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Phytochemical And GC-MS Screening of Leaf of *Gisekia Pharnaceoides* Linn. From Thar Desert, Rajasthan, India

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ABSTRACT
The present study was carried out to identify the phytochemical constituents present in the methanolic and ethyl-acetate leaf extract of Gisekia pharnaceoides Linn. belonging to family Molluginaceae. This angiospermic family mostly possess herbs, often creeping and well branched. Gisekia Pharnaceoides Linn. is a diffuse succulent, glabrous herb. The shade-dried leaf powder was extracted with solvents using soxhlet extractor and crude extract was used for GC-MS. The mass spectra of the compounds analysed in the extract was matched with the National Institute of Standards and Technology (NIST) library. Maximum % area is found for Mome Inositol, it is present in maximum amount (30.74%) with RT=15.273 min, followed by 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-(18.47 %) with RT=19.165 min in the methanolic extract. Tetracontane is present in maximum amount (39.97%) with RT=31.632 min, followed by 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-(14.64%) with RT=19.197min in ethyl acetate as solvent. The present study reveals that Gisekia pharnaceoides Linn. is biologically an important medicinal plant of Indian Thar Desert. The phytochemical constituents analysed shows antimicrobial, anti-tumor, antibacterial and anti-fungal activity. Thus the findings may create a platform to design bioactive compounds used to treat various ailments.
Keywords*: Gisekia pharnaceoides Linn., Gas Chromatography–Mass Spectroscopy, Retention Time, Therapeutic, Phytochemicals, Mass Spectra*

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INTRODUCTION

Medicinal plants have significant importance in individual's health and communities [1]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [2]. Due to their beneficial potentials, medicinal plants show presence of bioactive compounds constituting certain physiological and pharmacological activity. Plant based medicines have played significant role in primary health care needs of all the organisms, i.e. humans as well as animals [3]. Higher plants as source of bioactive compounds continue to play a foremost role in the protection of human health [4]. Arid zone of Indian Thar of Rajasthan is characterized by extremes of temperature, severe drought accompanied by high wind velocity, low relative humidity, evaporation far exceeding precipitation and too scanty rain fall to support any appreciable vegetation. Plants with only xerophytic adaptations are able to establish themselves. In the process of protection they accumulate some important secondary metabolites which have medicinal importance.

The present study helps in validating new source of efficiently useful secondary metabolites. Our research is there for being focussed towards elucidating apparent source of ethno medicinal plants using modern scientific examination like GC-MS. This is the best technique to identify the bioactive compounds of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compounds etc. [5]. *Gisekia* is a bitter kitchen herb generally known as 'sareli' in Rajasthan. The leaves of *G. Pharnacieoides* are simple, petiolate, ovate, with obtuse apex, and pinnate-reticulate venation [6]. It cures swellings, scabies, rhinitis, bronchitis, loss of appetite, heart troubles, leprosy, leucoderma and urinary disease [7]. Pharmacognosical analysis of the leaf of *Gisekia pharnaceoides* Linn. revealed occurrence of starch, protein, oil and calcium oxalate while the preliminary phytochemical screening has been reported to reveal the presence of tannins, alkaloids, resins, cardiac glycosides, flavonoids and carbohydrates [8].

The objective of this study is to investigate preliminary phytochemicals and bioactive constituents for the first time, using GC-MS analysis that is first step towards understanding nature of active principal behind medicinal nature of this plant.

