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Research Article

ASSESSMENT OF NUTRITIVE VALUES, PHYTOCHEMICAL CONSTITUENTS AND BIOTHERAPEUTIC POTENTIALS OF *EPIPHYLLUM OXYPETALUM*

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ABSTRACT

Epiphyllum oxypetalum is a species of cactus and one of the most commonly grown of the *Epiphyllum* species. It is one of the under-utilized resources available in the tropical regions of the globe and can be used as a substitute for digitalis. The Shoshone Indian tribe calls the night blooming Cereus "Pain in the heart" and used it for heart pain. Scanty work was reported on the phytochemical properties of leaf extract and no documented research work was reported on its leaf and flower for assessment of nutritive value and antimicrobial properties. Thus, the present investigation was carried out to access the nutritive values, phytochemical constituents and antimicrobial potentials of leaf extracts. The nutritive values of plant showed significant presence of proteins (14 mg/g), fatty acids (4.6 mg/g), and vitamins (0.18 mg/g), while carbohydrates were found to be absent. Phytochemical analysis of *Epiphyllum oxypetalum* showed the presence of saponins, phenolic compounds, steroids, glycosides, tannins, terpenoids, and resins while reducing sugars, alkaloids, flavanoids, sterols, phlobatanins and acidic compounds were absent. Since reducing sugars are absent, plant leaf can be used as a diet supplement to diabetic patients. Assessment of antimicrobial properties was done by using three solvent systems, (Petroleum ether, Acetone and Ethanol). All solvent systems at different concentrations were evaluated for antibacterial and antifungal capacity against selected bacterial and fungal pathogens; Maximum zone of inhibition was exhibited by acetone and petroleum ether (14mm) leaf extracts for *Escherichia coli*, acetone (14mm) for *Staphylococcus aureus*, acetone (11mm) and ethanol (10mm) for *Klebsiella pneumonia* and petroleum ether (12mm) for *Bacillus subtilis*. All the three leaf extracts were found to be ineffective against fungal strains (*Aspergillus niger*, *Aspergillus terreus*, *Aspergillus oryzae* and *Rhizopus oryzae*) tested.

Keywords: *Epiphyllum oxypetalum,* Nutritive value, Antibacterial activity, Phytochemicals, Reducing sugars, Zone of inhibition,

INTRODUCTION

Epiphyllum oxypetalum is a species of cactus and one of the most cultivated species in the genus. It is a variety of night blooming Cereus. Oxypetalum (Lat.) = with acute petals, refers to the acute petals of this species. *Epiphyllum oxypetalum* was the most commonly grown of the *Epiphyllum* species, and it is known under several common names including Night-blooming Cereus, Dutchman's Pipe, Queen of the Night, Wijaya Kusuma (Indonesian), Nishagandhi in Hindi and Marathi **(Table 1)**[1].

Table 1: Taxonomy of *Epiphyllum Oxypetalum*

Taxonomy	
Kingdom	**Plantae**
Sub Kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Cactaceae
Genus	*Epiphyllum*
Species	***E. oxypetalum***
Binomial name	
Epiphyllum oxypetalum	

The plant kingdom is a large reservoir of pharmacologically active molecules and large number of plant-derived medicines now commercially available[2]. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties[3]. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [4]. The bioactive compounds obtained from medicinal plants have been used to treat various ailments caused by microorganisms [5]. The most important of these bioactive principles are alkaloids, flavanoids, phenolic compounds and tannins that may be evolved in plants as self-defense against pests and pathogens[6]. Nature selects such type of plants and these plants are normally free from pests as well as pathogens[7]. Use of plants as traditional health remedies is very popular and important for 80% of the world's population in African, Asian, Latin America and Middle Eastern Countries. Their use is reported to have minimal side effects[8]. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants[9].The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources[10].

