Phytochemical Screening and GC-MS Analysis of *Epiphyllum oxypetalum* flower extracts

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ABSTRACT:
The present study was carried out in *Epiphyllum oxypetalum* flower to analyse the bioactive compounds present in it. *Epiphyllum oxypetalum* is a unique plant which blooms

KEYWORDS: *Epiphyllum oxypetalum,* phytochemical activity, bioactive compound, Gas Chromatography-Mass Spectrometry, Thin Layer Chromatography.

INTRODUCTION:

Epiphyllum oxypetalum:

Traditional medicines always play a vital role as important alternatives to conventional medicines. The use of plant for medicinal purpose dates back to an earlier record of human history. There has been an enormous increase in the demand of medicinal plant across the world for their chemical diversity and for the production of newer therapeutic moieties to control various diseases.[1] *Epiphyllum oxypetalum* belongs to the Cactaceae family. It is one of the most cultivated species of cactus. It is a variety of night blooming flower.

Epiphyllum oxypetalum was the most commonly grown of the *Epiphyllum* species, and it is commonly known with several names such as Brahma Kamal, Dutchman's pipe, Queen of the Night, Nishagandhi, Night blooming cereus. *Epiphyllum oxypetalum* flower has many medicinal values and it is commonly known as "Brahma Kamal". It is a very unique plant. The flower of *Epiphyllum oxypetalum* is used as a tonic for treating liver infections, choleocystitis, cancer, cholelithiasis, renal calculus and urinary infection, gynecological inflammation, freckles (anti-free radical) and hypercholesterolemia.[2] The biological activity of *Epiphyllum oxypetalum* flower is less explored and it is less proved experimentally in *Epiphyllum oxypetalum* flower extract. It is used as a traditional medicine widely for various bacterial infections.[3]

MATERIALS AND METHODS:

SAMPLE COLLECTION:

The Sample *Epiphyllum oxypetalum* was collected in the month of June - August from in and around Hosur, Krishnagiri district, Tamil Nadu. The collected flower was shade dried and then stored. The flower *Epiphyllum oxypetalum* was identified and authenticated by Dr. K. Rajagopal, Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Chennai, India.

EXTRACTION:

The extraction of *Epiphyllum oxypetalum* flower was done by maceration method with four different solvents separately [Ethanol, hexane, chloroform, aqueous]. The ethanol, hexane, chloroform and aqueous extract of *Epiphyllum oxypetalum* flower was extracted using 25g of coarse powder sample with 100ml of each solvent. The mixture was placed in the orbital shaker for 24 hours at 32℃ in room temperature. Four different solvent extracts were prepared and the extract was filtered with whatman filter paper.[4]

PHYTOCHEMICAL ANALYSIS:

Detection of Alkaloids:

1 ml of the extract was stirred with 1ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various reagents as follow:

Wagner's test:

The extract is added with 2ml of Wagner reagent by sides of the test tube. A reddish brown color precipitate confirms the test as positive.[5]

Wagner reagent:

Iodine (1.27g) and potassium iodide (2g) were dissolved in 5ml of water and made up to 100ml with distilled water

Detection of Carbohydrates:

Fehling's test: 1ml of the filtrate was boiled on a water bath with 1ml each of Fehling solution 1 and 2. Red precipitate indicates the presence of sugar.

Fehling reagent:

- Copper sulphate (34.66g) was dissolved in distilled water and made up to 500ml with distilled water
- Potassium sodium tartarate (173g) and sodium hydroxide (50g) was dissolved in water and created up to 500ml.[6]

Detection of Glycosides:

Borntrager's test:

To 2ml of the filtrate, 3ml of chloroform was added and shaken. chloroform layer was separated and 10% ammonia solution was added to it. The pink color indicates the presence of Glycosides.[7]

Detection of Saponins:

Foam test 1ml extract as dissolved in 2ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to test.[8]

Detection of Proteins:

Biuret test:

2ml of the filtrate was treated with few ml of 2% of copper sulphate solution. To this, 1ml of ethanol 95% was added followed by the excess of potassium hydroxide pellets. The pink color in the ethanolic layer indicated the presence of protein.[9]

Detection of phenolic compounds:

Ferric chloride test:

1ml of the extract was dissolved in 2ml of distilled water to this; few drops of neutral ferric chloride solution were added. A dark inexperienced color indicated the presence of synthetic resin compounds.[10]

Detection of Terpenoids:

To 2ml of extract, 1ml of chloroform was added and mixed well. A little of concentrated H_2SO_4 was carefully added to form a reddish brown layer.

