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Phytochemical Screening and GC-MS Analysis of Flower Extract of *Dillenia indica*

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ABSTRACT

Dillenia indica is an important medicinal tree. Its leaf and bark extracts are being used as medicine due to the presence of several pharmaceutically important phytochemicals, but presence of phytochemicals in its flowers is still unexplored and there is utmost need to check medicinal efficacy of flower extract. Phytochemical screening of flower extract showed the presence of coumarins, steroids, saponins, flavonoids. GCMS study was done for the identification of bioactive compounds from the methanolic extract of flowers, which showed the presence of various phytochemicals such as Tetrabutyl Titanate, Fucoxanthin, Chromone, Beclomethasone, Betamethasone Acetate, Demecolcine etc. Most of these compounds have many pharmacological activities such as antibacterial, antimicrobial, cancer radiotherapy, cytotoxic activity, antioxidant etc., whereas some chemicals are of industrial importance. Some toxic chemicals were also reported. Present work shows pharmaceutical importance of flowers of *Dillenia indica*.

KEY WORDS: GCMS, PLANT EXTRACT, PHYTOCHEMICAL, SECONDARY METABOLITES.

INTRODUCTION

Dillenia indica is commonly known as Elephant Apple, belongs to family Dilleniacae. It is an ethnomedical plant and is being used for the treatment of severe disease (Shahin et al, 2015). Its stem bark, leaves, fruit and seed extracts show the presence of various active compounds such as polyphenols, tannins, alkaloids, steroids, saponins and flavonoids. Extracts of *Dillenia* have shown antileukemic, antioxidant, anti-inflammatory, antiproliferative, antidiabetic, hepatoprotective, antimicrobial and other pharmaceutically important activities (Gandhi & Mehta, 2013). Due to over exploitation, it is considered as rare plant in Egypt (Khalifa and Loutfy, 2006) and as an endangered plant in China (Qin et al, 2017). It is indigenous to Indonesia. It also occurs in Bhutan, Malaysia, China, Sri Lanka, Myanmar, Nepal, Philippines, Vietnam and Bangladesh. In India, it is distributed in Assam, North Bengal, Bihar, Orissa, Madhya Pradesh and Gujarat. However, no qualitative and quantitative studies were made for the extract of flowers. Present work shows GCMS study of floral extract of *Dillenia indica* for detailed information of phytochemicals.

Gas chromatography-mass spectrometry (GCMS) is widely used in pharmaceutical industries for the identification and quantification of secondary metabolites (Rukshana et al, 2017). It is considered sensitive and suitable method for the analysis of natural organic substances. The analysis provides the details of bioactive compounds

ARTICLE INFORMATION

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present in the methanolic extract and retention time indicates the separation of compounds at different time interval. GCMS analysis of many plants has helped in the identification and characterization of phytochemicals present in their extracts (Socrates & Mohan, 2019; Eswaraiah et al. 2020).

Figure 1 (A): Tree of *D.indica* (B)Flower

MATERIAL AND METHODS

Flowers of *Dillenia indica* were collected from the plant growing in Ayurvedic garden, Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi (Fig.1).

Phytochemical Screening: Shade dried flowers were made into fine powder by mixer grinder. The powder of flower (5 gm) was added to 50 ml of distilled water and was boiled at 60° C for 10 min. Boiled extract was filtered with Whatman filter paper. Filtered solution was boiled till the formation of chocolate colored powder. This powdered plant extract was used for preliminary phytochemical screening. Aqueous plant extract was prepared by dissolving 25 mg of crude extract to 25 ml of double distilled water. Presence or absence of phytochemicals was observed by performing following tests with minor modifications: For the test of Coumarins (Rizk, 1982), aqueous plant extract (2 ml) was added to 3ml of 10% NaOH. The confirmation of coumarins was observed by the change in colour. Formation of yellow colour shows the presence of coumarins. To detect the presence of Saponins, foam test was done (Kumar et al, 2009). In this test, plant extract (2 ml) was mixed in 6 ml of DDW and was shaken vigorously.

