilin pigments

Introduction
Plants gives medicines, edible and non-edible oils, gums, resins and oleo-resins, perfumery oils, spices, insecticides, tannins and of course coloring matters known as dyes apart from wood which is major forest produce. The history of natural dye is about more than 4000 years ago. Natural dyes find use in the coloring of textiles, drugs, cosmetics, etc. Owing to their nontoxic effects, they are also used for coloring various food products. In India, there are more than 450 plants that can yield dyes. It has been recorded by ancient writers that there were at one time nearly a thousand different natural sources of dyes has the trade in dyes stuffs improved in the ancient world, there evolved a limited group of dye stuffs considered most desirable of these, there were about nine colors the most practical. There were indigo and blue cochineal and madder red log wood fustic, weld, safflower and yellow of these logwood is the only dye still being used commercially. The use of natural dyes is the safest way because they are not only eco-friendly but also prevent environmental pollution. Natural dyes are biodegradable, non-toxic, aesthetically appealing and may serve a better alternative to generate employment and utilize the wastelands. Natural dyes are obtained from plant, animal and mineral resources. Nowadays, fortunately, there is increasing awareness among people towards natural products. Due to their non-toxic properties, low pollution and less side effects, natural dyes are used in day-to-day food products.
Caesalpinia sappan is a species of flowering tree in the legume family, Fabaceae, that is native to Southeast Asia and the Malay archipelago. Red wood is locally abundant throughout the Philippines, India through Burma, Thailand, Indo-China, southern China to Malaysia at low and medium altitudes in dry thickets. It is originally produced from the Malay Archipelago and Southeast Asia. Indian Red wood Introduced, and probably of prehistoric introduction. Medicinally it refers to the dried heartwood of *Caesalpinia sappan* L. *Caesalpinia sappan* is an exotic throughout Indonesia, Papua New Guinea, Philippines, Solomon Islands, Sri Lanka, Taiwan, Province of China, United States of America. It is also a relative of Brazil wood (*Caesalpinia echinata*) and they are under a same genus. Hence, other common names of it include Sappan wood, "brazil wood", East Indian redwood, Sapanwood, Suou, Chekke

Sappanga, Sumu, sappan lignum, and so on. Common namesinclude Sappanwood, Sapanwood, PatangaChekkeSappanga (Kannada), Pathimukham (Malayalam),and Suō (JapaneseSappan wood (Engl.), Brazil wood (Engl.) Bukkum wood (Engl.) False sandalwood (Engl.) Indian redwood (Engl.) Su fang mu (Chin.) Trade name (sappan lignum, brazilin, sappanwood)

Sappan wood belongs to the same genus as Brazil wood (*C. echinata*), and was originally called "brezel wood" in Europe. The wood is somewhat lighter in color than Brazilwood and its other allies, but the same tinctorial principle appears to be common to all.

Under natural conditions *C. sappan* grows mostly in hilly areas with clayey soil and calcareous rocks at low and medium altitudes. In Peninsular Malaysia it grows best on sandy riverbanks. It does not tolerate too wet soil conditions. Biophysical limits *C. sappan* is Mean annual temperature: 24-28 deg C. Mean annual rainfall: 700-4 300 mm; Soil type: *C. sappan* tolerates sand and slope and soil pH of 5-7.5. Sapang is a small tree, 3 to 5 meters high, with scattered spines. trunk up to 14 cm in diameter; bark with distinct ridges and many prickles, greyish brown; young twigs and buds hairy, brownish. Leaves are compound, up to 50 centimeters long. This is a small evergreen tree, with a few small thorns and bipinnate, stipulate, alternate, 20-45(-50) cm long, 10-20 cm broad, with 8-16 pairs of up to 20 cm long pinnae; pinnae with prickles at the base and with 10-20 pairs of oblong, 10-20 mm x 6-10 mm long, subsessile leaflets, very oblique at base, rounded to emarginated at apex. Pinnae are about 20, opposite, and 10 to 20 centimeters long. Leaflets are 20 to 30, obliquely oblong to oblong-rhomboid. Flowers in terminal panicles, racemes pubescent, primary penducles 30- 40 cm long, the flowering 9-15 cm long, bracts ovate-acuminate, about 6 mm long, flowers fragrant, 2-3 cm long, 5-merous; sepals glabrous, petals pubescent, the superior one smaller; calyx tube 3 mm long; corolla yellow, uppermost lobes cuneate, other obovate, all clawed and gland-punctate; stamens 10, filaments densely tomentose in the lower half; ovary superior, pubescent. Flowers are yellow, on terminal panicles, 2 to 2.5 centimeters in diameter with densely wooly filaments. It blooms from June to September each year. Yellow flowers have 5 petals and form panicles. Flowering can occur after 1 year of growth and usually during the rainy season, fruiting about 6 months later. The tree flowers in August in Myanmar and in Indonesia pods are produced 13 months after planting.

