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GC-MS Analysis of Bio-active Compounds extracted from various extracts of the Bark of *Dolichandrone falcata plant.*

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A B S T R A C T.

Dolichandrone falcata is being used as popular Ayurveda and siddha medicine in treatment of many diseases. The objective of this investigation was to isolate and identify bio-active compounds from the bark of this plant. On investigation, the extracts of Dolichandrone falcata revealed the presence of phenols, tannins, flavonoids, alkaloids, saponins, glycosides, terpenoids and steroids. The GC-MS analysis disclosed presence of 83 compounds in methanol extract, 38 compounds in chloroform extract and 2 compounds in petroleum ether extract.

Key words- *Dolichandrone falcata*, GC-MS analysis, bioactive compounds.

INTRODUCTION

Medicinal plants are known to be the main source of drug therapy in traditional medicine. It is an alternative to western medicine and is strongly linked to religious beliefs and practices of Indigenous cultures. India is blessed with thousands of Ayurvedic and household formulations to treat various disorders, including anxiety, depression, arthritis, high blood pressure, hormonal imbalances, insomnia, migraine, skin problems, and other disorders [1]. The medicinal property of a plant depends upon the physiologically active biochemical compounds called secondary metabolites. Plants have an almost limitless ability to synthesize secondary metabolites which are present in the plant's parts like leaves, fruits, buds, stems, flowers, bark, roots, etc[2].

Dolichandrone falcata commonly known as "Medhshing" in Hindi, "Mesasninghi" in Sanskrit (meaning like a sheep horn) it is a medium size tree with dense foliage and profuse flowering, belonging to the family Bignoniaceae. Highly scented, creamish white flowers bloom in the evening and fall in the morning. Ripe fruits of previous year remain on the tree when the flowers of next year are in bloom. It is a rare plant found in the rocky areas of forest. The bark, leaves and fruits have medicinal properties [3].

The whole plant and its specific parts like leaves, stem and roots are known to have medicinal properties. It has long history of use by Indigenous and tribal people of world and India. 1

The decoction of bark is given in the treatment of nodules by the Bhil tribes of the Kota district in Rajasthan. Leaves paste is mixed with neem leaves and applied to curb swelling [4]. Fruit paste and bark paste are applied with water three times to get relief from scorpion bite and snake bite. Leaves juice taken orally with water acts as an antidote to snake bite by tribals of Kota region. Patil et al (2021) have reported that this plant has anti- snake venom activity, which is also used in the treatment of liver disorder.

Plants contain different phytochemicals, also known as secondary metabolites. Phytochemicals are useful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health. Phytochemicals are vital in pharmaceutical industry for development of new drugs and preparation of therapeutic agents [5]. The development of new drugs starts with identification of active principles from the natural sources.

The screening of plant extracts is a new approach to find therapeutically active compounds in various plant species. Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhoea, anti-ulcer, and anticancer activities, among others [6].

Collection of plant material

Dolichandrone falcata plant was collected from the Gulbarga university campus. Karnataka India, the taxonomic identity was confirmed in Department of Botany, Gulbarga university. Kalaburgi (Vocher specimen no GCAK-117).

Crude extract preparation

The bark of *Dolichandrone falcata* was cleaned and shade dried at room temperature and finely powdered in the domestic mixer. 30gm of coarsely powdered sample was subjected to Soxhlet extraction with different solvents like methanol, chloroform, and petroleum ether. The solvents were evaporated to obtain semisolid masses, which were further subjected to GC-MS analysis.

Preliminary phytochemical screening

Phytochemical profiling of methanol, chloroform, and pet ether extracts of *Dolichandrone falcata* plant bark were carried out using the procedures as described by Harborne, Trease, and Evans, Harborne, Soni and Sosa (11,12).

