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Antioxidant and antimicrobial potential of *Canavalia gladiata* (Jacq.) DC. leaves and seeds: GC-MS based metabolic profiling

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Canavalia gladiata (Sword Bean; family Fabaceae) is a fast-growing climber crop that has been widely cultivated in tropical and subtropical areas for its edible seeds and legumes. It has also been used as grain legume and medicinal plant in China for thousands of years. The metabolites from leaves and seeds of *C. gladiata* were extracted in petroleum ether and chloroform. Further, extracts were subjected to GC-MS analysis for metabolite profiling. Total of 31 phytoconstituents were identified; 14 compounds were reported to have biological activities. The Tridecane reported from leaves and seeds petroleum ether extract showed 10.67 and 2.72% of relative quantity of compound respectively and has antioxidant and antimicrobial activity. Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,11,15,15-hexadecamethyl (3.42%) found in petroleum ether leaves extracts showed antimicrobial activity. Moreover, 1- Hexadecane constituted 10.23% in seeds and 3.71% relative quantity in leaves extracted with chloroform showed antibacterial activity. The seeds and leaves extracted with chloroform reported 1-Tetradecene in 6.59 and 6.03% relative quantity respectively has antifungal potential. While Phenol 2, 4-bis [1, 1-dimethylethyl] reported in leaves (6.12%) and seeds (6.47%) chloroform extract act as antioxidant and antimicrobial agents. The 1-Nonadecene in seeds (3.47%) and leaves (2.14%) chloroform extracts showed antifungal activity while 1-Docosene in seeds (2.98%) and leaves (1.71%) of chloroform extract showed antimicrobial potential. Nonadecane constituted 3.06% in seeds and 2.78% in leaves chloroform extracts and has antioxidant potential. The present study revealed that the leaves and seeds of *C. gladiata* are a good source of antioxidants and other biologically active ingredients. However, petroleum ether and chloroform extracted different metabolites from seeds and leaves. Therefore, these findings highlighted the importance of solvent in phytochemical extractions and further bioactivity of *C. gladiata* metabolites creates an attention of researchers for their use in the field of paramedical industries and herbal medicine.

Keywords: Antimicrobial activity, Antioxidant, Bioactive compounds, *Canavalia gladiata*, GC-MS analysis.
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Introduction

From ancient time, the villagers have been using plants to cure various diseases due to promising source of phytoconstituents and therapeutic properties of the plants[1-2]. The plants derived medicinal compounds are the major source for pharmaceutical industries to develop new drugs and medicines for curing challenging health issues and diseases[3]. The antioxidants of the plants have great potential to detoxify the effect of oxidants or free radicals; most of the human diseases are arising due to less level of natural antioxidants in the body[4-5]. The isolation and identification of plant-derived bioactive compounds is a present need to fight against various environmental stresses[6]. The natural antioxidant gain popularity over synthetic antioxidants in food and pharmaceutical industries due to their effective nature. The edible and non-edible plants are rich sources of polyphenolic compounds and they have been reported with a variety of biological effects including antioxidant activity[8-9].

Canavalia gladiata (Jacq.) DC. (family Fabaceae) is widely cultivated in tropical regions like Africa, South and Southeast Asia (Sri Lanka, India, Indonesia, China, Korea and Japan). It is called 'sword bean' since its fruits have a shape similar to a straw cutter[10]. The *C. gladiata* is considered as being an underutilized legume although it has a good nutraceutical and medicinal potential as well as good agronomical status for cultivation[11]. It is commonly grown in Asia but has now spread to the tropical region of West Indies, Africa, South America, and Australia as a supplement of carbohydrates, proteins, vitamins, and minerals[11-12]. It has been used to treat nausea, vomiting, back pain, abdominal discomfort,