Detection of Steroids:

2ml of chloroform was added to the extract and a few drops of acetic acid were added. Followed by concentrated H_2SO_4. A mixture of blue and green color formation showed the presence of steroids.[11]

Detection of flavonoid:

1ml of extract was added with 2ml of ammonia solution. A mixture of yellow color showed the presence of flavonoid.[12]

Detection of tannin:

1ml of extract was added with 2ml of water and 2 drops of ferric chloride was added to it. A green color indicated the presence of tannin compound.[13]

Thin –Layer Chromatography:

Thin layer chromatography is a method for analyzing mixtures of compounds by separating the compound mixture. Thin layer chromatography (TLC) is used to determine the number of components in a mixture. The TLC plate was made by dissolving 30g of TLC silica gel in 60ml of distilled water and poured in the TLC plate without any air block or crack. Then the prepared plate was placed in TLC tank which contains the solvent system of benzene, ethyl acetate and acetic acid in the ratio of 5: 1.5: 0.25. TLC plate was taken out after 10 minutes, dried and viewed under the UV-light on 256nm and 364nm. The extract with good band formation will be then taken for purification process.[15]

Column Chromatography:

Column chromatography was performed to separate the phytochemical based on polarity. In column chromatography method, 30g of silica gel was made into a homogeneous suspension with hexane (1st eluent). The bottom was plugged with little cotton to prevent the adsorbent to pass out, and then the silica gel suspension was

poured into the column. The column set up was washed and dried. Then, the dried column was filled with hexane up to two-third of the column length. The prepared silica gel with the hexane has been poured into the column and allowed to settle down without any air space or bubble during package. After packing, the solvent level should be 3cm above the silica layer to avoid cracking and air block. The extracted sample up to 5ml was mixed with silica gel and loaded on the top of the column and it was run by hexane. The ratio of hexane and ethyl acetate was frequently changed by increasing the polarity (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9). All the fractions were collected and finally the column turned colorless.[16]

Gas Chromatography- Mass Spectrometry (GC-MS):
The *Epiphyllum oxypetalum* flower extract was dissolved in ethanol and then subjected to GC-MS analysis. The GC-MS analysis of sample was carried out in sophisticated instrument facility (SIF) at VIT University, Vellore, Tamil Nadu. GC-MS made by Perkin Elmer, Clarus 680 instrument was used for GC and Clarus 600 (EI) instrument. The Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. 1μL of the extract sample injected into the instrument; the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min−1; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec with the fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.[17,18]

RESULTS AND DISCUSSION:

The *Epiphyllum oxypetalum* flower extract was made by maceration method with different solvents based on polarity (hexane, chloroform, ethanol, aqueous). It was kept in orbital shaker for 24 hrs at room temperature. After 24 hrs the extract has been taken and filtered using whatman filter paper. The extract was dried and then stored in refrigerator.

Phytochemical Analysis:
The phytochemical activity was checked for 4 different solvent extract. It showed the presence of alkaloids, saponin, protein, terpenoids, flavonoids, tannin, and steroids in 4 different extract of *E.oxypetalum* flower. The phytochemical constituents present in this flower extract may be used as a bio therapeutic agent. These phytochemical have many medicinal uses which help to treat many diseases.[17]

The preliminary test for phytochemical analysis showed the presence of alkaloid (Hexane, ethanol), saponin (hexane), protein (chloroform), flavonoid (Aqueous), tannin (Aqueous) and terpenoids (Hexane, chloroform, ethanol, aqueous) (Table 1). Alkaloids play a major role in pharmacological activity such as anti-cancer, anti-malarial, anti-hyperglycemic. Saponin present in this flower extract was known for the anti-bacterial activity and anti-oxidant activity. Tannins are known to be responsible for antibacterial activity. The flavonoids contain antioxidant property which helps to protect the body cells from harmful free radicals.[17,20]

Thin Layer Chromatography:
Thin layer chromatography was done with the crude extract of *E. oxypetalum* with the different extracted samples. The extract with good band formation has been taken for partial purification process.[21] After viewing under UV light, it showed the presence of 4 bands in the ethanol extract for which retention factor was calculated. The values were 0.35, 0.42, 0.71, 0.78. In hexane and aqueous extract we got 2 bands and the retention factor was calculated. The values are 0.64, 0.71 and 0.71, 0.78. The number of bands that separated in ethanol is higher as compared to other extract so we carried out ethanol for further partial purification (Figure 1).

