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Rapid Phytochemical Profiling of *Moringa oleifera* and *Mucuna pruriens* leaf Extracts for Their Pharmacological Activity by GC-MS

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ABSTRACT

Mucuna pruriens and *Moringa oleifera* commonly known as velvet beans and drumstick plant belong to fabaceae and moringaceae family respectively. Purposely in the current research work, leaves are used for preparation of extracts because of their availability in abundance and lot of aspects which are still to be discovered. Gas Chromatography-Mass Spectrometry (GC-MS) is a well-known green chemistry technique as there is lesser solvent consumption for analysis of volatile organics with the help of library. Such efficient use of analytical techniques in the world of Natural Therapeutics for revealing the phytopharmaceutical aspect of plants is considered to be a sustainable & ecofriendly approach. Some of the novel components from the said plants having significant medicinal & pharmacological activity are reported in the current research. Amyrins, Lupeol, Sigmasterols and Sitosterols are some of the significant phytoconstituents found from the aforementioned plants.

Keywords: Green chemistry, Phytoconstituents, Extraction, Sustainable, Therapeutics.

INTRODUCTION

Traditional knowledge systems have repeatedly proven their roots in healing multiple diseases/disorders/discomforts in the human mankind by using herbal alternatives instead of modern medicine. Preliminary screening of such herbal remedies in the form of a whole plant or in parts is hence an essential aspect of standardization. Such screenings are performed in multiple ways as per the guidelines of World Health Organization (WHO). Post this evaluation; assessment of bioactivities and proving those therapeutic values by using in-vivo or in-vitro techniques becomes crucial. Phytoconstituents or secondary metabolites are the medico-therapeutic agents of plants.

Mucuna pruriens and *Moringa oleifera* belong to the Fabaceae and Moringaceae family respectively from kingdom plantae. These two families are known for their distinguished medicinal properties. Anti-inflammatory, antidiabetic, Antibacterial,

Antioxidant, etc. are some of the few prominently known activities. Herbal plants are the good source of medicinal ingredients having potency in developing new drugs which begins with identifying active ingredients from plant sources. The screening of plant extracts is a novel approach to evaluate therapeutically active phytoconstituents in the plant samples.[1]

Variety of plant components with multiple pharmacological activity are screened in preliminary physical and chemical investigations. These molecules have fundamental functional groups such as hydroxyl, alcohols, aldehydes, benzene rings, steroids.[2]

Plant samples are to be prepared via suitable extraction techniques with optimized suitable solvent for getting maximum components for analysis. Extraction is a crucial first stage when the sample has to be analyzed by modern analytical techniques. Suitable extraction protocol is a key to precise separation, identification and analysis of active ingredients.[3] Post extraction separation and simultaneous identification or characterization has to be done. Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), etc. are known to be the most used chromatographic techniques for preliminary screening of components.

In herbal research chromatographic fingerprints are the most often and prominently used for quality control purposes to unveil the phytochemical profile of the plant.[4]

GC-MS is another such tool which has brought revolution in the field of analytical chemistry. This amalgamation of chromatography and spectroscopy gives us new dimensions of information on our samples. Samples in volatile state are easily analyzed by GC-MS whereas the ones which are not volatile in the original state can be converted to volatile organics by derivatization techniques.

MATERIALS AND METHODS

Collection of plant material: Fresh Moringa oleifera and Mucuna pruriens leaves are collected from Fort area, Mumbai, Maharashtra.

Preparation of extract: Both the plant leaves are sun dried and grounded in mixer grinder. 1 g of these finely grounded plant leaves powder was weighed separately and following extracts were prepared using 10 mL of the respective solvent in separate labelled flasks as per the conditions.

