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Phytochemical analysis of bioactive constituents from normal leaf, insect induced midrib gall and petiole gall on *Eucalyptus tereticornis* smith

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Abstract
Plant galls caused by "insect" are unique example of host-pathogen interaction in which a complex association and mutual adaptation between the host and the pathogen takes place. Galls are characterized by cellular hypertrophy and hyperplasia. *Eucalyptus tereticornis* Smith, (Myrtaceae) is an important source of essential oil. Insect *Leptocybe invasa* infect stem, leaf midrib and petiole of *Eucalyptus tereticornis* which results into the gall formation. The present investigation deals with the evaluation of phytochemical compounds present in the normal leaf and the alteration that is observed in the midrib gall and petiole gall on *Eucalyptus tereticornis* through Gas Chromatography-Mass Spectroscopy technique. Methanolic extract of plant samples were used for the study of phytochemical constituents. GC-MS analysis of normal leaf, midrib gall and petiole gall of *Eucalyptus tereticornis* showed the presence of sixty six, thirty nine and forty four phytochemical compounds respectively. Further investigation on the effectiveness of these phytochemical constituents can result into various findings and discovery of novel and useful secondary metabolites which can be used as various purposes in medicinal and other related industries.

Keywords: plant galls, host-pathogen interaction, *Eucalyptus tereticornis*, *Leptocybe invasa*, phytochemical, GC-MS

Introduction

Eucalyptus tereticornis Smith is an evergreen tree of Myrtaceae family and is commonly known as mysore gum, forest red gum and blue gum. Trade name for this tree is Mysore gum. *Eucalyptus* is a native plant to Australia and few species are native to Papua New Guinea, Indonesia (Leicach *et al*., 2012) [18] and now it has spread to other parts of the world including India, South Africa and Europe. In India it is widely distributed in Tamil Nadu, Andhra Pradesh, Gujrat, Haryana, Mysore, Kerala, Nilgiri Hill and in the Semi-arid region of Rajasthan. *E. tereticornis* has the broadest latitudinal distribution of any other species in the genus *Eucalyptus.* It can survive over a wide range of climatic conditions (Orwa *et al*., 2009) [24]. *Eucalyptus* is used in various medicines. The essential oil of the *Eucalyptus* have 70-85% 1,8 cineole (eucalyptol). It brings relief to the patients of bronchitis and asthma. It is an excellent remedy for aching joints and rheumatism. 1,8 cineole (eucalyptol) content of *Eucalyptus* oil have antimicrobial activity against many viruses, bacteria and fungi. It also includes many medicinal properties such as anti-inflammatory, antioxidant, analgesic and immune stimulatory effects (Sadlon & Lamson 2010; Vecchio *et al*., 2016) [33, 42]. It is largely used in the cosmetics industry and has well-known insecticidal (Maciel *et al*., 2010) [21] and herbicidal properties (Vaughn and Spencer, 1993) [40]. *Eucalyptus* plantation infected with galling insect *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae: Tetrastichinae) was reported from Andhra Pradesh, Gujarat, Delhi, Maharashtra, Madhya Pradesh, Kerala, Pondicherry, Uttar Pradesh and Goa (Kumar *et al*., 2007) [17]. The stem, midrib and petiole are infested by the insect, *Leptocybe invasa* Fisher and La Salle. Feeding action of gall causing insects stimulate the tissue of host plant to develop into galls, whereas Hymenopteran insects trigger gall development by oviposition. During the process of gall formation vascular tissues can be modified for the optimum supply of nutrients and water, sub serving the needs of the insect (Meyer, 1969; Wool *et al*., 1999; Raman *et al*., 2006) [22, 44, 29]. Stress caused due to insect infestation can result into a variety of plant responses and also alters the metabolism of plant. This alteration in metabolism include production of various secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds that are very important phytochemical constituents and also help into stress tolerance. In the present investigation GC-MS technique was used for the comparative analysis of phytochemical compounds in normal leaf, midrib gall and petiole gall of *Eucalyptus tereticornis* that will help to knowing which phytochemical will part of biotic stress tolerance or mutual adaptation.

Materials and Methods

Dry Powder Preparation

Plant samples viz. normal leaf, midrib gall and petiole gall of *Eucalyptus tereticornis* were collected from the Jaipur and adjoining areas. All the samples were washed with tap water for removal of dirt and debris and dried in shade separately. Dried samples were pulverized to powder form using mechanical grinder**.**

Preparation of Extract

Powdered plant samples were extracted with methanol at 70–80°C by hot continuous percolation method in Soxhlet apparatus for 24 hours. The extract was filtered through Whatmann filter paper. The extract was then evaporated using rotary evaporator to obtain extract. The obtained methanolic extracts were used for the detection of preliminary phytochemical constituents through GC-MS analysis.

GC-MS Analysis

The GC-MS analysis of methanolic extracts of normal leaf, midrib gall and petiole gall of *Eucalyptus tereticornis* were performed using Shimadzu QP-2010 plus with thermal desorption system TD 20. It contains auto sampler and a gas chromatograph which interfaced to a mass spectrophotometer. The column size of this system is 30m × 0.25mm i.d × 0.26µm with a film thickness of 0.26mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml per min. The 2µl injection volume of sample was employed with split ratio of 10:1. The injector temperature was programmed initially at 280°C, the Ion-source temperature was 200°C. The oven temperature was programmed from 110°C (for 4 minutes), with an increase of 10°C/minute to 200°C, then 5°C/minute to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were studied using electron impact ionization at 70 eV. The total GC running time for each sample was 45 minutes.