GC-MS analysis

Gas chromatography mass spectrometer (GCMS) analysis of methanol, chloroform and pet ether extracts obtained from the bark of *Dolichandrone falcata* was performed using the *thermo GC-trace* ultra version- 5.0. Experimental conditions of GC-MS system were as follows- DB35- MS capillary standard non-polar column with dimensions of column 30 mm x 0.25 mm x 0.25 mm film. The GCMS instrument used for this study was fully automatic and computer interfaced. The Helium gas at rate of 1.0 mil min was used as carrier gas in the

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equipment. The injector was operated at an elevated temperature of 250°c and the oven temperature was programmed at 60°c for 15 min, then gradually increased in suitable steps up to 280°c at 3min.

Identification of compounds

In the last few years GC-MS has become established as a key technological platform for profiling of secondary metabolites. It is the best sensitive technique used for separation and identification of many structurally complex components that are present in plant extracts [7]. There is a scanty literature on GC-MS analysis of *Dolichandrone falcata.* The aim of present investigation was to investigate and identify the bioactive compounds extracted from bark of *Dolichandrone falcata* with different solvents like methanol, chloroform, and pet ether.

Results

Phytochemical screening of methanol, chloroform, and pet ether extract of *Dolichandrone falcata* bark revealed the presence of phenols, tannins, flavonoids, alkaloids, saponins, Glycosides, terpenoids, steroids. As shown in table

Table 1. **Phytochemical constituents of methanol, chloroform and pet ether extracts of *Dolichandrone falcata bark***

Phytoconstituents	Methanol extract	Chloroform extract	Pet ether extract
Total phenol	+++	+++	+
Tannins	++	++	++
Flavonoids	+++	++	+
Alkaloids	++	++	+
Saponins	+	+	+
Glycosides	+++	——	——
Terpenoids	++	++	+
Steroids	+++	+++	+

+++ Strongly present ++Present +Weakly present ---Absent

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GC-MS profiling of various extracts of *Dolichandrone falcata* bark

GCMS of *Dolichandrone falcata* bark extract showed presence of various components with different retention times and peak areas when extracted with three different solvents. The large compound got fragmented into small fragments giving rise to sharp peaks at different m/g ratio. These spectra are fingerprints of the compound which can be identified from the data library [8,9].

GCMS of methanolic extract showed presence of 83 peaks indicating the presence of 83 compounds of which 9 peaks referred to major bio-actives , viz ,Naphthalene ,2,6-bis (1,1 dimethyl ethyl) , 9,12, octadeconoic acid

, Phanazine, 1-amino-3-methoxy, 1,3,5 cycloheptatriene 7,7 -dimethyl -2,4-diphenyl, stigmasterol, Gama-sitosterol and Ergosta-4,6,8,22tetra-en-3-one(Table 1,Figure 1).

GCMS of chloroform extract showed the presence of 38 peaks relating to 38 compounds, of which 9 peaks referred to 9 major bio actives, viz, Methylene chloride, Methane oxybis dichloro, Trichloro methane, Toluene, Dibutyl phthfriedelan-alata, Docosenamide, 9-O-pivaloye-N-acetyl colchinol, Friedelan-3-one and Silane-dimethyl-2-hepthyloxy (Table 2, Figure 2).

GCMS of pet ether showed presence of two compounds, Trichloromethane and Dimethylsulfoxide (Table 3, Figure 3).

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Table No-2 Compounds identified in the methanol extract of *Dolichandrone falcata* *bark in GC-MS*