Initially *C. sappan* grows straight but at about 2.5 m height. For use as dyewood the tree is harvested every 6-8 years and for firewood every 3-4 years when the Trunk has attained 5-6 cm diameter. The wood is used for firewood and its energy value is about 25 000 kJ/kg. Timber: The tree is the source of the commercial redwood or Brazil wood. Sapwood is white, heartwood makes up to 90% of the total volume, is yellow or deep orange when fresh turning to dark red. The wood is straight grained with a fine to moderately fine texture, fairly heavy (600-780 kg/m^3), hard and lustrous. It is difficult to dry and susceptible to warping and collapse, but moderately easy to work; it takes high finish and is tough and resistant to termite attack. It is used for inlaying work, cabinet making, violin bows and for walking sticks. Gum or resin: The stem produces a gum. Tannin or dyestuff: The heartwood yields a valuable red crystalline dye, brazilin, used on cotton, silk and wool fabrics. Bakam gives bright red and violet shades, and with garcine produces a chocolate tint. Bark and pods yield similar dyes, pods contain ca. 40% tannin used for production of light leather goods. Roots give a yellow dye. Essential oil: Leaves contain a pleasant smelling volatile oil.

Caesalpinia sappan is found to be a potential source of natural dye. There is a growing demand for eco-friendly and nontoxic colorants. Since natural dyes are biodegradable and less toxic and allergenic than synthetic dyes, dyes derived from natural sources are regaining popularity for applications not only in coloration of textiles, but also as food ingredients and cosmetics. It is reported that sappan tree barks and heartwood are rich in tannin and can produced for use in historical paintings and Textiles. This plant is being used worldwide for various purposes including medicinal properties. The heartwood is found to be cytotoxic, antitumor, anti-microbial, anti-viral, immune stimulant etc. The identification and characterization of *Caesalpinia sappan* as a potential source of dye is found to be relevant to this modern era. The present study deals to know the dyeing properties and chemical characterization of *Caesalpinia sappan* for natural dye.

Materials and Methods

Location

The Brazilin dye extraction experiment was conducted at Forest College and Research Institute, Mettupalayam, located at $11^0$19’N latitude and $77^0$56’E longitude and at a an altitude of 300m MSL, and GC-MS analysis was conducted at The South India Textile Research Association, Coimbatore located at 11.0375654 latitude and 77.0371202 longitude

Weather and Climate

The climate is semi-arid tropical type with hot summer and cold winter. The dry season starts from early February to mid June and wet season from mid-august to early November. The mean annual rainfall in the region is 895 mm distributed over 49 rainy days with north east monsoon contributing to 60 percent and the balance through summer showers and southwest monsoon (Swaminathan *et al.*, 1991). The mean maximum and minimum temperatures are 30 °C and 20.5 °C respectively.

Targeted Species

Study is restricted to one targeted species *Caesalpinia sappan* that are commonly available in various parts of Kerala and North eastern India. This targeted species considered as treatment.

Extraction Methods

Soxhlet Apparatus

The method described by soxhlet in 1879 is the most commonly used method for extraction of dye from plant material. According to soxhlet‘s procedure, dye are extracted from repeated washing with an organic solvent usually hexane or petroleum ether, under reflux in special glass ware. In this method the sample is dried, grounded into smaller particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below the condenser. The flask is heated and solvent evaporates and moves up into the condenser where it is converted into liquid that trickles back down into boiling flask. At the end of extraction procedure which last a few hour the flask containing solvent is removed by evaporation remaining dye is calculated.

Chemicals Used

1. Tap water
2. Methanol

Extraction Methods – solvent extraction

The colour compounds in plants may vary in polarities depending on their chemical structure; therefore they need different solvents for extraction. So the parts are repeatedly extracted with various organic solvents. The least polar solvents (eg. carbon tetra chloride) are used first and then proceeded towards higher polar solvents (eg. n-butanol) in succession and finally with water. The water extract mostly contains the glycosides of the colouring principles along with other non-colouring organic compounds, minerals etc. The organic solvent extracts consist of the compounds in non-glycosidic form. The glycosidic linkage is opened up by hydrolysis of the water extracts with mineral acids to yield colour constituents (Sivakumaran, 1989) [27].

Natural dyes are extracted by boiling the dye yielding parts of the plant with water for appropriate period of time and pH. In the aqueous method, dyes can be extracted either in alkaline, neutral or acidic medium.

These are the extraction process are involved

Aqueous Extraction: Neutral medium

Alcoholic Extraction: Alcohol medium

Alcoholic Extraction: Alcohol medium

Natural coloring matters depending upon their nature can also be extracted by using organic solvents such as acetone, petroleum ether, chloroform, ethanol, methanol, or a mixture of solvents such as mixture of ethanol and methanol, mixture of water with alcohol, and so on. Feer (1891) reported extraction of finely powered madder with the treatment of six times its weight of alcohol, containing 10% HCL When the dissolution is complete, the alcoholic solution is concentrated and is precipitated by adding water. The precipitate is filtered and dried at low temperature.

