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Terminalia arjuna leaf gall : A potent Biofuel

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ABSTRACT
Terminalia arjuna (Roxb.) Wight and Arn, a well known plant for treating cardiovascular diseases belong to the family Combretaceae. There is ample evidence of this plant in the treatment of coronary artery disease, heart failure, hypertension and hypercholesterolemia. A hemipteran insect, Trioza fletcheri minor induces leaf galls on T. arjuna. Gall formation is the result of host-pathogenic interaction involving the offensive nature of the attacking insect and the defensive response of the plant. As a result, the infected plant suffers from stress conditions which leads to the production of secondary metabolites. To find out various secondary metabolites produced in response to gall induced stress, GC-MS (Gas Chromatography and Mass Spectroscopy) analysis has been carried out. As per the findings the normal leaf showed the presence of 21 compounds while the galled leaves are found with 57 compounds. The galled leaf showed the production of a new compound which is apotent biofuel and can be a good substitute of fossil fuels in this era of fuel crisis.
Keywords – *Terminalia arjuna,Trioza fletcheri minor*, leaf gall, secondary metabolites, GC-MS.

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INTRODUCTION

The drastic climate change, rapid depletion of nonrenewable fossil fuels like petroleum, and the high volatility in crude oil price, led to enormous pressure on the global economy [1]. Global climate change forces us to seek alternate, low carbon sources of fuel and energy which are environment friendly, substituting fossil fuels with renewable sources like biofuels is one such step. Biomass is evenly distributed worldwide that's why biofuels help to enhance and safeguard energy security. The biorefinery is an essential approach, that can replace the present dependence on fossil-fuel resources in near future [2]. The driving factors for biofuel generation are not only limited to developing new energy platforms and CO2 minimization, but also creating opportunities to secure the local supply of energyand support agricultural economics [3, 4]. In this context, lignocellulosic biomass-derived 5-hydroxymethylfurfural (HMF) has emerged as an important chemical to meet the chemical and fuel needs of the upcoming generation [1, 5].

Among other primary renewable building blocks, 5-hydroxymethylfurfural (HMF) is considered an important intermediate due to its rich chemistry and potential availability from carbohydrates such as fructose, glucose, sucrose, cellulose and inulin [2].

Terminalia arjuna plant is attacked by a hemipteran insect *Trioza fletcheri minor* which leads to leaf gall formation and various biological, anatomical and biochemical changes in the plant. This insect attack is a type of a biotic stress for the plant, so in response the plant produces various secondary metabolites/bioactive compounds. Accumulation of secondary metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules[6].

In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (e.g., carbohydrates, lipids and amino acids) and are involved in the plant defense against herbivores and pathogens. Often they may confer protection against environmental stresses [7].

To find out the production of various bioactive compounds in *Terminalia arjuna* after the insect attack , GC-MS analysis of the leaf and gall extract was carried out, and 21 compounds in the leaf whereas 57 compounds in the leaf galls were registered. In the leaf gall, the most abundant compound observed was 2-Furancarboxaldehyde, 5-(hydroxymethyl)- representing 46.14% of the total area. This compound is commonly known as 5-Hydroxyl Methyl Furfural (HMF) and has its wide application as a potent biofuel. Biomass derived 5-hydroxymethylfurfural (HMF) has emerged as an important platform chemical for the production of value added chemicals and liquid fuels that are currently obtained from petroleum [8].

MATERIAL AND METHODS

Dry Powder Preparation

Normal and infected leaves of the plant were collected from various localities of Jaipur, Rajasthan in the months of June, July, 2017. Normal and infected (galled) leaves were then shade dried separately. They were then pulverized to powder with the mechanical grinder.

Preparation of Extract-

5 gm powder of normal and galled leaves was weighed and was extracted with methanol (70-80ºC) by hot continuous percolation method in soxhlet apparatus for 24 hours. The extract was taken and filtered through whatmann filter paper. Then extract was concentrated by rotary evaporator to obtain extract.

GC-MS (Gas Chromatography and Mass Spectroscopy) analysis

The GC-MS analysis of methanolic extract of normal and galled leaves of Terminalia *arjuna* was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. It includes 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 carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized 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 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were analyzed using electron impact ionization at 70 eV. The total running time for each sample was 45 min.

Identification of phytochemical

Interpretation of phytochemical present in the sample was conducted using NIST, having more than 62,000 patterns and Wiley8 Library. The comparison of unknown spectrum with known spectrum of various components was done by stored spectrum of NIST library and Wiley8 Library. The name, molecular weight and structure of the components were ascertained.