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hiccups and asthma in Korea[12]. The metabolites such as urease, hemagglutinin, canavanine, and *Canavalia* gibberellin I and II are reported from plant and among these, canavanine and its isoform have known effects on T cell proliferation and immune enhancement[13-14]. It also has anti-allergic, anti-inflammatory, antibacterial, anticancer, antioxidant, hepato-protective, and immunomodulatory activity[12-15]. Gan *et al.*, has reported that *C. Gladiate* contains the highest amount of antioxidant polyphenols among 42 edible beans utilized in China, and its seed coats are rich in gallotannins (gallic acids), which is a distinctive attribute among legume polyphenols[16]. Usually, different solvents are used for extracting metabolites from various parts of the plants. Besides, extraction and yield of phytochemicals mainly depends on the type of solvents and the method of extraction used[17]. There is a research gap on *C. Gladiate* on investigation of secondary metabolites from different plant parts with variable use of solvents for extraction. Therefore, this study also focussed on solvent dependent plant metabolites extraction and plant part dependent variability in isolated metabolites.

The literature survey exhibited meagre information on metabolites of seed and leaves of *C. gladiata.* Therefore, the present investigation focused on the identification of metabolite from leaves and seeds of *C. gladiata.*

Materials and Methods

Collection and identification of plant material

The mature fresh leaves and green pods of *C. gladiata* (Fig. 1) were collected in the month of December 2016 from Sant Gadge Baba Amravati University Campus, Amravati (India). It was identified and authenticated from the Department of Botany, Sant Gadge Baba Amravati University, Amravati with the help of standard floras; the flora of British India, Flora of Amravati District[18], (Specimen voucher no. SGBAU/BOT/107/2016).

Sample preparation

The seeds were removed from mature green pods and immature, diseased seeds were sorted out. The fresh and clean seeds were crushed into liquid nitrogen till a fine powder was obtained. The mature disease-free leaves were separated and cleaned under running tap water followed by distilled water. It was blot dried using tissue paper. These leaves were crushed into liquid nitrogen. The resultant fine powder was soaked into 180 mL of petroleum ether and chloroform (Himedia, India). The mixture was

Fig. 1 — *Canavalia gladiata*, a) habitat, b) flowering twig, c) pods, and d) seeds

shaken on rotary shaker for 24 hours followed by sonication for 25 minutes (CD-4820 Ultrasonic cleaner). To the mixture, 2 g of sodium sulphate was added and the mixture was then filtered using Whatman filter paper no. 1 pre-wetted with respective solvent. The filtrates were further filter through 0.2 µm syringe micro-filters with PTFE membrane, Sigma Aldrich and stored in small sterile airtight bottles at 4 °C temperature for further experiment[19].

GC-MS analysis

The seed and leaves extracts derived from petroleum ether and chloroform were analysed at the Department of Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT) Bombay, Maharashtra (India). Exactly 2 µL of previously concentrated extract was employed for GC-MS (Model AccuTOFGCV; Specifications: EI/ CI Source: Time of Flight Analyser: Mass range -10 - 2000 amu: Mass resolution - 6000) analysis. The analysis was carried out using an Agilent 7880 with 30 mm column of 30 mm length 0.25 mm I.D., 0.32 µ film thickness. The helium gas was used as the carrier gas at constant flow rate of 1 mL/min. Injector temperature was set at 100 °C. The oven temperature was programmed from 50 to 280 °C at 10 °C/min up to 200 °C, and then at 10 °C /3 min up to 250 °C ending with a 5-minute isothermal at 280 °C. The sample was injected in split mode as 50: 1. The MS was taken at 70 eV[19].

Identification of compounds

The chromatogram, retention times, fragmentation patterns along with m/z value base peak, mass peak, and peak intensities were obtained through GC-MS analysis. The identification of compounds was based on retention time, fragmentation patterns along with the m/z values. The mass spectra of the unknown compound obtained from sample extract by GC-MS were matched with mass spectra of the known compounds stored in the database of the National Institute Standard and Technology (NIST) library. Their structures were defined by the per cent similarity values. The name, molecular weight, molecular formula, and structure of the compounds were identified. The biological activities of compounds were determined by comparing with Dr. Duke's Phytochemicals and Ethno-botanical database[20].

