ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical Examination And GC-MS Analysis of Methanol and Ethyl-Acetate Extract Of Root And Stem Of *Gisekia Pharnaceoides* Linn. (Molluginaceae) From Thar Desert, Rajasthan, India

Arora Sunita*, and Saini Manju.

* Professor, Department of Botany, Jai Narain Vyas University, Jodhpur (Raj.), India

ABSTRACT

Gisekia pharnaceoides Linn. belongs to family Molluginaceae and commonly known as sareli in Rajasthan. It is used against scabies, rhinitis, bronchitis, loss of appetite, heart troubles etc. The present investigation was designed to determine the bioactive constituents present in methanolic and ethyl-acetate extract of root and stem of Gisekia pharnaceoides using Perkin-Elmer Gas Chromatography-Mass Spectrometry. The GC-MS provided different peaks in methanolic extract of root and stem, revealed the presence of 36 and35 compound and ethyl-acetate extract shows 60 and 55 compound in root and stem with valuable biological activities. Gisekia pharnaceoides revealed the Cis-Vaccenic acid(27.21%),RT=19.124 is maximum and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione(0.10%), RT=17.117 is showing minimum % area in methanol extract of root. Mome inositol (53.62%),RT=15.742 is maximum and 1-Tetradecene(0.07%),RT=10.809 is in minimum amount in stem. The ethyl-acetate extract shows Pentadecanoic acid(21.69%),RT=17.420 as major component and Decane,3,3,5-trimethyl(0.05%),RT=16.300 is minor in amount in root and Tetracontane(42.41%),RT=31.610 is major and Cyclopentane,1,1'-[4-(3-cyclopentylpropyl)-1,7-heptanediyl]bis-(0.07%), RT=15.620 is minor compound analysed from stem. Presence of various chemical compounds confirms the application of G.pharnaceoides as a plant of medicinal value. The results of this study offer a platform of using root and stem of this plant as herbal alternative for various diseases.

Keywords: Medicinal plant, Molluginaceae, phytoconstituents, GC-MS analysis, Tetracontane, Mome-inositol.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Higher plants as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [1].Plants are used medicinally in different countries and are a source of many potent and powerful drugs[2]. Herbal drugs are widely prescribed, even when their biological components are not known due to their effectiveness, fewer side effects and relatively low cost [3]. It is safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Natural product, which come out from plants are important for pharmaceutical research and for drug development as a source of therapeutic agents [4]. The secondary metabolites of plants provides numerous biologically active products which have been used extensively as drug, food, additives, flavours, insecticides colorants, fragrances and chemicals [5],examples of these compounds include flavonoides, phenols, phenolic glycosides, saponins, and cyanogenic glycosides [6].In the recent years, there has been increasing focus on several plant derived polyphenolic compounds that may possess antimicrobial, antioxidants, anticancer and apoptosis inducing properties [7]. Molluginaceae consists of about 14 genera and 120 species of plants which are annual or perennial sub-shrubs, shrubs and herbs [8]. *Gisekia pharnaceoides* Linn is a creeping and well branched annual herb of sand dunes [9]. It acts as powerful anthelmintic in case of taenia and also given for chest disorders, warm infestation and mental disorders [10-13]. It is a high value nutritive source and can be used as a dietary supplement to prevent malnutrition especially in rural population [14] .Traditionally it is used in treatment of swelling and asthma. Medicinal plants are directly analysed for their existing compounds by GC-MS technique [15]. This technique is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [16].The aim of this study is to determine the bioactive compounds presents in the active fraction of *Gisekia pharnaceoides* (root and stem extract)using methanol and ethyl-acetate as solvent with the help of Gas chromatography-Mass spectrometry.

MATERIALS AND METHODS

Collection of the Plant material

Plants 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. Required plant part, that is root and stem were separated from whole plant. The samples were washed with sterile distilled water, shade dried and grounded into fine powder and stored in air tight polythene bags.

2g required sample 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. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids and alkaloids [17].

Preliminary Phytochemical Screening & GC-MS

Standard analytical procedures were adopted 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 vegetative part were finally identified, eluted by GC-MS analysis & their RT, % area & biological activity was known.