Identification of Phytochemical Components

Interpretation of phytoconstituents present in the sample was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown constituent was compared with the spectrum of the known constituents stored in the NIST library. The name, molecular weight and structure of the components of the test samples were ascertained**.**

Results

Gas Chromatography-Mass Spectrophotometry (GC-MS) is a combined technique of Gas Chromatography with Mass Spectrophotometry. Mass Spectrophotometry is wide ranging analytical technique, useful in the identification of the charged species according to their mass to charge ratio (M/Z). GC-MS is an excellent technique to identify the phytoconstituents of volatile compounds. The GC-MS analysis of normal leaf, midrib gall and petiole gall of *Eucalyptus tereticornis* showed the presence of sixty six (Fig 1), thirty nine (Fig 2) and forty four (Fig 3) phytochemical compounds respectively. Phytochemical constituents were identified on the basis of the peak area, retention time and molecular formula. The active principles with their retention time (RT), area %, compound name, of normal leaf, midrib gall and petiole gall are presented in Table 1, 2 and 3 respectively.

Discussion

The GC-MS analysis revealed that the methanolic extract of normal leaf of *Eucalyptus tereticornis* had more compounds than the midrib gall and petiole gall extract of the plant. Major Phytochemical compound identified in the GC-MS analysis of methanolic extract of normal leaf of *Eucalyptus tereticornis* was 2-Naphthalenemethanol, Decahydro-.Alpha.,.Alpha.,4a-Trimethyl-8-Methylene (Beta-Selinenol) with 17.19 % peak area. It was also present in midrib gall and petiole gall extract with 14.97% and 12.36% peak areas respectively. Beta-Selinenol is an important bioactive compound present in the essential oil of *Melaleuca cajuputi* and has anticoronavirus activity (My *et al*., 2020) [23]. The next highest compound identified in the normal leaf was (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol (7.57%). Major compounds identified in the midrib gall extract were Glycerin (17.22%), followed by (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-ol (15.24%). Major compounds identified in the petiole gall extract were (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol (15.03%), followed by 2-Naphthalenemethanol, Decahydro-.Alpha.,.Alpha.,4a-Trimethyl-8-Methylene/Beta-Selinenol (12.36%) and Glycerin (9.73%). Glycerin was only present in the midrib gall and petiole gall extract. Glycerin was not found in the normal leaf extract. This may be due to the insect feeding requirements. Glycerin is a simple polyol compound and can be used commercially. Glycerin has a potential for new biodiesel formulation (Jaecker-Voirol *et al*., 2008) [14]. It is also largely used in the food industry as a sweetener and in pharmaceutical formulations as a humectant. 5-Hydroxymethylfurfural (HMF) was only found in normal leaf extract. 5-HMF has been found to be useful in the treatment of sickle cell disease where it bind specifically with intracellular sickle haemoglobin (Abdulmalik *et al*., 2005) [1] and it has also several beneficial properties including anti-oxidative, anti-allergic, anti-inflammatory, anti-hypoxic, and antihyperuricemic effects which are useful for human health (Zhao *et al*., 2013) [47]. 5-HMF displayed antiproliferative activity on human melanoma A375 cells than other cell lines and it also have potential applications in cancer chemoprevention. It can also be developed as novel natural antioxidant (Li *et al*., 2009) [19]. Studies have showed that the presence of HMF protected the human liver cell line-LO2 against exposure to hydrogen peroxide, because it prevented caspase-3 activation, nitric oxide production and arrest of the cells in the S-phase of the cell cycle (Ding *et al*., 2010) [11]. 