



Analysis of impulsive constituents in normal leaf and insect stimulated leaf gall of *Mitragyna parvifolia*

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Abstract

The plant galls are a distinctive instance of multifaceted relation and conjoint adaptation involving the plant and the insect trigger cellular hyperplasia and hypertrophy. *Mitragyna parvifolia* (Kadamba) is a plant of religious standing. *Mitragyna parvifolia* has a prominent place in the diverse areas of human-usage. Till date, any sort of analyses of galls instigated on the leaves of *Mitragyna parvifolia* has not been there. The existing investigation was underway to compute the major as well as the minor phytochemical compounds present in normal leaves and the deviations that befall in leaf galls of *Mitragyna parvifolia*. The methanolic extracts of both the tissues were analyzed through gas chromatography-mass spectrometry technique aimed at the examination of the major components. The investigation primes to the conclusion that under biotic stress large number of polysaccharides, fatty acids and secondary metabolites are formed, and the leaf gall extract had a much greater unsaturated fatty acid content than a normal leaf extract. The fatty acids were obtained in greater expanse in leaf gall extract than normal leaf extract. The existence of this gall has not been recognized earlier. The investigate aims to scrutinize the host-pathogen interface, with the help of various biochemical facets triggered or altered during irritation caused by insect.

Keywords: *Mitragyna parvifolia*, leaf gall, phytochemicals, methanolic extract, GC-MS

Introduction

Mitragyna parvifolia (Family: Rubiaceae) stands one of the largely implanted tree inwards the tropical region. Kadamba is planted to along pavements and roads for shadow. An entirely developed Kadamba tree is capable of reaching up and around 45 meters stature. It is a huge tree by means of a wide-ranging pinnacle and orthodox cylindrical bole. This is a fast-growing tree, rises hastily in primary six to eight years. The bole holds width of about 1-1.6 meters, but arche typically not as much as that. Leaves are 13-32 centimeter extended. Blossoming typically commences when the tree is four/five years old. Kadamba florae are pleasantly perfumed, sore to carrot in shade, happen in thick, bulbous bonces of about 5.5 centimeter in width. The fruit of *Mitragyna parvifolia* an use in trivial, fleshy containers filled strictly collected to materialize a fleshy yellow-orange frutescence. On maturing, the fruit ruptures apart, freeing the seeds which are detached by rain or wind. The fruits produced by the tree are edible and can be consumed by humans well; the leaves of Kadamba are also used as fodder to feed cattle. The tree also serves as a decorative tree and supplier intended for low-quality timber^[1]. The timber then is used in countless human industries, like ply-wood and furniture. Hand-sheets of rational brightness and performance are produced from the pulp of Kadamba tree. Preservation of the Kadamba wood can be easily done with the help of open tank or pressure-vacuum systems. Also, a certain type of blonde colorant is attained from the bark. 'Attar' an Indian perfume has Kadamba flowers as an important raw material along with sandalwood base. Also, the extracts of Kadamba leaves are used for mouth gargles. Galls outcomes from relations between two entities and their genomes and ensues when a gall-inducing species outbreaks an appropriate host^[2]. Habitually, galling imitates

a parasitic association, in which the gall inducer changes assets of the host to be more easily retrieved or used up. They ascend due to the reactions of plants to the outbreak of insects. A distinct characteristic of higher plants is their capacity to yield numerous secondary metabolites. The major significant bio-active ingredients of the plant are alkaloids, tannins, flavonoids and phenolic complexes. In usual, these subordinate metabolites are an imperative cause with an assortment of operational measures and properties. Abiotic stress is instigated by insect to the plant^[3]. Stresses activate an extensive series of plant responses. Stress can have a disturbing impression on plant development and produce or can consequence into improvement of manufacture of secondary metabolites^[4]. The secondary metabolites are proficient of activating vagaries^[5] into plant cell which helps to surmount the stresses^[6]. In the current investigation the relative analysis of the precarious constituents in methanolic^[7] extracts of the normal leaf and leaf gall of *Mitragyna parvifolia* was done by GC-MS method.

