

GC-MS Analysis of Bioactive Phytochemicals in Methanol Extract of Aerial Part and Callus of *Dipterygium glaucum* Decne

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ABSTRACT

Introduction: This study was designed to evaluate the phytochemicals present in aerial part and *in vitro* induced callus methanol extracts of *Dipterygium glaucum* by Gas Chromatography-Mass Spectrometry (GC-MS) technique. **Methods:** GC-MS analysis of aerial part sample and callus produced from leaf of *D. glaucum* extracted in methanol solvents was performed using GC-MS QP 2010 Plus (Shimadzu, Japan) system comprising an auto sampler (AOC-20i) and a gas chromatograph interfaced to a mass spectrometer. **Results:** This study was carried out to identify and comparative analysis of bioactive phytochemicals from aerial part extract and callus extract of *D. glaucum*. This analysis revealed that both the extracts have 69 different types of phytochemical components in varying quantities. Some of the important phytochemical compounds were Stigmasterol, β -sitosterol, γ -sitosterol, Campesterol, Squalene, n-Hexadecanoic acid, Stearic acid, Myristic acid, Quinazoline, Linalyl acetate etc. These chemical compounds have anticancer, antitumor, anti-inflammatory, antidiabetic and antioxidants properties. **Conclusion:** This study represents the detection and identification of different phytochemical compounds from aerial part and callus extract of *D. glaucum*. Thus, due to the presence of various important bioactive phytochemicals this plant is recommended as a pharmaceutically important plant.

Key words: Callus, Campesterol, Capparidaceae, Fatty acid, Stigmasterol, Terpenoids.

INTRODUCTION

Plants are an important component of the health care system and are being used as a source of medicine due to their therapeutic potential since ancient times. The presence of the phytochemicals such as alkaloids, flavonoids and phenolic compounds in medicinal plants have been part of phytomedicine.¹ Phytochemicals are natural bioactive plant derived chemicals which play a vital role in the treatment and prevention of several diseases. The potential efficacy and safety of many plant-based drugs have already been proven.² The phytochemicals in the plant extract target the biochemical pathway, therefore the traditional drugs are safer than synthetic drugs.³ Now-a-days, synthetic medicines are widely used which may cause severe side effects in body and these consequences are sometimes more serious than that of disease itself. Therefore, to overcome this situation, pharmaceutical companies are spending a lot of time and money on the medicinal plant extracts for the formulation of natural drugs which are safe and cost effective i.e., affordable for common people.⁴ World Health Organization (WHO) in 2013, launched 'WHO Traditional Medicine Strategy 2014-2023' which emphasized to promote universal healthcare by joining traditional and complementary medicines together and to ensure the quality, effectiveness and safety of such medicines.⁵ Plants are rich source of secondary metabolites that have diverse biological activities and these metabolites serve as active medicines against several diseases.^{4,6} Nearly 20 % of plant species have been examined

in both biological or pharmacological applications to confirm their benefits and safety.⁷ In the recent times, a wide attention has been given to the studies on phytoconstituents of medicinal plants and its pharmacological activities.^{8,9} Various solvent system like water, ethanol, methanol, chloroform and ethyl acetate have been used for the extraction of secondary metabolites, such as steroids, terpenes, alkaloids, tannins and phenols.⁴ Many advanced techniques are adapted for the identification and quantification of bioactive compounds in plant materials and one of them, is Gas Chromatography-Mass Spectrometry (GC-MS) technique. GC-MS technique has become a key of technological platform for profiling of secondary metabolite¹⁰ and direct analysis of unknown components existing in medicinal plants.¹¹

A monotypic genus with one species, *Dipterygium glaucum* Decne., locally known as "Phel" belongs to the family Capparidaceae is distributed across the N.E. Africa, Arabia, Sind and India.¹² In India it is distributed on gravelly grounds and sand dunes in the desert areas of NW Rajasthan and Gujarat.¹³ *D. glaucum* is an important medicinal perennial shrub and used to cure respiratory diseases, skin diseases, wounds and chronic fever.¹⁴ This plant is a source of various phytochemicals such as volatile alkaloids, cyanides, coumarins and flavonoids¹⁵ with multiple biological activities like insecticidal, anthelmintic, antileishmanial, antibacterial and antifungal.¹⁶ The aerial plant parts of *D. glaucum* is used in bronchial asthma as bronchodilator¹⁷ while the whole plant is used as decoction and infusion for jaundice, psoriasis

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and ring worms.¹² *D. glaucum* have significant antioxidant activities and shows cytotoxic activity against cervical and breast cancer carcinoma cells.¹⁸ Thus, *D. glaucum* have enormous therapeutic potential and these properties demand phytochemical analysis, evaluation and validation for natural product development.

The present study is focused to evaluate and analyse the presence of phytoconstituents in methanolic extracts of aerial part and *in vitro* grown callus of *Dipterygium glaucum* in order to identify and characterize bioactive compounds with their concentration through a GC-MS technique.

MATERIALS AND METHODS

Instrument and chemicals

Gas Chromatograph-Mass Spectrometer system (GCMS QP 2010, Shimadzu, Japan), Shaker, Borosil beaker (500 ml, Borosil Glass works Ltd., Mumbai, India), Methanol (Analytical grade, Murashige and Skoog (MS) medium PT-100 (Murashige and Skoog 1962), 6-Benzyladenine (BA; Sigma-Aldrich) and 2,4-dichlorophenoxyacetic acid (2,4-D; Sigma-Aldrich*).