RESULT AND DISCUSSION

The preliminary phytochemical screening of root and stem in methanol and ethyl-acetate as solvent showed the presence of various metabolic compounds like alkaloids, tannins, steroids, flavonoids and carbohydrates. The compounds have shown affirmative and strong response in methanol as compared to ethyl-acetate (table-1).

ISSN: 0975-8585

Table1: Phytoconstituents extracted from vegetative parts (in methanol and ethyl-acetate extract)

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

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

The phytochemical constituents present in root and stem extract were identified by GC-MS using selected solvent that is methanol and ethyl-acetate. The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS)[18].The name, retention time, peak area, molecular weight and molecular formula of the components of the test materials were ascertained and biological activity of the identified compounds are investigated under our study. On comparison of the mass spectra of the constituents with the NIST library, different peak were obtained. All the phytoconstituents with their retention time (RT) were characterized and identified. Methanolic extract of root revealed 40 peaks (fig.-1) indicating 36 compounds, in which Cis-vaccenic acid(27.21%),RT=19.124 with highest percentage area showing hypolipidaemic, cosmetic, antihypertensive, anti-inflammatory effects followed by Cyclopentanol(16.00%), and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione(0.10%),RT=17.117 in minimum amount, as compared to ethyi-acetate extract of root that revealed 68 peaks(fig.-3) indicating 60 compounds out of that Pentadecanoic acid(21.69%),RT=17.420, is used as lubricants and adhesive agents is in maximum amount; followed by Cis-vaccenic acid(17.45%), and Decane,3,3,5-trimethyl(0.05%),RT=16.300, is in minimum amount. Methanolic extract of stem shows 39 peaks (fig.-2) indicating 35 compounds, where Mome inositol(53.62%),RT=15.742 possess antialopecic, anticirrhotic, antineuropathic, cholesterolytic and lipotropic activity, is a major compound followed by 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-(12.23%), and 1-Tetradecene(0.07%),RT=10.809 is a minor compound as compared the ethyl-acetate extract of stem, showing 69 peaks(fig.-4) indicating 55 compounds where Tetracontane(42.41%),RT=31.610 showing anti-inflammatory and analgesic activity followed by 7-Tetradecenal,(Z)-(12.27%) is in higher amount and **Cyclopentane,1,1'-[4-(3-cyclopentylpropyl)-1,7-heptanediyl**]bis-**(0.07%)**,RT=15.620 in minimum amount. 16 compounds are common in methanolic extract of root and stem where 12 compounds shows biological activity (table-2),34 compounds are similar in ethyl-acetate extract of root and stem, where 19 show various biological activity (table-3).

Table2: Bioactive compounds from vegetative parts (in methanol extract)

S. No.	Plant part	R.Time	Compounds	% area	M.F.	M.W.	Biological Activity
1.	Root Stem	7.343 7.346	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran	1.31 1.15	$C_6H_8O_4$	144	Antimicrobial, anti-inflammatory
2.	Root Stem	8.052 8.053	Naphthalene	0.43 0.23	$C_{10}H_8$	128	Antiseptic, carcinogenic
3.	Root Stem	10.808 10.809	1-Tetradecene	0.24 0.07	$C_{14}H_{28}$	196	Anti- tuberculosis
4	Root Stem	12.968 12.966	Dodecanoic acid	0.26 0.08	$C_{12}H_{24}O_2$	200	Pharmaceuticals activity
5.	Root Stem	13.347 13.348	9-Eicosene,(E)-	0.48 0.14	$C_{20}H_{40}$	280	Antimicrobial and cytotoxic

