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GC-MS analysis for the Determination of Chemical Constituents and Pharmacological Activities of Gum Karaya (*Sterculia urens* Roxb.,) Essential Oil

Chaitanya Darapureddy [1,*] K R S Prasad[2,*], Phani R S Ch[3]

[123]Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur District, Andhra Pradesh-522502

Abstract

Essential oils are produced by plants for many reasons including protection against various bacterial, fungal and viral infections. Numerous essential oils and their major constituents are known to exhibit promising pharmacological and antimicrobial activity and can therefore be a good source of biologically active molecules and/or fractions. It is generally accepted that a crude phytomedicine needs to be evaluated holistically and the research method best suited for this approach is metabolomics. In this study a non-targeted metabolomic approach was followed to explore the pharmacological and antimicrobial activity and chemistry of essential oil extracted from Gum Karaya (*Sterculia urens* Roxb.,). Essential oil was extracted by hydrodistillation and then analysed by gas chromatography coupled to mass spectrometry (GC–MS). The GC-MS analysis results confirms 30 compounds that accounted for 98.84 % of the oil, which included 6 compounds as major compounds. The major compounds identified in the essential oil are Diethyl Phthalate (37.57 %), 1,4,5,6-Tetrahydrocyclopentapyrazole-3-carboxylic acid, (1-adamantan -1-ylethylidene) hydrazide (19.83 %), 1,6-Octadien-3-ol, 3,7-dimethyl-,2-aminobenzoate (17.48 %), 1,3-Cyclopentadiene, 5,5-dimethyl- 2-propyl- (6.58 %), Bicyclo[2.2.1]heptane, 2-ethyliden-1,7,7-trimethyl-, (Z)- (4.28 %), and 1-Isopropenyl-3-propenylcyclopentane (4.55 %). Further the pharmacological activities such as DPPH free radical scavenging assay (antioxidant), α-amylase inhibition assay (anti-diabetic), albumin denaturation assay (anti-inflammatory) and well diffusion method (antibacterial) of the extracted essential oil was evaluated and found to be having high activity. Hence it can be concluded that the essential oil extracted from Karaya gum could be used as a source of natural antioxidant, antidiabetic, anti-inflammatory and antibacterial agents.

Keywords: *Sterculia urens* Roxb, Gum karaya, Essential oil, GC-MS analysis, pharmacological activities

INTRODUCTION

Sterculia urens Roxb (karaya plant) is a small to medium size tree belongs to family *Malvaceae*. It is proved to be having antifungal [1], antioxidant and antimicrobial [2] properties. The gum obtained from karaya plant was used as a laxative and used in the preparation of hydrophilic matrix tablets [3]. Karaya gum is an anionic, branched polysaccharide composed of galactose, rhamnose, glucuronic acid, and galacturonic acid and is reportedly contains 8% acetyl groups, 1.2–1.63% protein, and 1–2% of lipids [4]. It is having adhesive and binding agent and is having ability to absorb large amounts of water and is widely used in the food industry owing to its high availability, compatibility, physicochemical properties, and low cost [5].

Volatile oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehyde, esters, ethers, ketones, phenols and oxides) [6]. Other volatile compounds include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, the oil composition is a balance of various compounds although in many species one constituent may prevail over all others. In nature, essential oils plays an important role in the protection of plants such as antibacterial, antiviral, antifungal, insecticide and also against herbivores, reducing their appetite for these plants. They are also responsible for the characteristic smell of plants, which can attract some insects to favor the dispersal of pollen and seeds or repel other undesirable ones [7]. Essential oils having commercial

importance in the cosmetic, food and pharmaceutical industries, and in agriculture. Therefore, they are generally recognized as safe by the FDA (Food and Drug Administration). Its composition can vary considerably between species of aromatic plants and varieties and within the same variety of different geographical areas.

The literature survey, it can be confirmed that the chemical composition and pharmacological properties of the essential oil extracted from the gum of *Sterculia urens* Roxb was not explored till now. In view of the above, the present work is aimed to evaluate the phytochemical composition of the essential oil extracted from karya gum. Further the pharmacological activities such as antioxidant by DPPH free radical inhibition activity, antidiabetic activity by alpha amylase enzyme activity inhibition study, anti-inflammatory by albumin protein denaturation inhibition activity study and anti-bacterial activity by agar plate well diffusion method was studied for the extracted essential oil.

MATERIALS AND METHODS

Collection of Plant material

The fresh Karaya gum was collected in Paderu village, Visakhapatnam, district, Andhra Pradesh in January 2018. The collected gum was surface cleaned with tissue paper and sterile cotton. Then the gum was cut into very small pieces and preserved in an amber colour bottle for further study.