Plant material and callus culture

The fresh aerial parts of *Dipterygium glaucum* were collected in the month of February from Jaisalmer, an arid region of the Thar desert of Rajasthan, India (Figure 1). The aerial parts were rinsed thoroughly with autoclaved distil water to remove dust particles and dried at room temperature under the shade for nearly 20 days. The dried plant parts were pulverized to fine powder using mechanical grinder and kept in air tight polybags till further use. To analyse the callus culture of *D. glaucum*, *in vitro* callus culture has been established from leaf explant. The desired amount of callus was produced on MS medium augmented 0.5 mg l⁻¹ each BA and 2,4-D (Figure 2).



Figure 1: *Dipterygium glaucum* in its natural habitat.



Figure 2: *In vitro* callus culture establishment from leaf of *Dipterygium glaucum* on MS medium augmented with 0.5 mg l⁻¹ each of BA and 2,4-D. BA: 6-Benzyladenine and 2,4-D: 2,4-dichlorophenoxyacetic acid. (Scale bar = 10 mm)

Preparation of extracts

The powdered samples (aerial part and callus) of *D. glaucum* were extracted with methanol at room temperature. 30 g of aerial part powder and 10 g of callus were weighed, treated with 500 ml of methanol in a beaker covered with aluminium foil and kept on shaker for 2-3 days with occasional stirring. The extracts were filtered through muslin cloth followed by Whatman filter paper No. 1. and to obtain clear solution, the filtrate was further centrifuged at 2500 rpm for 10 minutes. The clear filtrate was evaporated to dryness to obtain the final concentrated extract and that extract was analysed by using GC-MS.

Gas chromatography-mass spectrometry (GC-MS) analysis

For GC-MS analysis, 1 µl of stock solution sample (1 mg/ml crude extract in methanol) was used. GC-MS analysis was performed at USIC, AIRF, Jawaharlal Nehru University, New-Delhi with GC-MS QP 2010 Plus (Shimadzu, Kyoto, Japan) system comprising an auto sampler (AOC-20i) and gas chromatograph interfaced to a mass spectrometer with an ion source temperature (220°C) and interface temperature (270°C). For GC-MS detection, a threshold desorption system was operated with an ionization energy of 1000 eV and mass range of 50-650 m/z purposed. The column oven temperature was 60°C and injection temperature 260°C at a pressure of 93.1 kPa. The carrier gas used was helium (99.99%) as a constant flow rate-total flow: 19.5 ml/min and column flow: 1.50 ml/min. The GC-MS program total running time was 50 minutes. The software used for the analysis of mass spectra and chromatograms was a Turbo mass and the relative percentage of each component was calculated by comparing its average peak area to the total area.

Identification of phytochemicals

The phytochemical components extracted were identified by comparing the retention time and molecular weight of the detected components with the mass spectra of the known components available in the Wiley libraries and National Institute of Standards and technology (NIST) library.

RESULTS

The GC-MS chromatograms spectra of the methanolic extract of aerial part and callus revealed the richness of phytochemical compounds in *Dipterygium glaucum* and contribute to the medicinal quality of the plant (Figures 3 and 4). The GC-MS analysis of aerial part extract and

