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Research Article

Phytocompounds Profiling in Cell Suspensions Extracts of *Gnidia glauca* (Fresen.) Gilg

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ABSTRACT

Phytocompounds enable plants to play a remarkable role as traditional remedies for human ailments. Due to their extremely low side effects, the ancient herbal medicinal systems are more beneficial than the modern medical systems. Gnidia glauca (Fresen.) Gilg has many biological properties, including antifungal, anticancer, antibacterial, and traditional medicine for swelling, indigestion, and snake bites. Because of the plant's impressive qualities and status as an endangered plant species, the callus suspension method was used as an alternative technique for phytocompounds enhancement. In order to screen the presence of phytocompounds, qualitative estimation was used, and ten phytoconstituents were identified by Gas Chromatography-Mass Spectroscopy (GC-MS) analysis in the cell suspension extracts. The present study's findings highlight the need for more investigations into the large-scale production and purification of bioactive substances.

Keywords: *Phytocompounds, GC-MS, Cell suspension culture, G. Glauca (Fresen) Gilg.*

INTRODUCTION

Since ancient times medicinal herbs have been utilized to treat human illnesses. Numerous medicinal plants and their extracted components have proven beneficial therapeutic potentials (Gowrish et al., 2013). *Gnidia glauca* (Fresen.) Gilg belongs to the *Thymelaeaceae* family and has been found to possess various traditional agrochemical and phytomedicinal applications.

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The leaves, roots and bark of *G. glauca* are rich sources of phytocompounds like saponins, terpenoids, steroids, alkaloids, phenols, flavonoids, tannins and glycosides. *G. glauca* leaf saponin fraction exhibits hemolytic activity (Sannabommaji et al., 2018) and cytotoxic activity on two human cancer cell lines, HT-29 and A-549 (Gowrish et al., 2013). In Africa, *G. glauca* is used for the treatment of abdominal pain, cancers, wounds, snake bites, sore throat and burns. It is also well known for its piscicidal, insecticidal, molluscicidal, and even homicidal activity for its use as arrow poison (Ghosh et al., 2015). Antidiabetic activity of *G. glauca* has been reported (Jamdade et al., 2019), and is a bactericidal agent for controlling/managing the bacterial blight disease of paddy (Sannabommaji et al., 2019). All parts of the plant possess immense medicinal properties, but it is on the endangered plants list; hence alternative method was conducted, establishing cell suspension cultures. Elicitor and precursor were treated to the pre-production production phytochemicals in the cell suspension method. The present study investigates the profiling (identification) of phytochemicals present in the cell suspensions extracts of *G. glauca* in the presence of various elicitors and precursors treated suspensions extracts like methyl jasmonate-fructose (MJ-F) combination-treated, methyl jasmonate (MJ) alone treated, ketoconazole-glucose (KCZ-G) combined treated, and ketoconazole (KCZ) alone treated cell suspensions extracts exhibited the presence of a higher number of compounds, compared to KCZ-G and KCZ treated extracts. The present study is helpful to further compound analysis, purification and investigation of biological activities of the compounds identified by the GC-MS method. Different extracts identified similar compounds in both MJ-F combination and MJ alone treated extracts.

MATERIALS AND METHODS

Plant collection and identification

G. glauca (Fresen) Gilg plant material was collected from the Western Ghats spanning Shivamogga district, Karnataka, in the month of July. The plant was authenticated by Dr. Kumaraswamy Udupa, renowned plant taxonomist, Department of Botany, Sri JCBM College, Sringeri, Chikkamagalore district, Karnataka, and a voucher specimen (No. FSB-0982) was preserved in herbarium of the department.