Presence of foam is confirmation for saponins. To observe the presence of phenol (Gibbs, 1974) in plant extract, 2 ml of aqueous extract was added to 2 ml 5% of aqueous ferric Chloride (FeCl3). If deep blue or black color is observed, then it indicates the presence of phenol. Presence of Flavonoids was observed by Alkaline Reagent Test and 2 ml plant extract was taken and few drops of 1N NaOH were added. Presence of flavonoids will be observed if it turns to yellow color and becomes colorless after adding dilute HCl. For detection of Tannins (Braymer's Test, Ugochukwu et al, 2013), 2 ml extract was added to 3 ml 10% alcoholic ferric chloride solution. Formation of blue or greenish color will indicate the presence of tannins. Test for Quinones (Ramya et al., 2015) was done by treating 2 ml plant extract with 4 ml of dilute Sodium hydroxide. If blue green or red color is formed, it will show the presence of quinone. To see the presence of Phlobatannins (Precipitate Test, Auwal et al, 2014),

1ml of extract was treated with 2ml of 1% hydrochloric acid. Then mixture was boiled. Observation of red precipitation will confirm the presence of phlobatannins. In the test for Alkaloids (Mayer's Test, Auwal et al, 2014), 2ml of extract was added to 0.5 ml of Mayer's reagent. White creamy precipitate formation is indicative of the presence of alkaloids. Fehling solution test was done to observe the presence of carbohydrate. Mixture of equal volume of Fehling solution A and B was made. Extract (2 ml) was added to 2 ml of Fehling solution and then it was boiled in water bath for 30 minute. Red precipitate will indicate the presence of carbohydrate. For observing the presence of Anthocyanins (Paris and Moyse, 1969), 2 ml extract was treated with 2N Hydrochloric acid. If pink-red color appears, which turns into blue-violet after addition of Ammonia, then it shows the presence of anthocyanins. For detection of fatty acid (Ayoola, 2008), 1 ml of extract was mixed with 3 ml of ether. It was poured on the filter paper and was evaporated till the filter paper becomes dried. Transparent filter paper indicates the presence of fatty acid. For confirmation of Steroids (Salkowski reaction, Shear & Kramer, 1926), 1ml of extract was dissolved in 10 ml chloroform. Concentrated sulfuric acid was added by side of test tube wall, appearance of red and yellow color in the upper and lower layer, respectively along with green florescence indicates the presence of steroids.

Table 1. Phytochemical analysis of *D. indica* flower

S.N.	Phytochemicals	Flower
1.	Tannins	-
2.	Coumarins	+
3.	Flavonoids	+
4.	Steroids	+
5.	Anthocyanin	-
6.	Saponin	+
7.	Quinones	-
8.	Phlobatnins	-
9.	Fatty Acid	-
10.	Phenolics	-
11.	Alkaloids	-
12.	Carbohydrates	-

Preparation of extract and methodology of GCMS: Shade dried flowers were made into fine powder by mixer grinder. Flower powder was incubated with 25ml 95% methanol and kept for 72 hour. Methanolic extract was filtered by using Whatman filter No.1 (pore size 0.4 µm). GCMS analysis of filtered extract was done to get the details of bioactive compounds.Methanolic extract was injected in the port of the Gas chromatography (GC) device. Here the extract got vaporized and spectral peaks of various phytochemicals was recorded on chromatogram. GCMS analysis of methanolic extract of flower of the plant was performed using

a THERMOSCEINTIFIC Gas Chromatography-TRACE ULTRA VER: 1100. and mass spectrometry- TSQ Duo. The oven temperature was maintained at 220°C at a rate of 6°C/min and flow rate of carrier gas was adjusted at 1 ml/min. The column of the GC was TG-5MS. Different parameters of the column were as such- the length of the column: 30 m, the diameter: 0.25nm and the thickness of the film: 0.25µm. The GCMS programming were done as follows: Injector temperature 215°C,

Transfer line 218o C, oven temperature program 80-280°C with ramping 5°C per min, carrier gas: Helium at 1.5 mL/min, individual components were identified by NIST MS 2.0 structural library.The split sampling technique was used to inject the sample in the ratio of 1:10. The time elapsed between elution and injection was recorded as the "retention time".