Extraction of essential oil from gum

Hydro-distillation technique was utilised for extracting the essential oil from Karaya gum. A Clevenger-type apparatus (10 mL volume capacity, lighter than water, and fitted with a condenser), 2000 mL round bottom flask, and heating mantle (2000 mL) were used for the extraction of essential oil. The small pieces of gum were transferred into the round bottom flask, 1 L distilled water was added, the apparatus was fixed, and the temperature was adjusted to 70 °C. The process was continued for 3 h, the volume of extracted essential oil was recorded, and the percentage yield was calculated. The experiment was repeated 3 times, and the percentage ± standard deviation was determined. The extracted essential oil was then dried using anhydrous Na_2CO_3 and stored in amber coloured borosilicate glass vials at 4 °C for further analysis of its chemical composition and pharmacological activities [8].

Analysis of the essential oil by GC-MS

The essential oil from Karaya gum was subjected to GC–MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5975 inert mass selective detector (EIMS, electron energy, 70 eV, scan range 50–1000 amu, and scan rate 2 scans/s), and an Agilent Chem Station data system. The GC column was an DB-5 ms, fused silica capillary with a (5% phenyl)-methyl poly siloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 mL/min. Inlet temperature was 230 °C and MSD detector temperature was 230 °C. The GC oven temperature program was used as follows: 70 °C @ 5 °C/min, final temperature 120 s ramp @ 10 °C/min, final temperature 280 °C for 20 min. The sample was dissolved in 10 mL of methanol:acetone (1:1) mixture. 1 µL injections using a split less injection technique was used.

Identification of essential oil components was achieved based on their retention indices, and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chem station data system (D.02.00.275, version 2.0d)].

Pharmacological activities

Antioxidant activity

DPPH free radical scavenging assay was studied for the determination of antioxidant activity of essential oil extracted from Karaya gum. The assay was performed based on the procedure described by Djacbou et al., 2014 [9] with slight modification. In the typical experiment, 3 mL of selected concentration of essential oil extracted from Gum karaya was mixed with 2 mL of methanolic DPPH (0.1 mmol/L) solution. The content was incubated in dark at room temperature for 30 min. Then after incubation, the optical density (OD) of the solution was measured at 517 nm using double UV-visible spectrophotometer (TECHOMP UV 2301, Japan). The radical scavenging activity of the bark extracts was calculated using eqn. 1:

$$\% \text{ radical scavenging activity} = [(OD_{control} - OD_{sample})/OD_{control}] \times 100 \text{ ---------- (1)}$$

The ascorbic acid as reference standard and the results were expressed as percentage inhibition of DPPH radical. The effective concentration of the essential oil that scavenged the DPPH radicals by 50 % (IC_{50}) is calculated by interpolation from linear regression analysis of the obtained results. The Ascorbic acid (AA) was considered as reference standard and from the obtained IC_{50} values, the AA equivalent (AAEQ) was calculated ($AAEQ = IC_{50,AA}/IC_{50,oil}$).

Anti-diabetic activity

α-amylase inhibition assay was performed for the determination of anti-diabetic activity of the essential oil extracted from Karaya gum. The assay was performed based on the procedure described by *Shettar et al*., 2017 [10] with slight modification. In brief, 0.5 mL different concentrations of essential oil were mixed with 0.5 mL (0.5 mg/mL) α-amylase solution with pH 6.9 sodium phosphate buffer (0.02 M). Then the reaction mixture was incubated for 10 min at room temperature and 0.5 mL (1%) starch solution in pH 6.9 sodium phosphate buffer (0.02M) was added. The resulting solution was incubated for 10 min at room temperature, then heated on a water bath at 100 °C for 5 min. Then the reaction was terminated by adding 1 mL of dinitrosalicylic acid colour reagent and then cooled to room temperature. The final volume in all the test tubes were made up to 10 mL and the absorbance was measured at 540 nm. Similar experiment was performed by replacing extract and α-amylase with buffer solution and was considered as blank. The control solution was prepared by replacing essential oil with buffer. The standard drug Acarbose is considered as standard, and the α-amylase inhibition activity was calculated using eqn. 2:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100 \text{ ---------- (2)}$$

Acarbose is considered as standard, and the results observed in the study was used for calculating the IC_{50} concentrations and less IC_{50} concentrations of the essential oil proved to be having more activity.