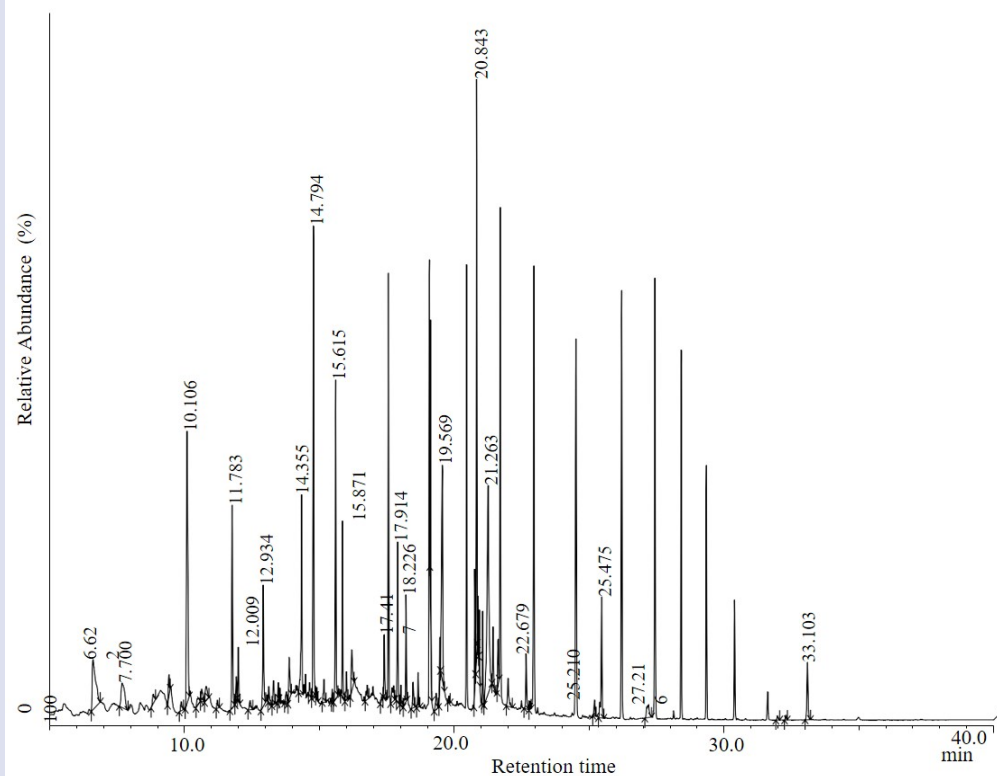


Figure 3: GC-MS chromatogram of methanolic extract of aerial part showing relative abundance and retention time of *Dipterygium glaucum*.

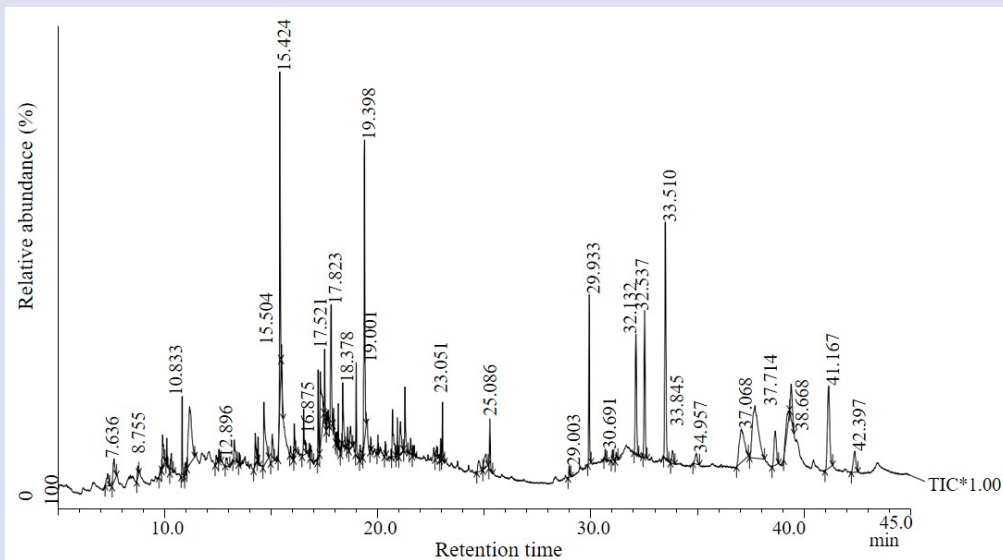


Figure 4: GC-MS chromatogram of methanolic extract of callus showing relative abundance and retention time of *Dipterygium glaucum*.

callus extract indicated the presence of 33 and 36 bioactive compounds respectively. The name and nature of the identified phytochemical compounds in aerial part and callus with their retention time, molecular formula, molecular weight and abundance (peak area in %) are presented in Tables 1 and 2 respectively. The major compounds in aerial part extract were- 9,12-Octadecadienoyl chloride, (Z,Z)- (8.86%),

Dibenzofuran (8.40%), 9-Octadecenoic acid (Z)-, methyl ester (8.18%), Azulene (8.17%), n-Hexadecanoic acid (5.54), Fluorene (5.36%), Acenaphthylene (4.07%) Benzenesulfonic acid, 4-Hydroxy (3.65%), β -Sitosterol (1.43%) and Phytol (0.68%). The callus cells also expressed good amount of phytoconstituents which was proved by the GC-MS analysis of the callus produced from leaf explant of *Dipterygium*

Table 1: List of compounds identified from different classes of bioactive phytochemicals by GC-MS of methanolic extract of aerial part of *Dipterygium glaucum*.