For quantitative analysis, the relative quantity of each compound was calculated by formula as follows[20].

$$\text{Relative quantity of compounds} = \frac{\text{Chromatographic peak area of each compound}}{\text{Total peak area of all chromatographic peaks}} \times 100$$

Results and Discussion

The assessment of bioactive potential of medicinal plants has become a prime importance due to continuous increase in health challenges in the world. The plants have an ability to withstand the changing environmental conditions and fighting against variable environmental stresses. As reported by Gan *et al.* the 'Red sword bean' coats are an excellent natural source of gallotannins and their gallotannin-rich extracts can be utilized as a natural antioxidant and antibacterial agents with potential health benefits as well as application in food industry[16]. The present study was conducted to evaluate the metabolites bioactive properties of *C. gladiata* leaves and seeds extracted with petroleum ether and chloroform by GC-MS analysis (Fig. 2-5). Total of 31 phytoconstituents were extracted from the samples

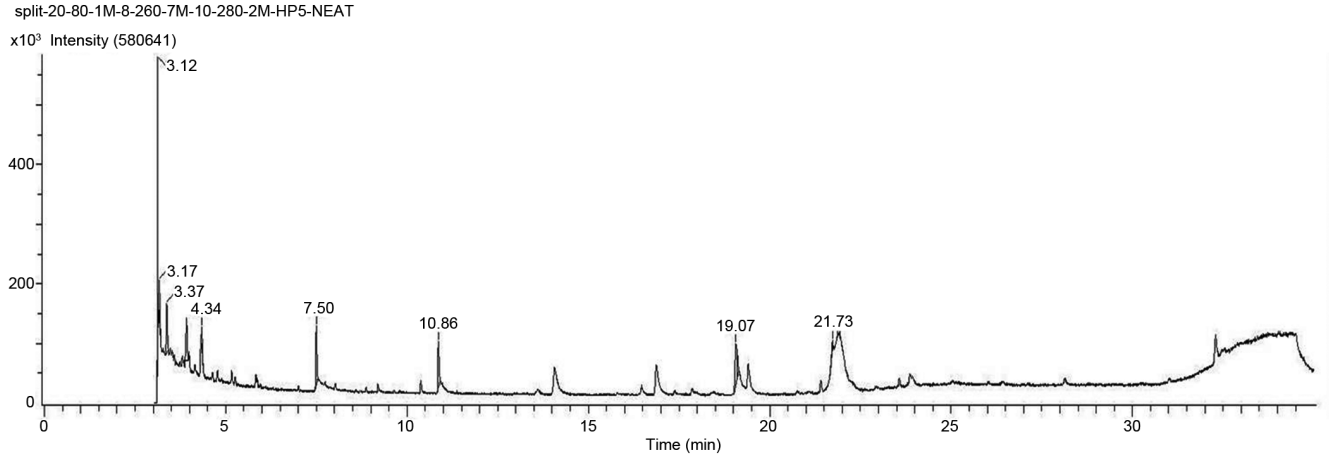

Fig. 2 — Chromatogram obtained from petroleum ether extract of *Canavalia gladiata* leaves by GCMS

