

Phytochemical Screening of Ethanol Extract of *Eugenia Jambolana* Lam.

AUTHORS: Sindhuja G, Rebecca Vinolia, A.F.Philomina, Yasmin M.

CORRESPONDING AUTHOR: Dr.A.Mary Agnes

Corresponding Author: Associate Professor

Authors: Research Scholar, Pg & Research, Department Of Zoology, Auxilium College
(Autonomous), Katpadi, Vellore-6, TN, And India

Corresponding Author: Dr.A.Mary Agnes

Agnesanthonyv14@gmail.com

Abstract

The aim of the present study is to identify the important functional groups and Phytochemical constituents of the ethanol extract of *Eugenia jambolana*, which was analyzed by GC-MS. The GC-MS analysis of the ethanol extract from the seed part of *Eugenia jambolana* detected the presence of 17 different compounds in each extract. Both extracts exhibit different compounds and biological activity. Results revealed various major compounds among which the Phytochemical compounds were identified: Hexadecanoic acid, Dotriacontane, Oleic acid, Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl), Hexadecanoic acid, Ethyl ester, T-Butyl Cyclopentaneperoxy-carboxylate, 2,3-Anhydro-D-Mannosan, 2,3-Anhydro-D-Galactosan, Alpha-Cadinol, Tau-Murol, Diglycerol. GC-MS analysis revealed that the extracts contain a high concentration of phytoconstituents, which may be bioactive components involved in the plant's therapeutic properties. GC-MS analysis revealed that the extracts contain a high concentration of phytoconstituents, which may be bioactive components involved in the plant's therapeutic properties. These results can be used as a good preliminary indication for future applications of antifungal, antibacterial, antimalarial, antidiabetic, anti-inflammatory, antimicrobial, preservative, and antioxidant.

Keywords: GC-MS analysis, *Eugenia jambolana*, Seed part, phytochemical compounds

Introduction

Medicinal plants have been the basis of traditional medicine (TM) used for the treatment of various diseases in diverse cultures around the world. For many centuries, herbs and herbal-derived medicines have played a crucial role in health and disease management. Many ancient civilizations have shown documented evidence for the use of herbal extracts, concoctions, and various forms of plant preparations for the treatment of different kinds of diseases and ailments (Obidike I, et al., 2013). In India, from ancient times, different parts of medicinal plants (80,000 species) have been used as traditional medicines in different systems of Indian medicine for treatments of various diseases. At present, about 25% of the active constituents have been identified from medicinal plants that have been used as prescribed medicines. Certain reports have estimated that over 25,000 actual plant-based formulations are available in the Indian systems of folk and traditional medicine, which are prescribed by about 1.5 million practitioners in preventive, persuasive, and healing applications. Most of these plants, in addition to their medicinal values, have obvious economic, cosmetic, and social applications. These plants contain diverse secondary metabolites or constituents that are

responsible for their pharmacological and toxicological effects on both humans and animals. These secondary metabolites are also potential sources of new drugs or lead compounds in the development of new drug molecules. Despite the growing market demand for herbal medicines, there are still concerns associated with not only their use but also their safety. Reports state that less than 10% of herbal products in the world market are truly standardized to known active components (chacondoT,et al.,2012)

Only medicinal plants proved to possess good pharmacological activity without obvious significant adverse effects, cytotoxicity, organ toxicity, and pathological effects could qualify as candidates for clinical studies in humans. Acute toxicity tests or acute lethality (LD50), sub acute, sub chronic, and chronic toxicological tests are among the required tests. Such studies or tests are appropriate in order to ascertain the relative safety of humans based on animal studies. (Obidike I, et al., 2013).

Various bioactive compounds of medicinal plants exhibit stimulating pharmacological actions like antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant properties. The potential of these bioactive compounds for use in the treatment of various ailments should be investigated. Plant-based medicines are often prepared from crude plant extracts comprising a complex mixture of different phytochemicals. Test phytochemicals have unique and complex structures and are used in treating prolonged as well as contagious diseases. An enormous pool of bioactive secondary metabolites exists in various plant species, but merely a small proportion of them have been examined and found to be significant sources of bioactive agents. The development of suitable screening methods is very important in the search for new compounds and also for quality control.