Sl. No.	Peak No	Name of the Compound	Mol Formula	Mol Weight	Retention Time	Peak Area%	Reported Biological Activity
1.	25	Naphthalene,2,6-bis (1,1 dimethyl ethyl)	$C_{18}H_{24}$	240	22.416	5.32%	------------
2.	26	Naphthalene,2,6-bis (1,1 dimethyl ethyl)	$C_{18}H_{24}$	240	22.855	5.74%	--------------
3.	32	9,12, octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	25.175	4.46%	Antiinflammatory activity. A drug used for the treatment or prevention of cardiac arrhythmias
4.	40	phenazine, Ph1-amino-3-methoxy	$C_{13}H_{11}N_3O$	225.25	28.566	12.49%	It has insecticidal, antimicrobial, anti-parasites, and anti-cancer activity
5.	43	1,3,5, cycloheptatriene 7,7-dimethyl-2,4-diphenyl			29.554	3.30%	Used as a triplet quencher for rhodamine 6G dye lasers
6.	70	Stigmasterol	$C_{29}H_{48}O$	412.7	36.865	4.04%	It can inhibit the development of various cancer cells by inhibiting the promotion and growth of apoptosis of cancer cells.
7.	72	Gama-sitosterol	$C_{29}H_{52}O_2$	432.7	37.550	9.97%	It is used for lowering cholesterol level and reduces glucose level.
8.	75	Ergosta-4,6,8,22 tetra-en-3-one	$C_{28}H_{40}O$	392.6	38.310	3.60%	--------------
9.	78	Stigmast-4-en-3-one	$C_{29}H_{48}O$		39.163	3.32%	Antimicrobial and Hypoglycemic activity

Table No-3 Compounds identified in the chloroform extract of *Dolichandrone. falcata bark in GC-MS.*

Sr. No.	Peak No	Name Of the Compound	Mol Formula	Mol Weight	Retention Time	Peak Area	Reported Biological Activity
1	1	Methylene chloride	CH_2Cl_2	84.93	1.418	11.32%	-----------
2	2	Methane oxybis dichloro	$C_2H_2Cl_4O$	183.84	1.598	25.86%	------------
3	3	Trichloromethane	$C_2H_5Cl_3$	135.42	1.705	18.56%	Used for purification of antibiotics, alkaloids, and vitamins
4	6	Toluene	C_7H_8	92.14	2.201	14.07%	Used in veterinary medicine as a treatment for various parasites in dogs and cats
5	18	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	22.683	4.36%	------------
6	29	Docosenamide	$C_{22}H_{43}NO$	337.6	32.190	1.91%	Anti-inflammatory, Renoprotective effects, Androgenic properties and antioxident effect.
7	36	9-O-pivaloye-N-acetyl colchinol	$C_{25}H_{31}NO_6$	441.5	38.385	3.69%	-----------
8	37	Friedelan-3-one	$C_{30}H_{50}O$	426.7	40.227	1.94%	Used to treat pain and Inflammation
9	38	Silane-dimethyl-2-(napthoxy)hepthyloxy	$C_{19}H_{28}O_2Si$	316.5	41.061	4.39%	------------

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Table No-4 Compounds identified in the petroleum ether extract of *Dolichandrone falcata bark in GC-MS.*

Sl. No	Peak No	Name Of the Compound	Mol Formula	Mol Weight	Retention Time	Peak Area	Reported Biological Activity
1	1	Trichloromethane	$C_2H_5Cl_3$	135.42	1.625	0.24%	Used for purification of antibiotics, alkaloids, and vitamins
2	2	Dimethylsulphoxide	C_2H_6OS	78.13	5.033	99.60%	Used as a typical analgesic, anti-inflammatory and an antioxidant activity.