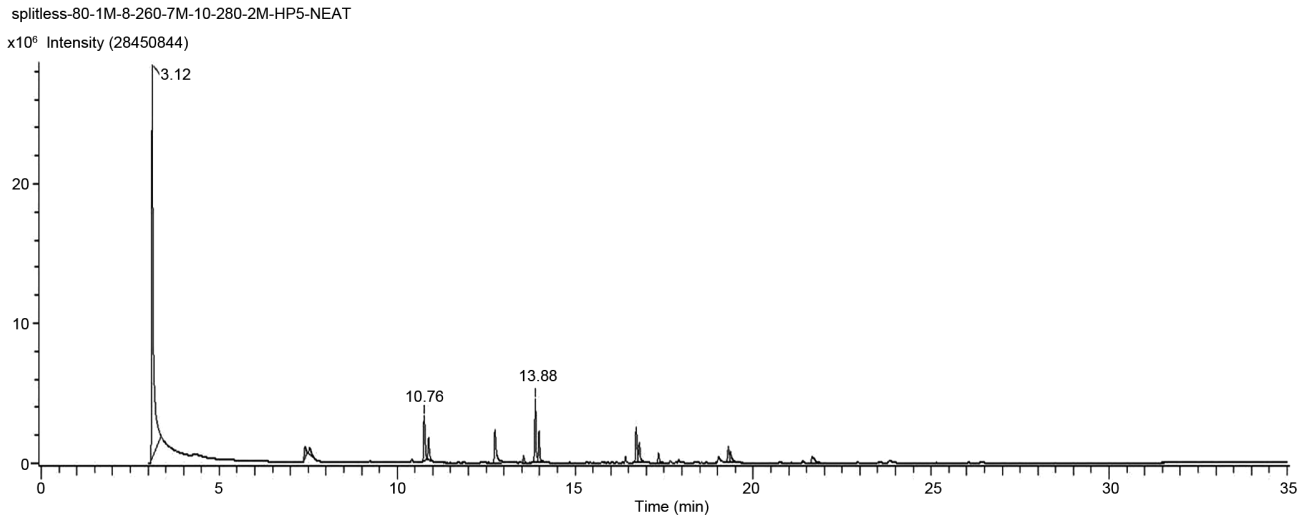

Fig. 3 — Chromatogram obtained from chloroform extract of *Canavalia gladiata* leaves by GCMS

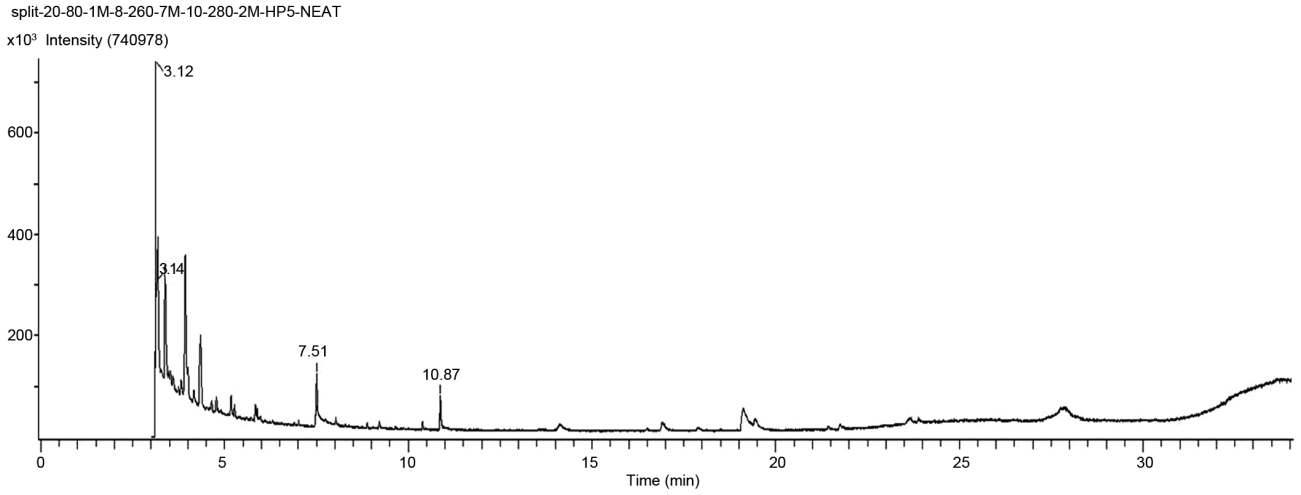

Fig. 4 — Chromatogram obtained from petroleum ether extract of *Canavalia gladiata* seeds by GCMS