ISSN: 0975-8585

6	Root Stem	15.266 15.742	Mome inositol	8.67 53.62	$C_7H_{14}O_6$	194	Antialopethic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic,
7.	Root Stem	16.131 16.136	2,6,10,trimethyl,1 4-ethylene-14-pentadecene	0.18 1.05	$C_{20}H_{38}$	278	Antiproliferative
8.	Root Stem	16.333 17.399	Pentadecanoic acid	15.96 7.63	$C_{15}H_{30}O_2$	242	Lubricants, additives, adhesive agents
9	Root Stem	17.012 17.015	Hexadecanoic acid, methyl ester	1.04 0.63	$C_{17}H_{34}O_2$	270	Antioxidant, hypocholesterolenic, antiandrogenic, flavour, nematicide, hemolytic5-alpha reductaee inhibitor
10	Root Stem	18.690 18.693	9,12-Octadecadienoic (Z,Z)-,methyl ester	0.62 0.36	$C_{19}H_{34}O_2$	294	Hepatoprotective, anti-histaminic, Antieczemic , Hypocholesterolemic
11	Root Stem	19.289 19.292	Octadecanoic acid	1.51 0.79	$C_{18}H_{36}O_2$	284	Antibacterial, Cosmetic, Lubricant Hypocholestrolemic, perfumery
12	Root Stem	21.620 21.621	9-octadecenamide	0.19 0.22	$C_{18}H_{35}NO$	281	Good therapeutic agent for the treatment of sleep disorders and pain

Table 3: Bioactive compounds from vegetative parts (in ethyl-acetate extract)

S. No.	Plant part	R.Time	Compounds	% area	M.F.	M.W.	Biological Activity
1.	Root Stem	4.915 4.912	1,2-ethanediol,diacetate	0.22 0.22	$C_6H_{10}O_4$	146	Fragrances, cleaners, and detergents
2	Root Stem	9.531 14.591	Heptadecane	1.37 0.56	$C_{17}H_{36}$	240	Antioxidant
3	Root Stem	10.910 10.911	Tetradecane	0.32 0.18	$C_{14}H_{30}$	198	Antifungal, Antibacterial, Nematicidal
4.	Root Stem	12.435 12.437	Phenol,2,4-bis(1,1-dimethylethyl)	1.12 0.59	$C_{14}H_{22}O$	206	Antioxidant, Antibacterial
5	Root Stem	12.740 12.740	Eicosane	0.34 2.02	$C_{20}H_{42}$	282	Antifungal, Antibacterial, Antitumor, Cytotoxic
6	Root Stem	13.432 13.432	Hexadecane	1.09 0.56	$C_{16}H_{34}$	226	Antifungal, Antibacterial, Antioxidant
7	Root Stem	13.497 13.499	1,2-Benzenedicarboxilic acid, diethyl ester	0.90 0.30	$C_{12}H_{14}O_4$	222	Cosmetics, Insecticide, Aspirin, plasticizer
8	Root Stem	13.751 13.752	Dodecanoic acid, 1 methylethyl ester	0.38 0.19	$C_{15}H_{30}O_2$	242	Cosmetics, Lubricants
9	Root Stem	14.172 14.173	2-Methyl tetracosane	0.19 0.18	$C_{25}H_{52}$	352	Free radical scavenging
10	Root Stem	15.295 15.291	Tetradecanoic acid	4.99 2.23	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lybricant, Hypocholesterolemic
11	Root Stem	15.383 15.384	Heptadecane,3-methyl-	0.16 0.08	$C_{18}H_{38}$	254	Pest repellent, sex pheromone
12	Root Stem	16.737 15.740	Nonadecane	1.06 0.53	$C_{19}H_{40}$	268	Cytotoxic effect, antimicrobial

ISSN: 0975-8585

13	Root Stem	17.116 17.116	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1.47 0.80	$C_{17}H_{24}O_3$	276	Antimicrobial activity
14	Root Stem	17.420 17.419	Pentadecanoic acid	21.69 12.21	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
15	Root Stem	19.317 19.317	Octadecanoic acid	9.90 5.73	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
16	Root Stem	20.696 23.242	Tetratetracontane	0.22 1.04	$C_{44}H_{90}$	618	Hypoglycaemic, Antioxidant activity
17	Root Stem	25.317 31.797	1-Heptacosanol	1.35 2.01	$C_{27}H_{56}O$	396	Nematicidal, anticancer, antioxidant and antimicrobial
18	Root Stem	27.008 27.010	Squalene	0.84 0.57	$C_{30}H_{50}$	410	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, Pesticide, Chemo preventive, Lipoxygenase-inhibitor
19	Root Stem	37.162 31.610	Tetracontane	4.95 42.41	$C_{40}H_{82}$	562	Anti-inflammatory, analgesic activity