Methanolic extract of the natural dye source is prepared by extracting the raw material in methanol in soxhlet for suitable time period and then evaporated under reduced pressure to make concentrated extract. Methanolic extract of Eucalyptus bark is extracted by supercritical extraction technique. This technique enhances mass transfer in supercritical fluid extraction by using modifier for successful extraction of natural products. Natural colourants such as quercetin, rhamnetin and other flavonoids are isolated by this technique. In a study, balsam flowers are extracted in aqueous as well as methanolic medium. Flowers are extracted in methanol for 4 hrs in soxhlet to yield the dye (Tiwari *et al*., 2000b) [20]. Annato dye is purified by soxhelation process using methanol at 55 °C for 36 hrs. After soxhelation, methanol is evaporated and the dye crystallized from methanol solution, dried and stored (Gulrajani *et al* 1999).

Natural dyes are extracted by boiling the dye yielding parts of the plant with water for appropriate period of time and pH. In the aqueous method, dyes can be extracted either in alkaline, neutral or acidic medium.

These are the extraction process are involved

Aqueous Extraction: Neutral medium

Aqueous Extraction: Neutral medium

Paul *et al*. (1996) reported dye extraction from dried rhizomes of turmeric using aqueous method of dye extraction. Same method is used for fruits of kapila. Dried rhizome/fruits are powdered and boiled in water for 45 min. The extract is filtered and used for dyeing. In a sjfcwiy Carina flowers are crushed and dissolved in distilled water and allowed to sitr in sonicator for quick extraction for lhour. (Ghorpade *et al*. 2006). 'Catechin is extracted from the dried and ground cutch in aqueous medium by adding cold water in it. The solution is filtered, the residue is dissolved in hot water and charcoal is added, the solution is boiled and is filtered hot. The filtrate is cooled when crystals of catechin crystallizes. The cochineal dye is obtained by boiling the cochineal insect in water or by placing on a hot oven or by exposing to sun. The latter method produces the highest quality dye. To produce 1 kg of dye 1,50,000 dried insects reared on 0.16 hectares of cactus are required (Glover & Pierce, 1993) [21]. For dye extraction from mesta calyx, four methods such as, aqueous, alkaline, acid and alcohol are tried by Katyayini & Jacob 1999 [22]': however aqueous method is found best to extract the pure form of dye the wood can be chipped and powdered and extracted under soxhlet apparatus.

5g of the coarsely powdered bark was used for the solvent extraction process. The 5g powdered bark was filled in the thimble of Soxhlet apparatus using methanol as a solvent. The process of extraction continued for an hour at 55 °C. The solvent extracts collected in airtight containers were preserved in refrigerated condition at 5 °C for further use

Chemical composition analysis

The Gas chromatography - Mass spectroscopy (GC-MS) analysis affords the advantage of identifying the chemical entities present, which constitutes the chemical picture of a plant extract and the whole mixture can be resolved in to individual components. GC-MS analysis of the methanol extract of *C. sappan* was performed using equipment with specications as follows

Equipment: THERMO GC - Trace Ultra Ver: 5.0, Thermo MS DSQ II

Column: DB 35 - MS Capillary Standard Non - Polar Column

Dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 µm

Carrier Gas: He, Flow: 1.0 ML/Min

Temp Prog: OVEN TEMP 70 C RAISED TO 260 C AT 6 C /MIN

Injection Volume: 1 Micro Liter

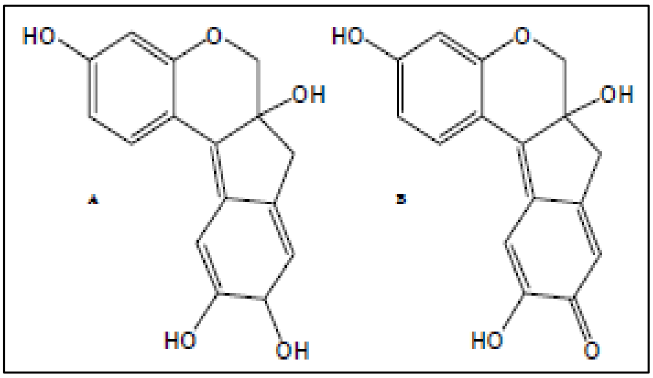
The dye stuff components in dried heart wood of *Caesalpinia sappan* were extracted with water, alkaline medium and acidic medium are feed to GC-MS (gas chromatography and mass spectroscopy) to analyze the chemical components of the heart wood (Lioe H.N. *et al*). The extract from the soxhlet apparatus is collected and analyzed under Gas Chromatography and mass spectroscopy for the chemical characterization. The extract fed in to the equipment and the total running time is of 42 minutes.

Results and Discussion

Brazil wood has been traditionally used as food and beverage colorant all around the world. The dried heartwood is widely used in oriental medicine, particularly against inflammation. The methanol extract were subjected to GC-MS analysis.