Eugenia jambolana:

Scientific Classification

Kingdom	Plantae
Sub-Kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Eugenia
Species	jambolana

Vernacular names and etymology

Syzygiumjambolana, Eugenia cumini, Syzygiumcumini, Eugenia jambolana.

Assamese: Jamu, kala jamu

Bengal: Kala-jam

Jamun, Damson plum, Duhat plum, Indian blackberry, jambolan, jambolana plum, Java plum, Malabar plum, Portuguese plum, Black plum tree, Indian blackberry, jambolan-plum, Java plum, Malabar plum, Portuguese plum, Jamun, Black plum tree, Indian blackberry, jambolan-plum, Java plum, Malabar plum, Portuguese plum, Jamun, Black plum tree,

Kannad: Ama-phala, Jambunera, Nayinera

Konkani: Jambul.

Jamun, jam, Phalinda, Jemni-paiman, Jamoom

Naval Malayalam

Manipuri: Gulamchat, jam.

Marathi: jambool

Mizo: Hmuipuri

Babla.Babul, Babur, and Kikar

In Sanskrit: Nilaprala, Rajaphala, Jambu, Jambula, Megha-varna

Nagun, Navel, Nairuri, Nawar, Narvel, Naga, Naval

Naeraedu, Pedda-neredu, Nairuri, Racha-neredu, Nareyr, Nasodu

Jamun (Baliga et al., 1776–1789)

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Jamun (Baliga et al., 1776–1789)

Habitat and Description: Jamun is a large evergreen tree of around 30 m in height. Bark is pale brown in color and rough in texture, mostly found on old stems. The leaves are leathery and 6 to 12 centimeters long with a broad tip and less acuminate (may vary in shape, smooth and glowing with numerous nerves which unite in the margin), the tip being broad and less acuminate. Panicles measuring 4 to 6 cm arise mostly from branchlets that are below the leaves. Flowers are scented, greenish-white, 7.5–13 mm across in branched clusters at stem tips; calyx cuplike; 4 petals fused into a cap; many stamens. The calyx is funnel-shaped, about 4 mm long, and toothed. The fruits are found in clusters of four to twenty and do not ripen simultaneously. Fruits are variable, up to 1.5–3.5 cm long, ellipsoid or oblong, black with pink juicy pulp. These fruits are dark-purple or nearly black, luscious, fleshy, and edible. They contain a single large seed. *Eugenia jambolana* (E. jambolana) Lam., commonly known as Jamun or black plum, is an integral part of the indigenous medicine system of India to treat various diseases. Traditionally, all parts of Jamun, such as fruits, leaves,

seeds, and bark, are used in Ayurvedic medicine. The Jamun plant has been reported to have numerous medicinal properties, including antioxidants. Sagrawat H, et al. (2006) discovered that it has gastro protective, anti-ulcer, and radio protective properties.

The pulp of the Jamun berry contains anthocyanin, delphinidin, petunidin, and malvidin-glucosides, which impart its bright purple color. Both the fruit pulp and seed extracts of the Jamun berry have a long history of medicinal use, and they have been extensively studied for their anti-diabetic properties. Despite a growing body of evidence supporting the anticancer properties of anthocyanin-rich berry extracts (Sharma B, et al., 2008),

(Nadkarni, 1944), According to them, the current research programme represents the therapeutic potential of *E. jambolana* stem bark based on significant data on its efficacy for various pharmacological properties, such as antihyperglycemic, antioxidant, hepatoprotective, analgesic, anti-inflammatory, and antimicrobial activities. A glucoside called jamboline, a novel phenolic compound, a trace of pale yellow essential oil, chlorophyll, fat, resin, and albumen are all found in the seed. tannins (19%) (The Wealth of India, 1982), phenolics such as ellagic acid, gallic acid (1-2%), caffeic and ferulic acids and derivatives, guaiacol, resorcinol ethyl ether and corilagin (Williamson, 2002). The seeds are fairly rich in protein and have 28 calcium phosphate (The Wealth of India, 1982). Monoterpenoids like B-pinene, terpinene, terpinolene, borbeneol, B-phellandrene, a-terpineol, and eugenol (Williamson, 2002) and flavonoids such as rutin and quercetin (Sharma et al. 2008). B-sitosterol is also present in *E. jambolana* seed (Gupta and Agrawal, 1970).

