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ORIGINAL ARTICLE

Quantitative Phytochemical Analysis on Leaf Extract of *Calanthe masuca* (D.Don) Lindl., an Endangered Medicinal Plant (Orchidaceae)

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Calanthe masuca (D.Don) Lindl plant leaves have medicinal properties. It is an endangered and terrestrial orchid. They are distributed in evergreen forest; plant collection is very difficult for researches. That's why very rare because of group of plants not widely represent in the forest. The present study has aimed to evaluate the phytochemical analysis using three different solvents Methanol, Aqueous and Petroleum Ether. The secondary metabolites have estimated into phenols, flavonoids, alkaloids, tannins, saponins, steroids and terpenoids. The result showed that the methanol leaf extract shows as a high concentration. Aqueous extract showed moderate concentration and Petroleum ether extract showed low concentration. Methanol extract contained secondary metabolites such as the total phenol (31.50±0.22 mg/g) and tannins (24.60±0.02 mg/g) as mg, alkaloids (54.40±0.33 mg/g) as mg, flavonoids (73.20±0.62 mg/g) as mg, saponins (35.00±0.05 mg/g), Steroid (60.95±0.57 mg/g) and Terpenoids (14.00±0.26 mg/g). The *Calanthe masuca* is a medicinal plant based on the secondary metabolites presence of phenols, flavonoids, alkaloids, tannins, saponins, steroids and terpenoids. They are used as a source of potentials therapeutic medicinal values and curing various infectious diseases.

Key words: Medicinal plant, therapeutic drugs, secondary metabolites, Orchidaceae

The phytochemical compound synthesized into the secondary metabolisms. Secondary metabolites have a vital role in the reinforcement of many plant tissues. This metabolite doesn't essential for growth and they produce to protect against insects, diseases and plant regulation and plant hormones (Ruchi *et al.,* 2012, Ruthisha *et al.,* 2017). The secondary metabolites contain a wide range of chemical structures and biological activities (Rajarajeshwari and Nandakumar, 2015).

They are play an important role to in many bioactive profiles of plants and other interesting microorganisms, several phytochemical screenings have been carried out by the various groups of naturally occurring phytochemicals (Hurkan *et al.,* 2019, Sasikala *et al.,* 2017). This bioactive compound have been considered in many biological activities of some phytochemical including alkaloids, flavonoids, bibenzyl derivatives and phenanthrenes have been found in the orchids and it is suggested various medicinal properties (Dawande and Gurav 2017).

The medicinal plants have significant places in the daily healthcare regimes of humans and animals (Madike *et al.,* 2017). They are called as backbone of the traditional system of medicine because thousands of drug plants have much pharmacological properties. In orchids, more than 25,000 species and 850 genera are presented in the universe. The medicinal plants as re-budding health assistance have been fueled by the raising of prescription drugs in safeguarding personalized health and well-being and the bio-prospecting of new plant-derived drugs (Pramanick 2016, Singh *et al.,* 2015 & Mythil *et al.,* 2014). Which are aesthetically and medicinally important, an also regarded as ecological indicators (Kumari and Pathak, 2020; Houqu *et al.,* 2021).

The present study *C. masuca* is an orchid plant genus name *Calanthe* and the specie *masuca* belongs to the family of *Orchidaceae.* This plant is an endangered orchid and they are rarely growing in evergreen terrestrial and forest areas with pink and white flowers. Usually the plant height is 180 cm tall in the southern part of India. The tribal people told it have medicinal importance to cure headaches, cough, snack bite and bone-broken disease. Hence the present study to find out the secondary metabolites of leaf extract in a different solvent of *C. masuca*.

MATERIALS AND METHODS

Plant collection and extract preparation

The *C. masuca* plant is an endangered orchid plant materials have collected from Kolli Hills in the Solakadu area in the Namakkal District of Tamil Nadu, India. The plant materials have been washed in two to three times with running water and one time sterile with distilled water. The leaf portion only was cut into small pieces keep in shade dried and coarsely powdered separately and stored in a well-closed bottle for further analysis in the laboratory.

Plant Authentication

The Botanical identification confirmed were by Dr.V.Ravichanthiran scientist's" & Head of the Office in Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu. The voucher specimen has been submitted to the Department of Botany, National College (Autonomous), Tiruchirappalli – 620 001, Tamil Nadu, India.

Chemical Reagents

The present study used various chemical compounds such as Sodium carbonate, Acetic acid, ammonium hydroxide, sulphonic acid, diethyl ether, sodium chloride, sulphuric acid, Iron (III) chloride, potassium hexacyanoferrate (III) solution and Folin – Ciocalteu phenol reagents has been obtained from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy in Sastra Deemed University.