- **Extract I:** Steady maceration Methanol MPL
- **Extract II:** Accelerated Maceration Methanol MPL
- **Extract III:** Accelerated Maceration Ethanol MPL
- **Extract IV:** Soxhlet Methanol MPL
- **Extract V:** Steady maceration Methanol MOL
- **Extract VI:** Accelerated maceration Methanol MOL
- **Extract VII:** Soxhlet Methanol MOL

The accelerated extract was exposed to ultrasonication at the temperature of 45°C for 30 min and was kept for overnight extraction at steady state. After 24 h of incubation the filtered extract was further diluted 1:100 times and 1 l volume is injected in the GC-MS system. The sound vibrations are generated in a sonicator the accelerated extraction process is expected to give good yield of the extract.

GC-MS analysis

Different extracts were prepared and filtered using 0.2 micron syringe filters and injected in the GC-MS system with an injection volume of 1 l for all the samples. The temperature used was a programmed method for the present work. Sample injector was kept at 240°C temperature. The Carrier gas flow was 1.0 mL/minute. Rtx-5sil MS capillary column was used for the analysis. The initial column temperature was 70°C and was kept constant for 2 min, it was then increased up to 280°C at the rate of 10°C/min followed by a hold at the said temperature for 10 minutes. The total run time was 33 minutes. The split mode of injection was also applied and Interface was at 270°C. MS was operated on scan mode for Retention time (R_t in minutes) determination of reference standards with a mass range of (m/z) 35-600.

RESULTS

Table 1: Prominent molecules found in GC-MS screening and approximate %content

Name of the components	Mol. Weight	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	Extract 6	Extract 7
Octadecanoic acid	284.47g/mol	3.59	11.85		25.18	9.87	18.90	16.98
Hexadecanoic acid	256.42g/mol	4.07	13.79		15.48	13.32	18.18	20.67
Beta Amyrin	426.72g/mol	2.66	5.92	2.88	5.01	4.60	3.74	3.64
Alpha Amyrin	426.72g/mol	7.77	18.23	7.57	15.60	14.44	11.16	11.12
Gamma sitosterol	414.70g/mol	1.34	3.80		3.34	1.80	1.85	1.64
Lupeol	426.71g/mol			2.75				

Table 2: Name of the component with the structure and their activity

Name of the component	Activity
Octadecanoic acid	Antitumor, Antiviral, Anti-inflammatory, and Acaricidal properties[5]
Hexadecanoic acid	Antioxidant, Antibacterial, and Anti-inflammatory properties[6,7,8]
Beta Amyrin	Anti-inflammatory, Anti-microbial, Anti-oxidative, Chemoprotective and neuroprotective properties[9,10]
Alpha Amyrin	Anti-inflammation, Antimicrobial and Antifungal[11,12,13]
Gamma sitosterol	Antidiabetic, Anti-inflammatory, Anticancer and antioxidant activity[14,15]
Lupeol	Anticancer and Anti-inflammatory[16]

Table 3: Different components identified from Mass spectra of Extract 1

Peak	R. Time	Area	Area %	Height	Name
1	2.020	300570	0.04	445011	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	13.967	700346	0.14	228322	Tetradecanoic acid
3	15.628	3152892	0.45	1805240	Hexadecanoic acid, methyl ester
4	15.895	1167941	0.17	253336	Cis-9-Hexadecenoic acid
5	16.120	28635335	4.07	8208654	n-Hexadecanoic acid
6	16.760	962296	0.14	418822	Methyl abietate
7	17.322	4796680	0.68	2841694	9,12-Ocetadecadienoic acid (Z,Z)-,methyl ester
8	17.373	2329948	0.33	1401813	11-Octadecenoic acid, methyl ester
9	17.611	987954	0.14	448269	Methyl stearate
10	17.808	25217114	3.59	7124217	9,12- Octadecadienoic acid (Z,Z)-