Antimicrobial activity

Antibiotic resistance has grown significantly in the previous decade, presents a serious threat to the treatment of infectious diseases. Drug resistance is one of the most important global problems to the treatment of infectious diseases (Picazo et al, 2006; Kiffer et al, 2007). Antibiotic resistance is degrading our therapeutic armamentarium, in addition to significantly increasing the costs and toxicity of newer antibiotics. Bacterial resistant strains are increasing in number and variety; however no materially different modern drugs are currently accessible. Infections produced by resistant bacteria have become extremely difficult to treat. Because they are resistant to several antibiotics, treatment choices are restricted. As a result, other therapy options are being explored. Medicinal herbs have been used for centuries.

Medicinal plants have long served as examples for many therapeutically proved treatments, and they are now being reviewed as antibacterial agents. Thousands of plant species have been studied in vitro on thousands of microorganisms, and many traditional medicines are potent against gram-positive and gram-negative bacteria.

However, only few of these medicinal plant extracts have been tested against microbial resistance against them. Plants have been a great source of natural compounds for sustaining healthcare for a long time, particularly in the recent decade, with more extensive investigations for natural remedies. Plants create a diverse range of organic molecules, which are mostly secondary metabolites, which, in addition to giving distinctive odour, colour, and flavour traits, can also have antibacterial properties (Cowan, 1999).

Anti-inflammatory and Analgesic activity

Inflammation is regarded as a basic physiologic protective measure that aids the body in protecting itself against infection, bacteria, toxic substances, allergies, and other unpleasant stimuli. Most of these disease conditions could be produced by unregulated but chronic inflammation (Kumar et al, 2004). Although it is a protection mechanism, the complex events and mediators involved in the inflammatory response can cause, sustain, or worsen numerous diseases (Sosa et al., 2002).

Antioxidant activity

The liver is the most essential organ in the human body. It is crucial in the regulation of several physiological functions. It also performs various important processes like as metabolism, secretion, and storage. It has a high capability for detoxication and the synthesis of beneficial principles (Shanani, 1999; Subramoniam and Pushpangadan, 1999). It contributes to the body's upkeep, performance, and homeostasis regulation. It is involved in practically all of the metabolic pathways that allow plants to grow, resist disease, provide nutrients, energy, and reproduce. Furthermore, it promotes glucose, protein, and fat metabolism, as well as detoxification, bile secretion, and vitamin storage (Ahsan et al, 2009).

Antihyperglycemic activity

Diabetes mellitus is a clinical disease defined by excessive hyperglycemia induced by a relative or total insulin shortage or physiological resistance to insulin activity. It is the most widely accepted disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. Diabetes has served as a clinical model for other fields of medicine. The basic flaw in food metabolism causes extensive, multiorgan consequences that eventually affect nearly every system of the body and every specialty of medicine. Diabetes, it has been remarked, is associated with medicine and health care. Although this is true clinically, our growing understanding of the syndrome's pathophysiology, as well as the mechanisms of longterm consequences, has pushed diabetes research to the forefront of immunology and molecular biology (Debra-Haire-Joshu, 1991).

Extractions and characterizations of numerous such bioactive compounds from various medicinal plants have led to the delivery of certain medicines with a high-activity profile. The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which helps to select the biologically active plants. In recent years, gas chromatography-mass spectrometry (GC-MS) has commonly been employed for the detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants.

MATERIALS AND METHODS

Sterilization of glassware: Glassware was soaked overnight in cleaning solution and washed thoroughly with running tap water. Then it was cleaned with a detergent solution and rinsed several times with tap water before being rinsed with distilled water and air dried.

Chemicals: 90% ethanol.

Collection of plants: Mature fruits of *E. jambolana* were collected from trees in the Vellore area (Tamilnadu) in April 2019. The collected seeds were identified and used for Phytochemical analysis.