5-HMF is a furan derivative and has potential to be sustainable substitutes for petroleum-based building blocks which can be used in the production of plastics and fine chemicals (Chheda *et al*., 2007) [9]. Other important phyto-constituent found in the methanolic extract of normal leaf was 2-Methoxy-4-Vinylphenol that has anti-inflammatory effect by inhibiting NF-κB and MAPK Activation and Acetylation of Histone H3 (Jeong *et al*., 2011) [15]. Alpha-Terpinyl Acetate can be used as a suitable lead for the development of a molecule that might have disease amelioration effects in Alzheimer's disease (Chowdhury and Kumar, 2020) [10]. Tetradecanoic Acid (Myristic Acid) is used in soaps and cosmetic industries. Myristic Acid also has antimicrobial potential against *Listeria monocytogenes* in milk (Chen *et al*., 2019) [8]. Neophytadiene have showed antifungal, anti-inflammatory, antipyretic and analgesic properties (Raman *et al*., 2012) [30]. 8-Octadecanone and 9-Undecenal, 2,10-Dimethyl displayed antibacterial activity (Tayung *et al*., 2011 and Tajudin, 2020) [38, 37]. Linoleic Acid is present in normal leaf extract. (Z,Z)-6,9-Cis-3,4-Epoxy Nonadecadiene have antibacterial properties (Patra *et al*., 2015) [27]. Phytol has shown Antinociceptive, Antioxidant (Santos *et al*., 2013) [35] and Antimycobacterial Activities (Rajab *et al*., 1998) [28]. Vitamin E prevents Platelet hyperaggregation, which can lead to atherosclerosis. Vitamin E also helps to decrease the production of prostaglandins such as thromboxane, which cause platelet clumping. Additionally Vitamin E also has beneficial effects on skin and human health (Rizvi *et al*., 2014) [32]. Gamma.-Sitosterol has anticancerous (Sundarraj *et al*., 2012) [36], Cytotoxic (Endrini *et al*., 2014) [13], Antidiabetic and antihyperlipidemic activities (Balamurugan *et al*., 2011) [3]. Benzene, 1-(1,5-Dimethyl-4-Hexenyl)-4- Methyl displayed Anti-inflammatory, Anticancer, Antioxidant properties (Pakkirisamy *et al*., 2017) [25]. Dibutyl Phthalate have been widely used as plasticizers. Hexadecanoic Acid was present in both normal leaf and midrib gall extract. Hexadecanoic Acid might serve as a plausible inhibitor against COVID-19 Main Proteases (Alam *et al*., 2021) [2]. Hexadecanoic Acid has displayed potential antifungal and antibacterial activity (Begum *et al*., 2016) [4]. Some phytoconstituents were found only in midrib gall and petiole gall extract such as Cryptomeridiol that has a significant medicinal value because it has anti-Alzheimer properties and antispasmodic in nature (Locksley *et al*., 1982 & Tebbaa *et al*., 2011) [20, 39]. Exo-2-Hydroxycineole was isolated from eucalyptus honey (66 mg/kg) in higher amount (Vázquez *et al*., 2006) [41]. 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-Octahydro-.Alpha.,. Alpha.,4a,8-Tetramethyl-, (2R-Cis)- displayed growth inhibition of *Staphylococcus aureus* and colibacillus. It can also be used as cosmetics and food additives in industrial production (Wang *et al*., 2013 & Chen *et al*., 2018) [43, 7]. Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)- (Elemol) is a fragrance ingredient and used in fine fragrances, decorative cosmetics, shampoos as well as in non-cosmetic products such as detergents and household cleaners (Bhatia *et al*., 2008) [5]. Some phytochemical compounds were found only in petiole gall extract such as Succinic Acid (SA) which seems to be a good inhibitor against the coronavirus epidemic (Sagaama *et al*., 2020) [34]. 1-Cyclohexene-1-Carboxylic Acid, 4-(1-Methylethenyl)- (Perillic acid) has showed immunomodulatory activity (Raphael *et al*., 2003) [31] and it has been found to induce apoptosis and cell cycle arrest in non small cell lung cancer cells (Yeruva *et al*., 2007) [46]. Petiole gall extract also has 1-Methyl-4-(5-Methyl-1-Methylene-4-Hexenyl)-1-Cyclohexene (Beta-Bisabolene) and Caryophyllene Oxide which were found to be promising candidates in the inhibition of the activity of SARS-CoV-2 molecular target proteins (Duru *et al*., 2021) [12]. β-Caryophyllene Oxide had anticancer (Park *et al*., 2011) [26], analgesic, anti-inflammatory (Chavan *et al*., 2010) [6], antifungal (Yang *et al*., 2000) [45] and cytotoxic properties (Jun *et al*., 2011) [16].

