International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 12-09-2021, Accepted: 28-09-2021, Published: 13-10-2021
Volume 6, Issue 5, 2021, Page No. 1210-1212

Identification of phytochemicals in methanolic extract of *Caesalpinia sappan* L leaves by GC-MS analysis

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Abstract

Caesalpinia Sappan L belonging to the Fabaceae family and is well-known for its ethnomedicinal applications. The wood of the plant yields a red dye, which can be used for medicinal, cosmetic purposes and as a natural dyeing agent. In the present investigation methanolic extract of Caesalpinia, Sappan leaves were analyzed by GC –MS technique to identify important phytochemical constituents present in it. Twelve phytochemical components were identified by GC-MS chromatogram. Results of this study can be a way for the invention of newer herbal medicines from Caesalpinia Sappan leaves which can be used for several ailments.

Keywords: Caesalpinia Sappan leaves, Gas chromatography-mass spectrometry (GC–MS), Caryophyllene, epoxide, Aromadendrene oxide-(2), (-)-.Beta.-caryophyllene oxide, neophytadiene, (e)-Phytol

Introduction

Phytoconstituents are chemical compounds naturally present in the plants which protect plants from various diseases and predatory animals [1]. Synthesize of phytoconstituents in plants is by primary and secondary metabolism and may be grouped as active drug and inactive nondrug constituents. These potentially valuable chemical compounds can be used to produce drugs for different incurable diseases, important active components have been discovered from plants includes alkaloids, terpenoids, glycosides, gums and mucilage, volatile oils, tannins, and other essential constituents like vitamins, sugars, organic acids, lipids, and antibiotics [2].

The selected medicinal plant namely Caesalpinia sappan L belongs to the Fabaceae family. The tree is native to the Indo-Malayan region [3]. It is cultivated in parts of India. Caesalpinia Sappanan is used in Ayurveda as an emmenagogue, hemostatic, anti-inflammatory, and antidiarrhoeal agent. The heartwood of the plant is one of the chief constituents of indigenous preparation 'Lukol' which is orally administered for the treatment of non-specific leucorrhoea [4]. To stimulate menstrual flow and for a fast recovery for women after childbirth C. Sappan wood decoction is given as tonic. The sappan wood is also used in the treatment of thrombosis (blood clots within the vessel) and bruise [5]. The objective of this work is to determine the Phytochemicals found in the extract of Sappan leaves (methanolic) by GC-MS analysis. Recently GC-MS is used as one of the technical platforms for the fingerprint analysis of phytocomponents presents both in herbal and non-herbal species [6]. Knowledge of these phytochemical constituents can be utilized for the development of newer drug molecules that may be used for curing different ailments.

Fig 1: Caesalpinia Sappan

Materials and Methods

Plant materials

Caesalpinia Sappan leaves (Pathimugam in Malayalam) were collected from a single tree from Kottayam, Kerala, India. Identification and authentication were done (BSI/ SRC/ 5/ 23/ 2020/ Tech/63) by Dr. M.U. Sharief, The Scientist 'E' & Head of office, Botanical Survey of India (Southern region) Coimbatore.

Preparation of extracts

Caesalpinia Sappan leaves were collected and washed with water to discard all the possible contaminants present in them. Sappan leaves were dried in shade and powdered coarsely, and then the powder was extracted by continuous hot percolation using methanol. The obtained extract was dried (under reduced pressure) and reserved in a vacuum desiccator till further studies.

Extraction: The shade dried leaf powdered and extracted (100 g) successively with 600 ml each of petroleum ether, chloroform, ethyl acetate and methanol in a Soxhlet extractor for 20hr. All the extracts were preserved in a refrigerated condition till further use. These extractive values are shown in Table-1

Table 1: Phytochemical Analysis of Successive Extracts of Caesalpinia sappan leaves

Sl. No.	Phytoconstituents	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol
1	Alkaloids	-	-	-	-
2	Carbohydrates	-	-	-	+
3	Glycosides	-	-	-	+
4	Proteins &Aminoacids	-	-	-	-
5	Steroids	+	+	-	-
6	Saponins	-	-	+	+
7	Flavanoids	-	+	+	+
8	Tannins &Phenolics	-	-	+	+
9	Fixed oils	+	+	-	-
10	Triperpenoids	-	-	+	+

GC-MS analysis

From the phytochemical analysis, it is clear that all most all the phytoconstituents are present in the methanolic extract, so further GC-MS studies were conducted on methanolic extract of Caesalpinia sappan to identify the phytoconstituents are present in it.

Shimadzu GC-MS (Model Number: QP2010S) with GC – MS solutions software was used to carry out GC-MS profiling of C.Sappan leaves extract.Chromatographic conditions: Elite-5 MS column(fused silica) of 30 mm length, 0.25mm internal diameter and 0.25µ thickness was used. The carrier gas used was Helium at a flow rate of 1 ml /min and the injection volume of the sample was 1.0 microlitre. The oven temperature is maintained at 280^0 C. The total time taken for GC running was 50min. The relative percentage amount of each component was calculated by compairing the average peak area to the total area.