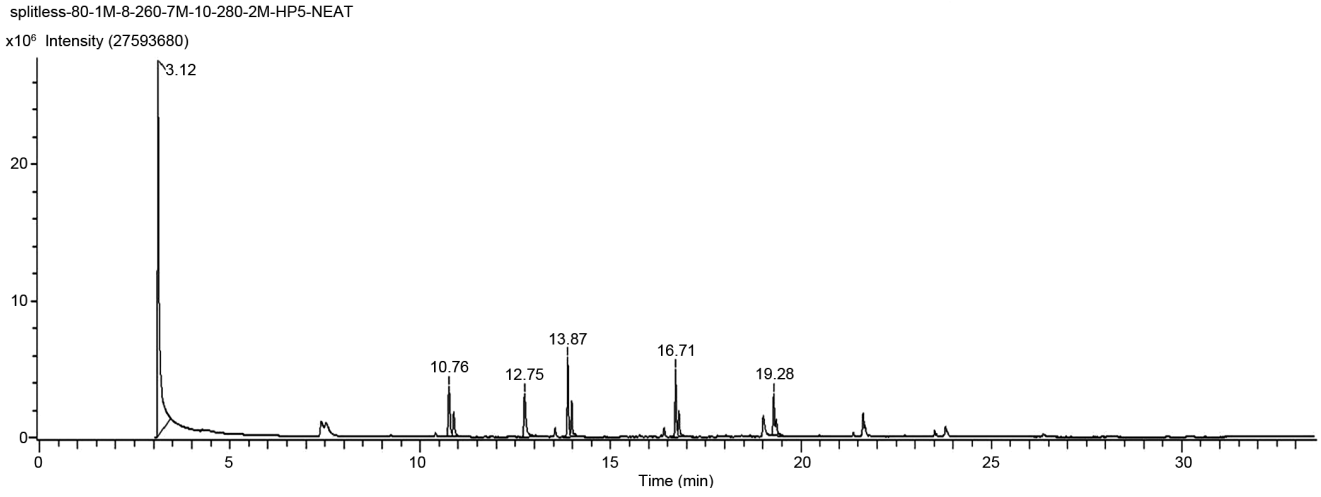

Fig. 5 — Chromatogram obtained from chloroform extract of *Canavalia gladiata* seeds by GCMS

under study (Table 1-4). About 14 different compounds were identified with bioactive potential showing antioxidant and antimicrobial properties. Tridecane was extracted from leaves petroleum ether found to be 10.67%, whereas, the seeds extract showing 2.72% having antioxidant and antimicrobial activity[21]. Moreover, 1- Hexadecane constituted 10.23% in seeds and 3.71% in leaves extracted with chloroform, has been reported to have antibacterial activity[22]. 'Jack bean' (*Canavalia ensiformis* (L.) D.C.) is a potential source of flavonoids, especially kaempferol as earlier demonstrated by Babushok *et al.*[23]. Another phytoconstituent namely, 1-Tetradecene observed in the seeds (6.59%) and leaves (6.03%) extracted with chloroform has been shown to possess anti-tuberculosis and antifungal potential[24]. Phenol 2, 4-bis [1, 1-dimethylethyl] present in the leaves (6.12%) and seeds (6.47%) extracted with chloroform has also

Table 1 — Bioactive compounds identified in petroleum ether leaves extracts of *Canavalia gladiata* (Jacq.) DC.