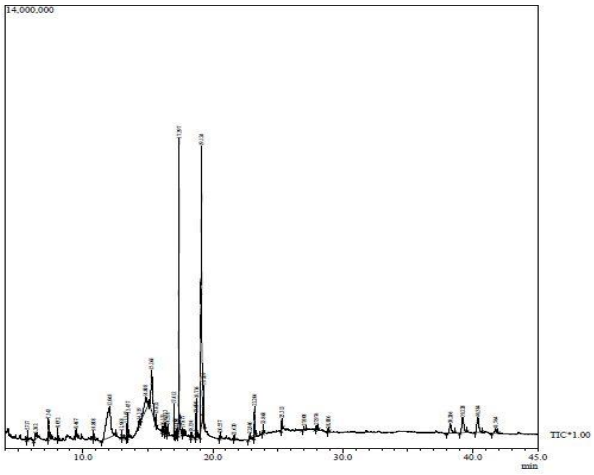

Fig. 1: GC-MS Chromatogram of the methanolic root extract

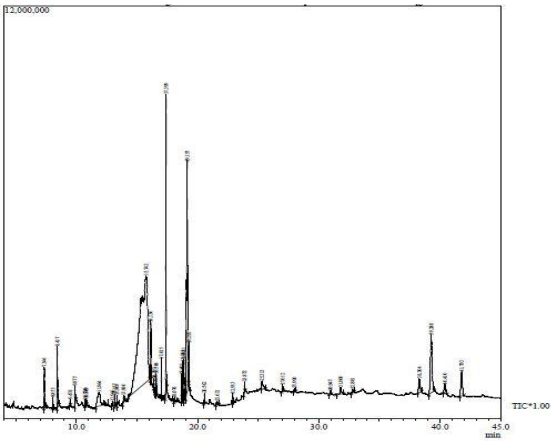

Fig. 2: GC-MS Chromatogram of the methanolic stem extract

ISSN: 0975-8585

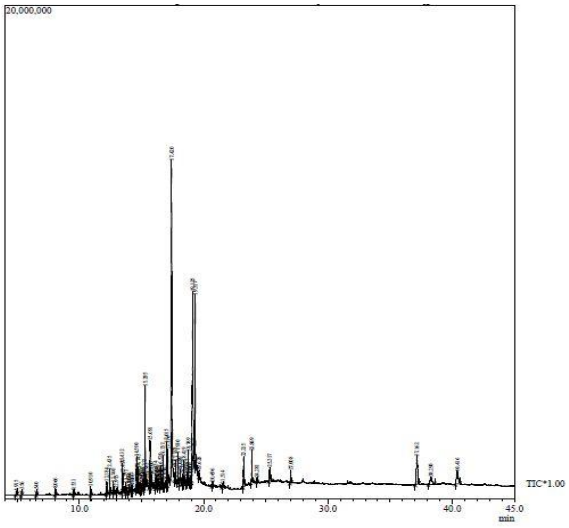

Fig. 3: GC-MS Chromatogram of ethyl-acetate root extract

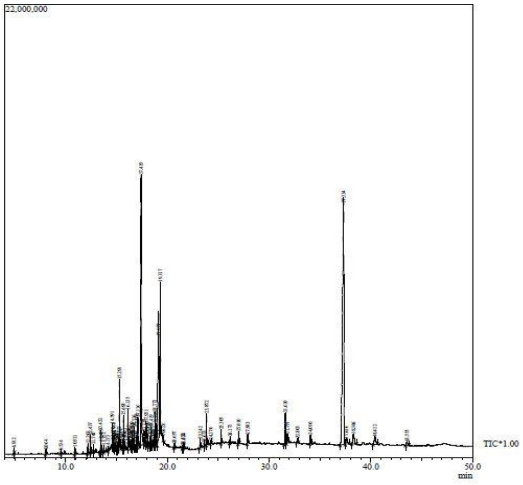

Fig. 4: GC-MS Chromatogram of ethyl-acetate stem extract

CONCLUSION

The present study has been found useful in the identification of several constituents present in the methanolic and ethyl-acetate extract of root and stem of *Gisekia pharnaceoides.* It could be concluded that this plant is of phytopharmaceutical importance. From the above discussion it has been revealed that *Gisekia pharnaceoides* is an important herb, with regard to its medicinal and nutritional properties. The study revealed presence of various important bioactive compounds and proved that the root and stem are medicinally important. further research is necessary to identify and purify the active principle responsible for therapeutic activity with ethics and legal issues. This report is first to screen many bioactive components that can be used to treat many diseases.