Figure 2: GCMS analysis and Chromatogram of methanolic extract of D. indica Flower

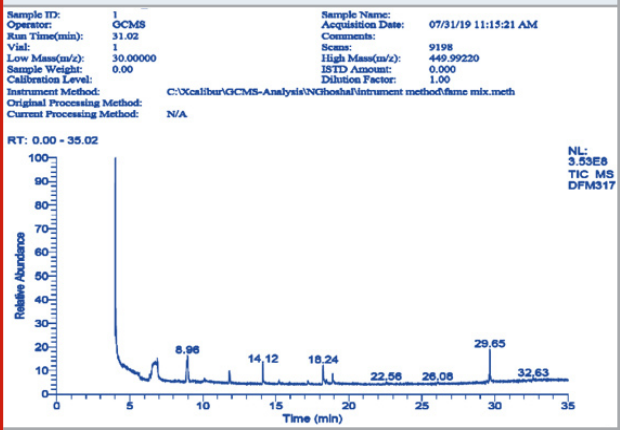
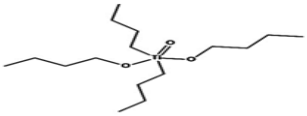
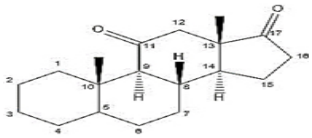
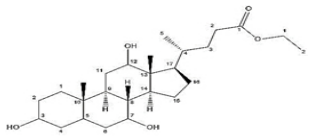
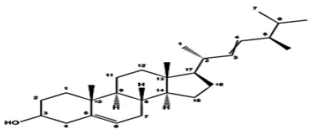
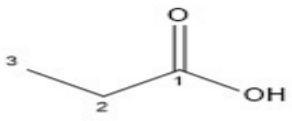
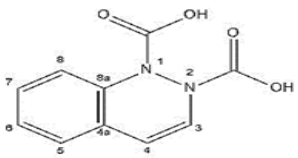
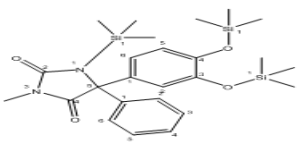
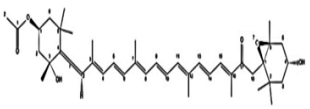
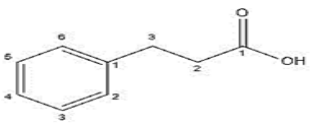


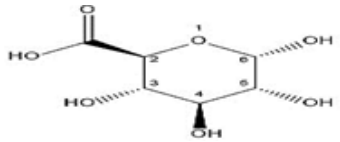
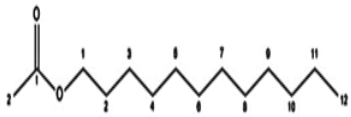
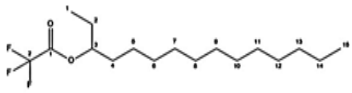
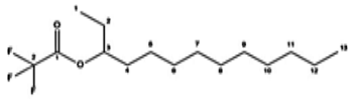
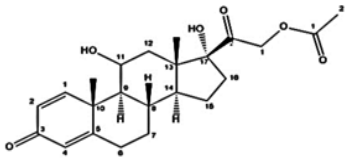
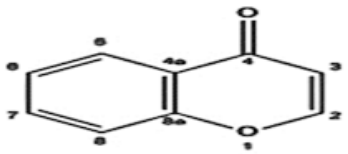
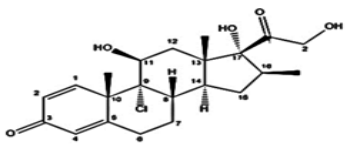
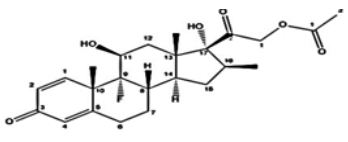
Table.2 Bioactive compounds present in methanolic extract of *D.indica* Flowers