Phyto constituents are the natural bioactive compounds found in plants. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on[11]. The phytochemical screening of the *Euphorbia balasamifera* showed the leaves, stems and root were rich in most of the secondary metabolites[12].Plant-based antimicrobials have enormous therapeutic potential and poses lesser side effects than the synthetic antimicrobials[13]. Tannins (tannic acids) and saponins are responsible for the antibacterial activity of the plant seed extracts[14]. The extract of *Rhododendron setosum* and the essential oil of *Eucalyptus globulus* were most effective against *Escherichia coli*, *Staphylococcus aureus* respectively and the extracts of *Azadiracta indica* and *Elsholtzia fructicosa* were most effective against *Klebsiella species* [15]. The antibacterial activity of the leaves of *Annona muricata* extracts by agar cup method against eight bacterial species showed antibacterial activity; these promissory extracts open the possibility of finding new clinically effective antibacterial compounds[16]. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases. The present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems[17].

MATERIALS AND METHOD

Plant material *(Epiphyllum oxypetalum)*

The leaves of *Epiphyllum oxypetalum* were collected from in and around the city of Bangalore, India in December 2011. The plant was authenticated by Department of Botany, Indian Institute of Science (IISc); Bangalore, India **(Figure 1).**

Fig. 1: *Epiphyllum Oxypetalum leaves and flowers*

Chemicals and Instruments

All the chemicals and reagents used for the experiments were analytical grade.

Nutritive Analysis of *Epiphyllum oxypetallum*

Protein estimation was done by using Lowry's method. Estimation of Starch was done by using Iodine test method. Determination of Lipids was performed by using phosphovanillin reagent method[18, 19].

Estimation of Vitamin niacin

5gms of sample was grinded in 30ml of sulphuric acid (4N), steamed for 30mins, cooled and volume was made up to 50ml with distilled water. Extract was filtered using Whatman no.1fiter paper, to the filtrate 60% basic lead acetate was added and pH was adjusted to 9.5 with the pH meter using 10N NaOH and centrifuged. To the supernatant 2ml of concentrated sulphuric acid was added and allowed to stand for 1 hour and centrifuged. Supernatant was collected, and 5ml of 40% zinc sulphate was added and pH was adjusted to 8.4 with 10N NaOH, centrifuged and supernatant was collected, pH was adjusted to 7.0. Estimation was carried out by UV Spectrometric method[19].

Fluorescent analysis of leaf powder

Fluorescence analysis was determined by treating the plant leaf powdered with reagents such as Iodine, 25% ammonia, NaOH, $FeCl_3$, $H2SO_4$, HCl and 50% HNO3 placed on a micro slide. They were observed under UV 366nm, UV 254nm and in day light[20].

Preparation of solvent extracts for phytochemical and antimicrobial analysis

The leaves were shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder with the help of a blender. 25g of powder was filled in thimble and extracted successively with 250 ml petroleum ether, acetone and ethanol using a soxhlet extractor for 8 h at a temperature below the boiling point of the solvent. All the extracts were concentrated using rotary evaporator and preserved at 5°C in air tight bottle until further use[21].

Preliminary phytochemical analysis

Preliminary phytochemical analysis was carried to identify the presence of alkaloids, flavonoids, saponins, tannins, glycosides (Keller-Killani test), terpenoids (Salkowski test), phlobatanins, reducing sugars, proteins and amino acids (Ninhydrin test), phytosterols (Libermann-Burchard's test), phenolic compounds (Ferric chloride test), resins and acidic Compounds[21].

Collection and maintenance of microbial strains

The antimicrobial activity of the plant leaf extracts was carried out on the following bacterial and fungal starins respectively; *E. coli* MTCC 443, *Bacillus subtilis* MTCC 9003, *Staphylococcus aureus* MTCC 3163 and *Klebsiella pneumonia* MTCC 432 and *Aspergillus terreus* MTCC 11045, *Aspergillus niger* MTCC *282, Aspergillus oryzae* MTCC 634 *and Rhizopus oryzae* MTCC 553. All the bacterial & fungal strains were procured from the Institute of Microbial Technology, Chandigarh, India. The strains were first checked for purity on the basis of standard microbiological, cultural and biochemical tests and then used for their sensitivity on test samples.