Table 4: Different components identified from Mass spectra of Extract 1

Peak	R. Time	Area	Area %	Height	Name
35	24.254	22358753	3.18	3935885	Urs-12-en-28-al
36	24.419	46729343	6.65	7870493	Urs-12-en-28-al
37	24.866	917279	3.13	212708	.alpha.-Amyrin
38	25.234	22818233	3.25	2870784	Urs-12-en-28-al
39	25.350	10515941	1.50	2357818	Urs-12-en-28-al
40	25.473	3710549	0.53	1275851	Stigmasta-4,7,22-trien-3.beta.-ol
41	25.641	18716141	2.66	5348819	.beta.-Amyrin
42	26.218	54611027	7.77	12961171	.alpha.-Amyrin
43	28.684	2034344	0.29	193722	Urs-12-en-28-al,3-(acetyloxy)-,(3.beta)-
44	29.753	9433972	1.34	1768997	Gamma sitosterol
45	30.054	17497427	2.49	3099064	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl
46	31.0833	15985164	2.27	1501432	2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro
47	31.991	1186471	0.17	202515	Humulane-1,6-dien-3-ol

Table 5: Different components identified from Mass spectra of Extract 2

Peak	R. Time	Area	Area %	Height	Name
1	2.020	215934	0.16	332516	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	13.454	149643	0.11	77409	Methyltetradecanoate
3	13.983	1049633	0.76	140301	Tetradecanoicacid
4	15.628	5750905	4.17	3351896	Hexadecanoicacid,methylester

5	15.895	755708	0.55	156298	cis-9-Hexadecenoicacid
6	16.101	19006608	13.79	6299841	n-Hexadecanoic acid
7	16.761	553268	0.40	252274	Methylabietate
8	17.324	10090766	7.32	6058912	9,12-Octadecadienoicacid(Z,Z)-,methylester
9	17.374	4059976	2.95	2492641	11-Octadecenoicacid,methylester
10	17.610	815759	0.59	475545	Methylstearate
11	17.792	16328784	11.85	5414128	9,12-Octadecadienoic acid (Z,Z)-
12	17.827	14098305	10.23	4769289	9-Octadecenoicacid, (E)-
13	18.018	2661142	1.93	901942	Octadecanoic acid
14	18.439	269307	0.20	111255	2,6-Di(2-furylmethylidene)cyclohexan-1-one
15	20.114	372482	0.27	200375	Methylabietate
16	20.974	992960	0.72	384524	Abietic acid
17	21.922	1851112	1.34	186623	Humulane-1,6-dien-3-ol
18	22.921	921872	0.67	141332	6a,14a-Methanopicene,perhydro-1,2,4a,6b,9,9,12a-
19	24.033	1322081	0.96	142767	Urs-12-en-28-al
20	24.944	1373714	1.00	163210	Urs-12-en-28-al
21	25.474	1912817	1.39	611697	Stigmasta-4,7,22-trien-3.alpha.-ol
22	25.632	8160094	5.92	2459198	.beta.-Amyrin
23	26.191	25123448	18.23	6961087	.alpha.-Amyrin
24	27.488	506068	0.37	146680	1-(Dimethyldodecylsilyloxy)butane
25	28.188	662802	0.48	97580	Ergost-5-en-3-ol,(3.beta.)-
26	29.161	1469020	1.07	257683	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b
27	29.754	5242820	3.80	1044182	.gamma.-Sitosterol

Table 6: Different components identified from Mass spectra of Extract 3

Peak	R. Time	Area	Area %	Height	Name
15	24.475	10917456	0.67	3277035	Urs-12-en-28-al, 3-(acetyloxy)-,(3.beta.)-
16	25.010	8309770	0.51	2570577	Urs-12-en-28-al
17	25.266	150974256	9.20	12160822	Urs-12-en-28-al
18	25.495	10894013	0.66	3650897	5.alpha.-Pregnane-12,20-dione,cyclic 12-(ethylene
19	25.679	47196386	2.88	12395631	.beta.-Amyrin
20	25.896	14814008	0.90	2621430	Urs-12-en-28-al
21	26.120	30475279	1.86	5406467	3.beta-Myristoylolean-12-an-28-ol
22	26.286	124208022	7.57	22218438	.alpha.-Amyrin
23	29.678	12631680	0.77	2463115	6,beta, Bicyclo[4,3,0]nonane,5.beta.-iodomethyl-1.b
24	30.152	45060470	2.75	9224909	Lupeol
25	31.698	56270920	3.43	3041374	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a- octahydro