Anti-inflammatory activity

Albumin denaturation inhibition assay was performed for the determination of anti-inflammatory activity of the essential oil extracted from Karaya gum. The assay was performed based on the procedure described by Murthuza et al., 2018 [11] with slight modification using diclofenac as standard. The results observed in the study was used for calculating the IC_{50} concentration of the extracted essential oil and the results were compared with diclofenac standard. In the experiment, 1 mL of the selected concentration of the essential oil solution prepared in methanol was mixed with 1 mL of albumin (1mM) solution in phosphate buffer (0.2 M). The reaction mixture was incubated at room temperature for 10 min. Then the turbidity of the reaction mixture was measured spectrophotometrically at 660 nm. The % albumin denaturation inhibition activity was calculated using eqn. 3:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100 \text{ ---------- (3)}$$

Anti-bacterial activity

Agar plate well diffusion method as per the procedure described by Dalir et al., 2020 [12] with slight modification was studied for the evaluation of the anti-bacterial activity of the essential oil extracted from Karaya gum. In this study, two gram positive bacteria namely *Bacillus subtilis* (MTCC – 1427) and *Staphylococcus aureus* (MTCC – 1430), two-gram negative bacteria namely *Escherichia coli* (MTCC – 294) and *Pseudomonas aeruginosa* (MTCC – 1748) were selected. Gentamycin and distilled water were considered as positive and negative controls respectively and the results were expressed as millimetre (mm) of zone of growth inhibition observed for the studied concentration of the sample.

In a sterile petri dish, 10 mL of Mueller- Hinton agar medium was poured as a basal layer followed with 15 mL of seeded medium previously inoculated with selected bacterial suspension (100 mL of medium/1 mL of 10^7 CFU) to attain 10^5 CFU/ml of medium. Then wait till the complete solidification of the medium in the petri plate and wells were prepared using sterilized stainless-steel cork borer. In each well 25 µL of selected concentration of gum karaya essential oil and Gentamycin (standard) were loaded with sterile micro-pipette. Simultaneously in a separate petri dish, water was loaded and served as negative control and plates were grown at 37 °C for 24 H. Then the zone of inhibition of standard and gum karaya oil was measured in mm (Millimetre) by comparing with negative control.

RESULTS AND DISCUSSION

Essential oils are the complex mixtures of volatile organic compounds produced in the form of secondary metabolites in plants. The essential oil from Karaya gum was obtained by hydro distillation and was analysed by using gas chromatography–mass spectrometry (GC–MS). The % composition of essential oil was observed to be 9.66±0.151 % and confirms that the hydro distillation process of extraction extracts high quantity of the essential oil from gum. This method also having the advantage of non – usage of hazardous and costly chemicals and the extraction process is economical. The GC MS chromatogram obtained for the essential oil extracted from Karaya gum was given in fig 1.

The GC-MS analysis results confirms 86 compounds that accounted for 99.82 % of the oil (Table 1). which included 3 compounds as major compounds that are present more than 10 % of the total composition of the essential oil. The major compounds identified in the essential oil are Caryophyllene (22.59 %), Beta Myrcene (19.24 %) and Bicyclo [3.1.1] heptane, 6,6-dimethy l-2-methylene (12.79 %). One compound namely Naphthalene, 1,2,4a,5,8,8a- hexahydro- 4, 7- dimethyl -1 - (1- methylethyl) - [1S-(1. alpha.,4a. beta.,8a. alpha)] was found to be more than 5 % in the extracted essential oil. Among the compounds identified using GC - MS analysis, eleven compounds were observed to be present more than 1 % of the composition in the oil. The eleven compounds that are present more than 1% are 1,6-Cyclodecadiene, 1- methyl- 5- methylene- 8- (1-methylethyl)-, [S-(E,E)]-, Beta.- Bisabolene, 1,6-Octadien- 3-ol 3,7- dimethyl-, Humulene, 1,6- Cyclodecadiene, 1- methyl – 5- methylene - 8- (1-methylethyl)-, [S-(E,E)], Caryophyllene oxide, D-Limonene, Cyclohexane, 1- ethenyl - 1- methyl- 2- (1- methylethenyl) - 4 - (1-methylethylidene)-, Ledol, (E,E) - 7,11,15-Trimethyl – 3 – methylene – hexadeca - 1,6,10,14 - tetraene and Cyclohexane, 1 – ethenyl – 1 – methyl -2 - (1- methylethenyl)- 4- (1- methylethylidene). In the compounds identidied, 42 compounds were observed to be very and the % composition of these compounds was found to be less than 0.01 %.

Caryophyllene is the major compound detected in Karaya gum essential which is a bi-cyclic hydrocarbon sesquiterpene that occurs in essential oils of several plants, including *Piper nigrum* L. (*Piperaceae*), *Baccharis* spp. (*Asteraceae*) and *Copaifera* spp. (*Leguminoseae*). A variety of biological activities, including anti-carcinogenic, anti-inflammatory, and antioxidant have been related to this hydrocarbon sesquiterpene. Moreover, due to its woody and spicy odor, β-caryophyllene is commonly used as a flavoring agent [13].