Assessment of Antibacterial activity

Stock cultures were maintained on nutrient agar slants at 4°C and then sub-cultured in nutrient broth at 37°C prior to each antimicrobial test. The agar disc diffusion method was employed to determine the antimicrobial activities of the plant. A suspension of each tested micro-organisms were spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). Filter paper discs (6 mm in diameter) were soaked in extract and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters [22, 23].

Assessment of Antifungal Activity

The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture by point inoculation. The 5mm filter paper discs were impregnated with the extracts and placed on test organism-seeded plates. Ethanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm[24].

RESULTS:

Nutritive Analysis of *Epiphyllum oxypetalum*

The concentration of protein was found to be, 14 mg/g of leaf dry powder. No deep blue colour was observed, starch was absent. Pink colour develops and the sample was found to contain 4.6 mg/g lipids of leaf dry powder. Niacin was estimated by UV spectrophotometric method and the concentration of niacin was found to be, 0.18 mg / g leaf dry powder.

Fluorescent analysis of leaf powder

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. The result of fluorescence studies of *Epiphyllum oxypetalum* powder using different reagents under ultraviolet and normal light signified the presence of phytochemicals (**Figure 2 & Table 2).**

Table 2: Fluorescent Analysis of *Epiphyllum Oxypetalum*

S. No.	Reagents	Day light	UV 254nm	UV 366nm
1	Powder as such	Light green	Dark green	Black
2	Ferric chloride	Brown	Yellowish green	Black
3	50% Nitric acid	Brown	Green	Black
4	Sulphuric acid	Orange	Yellowish green	Black
5	Hydrochloric acid	Light green	Dark green	Black
6	NaOH	Light green	Dark green	Black
7	25 % Ammonia	Light green	Dark green	Black
8	Iodine	Yellow	Light green	Black

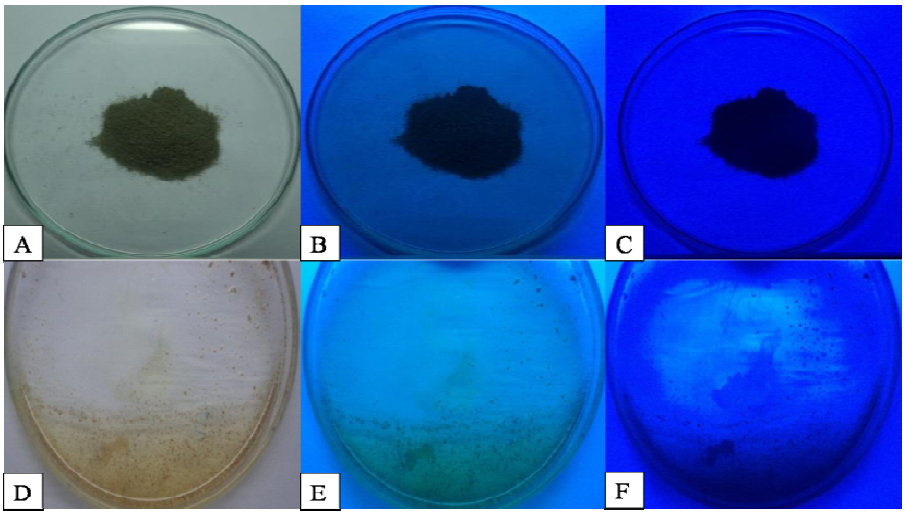

Fig. 2: Fluorescent Analysis

A. Leaf powder under visible light – light green .B. Leaf powder under UV 254(nm) – Dark green. C. Leaf powder under UV (366nm)-Black. D. Leaf powder treated with Sulphuric acid under visible light – Orange. E. Leaf powder treated with Sulphuric acid under UV 254nm – Yellowish green F. Leaf powder treated with Sulphuric acid under UV 366nm-Black.