S. No.	RT	Phytochemicals	Rel %	Class	MF	MW	Activity
1	10.86	Tridecane	10.67	Alkane	$C_{14}H_{30}$	198	Antioxidant, Antimicrobial[21]
2	16.85	Hexadecane	5.17	Alkane	$C_{16}H_{34}$	226	Antioxidant, antimicrobial[22]
3	21.63	Silane,dimethyl [docosyloxy]butoxy	3.21	Silicon ether	$C_{28}H_{60}O_2Si$	456	Antimicrobial[31]
4	32.47	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,1 1,15,15-hexadecamethyl	3.42	Silicon ether	$C_{20}H_{48}O_7Si_8$	577	Antimicrobial[29]
5	3.17	1,6-Anhydro-3,4-dideoxy-β-D-manno-hexapyranose	8.43	Sugar	$C_6H_{10}O_3$	130	Not reported
6	3.37	4-Heptanol	11.79	Alcohol	$C_6H_{16}O$	116	Not reported
7	3.92	Benzene (1,3,3-Trimethylnonyl)	8.66	Aromatic Hydro-carbon	$C_{18}H_{30}$	246	Not reported
8	4.34	Benzene 1,3,5 Trimethyl	4.17	Aromatic Hydro-carbon	C_9H_{12}	120	Not reported
9	7.50	3-Hexanone,2,4-dimethyl	11.91	Ketone			Not reported

RT=Retention time, Rel%=Relative percent, MF=Molecular formula, MW=Molecular weight

Table 2 — Bioactive compounds identified in chloroform leaves extracts of *Canavalia gladiata* (Jacq.) DC.

S. No.	RT	Phytochemicals	Rel. %	Class	MF	MW	Activity
1	7.41	1-Dodecene	1.65	Alkene	$C_{12}H_{24}$	168	Antioxidant, antimicrobial[32]
2	7.54	Oxalic acid,4-chlorophenyl tetradecyl ester	1.63	Carbo-xylic acid	$C_8H_7ClO_2$	170	Not reported
3	10.76	1-Tetradecene	6.03	Alkene	$C_{14}H_{28}$	196	Antituberculosis Antifungal[24]
4	12.75	Phenol,2,4-bis[1,1-dimethylethyl]	6.12	Phenol	$C_{14}H_{22}O$	206	Antioxidant, antimicrobial[22,25]
5	13.88	1-Hexadecene	3.71	Alkene	$C_{16}H_{32}$	224	Antibacterial[22]
6	13.98	Nonadecane	2.78	Alkane	$C_{19}H_{14}$	268	Antioxidant[28]
7	19.30	1-Nonadecene	2.14	Alkene	$C_{19}H_{38}$	266. 50	Anticancer, antifungal[26,33]
8	21.66	1-Docosene	1.71	Alkane	$C_{22}H_{44}$	308	Antimicrobial, Anticarcinoma[21,22]

RT=Retention time, Rel%=Relative percent, MF=Molecular formula, MW=Molecular weight

Table 3 — Bioactive compounds identified in petroleum ether seed extracts of *Canavalia gladiata* (Jacq.) DC.

S. No.	R.T.	Phytochemicals	Rel. %	Class	MF	MW	Activity
1	3.14	Bicyclo [2,1,1,]hexane-2-ol,2-ethenyl	18.02	Terpene	$C_8H_{12}O$	124	Not reported
2	3.38	4-Heptanol,3-methyl	16.54	Alcohol	$C_8H_{18}O$	130	Not reported
3	4.34	Benzene1,2,3-trimethyl	9.19	Aromatic Hydro-Carbon	C_9H_{112}	120	Not reported
4	5.83	Heptane,2,6-dimethyl	3.23	Alkane	C_9H_{20}	128	Not reported
5	7.51	3-Hexanone, 2,4-dimethyl	6.93	Ketone	$C_8H_{16}O$	128	Not reported
6	10.87	Tridecane	2.72	Alkane	$C_{14}H_{30}$	198	Antioxidant, Antimicrobial[21]

RT=Retention time, Rel%=Relative percent, MF=Molecular formula, MW=Molecular weight

Table 4 — Bioactive compounds identified in chloroform seed extracts of *Canavalia gladiata* (Jacq.) DC.