Materials and Method

- 1. Plant material and sample collection:** The normal leaves and leaf galls were assembled from the Jaipur and adjoining areas. The leaves were recognized and validated by the herbarium of Department of Botany, University of Rajasthan, Jaipur (RUBL No. RUBL211741).
- 2. Extraction of plant sample in methanol:** Leaves and Leaf galls were desiccated in dark, pulverized discretely and mined with methanol for 24 hours by means of Soxhlet extraction. The extricate was then filtered through whatmann filter paper, evaporated further down condensed pressure and vacuum dried up to get

the dried powder. The methanolic extract of the plant was used for initial phyto-chemical and GC-MS analysis.

- 3. GC-MS Analysis and Compound Identification:** The GC-MS analysis of methanolic citation of normal leaf and galled leaves of *Mitragyna parvifolia* was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. Biochemical essential gears of the citations were recognized by corresponding the peaks by mainframe NIST MS collections and established by associating mass spectra of the peaks. Mass spectrometry is wide ranging analytical technique to identify charge species according to their mass to change ratio (M/Z). Thirty-four phytochemical compounds were present in normal leaf of *Mitragyna parvifolia* whereas thirty-three phytochemical in leaf gall of this plant species. These compounds were confirmed by the peak area, Retention Time (RT) and molecular formula. These compounds of normal and leaf galls are presented in table (2).

Results

Phyto-chemical examination of the methanolic extracts of the normal leaf and displayed the existence of carbohydrates, steroids, proteins, and various other amino acids while galled leaves disclosed the incidence of carbohydrates, steroids, glycosides, tannins, proteins, flavonoids and alkaloids, these have antimicrobial activity. The same is depicted in the tables and diagrams. The (-)

denotes absent whereas (+) denotes present of phyto-constituents in methanolic extracts of normal and leaf gall result showed in (Table No. 1).

In GC-MS thirty-four phytochemical compounds were present in normal leaf of *Mitragyna parvifolia* whereas thirty-three phytochemical in leaf gall of this plant species. Out of them some are very medicinally and economically important. Valeric acid used in perfume and cosmetic which was present only in normal leaf but absent in galled leaf. Ascorbic acid (RT-19.302) was found to be higher in GCMS profile of galled tissues. It was recorded 3.98% in gall but 2.69 in normal leaf tissues out of complete volatile profile. 24-Noroleana-3, 12-diene and 2-Norursa-3,12-diene are used in traditional medicines and in spices were decreased in hypertrophical new formed gall tissues but these are present in very good amount in earlier to infestation profile. Lanosterol 1.15 area% was recorded after gall induction in leaf which is used in drugs to control cholesterol level in body. 10.87% Ajmalicine was recorded in galled leaf but absent in normal leaf. insect irritation caused stimulation of this compound synthesis in hypertrophied tissues. Presence of Alpha 1 and rano-receptor antagonistic in this compound used to treat high blood pressure. Formosanan-16 carboxylic acid was found elevated (11.82%) in galled leaf in comparison to normal leaf (1.41%). Phthalic acid (RT-25.208) was recorded in gall but found in absent in normal leaf Alpha-Amyrin (RT-38.874) having antioxidant, anti-inflammatory, antipurative and gastroprotective properties was recorded 2.41% in gall.

Table 1: Preliminary profile of phytoconstituents in Leaf and leaf gall extracts

S. no	Phytoconstituents	Methanolic extracts of normal leaf	Methanolic extracts of leaf gall
1	Saponin	-	-
2	Alkaloids	-	+
3	Tanins	-	+
4	Steroids	+	+
5	Carbohydrate	+	+
6	Glycerides	-	+
7	Proteins and Amino Acids	+	+
8	Flavonoids	-	+

Table 2: Comparative profile of compounds identified from methanolic extract of Normal Leaf and leaf galls of *Mitragyna parvifolia* using GC-MS analysis.