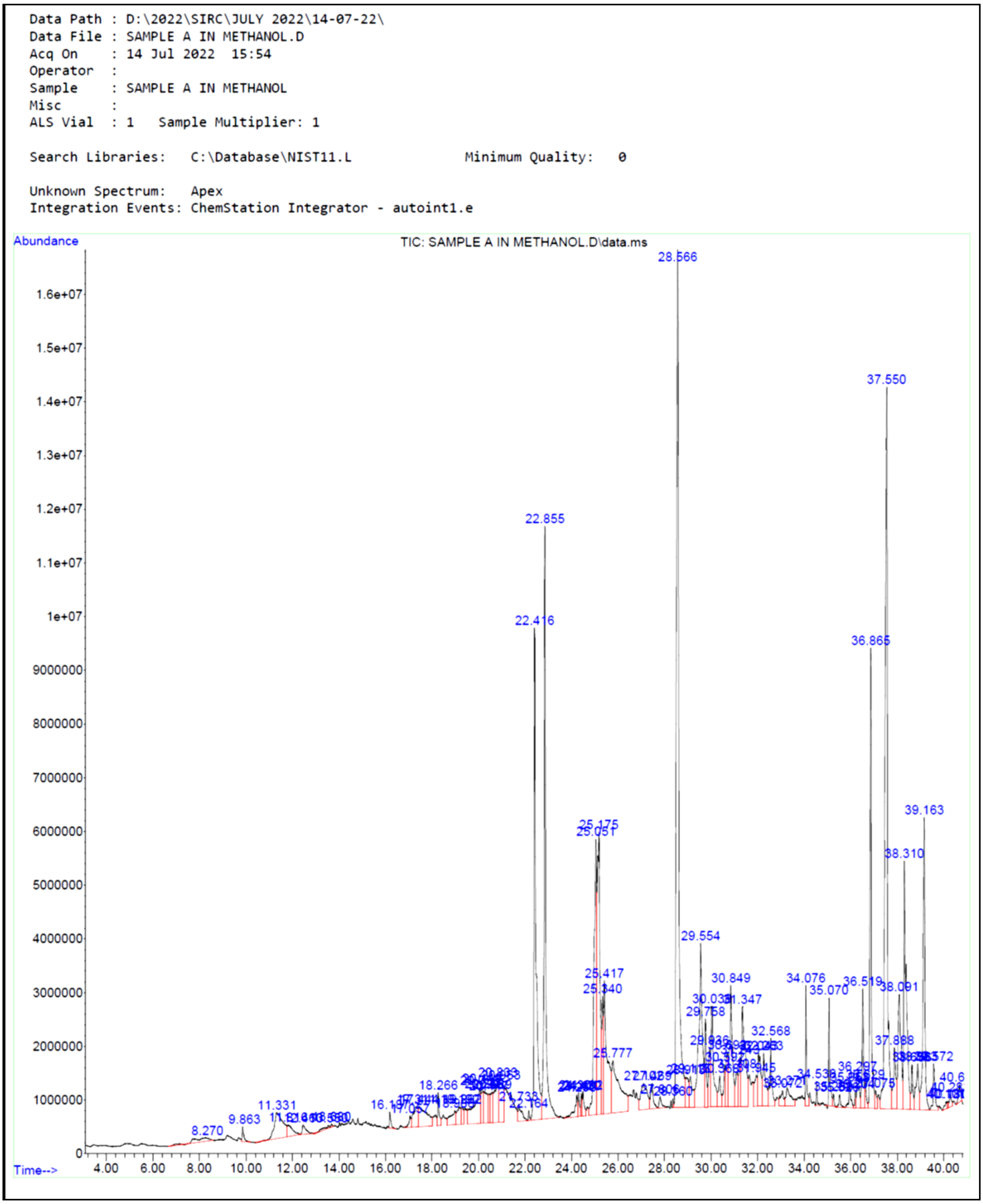

Fig.1 Chromatogram of methanol extract of *Dolichondrone falcata* bark