The second major compound detected is β-Myrcene which occurs naturally as a major component in the essential oils of many plants such as hops, bay leaf, and lemongrass. It is used as a flavouring agent, for example, in foods and beverages. It is also used widely in cosmetics, soaps, and detergents as well as other fragranced products such as perfumes, air care products, polishes, wax blends, adhesives, disinfectants, biocides, paints, plasters, fuels, inks, and toners [14].

Another major compound Bicyclo [3.1.1] heptane, 6,6-dimethy l-2-methylene is a bicyclic terpene compound. Its structure is similar to β-pinene which is the most common terpene and having the ability to increase mental alertness, clarity, and overall cognitive functions. It is also having anti-inflammatory, antidepressant, (targeted at treating conditions such as arthritis and fibromyalgia), antiseptic, and antioxidant activities [15]. The compounds that are present in the less quantity were also having various pharmacological activities.

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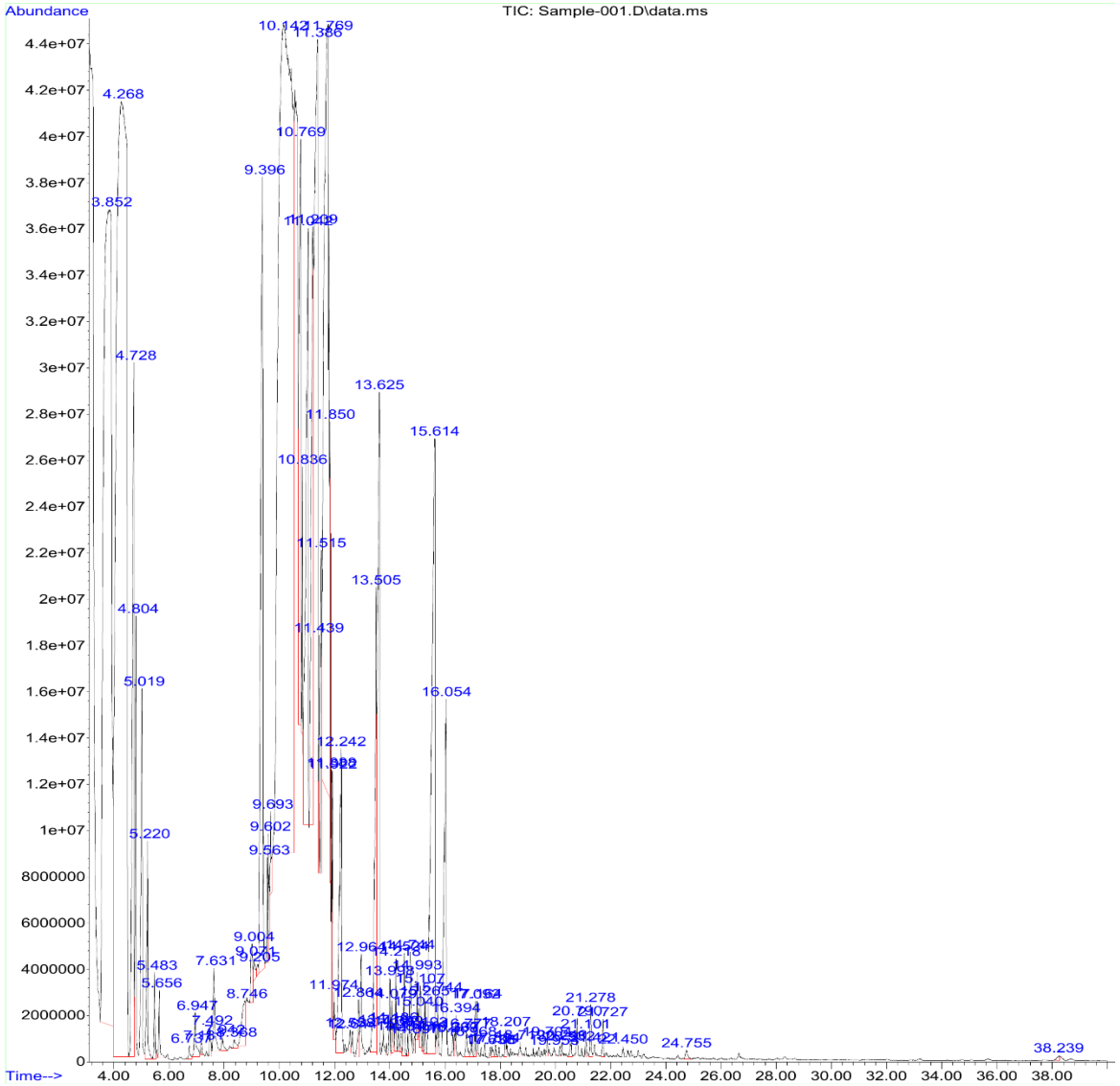