Peaks#	Name of Compounds	Retention Time	Area % in Normal Leaf	Area % in Leaf Gall
1	Glycerin	6.070	4.71	-
2	1-Hetanol, 2- propyl	10.228	0.70	-
3	4-Chlorolysine	10.658	0.75	-
4	1,3-Benzenediol, 2-hydroxy-4-methyl	12.756	0.82	-
5	Benzaldehyde,2-hydroxy-4methyl.	13.547	2.53	-
6	D-Allose	14.154	1.09	-
7	2- Butenediolic acid (Z), dibutyl ester	14.606	0.74	-
8	Phthalic acid, ethyl neopentyl ester	15.307	0.48	-
9	3-O Methyl I-d glucose	16.158	30.86	-
10	6-Hydroxy-4,4 7n-trunethyl 5,6,7,7a-tetrahydr	17.441	0.45	-
11	2-Cyclohexen-1-one, 4-hydroxy-3,5, 6, -trimethy	17.617	1.07	-
12	3-Piperidinecarboxylic acid, 1- ethyl-4-oxo	17.978	2.40	-
13	1H-Pyriolo[2,1-b] pyridine-2,6diane,3,3a,4,5.	18.231	4.05	-
14	3,7,11, Trimethyle-8,10-dodecedienylacetate	18.458	-	1.18
15	3,7,11, Trimethyle-8,10-dodecedienylacetate	1.583	-	8.51
16	3,7,11, Trimethyle-8,10-dodecedienylacetate	18.815	0.18	-
17	Hexadecanoic acid, methyl ester	18.964	-	0.84
18	Hexadecanoic acid, methyl ester	18.964	0.87	-
19	1-(+ Ascorbic acid 2,6 dihexadecanoate	19.307	2.69	3.98
20	1,4-Dimethyleleaehydroguinolin4-ol	20.418	0.86	-
21	10-Phenyldecanoic acid	20.515	1.13	-

22	Acetamide,2-3-hydroxy-8-azabicyclo [3,2,1]	20.608	2.10	-
23	Phytol	20.762	11.28	-
24	Cis, cis, cis-7,10,13-Hexadocalienal	21.001	0.66	-
25	Octadecanoic acid	21.211	0.19	-
26	Octadecanoic acid,2-hydroxyethyl ester	21.906	0.24	-
27	Hexadecanoic acid,2-hydroxy-1-(hydroxymel)	24.909	2.26	-
28	Sulfurousacid,hexyltetradecyl ester	28.576	2.19	-
29	Valeric acid,4- nitrophenyl ester	29.180	0.31	-
30	Valeric acid,4- nitrophenyl ester	29.327	2.06	-
31	Oxayohimban-16-carboxylic acid 16,17dideh	31.905	0.43	-
32	Formosanan-16-carboxylic acid19-methyl-2	33.205	1.41	-
33	Alpha-tocopherol-betaD-mannoside	33.859	0.40	-
34	3S,8S,9S,10R,13R,14S,17R)-17-(2R,5R)-5-	37-872	6.18	--
35	24-Noroleana-3,12-diene	38.870	8.24	-
36	24-Norursa-3,12-diene	40.185	-	2.85
37	24-Norursa-3,12-diene	40.194	11.95	-
38	9,19-cyclolanosl-24en-3-ol, acetate, (3, beta)	42.710	2.41	-
39	1,2-Cyclopentanedione	5.212	-	0.54
40	Pentane	8.303	-	0.09
41	3 acetoxydodecane	8.935	-	0.07
42	4-H- PYRAN-4-one,2,3-dihydro-3,5dihydroxy-6	9.144	-	0.14
43	Benzoic acid	9.388	-	0.08
44	1-heptanol,2-propyl	10.222	-	0.05
45	Silane,dimethyl di (but-2-enyloxy	14.500	-	0.10
46	Dodecanoic acid,3 hydroxy-	15.675	-	3.24
47	Alpha-d-6,3Furanose, Methyl-Beta-d-glucohydroxy	16.633	-	38.80
48	Tetradecanoic acid	19.949	-	0.061
49	9,12- Octadecadienoic acid (Z, Z)-methylester	26.600	-	1.43
50	9,12., 15- Octadecadienoic acid (Z, Z)-methylester	20.656	-	1.44
51	Phytol	20.761	-	1.45
52	Linoclaidic acid	20.947	-	0.32
53	Cis, cis, cis-7,10,13- Hexadecalrienal	21.000	-	0.52
54	Thunbergol	21.260	-	1.16
55	9-Octadecenamide, (Z)	23.087	-	0.15
56	Hexadecanoic acid2-hydroxy-1-(hydroxymethyle)	24.902	-	0.49
57	Phthalicacid,di(2- propylpentyl) ester	25.208	-	1.38
58	Ajmalicine	31.913	-	10.87
59	Stagmasta-5,22-dien-3-ol, acetate, (3 beta)	32.205	-	0.31
60	Formosanan-16carboxylic acid,19 methyl-2-	33.215	-	11.82
61	Ergost-5-en-3-ol, (3beta)-	35.788	-	6.04
62	Stigmasterol	6.390	-	1.12
63	Gamma- sitosterol	37.883	-	3.82
64	Alpha amyirin	38.874	-	2.41
65	Alpha tocopheryl acetate	41.797	-	0.59
66	lanosterol	42.687	-	1.15