The matured fruit pulp of *Eugenia jambolana* was collected. The fruit was washed in running tap water to remove dirt before being dried in the shade to remove the pulp and moisture content of the seed. The dried seeds were powdered, then subjected to solvent extraction using ethanol solvents in two different methods. The solvent used for these experiments was ethanol. In the first method, 5g of each sample was soaked in 100 ml of 95% ethanol. The prepared solvent extracts were allowed to boil at a temperature of about 60 °C for 15 minutes and then filtered after overnight incubation at room temperature using Whatmann No. 1 filter paper and were parched and stored. In the second method, 5g of each sample was soaked in 100 ml of 95% ethanol at room temperature for 24 h. The extract was filtered using Whatmann filter paper; the filtrates were collected and stored at room temperature for further study.

METHODS

The Clarus 680 GC was used in the analysis. It employed a fused silica column, packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m 0.25 mm ID 250 m df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1L of extracted sample injected into the instrument at the oven temperature was as follows: 60 °C for 2 minutes, followed by 300 °C at a rate of 10 °C min⁻¹; and 300 °C for 6 minutes. The mass detector conditions were: transfer line temperature of 230 °C; ion source temperature of 230 °C; and ionisation mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan interval of 0.1 sec. The fragments range from 40 to 600 Da. The spectrums of the components were compared with the database of spectra of known components stored in the GC-MS NIST (2008) library.

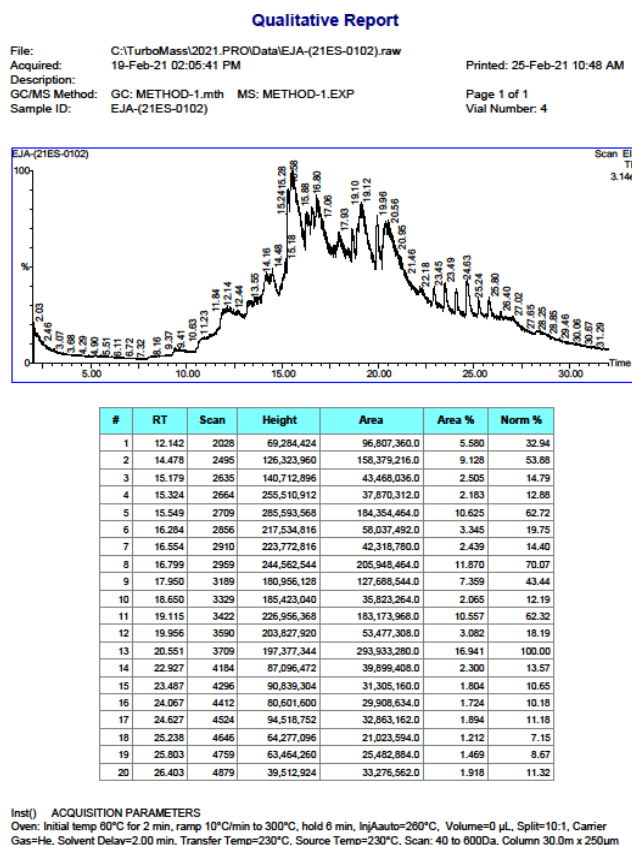
Results

In GC-MS analysis of medicinal *Eugenia jambolana*, 17 major components were found in each extracts (Fig 1). In both methods ethanol extract exhibit higher activity but different compounds were found in different quantities (Fig). The major percentage of compounds present in the first method were 1,1-Dodecanediol ,Diacetate,Hexatricontane,Nonadecane,1 Chloro, Dotriacontane, Hexatriacontane,1-Heptadecanamine,Oleic acid,Cyclohexane,1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl),N-Hexadecanoic acid, Hexadecanoic acid, Ethyl ester,T-Butyl Cyclopentaneperoxy-carboxylate ,5-Isopropyl-6-Methyl-Hepta-3,5-Dien-2-OL , 2,3-Anhydro-D-Mannosan,2,3-Anhydro-D-Galactosan ,Alpha-Cadinol ,Tau-Muurolol, Ledene Oxide (II) Diglycerol In second method also 17 major compounds were present but different compound were found 2,6,6- Trimethyl-Bicyclo(3.1.1)Hept-3-ylamine ,1,1-Dodecanediol,Diacetate ,1,6;3,4-Dianhydro-2-Deoxy-Beta.-D-Lyx-Hexopyranose, 2,6,6,-Trimethyl-Bicyclo(3.1.1) Hept-3-Ylamine , 1-Hexyl-2-Nitrocyclohexane ,1-Hexyl-2-Nitrocyclohexane ,Eicosanoic acid , T-butyl cyclopentaneperoxy-carboxylate ,2,3-Anhydro-D-Galactosan ,2,3-Anhydro-D-Mannosan, 1,6;2,3-Dianhydro-4-O-Acetyl -Beta-D-Allopyranose , Alpha-Cadinol,Pregnan-3,11-Diol-20-One,Propanal,2,3-Dihydroxy,Diglycerol was reported for many pharmacological properties.

