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Research Article

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF LEAF, ROOT, AND BARK PARTS IN METHANOLIC EXTRACTS OF *SEMECARPUS ANACARDIUM L.*

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ABSTRACT

Natural resources are considered the treasure of the earth. Medicinal plants are the major source of natural products having a wide range of therapeutic applications due to secondary metabolites which are potential sources of drugs. Green plants release thousands of bioactive compounds from their various parts such as leaves, stems, and roots, and there are various secondary metabolites. In India, *S.anacardium* is used in Ayurvedic and Unani preparations as a traditional folk medicine and remedy for several illnesses. The screening of primary phytochemicals, crude extracts of leaf, stem bark, and roots of *S.anacardium* revealed the presence of important phenolic compounds, alkaloids, fatty acids, saponins, flavonoids, many secondary metabolites, and significant bioactive compounds. The present investigation was carried out to detect and identify possible bioactive compounds present in the different plant parts of the *S.anacardium* plant by Gas chromatography- Mass spectroscopy (GC-MS) technique. Twenty bioactive compounds were identified in the methanolic extract of *S. anacardium*. Among them 9 compounds were found in methanolic extracts of leaf, 8 compounds in methanolic extracts of stem bark, and 3 compounds in methanolic extracts of root. The results revealed the presence of various phytochemical compounds in all parts of the plants in methanolic extracts.

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INTRODUCTION

India has a rich biodiversity, and plants have been used in all sectors of the economy viz agriculture, food, fodder. Since antiquity, scientists have created wonderful drugs in Ayurveda using herbal medicines developed between 2000-500 B.C. Plants have formed the source of sophisticated traditional medicine systems that have existed for thousands of years and continue to provide mankind with novel remedies (Gurib Fakim., 2006). Medicinal plants are solar-powered biochemical factories and many people use them as a treatment for several diseases. Plants are the source of new drugs because of their safety, efficacy, less expensive, readily available, lesser side effects, and fruitful life in the future (Arun *et al.*, 2009; Ikegami *et al.*, 2012; Prakash *et al.*, 2018; Yadav *et al.*, 2011). Ancient texts including the Holy Bible and Quran, Vedas, and other religious books describe the general usage of traditional herbs and medicinal plants. Bhalla taka (*S.anacardium*) is one of the ancient traditional herbs and its benefits have been revealed in Valmiki Ramayana, one of the greatest holy books (Balapure *et al.*, 1987). People are protected under nature's umbrella and Green plants are considered universal food

providers to all organisms. Natural products are still one of the major sources of the invention of new drug molecules today (Newman *et al.*, 2012). The crude extracts and isolated compounds have been found to possess various biological activities, particularly in antimicrobial, antidiabetic, anticancer, anti-inflammatory, and antioxidant and skin infections. The screening of plant extracts and plant products for antibiotic prototypes (Aflon *et al.*, 2003).

Phytochemicals are responsible for the medicinal activity of plant species. Phytochemicals are biologically active, naturally occurring chemical compounds rich in plants, which give health to humans (Hasler *et al.*, 1999). Secondary metabolites are of great value both industrially and medicinally. *Anacardiaceae* plants constitute a major source of natural organic compounds and secondary metabolites in different parts of the plant are used as medicine in different countries for curing illnesses (Salehi *et al.*, 2020). Medicinal plants are the source of potential antimicrobial properties including natural antioxidants (Carotenoids, alkaloids, phenolic acids, anthraquinones, glycosides, lignins, flavonoids, sterols, and saponins) are responsible for preventing oxidative stress-

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associated illness. Several secondary metabolites are present in its leaves, fruits, and other parts of the plant and show antioxidant, anti-inflammatory, anticancer, and antimicrobial activities that can recover the healing process of sickness (Tyagi *et al.*,2017; Moses *et al.*,2019). Secondary metabolites of plants contain useful properties to cure several infections for the enhancement of our life span. Flavonoids are a cluster of normal constituents with adjustable phenolic structures and are found in fruits, vegetables, grains, bark, roots, and branches (Middleton, 1998). Extraction is the main step for the recovery and separation of bioactive compounds from plant resources, before component analysis (Karimi and Jaafar, 2011).

MATERIALS AND METHODS

Collection of plant material

The leaves, bark, and roots of *S.anacardium* were collected from the 7-10-year-old trees growing widely in different forest areas of Warangal and Adilabad districts in Telangana.