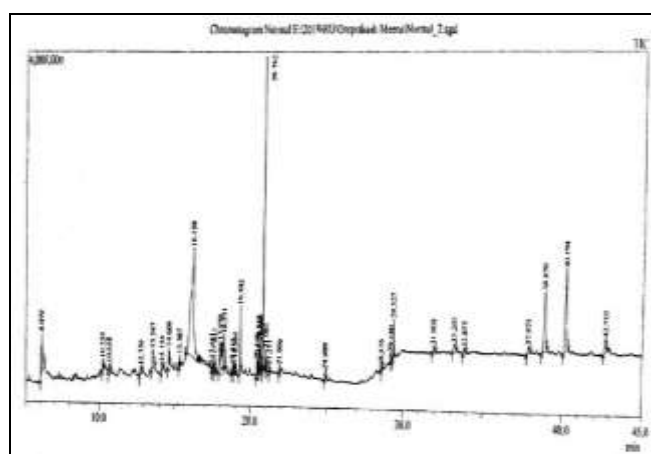


Fig 1: Showing GC-MS Chromatogram of Leaf Gall extract of *Mitragyna parvifolia* Peak Report TIC

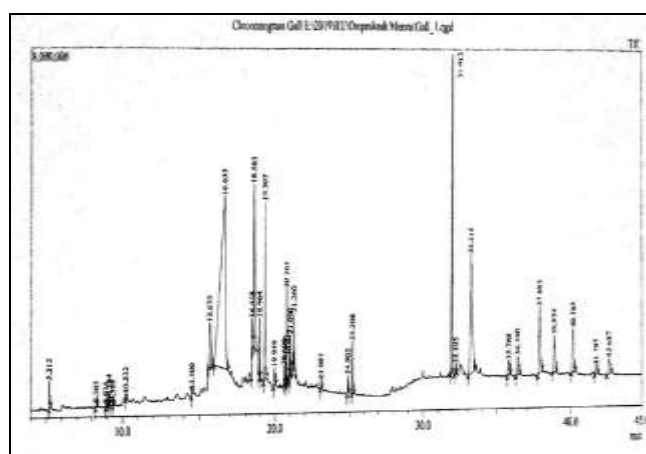


Fig 2: Shows GC-MS Chromatogram of Normal Leaf Part of *Mitragyna parvifolia* Peak Report TIC

Discussion

The plants are sources of several bio-active compounds that also produce large number of secondary metabolites^[8] and substances of medicinal and industrial importance. Several studies have proved that the phytoconstituents are widely responsible for the therapeutic^[9, 10] and industrial potential of the plant. The seclusion of the distinct phytochemical constituents can be ensued to treasure a drug^[11] for certain diseases^[12]. Gallic acid was successfully isolated from various leaf galls of *Madhuca longifolia*^[13] Carbohydrates, steroids, glycosides, tannins, proteins, flavonoids and alkaloids have antimicrobial activity^[14]. GC-MS analysis of normal and galled flower of *Crataeva religiosa* eighty-four and sixty-four phytochemical compounds were present in normal and flower gall respectively^[15]. Oleic acid is only compound which is not found in normal leaf but in galled leaf is found in highest amount under stressed conditions and can be exploited commercially^[16].

Mitragyna parvifolia is important medicine. Plant has vast array of phytoconstituents which indicate that pathogenesis induces a different profile of compounds in diseased parts. Gall leaves have very important constituents like ajmalicine and lanosterol which were absent in normal leaf. These compounds help to control the blood pressure and cholesterol level in body.

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