S.No.	Compound name	Molecular Formula	Molecular weight (g mol ⁻¹)	Retention time (min)	Peak Area (%)	Compound type
1.	Benzenesulfonic acid, 4-Hydroxy	C ₆ H ₆ O ₄ S	174	6.62	3.65	Phenolic
2.	4H-Pyran-4-one, 3-Hydroxy-2-methyl	C ₆ H ₆ O ₃	126	8.85	0.39	Organic compound
3.	1,5-Anhydro-6-deoxyhexo-2,3-diulose	C ₆ H ₈ O ₄	144	9.44	0.56	Glycoside
4.	Phenol, 2,4-dichloro-	C ₆ H ₄ Cl ₂ O	162	9.89	0.30	Phenolic
5.	Azulene	C ₁₀ H ₈	128	10.10	8.17	Hydrocarbon
6.	1,2-Benzenediol	C ₆ H ₆ O ₂	110	10.48	0.37	Phenolic
7.	2,3-Dihydro-benzofuran	C ₈ H ₈ O	120	10.65	0.43	Phenolic
8.	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	10.81	0.62	Benzyl or furfuryl alcohol
9.	Naphthalene, 1-methyl-	C ₁₁ H ₁₀	142	11.78	4.00	Hydrocarbon
10.	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	11.93	0.44	Phenolic
11.	1,7,7-Trimethyl bicyclo[2.2.1]heptan-2-one	C ₁₀ H ₁₄ D ₂ O	154	12.44	0.33	Terpene
12.	Naphthalene, 2,6-dimethyl-	C ₁₂ H ₁₂	156	13.31	0.97	Hydrocarbon
13.	Acenaphthylene	C ₁₂ H ₁₀	154	14.35	4.07	Hydrocarbon
14.	Dibenzofuran	C ₁₂ H ₈ O	168	14.79	8.40	Phenol
15.	1,2-Benzenedicarboxylic acid	C ₂₄ H ₃₈ O ₄	390	15.49	0.21	Phenol
16.	Fluorene	C ₁₃ H ₁₀	166	15.61	5.36	Hydrocarbon
17.	Phenanthrene	C ₁₄ H ₁₀	178	17.91	2.75	Hydrocarbon
18.	Neophytadiene	C ₂₀ H ₃₈	278	18.22	1.67	Terpenoid
19.	Tridecanal	C ₁₃ H ₂₆ O	198	18.48	0.62	-
20.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	18.67	0.54	Terpene
21.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	19.13	2.85	Fatty acid ester
23.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	19.56	5.54	Fatty acid
24.	9,12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	20.77	2.42	Fatty acid ester
25.	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	20.84	8.18	Fatty acid ester
26.	Phytol	C ₂₀ H ₄₀ O	296	20.93	0.68	Terpene
27.	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298	21.06	1.30	Fatty acid ester
28.	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	21.26	8.86	Fatty acid
29.	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	21.44	1.12	Fatty acid
30.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	25.21	0.36	Fatty acid ester
31.	Di-n-octyl phthalate	C ₂₄ H ₃₈ O ₄	390	25.47	2.60	Ester
32.	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	31.99	0.11	Alkaloid
33.	β-Sitosterol	C ₂₈ H ₅₀ O	414	33.10	1.43	Steroid

glaucum. The major compounds of callus extract were-, Stigmasterol (7.51%), Cholesta-3,5-dien-7-one (6.42%), n-Hexadecanoic acid (6.47%), Ergost-5-en-3-ol, (3.β., 24R)- (4.18%), Cholesta-4,6-Dien-3-ol, Benzoate, (3.β.)- (3.95%), Squalene (3.17%), Phenanthrene (3.14%), Tetradecanoic acid (3.07%), Octadecanoic acid (1.50%), γ-sitosterol (1.20%). The components found similar in both the extracts were 1,2 Benzenedicarboxylic acid, n-Hexadecanoic acid, Phenanthrene, Hexadecanoic acid, Methyl ester and Fluorene.

DISCUSSION

Gas chromatography coupled with mass spectrometry (GC-MS) is preferred for more precise information in both qualitative analysis and for quantitative determination.¹⁹ The *D. glaucum* plant was found to be rich in steroids, terpenes and terpenoids, fatty acid and their esters, hydrocarbons, phenolic and alcoholic compounds. Present study shows the medicinal values of *D. glaucum* by identifying various phytochemical compounds through GC-MS analysis of aerial part and callus. The bioactive phytochemical constituents reported in *D. glaucum* through the present study are from group of Terpenes and Terpenoids (Eucalyptol; Neophytadiene; 3,7,11,15-Tetramethyl-2-

hexadecen-1-ol / Phytol; Linalyl acetate; Alpha-guaiene, etc.), Steroids (β-sitosterol; γ-sitosterol; Stigmasterol; Ergost-5-en-3-ol, (3.β., 24R)- / Campesterol; Squalene; etc.), Fatty acids and esters (n-Hexadecanoic acid; Hexadecanoic acid, methyl ester; 9-Octadecanoic acid, methyl ester; Tetradecanoic acid; Oleic acid; etc.), Phenolic compounds (1,2-Benzenediol; 2-Methoxy-4-vinylphenol; 2,3-Dihydro-benzofuran; etc.), Alkaloids (Quinazoline) and Hydrocarbons (Azulene; etc.). The biological activities and uses of some important phytochemical constituents are summarized in Table 3.

Steroids

Overall, the richness of steroids is more in callus extract than the aerial part of *D. glaucum*. Various steroids were present in callus extract such as Squalene, Stigmasterol, Campesterol, γ-sitosterol. Similar to our observation, presence of good amount of steroid in callus culture has been reported by Galanes²⁰ in *Solanum aviculare*. The Squalene compound has antitumor, antioxidant, anticancer, antimicrobial, chemo-preventive, pesticide and sun-screen properties.²¹ Squalene has also been reported as an important precursor for the synthesis of phytosterols such as Sitosterol, Campesterol and Stigmasterol.²²

Table 2: List of compounds identified from different classes of bioactive phytochemicals by GC-MS of methanolic extract of callus of *Dipterygium glaucum*.