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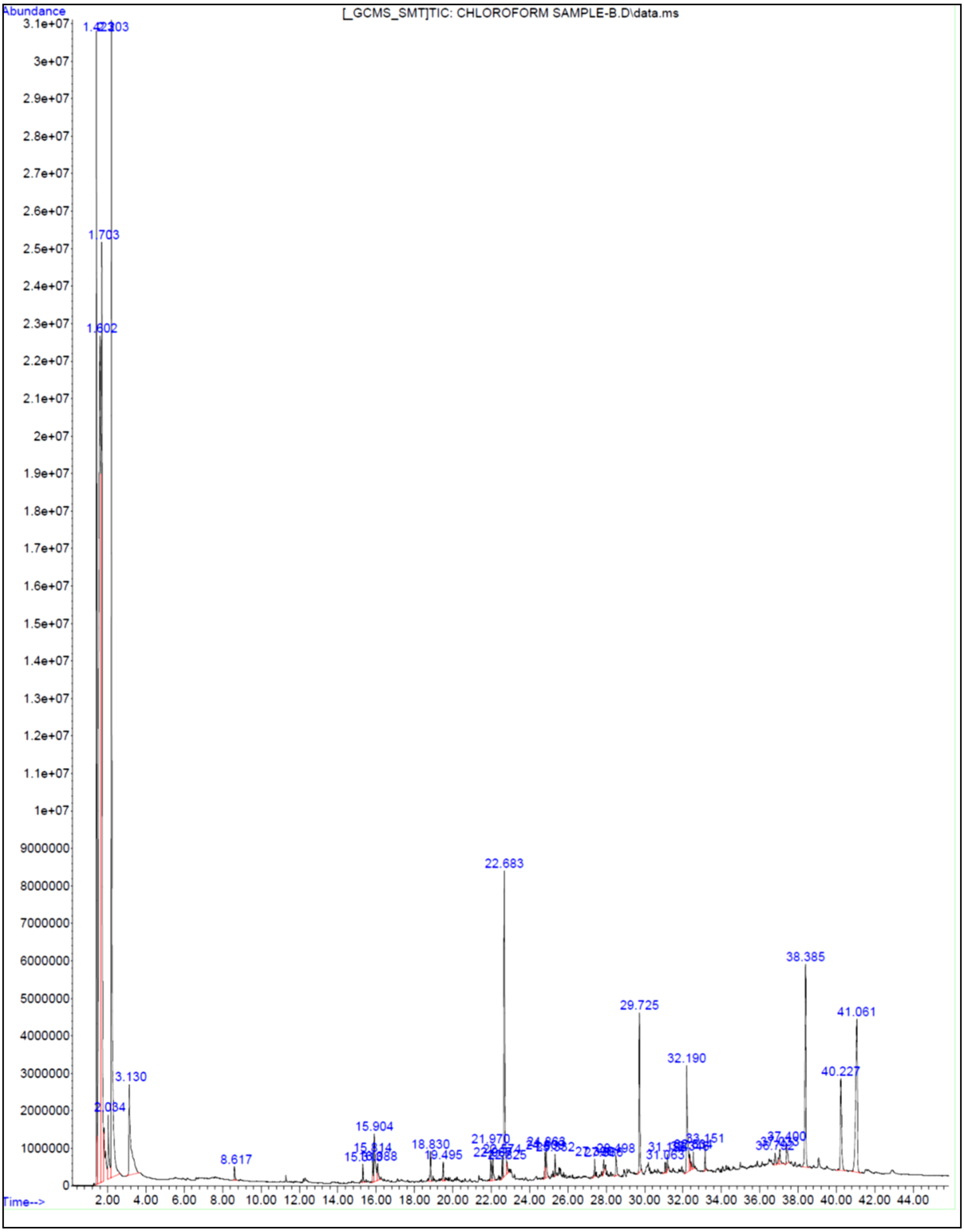