RESULT

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. MS is wide ranging analytical technique, which identify the charged species according to their mass to charge ratio (M/Z). GC-MS is one of the best techniques to identify the constituents of volatile compounds. The GC-MS analysis of *T. arjuna* normal leaf showed the presence of twenty one compounds and galled leaf showed the presence of fifty seven compounds. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), area percentage, compound.

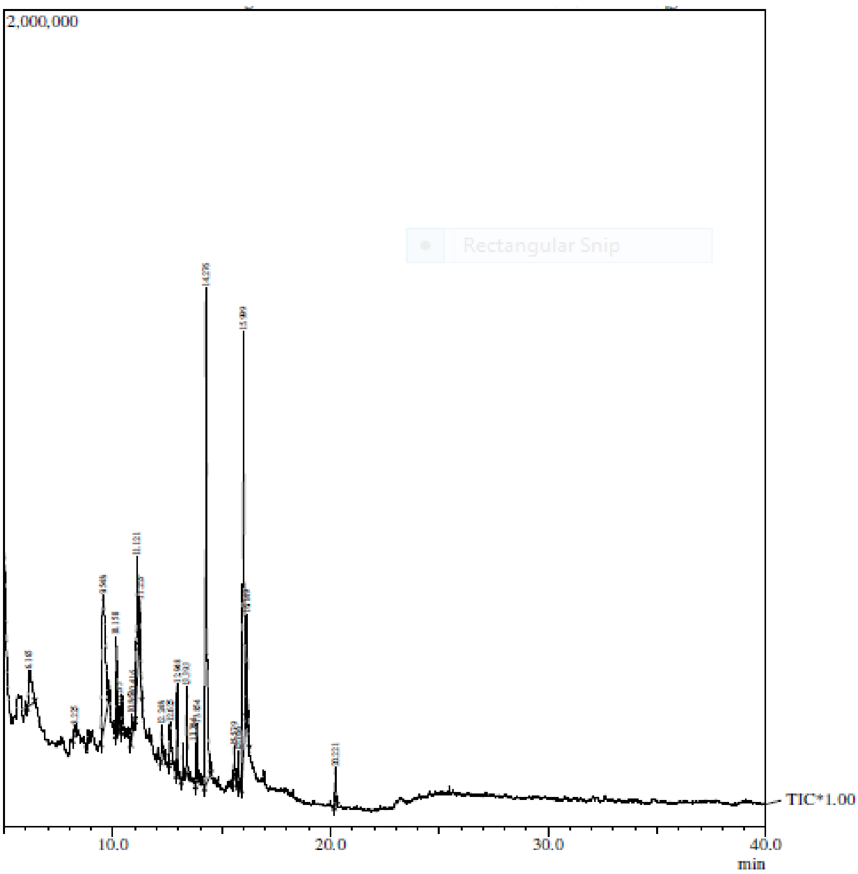

Figure 1:- Shows GC-MS Chromatogram of Normal leaf of *Terminalia arjuna.*

Table 1:- Compounds identified from methanolic extract of Normal leaf of *Terminalia arjuna* using GC-MS analysis.

Peak#	R.Time	Area	Area%	Name
1	6.165	893997	4.21	
2	8.225	144980	0.68	(3S)-(-)-3-Acetamidopyrrolidine
3	9.548	3715795	17.51	.beta.-D-Glucopyranose, 1,6-anhydro-
4	10.158	716706	3.38	3,6-DIMETHYL-3-OCTENE-2,7-DIONE
5	10.275	178361	0.84	1,5,5-Trimethyl-6-[3-acetoxybutyl]-3,6-epidioxycyclohexene
6	10.416	539218	2.54	
7	10.867	185117	0.87	cis-Z-.alpha.-Bisabolene epoxide
8	11.121	1437993	6.78	1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-,
9	11.227	553417	2.61	3-BUTEN-2-OL, 4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-
10	12.248	340121	1.60	TETRADECANOIC ACID
11	12.625	473449	2.23	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-H
12	12.948	435132	2.05	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
13	13.204	196003	0.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	13.393	520366	2.45	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-,
15	13.854	363537	1.71	Pentadecanoic acid, 14-methyl-, methyl ester
16	14.276	4128398	19.45	n-Hexadecanoic acid
17	15.579	251098	1.18	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
18	15.766	185680	0.87	OCTADECANOIC ACID, METHYL ESTER
19	15.999	4844516	22.83	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
20	16.149	808161	3.81	Octadecanoic acid
21	20.221	310617	1.46	Di-n-octyl phthalate
		21222662	100.00	