S.No.	Compound name	Molecular Formula	Molecular weight (g mol ⁻¹)	Retention time (min)	Peak Area (%)	Compound type
1.	Eucalyptol	C ₁₀ H ₁₈ O	154	7.35	0.56	Terpene
2.	2-Butanone,3-methyl-1-phenyl-	C ₁₁ H ₁₄ O	162	7.63	1.02	Phenolic
3.	Linalyl acetate	C ₁₂ H ₂₀ O ₂	196	10.83	1.30	Terpenes
4.	Quinazoline	C ₈ H ₆ N ₂	130	11.00	0.29	Alkaloids
5.	Z,Z,Z-1,4,6,9-Nonadecatetraene	C ₁₉ H ₃₂	260	12.43	0.24	Alkene
6.	n-Decanoic acid	C ₁₀ H ₂₀ O ₂	172	12.57	0.26	Fatty acid
7.	1,3-Isobenzofurandione, 3a,4,7,7a-tetrahydro-5-methyl-	C ₉ H ₁₀ O ₃	166	13.28	0.76	Esters
8.	Alpha-guaiene	C ₁₅ H ₂₄	204	13.51	0.16	Sesquiterpenes
9.	Phenol,3,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	14.39	0.42	Phenolic
10.	Fluorene	C ₁₃ H ₁₀	166	15.50	0.28	Hydrocarbon
11.	1,6-methanonaphthalen 1(2H)-ol, octahydro 4,8A,9,9-tetramethyl-, (1R,4s,4as,6R,8as)	C ₁₅ H ₂₆ O	222	16.54	1.16	Sesquiterpenes
12.	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	17.32	3.07	Fatty acid
13.	Benzyl Benzoate	C ₁₄ H ₁₂ O ₂	212	17.52	1.32	Esters
14.	Phenanthrene	C ₁₄ H ₁₀	178	17.82	3.14	Hydrocarbon
15.	Isopropyl myristate	C ₁₇ H ₃₄ O ₂	270	17.94	0.35	Esters
16.	2-Pentadecanone,6,10,14-trimethyl	C ₁₈ H ₃₆ O ₂	268	18.15	0.53	Diterpenoids
17.	1-Octadecanol	C ₁₈ H ₃₈ O	270	18.73	0.86	Fatty alcohol
18.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	19.00	1.43	Fatty acid ester
19.	Palmitoleic acid	C ₁₆ H ₃₀ O ₂	254	19.19	0.43	Fatty acid
20.	n- Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	19.39	6.47	Fatty acid
21.	Ethylene brassylate	C ₁₅ H ₂₆ O ₄	270	20.02	0.28	Ester
22.	Heptadecanoic acid	C ₁₇ H ₃₄ O ₂	270	20.36	0.24	Fatty acid
23.	9-Octadecanoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	296	20.70	1.49	Fatty acid ester
24.	Oleic acid	C ₁₈ H ₃₄ O ₂	282	21.07	1.28	Fatty acid
25.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	21.29	1.50	Stearic acid
26.	10-Nonadecanol	C ₁₉ H ₄₀ O	284	22.96	0.35	Alcohol
27.	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324	23.05	0.99	Terpenes
28.	1,2 Benzenedicarboxylic acid	C ₂₄ H ₃₈ O ₄	390	25.27	1.09	Phenolic
29.	Squalene	C ₃₀ H ₅₀	410	29.93	3.17	Steroid
30.	Tetrapentacontane	C ₅₄ H ₁₁₀	759	31.02	0.22	-
31.	Cholesta-4,6-Dien-3-ol, Benzoate, (3.beta.)-	C ₃₄ H ₄₈ O ₂	488	32.13	3.95	Steroid
32.	Ergost-5-en-3-ol, (3.beta., 24R)-	C ₂₈ H ₄₈ O	400	37.06	4.18	Steroid
33.	Stigmasterol	C ₂₉ H ₄₈ O	412	37.71	7.51	Steroid
34.	γ-Sitosterol	C ₂₉ H ₅₀ O	414	39.26	1.20	Steroid
35.	Cholesta-3,5-dien-7-one	C ₂₇ H ₄₂ O	382	41.16	6.42	Steroid
36.	Stigmast-4-en-3-one	C ₂₉ H ₄₈ O	412	42.39	1.23	Steroid

Phytosterols have antitumoral, anticancerous, anti-inflammatory, anti-atherogenic, anti-ulcerative, antifungal and antibacterial activities.²³⁻²⁵ In addition, Stigmasterol (7.51%), a major compound present in callus extract, is an unsaturated phytosterol with multiple medicinal properties like anti-osteoarthritic, cholesterol lowering,²⁶ thyroid inhibitory, hypoglycemic, antiperoxidative,²⁷ anti-inflammatory, antiviral, antihepatotoxic, cancer preventive activities,²⁸ antioxidant and it also shows antibacterial activity against multidrug resistant mycobacterium.²⁹ Similarly, Cheong et al.²² reported the presence of Stigmasterol in the callus extract of *Strobilanthes crispus*. Ergost-5-en-3-ol,(3.beta., 24R)- (4.18%) also known as Campesterol is a cholesterol absorption reducing agent and possess antioxidant and anticancerous activities.³⁰ The presence of γ-sitosterol or Clionasterol was detected in callus extract of *D. glaucum* and shows anti-cancerous,³¹ hepatoprotective, antihyperglycemic activity and act as an antidiabetic drug.^{32,33} β-sitosterol (1.43%) was the only steroid present in the aerial part extract and shows different biological activities like antitumor,³⁴ antiproliferative,³⁵ antioxidative, antiviral, immunomodulatory and hepatoprotective activity.³⁶ Some other steroidal compounds present in callus extract were Cholesta-3,5-dien-7-one (6.42%), Cholesta-4,6-

dien-3-ol, Benzoate, (3.beta., 24R)- (3.95%) and Stigmast-4-en-3-one (1.23%).