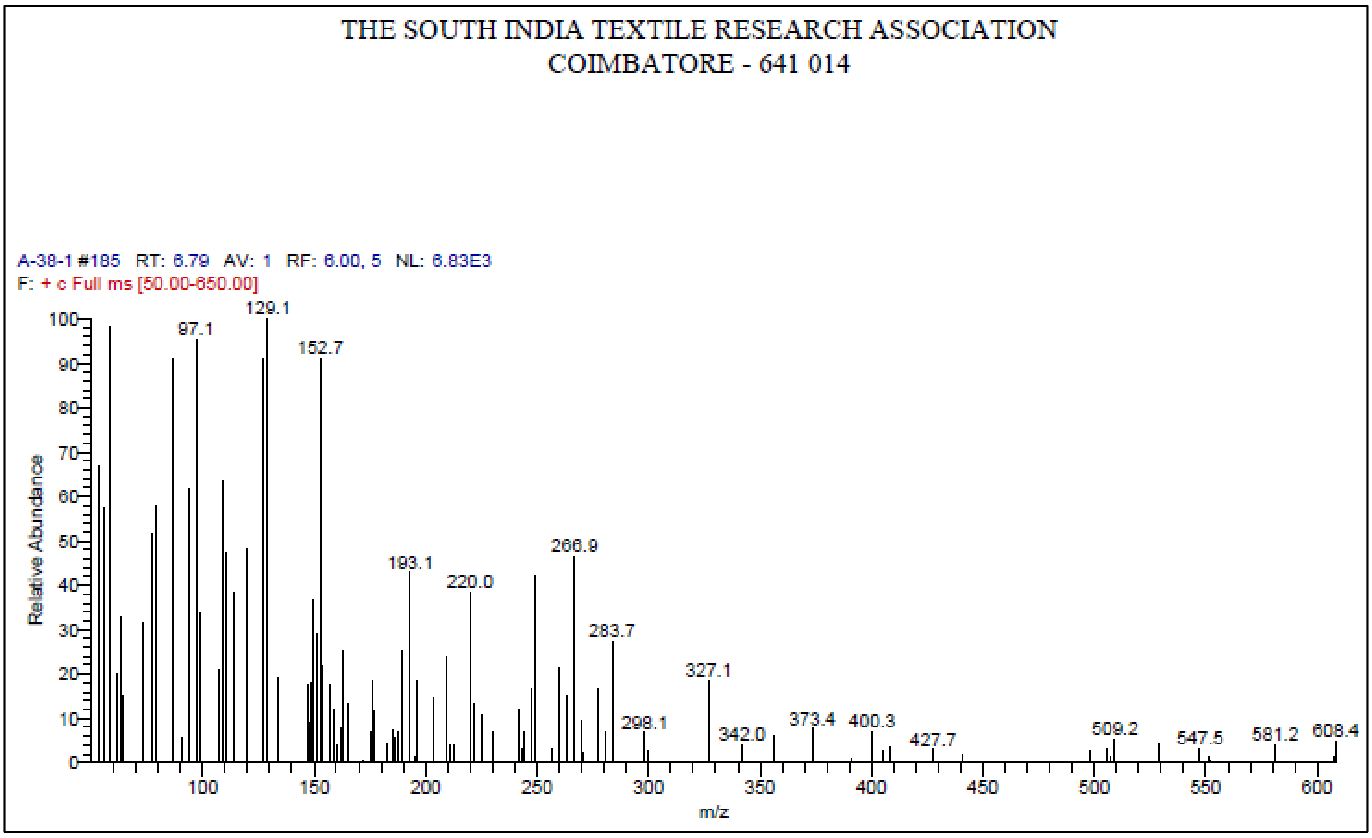
The methanol dye extract of brazil wood after crystallization has a reddish brown color. The color of the extract is varies from red to violet color (Kim *et al*., 1997; Ye *et al*., 2006) [23, 24]. The color pattern as follows Mephisto, Rose Soiree, Sundown Yesenrite, Onion Skin Pink, Mellow glow, Mineral r, Bois de rose, London smoke (Chandraprabha *et al*., 2005) [25]

The variation in colour pattern of Brazil wood is found to the presence of dye stuff component called "Brazilin and Brazilein ". The major coloring component of the *Caesalpinia sappan* L. is Brazilin but Brazilein which is the oxidised product of Brazilin. The molecular weight of Brazilin is found to be 286 has been found to be the major dyestuff component of the extract, however this component can be easily oxidized by air and light to produce Brazilein with a molecular weight of 284 (Rosenburg, 2008) [26].