Preparation of extracts:

Fresh leaves, bark, and roots of *S.anacardium* were collected and washed thoroughly under running tap water until the surface dust particles were removed. These plant materials were shade-dried at room temperature grind into a fine powder and preserved in a container for further use. About, 5g of grinded leaf, bark, and root powders were mixed in 50 ml of 90% methanol and incubated for 48hrs in an orbital shaker at 130 rpm. The supernatant was collected using Whatman no 1 filter paper and evaporated under laboratory conditions to obtain a crude extract of leaf, bark, and root of methanolic extracts. The final extract was prepared and maintained for further studies. The extracts were stored in a refrigerator at 4°C for further use.

GC-MS analysis

Gas chromatography-mass spectroscopy has proved to be a valuable technique for the identification and analysis of bioactive compounds. GC-MS analysis was carried out by

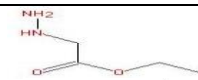
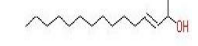
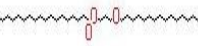
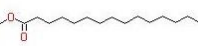
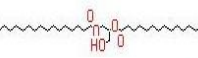
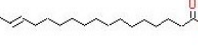

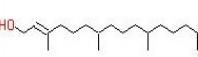
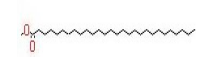
using the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. For Gas chromatography an (Agilent 7890 instrument was used, and for Mass Spectrometer Joel, Accu TOF GCV) instrument was used, and fused with a silica capillary column (30m ×0.25mm df × 0.25µm film thickness) GCMS method is one of the greatest methods used in identifying bioactive compounds in plants. Helium gas was used (99.99%) as carrier gas with a flow rate of 1 ml/min. Detector and injector temperatures were 300 and 250°C, respectively. The compounds were identified by comparing their retention times with those of pure standards registered in the NIST library software associated with the instrument. The structure of the compound, molecular weight, spectral data, retention time, molecular formula, category, and percentage of each bioactive compound of methanolic extracts was calculated by the total peak area of all components in the chromatogram. Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry and most significant tool for the identification and quantification of volatile and semi-volatile organic compounds in complex mixtures. (Hima bindu *et al.*,2003). Hence the present study is carried out to find out the bioactive compounds present in methanolic leaf, bark, and root extracts of *S.anacardium* by using GC-MS Gas chromatography-mass spectroscopy analysis. GC-MS plays a vital role in the identification of individual compounds present in the species.

RESULTS AND DISCUSSION

Components detected in methanol leaf extracts

GCMS analysis of methanol leaf extracts showed nine bioactive compounds which were presented in (Table 1, Fig 1). The compounds are Acetic acid hydrazino, ethyl ester, E-3-Pentadecan -2-0l, Stearic acid, and 3-(octadecyl oxy) propyl ester it is reported to use as a cosmetic 5-alpha-reductase-inhibitor, flavor, hypo cholesterolemic, lubricant, perfumery, propepic, suppository activities in *Schinus molle* (Salem *et al.*, 2016).

Table 1 Phytochemicals detected in the leaf extract of *S. anacardium*.

Peak no	R.T	Chemicals identified	Structure of the compound	MF	MW (g/mol)	Peak Area %
01	5.83	Acetic acid hydrazino, ethyl ester		C ₄ H ₁₀ N ₂ O ₂	118	7.24
02	18.92	E-3-Pentadecan -2-0l		C ₁₅ H ₃₀ O	226	7.22
03	19.05	Stearic acid ,3-(octadecyloxy) propyl ester		C ₃₉ H ₇₈ O ₃	594	1.89
04	20.24	Hexadecanoic acid, methyl ester		C ₁₇ H ₃₄ O ₂	270	8.10
05	20.93	Hexadecanoic acid 1-(hydroxy methyl) 1-2-ethanediyl ester		C ₃₅ H ₆₈ O ₅	568	3.90
06	22.61	E-15 .Heptadecenoic acid		C ₁₇ H ₃₂ O ₂	268	0.75
07	22.70	9,12,15-Octadecatrienoic acid,2-phenyl-1,3-dioxan-5yl ester		C ₂₈ H ₄₀ O ₄	440	2.23
08	22.87	Phytol		C ₂₀ H ₄₀ O	296	66.05
09	23.00	Octacosanoic acid ,Methyl ester		C ₂₉ H ₅₈ O ₂	438	2.56

Retention time (RT), Molecular formula, and Molecular weight (MW).