Eucalyptus tereticornis is used in a variety of traditional Indian medicines. Insect infestation on *Eucalyptus tereticornis* altered the phytochemical profile of galled parts as seen in the GC-MS analysis. Some important phytoconstituents were identified in the methanolic extracts of normal leaf, midrib gall and petiole gall like Beta-Selinenol, 5-Hydroxymethylfurfural, Gamma.-Sitosterol, Hexadecanoic Acid, Cryptomeridiol. These compounds have important medicinal properties.

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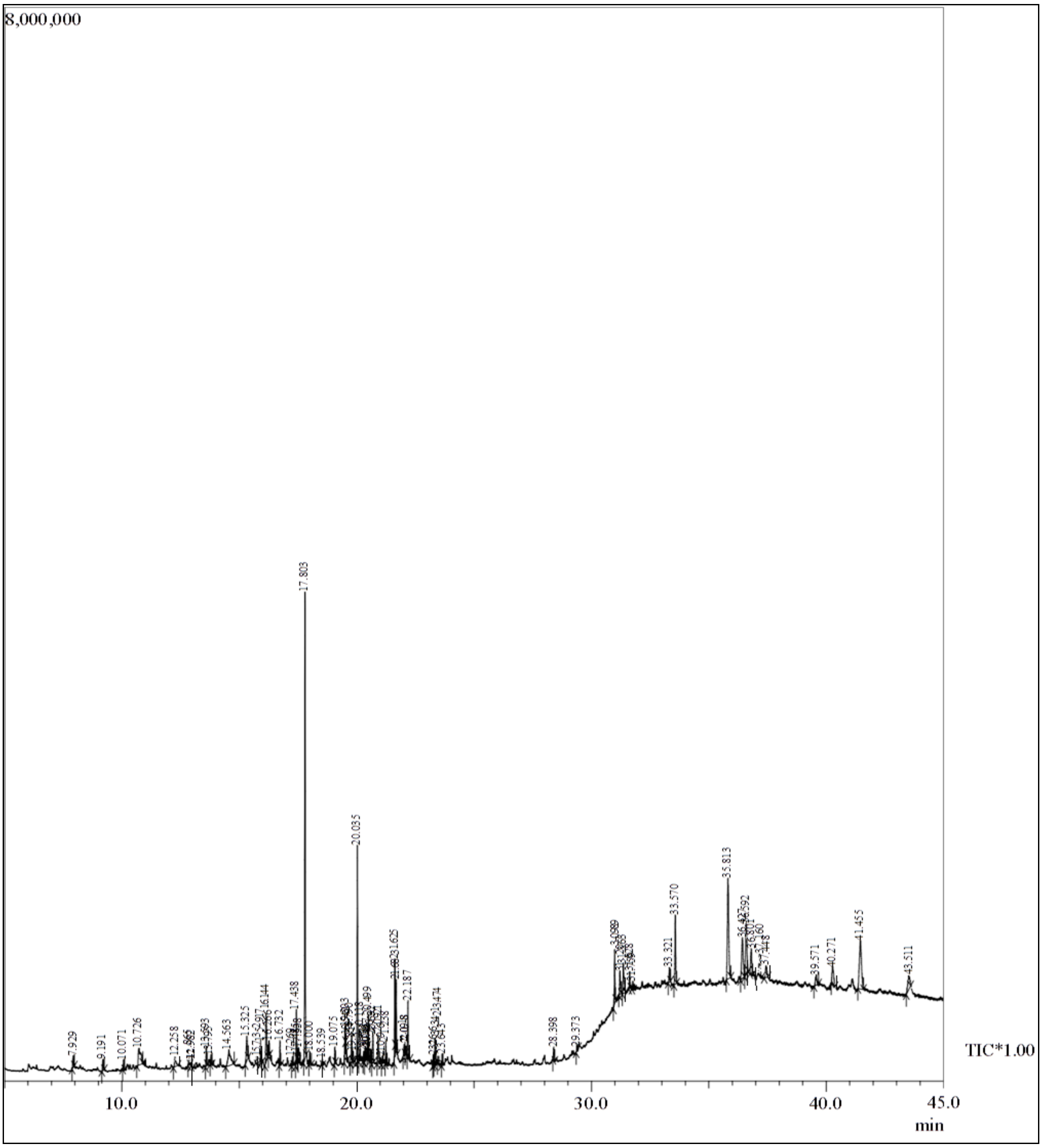

Fig 1: GC-MS Chromatogram of Normal Leaf Part of *Eucalyptus tereticornis*

Table 1: Phytocomponents identified from methanolic extract of Normal Leaf Part of *Eucalyptus tereticornis* using GC-MS analysis.