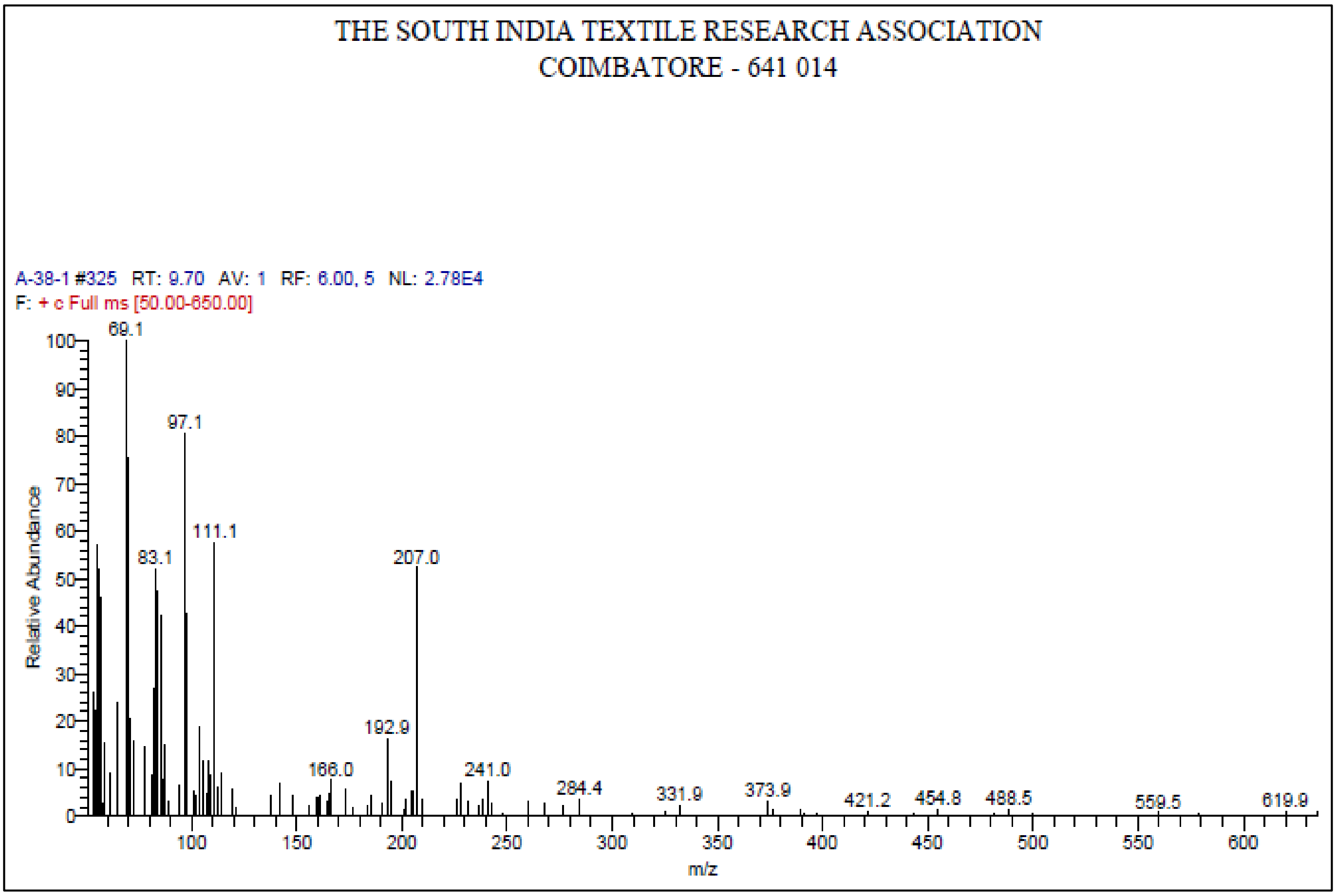


GC-MS analysis of *Caesalpinia sappan* L. were conducted at The South India Textile Research Association, Coimbatore shows that the sample also contains the major dye stuff component called Brazilein with a molecular weight of 284. The GC-MS generated graphs shows the peak value of molecular weight (Graph No: 1) 283.7 at the RT of 6.79. The Graph No: 2 shows the peak value with a molecular weight of 284.4 also confirm the presence of Brazilein which have a molecular weight of 284 at the Retention time of 9.70.