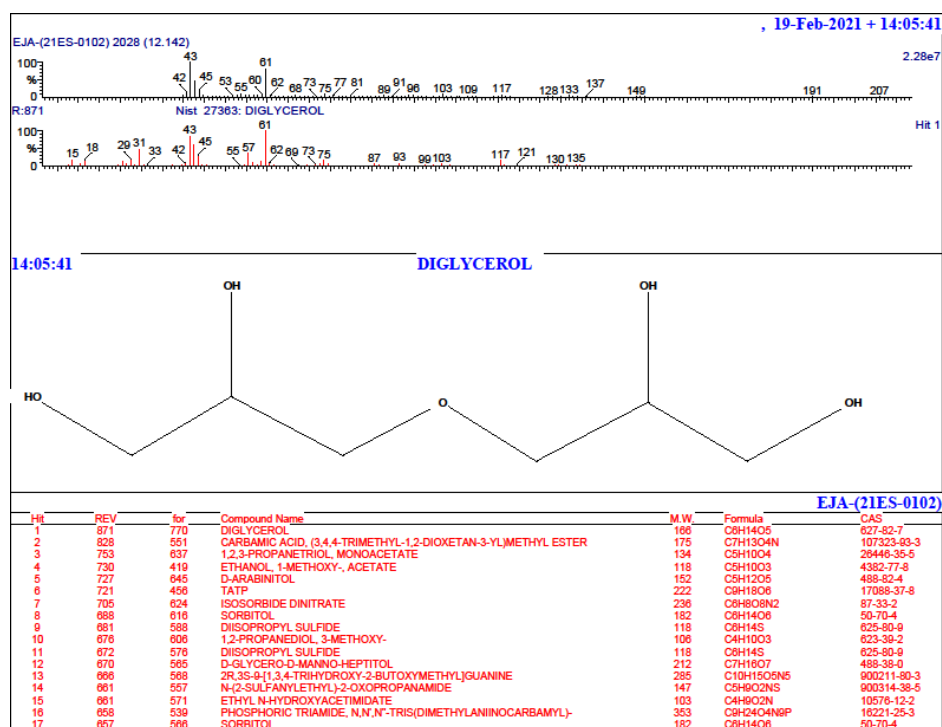
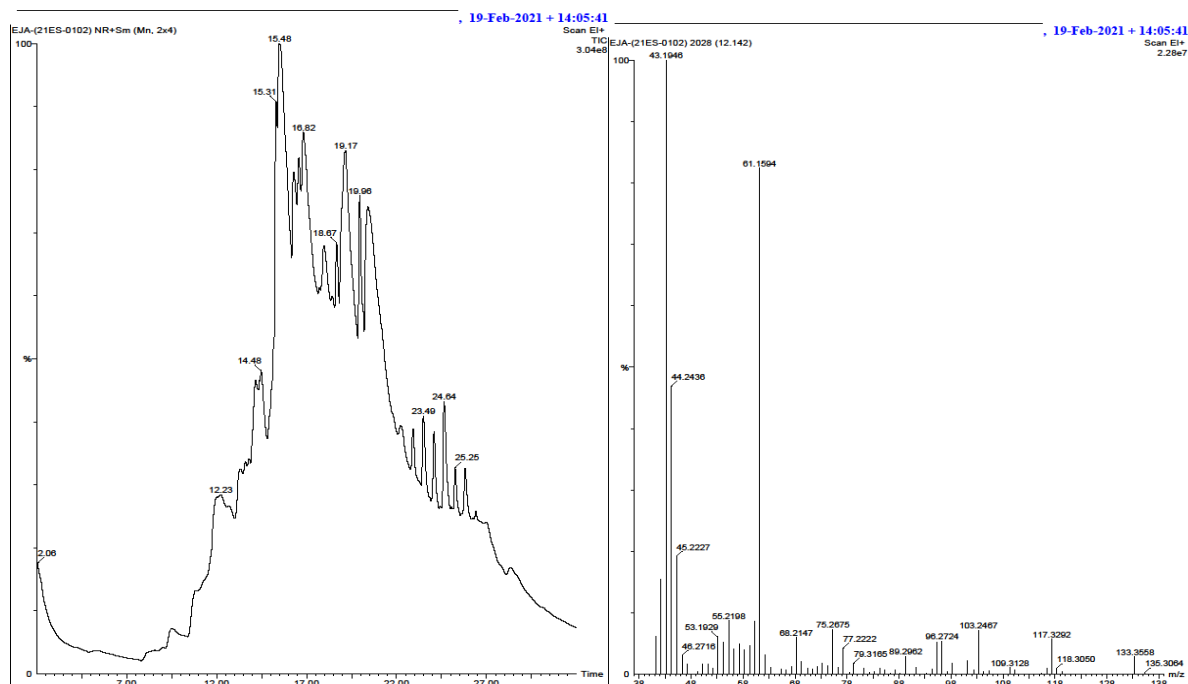
Conclusion