Peak#	R. Time	Area	Area%	Name
1	7.929	233149	0.55	1,3,5-Triazine-2,4,6-Triamine
2	9.191	250810	0.59	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-
3	10.071	144047	0.34	Cyclooctane, 1,2-Diethyl-
4	10.726	858272	2.01	5-Hydroxymethylfurfural
5	12.258	288546	0.68	2-Methoxy-4-Vinylphenol
6	12.865	121441	0.29	5-Caranol, (1S,3R,5S,6R)-(-)-
7	12.982	143112	0.34	2h-Pyran-3-Ol, 6-Ethenyltetrahydro-2,2,6-Trimethyl-
8	13.593	257326	0.60	Cetene
9	13.795	148291	0.35	2H-Pyran-3-Ol, 6-Ethenyltetrahydro-2,2,6-Trimethyl-
10	14.563	916416	2.15	2-Octen-1-Ol
11	15.325	558938	1.31	Hydroxy-.Alpha.-Terpenyl Acetate
12	15.732	78245	0.18	Acetic Acid, 1-Methyl-1-(4-Methyl-5-Oxo-Cyclohex-3-Enyl)Ethyl Ester
13	15.917	345342	0.81	Hydroxy-.Alpha.-Terpenyl Acetate
14	16.144	1031356	2.42	2-Butanone, 4-(4-Hydroxyphenyl)-
15	16.266	177204	0.42	3',5'-Dimethoxyacetophenone
16	16.732	312518	0.73	1-Heptadecene
17	17.269	153983	0.36	(-)-Tricyclo[6.2.1.0E4,11]Undec-5-En, 1,5,9,9-Tetramethyl- (Isocaryophyllen-I1)
18	17.438	688942	1.62	Agarospirol
19	17.485	59291	0.14	(-)-Aristolene
20	17.538	296815	0.70	11,11-Dimethyl-4,8-Dimethylenebicyclo[7.2.0]Undecan-3-Ol
21	17.803	7324653	17.19	2-Naphthalenemethanol, Decahydro-.Alpha.,.Alpha.,4a-Trimethyl-8-Methylene-
22	18.000	228550	0.54	Tricyclo[4.4.0.0(2,7)]Dec-8-Ene-3-Methanol,.Alpha.,.Alpha.,6,8-Tetramethyl-
23	18.539	70039	0.16	Cubenol
24	19.075	214025	0.50	Tetradecanoic acid
25	19.503	278607	0.65	7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol
26	19.542	117137	0.27	Undecanenitrile
27	19.790	501096	1.18	2-Hydroxymethyl-2,6,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]Undecane
28	19.854	171855	0.40	2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-Octahydronaphthalen-2-Yl)Propan-2-Ol
29	20.035	3224294	7.57	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol
30	20.118	423897	0.99	Neophytadiene
31	20.301	141511	0.33	2,2,4a,7a-Tetramethyldecahydro-1H-Cyclobuta[e]Inden-5-Ol
32	20.367	196255	0.46	Terpinyl Formate
33	20.457	21884	0.05	1,16-Hexadecanediol
34	20.499	461057	1.08	Tricyclo[4.4.0.0(2,7)]Dec-8-Ene-3-Methanol,.Alpha.,.Alpha.,6,8-Tetramethyl-
35	20.596	179414	0.42	8-Octadecanone
36	20.692	513962	1.21	9-Undecenal, 2,10-Dimethyl-
37	20.921	545203	1.28	2-Butanone, 4-(2,6,6-Trimethyl-1-Cyclohexen-1-Yl)-
38	21.096	122347	0.29	Cyclopentaneethanol, 4-(Acetyloxy)-2-[(Acetyloxy)Methyl]-.Beta.,3-Dimethyl-,Acetate
39	21.238	326752	0.77	Hexadecanoic Acid, Methyl Ester
40	21.625	1156616	2.71	Dibutyl Phthalate
41	21.683	917451	2.15	n-Hexadecanoic Acid
42	22.018	165852	0.39	p-Camphorene
43	22.098	132537	0.31	Trifluoroacetic Acid, Pentadecyl Ester
44	22.187	843328	1.98	Terpinyl Formate
45	23.266	134573	0.32	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
46	23.341	412249	0.97	(Z,Z)-6,9-Cis-3,4-Epoxy-Nonadecadiene
47	23.474	684074	1.61	Phytol
48	23.643	152279	0.36	Methyl Stearate
49	28.398	227257	0.53	1,2-Benzenedicarboxylic Acid
50	29.373	39953	0.09	1-Hydroxymethyl-5,8,9-Endo-10-Exo-Tetramethyltricyclo[6.3.0.0(5,11)]Undecane
51	30.989	760611	1.79	2,6,10,14,18-Pentamethyl-2,6,10,14,18-Icosapentaene
52	31.207	519897	1.22	.Alpha.-Tocospiro B
53	31.363	708778	1.66	.Alpha.-Tocospiro B
54	31.628	243510	0.57	1-Pentatriacontanol
55	31.759	110128	0.26	Oxirane, 2,2-Dimethyl-3-(3,7,12,16,20-Pentamethyl-3,7,11,15,19-Heneicosapentaenyl)-
56	33.321	288459	0.68	Stigmast-5-En-3-Ol, Oleate
57	33.570	1374987	3.23	Vitamin E
58	35.813	3067831	7.20	.Gamma.-Sitosterol
59	36.592	1566081	3.68	3-(1,5-Dimethyl-Hexyl)-3a,10,10,12b-Tetramethyl-1,2,3,3a,4
60	36.801	697090	1.64	1,4-Dimethyl-8-Isopropylidenetricyclo[5.3.0.0(4,10)]Decane
61	37.160	628073	1.47	.Alpha.-Amyrin
62	37.448	392807	0.92	Vitamin E
63	39.571	299066	0.70	Benzene, 1-(1,5-Dimethyl-4-Hexenyl)-4-Methyl
64	40.271	654479	1.54	Urs-12-En-28-Al, 3-(Acetyloxy)-, (3.Beta.)-
65	41.455	2297927	5.39	Ursolic Aldehyde
66	43.511	645425	1.51	3.Beta.-Myristoylolean-12-En-28-Ol