Preparation of extracts for analysis

Fresh tender leaves of *G. glauca* were used as explants for callus establishment, inoculating horizontally on the surface of MS media supplemented with 2,4-D (2 mg/l); cultures were incubated at 16/8 hrs (light/dark), harvested up to the callus induction. The white friable 30 days old callus (500 mg) and the same growth regulators with liquid MS media were used for the establishment of cell suspension culture. Elicitors and precursors were filter-sterilized and supplemented at different concentrations. Elicitors methyl jasmonate (50 µM, 100 µM, 200 µM), and carbohydrate/sugar precursors fructose (4%), glucose (4%), and also another molecule ketoconazole (50 µM, 100 µM, 200 µM), were prepared supplemented in the medium, and pH was adjusted to 5.7. Elicitors and precursors were supplemented into 50 ml nutrient liquid media containing 250 ml Erlenmeyer conical flasks and were kept in a gyratory rotatory shaker incubator at 120 rpm speed, 26°C temperature in dark condition. Then cultures were harvested at regular time intervals (6, 12, 18, and 24 days). After the completion of each successive culture, day, elicited culture was collected through sterile muslin cloth and oven dried at 110 °C; extracts were collected and stored for further parameters study. The best results were obtained from 100 µM at18th day extracts; this was confirmed by qualitative analysis, which is further helpful in conducting GC-MS analysis. The elicited cell suspension extracts were dried, powdered and dissolved in 80% HPLC grade methanol, and filtered through a 0.22 µ nylon membrane filter; this filtrate was subjected to qualitative analysis by standard protocols and GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The Phytocompounds analysis was got done by GC-MS analysis at Centre Analytical Instrumentation-Kerala (CAI-K) using Shimadzu GC-MS Model Number-QP2010S. ELITE-5MS 30-meter length, 0.25 mm ID, and thickness 0.25 µm column were used, the column oven temperature was maintained at 80.0 °C with injection temperature 260.00 °C, the split mode used for injection. The pressure rate was 65.0 kPa, the total flow rate was 24.0 mL/min, and the column flow rate was 1.00 mL/min in a linear velocity of 36.8 cm/sec. For the GC–MS detector gained by 1.11 kV +0.20 kV) NIST 11 and WILEY 8 library and software databases were used for the interpretation of GC-MS results in data compound identification.

RESULTS AND DISCUSSION

Preliminary phytochemical screening by qualitative analysis:

The qualitative phytochemical screening results of the cell suspension extracts are shown in Table 1. Positive results of the phytochemical screening by qualitative analysis indicated the presence of saponins, terpenoids, alkaloids, phenolics, steroids, and glycosides in the cell suspension extracts of *G. glauca* treated with elicitors and precursors. Similar reports were reported in other plants' phytochemical extracts (Sannabommaji et al., 2019, & Ramachandran et al., 2022).

Results obtained from GC-MS analysis were provided in Figure 1, and identified phytocompounds were listed in Table 2. and various phytocompounds present in elicitors and precursors treated cell suspension extracts of *G. glauca* and these phytocompounds. These compounds have been reported to possess different biological activities in the literature. Similar results were reported for phytochemical extracts of other plants (Ramachandran et al., 2022, Patil & Khan, 2021, & Bunrathep et al., 2006).

Identification of phytocompounds by GC-MS analysis:

The compounds present in various cell suspensions extracts of *G. glauca* possess different biological activities, the four types of extracts GC-MS chromatograms represented in Figure. 1 (a, b, c, and d). The compound 1,2-benzene dicarboxylic acid, bis (2-methyl propyl) ester, is present in all four types of extracts (Peak No-1; RT-27.249, 27.285, 27.395, 27.306). This compound possesses anticancer activity (Kumar et al., 2021). While 1,2 benzene dicarboxylic acid, dioctyl ester (Peak No-2; RT-39.093) from MJ-F extract (Fig. 1a) has been reported to possess antioxidant and antibacterial activity (Salem et al., 2016). The compound Myristic acid vinyl ester from MJ-F (Peak No-3, RT-41.571) and MJ (Peak No-2, RT-41.781) has been reported to possess antimicrobial and anticancer activity (Sujatha et al., 2020). While, the compound 2-methyltetracosane (Peak No-4, RT- 43.590) from MJ-F (Fig. 1a) has been reported to possess anticancer, antioxidant, antimicrobial, anti-androgenic and anti-inflammatory activities (Momodu et al., 2022). In the same extract, the compound Nonadecane (Peak No-5, RT-44.790) has been reported to possess anticancer activity in tumoral human cell lines HeLa and MCF-7 and antimicrobial activity (Hsouna et al., 2011). The compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Peak No-3, RT-45.511) from MJ extract (Fig. 1b) showed anticancer activity (El-fayoumy et al., 2021), and the same extract the compound Eicosanoic acid, 2-[(1-oxohexadecyl) oxy]-1-[[(1-oxohexadecyl) oxy] methyl] ethyl ester (Peak No-4, RT-46.641) has antimicrobial activity (Ramya et al., 2019). While the compound palmitic acid vinyl ester, in MJ (Peak No-5, RT-49.231) and KCZ-G (Peak No-2, RT-41.173) (Fig. 1 b and c) has been reported to possess antimicrobial activity (Ramya et al., 2019). The compound 1,2-benzene dicarboxylic acid (Peak No-2, RT-39.106) in KCZ extract (Fig.1 d) has been reported to possess antimicrobial activity (Yabesh et al., 2018) and cytotoxic activities (Krishnan et al., 2014). Among all the ten phytocompounds identified in all the four cell suspensions extracts of *G. glauca* by GC-MS analysis peak area % is high in 1,2-benzene dicarboxylic acid, bis (2-methyl propyl) ester (Fig. 1d; peak No-1, peak area%-91.41), and Palmitic acid vinyl ester (Figure 1c, peak Area%-90.90). The results obtained by GC-