Fig. 1: GC-MS chromatogram of Karaya gum essential oil

Table 1: GC-MS results of Karaya gum essential oil

Peak No	RT (min)	Area %	Name of the compound
1	3.85	12.79	Bicyclo [3.1.1] heptane, 6,6- dimethy l-2- methylene
2	4.27	19.24	.beta.-Myrcene
3	4.73	2.22	D-Limonene
4	4.80	0.78	Bicyclo [3.1.0] hex- 2- ene, 4- methyl- 1- (1-methylethyl)-
5	5.02	0.89	trans-. beta.- Ocimene
6	5.22	0.48	.beta.- Ocimene
7	5.48	0.16	o- Cymene
8	5.66	0.14	Cyclohexene, 1- methyl- 4- (1-methyle thylidene)-
9	6.74	0.04	1-Trimethylsilylpent -1 -en- 4- yne
10	6.95	0.16	2,4,6- Octatriene, 2,6- dimethyl-, (E,Z)-
11	7.18	0.03	2- Nonanone
12	7.49	0.08	Benzene, 1- methoxy- 2-methyl-
13	7.63	0.27	Furan, 3- (4- methyl- 3-pentenyl)-
14	7.94	0.05	cis- Linaloloxide
15	8.37	0.04	Bicyclo [4.1.0] heptane, 7-(1-methyl ethylidene)-
16	8.75	0.13	Ylangene
17	9.00	0.20	Propane, 1,1'-oxybis 3-chloro-
18	9.07	0.07	1,6-Cyclodecadiene, 1- methyl- 5- methylene- 8- (1-methylethyl)-, [S-(E,E)]
19	9.20	0.03	Copaene
20	9.40	3.29	1,6- Octadien- 3-ol 3,7- dimethyl-
21	9.56	0.12	Naphthalene, 1,2,3,5,6,7,8,8a- octa hydro-1,8a- dimethyl-7- (1-methylethenyl)-, [1S-(1. alpha.,7. alpha., 8a. alpha.)]-
22	9.60	0.18	(Z,Z)-.alpha.- Farnesene
23	9.69	0.14	Bicyclo [7.2.0] undec- 4- ene, 4,11,11 -trimethyl- 8-methylene-
24	10.14	22.59	Caryophyllene
25	10.76	2.05	Cyclohexane, 1-ethenyl- 1-methyl- 2- (1- methylethenyl)-4-(1-methylethylidene)-
26	10.83	0.32	Patchoulene
27	11.04	2.89	Humulene
28	11.27	2.74	1,6- Cyclodecadiene, 1- methyl- 5-methylene- 8- (1-methylethyl)-, [S-(E,E)]
29	11.39	4.58	1,6- Cyclodecadiene, 1- methyl- 5- methylene- 8- (1-methylethyl)-, [S-(E, E)]-
30	11.44	0.29	10s,11s- Himachala-3(12),4-diene
31	11.51	0.59	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
32	11.77	7.29	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1.alpha.,4a.beta.,8a.alpha)]-
33	11.85	0.56	Benzene, 1-(1,1-dimethylethoxy)-4- methyl-
34	11.88	0.11	Selina-3,7(11)-diene
35	11.92	0.24	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1.alpha.,4a.beta.,8a.alpha)]-
36	11.97	0.08	2H-3,9a- Methano- 1-benzoxepin, octahydro -2, 2, 5a, 9-tetramethyl-, [3R- (3. alpha., 5a. alpha., 9. alpha., 9a. alpha.)]-
37	12.24	1.28	Cyclohexane, 1- ethenyl- 1- methyl - 2- (1-methyl ethenyl)- 4- (1-methylethylidene)-
38	12.56	0.05	.beta.-copaene
39	12.64	0.04	6-epi-shyobunol
40	12.86	0.14	1, 1, 5- Trimethyl-1, 2-dihydronaphthalene
41	12.96	0.19	Cycloheptane, 4- methylene- 1-methyl-2- (2- methyl- 1-propen-1- yl)- 1- vinyl-
42	13.50	1.65	Ledol
43	13.62	2.40	Caryophyllene oxide
44	13.77	0.07	1,6,10- Dodecatrien -3-ol, 3, 7, 11- trimethyl-, [S-(Z)]-
45	14.00	0.14	12-Oxabicyclo [9.1.0] dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-