Preparation of solvent extracts for phytochemical and antimicrobial analysis

Percentage of yields of different solvent extracts of *Epiphyllum Oxypetalum* leaf was presented in **(Table 3).**

Table 3: Yield % of plant leaf extracts

S. No.	Solvent	% of Yield
1.	Petroleum ether	4.51
2.	Acetone	5.75
3.	Ethanol	7.204

Preliminary phytochemical analysis

The petroleum ether, acetone and ethanol extracts of *Epiphyllum Oxypetalum* revealed the presence of Glycosides, Saponins, Steroids, Phenols, Proteins, Resins, Tannins and Terpenoids while reducing sugars, alkaloids, flavonoids, sterols Phlobatanins and acidic compounds were absent **(Figure 3) & (Table 4).**

Assessment Antibacterial activity

All solvent systems at different concentrations were evaluated for antibacterial capacity against selected bacterial pathogens; Maximum zone of inhibition was exhibited by acetone and petroleum ether (14mm) leaf extracts for *Escherichia coli,* acetone (14mm) for *Staphylococcus aureus*, *acetone* (11mm) and ethanol (10mm) for *Klebsiella pneumonia* and petroleum ether (12mm) for *Bacillus subtilis*. All positive control had not shown any zone of inhibition on all the test organisms. The zone of inhibition by Ethanol, Acetone and petroleum ether extracts of *Epiphyllum oxypetalum* leaf tested against selected bacterial pathogens at different dilution is presented in **(Figure 4 & Figure 5).**

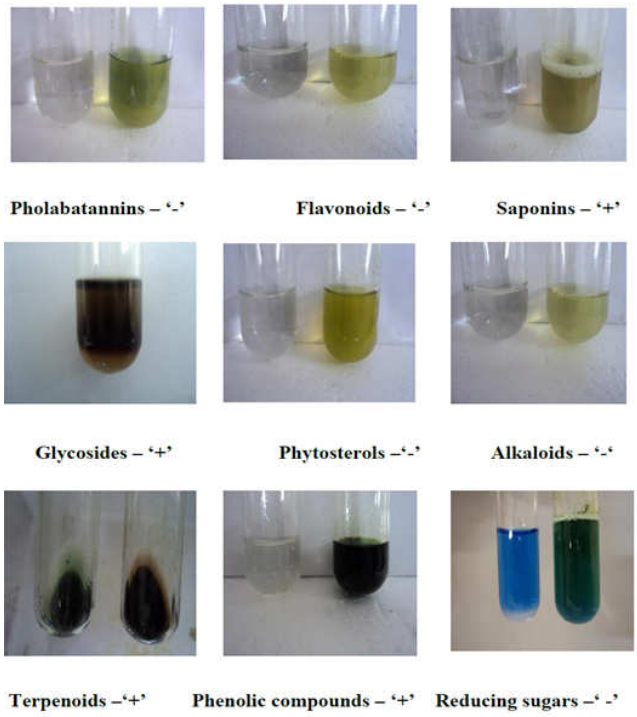

Fig. 3: Preliminary phytochemical analysis of leaf extract of *Epiphyllum Oxypetalum.*

Table 4: Preliminary phytochemical analyses of leaf extracts of *Epiphyllum Oxypetalum*

S. No.	Test	Leaf extract		
		Ethanol	Acetone	Petroleum ether
1.	Saponins	+	+	-
2.	Alkaloids	-	-	-
3.	Flavonoids	-	-	-
4.	Glycosides	+	+	+
5.	Proteins	+	+	+
6.	Steroids	+	+	+
7.	Reducing sugars	-	-	-
8.	Terpenoids	+	+	+
9.	Sterols	-	-	-
10.	Phlobatanins	-	-	-
11.	Phenolic compounds	+	+	-
12.	Resins	+	+	+
13.	Acidic compounds	-	-	-
14.	Tannins	+	+	-