Hexadecanoic acid, methyl ester has anti-inflammatory, hypo-cholesterol emic, cancer-protective nature, lubricant, hepatoprotective activity, antioxidant, nematicide, insecticide, anti-histaminic, anti-eczemic, anti-acne, alpha-reductase inhibitor, Nematicide, anti-androgenic, anti-arthritis, decrease blood cholesterol, hemolytic, anti-coronary activities (Krishnamoorthy *et al.*,2014; Belakhdar *et al.*,2015; Gnana Sundaram *et al.*,2017; Arora *et al.*,2017). Hexadecanoic acid, methyl ester is considered a fatty acid ester and it is treated as a pesticide, flavor agent, and anti-androgenic also reported in *Neolamarckia cadamba* (Zayed *et al.*,2014).

acid, 2-phenyl-1, 3-dioxan-5yl ester. (Al-Marzoqi *et al.*, 2016) reported that 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester has Antiviral and anti-obesity properties. Phytol has anti-microbial, anti-cancer, diuretic, anti-inflammatory, cytotoxicity, anxiolytic, immunomodulators, and induction of apoptosis activities. (Parthipan *et al.*, 2015 ; Rajalakshmi *et al.*,2016; Sudha *et al.*,2013; Islam *et al.*, 2018). Octacosanoic acid, Methyl ester. Of the nine compounds identified in the leaf extract of *S.anacardium*, the activity of three compounds were not reported.

GCMS investigation of methanolic extracts of bark

Stem bark extracts show only eight chemical compounds. The Ion chromatogram outcomes of this study are shown in (Fig 2). Stem bark extract analysis known 08 compounds are demeclocycline. Demeclocycline is officially indicated for the treatment of various types of bacterial infections and is used as an antibiotic in the treatment of Lyme disease, acne, and bronchitis. Demeclocycline used in organic chemistry and a phosphocholine derivative has antifungal activity. Pentadecanoic acid, 14 methyl, methyl ester shows anti-oxidant, hypo chloesterolemic, nematicide, pesticide, anti-fungal, and anti-microbial activities reported by (Rangel-Sanchez *et al.*,2014; Alwin Beschi *et al.*,2021; Elezabeth *et al.*,2014; Elaiyaraja *et al.*, 2016). Trilinolein has been reported to provide several beneficial effects including increasing erythrocyte deformability, anti-ischemic, anti-arrhythmic, and

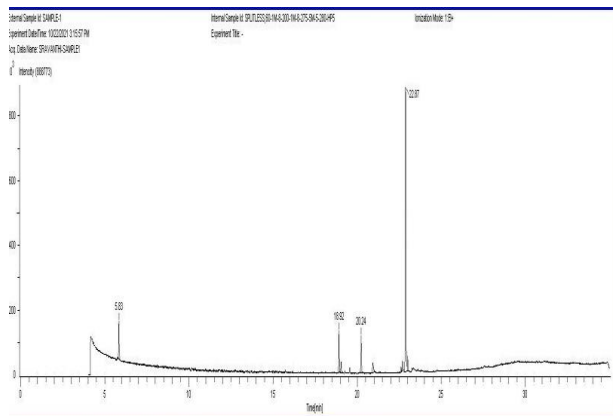


Fig 1. Ion Chromatogram (GC-MS) of methanolic leaf extract of *S.anacardium*

Table 2 Bioactive compounds are found in the stem bark extract of *S.anacardium*.

Peak no	RT	Chemicals identified	Structure of the compound	MF	MW (g/mol)	Peak Area %
01	5.87	Demeclocycline		C ₂₁ H ₂₁ ClN ₂ O ₈	464	11.70
02	20.23	Pentadecanoic acid,14 methyl, methyl ester		C ₁₇ H ₃₄ O ₂	270	19.59
03	22.61	Trilinolein		C ₅₇ H ₉₈ O ₆	878	2.31
04	22.69	9,12-Octadecadienoic acid, methyl ester, (E, E)		C ₁₉ H ₃₄ O ₂	294	5.68
05	23.00	Docosanedioic acid, dimethyl ester		C ₂₄ H ₄₆ O ₄	398	4.43
06	24.08	10, Acetoxy,hydroxy1,2,6a,6b,9,9,12a-hepatamethyl,1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12, 12a,12b,13,14b,octadecahydro-2H-Picene-4a-carboxylicacid, methyl		C ₃₃ H ₅₂ O ₅	528	12.60
07	28.65	Ethylisoallcholate		C ₂₆ H ₄₄ O ₅	436	31.47
08	31.13	Ethylisoallcholate		C ₂₆ H ₄₄ O ₅	436	12.35

Retention time (RT), Molecular formula, and Molecular weight (MW) .