S. No.	R.T.	Phytochemicals	Rel. %	Class	MF	MW	Activity
1	7.41	1-Dodecene	1.62	Alkene	$C_{12}H_{24}$	168	Antioxidant, antimicrobial[32]
2	7.53	Oxalic acid,4-chlorophenyl tetradecyl ester	2.93	Ester	$C_8H_7ClO_2$	170	Not reported
3	10.76	1-Tetradecene	6.59	Alkene	$C_{14}H_{28}$	196	Anti-tuberculosis, antifungal[24]
4	10.88	Tetradecane	3.30	Alkane	$C_{14}H_{30}$	198	Antioxidant, preservative[34]
5	12.76	Phenol 2,4-bis[1,1-dimethylethyl]	6.47	Phenol	$C_{14}H_{22}O$	206	Antimicrobial, antioxidant[22,25]
6	13.88	1-Hexadecene	10.23	Alkene	$C_{16}H_{32}$	224	Antibacterial[22]
7	13.98	Nonadecane	3.47	Alkane	$C_{19}H_{14}$	268	Antioxidant[28]
8	16.40	Heptadecane,3-methyl	0.73	Alkane	$C_{18}H_{38}$	254	Antifungal[33]
9	19.28	1-Nonadecene	3.06	Alkene	$C_{19}H_{38}$	266	Anticancer, antifungal[26]
10	21.63	1-Docosene	2.98	Alkene	$C_{22}H_{44}$	308	Antimicrobial, Anticarcinoma[21]

RT=Retention time, Rel%=Relative percent, MF=Molecular formula, MW=Molecular weight

been shown to have antioxidant and antimicrobial properties[22,25]. 1-Nonadecene found to be 3.47% in the seeds and 2.14% in the leaves chloroform extracts has shown to possess anticancer and antifungal activities[26]. The antitumor potential of *C. gladiata* has been evaluated against Daltons Lymphoma Ascites (DLA) in *in-vitro* and *in-vivo* studies[27]. 1-Docosene was observed as 2.98% in seeds and 1.71% in leaves of chloroform extracts are reported to have antimicrobial and anticarcinogenic potential[21]. Nonadecane constituted 3.06% in seeds 2.78% in leaves chloroform extracts with antioxidant potential[28]. Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,11, 15,15-hexadecamethyl found to be 3.42% in petroleum ether leaves extracts possesses antimicrobial activity[29]. The nematotoxic compounds were reported in *Canavalia ensiformis* seeds which are observed to be effective in the control of the root knot nematode *Meloidogyne incognita*[30]. *C. gladiata* has shown antioxidant, tyrosinase inhibition and antibacterial activities[10]. *C. gladiata* extracts may be useful for the development of skin whitening and anti-bacterial agents as reported earlier by Kim *et al.*[10]. The Tridecane, Hexadecane, Silane, dimethyl [docosyloxy]butoxy, Octasiloxane,1,1,3,3,5,5,7,7,9,9, 11,11,11,15,15-hexadecamethyl, 1-Dodecene, 1-Tetradecene, Phenol,2,4-bis[1,1-dimethylethyl], 1-Hexadecene, Nonadecane, 1-Nonadecene, 1-Docosene, Tridecane, Tetradecane, Heptadecane, 3-methyl compounds first time reported in *C. gladiata* has biological activities. The petroleum ether seeds and leaves extract reported Benzene1,2,3-trimethyl, 3-Hexanone, 2,4-dimethyl and Tridecane a similar compound while chloroform seed and leaves extract also showed similar metabolite profiling except Heptadecane, 3-methyl and Tetradecane in seed extract. Therefore, petroleum ether and chloroform showed variation in metabolite extraction.

Conclusion

The present study revealed that the leaves and seeds of *C. gladiata* is a good source of biologically active ingredients. However, petroleum ether and chloroform extracted different metabolites from the seeds and leaves. Therefore, these findings highlighted the importance of solvent in phytochemical extractions and the bioactive potential of *C. gladiata* metabolites highlights their importance in pharmaceutical application. Further study can be extended to screen metabolites and other biological effects. Moreover, these tested plant extracts could be checked for a better understanding of their safety and efficacy. The existence of different bioactive metabolites justifies the application of whole plant as an antimicrobial and antioxidant agent.

Conflict of interest

All authors declared there is no conflict of interest.

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