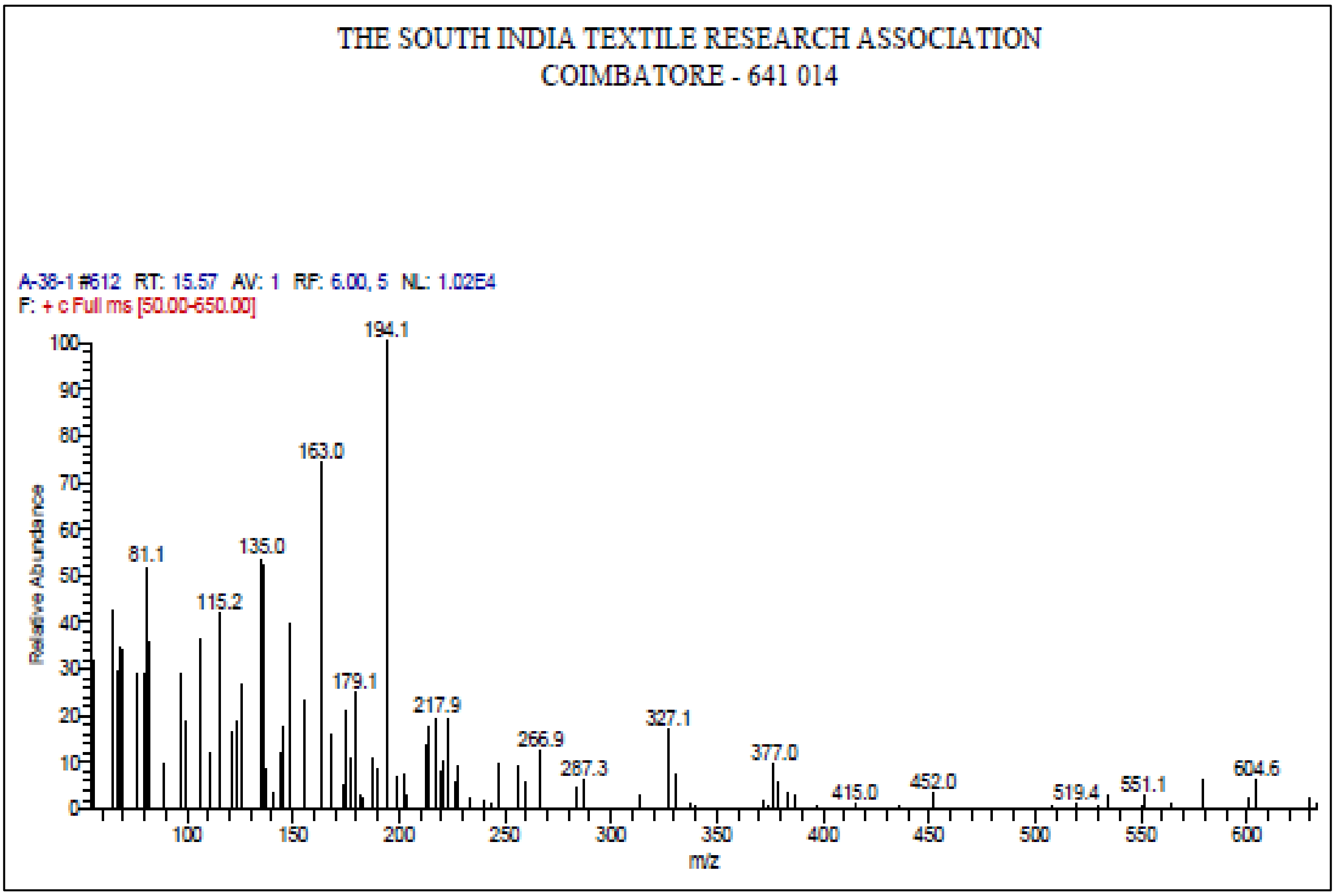
The Graph No: 3 shows the peak value of the molecular weight 287.3 i.e., the presence of Brazilin in the sample which is not oxidized at the Retention time of 15.57. Graph No:4 shows the peak value of molecular weight 283.8 which confirms the presence of Brazilein which have a molecular weight of 284 at the Retention time of 19.93.The Graph No:5 shows the peak value of molecular weight of 286.1 which confirms the presence of Brazilin of molecular weight 286 at the Retention time of 28.98. The Graph No: 6 confirm the presence of Brazilin with the peak of molecular weight of 284.2 at the retention time of 35.04. So the presence of Brazilin and Brazilein is confirmed in the sample subjected to study. The molecular weight of Brazilin and Brazilein is 284 and 286 respectively which confirmed through the various peaks in the graphs generated through GC-MS analysis of the sample subjected for study. *Caesalpinia sappan* L. is a potential source of dye with good dye stuff components so it can be used in textile industry as a natural dye. The extraction of dye from *Caesalpinia sappan* can be done easily and a wide spectrum color range is also available which will be helpful to replace the synthetic dyes. This study may give information to the possible production of natural dyestuff component called Brazilin from *Caesalpinia sappan* in a large scale as a source of natural dye