MATERIALS AND METHODS

The Leaves were collected from habitat comprising of sandy soil from Jodhpur, Pali, Barmer, Churu and Jhunjhunu districts of Rajasthan in the month of July-Oct.2015.The specimen authentication and recognition was done by Botanical Survey of India(BSI) Jodhpur, Rajasthan. The samples were washed with sterile distilled water they were shade dried and grounded into fine powder and stored in air tight polythene begs. Following procedure was adopted for extraction and screening of bioactive components-
2g of leaf powder was transferred to round bottom flask each containing 100ml of selected solvent i.e. methanol and ethyl-acetate, boiled at 65°-75°C for 6 hours using soxhlet assembly. Extract were filtered, evaporated to dryness. The ultimate residue obtained was then subjected to GC-MS analysis and stored at 4°C for future use. The GC-MS analysis was performed at AIRF (Advanced Instrumentation Research Facility) JNU, Delhi.
Standard analytical procedures [9] were used for screening of preliminary phytochemicals i.e. Wagner's Test (for alkaloids), Braymer's Test (for tannins), Salkowski's Test (for steroids), Sodium Hydroxide Test (for Flavonoids), Frothing Test (for saponins) and Molisch's Test (for carbohydrates). The compounds present in both the extract of leaf were finally identified, eluted by GC-MS analysis & their RT, % area & biological activity was known.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of leaf extract in methanol and ethyl-acetate as solvent showed the presence of various metabolic compounds like alkaloids, tannins, steroids, flavonoids and carbohydrates (table1).The compounds have shown affirmative and strong response in methanol as compared to ethyl-acetate. The compounds present in both the extract of leaf were identified by GC-MS analysis (table 2-4) revealing 46 and 85 peaks (fig.1, 2) indicating the presence of 42 and 69 compounds in methanol and ethyl-acetate extract respectively.
We identified the concentration of compounds (% area) in methanol and ethyl-acetate extracts, with their retention time (RT) in minute. Mome inositol is present in maximum amount (30.74%), followed by 9,12,15-octadecatrienoic acid,(Z,Z,Z)-(18.47%) and 1-Nonadecene (0.09%) was present in minimum amount in methanolic extract of leaf of this plant. Tetracontane is present in maximum amount (39.97%), followed by 9,12,15-octadecatrienoic acid,(Z,Z,Z)- (14.64%) and 9-Octadecenamide (0.07%) was present in amount equivalent to 9-eicosene,(E) with ethyl-acetate extract.
The compounds were recognized through mass spectrometry attached with GC. It is the best technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. [4]. The mass spectra of the compounds obtained was identified and compared with NIST (National Institute of Standards and Technology) library. Structure of compounds were confirmed by study of base peaks, retention time (RT) and molecular weight (MW). We identified 10 compounds common in both the extract. 32 and 59 compounds are non-common in methanol and ethyl-acetate extract respectively. An important characteristic of plant extract and their compounds is their hydrophobicity [10].
Among the identified phytochemicals, Phytol, squalene and vitamin E were detected in leaf extract with ethyl-acetate. The compounds have been reported effective against cancer, heart and asthematic disorders. Phytol is a promising pharmaceutical, active against various microbes, inflammations and carcinomas. Squalene, another compound that prevents cancer at earlier stages is a polyunsaturated hydrocarbon, an outstanding quencher of active singlet oxygen molecules with high potential in nutraceutical and pharmaceutical industries. Vitamin E, soluble in fat acts as an antioxidant, balances cholesterol, fights free radicals, inflammation, repairs damaged skin and hair, improves vision and strength of muscles.
Conclusively the investigations revealed the stronger extraction capacity of ethyl-acetate as compared to methanol extract. Presence of various bioactive compounds justifies the use of leaf of this plant to treat many incurable diseases. It can also be concluded that these findings on biological systems can open up new platform for natural and herbal components that can be employed for clinical trials in future. The accurate pharmacognosical description and research (isolation, quantification and purification) together with safety profile is required for future therapeutic utilization.

Table1: Phytochemical constituents of the leaf extract of *Gisekia pharnaceoides* Linn.

S.No.	Phytochemicals	Tests	Methanol	Ethyl-acetate
1.	Alkaloides	Wagner's	++	+
2.	Tannins	Braymer's	++	++
3.	Steroids	Salkowski's	+	++
4.	Flavonoids	Sodium hydroxide	+	++
5.	Saponins	Frothing	++	-
6.	Carbohydrates	Molisch's	++	++

Key: (-) absent, (+) present, (++) abundantly present

Table2: Phytocomponents common in both extract, showing biological activity of the leaf

S.No	Solvents	R. Time	Compounds	% area	M.Formula	M.Wt.	Biological Activity
1.	Methanol Ethyl-acetate	**13.353** 14.469	9-Eicosene, (E)-	0.23 0.07	$C_{20}H_{40}$	280	Antimicrobial and cytotoxic
2.	Methanol Ethyl-acetate	16.139 16.139	2,6,10,trimethyl,14-ethylene-14-pentadecene	2.21 3.39	$C_{20}H_{38}$	278	Antiproliferative
3	Methanol Ethyl-acetate	16.591 16.395	2-hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]	0.76 1.31	$C_{20}H_{40}O$	296	Antimicrobial, Sedatives and anaesthetics
4.	Methanol Ethyl-acetate	17.398 17.438	Pentadecanoic acid	11.67 12.95	$C_{15}H_{30}O_2$	242	Lubricants, additives, adhesive agents
5	Methanol Ethyl-acetate	18.884 18.883	Phytol	4.22 2.72	$C_{20}H_{40}O$	296	Antimicrobial, anticancer, diuretic, Anti-inflammatory
6	Methanol Ethyl-acetate	19.165 19.197	9,12,15-Octadeca trienoic acid,(Z,Z,Z)-	18.47 14.64	$C_{18}H_{30}O_2$	278	Antiinflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary
7	Methanol Ethyl-acetate	19.295 19.332	Octadecanoic acid	0.70 3.47	$C_{18}H_{36}O_2$	284	Cosmetic, flavor,hypocholesterolemic,lubricant,perfumery,propecic,suppository
8	Methanol Ethyl-acetate	27.015 27.016	Squalene	0.85 1.03	$C_{30}H_{50}$	410	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant
9	Methanol Ethyl-acetate	32.809 32.816	Vitamin E	0.50 0.87	$C_{29}H_{50}O_2$	430	Antiaging, analgesic, antidiabetic, Anti-inflammatory, antioxidant, antidermatitic, antileukemia, antitumor, anticancer, hepatoprotective, hypo-cholesterolemic, Antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary

Table 3: Bioactivity of phytochemicals identified in the methanol leaf extract

S.No.	R.Time	Compound	% area	M.Formula	M.W.	Biological activity
1.	7.355	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran	0.50	$C_6H_8O_4$	144	Antimicrobial, anti-inflammatory
2.	8.061	Naphthalene	0.31	$C_{10}H_8$	128	Antiseptic, carcinogenic
3.	8.423	2,3-Dihydro-benzofuran	3.47	C_8H_8O	120	Antilipidemic
4.	9.880	2-methoxy-4-vinylphenol	0.71	$C_9H_{10}O_2$	150	Antibacterial
5.	10.733	5-oxo-pyrrolidine-2-carboxylic acid methyl ester	0.21	$C_6H_9NO_3$	143	Anti-inflammatory, antiarthritis
6.	15.273	Mome inositol	30.74	$C_7H_{14}O_6$	194	Antialopethic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic, sweetener
7.	16.397	3,7,11,15-tetramethyl-2-hexandecan-1-ol	0.49	$C_{20}H_{40}O$	296	Antimicrobial, Anti-inflammatory, anticancer diuretic
8.	17.020	Hexadecanoic acid, methyl ester	2.71	$C_{17}H_{34}O_2$	270	Antioxidant, hypocholesterolenic, antiandrogenic, flavour, nematicide, hemolytic5-alpha reductase inhibitor
9.	17.683	1-Nonadecene	0.09	$C_{19}H_{38}$	270	Anti-fungal activity
10.	18.767	9,12,15-octadecatrienoic acid, methyl ester,(Z,Z,Z)	4.19	$C_{19}H_{32}O_2$	292	Anti-inflammatory, hypo-cholesterol, cancer preventive, hepatoprotective
11.	23.874	1,2-Benzenedicarboxylic acid	0.10	$C_{24}H_{38}O_4$	390	Antioxidant, antimicrobial, antifouling

Table 4: Bioactivity of phytochemicals identified in ethyl-acetate leaf extract

S.No	R.Time	Compound	% area	M. Formula	M.Wt.	Biological activity
1.	4.914	1,2-ethanediol,diacetate	0.18	$C_6H_{10}O_4$	146	Fragrances, cleaners, and detergents
2.	9.534	Heptadecane	0.05	$C_{17}H_{36}$	240	Antioxidant
3.	10.912	Tetradecane	0.11	$C_{14}H_{30}$	198	Antifungal and antibacterial
4.	12.208	Pentadecane	0.29	$C_{15}H_{32}$	212	Sugar-phosphatase inhibitor, acrocylindropepsin inhibitor, chymosin inhibitor, Antibacterial
5.	12.440	Phenol,2,4-bis(1,1-dimethylethyl)	0.43	$C_{14}H_{22}O$	206	Antioxidant
6.	12.741	Eicosane	0.37	$C_{20}H_{42}$	282	Antifungal, antitumor antibacterial, larvicidal, antimicrobial and cytotoxic effects
7.	12.912	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.10	$C_{11}H_{16}O_2$	180	Antifungal, antialgal antibacterial, antioxidant
8.	12.976	Dodecanoic acid	0.09	$C_{12}H_{24}O_2$	200	Antibacterial,cox-1 and cox-2 inhibitor antioxidant, antiviral, hypocholesterolemic,
9.	13.433	Hexadecane	0.34	$C_{16}H_{34}$	226	Antifungal, antibacterial, antioxidant
10.	13.753	Dodecanoic acid, 1-methylethyl ester	0.12	$C_{15}H_{30}O_2$	242	Cosmetic and lubricants
11.	14.175	Docosane	0.70	$C_{22}H_{46}$	310	Antibacterial
12.	15.294	Tetradecanoic acid	1.30	$C_{14}H_{28}O_2$	228	Antifungal, antibacterial, antioxidant, cancer preventive and nematicide, hypercholesterolemic,lubricant
13.	15.692	Nonadecane	0.76	$C_{19}H_{40}$	268	Cytotoxic effect, antimicrobial
14.	16.531	1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester	1.31	$C_{16}H_{22}O_4$	278	Antimicrobial, antifouling
15.	17.118	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.45	$C_{17}H_{24}O_3$	276	Antimicrobial
16.	21.628	9-octadecenamide	0.07	$C_{18}H_{35}NO$	281	Good therapeutic agents for the treatment of sleep disorders and