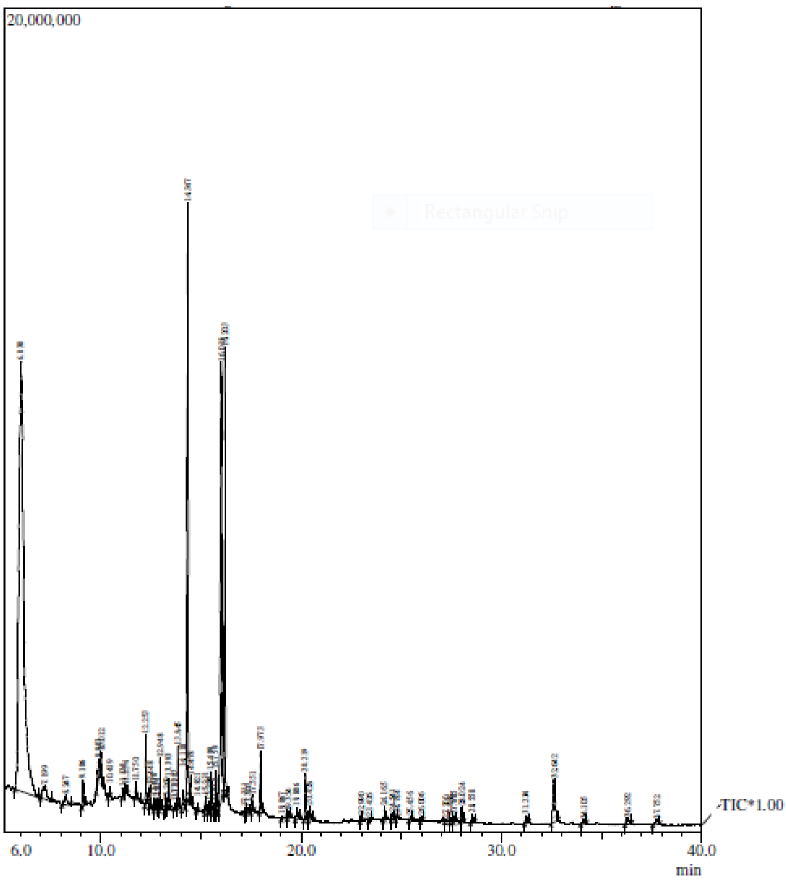

Figure 2: Shows GC-MS Chromatogram of Galled leaf of *Terminalia arjuna.*

Table 2:- Compounds identified from methanolic extract of Galled leaf of *Terminalia arjuna* using GC-MS analysis.

Peak#	R.Time	Area	Area%	Name
1	6.030	181567834	46.14	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
2	7.199	4715760	1.20	1-[N-Methylpiperazine]ethanol
3	8.247	2236383	0.57	1-Isopropoxy-2,2,3-trimethylaziridine (sin)
4	9.106	2619147	0.67	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-
5	9.903	2567258	0.65	
6	10.012	1476144	0.38	N,N-BIS(2-HYDROXYETHYL)DODECANAMIDE
7	10.419	287961	0.07	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
8	11.120	430107	0.11	1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-,
9	11.254	348870	0.09	Tricyclo[5.1.0.0(2,4)]octane-5-carboxylic acid, 3,3,8,8-tetram
10	11.750	1717632	0.44	1-Oxetan-2-one, 4,4-diethyl-3-methylene-
11	12.253	4310684	1.10	TETRADECANOIC ACID
12	12.448	917476	0.23	1-HEPTADECENE
13	12.678	1137234	0.29	2,3-Dioxabicyclo[2.2.2]oct-5-ene, 1-methyl-4-(1-methylethyl)-
14	12.814	325853	0.08	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-bu
15	12.948	2388381	0.61	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
16	13.202	660943	0.17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	13.269	362104	0.09	Pentadecanoic acid
18	13.393	2034438	0.52	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	13.731	299876	0.08	6,6,7-Trimethyl-octane-2,5-dione
20	13.845	3006273	0.76	Hexadecanoic acid, methyl ester
21	14.118	1812239	0.46	9-Hexadecenoic acid
22	14.347	51746766	13.15	n-Hexadecanoic acid
23	14.478	1153426	0.29	1-Nonadecene
24	14.821	287976	0.07	EICOSANOIC ACID, METHYL ESTER