GC-MS is one of the best, fast, and accurate techniques to detect various compounds, including alcohols, alkaloids; nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and

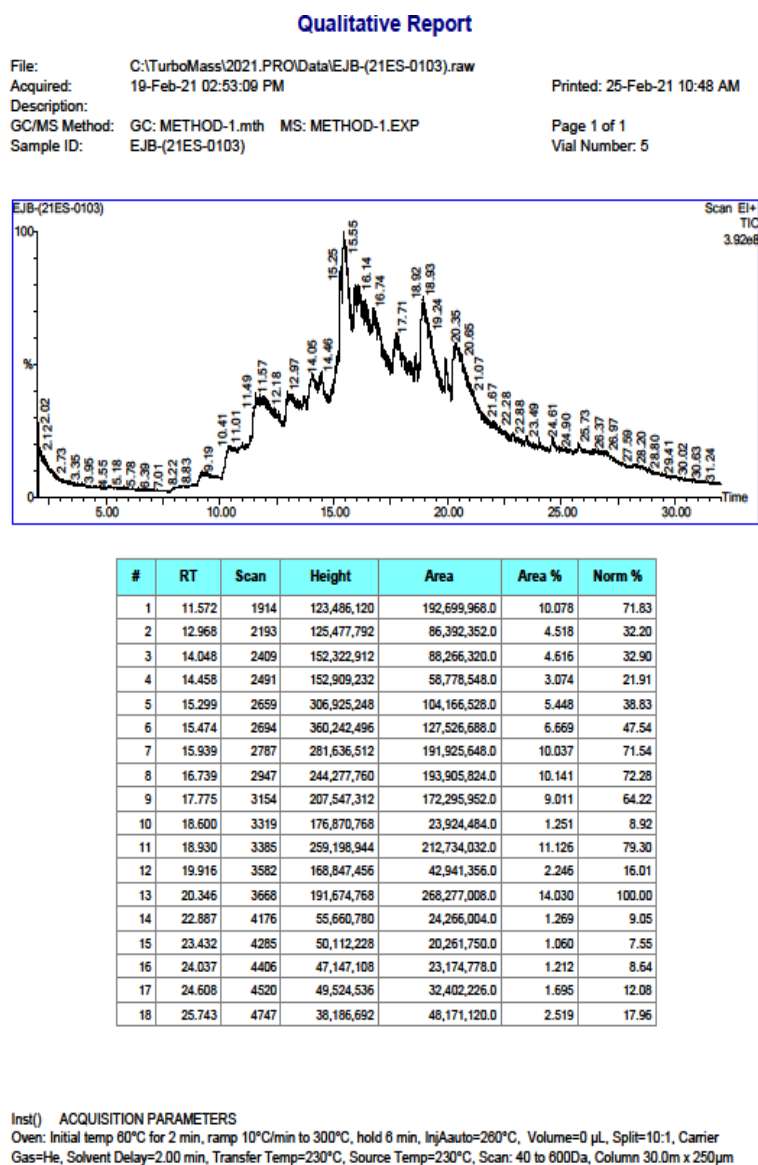
amino acids, and requires a small volume of plant extracts. Hence, in the present study, the GC–MS technique was adopted for the detection and identification of Phytochemical compounds present in the medicinal plant. GC-MS analysis revealed that the extracts contain a high concentration of phytoconstituents, which may be bioactive components involved in the plant's therapeutic properties. These results can be used as a good preliminary indication for future applications of antifungal, antibacterial, antimalarial, antidiabetic, anti-inflammatory, antimicrobial, preservative, and antioxidant.