Table 7: Different components identified from Mass spectra of Extract 4

Peak	R. Time	Area	Area %	Height	Name
1	2.020	244247	0.13	366467	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	13.452	705287	0.39	398570	Methyltetradecanoate
3	15.417	655613	0.36	384414	9-Hexadecenoicacid,methylester,(Z)-
4	15.639	28265960	15.48	15918033	Hexadecanoic acid, methyl ester
5	16.069	5181021	2.84	1788882	n-Hexadecanoicacid
6	16.760	654601	0.36	300004	Methylabietate
7	17.341	45980328	25.18	22335999	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	17.387	22481994	12.31	13516953	9-Octadecenoicacid,methylester,(E)-
9	17.430	169581	0.09	211674	11-Octadecenoicacid,methylester
10	17.611	3758022	2.06	2239325	Methylstearate
11	17.757	1962714	1.07	936027	9,12-Octadecadienoicacid(Z,Z)-
12	17.797	3760771	2.06	1018431	22-Tricosenoicacid
13	18.402	227873	0.12	151328	10-Nonadecenoicacid,methylester
14	19.427	514597	0.28	279062	Methyl18-methylnonadecanoate
15	19.696	259670	0.14	161280	2-Ethyl-1-cyclohexyldimethylsilyloxyhexane
16	19.828	627120	0.34	255764	Methyl(11R,12R,13S)-(Z)-12,13-epoxy-11-methox

17	20.112	524951	0.29	271561	Methylabietate
18	20.801	595502	0.33	313190	Methyl(11R,12R,13S)-(Z)-12,13-epoxy-11-methox
19	20.975	787790	0.43	286745	Abietic acid
20	22.917	392004	0.21	83431	9,19-Cyclolanost-24-en-3-ol,acetate,(3.beta.)-
21	24.034	2245815	1.23	270738	Urs-12-en-28-al
22	24.941	1779705	0.97	230911	Urs-12-en-28-al
23	25.469	2242119	1.23	714488	Stigmasta-4,7,22-trien-3.beta.-ol
24	25.630	9156044	5.01	2810520	.beta.-Amyrin
25	26.192	28491141	15.60	7696491	.alpha.-Amyrin
26	27.490	366851	0.20	116954	Linaloloxide,trimethylsilylether
27	28.182	496158	0.27	96070	Ergost-5-en-3-ol,(3.beta.)-
28	29.152	1134913	0.62	233790	.beta.-Amyrin
29	29.746	6098868	3.34	1181277	.gamma.-Sitosterol

Table 8: Different components identified from Mass spectra of Extract 5

Peak	R. Time	Area	Area%	Height	Name
1	2.020	277506	0.11	412670	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	9.081	74317	0.03	42884	Decanaldimethylacetal
3	10.742	128069	0.05	58356	Octanoicacid,6,6-dimethoxy-,methylester
4	11.392	272576	0.10	86128	Nonanedioicacid,dimethylester
5	11.991	728467	0.28	393770	Decanaldimethylacetal
6	13.451	959254	0.36	552541	Methyltetradecanoate
7	15.417	812880	0.31	469357	9-Hexadecenoicacid,methylester,(Z)-
8	15.641	35042038	13.32	19072014	Hexadecanoic acid, methyl ester
9	16.067	5033074	1.91	1674141	n-Hexadecanoicacid
10	16.761	830396	0.32	371387	Methylabietate
11	17.333	25966196	9.87	14212903	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
12	17.385	25220367	9.59	14533767	9-Octadecenoicacid,methylester,(E)-