Determination of Total Phenol content (followed by Folin-Ciocalteu methods, 1997)

In this investigation of the leaf extract here using the observed Folin-Ciocateu strategies. The response combination includes 1 ml of extract and 9 ml of distilled water taken in a volumetric flask (25 ml). The 1 milliliter of Folin-Ciocalteu phenol reagent changed with the combination well-shaken properly. After 5 minutes, 10 ml of 7% Sodium carbonate (Na2CO3) answer becomes handled to the aggregate. The answer becomes made up of 25 ml. A set of trendy answers of gallic acid (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 mg/ml) have been

prepared in the identical way described in advance. Incubated for 90 min at room temperature for the absorbance for taking a look at widespread solutions decided towards the reagent clean at 750 nm with an Ultraviolet (UV) /visible spectrophotometer. The phenol content material was expressed as gallic acid equivalent (mg/ml).

Determination of Alkaloids (followed by Harbone methods, 1973)

The determinations of alkaloids were detected into leaf by Harbone methods. 200ml of 10% acetic acid in ethanol was added leaf powder sample (2.5g) in a 250ml and allowed to stand for 4 hours. The extract was filtrated in muslin cloth. The leaf extract was concentrated in a water bath at 60° C to one-quarter of the original values followed by the addition of 15 drops of concentrated ammonium hydroxide, wait for drop clever to the extract till the precipitation because complete immediately after filtration. After 3 hrs of mixture sedimentation, the supernatant removed on precipitates was washed with 20ml of 0.1M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm).The electronic weighing balance Model B-218 were used to measure the sample. The residue was dried in an oven the percentage of the alkaloid weight of the sample was analyzed.

Determination of flavonoids (followed by Boham and Kocipai method, 1974)

The determination of flavonoids was determined in leaf by the followed by Ejikeme methods; Boham & Kocipai methods. 50 ml of 80% aqueous methanol added 2.5g of sample were added in a 250ml beaker, covered, and allowed to stand with 24 hours at room temperature. In this method discarding the supernatant, the residue again and again repeated three times with the same volume of ethanol here after it was filter and collected with Whatman filter paper. It was transferred into crucible evaporated to dryness over a water bath & cooled in desiccators and weighed until a constant weight was obtained. The flavonoids present in the sample.

Determination of Tannins (followed by Rajpal methods, 1992)

Tannin was determined into leaves followed by Rajpal methods. 1g of sample was digested with 50 ml of water and heated in a water bath for 30 min with frequent stirring. The supernatant was collected into a volumetric flask, and the extraction has repeated until the solution became colorless. The solution was cooled and made up to a volume of 100ml with distilled water from which 25ml was taken with 750 ml of water and 25ml of the indigo sulphonic acid solution was constant stirring until golden yellow color appears. A clean change into additionally accomplished without the sample. Each ml of 0. l M potassium permanganate solution has equivalent to 0.004157g tannins. They based on the titration value; the tannin content was calculated.

Determination of Saponin (followed by Ejikeme, 2016)

Saponins has been determined in the plant extract followed by Ejikeme and Obadoni; Ochuko methods. A hundred ml of 20% aqueous ethanol have delivered to five g of every leaf powder sample in a 250 ml conical flask. The sample solution was heated over a hot water bath for 4 hrs with continuous stirring at temperature of 55° C. The residue of the mixture was re-extracted with another 100ml of 20% aqueous and ethanol added after filtration and heated for 4 hrs at a constant temperature of 55^0C with constant stirring. The sample extract was evaporated to 40ml over a water bath at 90° C. 20ml of diethyl ether is added to the concentration in a 250ml separator funnel and vigorously agitated. The aqueous layer has recovered while the ether layer is discarded. This purification process was repeated twice; 60 ml of n-butanol was added and extracted twice with 10 cm3 of 5% sodium chloride. After removing the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes after that solution was transferred into a crucible and dried in an oven to a constant weight.

Determination of Steroids

The compound of Steroids have been decided into 1 ml of leaf extract decete of steroid answer becomes transferred with 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5 % w/v, 2 ml) have been added, accompanied using potassium hexacyanoferrate (III) answers (0.5 % w/v, 0.5 ml). The mixture turned too heated in a water bath maintained at 72°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance has

measured at 780 nm against the reagent with clean.

Determination of Terpenoids (followed by Indumathi methods; 2014)

Terpenoids was determined the leaf extract followed by Indumathi methods, 100 mg (wi) leaf powder was taken and soaked in 9 ml of ethanol keep with 24 hrs. The sample solution was filtrated, after being extracted with 10 ml of petroleum ether using a separating funnel. In the process, ether extract was separated into pre-weighted glass vials and waited for its complete drying (wf). Ether has been evaporated and the yields (%) of terpenoid contents have measured by the basic formula (wi-wf/wi × 100).

RESULTS AND DISCUSSION

The present studies on the quantification of phytochemical compounds have been carried out in leaf samples of *Calanthe masuca*. They are evaluated into three different solvents such as aqueous, methanol and petroleum ether. *Calanthe masuca* of the leaf extract has shown the presence of various phytochemical compounds. A high concentration of secondary metabolites is present in the methanol extract alkaloids (54.40±0.33 mg/g), flavonoids (73.20±0.62 mg/g), total phenol (31.50±0.22 GAE mg/g), tannins (24.60±0.02 mg/g), saponins (35.00±0.05 mg/g), Steroid (60.95±0.57 mg/g) and Terpenoids (14.00±0.26 mg/g). A moderate concentration has been shown in the aqueous extract and low concentrations represent Petroleum Ether (Table: 1).