Hexadecenoic acid 1-(hydroxy methyl) 1-2-ethanediyl ester is reported to have Antioxidant, hypo cholesterolemic, nematicide, pesticide, lubricant, emollients, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor (Prabha *et al.*,2019; Jiji *et al.*, 2017). Sharmila *et al.*, (2021) reported that hexadecenoic acid 1-(hydroxy methyl) 1-2-ethanediyl ester shows acidifier, arachidonic acid inhibitor, increased aromatic amino acid decarboxylase activity. Kadhim *et al.*, (2017) reported that hexadecenoic acid 1-(hydroxy methyl) 1-2-ethanediyl ester shows anti-microbial activity found in *Vitis vinifera*. E-15. Heptadecenoic acid. 9, 12, 15-Octadecatrienoic

antioxidant properties, and cardio-protective effects (Chan *et al.*,2005; Srivastava *et al.*,2017).9,12-Octadecadienoic acid, methyl ester (E, E) it is reported to have anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, anti-androgenic, antiarthritic, anti-coronary, insectifuge, antieczemic and antiarthritic (Chinnasamy *et al.*, 2018; Manonmani *et al.*,2015; Ganesh *et al.*,2017). Docosanedioic acid, dimethyl ester.

Prakash *et al.*, (2019) reported that Docosanedioic acid, dimethyl ester has anti-fungal activity. 10, Acetoxy,

hydroxyl,2,6a,6b,9,9, 12ahepatamethyl, 1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12,12a, 12b, 13, 14b, octadecahydro - 2H-Picene - 4a-carboxylicacid, methyl. Ethylisoallocholate is reported to have antioxidant, anti-inflammatory, anti-arthritis, antiasthma, anticancer, diuretic, and anti-microbial activity (Khan *et al.*, 2019; Malathi *et al.*, 2016; Muthu Lakshmi *et al.*,2012; Sudha *et al.*, 2013; Imtair *et al.*,2019). Balabhaskar *et al.*,(2020) reported that Ethyl isoallocholate shows anti-tumour activity found in *Bauhinia tomentosa*. Hussein *et al.*,(2016) reported that Ethyliso-allocholate shows anti-inflammatory activity and anti-infective effects in *Quercus infectoria*. Among the nine compounds identified in the bark extract of *S.anacardium*, the activity of one compound was not reported.

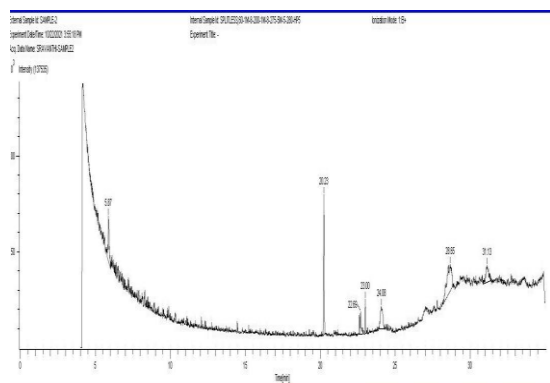


Fig 2. Ion Chromatogram (GC-MS) of methanolic bark extract of *S.anacardium*.

were identified from root extract by gas chromatography–mass spectrometry (GC-MS) analysis.

Several experiments have shown that *S.anacardium* has a great diversity of phytochemicals such as naphthoquinones, iridoids, and other compounds with desirable pharmacological effectiveness. The Indian pharmaceutical industry is the third largest industry in the world in terms of the manufacture of drugs and pharmaceuticals.

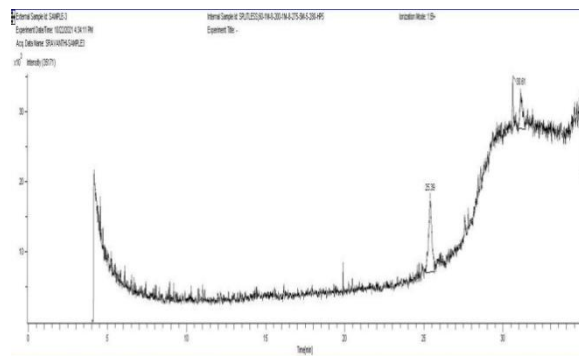


Fig 3 Ion Chromatogram (GC-MS) of methanolic root extract of *S.anacardium*.