25	15.231	894235	0.23	Heptadecanoic acid
26	15.394	593932	0.15	1-Hexadecanol
27	15.499	4154272	1.06	9,12-Octadecadienoic acid, methyl ester, (E,E)-
28	15.678	428015	0.11	Phytol
29	15.759	1910683	0.49	Octadecanoic acid, methyl ester
30	16.018	57968644	14.73	6-Octadecenoic acid, (Z)-
31	16.203	27605528	7.02	Octadecanoic acid
32	17.211	340379	0.09	2,5-METHANO-1H-INDEN-7(4H)-ONE, HEXAHYDRO-
33	17.316	573705	0.15	1-OCTADECANETHIOL
34	17.551	897343	0.23	EICOSANOIC ACID, METHYL ESTER
35	17.973	4140030	1.05	EICOSANOIC ACID
36	19.067	332973	0.08	NONADECANOIC ACID
37	19.354	615304	0.16	Octadecyl trifluoroacetate
38	19.806	1234554	0.31	DOCOSANOIC ACID, METHYL ESTER
39	20.219	3333687	0.85	Di-n-octyl phthalate
40	20.428	1468602	0.37	Docosanoic acid
41	22.990	356369	0.09	TETRACOSANOIC ACID, METHYL ESTER
42	23.426	205920	0.05	1-Hexadecanol, 2-methyl-
43	24.165	800533	0.20	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-
44	24.541	933976	0.24	17-Pentatriacontene
45	24.784	380531	0.10	FURAN, 4,5-DIETHYL-2,3-DIHYDRO-2,3-DIMETHYL-
46	25.456	307205	0.08	9-Octadecenal, (Z)-
47	26.006	360320	0.09	.beta.-Sitosterol
48	27.200	251109	0.06	.beta.-Tocopherol
49	27.466	321326	0.08	.beta.-Sitosterol
50	27.685	538717	0.14	CHOLESTA-4,6-DIEN-3-OL, BENZOATE, (3.BETA.)-
51	28.024	1447262	0.37	.beta.-Sitosterol
52	28.558	864567	0.22	dl-.alpha.-Tocopherol
53	31.234	1065609	0.27	Stigmasterol
54	32.642	7461886	1.90	.beta.-Sitosterol
55	34.105	710704	0.18	d-Norandrostane (5.alpha.,14.alpha.)
56	36.292	1404330	0.36	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B
57	37.752	1183488	0.30	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-
		393496503	100.00	

DISCUSSION

The GC-MS analysis showed that the methanolic extract of normal leaf had fewer compounds (table 1) than the leaf gall extract of *Terminalia arjuna*(table 2). In the leaf gall, the most abundant compound observed was 2-Furancarboxaldehyde, 5-(hydroxymethyl)- representing 46.14% of the total area (table 2). This compound is commonly known as 5-Hydroxyl Methyl Furfural (HMF) and has its wide application as a potent biofuel.HMF has the potential to be a sustainable substitute for petroleum-based building blocks used in production of fine chemicals and plastics[9].Considerable efforts have been made on the transformation of carbohydrates into HMF in recent years. HMF is extremely useful not only as intermediate for the production of the biofuel dimethylfuran (DMF) and other molecules, but also for important molecules such as levulinic acid, 2,5-furandicarboxylic acid (FDA), 2,5 diformylfuran (DFF), dihydroxymethylfuran and 5-hydroxy-4-keto-2-pentenoic acid [8]. Its wide application as a potent biofuel makes it an economically and ecologically important compound in near future.

CONCLUSION

The chemical compound 5-HMF which was formed in the *T. arjuna* leaf post infection, is of immense use in bio refinery, a good substitute of fossil fuels in the present era of fuel crisis.

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REFERENCES

1. Werpy T. and Peterson G.(2004). Top Value Added Chemicals from Biomass, Pacific Northwestern National Laboratory, 2004,vol. 1.
2. Rosatella A.A. , Simeonov S.P., Frade R.F.M. and Afonso C.A.M. (2011). 5-Hydroxymethylfurfural (HMF) as a building block platform: Biological properties, synthesis and synthetic. Green Chem., **13**, 754-793.
3. Lange J.P. (2007). Catalysis for Renewables: From Feedstock to Energy Production, ed. G. Cento and R. A. van Santen, Wiley-VCH, Weinheim, 2007, pp. 21–51.
4. Lange J.P. (2007).Biofuels, Bioprod. Biorefin., 2007, 1, 39.
5. Wettstein S.G., Alonso D.M., Gürbüz E.I. andDumesic J.A.(2012). Chem. Eng., 2012, 1, 218.
6. Akula R and Gokare R.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling and Behavior. 1; 6(11): 1720–1731.
7. Seigler D.S. Plant Secondary Metabolism.(1998). Chapman and Hall, Kluwer Academic Publishers, Boston.711.
8. Saha B. and Abu-Omar. M.M. (2014). Advances in 5-hydroxymethylfurfural production from biomass in biphasic solvents. Green Chem..16, 24.
9. Juben N. Chheda, Yuriy Roma´n-Leshkov and James A. Dumesic. (2007): Production of 5-hydroxymethylfurfural and furfural by dehydration of biomass-derived mono- and poly-saccharides.Green Chem. 9, 342–350.

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