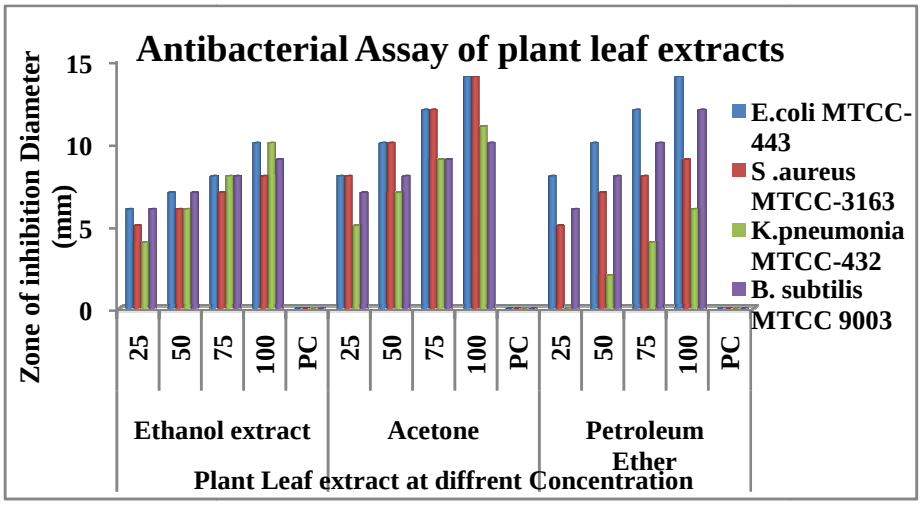

Fig. 4: Antibacterial activity of *Epiphyllum Oxypetalum* Leaf Extracts

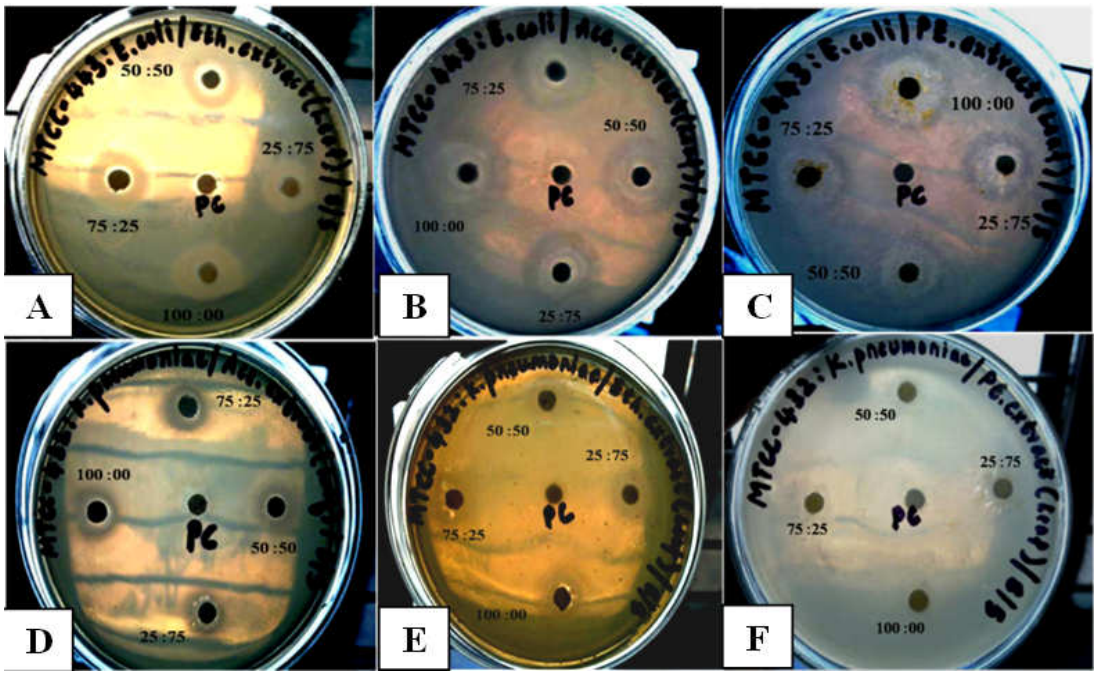

Fig. 5: Zone of inhibition of *Epiphyllum Oxypetalum* leaf extracts on selected bacterial strains

A. Zone of inhibition of Ethanol leaf extract on *E. coli* MTCC 443, (PC) Ethanol .B. Zone of inhibition of Acetone leaf extract on *E. coli* MTCC 443, (PC) Acetone. C. Zone of inhibition of Petroleum ether leaf extract on *E. coli* MTCC 443, (PC) Petroleum ether. D. Zone of inhibition of Acetone leaf extract on *K. Pneumonia* MTCC 432, (PC) Acetone. E. Zone of inhibition of Ethanol leaf extract on *K. Pneumonia* MTCC 432, (PC) Ethanol. F. Zone of inhibition of Petroleum ether leaf extract on *K. Pneumonia* MTCC 432, (PC) Petroleum ether.

Assessment Antifungal activity

All three extracts (Ethanol, Acetone and Petroleum ether) were found to be ineffective against the tested fungal pathogens. The study shall be extended to screen the inhibitory activity of *Epiphyllum oxypetalum* leaf extracts on soil pathogenic fungi.

DISCUSSION

The present study was carried out on leaf part of the plant *Epiphyllum oxypetalum*. The nutritive values of plant were shown significant presence of proteins (14 mg/g), while carbohydrates were found to be absent, which were considered as an important aspect of the plant. It also showed the presence of fatty acids (4.6 mg/g) and vitamin (0.18 mg/g) niacin which are added positives of the leaves of the plant. The preliminary phytochemical screening of the plant leaf was carried out to detect the presence of secondary metabolites. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. Different solvent extracts of *Epiphyllum oxypetalum* leaf powder and dry leaf powder were treated with different chemical reagents, they were observed under Visible and UV light and they exhibited characteristic coloration and indicate the presence of various secondary metabolites.