Column Chromatography:
In 4 different crude extracts of *E. oxypetalum* flower, separation of band was seen in TLC plate and good separation of band in ethanol extract. So, further partial purification of ethanol extract in the column chromatography was performed. The extracted sample of 5ml mixed with silica gel was loaded on the top of the column and it was run by the hexane. The ratio was frequently changed by increasing the polarity (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) using different solvent ratios of hexane: ethyl acetate mixture and the eluted fraction were shown in the Figure 12. All the fractions were collected and finally the column turned colorless and further we checked in TLC with the collected fraction. The eluted fraction with solvent ratio 3:7 showed a good band separation as shown in Figure 2.[22]

Gas Chromatography-Mass Spectrometery (GC-MS):
The GC-MS is one of the best mechanisms to identify the phytoconstituents of alcohols, long chain, volatile matter, acids, esters, etc., It showed the presence of various phytochemicals which was found to be similar as present in the leaf extract of *E. oxypetalum*.[3,29,33] The first compound identified with less retention time (2.86 min) was Hexadecanoic acid, ethyl ester and the last compound which took long retention time (30.81min) was Pterin-6-carboxylic acid. The GC-MS chromatogram of ethanol extract of *E. oxypetalum* flower was shown in Figure 3. Hexadecanoic acid, Oleic acid, Nonadecanoic acid, and 1-octadecyne were found in both leaf and flower extracts.[31,32,34] The flavonoid compound coumarin present in the flower was proved for biological activity such as antifungal, antitumor, and anti-inflammantory properties.[22-26] The presence of bioactive compounds justifies the use of *E. oxypetalum* flower with different therapeutic activities [26,2728]

CONCLUSION:

The *Epiphyllum oxypetalum* flower showed the presence of numerous phytochemical or bioactive compounds such as flavonoids, alkaloids, saponins, tannins, proteins, aminoacids and terpenoids. The crude ethanol extract of *E.oxypetalum* characterized through GC-MS analysis showed the presence of flavonoids compound [7-hydroxy-3(1,1-dimethyl prop-2enyl) coumarin and some other compounds such as Oleic acid, Nonadecanoic acid and Hexadecanoic acid which were also reported in the leaf extract of *E. oxypetalum*. Thus these bioactive components could be isolated in future from *E. oxypetalum* flower to explore their unique biological activity against pathogenic organisms.

Table 1: Phytochemical analysis of *Epiphyllum oxypetalum* flower [+ ve indicates the presence of components and the –ve indicates the absence of components]

TEST	Hexane	Chloroform	Ethanol	Aqueous
Alkaloids	+ve	-ve	+ve	-ve
Carbohydrates	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve
Saponins	+ve	-ve	-ve	-ve
Protein and amino acids	-ve	+ve	-ve	-ve
Phenol	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	-ve
Steroids	-ve	-ve	-ve	+ve
Flavanoids	-ve	-ve	-ve	+ve
Tannins	-ve	-ve	-ve	+ve

Table 2: Thin layer chromatography in crude extract of *Epiphyllum oxypetalum* flower

Sample extract	No. of Spots Detected	R_f Value
Ethanol	4	0.35, 0.42, 0.71, 0.78
Chloroform	1	0.78
Aqueous	2	0.71, 0.78
Hexane	2	0.64, 0.71

Table 3: GC-MS analysis of phytochemical compounds and its activity

S. No	Retention Time	Chemical compounds	Biological Activity
1	18.730	Hexadecanoic acid, ethyl ester	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductaseinhibitor[22]
2	19.455	Nonadecanoic acid	Lubricant, Saturatedfattyacid, Anticancer, Antifungal[26]
3	19.865	Oleic acid	Hypochlorestol, Anticancer, Emulsing agent[7]
4	21.056	11-tridecen-1-ol	No activity reported
5	21.296	1-octadecyne	Antibacterial, Anticorrosion Lubricant
6	21.686	Oleic acid	Hypochlorestol, Anticancer Emulsing agent[22]
7	22.0016	Hexadecanal	Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic[27]
8	22.966	Spiro[androdt-5-ene-17,1-cyclobutan]-2-1-3-hydroxy	Antiarthritic, Hepatoprotective, Antiasthama, Diuretic, Anti-inflammatory[28]
9	23.912	Oleic acid	Hypochlorestol, Anticancer, Emulsing agent[29]
10	24.792	1,6;3,4-dianhydro-2-deoxy-. beta.-D-Lyxo-hexopyranose	Antimicrobial and Antibacterial activity[24]
11	25.117	1-octadecyne	Antibacterial, Anticorrosion, Lubricant[30]
12	26.013	di-n-decylsulfone	Antigonistic, Antifungal, Antimicrobial, Anthelminti [31]
13	26.688	7-hydroxy-3(1,1-dimethyl prop-2enyl) coumarin	Antifungal, Antitumor, Antiinflammantory[32]
14	28.474	Pterin-6-carboxylic acid	Antipsychotic, Moodstabilizer and Antiparasite[33]

Figure 1: Ethanol extract in TLC viewed under UV cabinet

Figure 2: TLC plate of fraction 3:7 under UV -cabinet

Figure 3: The GC-MS chromatogram of ethanol extract of *Epiphyllum oxypetalum* flower.

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