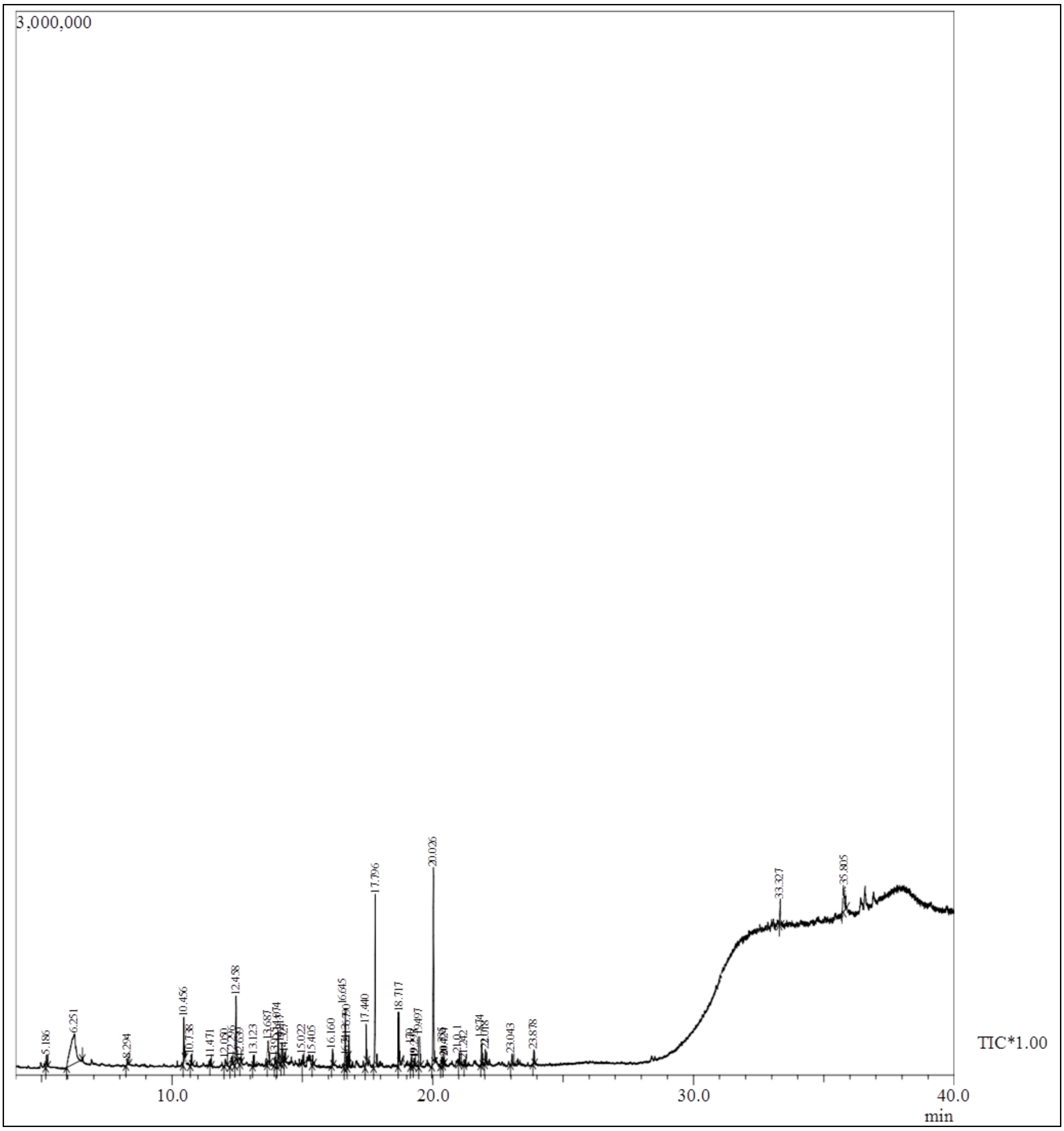

Fig 2: GC-MS Chromatogram of Leaf Midrib Gall extract of *Eucalyptus tereticornis*

Table 2: Phytocomponents identified from methanolic extract of Leaf Midrib Gall of *Eucalyptus tereticornis* using GC-MS analysis.

Peak#	R.Time	Area	Area%	Name
1	5.186	62245	0.88	2-Cyclopenten-1-One, 2-Hydroxy-
2	6.251	1220408	17.22	Glycerin
3	8.294	35335	0.50	1,3-Cyclohexane-1,3-D2-Diamine, Cis-
4	10.456	225580	3.18	1-Heptanol, 2-Propyl-
5	10.738	53374	0.75	1-Isopropyl-4-Methyl-7-Oxabicyclo[2.2.1]Heptane
6	11.471	18060	0.25	2,6-Octadienal, 3,7-Dimethyl-
7	12.050	48105	0.68	4-Hydroxy-2-Methylacetophenone
8	12.296	50783	0.72	Exo-2-Hydroxycineole
9	12.458	366404	5.17	Exo-2-Hydroxycineole
10	12.639	21455	0.30	Oxirane, Butyl-
11	13.123	50779	0.72	Oxirane, (3-Methylbutyl)-
12	13.687	152449	2.15	3,5-Dimethyl-Cyclohexanol
13	13.955	35737	0.50	4-Cyclopentene-1,2,3-Triol, (1.Alpha.,2.Alpha., 3.Alpha.)-
14	14.074	223534	3.15	3,4,8-Trimethyl-2-Nonenal
15	14.217	110341	1.56	2-Cyclohexen-1-One, 4-Hydroxy-3-Methyl-6-(1-Methylethyl)-, Trans-
16	14.327	50244	0.71	1,5-Cyclooctadiene, 3-Methyl-
17	15.022	42706	0.60	5-Isopropenyl-3,8-Dimethyl-1,2,3,3A,4,5,6,7-Octahydroazulene
18	15.405	36803	0.52	5-Isopropenyl-3,8-Dimethyl-1,2,3,3A,4,5,6,7-Octahydroazulene
19	16.160	84688	1.20	5-Hepten-3-Yn-2-Ol, 6-Methyl-5-(1-Methylethyl)-