						pain
17.	23.244	Tetratetracontane	0.69	$C_{44}H_{90}$	618	Hypoglycemic, antioxidant
18.	31.632	Tetracontane	39.97	$C_{40}H_{82}$	562	Anti-inflammatory, analgesic activity
19.	37.660	1-heptacosanol	1.39	$C_{27}H_{56}O$	396	Nematicidal, anticancer, antioxidant and antimicrobial
20.	43.593	Stigmast-4-EN-3-one	0.53	$C_{29}H_{48}O$	412	Hepatoprotective, hypoglycemic, Antimicrobial, antioxidant, antiasthmatic, diuretic.
21.	48.929	Phytol, acetate	0.66	$C_{22}H_{42}O_2$	338	Antimicrobial, anti-inflammatory, diuretic, anticancer.

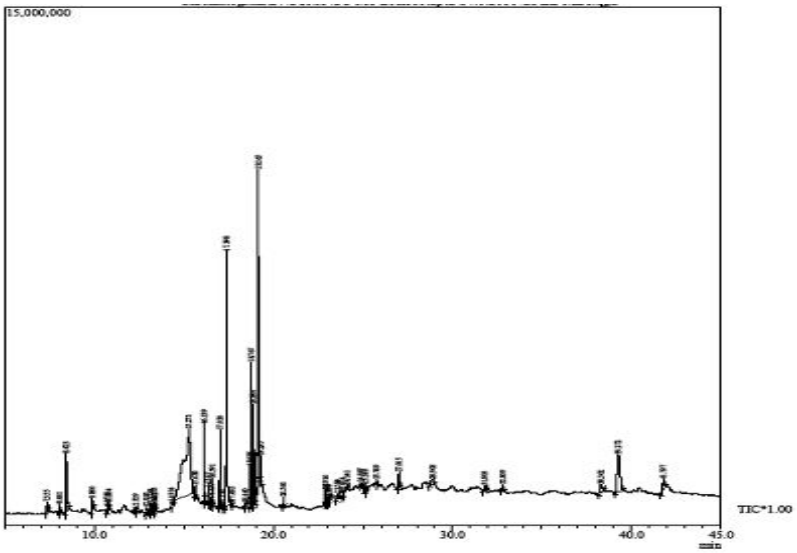

Fig. 1: GC-MS Chromatogram of the methanol leaf extract

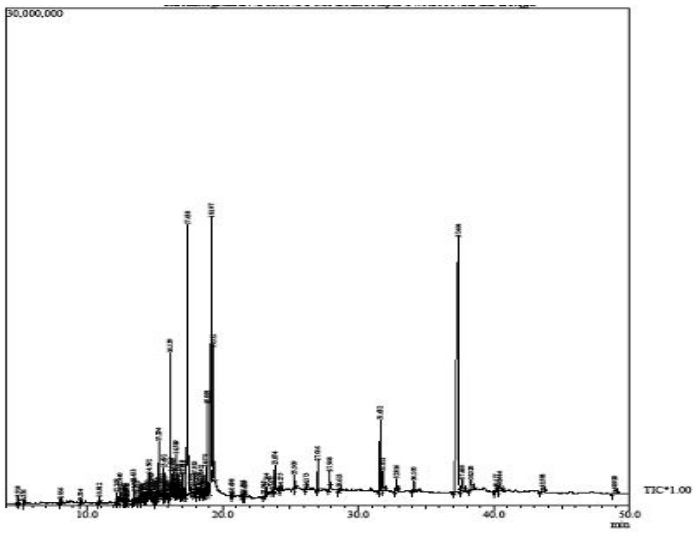

Fig.2: GC-MS Chromatogram of the ethyl-acetate leaf extract

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