46	14.08	0.09	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
47	14.14	0.03	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
48	14.22	0.16	Guaia-1(10),11-diene
49	14.31	0.05	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-
50	14.41	0.04	1H- Cycloprop[e] azulene, 1a,2,3,4,4a,5,6,7b- octahydro-1,1,4,7-tetramethyl-, [1aR-(1a. alpha.,4.a lpha.,4a., beta.,7b. alpha.)]-
51	14.52	0.21	Tricyclo [4.1.0.0(2,4)] heptane, 3,3,7,7- tetramethyl- 5- (2-methyl-1-pro
52	14.65	0.03	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,
53	14.74	0.25	cis-.beta.-Farnesene
54	14.90	0.04	.beta.-bisabolol
55	14.93	0.12	1,3,6,10- Cyclotetradecatetraene, 3,7,11- trimethyl-14-(1-methylethyl)
56	15.04	0.04	1H- Cycloprop [e] azulene, 1a,2,3,5,6,7,7a,7b- octahydro- 1,1,4,7-tetramethyl-, [1aR- (1a.alpha.,7. alpha.,7a .beta., 7b.alpha.)]-
57	15.11	0.12	Bicyclo [4.4.0] dec- 1- ene, 2- isopropyl- 5- methyl- 9-methylene-
58	15.19	0.03	Cyclohexene, 6- ethenyl- 6- methyl- 1- (1- methylethyl)-3- (1-methylethylidene)- (S)-
59	15.26	0.10	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b- octahydro-1,1,4,7-tetramethyl-, [1aR- (1a. alpha., 4. alpha., 4a. beta., 7b. alpha.)]-
60	15.61	3.89	.beta.-Bisabolene
61	15.74	0.11	.alpha.-Cadinol
62	16.05	1.55	(E,E)-7,11,15- Trimethyl- 3- methylene- hexadeca-1,6,10,14-tetraene
63	16.29	0.04	Alloaromadendrene oxide-(1)
64	16.34	0.04	Tricyclo [4.4.0.0(2,7)] dec- 3- ene- 3-methanol, 1-methyl-8- (1-methylethyl)-
65	16.39	0.07	Longifolene
66	16.78	0.08	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-
67	16.97	0.04	6-Isopropenyl- 4,8a- dimethyl-1,2,3,5,6,7,8,8a- octahydro-naphthalen - 2- ol
68	17.09	0.08	Benzene, 1-methyl-3-[(1-methylethylidene) cyclopropyl]-
69	17.16	0.09	1-Methyl- 6- methylenebicyclo [3.2.0] heptane
70	17.64	0.02	1,6,10- Dodecatrien- 3-ol, 3,7,11- trimethyl-, [S-(Z)]-
71	17.71	0.02	(S)-N- (Alpha- methylbenzyl) methacrylamide
72	17.83	0.02	trans- Geranyl geraniol
73	18.21	0.06	1,6,10,14- Hexadecatetraen- 3- ol, 3,7,11,15- tetramethyl- (E,E)-
74	18.72	0.04	4- Hexadecen- 6- yne, (Z)-
75	19.70	0.03	(-)-Neoclovenen -(I), dihydro- Anthracene, 9-butyl-
76	19.95	0.03	(1Ar-(1a alpha,4a beta,8a s (*)))-4a, 8,8 trimethyl octahydro benzo (c) cyclopropa (d) pyran 2,4-dione
77	20.24	0.02	Bicyclo [7.2.0] undec -4- ene, 4,11,11-trimethyl-8-methylene-
78	20.53	0.04	1-Formyl-2,2,6- trimethyl- 3-(3- methyl- but- 2- enyl)-6-cyclohexene
79	20.79	0.08	Androsta-1,4- dien-3- one, 17-hydroxy-17-methyl-, (17.alpha.)-
80	21.101	0.04	3,4-Dimethylphenyl heptyl ether
81	21.28	0.11	1-Cyclohexene- 1- propanal, 2,6,6-trimethyl-
82	21.42	0.03	Thunbergol
83	21.73	0.08	Gamma.-Gurjunenepoxide-(2)
84	22.45	0.02	trans-Geranylgeraniol
85	24.75	0.04	Thiourea, 1-tert-butyl-3-(4-methoxyphenyl)-
86	38.24	0.02	5(1H)- Azulenone, 2,4,6,7,8,8a- hexahydro-3,8-dimethyl-4- (1-methylethylidene)-, (8S-cis)-

The DPPH free radical scavenging activity of essential oil extracted from Karaya gum was studied in the concentration range of 5-40 µg/mL and the results were compared with standard ascorbic acid. In the very high concentration studied i.e at 40 µg/mL the DPPH radical inhibition was observed to be 98.77±0.102, 71.09±0.207 % respectively for ascorbic acid standard and extracted essential oil respectively. The IC_{50} was observed to be 22.20±0.03, 32.11±0.035 µg/mL for ascorbic acid standard and essential oil respectively confirms that the essential oil extracted from Karaya gum was observed to be having high DPPH radical scavenging activity. The results were shown in fig 1.