Table 9: Different components identified from Mass spectra of Extract 5

Peak	R. Time	Area	Area%	Height	Name
40	24.038	4013443	1.53	455042	Urs-12-en-28-al
41	24.943	2737178	1.04	350446	Urs-12-en-28-al
42	25.473	2975679	1.13	939760	Stigmasta-4,7,22-trien-3.beta.-ol
43	25.633	12110093	4.60	3595217	.beta.-Amyrin
44	26.203	37987777	14.44	9807839	.alpha.-Amyrin
45	28.161	611561	0.23	89551	Ergost-5-en-3-ol,(3.beta.)-
46	28.614	590707	0.22	64959	Oleana-11,13(18)-diene
47	29.153	1225836	0.47	250889	.beta.-Amyrin
48	29.740	4738083	1.80	893668	.gamma.-Sitosterol
49	30.035	13081394	4.97	2338461	Aceticacid,3-hydroxy-7-isopropenyl-1,4a-dimethyl
50	30.321	814472	0.31	172149	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b

Table 10: Different components identified from Mass spectra of Extract 6

Peak	R. Time	Area	Area %	Height	Name
1	2.020	242959	0.14	355838	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	3.172	63548	0.04	51586	Hexanaldimethylacetal
3	11.993	352199	0.20	187365	Decanaldimethylacetal
4	13.452	834723	0.48	468700	Methyltetradecanoate
5	15.417	736184	0.42	424482	9-Hexadecenoicacid,methylester,(Z)-
6	15.640	31825069	18.18	18041742	Hexadecanoic acid, methyl ester
7	16.064	3568393	2.04	1194934	n-Hexadecanoicacid
8	16.762	534031	0.31	231923	Methylabietate
9	17.335	33085356	18.90	17577385	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
10	17.386	23312621	13.32	14105679	9-Octadecenoicacid,methylester,(E)-

Table 11: Different components identified from Mass spectra of Extract 6

Peak	R. Time	Area	Area %	Height	Name
35	25.466	1553858	0.89	503479	Stigmasta-4,7,22-trien-3.beta.-ol
36	25.626	6554670	3.74	2012664	.beta.-Amyrin
37	26.181	19528118	11.16	5534024	.alpha.-Amyrin
38	27.480	315991	0.18	98470	1-(Dimethyldodecylsilyloxy)butane
39	28.182	173315	0.10	48087	Ergost-5-en-3-ol,(3.beta.)-
40	28.614	118498	0.07	36124	Oleana-11,13(18)-diene
41	29.156	913881	0.52	203462	.beta.-Amyrin
42	29.741	3246131	1.85	662420	gamma.-Sitosterol
43	30.017	7443107	4.25	1352157	Aceticacid,3-hydroxy-7-isopropenyl-1,4a-dimethyl
44	31.614	196487	0.11	44719	Stigmasta-3,5-dien-7-one
45	31.980	704388	0.40	137658	9,19-Cyclolanost-24-en-3-ol,acetate,(3.beta.)-

Table 12: Different components identified from Mass spectra of Extract 7

Peak	R. Time	Area	Area %	Height	Name
15	15.416	507974	0.35	309848	9-Hexadecenoicacid,methylester,(Z)-
16	15.638	30305493	20.67	16672459	Hexadecanoic acid, methyl ester
17	15.939	178702	0.12	39957	cis-9-Hexadecenoicacid
18	16.062	1825597	1.25	589818	n-Hexadecanoicacid
19	16.639	203034	0.14	70187	Heptadecanoicacid,methylester
20	16.759	499772	0.34	211045	Methylabietate
21	16.897	73332	0.05	32390	Methylabietate
22	17.032	117271	0.08	54207	1,7,7-Trimethyl-3-phenethylidenebicyclo[2.2.1]hept
23	17.326	16446890	11.22	9195909	9,12-Octadecadienoicacid(Z,Z)-,methylester
24	17.383	24898433	16.98	14447355	9-Octadecenoic acid, methyl ester, (E)-
25	17.428	1421563	0.97	666840	11-Octadecenoicacid,methylester