Phyto constituents are the natural bioactive compounds found in plants. Screening of *Epiphyllum oxypetalum* showed the presence of saponins, phenolic compounds, steroids, glycosides, tannins, terpenoids, and resins while reducing sugars, alkaloids, flavonoids, sterols, phlobatanins and acidic compounds were found to be absent. The presence of saponins, known for their anticholesterol activity and phenolic compounds, known for their antibacterial activity, presence of these compounds shows the medicinal potentials of the plant. Since reducing sugars are absent plant leaf part can be used as a diet supplement to diabetic patients. This study clearly reveals the broad spectrum antibacterial nature of the plant and suggests that this plant could be exploited in the management of diseases caused by bacteria in human systems. Antifungal activity was absent in the plant. Further works can be carried out on detecting different kinds of phenolic compounds present, which may have significant therapeutic and medicinal value. Since the present study was only carried out on leaves different parts of the plant like flower, stem, roots can be studied further.

CONCLUSION

Present investigation revealed that *Epiphyllum oxypetalum* leaves could be a very useful resource as a biotherapeutic agent. As its leaf extracts possess god nutritive values and a broad spectrum of activity against pathogenic bacteria, its expended use as dried/dehydrated extracts and its blends could be worth exploiting from economic point of view. These efforts could open up the possibility of finding new clinically effective biotherapeutic agents. This indeed is a step towards its sustainable use and justifying its abundance. Attempts are also being made to screen the leaf fibers as prebiotic agents.

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