ACKNOWLEDGEMENT

The authors are thankful to AIRF, JNU, Delhi and CAS department of Botany JNVU, Jodhpur (Rajasthan) for providing infrastructure and technical support.

REFERENCES

[1] Farombi. EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African. J. Biotech. 2003; 2:662-671.

[2] Ponmozhi V, Pavithra S, Naga lakshmi M, Jaganathan J, Karthik L, Shree Devi M. S. Antibacterial activity of selected medicinal plants and GC-MS analysis of *Withania somnifera* .Malaya Journal of Bioscience. 2015; 2(3):160-165.
[3] Kumar R, Kumar S, Patra A, Jayalakshmi S. Hepatoprotective activity of aerial parts of *Plumbago zeylanica* Linn. against carbon tetrachloride-induced hepatotoxicity in rats. Int. J. Pharm. Sci. 2009; 1(1):171-175.
[4] Dhivya R and Manimegalai K. Preliminary phytochemical screening and GC-MS Profiling of ethanolic extract of *Calotropis gigantea* Linn.(Apocynaceae). Journal of pharmacognosy and phytochemistry .2013;2(3):28-32.
[5] Koduru S, Grierson DS, Afolayan A. Antimicrobial activity of *Solanum aculeastrum*. Pharm Biol. 2006; 44: 283-286.
[6] Shahidi F, Mc Donald J, Chandrasekara A, Zhong Y, Phytochemicals of foods, beverages and fruit vinegars: chemistry and health effects. Asia Pacific J Clin Nutr. 2008; 17:380-382.
[7] Clement MV. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 Signaling-dependent apoptosis in human tumor cells. Blood. 1998; 92:996-1002.
[8] Vishnoupriya C, Selvakumari E, Gopal V. A Comprehensive Review on *Gisekia pharnaceoides* Linn. (Molluginaceae). International Researach Journal of Pharmacy. 2013; 4(2) :10-12.
[9] Arora S, Saini M. Morphological studies of medicinally important plants of *Gisekia pharnaceoides* Linn. and *Corbichonia decumbens* (Forssk.)Exell of Molluginaceae from thar desert of Rajasthan, India. Biolife. 2016; 4(2):327-332.
[10] Kritikar KR, Basu BD."Indian Medicinal Plants". International Book. Distributors, Dehradun, India.1987; Vol 2 pp.1177-1182.
[11] Anonymous."The Wealth of India", CSIR, New Delhi. 1956; Vol 4 pp.131.
[12] Murugesa MKC, "Gunapada," Materia Medica, Directorate of Indian medicine, Chennai. 1998;pp 340.
[13] Yoganarasimhan SN. ¨Medicinal Plants of India" Regional Research Institute(Ayurveda),Bangalore. 2000;Vol 2 pp.245.
[14] Chinnasammi G, Bernard WCS, Paramasivan TP, Chellen R. Nutritional evaluation, in vitro free-radical scavenging and in vivo anti-inflammatory effects of *Gisekia pharnaceoides* and identification of kaempferol as a nutraceutical agent. British Biotechnology Journal. 2011;1(3):113-135.
[15] Arora S, Saini M. Biochemical screening of leaf extract of *Corbichonia decumbens*(Forssk.)Exell of Molluginaceae from Thar Desert of Rajasthan, India. Advances in Plant Sciences. 2016; 29(11):275-278.
[16] Manjamalai A, Yasaswine N, Aiswarya H, Berlin Grace VM., Antifungal, Anti-inflammatory and GC-MS analysis of methanolic extract of *Plectranthus amboinicus* leaf. International journal of current pharmaceutical Research. 2011; 3(2):129-136.
[17] Betz JM, Gay ML, Mossoba MM, Adams S and Portz BS: J AOAC Int. 1997; 80: 303.
[18] Cong Z, Meiling Q, Qinglong S, Shan Z, Ruonong FJ. Pharm. Biomed. Anal. 2007; 44: 464.