Table 13: Different components identified from Mass spectra of Extract 7

Peak	R. Time	Area	Area %	Height	Name
45	25.467	1204966	0.82	392506	Stigmasta-4,7,22-trien-3.beta.-ol
46	25.623	5342450	3.64	1661193	.beta.-Amyrin
47	26.177	16302101	11.12	4614304	.alpha.-Amyrin
48	29.745	2405666	1.64	494354	gamma.-Sitosterol
49	30.014	6431907	4.39	1198148	Aceticacid,3-hydroxy-7-isopropenyl-1,4a-dimethyl

DISCUSSION

The above tables are prepared from Total Ion Chromatograms (TICs) and Mass spectras by all the extracts run by GC-MS of the two aforementioned plants. Peak height and peak areas are also tabulated. Table 1 shows the approximate percentage content of distinguished components in the plants. As per the results, Octadecanoic acid, hexadecenoic acid, Amyrins, Sitosterol and Lupeol are found to be the prominent components in both the plants. Both the plants are widely distributed in nature and are effective to reveal many medicinal properties and other biological activities. Leaf extracts are considered for revealing other important phytochemicals which can be an asset for curing multiple other diseases or disorders.

Octadecanoic acid: Stearic acid is extracted in highest amount in moringa extract by accelerated maceration process. Soxhlet extract has worked best for extraction from MPL. Heating the extract at controlled rate increases the concentration of the components.

Hexadecenoic acid: Palmitic acid is found to be extracted in maximum concentration by Soxhlet extraction from both the plants. Repetitive continuous method of extraction with appropriate heat seems to have worked best for hexadecenoic acid.

Beta amyrin: Accelerated maceration with ultrasonication and heating gives more yield of beta amyrin. *Mucuna pruriens* is showing more concentration as compared to Moringa oleifera.

Alpha amyrin: Highest extracted analyte is alpha amyrin in both the plants. Alpha amyrin shows lowest solubility in ethanol extract. Velvet beans extracts are producing more amyrins considering their therapeutic importance.

Gamma sitosterol: Gamma sitosterol is extracted more by acceleration of extraction. It is non polar molecule which is extracted better with acceleration of sound waves being introduced.

Lupeol: Lupeol is also a significant non polar molecule which is extracted better in just Mucuna pruriens with Soxhlet extraction. Soxhlet is a continuous extraction process used for better yield of non-polar analytes.

CONCLUSION

Alcoholic accelerated maceration is proven to be the most suitable method for both the plants in-order to extract maximum number of phytoconstituents. Alcohol is an effective organic solvent having good extractive power and are stable agents for preserving the extracts for longer duration of time. GC-MS or any other instrumental techniques used for analysis are compatible with such organic solvents preferably alcoholic solvents as they are neither extremely non-polar nor they are highly polar, hence alcoholic extracts are most preferred. Soxhlet is also showing satisfactory results but considering the sustainable and greener approach; usage of low volumes of solvents is possible in maceration. Both the techniques of extraction have their pros and cons. Methanol is considered to be most useful solvent of choice. GC-MS is also considered as a greener technique considering its negligible consumption of solvents and ease of analysis. GC-MS uses gas as the mobile phase and does not involve any liquid for its use as it is not an environment friendly approach. Since, the injection volume is negligible the sample consumption required is also minimal. GC-MS is a tool showing high sensitivity and selectivity having the ability to provide qualitative and quantitative analysis of the data. It is known to be the most versatile tool which can analyze wide range of samples including volatile organic compounds, drugs, biological samples, pesticides. GC analyses volatile samples & MS works in vacuum hence, hyphenating these two techniques for identification of plant components is a much quicker and suitable approach in herbal chemistry. The NIST library reveals majority of components in the form of ions by GC-MS in both the plants. All the above components are predominantly best antioxidants and hence have crucial role in showing action against multiple diseases.

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Conflict of Interest

Neither the authors nor the institutions have any conflict of interest.

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