Terpenes and Terpenoids

Terpenes and terpenoids has important physiological as well as ecological roles and shows many biological activities such as hepato-protective, antioxidant and cholinesterase inhibitors.¹¹ Total ten different terpenes and terpenoids compounds were present in the extracts of *D. glaucum*. Neophytadiene (1.67%), a terpenoid compound, was found in the aerial part extract and has anti-inflammatory, antipyretic, antioxidant and antimicrobial properties.³⁷ It is also used in treatment of rheumatism, headache and skin problems.³⁸ 3,7,11,15- Tetramethyl-2-hexadecen-1-ol (0.54%), commonly known as Phytol, a monounsaturated diterpene alcohol present in aerial part extract. This compound known to possess anticancer, anti-inflammatory, antidiuretic, antimicrobial,²⁹ antioxidant, immunostimulant and cholesterol lowering properties.¹¹ Correspondingly, Ogunlesi et al.³⁹ and Satyal et al.⁴⁰ identified Phytol in leaves of *Euphorbia hirta* and *Cassia fistula*, respectively, which act as a precursor of Vitamins (E & K), including the cytotoxic activity against

Table 3: Some important phytochemical constituents identified in methanolic extracts of aerial part and callus of *D. glaucum* and their biological activities.

S.No.	Compound name	Compound class	Aerial part/ Callus	Uses / Biological activities
1.	β -Sitosterol	Steroid	Ae	Antitumor, ³⁴ antiproliferative, ³⁵ antioxidative, immunomodulatory, antiviral and hepatoprotective ³⁶
2.	γ -Sitosterol	Steroid	Ca	Anti-cancerous ³¹ , antihyperglycemic activity, hepatoprotective and antidiabetic drug ³²⁻³³
3.	Stigmasterol	Steroid	Ca	Anti-osteoarthritic ²⁶ , antiperoxidative, thyroid inhibitory ²⁷ , antihepatotoxic, anti-inflammatory, antiviral, cancer- preventive ²⁸
4.	Ergost-5-en-3-ol, (3.beta., 24R)-	Steroid	Ca	Antioxidant, anti-cancerous, hypocholesterolemic ³⁰
5.	Eucalyptol	Terpenes	Ca	Anti-inflammatory ⁴³
6.	1,2-Benzenediol	Phenolic	Ae	Antioxidant, anti-cancer and pesticides ⁵⁴
7.	Neophytadiene	Terpenoid	Ae	Antipyretic, antioxidant, antimicrobial and anti-inflammatory ³⁷
8.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene	Ae	Antimicrobial, anti-inflammatory, anti-cancer and anti-diuretic ²⁹
9.	n-Hexadecanoic acid	Fatty acid	Ae/Ca	Anti-cancerous ⁴⁷ , anti-inflammatory ⁴⁸ , antioxidant, anti-androgenic, hypo-cholesterolemic, hemolytic, 5- alpha reductase inhibitor, nematocides and pesticides ¹⁹
10.	2-Methoxy-4-vinylphenol	Phenolic	Ae	Anti-inflammatory, anti-microbial, analgesic and antioxidant ⁵¹
11.	2,3-Dihydro-benzofuran	Phenolic	Ae	Diabetic retinopathy and arthritis ³⁸
12.	Hexadecanoic acid, methyl ester	Fatty acid ester	Ae/Ca	Antitumor, immunostimulant ¹¹ , antioxidant, 5-alpha reductase inhibitor, hypo-cholesterolemic and pesticides ¹⁹
13.	Linalyl acetate	Terpene	Ca	Anti-inflammatory ⁴¹
14.	Quinazoline	Alkaloids	Ca	Antitumor ⁶⁵
15.	9-Octadecenoic acid, methyl ester	Fatty acid ester	Ae	Antiandrogenic, cancer preventive, anti-inflammatory, anemiagenic, dermatitigenic and insecticide ¹⁰
16.	Ethyl Iso-Allocholelate	Alkaloid	Ae	Anti-inflammatory and anti-microbial agents ⁶⁴
17.	Tetradecanoic acid	Fatty acid	Ca	Cancer preventive, antioxidant, ⁴⁹ antifungal and antibacterial ⁵⁰
18.	Oleic acid	Fatty acid	Ca/Ae	Anti-inflammatory, cancer preventive, anti-androgenic, anemiagenic, 5-alpha reductase inhibitor, dermatitigenic, hypocholesterolemic, insecticide ⁵¹
19.	Alpha-guaiene	Sesquiterpene	Ca	Anti-cancerous ⁴⁴
20.	Azulene	Hydrocarbon	Ae	Anti-inflammatory ⁶²
21.	Squalene	Steroid	Ca	Antioxidant, anticancerous, antitumor, antimicrobial, chemopreventive and pesticides ²¹

Ae: Aerial part; Ca: Callus.

breast cancer cell line. Linalyl acetate (1.30%), a monoterpenoid and 1,6-methanonaphthalen 1(2H)-ol,octahydro 4,8A,9,9-tetramethyl-, (1R,4s,4as,6R,8as) (Patchouli alcohol) (1.16%), a tricyclic sesquiterpene were present in the callus extract and shows anti-inflammatory activity.^{41,42} Eucalyptol (1.8 -cineol) (0.56%), saturated monoterpenes, a major constituent of eucalyptus oil shows anti-inflammatory activity and also used in remedy of sinusitis, bronchitis and colds.⁴³ Alpha-guaiene (0.16%), a constituent of Agarwood, a sesquiterpenoids was also present in callus extract and shows anti-cancerous activity.⁴⁴ The 4,8,12,16- tetramethylheptadecan-4-olide (0.99%), an isoprenoid γ -lactone is also found in callus extract.