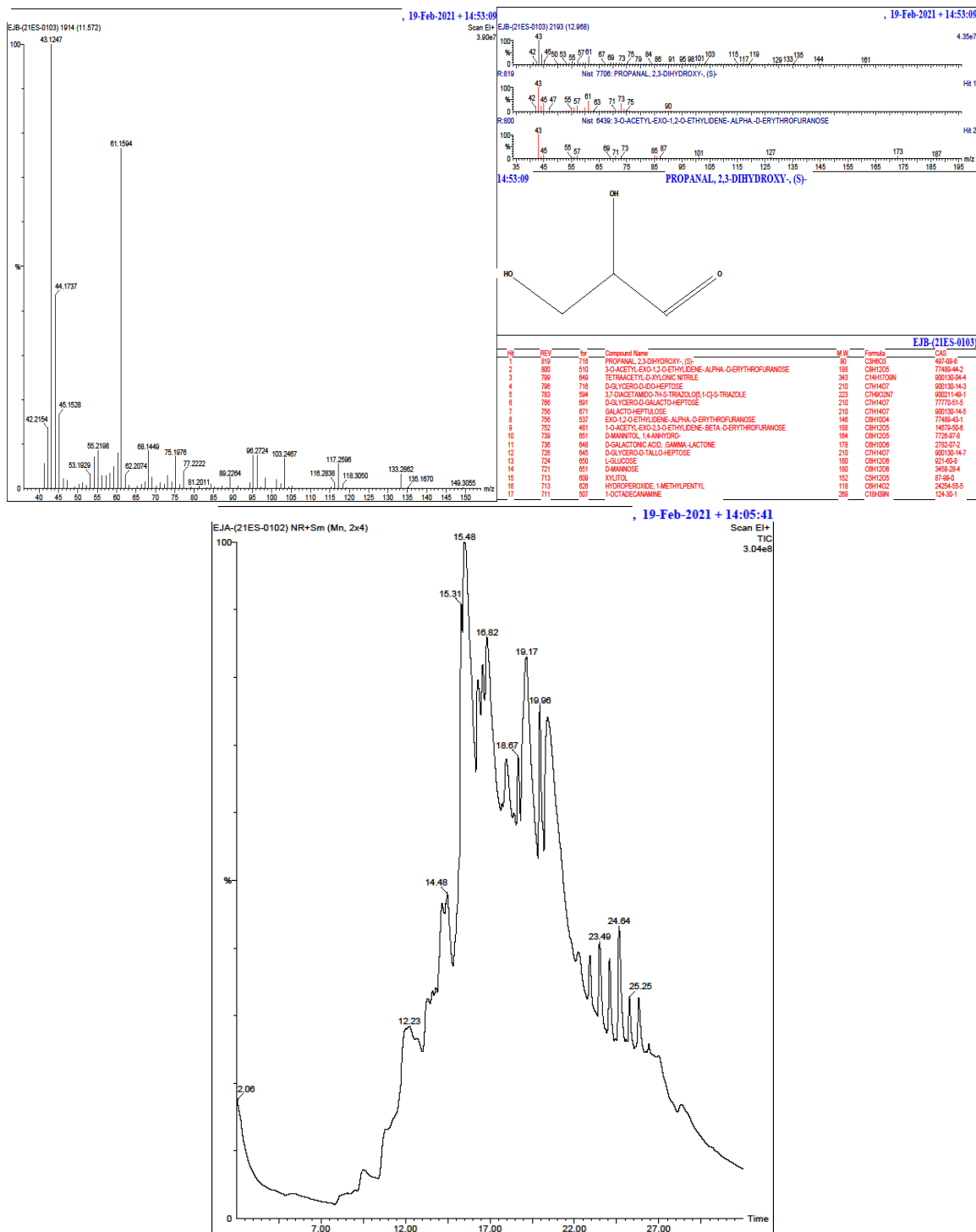
The results of GC-MS analysis revealed the tentative identity of 17 compounds present in Without heat Ethanolic extract *Eugenia jambolana* The active principles with their retention time, probability, molecular weight, and concentration (peak area %) are presented in Fig 1