MS analysis, the MJ-F combination, and MJ alone treated extracts resulted in a higher number of compounds than KCZ-G and KCZ treated extracts.

The ten different compounds were identified in four types of cell suspensions extracts of *G. glauca* that were treated with elicitors alone or elicitor-precursor combinations. There are very few reports on the use of cell suspension extracts for phytochemical analysis by the GC-MS method, and no prior reports employing the GC-MS method have been made on the phytocompounds found in these extracts from *G. glauca* cell suspension; this is the first report on the compounds identified by GC-MS in cell suspension extracts of *G. glauca* (Fresen.) Gilg. These bioactive compounds might be the reason for the medicinal potential of the plant. A cell suspension is a different approach to enhancing the production of phytoconstituents by treating the elicitors and precursors in the cell suspension method without disturbing the wild plant; this study promotes the conservation of medicinal plants that are in danger of extinction this is encouraging additional studies on the isolation of the discovered phytoconstituents. This study is helpful to further compound analysis and investigation of biological activity.

Table 1: Qualitative phytochemical screening results of cell suspensions extracts of *G. glauca*

Sl. No	Phytochemical tests	Extracts			
		MJ-F	MJ	KCZ-G	KCZ
1	Saponins (Salkowski test, Foam test)	+	+	+	+
2	Terpenoids (Liebermann-Burchard test)	+	-	+	-
3	Alkaloids (Wagner's test, Hager`s test)	+	-	+	-
4	Phenolics (Ferric chloride Test)	+	-	+	-
5	Steroids (Salkowski test)	+	+	+	+
6	Glycosides (Keller-Killian test)	+	-	-	+

+ present, - absent, MJ-F- Methyl Jasmonate-Fructose, KCZ-G- Ketoconazole-Glucose

Table 2: Compounds Identified by GC-MS analysis of cell suspension extracts of *G. glauca* (Fresen.) Gilg was treated with MJ-F, MJ, KCZ-G, and KCZ, respectively

Cell suspension extract	Peak No.	Identified compound	Molecular formula	Molecular weight	Base m/z	RT	Area %
MJ-F	1	1,2 benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278.34	149.00	27.249	4.80
	2	1,2 benzene dicarboxylic acid, dioctyl ester	$C_{34}H_{58}O_4$	530.8	149.00	39.093	0.43
	3	Myristic acid vinyl ester	$C_{16}H_{30}O_2$	254.41	71.10	41.571	46.55
	4	2-methyltetracosane	$C_{25}H_{52}$	352.7	71.10	43.590	11.37
	5	Nonadecane	$C_{19}H_{40}$	268.5	71.10	44.790	36.86
MJ	1	1,2-benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278.34	149.00	27.285	2.52
	2	Myristic acid vinyl ester	$C_{16}H_{30}O_2$	254.41	71.10	41.781	48.78
	3	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	71.10	45.511	12.32
	4	Eicosanoic acid, 2-[(1-oxohexadecyl) oxy]-1-[[(1-oxohexadecyl) oxy] methyl] ethyl ester	$C_{57}H_{108}O_6$	889.5	71.10	46.641	4.74
	5	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282.4614	71.10	49.231	31.62
KCZ-G	1	1,2-benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278.34	149.00	27.395	9.10
	2	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282.46	71.10	41.173	90.90
KCZ	1	1,2-benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278.34	149.00	27.306	91.41
	2	1,2-benzene dicarboxylic acid	$C_8H_6O_4$	166.13	149.00	39.106	8.59

MJ-F: Methyl jasmonate-fructose; MJ: Methyl jasmonate, KCZ-G: Ketoconazole-glucose; KCZ-Ketoconazole.