20	16.645	313502	4.42	1H-Cycloprop[e]Azulen-7-Ol, Decahydro-1,1,7-Trimethyl-4-Methylene-
21	16.743	25348	0.36	5,8,11,14-Eicosatetraenoic Acid, Methyl Ester
22	16.790	97479	1.38	1,1,4,7-Tetramethyldecahydro-1H-Cyclopro
23	17.440	214528	3.03	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-Octahydro-.Alpha.,. Alpha.,4a,8-Tetramethyl-, (2R-Cis)-
24	17.796	1061101	14.97	2-Naphthalenemethanol, Decahydro-.Alpha.,.Alpha.,4a-Trimethyl-8-Methylene-,
25	18.717	262928	3.71	1,3B,6,6-Tetramethyldecahydro-1H-Cyclopropa[7,8]Azuleno[4,5-B]Oxirene
26	19.170	46234	0.65	1H-3a,7-Methanoazulene, Octahydro-1,4,9,9-Tetramethyl-
27	19.293	42748	0.60	2-Methyl-3-Isobutenyl-4-Penten-2-Ol
28	19.497	173953	2.45	Cryptomeridiol
29	20.026	1080236	15.24	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol
30	20.335	49640	0.70	2-(4a,8-Dimethyl-2,3,4,5,6,8a-Hexahydro-1H-Naphthalen-2-Yl)Propan-2-Ol
31	20.424	21828	0.31	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester
32	21.021	50237	0.71	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)-
33	21.242	25169	0.36	Hexadecanoic Acid, Methyl Ester
34	21.874	119141	1.68	2-Methyl-5-(2,6,6-Trimethyl-Cyclohex-1-Enyl)-Pentane-2,3-Diol
35	22.018	84689	1.20	1,6,10-Dodecatrien-3-oL, 3,7,11-Trimethyl-
36	23.043	64451	0.91	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)-
37	23.878	67536	0.95	2-(2-(2-Butoxyethoxy)Ethoxy)Acetic Acid
38	33.327	159918	2.26	Stigmast-5-En-3-Ol, (3.Beta.)-
39	35.805	237096	3.35	Anhydropanaxadiol

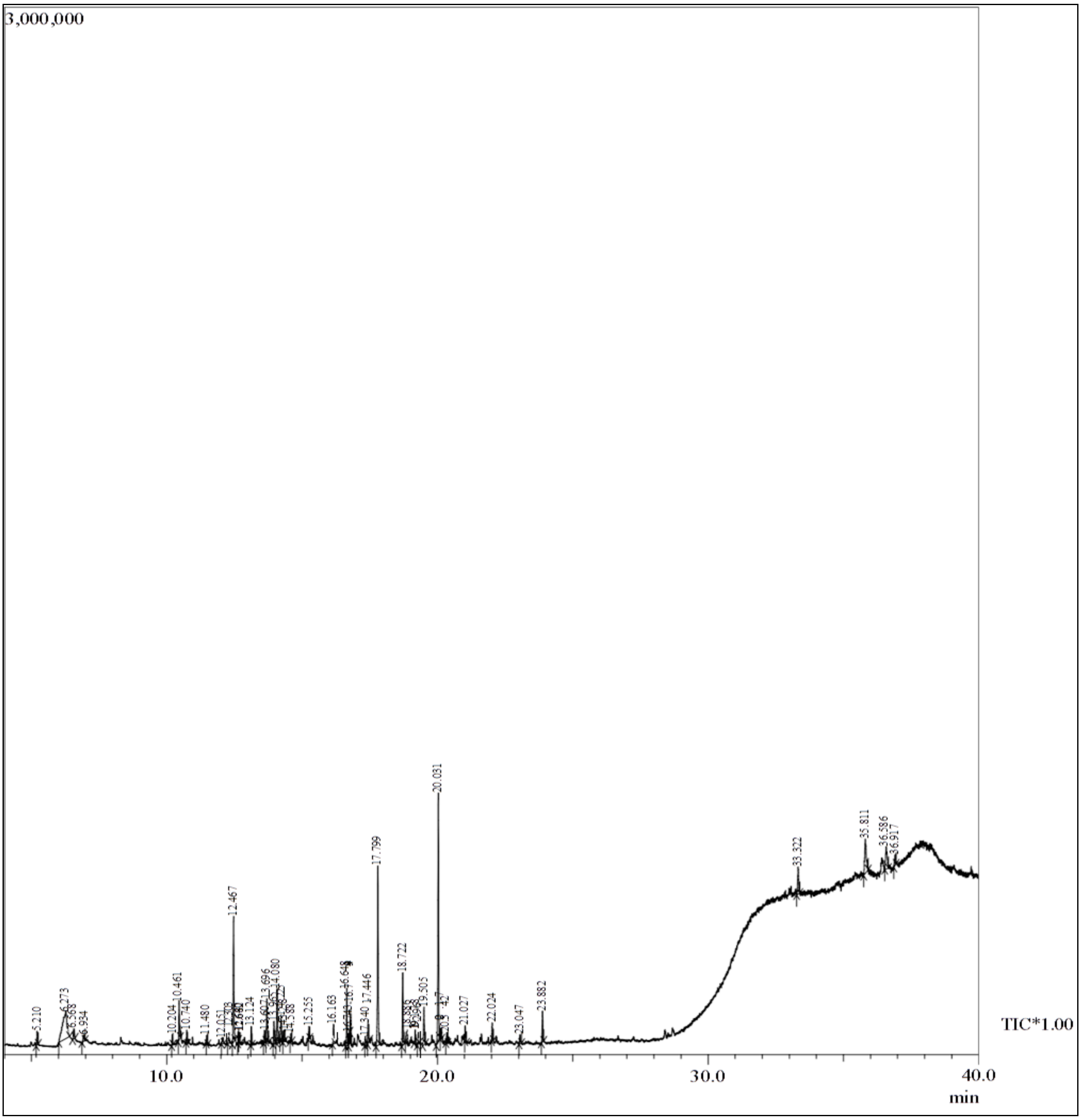

Fig 3: GC-MS Chromatogram of Petiole Gall extract of *Eucalyptus tereticornis*

Table 3: Phytocomponents identified from methanolic extract of Petiole Gall of *Eucalyptus tereticornis* using GC-MS analysis.