Chemical constituents from GC-MS analysis of without heat ethanol extract of *Eugenia jambolana*. GC-MS chromatogram of *Eugenia jambolana* extract Fig 2.

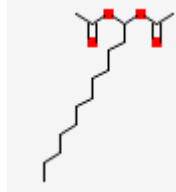
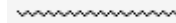


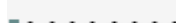
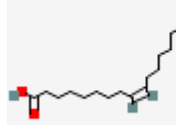
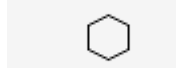


The results of GC-MS analysis revealed the tentative identity of 17 compounds present in with heat Ethanolic extract *Eugenia jambolana* The active principles with their retention time, probability, molecular weight, and concentration (peak area %) are presented in Fig 3

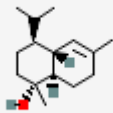
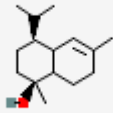



Chemical constituents from GC-MS analysis of with heat ethanol extract of *Eugenia jambolana*.GC-MS chromatogram of *Eugenia jambolana* extract Fig 4.

Activity of Phytocomponents identified in the ethanol extract of *Eugenia jambolana* seed part
(Table 1)

S.No	Compound Name	Molecular Weight	Formula	Structure	Biological Activity
	1,1-Dodecanediol	286	<u>$C_{16}H_{30}O_4$</u>		antioxidant, and antibacterial activities
	Hexatricontane	507	<u>$C_{36}H_{74}$</u>		antimicrobial activity
	Nonadecane	268.5	<u>$C_{19}H_{40}$</u>		Antimicrobial and anticancer
	1-Chloro, Dotriacontane	450.9	<u>$C_{32}H_{66}$</u>		Antibacterial activity, Antimicrobial activity,
	1-Heptadecanamine	255.5	<u>$C_{17}H_{37}N$</u>		Antibacterial Activity
	Oleic acid	282.5	<u>$C_{18}H_{34}O_2$</u> or $C_8H_{17}CH=CH(C_7H_{15})COOH$		Fatty Acids Analysis, Antioxidant, Antibacterial activity
	Cyclohexane	84.16	<u>C_6H_{12}</u>		Anticancer activity, antioxidant activity, cytotoxic activity, anti-inflammatory activity

	1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)	280.5	<u>C₂₀H₄₀</u>		Antimicrobial activity, human pathogenic bacteria.
	N-Hexadecanoic acid	256.4241	<u>C₁₆H₃₂O₂</u>		antioxidants, hypocholesterolemic, nematocide, and pesticide
	Ethyl ester	114.14	<u>C₆H₁₀O₂</u>		antimicrobial activity
	T-Butyl Cyclopentaneperoxycarboxylate	186.25	<u>C₁₀H₁₈O₃</u>		analgesic, antispasmodic and antibacterial activity
	5-Isopropyl-6-Methyl-Hepta-3,5-Dien-2-OL	168.28	<u>C₁₁H₂₀O</u>		Antimicrobial Activity
	2,3-Anhydro-D-Mannosan	144.12	<u>C₆H₈O₄</u>		NA
	2,3-Anhydro-D-Galactosan	144.12	<u>C₆H₈O₄</u>		Preservative

Alpha-Cadinol	222.37	$C_{15}H_{26}O$		Antimicrobial Activities
Tau-Muurolol, Ledene Oxide (II) Diglycerol	222.37	$C_{15}H_{26}O$		NA
1 Chloro	142.62	$C_8H_{11}Cl$		NA

Preparation of plant extract (Fig 5,6)



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