Fatty acids and their esters

Presence of fatty acids and their esters were in abundance in both the extracts of *D. glaucum* and 18 major fatty acids compounds were found. Fatty acids show antibacterial activity and widely used in drug preparations, fat emulsions, cosmetics and liposomes.^{45,46} Palmitic acid or n-Hexadecanoic acid, a saturated fatty acid was found in both, aerial part (5.54%) and callus extract (6.47%). It has anti-cancerous,⁴⁷ anti-inflammatory,⁴⁸ antioxidant, anti-androgenic, hypocholesterolemic, 5-alpha reductase inhibitor, nematocides and insecticides properties.¹⁹ In addition, another compound Hexadecanoic acid, methyl ester was also present in both aerial part extract (2.85%) and callus extract

(1.43%). It is a saturated fatty acid ester and shows antitumor, immunostimulant¹¹, antioxidant, 5-alpha reductase inhibitor, pesticides and hypocholesterolemic properties.¹⁹ Stearic acid and Myristic acid are saturated fatty acids and found in callus extract. Myristic acid or Tetradecanoic acid (3.07%) has cancer preventive, antioxidant,⁴⁹ antifungal and antibacterial properties.⁵⁰ Stearic acid or Octadecanoic acid (1.50%) shows antimicrobial activity.⁵⁰ Oleic acid or 9-Octadecenoic acid, a mono unsaturated fatty acid present in aerial part extract (1.12%) and callus extract (1.28%). It has anti-inflammatory, cancer preventive, anti-androgenic, 5-alpha reductase inhibitor, anemiagenic, insecticide, dermatitigenic and hypocholesterolemic properties.⁵¹ Moreover, it is also used in aerosol products as an emulsifying or solubilizing agent.⁵² Another saturated fatty acid found in the callus extract was Heptadecanoic acid (0.24%). It has anti-inflammatory, antibacterial, antimicrobial, hypocholesterolemic and cancer preventive activities.²⁹ In aerial part extract, a linoleic acid ester group compounds were identified, namely, 9- Octadecenoic acid (Z)- methyl ester (8.18%) and 9,12- Octadecadienoic acid, methyl ester (2.42%) which have anti-androgenic, anti-inflammatory, hypocholesterolemic, and cancer preventive properties.¹⁰ The presence of another compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.36%) in aerial part which has medicinal properties such as anti-oxidant, anthelmintic and anti-inflammatory.⁵³ Overall abundant fatty acids were present in *D. glaucum*.

Alcoholic and phenolic compounds

The aerial part extract has 8 alcoholic and phenolic compounds whereas 5 were present in callus extract. The phenolic compound, 1,2-Benzenedicarboxylic acid, also known as Phthalic acid, was found in both aerial part (0.21%) and callus extract (1.09%). Phenolic compounds present in the aerial part extract were 2-Methoxy-4-vinylphenol (0.44%), 2,3-Dihydro-benzofuran (0.43%) and 1,2-Benzenediol (0.37%). The phenolic compound, 2-Methoxy-4-vinylphenol possess anti-inflammatory, antimicrobial and antioxidant activity.⁵¹ 2,3-Dihydro-benzofuran or Coumaran an essential oil which is used to cure arthritis and diabetic retinopathy.³⁸ The presence of another important phenolic compound was Catechol or 1,2-Benzenediol which shows anticancer, analgesic and antioxidant properties.⁵⁴ It is also used as a raw material for drugs, pesticides, polymerization inhibitors, dyes, leather tanning, analytical reagents and deoxygenating agent.⁵⁵ 2-Butanone,3-methyl-1-phenyl (1.02%), 1-Octadecanol (0.86%) and 10-Nonadecanol (0.35%) compounds were present in the callus extract. 10-Nonadecanol, an alcoholic compound possesses nematocides and pesticides properties.⁵⁶

Esters

Besides fatty acid esters, a total of 5 ester compounds were also detected. Among the detected esters, the Benzyl benzoate (1.32%), a natural compound present in the callus extracts shows anti-hypertensive effects by suppressing the Ang-II induced hypertension.⁵⁷ Isopropyl myristate (0.35%), an ester of isopropyl alcohol and myristic acid and ethylene brassylate were used in cosmetics, perfumery⁵⁸ and pharmaceuticals ointments.⁵⁹

Hydrocarbons

In the extracts of *D. glaucum*, total 8 different hydrocarbons were detected. Among them Fluorene and Phenanthrene were present in both the aerial part and callus extracts. Fluorene, an aromatic hydrocarbon having medicinal values, used in production of drugs and in pharmaceuticals⁶⁰ whereas, Phenanthrene, a natural compound has been reported for its anticancer, anti-inflammatory, antimicrobial, spasmolytic, phytotoxicity, antiplatelet aggregation and antiallergic activities.⁶¹ The compound Azulene (8.17%) was found in the aerial part extract and shows anti-inflammatory activity.⁶²