S.N.	COMPOUND	M. W.	M. F.	%AREA	RT
1.	TETRABUTYL TITANATE	344.35	$C_{16}H_{40}O_4Ti$	0.35	4.35
2.	ANDROSTANE-11,17-DIONE	260.5	$C_{19}H_{32}$	0.07	6.21
3.	ETHYL ISO-ALLOCHOLATE	436.6	$C_{26}H_{44}O_5$	1.02	6.36
4.	ERGOSTA-5	394.6	$C_{28}H_{42}O$	1.02	6.36
5.	PROPANOIC ACID	74.08	C3H6O2	0.04	7.22
6.	1,2-CINNOLINEDICARBOXYLIC ACID	173.17	$C_{10}H_7NO_2$	0.04	7.22
7.	2,4-IMIDAZOLIDINEDIONE, 5--[3,4-BIS[(TRIMETHYLSILYL)OXY] PHENYL]-3-METHYL-5-PHENYL-1-(TRIMETHYLSILYL)	516	$C_{25}H_{40}N_2O_4Si_3$	0.05	7.29
8.	FUCOXANTHIN	658.91	$C_{42}H_{58}O_6$	0.09	7.36
9.	BENZENE PROPANOIC ACID	152.19	$C_9H_{12}O_2$	0.13	7.46
10.	α-D-GLUCOPYRANOSIDURONIC ACID	208.17	$C_7H_{12}O_7$	0.13	7.46
11.	LAURYL ACETATE	228.37	$C1_4H_{28}O_2$	0.47	8.34
12.	3-TRIFLUOROACETOXYPENTADECANE	324.4	$C_{17}H_{31}F_3O_2$	0.15	9.29
13.	3-TRIFLUOROACETOXYTRIDECANE	296.37	$C_{15}H_{27}F_3O_2$	0.15	9.29
14.	PREDNISOLONE ACETATE	402.5	$C_{23}H_{30}O_6$	0.19	11.21
15.	CHROMONE	146.14	$C_9H_6O_2$	0.19	11.21
16.	BECLOMETHASONE	408.9	$C_{22}H_{29}ClO_5$	0.06	11.34
17.	Z-11-PENTADECENAL	224.38	$C_{15}H_{28}O$	0.19	13.49
18.	BETAMETHASONE ACETATE	434.5	$C_{24}H_{31}FO_6$	0.21	16.49
19.	17-PENTATRIACONTENE	490.9	$C_{35}H_70$	0.1	20.27
20.	DEMECOLCINE	371.4	$C_{21}H_{25}NO_5$	0.1	23.06
21.	ACETIC ACID	60.052	CH_3COOH	0.13	23.28
22.	ISOXAZOLE	69.06	C_2H_3NO	0.02	26.22
23.	PHTHALIC ACID	166.14	$C_8H_6O_4$	6.67	29.65
24.	DIISOOCTYL PHTHALATE	390.55	$C_{24}H_{38}O_4$	6.67	29.65
25.	HEXA-T-BUTYLSELENATRISILETANE	505.90	$C_{24}H_{54}SeSi_3$	0.04	29.93
26.	NORMORPHINE	271.31	$C_{16}H_{17}NO_3$	0.05	29.98
27.	PROSTAGLANDIN	354.5	$C_{20}H_{34}O_5$	0.04	33.04