Fig. 2 Chromatogram of chloroform extract of *Dolichondrone falcata* bark

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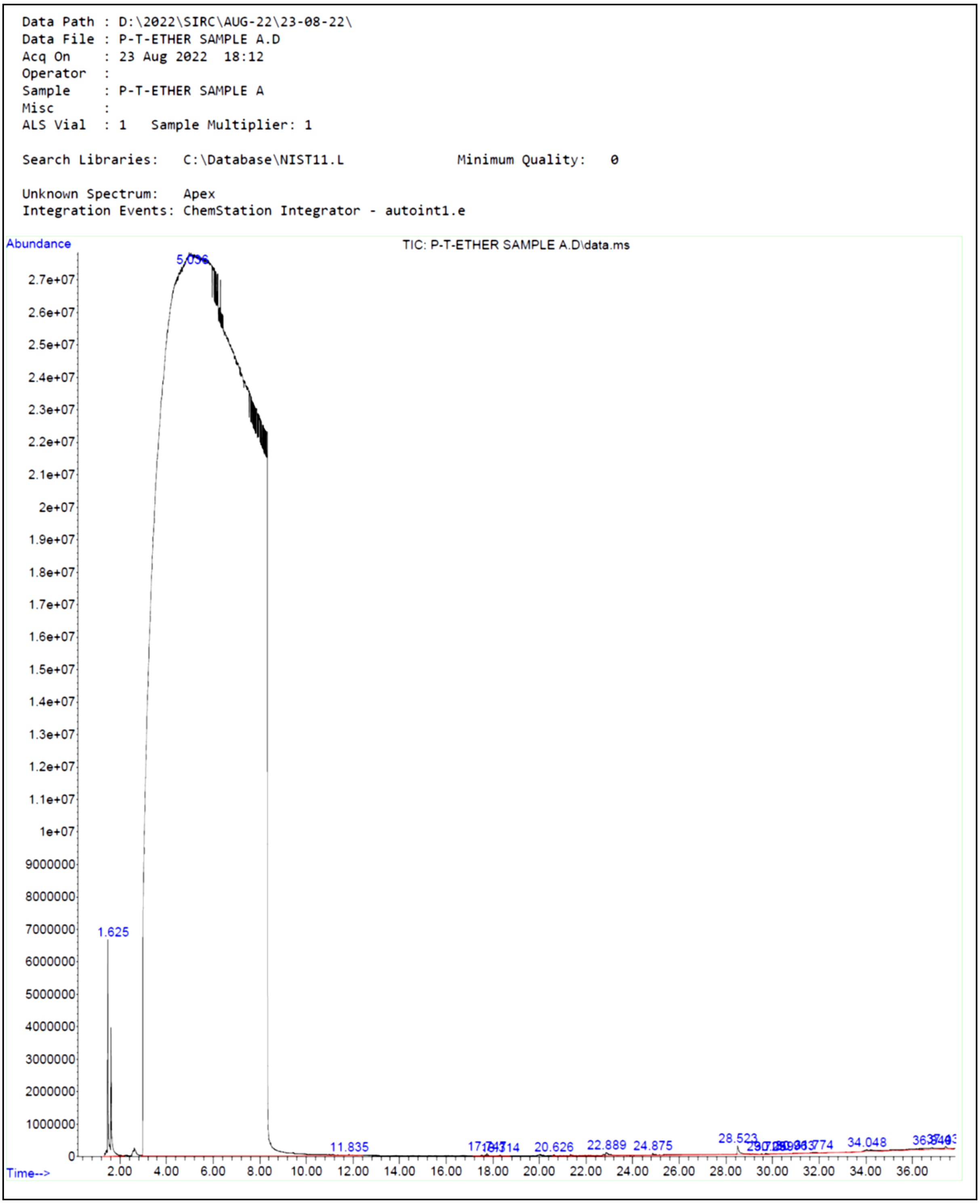

Fig. 3 Chromatogram of Petroleum ether extract *of Dolichondrone falcata* bark

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Phytochemical

screening of methanol chloroform and pet ether extracts of *Dolichandrone falcata* bark revealed the presence of phyto-compounds that have been documented to have antioxidant and other activities. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [10]. Flavonoids have anti-oxidative and mucosal protective effect [11, 12]. Flavonoid-rich vegetables are widely used functional foods since they can be used to treat cardiovascular diseases [13]. They are characterized by their good bioavailability and, hence, constant dietary consumption of flavonoids has been reported to give pharmacologically relevant plasma concentrations in humans [14]. In addition, several studies have reported the possible cardioprotective effects of flavonoids against ischemia reperfusion [15,16]. Saponins may activate mucous membrane protective factors, while tannins reduce the permeability of mucosa to chemical irritation. Consequently, they reduce inflammation, exert astringent and protective action on the stomach mucosa, and curb excess acidity. In addition, terpenoids and alkaloid compounds have also been reported to have potent activity against gastric ulcers. Terpenoids have been reported to relax cardiovascular smooth muscle by inhibition of Ca2+ influx in vascular smooth muscle or via quenching of reactive oxygen species (ROS) and stimulation of nitric oxide (NO) synthesis [17]. The presence of these phytochemicals in methanol, chloroform and pet ether extracts possibly indicates its numerous medicinal properties such as anti-inflammatory, anti-ulcer, and anti-oxidative properties, among others [18].