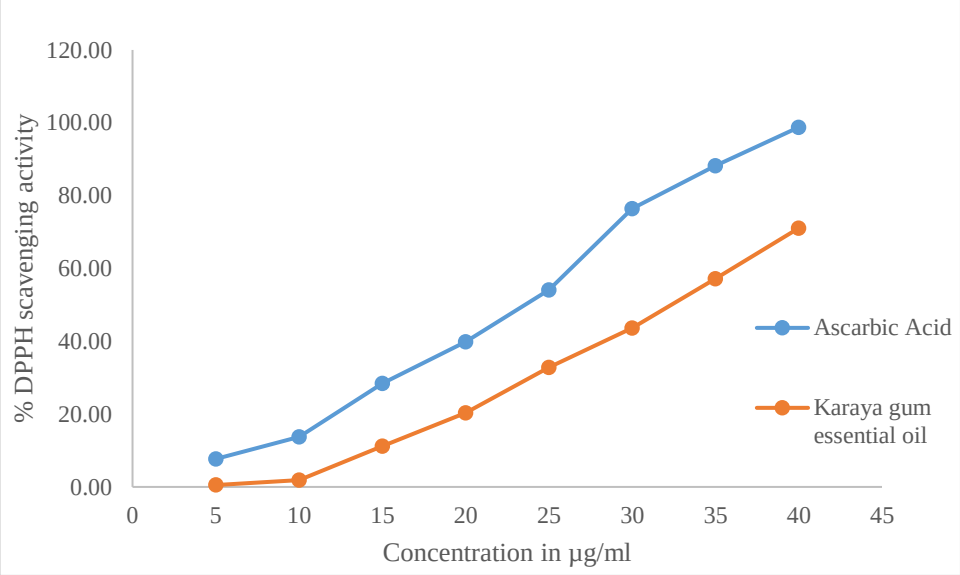

Fig 1: DPPH radical scavenging activity results

Anti-diabetic activity of the essential oil extracted from Karaya gum was determined by performing α-amylase inhibition assay using acarbose standard. The IC_{50} concentration was calculated as 71.51±0.08 for standard and 143.66±0.17 for extracted oil. Fig 2 gives the comparative α-amylase inhibition assay results of standard and essential oil extracted from karaya gum. The results confirms that the oil extracted from gum of karaya was found to be having α-amylase inhibition activity.

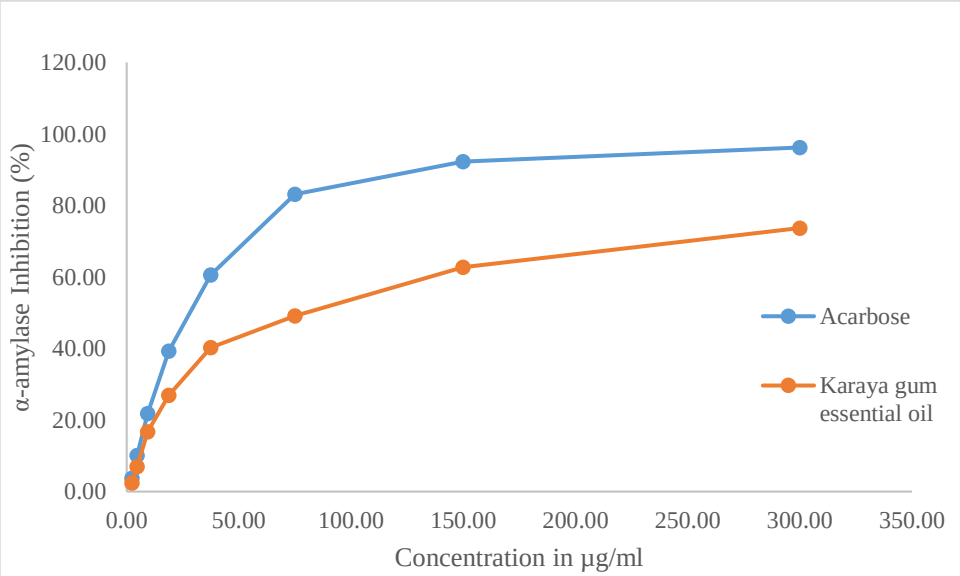

Fig 2: α-amylase inhibition assay results

The anti-inflammatory activity of essential oil extracted from karaya gum was evaluated by performing albumin denaturation inhibition assay and the results observed in the study was represented in Fig 3. The assay was performed in the concentration range of 25-200 µg/mL and the drug diclofenac is considered as the standard. The albumin denaturation inhibition activity was found to be very high for extracted oil with IC_{50} values of 138.63±0.25 which is near to the standard (107.13±0.13µg/mL).