Other compounds

Some other compounds were found in the aerial part extract of *D. glaucum* such as 1,5-Anhydro-6-deoxyhexo-2,3-diulose (0.56%), a glycoside which is used as preservative⁶³ and Ethyl iso-allocholate (0.11%), an alkaloid showing anti-inflammatory and antimicrobial properties.⁶⁴ Another compound of interest found in callus extract was an alkaloid Quinazoline (0.29%) which shows anti-tumour activity.⁶⁵

CONCLUSION

Dipterygium glaucum is a medicinal plant and represent rich source of phytochemical compounds having various biological activities. The results of GC-MS analysis presented a comparative study of various phytochemical compounds found in methanolic extract of aerial part and *in vitro* induced callus of *D. glaucum*. Total 69 bioactive compounds were identified with medicinal value as well as biological activity. Some important bioactive phytochemicals present in *D. glaucum* were Stigmasterol, Campesterol, β -sitosterol, γ -sitosterol, n-Hexadecanoic acid, Tetradecanoic acid, Quinazoline, Squalene, Oleic acid, etc. It could be concluded that besides aerial part, the *in vitro* produced callus also possesses various bioactive phytochemical compounds from different categories of bioactive compound such as terpenes, steroids, fatty acids, esters, hydrocarbons, phenolic, alcoholic, etc. Till now, no work has been carried out to identify the biological potential of this medicinal plant. Hence it is the first report on GC-MS analysis of methanolic extract of this medicinal plant of arid region.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to publish this manuscript.

SUMMARY

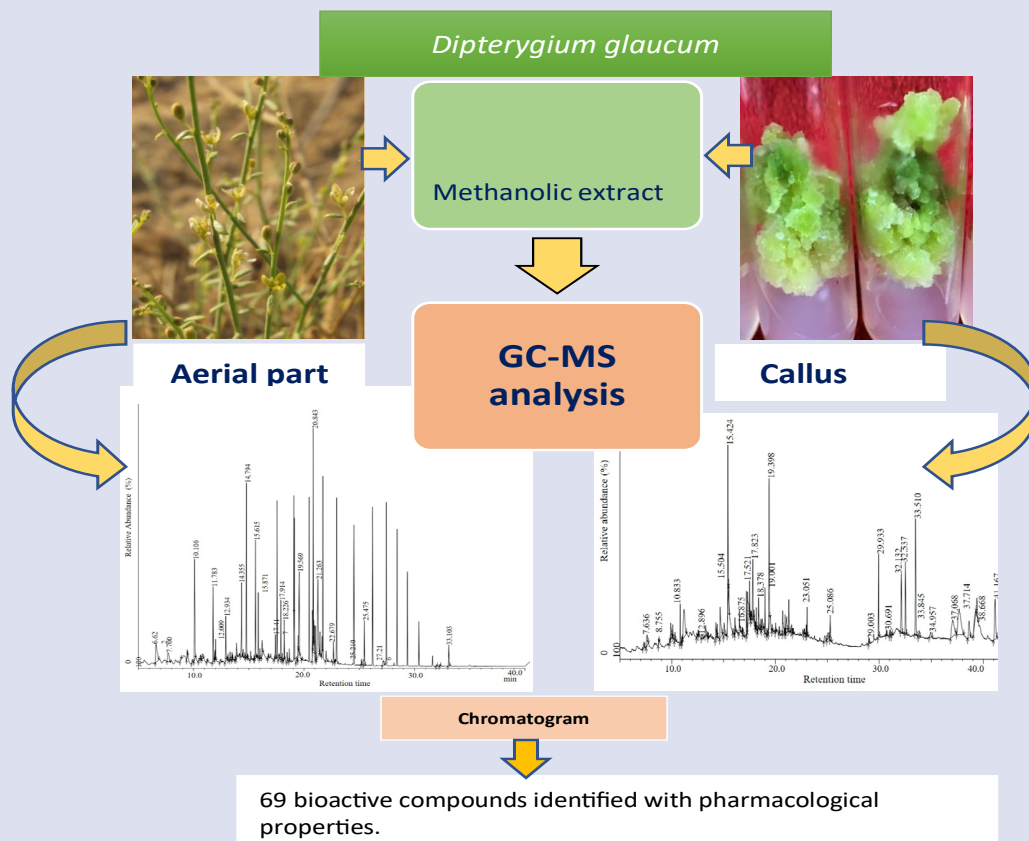
This study presented a comparative analysis of methanolic extract of aerial part and *in vitro* induced callus of *Dipterygium glaucum* using Gas Chromatography-Mass Spectrometry analysis. A total of 69 compounds identified belonging to various classes of compounds with different pharmaceutical properties. The outcome of this study presented that the callus extract also express good amount of bioactive compounds. Some of the important phytochemical compounds were Stigmasterol, γ -sitosterol, β -sitosterol, Campesterol, Squalene, Stearic acid, n-Hexadecanoic acid, Myristic acid, Quinazoline, Linalyl acetate etc. *Dipterygium glaucum* can become a good source for therapeutic studies.

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GRAPHICAL ABSTRACT



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