S.anacardium nut shows biologically active compounds that are used as remedies to develop new medicines in the pharmaceutical industry (Tiwari *et al.*, 2019).

Table-3 Phytochemicals found in the root extract of *S. anacardium*.

Peak no	RT	Chemicals identified	Structure of the compound	MF	MW (g/mol)	Peak Area %
01	25.39	Tetradecanoic acid ,3,3a,4,6a,7,8,9,10,10a,10b-dihydroxy-5(hydroxy methyl)-2,10-dimethyl-3-oxobenz(e)azulen-8-yl ester,(3Ar-(3aα,6aα,80		C ₁₃ H ₅₀ O ₆	518	62.65
02	30.61	3-Pyridinecarboxylic acid,2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a7a-epoxy-5H-cyclopenta(a)cyclopropa(f)cy		C ₃₂ H ₃₉ NO ₁₀	597	10.96
03	31.11	3-Pyridinecarboxylic acid,2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a7a-epoxy-5H-yclopenta (a) cyclopropa(f)cy		C ₃₂ H ₃₉ NO ₁₀	597	26.37

Bioactive compounds study from root in methanolic extract

GC-MS analysis of root extract reveals three compounds and is presented in Table 19. The peaks of these three compounds were reported in (Fig 3).

- 1) Tetra decanoic acid, 3, 3a, 4, 6a, 7, 8, 9, 10, 10a, 10b - dihydroxy -5 (hydroxymethyl) -2,10-dimethyl-3-oxobenz(e) azulen-8-yl ester, (3Ar-(3aα,6aα, 80
- 2) pyridine carboxylic acid, 2,7,10-tris (acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a7a-epoxy-5Hcyclopenta(a) cyclopropa (f) cyl .
- 3) pyridine carboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a7a-epoxy-5Hcyclopenta(a)cyclopropa(f)cyl.

In the present investigation, nine bioactive plant-based compounds were identified from leaf methanol extract, eight compounds from identified bark extract, and three compounds

S.anacardium is commonly used in ethnomedicine powerful remedies have been used by traditional healers in the treatment of many diseases, such as anti-atherogenic, anti-inflammatory, anti-oxidant, anti-microbial, anti-reproductive, CNS stimulant, hypoglycaemic, anti-carcinogenic and hair growth promoter (Semalty, *et al.*,2010).

Akare *et al.*, (2015) investigated the therapeutic importance of *S.anacardium* nut possessing some biologically active compounds such as sterols, galluflavanone, semecarpu flavanone, nallaflavanone, amentoflavone, an cardoside, etc. The fruit extracts of *S.anacardium* in phytochemical analysis showed the presence of saponins, flavones, tannins, and glycosides. Phyto constitutes gives defensive health profits to human beings. (Madhuri *et al.*, 2018). *S.anacardium* nut shows biologically active compounds that are used as remedies to develop new medicines in the pharmaceutical industry.(Tiwari *et al.*, 2019).

Solankar *et al.*, (2018) investigated the antioxidant activity of aqueous and ethanolic extracts of leaves of *S.anacardium*. According to their results presence of phytochemicals in leaves is considered as an active medicinal chemical constituent.

CONCLUSION

Herbal medicines are valuable sources of innumerable medicinal properties and therapeutic uses used for the preparation of potential drug molecules that can be used to treat harmful diseases. The GC-MS analysis pointed out extraordinary compounds such as Phytol, Trilinolein, and octacosanoic for fatty acids and octacosanol regarding fatty alcohols. The presence of various bioactive compounds is active with anti-cancer, anti-inflammatory, anti-diabetic, anti-allergic, lubricant, nematicide, anti-oxidant, anti-microbial, and anti-ischemic, hypercholesterolemic, immunosuppressive and hepatoprotective activities, anti-arrhythmic effects. These various bioactive compounds of *S.anacardium* are useful in treating various ailments and recommended phytochemicals used in the development of new useful drugs with pharmaceutical importance. This investigation makes a pathway for the development of novel and useful drugs.

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