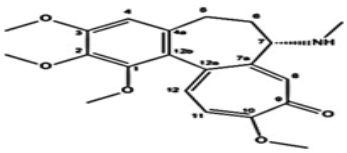
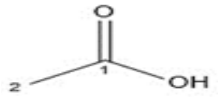
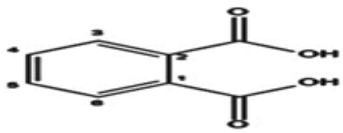
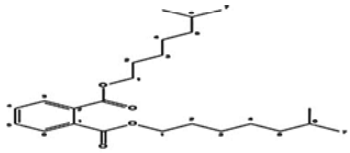
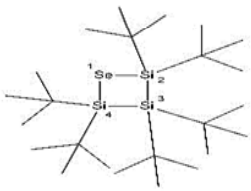
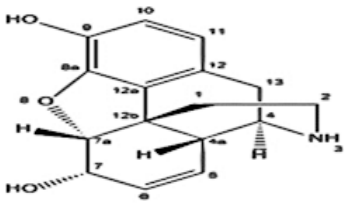
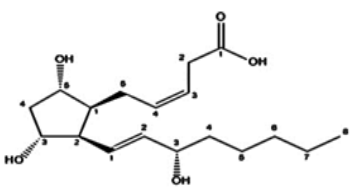
Table 3. Compound Structures with their Biological activities

S.N.	Compound Name	Biological Activity	Reference
1.	 TETRABUTYL TITANATE	Antibacterial	Zhang & Zhang,2014
2.	 ANDROSTANE-11,17-DIONE	Steroid	Hamalainen et al, 1991
3.	 ETHYL ISO-ALLOCHOLATE	Antimicrobial	Malathi & Ramaiah, 2017
4.	 ERGOSTA-5,22-DIEN-3-OL	Anti-tumor activity and immunomodul atory activity	Hussein et al, 2016
5.	 PROPANOIC ACID	Uses in the food industry but has recently found applications in the cosmetic, plastics and pharmaceutical industries.	Gonzalez- Garcia et al, 2017
6.	 1-,2-CINNOLINEDIC ARBOXYLIC ACID	Antitumor, antiviral, aestrogenic	Hassan et al, 2016
7.	 2,4-Imidazolidinedione, 5-[3,4-bis [(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-	Used as modulator of Kv3.1 and/or Kv3.2 for the treatment of depression, schizophrenia, mood disorders, sleep disorders, etc.	Alvero et al, 2012
8.	 Fucoxanthin	Antioxidant, anti-obesity, anti-diabetic, anticancer, and antimicrobial	Karpinski & Adamczak, 2019
9.		Treatment of Polycystic kidney disease	Mei, 2006

Continue Table 2

	BENZENEPROPANOIC ACID		
10.	 α-D-GLUCOPYRANOSIDURONIC ACID	Antibacterial	Lou & Cassidy,2010
11.	 LAURYL ACETATE	Flavoring agents, skin permeation enhancer, Industrial use	Burkoth et al, 1996
12.	 3-TRIFLUROACETOXYPENTADECANE	Anti-nephrotoxic and antioxidant activities	Hussein et al, 2016
13.	 3-TRIFLUROACETOXYTRIDECANE	Uses in chemical industry Toxic to aquatic life, harmful to skin and eyes	Anonymous, 2018
14.	 PREDNISOLONE ACETATE	Intraocular anti-inflammatory	Stanley, 2008
15.	 CHROMONE	Asthma, Antimicrobial, Anticancer, anti-inflammatory, Antioxidant	Tawfik et al, 2014
16.	 BECLOMETHASONE	Uses in chronic asthma	Adams et al,2002
17.	 Z-11-PENTADECENAL	Bioleum (industrial)	Wang, 2013
18.	 BETAMETHASONE ACETATE	Dry eye control	Shokoohi-Rad et al (2018)
19	 17-PENTATRIACONTENE	Antioxidant, antitumour, antiviral, hypolipidemic	Enema et al, 2019