Identification of components

Detection of phytoconstituents was done using National Institute Standard and Technology (NIST II) and WILEY 8 library. For characterizing phytoconstituents, parameters such as comparison of retention time and peak, computer matching, and the characteristic fragmentation patterns of the mass spectra were used.

Phytochemicals detected from the chromatogram are shown in Table 2. The chromatogram (Figure:1) disclosed the presence of various compounds like Cadina 1,3,5,tetraene, caryophylleneepoxide,alpha.calacorene,aromadendreneoxide-(2),(-)betacaryophylleneoxide,14-methyl-8-hexadecyn-1-ol,1,2,3,4-tetrahydro-3isopropyl-5-methyloxonaphthaalene, retinolacetate, 3,5,6,12 tetra hydroxyl ergostan-25-ylacetate,neophyte diene, (e)-phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol**.**

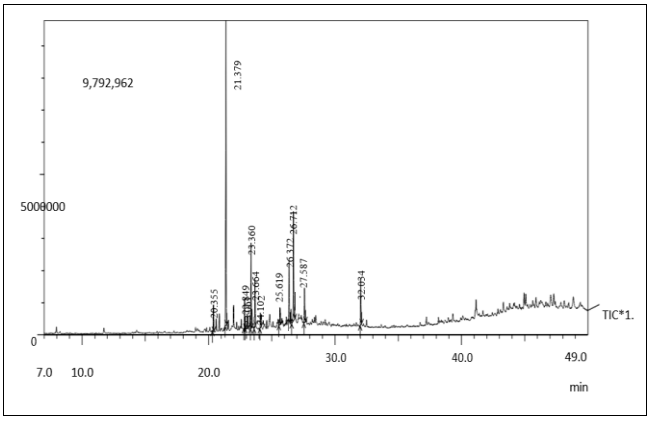

Fig 2: GC/MS profile of Caesalpinia Sappan leaves extract

Table 2: Components detected from GC/MS study of Caesalpinia Sappan leaves extract (in methanol)

Peak	Retention Time	Area%	Mol. formula	Molecular weight	Name
1	20.355	3.08	$C_{15}H_{20}$	200.325	Cadina-1,3,5,9-tetraene
2	21.379	36.52	$C_{15}H_{24}O$	220.35	aryophyllene, epoxide
3	22.849	3.72	$C_{15}H_{20}$	200.32	Alpha.-calacorene
4	23.061	2.12	$C_{15}H_{24}O$	220.35	Aromadendrene oxide-(2)
5	23.360	10.24	$C_{15}H_{24}O$	220.35	(-)-.beta.-caryophyllene oxide
6	23.664	5.72	$C_{17}H_{34}O$	254.5	14-methyl-8-hexadecyn-1ol
7	24.102	2.09	$C_{20}H_{22}N_4O_4$	382.4	1,2,3,4-tetrahydro-3 isopropyl-5-methyl-1-oxonaphthalene
8	25.619	2.80	$C_{22}H_{32}O_2$	328.5	Retinol acetate
9	26.372	8.22	$C_{30}H_{52}O_6$	508.7	3,5,6,12-tetrahydroxyergostan-25-yl acetate
10	26.712	15.18	$C_{20}H_{38}$	278.5	Neophytadiene
11	27.587	4.55	$C_{20}H_{40}O$	296.5	(e)-phytol
12	32.034	5.76	$C_{20}H_{40}O$	296.5	3,7,11,15-tetramethyl hexadec – 2-en-1-ol

Results & Discussion

GC/MS analysis of the Caesalpinia Sappan leaves methanolic extract detected 12 peaks which indicates that it contains twelve phytoconstituents. Terpenes are the chief constituents of C.sappan leaves and these got antioxidant [7, 8], anticancer [9, 10], wound healing property [11], anti-inflammatory [12], antimicrobial [8] and schistosomicidal activity [13]. Table 3 represents the activity of phytocomponents present in the extracts of Caesalpinia Sappan leaves.

Table 3: Pharmacological Activity of components present in the extract of C. Sappan leaves

Compound	Activity
Caryophyllene, Epoxide	Antifungal
Aromadendrene Oxide-(2)	Anticancer
(-)-.Beta.-Caryophyllene Oxide	Anticancer, Analgesic
Retinol, Acetate	Used in Retinitis Pigmentosa and Retinitis Pigmentosa.
Neophytadiene	An anti-inflammatory and antimicrobial agent
(E)-Phytol	Precursor for manufacturing synthetic Vitamin E & K1, schistosomicide.
3,7,11,15-Tetramethyl Hexadec – 2- EN-1-OL	Anticancer

Conclusion

GC-MS profiling of leaf extract of Caesalpinia Sappan showed that several active constituents having many biological activities can be extracted using the strong extractive power of methanol. These phytochemicals are found reasonably responsible for multi-therapeutic uses and various health disorders including cancer. The work is in progress to develop newer drug formulation from the plant and brighten the pharmacological profile of it in the arena of traditional medicine.

Acknowledgements

We would like to thank everyone who has contributed to this study.

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