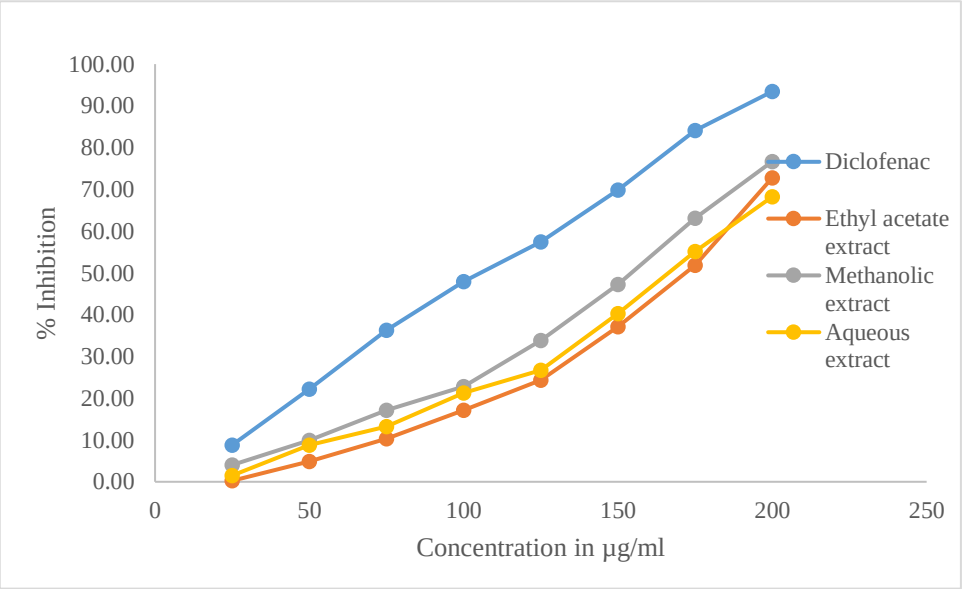

Fig 3: Anti-inflammatory activity by inhibition of albumin denaturation assay

The essential oil extracted from Karaya gum was screened for the determination of anti-bacterial activity by agar plate well diffusion method. Gentamycin standard and plant extracts at a concentration 1 µg/mL, 10 µg/mL and 50 µg/mL was studied against two gram positive and two-gram negative bacteria and results were presented in Table 2. The results confirm that the extracted oil having potentially effective in suppressing the growth of studied bacteria with variable potency. Among the bacteria studied, the extracts show more potent against the growth of gram-negative bacteria. The essential oil at a very low concentration of 1 µg/mL concentration shows the zone of inhibition against the growth of gram-negative bacteria studied. There is no zone of inhibition observed against gram positive bacteria at 1 µg/mL concentrations. At a concentration of 10 and 50 µg/mL, the karaya gum essential oil having potential growth inhibition zone against all the bacteria studied. The results of the anti-bacterial activity of the extracted essential oil were found to be very close to the standard studied.

Table 2: Anti-bacterial activity results

S No	Sample	Zone of growth inhibition observed in mm			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
1	KGEO at 1 µg/mL	-	-	2.67±0.15	2.20±0.10
2	KGEO at 10 µg/mL	3.70±0.20	2.70±0.20	4.80±0.10	4.27±0.15
3	KGEO at 50 µg/mL	6.77±0.15	6.10±0.20	8.37±0.15	7.17±0.12
4	GM at 1 µg/mL	4.70±0.20	4.13±0.15	5.33±0.15	3.73±0.21
5	GM at 10 µg/mL	6.77±0.15	6.00±0.10	8.37±0.15	8.77±0.15
6	GM at 50 µg/mL	11.30±0.20	10.57±0.21	12.53±0.06	13.37±0.25

KGEO = Karaya gum essential oil; GM = Gentamycin standard

All the value expressed as mean ± SD (n=3)

The extracted essential was found to be having 86 number of different chemical constituents and among the chemical constituent compounds identified, one compound was found to having more than 20 %, two compounds were found to be more than 10 % and 12 compounds were found to be in the range of more than 1 % and less than 10 % composition in the essential oil. The oil shows potential pharmacological activities such as DPPH free radical scavenging activity, α-amylase inhibition assay, albumin denaturation assay and anti-bacterial activities. The presence of large number of volatile bio active compounds in the extracted essential oil may be responsible for the pharmacological activities of the essential oil extracted from karaya gum.

CONCLUSION

The results presented in this study are the first given information's on the chemical composition of essential oil from gum of Karaya (*Sterculia urens* Roxb) plant. The oil yield was found to be 9.66±0.151 % (v/w). A total of 86 different chemical constituents were identified in the extracted essential oil, comprising of 99.82 % of the total oil. The essential oil having potential pharmacological activities such as DPPH free radical scavenging activity, α-amylase inhibition assay, albumin denaturation assay and anti-bacterial activities. Hence the study suggests that the essential oil from the gum of karaya plant could be used as another potential source for new drug development in treating various disorders caused by extreme oxidative stress.

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CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

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