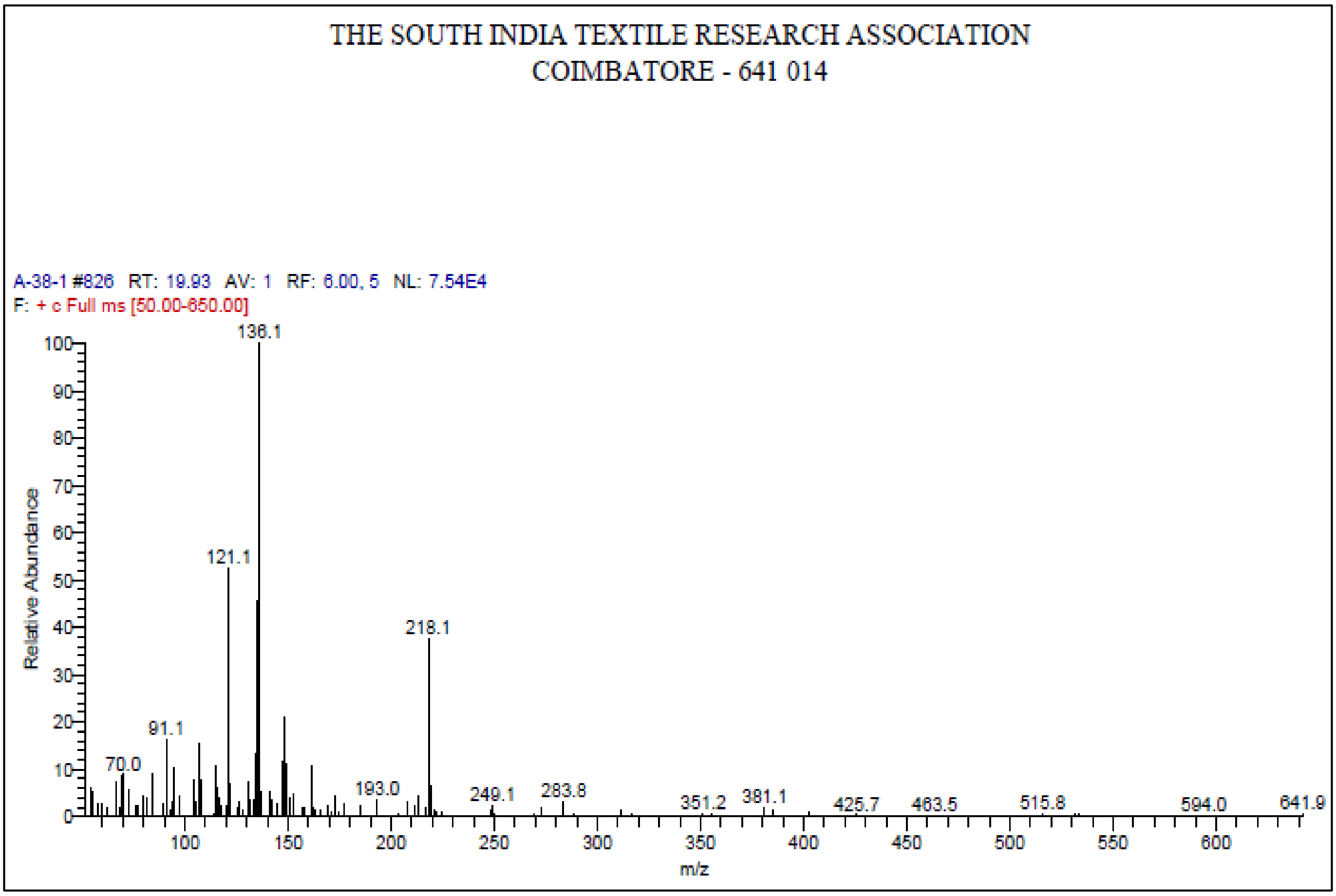
Graph 1: The presence of Brazilein at the peak of molecular Weight 283.7



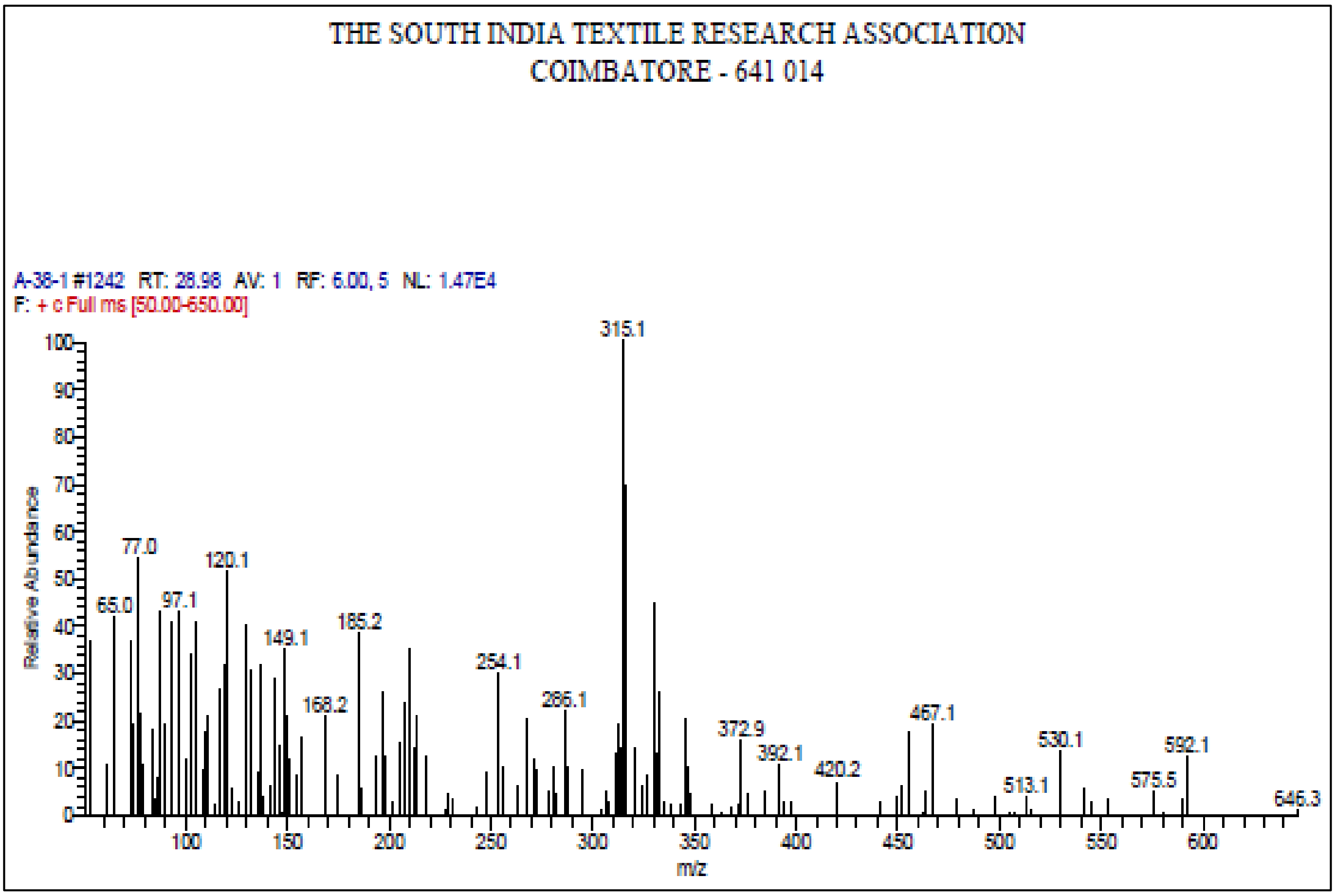
Graph 2: The presence of Brazilein at the peak of molecular Weight 284.4