Peak#	R. Time	Area	Area%	Name
1	5.210	66103	0.70	2-Cyclopenten-1-One, 2-Hydroxy-
2	6.273	915148	9.73	Glycerin
3	6.568	77557	0.82	Bicyclo[3.1.0]Hex-2-Ene, 2-Methyl-5-(1-Methylethyl)-
4	6.934	48088	0.51	Ethanone, 1-(3-Methylphenyl)-
5	10.204	49750	0.53	3-Cyclohexene-1-Methanol,.Alpha.,.Alpha.,4-Trimethyl-
6	10.461	208279	2.21	1-Heptanol, 2-Propyl-
7	10.740	68902	0.73	2-Oxabicyclo[2.2.2]Octane, 1,3,3-Trimethyl-
8	11.480	38445	0.41	2,6-Octadienal, 3,7-Dimethyl-
9	12.051	46643	0.50	4-Hydroxy-2-Methylacetophenone
10	12.303	66893	0.71	2-Oxabicyclo[2.2.2]Octan-6-Ol, 1,3,3-Trimethyl-
11	12.467	684241	7.27	Exo-2-Hydroxycineole
12	12.640	36906	0.39	2-T-Butylperoxy-2-Ethylbutan-1-Ol, Butyrate Ester
13	12.682	40787	0.43	Exo-2-Hydroxycineole
14	13.124	85826	0.91	R-(+)-Methyl-3-Isopropyl-6-Oxoheptanoate
15	13.607	74062	0.79	Succinic Acid, Tridec-2-Yn-1-Yl Tetrahydrofurfuryl Ester
16	13.696	281597	2.99	1,2-Cyclohexanediol, 1-Methyl-4-(1-Methylethenyl)-
17	13.965	127412	1.35	2-Cyclohexen-1-One, 4-Hydroxy-3-Methyl-6-(1-Methylethyl)-
18	14.080	339623	3.61	2-Cyclohexen-1-One, 4-Hydroxy-3-Methyl-6-(1-Methylethyl)-
19	14.225	173860	1.85	2-Cyclohexen-1-One, 4-Hydroxy-3-Methyl-6-(1-Methylethyl)-
20	14.328	75052	0.80	1-Cyclohexene-1-Carboxylic Acid, 4-(1-Methylethenyl)-
21	14.588	43749	0.47	2-Cyclohexen-1-One, 4-Hydroxy-3-Methyl-6-(1-Methylethyl)-
22	15.255	54510	0.58	Carbonic Acid, Nonyl Prop-1-En-2-Yl Ester
23	16.163	116917	1.24	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)-
24	16.648	304183	3.23	1H-Cycloprop[e]Azulen-7-Ol, Decahydro-1,1,7-Trimethyl-4-Methylene-
25	16.743	25725	0.27	Bicyclo[2.2.2]Oct-5-En-2-One, 1,4-Dimethyl-, (.+-.)-
26	16.794	152721	1.62	1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[E]Azulen-4-Ol
27	17.340	39315	0.42	1,4-Methano-1H-Cyclohepta[d]Pyridazine, 4,4a,5,6,7,8,9,9a-Octahydro-10,10-Dimethyl-
28	17.446	234978	2.50	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-Octahydro-.Alpha.,.Alpha.,4a,8-Tetramethyl-, (2R-Cis)-
29	17.799	1162431	12.36	2-Naphthalenemethanol, Decahydro-.Alpha.,.Alpha.,4a-Trimethyl-8-Methylene-
30	18.722	380057	4.04	Caryophyllene Oxide
31	18.886	122574	1.30	Cycloheptane, 4-Methylene-1-Methyl-2-(2-Methyl-1-Propen-1-Yl)-1-Vinyl-
32	19.178	76893	0.82	1,3-Bis-(2-Cyclopropyl,2-Methylcyclopropyl)-Buthen-2,-One-1
33	19.296	59212	0.63	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl--3-(1-Methylethenyl)-
34	19.505	248215	2.64	Cryptomeridiol
35	20.031	1413514	15.03	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-Yl)-1,4a-Dimethyldecahydronaphthalen-1-Ol
36	20.127	47962	0.51	1H-Cyclopropa[A]Naphthalen-4-Ol, 1A,2,4,5,6,7,7A,7B-Octahydro-
37	20.342	57128	0.61	(E,1'RS,2'RS,4'SR,7'SR)-1-(2',5',5'-Trimethyl-3'-Oxatricyclo[5.1.0.0(2,4)]Oct-4'-Yl)-
38	21.027	86109	0.92	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)-
39	22.024	97773	1.04	1-Methyl-4-(5-Methyl-1-Methylene-4-Hexenyl)-1-Cyclohexene
40	23.047	55674	0.59	Cyclohexanemethanol, 4-Ethenyl-.Alpha.,.Alpha.,4-Trimethyl-3-(1-Methylethenyl)-
41	23.882	165922	1.76	Monomethyl Monobutyl "Capped" Tetraethylene Glycol
42	33.322	199014	2.12	Stigmast-5-En-3-Ol, (3.Beta.)-
43	35.811	405131	4.31	Stigmast-5-En-3-Ol, (3.Beta.)-
44	36.586	261254	2.78	3-(1,5-Dimethyl-Hexyl)-3a,10,10,12b-Tetramethyl-1,2,3,3a,4

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