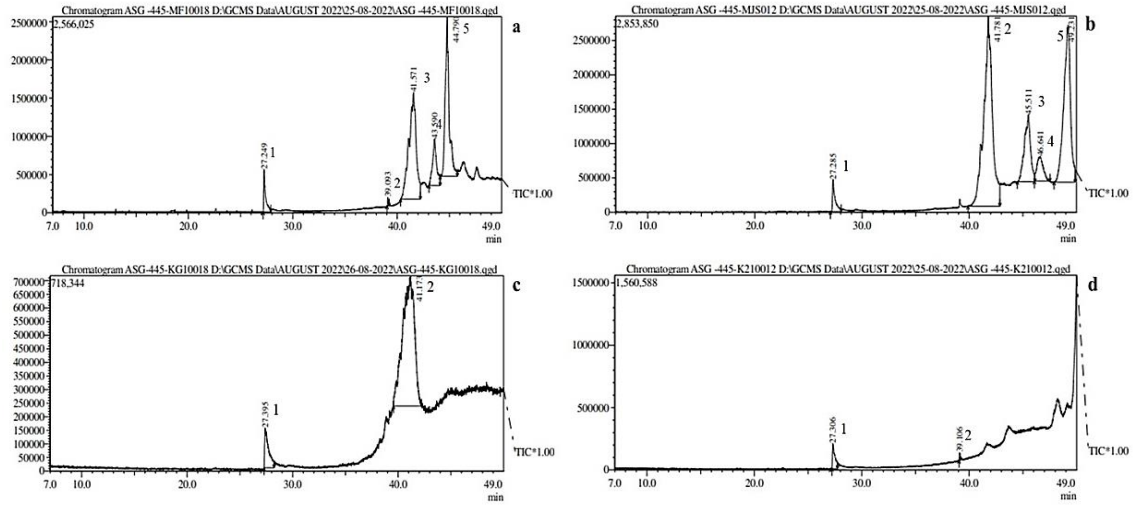

Figure 1: GC-MS chromatogram of various suspension extracts of *G. glauca*
a. MJ-F b. MJ c. KCZ-G d. KCZ
MJ-F: Methyl jasmonate-fructose; MJ: Methyl jasmonate, KCZ-G: Ketoconazole-glucose; KCZ-Ketoconazole

CONCLUSION

In the present study, phytochemical analysis was carried out by conventional methods like qualitative analysis and modern methods like GC-MS analysis. Cell suspension culture is an alternative method for the production or product enhancement of phytochemicals, especially in the case of endangered plants like *G. glauca*, to avoid destructive harvesting of the plant from its wild. In the present study, two elicitors, methyl jasmonate and ketoconazole, were supplemented in the medium either alone or in combination with sugar-precursors fructose with methyl jasmonate and glucose with ketoconazole. Of the four tested cell suspension extracts, MJ and MJ-F treated cultures extracts showed the presence of a higher number of compounds compared to KCZ or KCZ-G treated cultures. Of the ten phytochemicals identified by GC-MS, the majority of these compounds have been reported to possess various biological properties. The presence of these compounds in *G. glauca* might be responsible for the anticancer, antimicrobial and anti-inflammatory properties of *G. glauca.*

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Conflict of Interest:
The authors declare no conflict of interest.

Author Contribution:
Dr. Vadlapudi Kumar conceived the idea and planned the work along with Dr. Poornima D.V. and Dr. Anuradha C.M. The research work was carried out, and the manuscript was drafted by Vishala E. along with Ruksana F. and supported by Prathap H.M., Pratap G.K., Savitharani M., Manjunatha T. for execution and interpretation of results. The manuscript was corrected by Dr. Vadlapudi Kumar.

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