Dolichandrone falcata is known to be medicinal in traditional practices. In the present study attempts were made to find the chemical constituents of plant bark to know their medicinal importance by using gas chromatography mass spectroscopy approach [19]. The results obtained in the present study authenticate the earlier reports of therapeutic importance of plants. Patil And Biradar (2013) reported that bark of *Dolichandrone falcata* plant play a key role in the treatment of diseases without any side effects, and bark extract exhibit antimicrobial activity.

The compounds obtained showed 70% of metabolites which differed from each other. Phenazine, Ph1-amino-3-methoxy found in methanol extract with 12.49%concentration is known to possess insecticidal, antimicrobial, anti-parasites, and anti-cancer activity [20]. The bark is shown to have an anti-inflammatory effect, the compound responsible for this property is n-octadecadienoic acid (25.17%)[21]. The most important metabolite sterol was found to be present in both methanol and cchloroform extract with its highest concentration in methanolic extractt, for example stigmasterol in methanolic extract, Gamma sitosterol in chloroform extract [22].

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In the present study, among the identified phytochemicals from methanolic extract the compound 9,12, octadecadienoic acid not only possess anti-inflammatory activity but also it plays significant role in prevention of cardiac arrhythmias phenazine, Phl-amino-3-methoxy, has insecticidal, antimicrobial, anti-parasites, and anti- cancer activity. 1,3,5, cycloheptatriene is used as a triplet quencher for rhodamine 6G dye lasers. Stigmasterol can inhibit the development of various cancer cells by inhibiting the promotion and growth of apoptosis of cancer cells. Gama-sitosterol is used for lowering cholesterol level and reduces glucose level. Stigmast-4-en-3-one has antimicrobial and hypoglycaemic activity [23].

Trichloromethane and Toluene were obtained from pet ether extract. Trichloromethane is used for purification of antibiotics, alkaloids, and vitamins. Toluene is used in veterinary medicine as a treatment for various parasites in dogs and cats. Docosenamide has anti-inflammatory. reno protective effects. androgenic properties, and antioxidant effects. Friedelan-3-one is used to treat pain and inflammation [24].

Amongst the two compounds identified in the pet ether extract, Dimethyl sulfoxide is used as a typical analgesic, anti-inflammatory and antioxidant activity. Using Duke's phytochemical and ethnobotanical database (online), the biological activity of identified phytocomponents was ascertained [25].

India is a home to a variety of traditional medicine system that relay to an exceptionally substantial extent on native plant species for new drug materials [26]. The analysis of *Dolichandrone falcata* proves its potential as an alternative medicinal plant in pharmaceutical and drug formulations [27].

CONCLUSION

Dolicandrone falcata has highest importance for its valuable secondary metabolites.

GCMS analysis has shown the presence of different phytochemical compounds in methanol (83), chloroform (38) and pet ether (02) extracts. *Dolichandrone falcata* can be recommended as a store house of phytochemicals which in turn may constitute to some important biological activities.

Isolation of individual photochemical constituents and subjecting them to biological activity will give fruitful results and will open a new area of investigation of individual components and their pharmacological potency [28,29] 12

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Plant authentication.

The plant were identified and authenticated by the Department of Botany, Gulbarga University, Kalaburagi. The plant was deposited in the herbarium of Department of Botany, University of Gulbarga, with the voucher number GCAK-117.

Authors’ contributions

All the authors contributed to the design of the study. All the authors contributed to the phytochemical profiling of the plant fraction. All the authors contributed to the GC-MS evaluation of the plant sample, and in the interpretation of the results. All the authors contributed to preparing the manuscript. All the authors read and approved the manuscript.

Complaints about standard

Declarations

A) Conflict of Interest- The authors declare no financial or personal conflict of interest.

B) Ethical approval- This article does not contain any studies with human participants or animal performed by any of the authors.

C) Informed consent- None

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