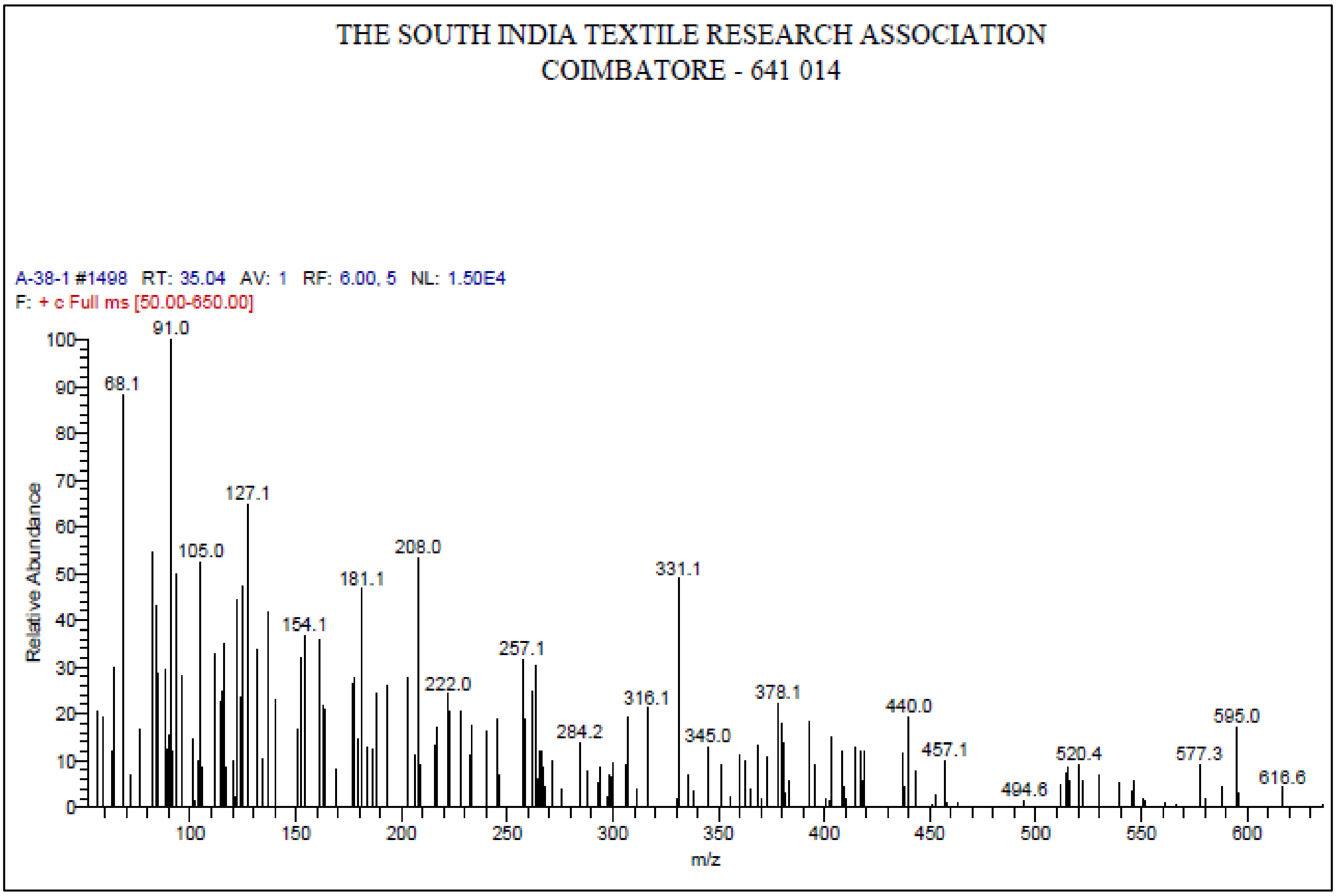
Graph 3: The presence of Brazilin at the peak of molecular Weight 287.3



Graph 4: The presence of Brazilein at the peak of molecular Weight 283.8



Graph 5: The presence of Brazilin at the peak of molecular Weight 286.1



Graph 6: The presence of Brazilein at the peak of molecular Weight 284.2

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