Continue Table 2

20.	 DEMECOLCINE	Chemical enucleant	Fernandes et al, 2007
21.	 ACETIC ACID	Effective disinfectant, wine & bakery industry	Togashi et al, 2004; Cortesia et al, 2014
22.	 ISOXAZOLE	Immunomodulatory	Zimecki et al, 2018
23.	 PHTHALIC ACID	Plasticizer, causes several human health hazards	Bang et al, 2011
24.	 DIISOOCTYL PHTHALATE	Plasticizer (industrial), Adhesive, Antiandrogenic activity	Saillenfait et al, 2013
25.	 HEXA-T-BUTYLSELENATRISILETANE	Use not reported in literature	
26.	 NORMORPHINE	Analgesic, neurotoxic	Oguri et al, 1989 Glare et al, 1990
27.	 PROSTAGLANDIN	Acute anterior uveitis, Abortifacient, Rheumatoid arthritis	Miller et al, 1973 Keirse,1992; Fattahi and Mirshafiey, 2012

The peak was measured from the base to the tip of the peak. Retention index of the compounds were identified by comparing the retention times and identification of each component was confirmed by the comparison of its retention index with data in the NIST library. Interpretation of Mass-Spectrum was carried out by using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns. Spectrum of the known compound which are stored in NIST library was used to compare the spectrum of unknown component. The molecular weight, name, chemical structure and molecular formula of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GCMS is a technique that combines the separation of phytochemicals by gas chromatography and their detection by mass spectroscopy (Chauhan et al., 2014). Various parts of *Dillenia indica* i.e.fruit, leaf and bark have shown the presence of many primary and secondary metabolites (Barua et al, 2018). Present work shows preliminary screening of phytochemicals of aqueous flower extract of *Dillenia indica* and the presence of some very important phytochemicals such as coumarins, flavonoids, steroids and saponin were observed (table-1).

The peak in GCMS of methanolic extract of the flower of *Dillenia indica* showed the presence of the secondary phytochemical compounds like phenolic and fatty acids and other medicinally important bioactive compounds. GCMS analysis of methanolic flower extract showed significant presence of 27 phytochemicals (Fig. 2 and Table-2). The most abundant 27 compounds found in the methanolic extract of flowers were TetrabutylTitanate, Fucoxanthin, Ergosta-5, Lauryl Acetate, Beclomethasone, Betamethasone Acetate, Demecolcine,Androstane-11,17-Dione, Ethyl Iso-Allocholate, 1,2-Cinnolinedicarboxylic Acid, 2,4-Imidazolidinedione, Benzene Propanoic Acid, α-D-Glucopyranosiduronic Acid, Lauryl Acetate, 3-Trifluoroacetoxypentadecane, 3-Trifluoroacetoxytridecane, Prednisolone Acetate, Chromone, Z-11-Pentadecenal, 17-Pentatriacontene, Acetic Acid, Isoxazole, Phthalic Acid, Diisooctyl Phthalate, Hexa-T-Butylselenatrisiletane, Normorphin, Prostaglandin. The structures of above compounds have been mentioned in Table-3.

Many of above compounds are pharmaceutically important and their medicinal efficacies have been reported by many researchers as antibacterial, anti-inflammatory, and antiviral activities (Table 3). Some compounds show their industrial applications, whereas few compounds also show toxicity (Table 3). GCMS is an integrative technique for separation, identification and quantification of chemicals in a given sample (Leary et al, 2019). Quantification is an important step for data analysis and different softwares are being used for the calculation of retention time corresponding to specific peaks (Johnsen et al, 2017). The technique has great applications in pharmaceutical industries as it helps in the identification of bioactive compounds as well as any impurities present in the plant extract (Chauhan et al., 2014). In present study, several compounds have been identified from flower extract. Pharmaceutical activities of many compounds have been reported earlier by several researchers (Table- 3), which indicates potential of flower for the production of medicines. Therefore, like leaves and bark, flowers of *D. indica* too has significant medical efficacy. Uses of other parts of *Dillenia indica* as traditional medicines are already in practice. Now, there is need to use flower extract as well.

CONCLUSION

In the present investigation, 27 bioactive compounds have been identified from the methanolic extract of D. indica by GC-MS. The presence of various bioactive compounds in D. indica proved pharmaceutical and medicinal importance. However, further research is needed in order to analyze its bioactivity and toxicity profile.

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