In *C. masuca* leaf, a flavonoid compound is one of the largest ubiquitous groups of secondary metabolites. The presence of flavonoid is 73.20±0.62 (QE) mg/g methanol extract in quercetin equivalent per gram, the aqueous extract was moderate concentration and the petroleum ether was present in low concentration. Flavonoids are water-insoluble and belong to the polyphenols family found in the leaf. It has the biological properties of antioxidants, anti-carcinogenic, anti-microbial and anti-tumor. *Dendrobium denudates* plants have reported that the antioxidant activity of flavonoids is affected by the location of the hydroxyl group in the B-ring of the molecule (Alba-Patino *et al*., 2021; Madhu *et al*., 2016; Tiwari *et al*., 2011).

Flavonoids are polyphenolic plant secondary metabolites. Which are synthesized by the polypropanoid pathway with phenylalanine as startup molecule and dietary component. Flavonoids are thought to have health-promoting properties due to their high antioxidant capacity both in vivo and in vitro systems and human protective enzyme systems. Now a day's number of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers and other age-related diseases (Dawande and Gurav, 2021).

The presence of Steroids is 60.95±0.57 mg/g in a second high concentration of methanol extract, in an aqueous moderate concentration and petroleum ether in a low concentration. Panwar *et al.,(*2012) have reported that of *Eulophia* species are thought to have health-promoting properties due to Hypolipidemic, hypoglycaemic, antioksidan, obesitas, tumor, afrodisiak, penambah nafsu makan, mengatasi permasalahan cardio.

The presence of alkaloids content was about 54.40±0.33 mg/g methanol extract in high concentration, the aqueous in moderate concentration and the petroleum ether its low concentration. Alkaloid compounds act as antibacterial and anti-diabetic properties and also for the blocking of pathogenic microbes. Alkaloids are the most powerful and effective compared to other secondary metabolites. The possession of alkaloid groups also affects the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant disease, infections, and malaria. It also helps to cure various infectious diseases. In alkaloids have been reported to exert analgesic, antispasmodic and antibacterial activities (Hurkan *et al*., 2019; Herrera *et al*., 2022).

The saponin content was a high concentration in methanol extract at about 35.00±0.05 mg/g, aqueous is moderate concentration and the petroleum ether its low concentration. Saponins are glycosides characterized by their ability to foam in aqueous solutions used as detergents. The saponins have used in hyper cholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss (Kornienko and Tesliuk, 2021; Vinha and Soares, 2012).

In methanol extract 31.50±0.22 mg/g in high concentration, aqueous in moderate concentration and petroleum ether in low concentration (Fig 1). Phenolic compounds have been effective in disinfections and bactericides. They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, cardiovascular protection and improvement of endothelial function. The phenolic compounds may contribute directly to anti-oxidative action. It implies that polyphenolic compounds have inhibitory effects on mutagenesis (Bidarnamani *et al.,* 2020; Thitikornnpong *et al.,* 2022). Phenol is a group of secondary metabolism. They are synthesized by plants and utilized as UV, wounding and infection protectant in the plants. The natural phenolic compounds play an important role in cancer prevention and its treatment. Presence of this compound from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. They have various bioactivities of phenolic compounds are responsible for their chemopreventive properties. (Dawande & Gurav, 2017; Hossain *et al*., 2020).

The Tannin content was about 24.60±0.02 mg/g present in methanol. Whereas in aqueous and petroleum ether extract quantity it's low concentration. Tannin is an anti-nutrient that belongs to the polyphenols group. Tannins are well known for their anti-oxidant and antimicrobial properties, as well as for soothing relief, skin regeneration, anti-inflammatory and diuretics (Kotha *et al.,* 2020; Deyab *et al*., 2016)

The saponin content was a high concentration in methanol extract at about 35.00±0.05 mg/g, aqueous is moderate concentration and the petroleum ether its low concentration. Saponins are glycosides characterized by their ability to foam in aqueous solutions used as detergents. The saponins have used in hyper cholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss (Karoojee *et al.,* 2021). The presence of Steroids is 60.95±0.57 mg/g in a high concentration of methanol extract, in an aqueous moderate concentration and petroleum ether in a low concentration. Janaki *et al*, have revealed that the presence of steroids, which are known to produce an inhibitory effect on inflammation. The terpinoid content was about 14.00±0.26mg/g present in methanol in high concentration, aqueous and petroleum ether low concentrations. Kenyan Wild Orchids have demonstrated flavonoids, saponins, alkaloids, tannins, terpenoids, steroids and glycosides with varying degrees of product yield (%) (Chagona *et al*., 2021; Víctor *et al*., 2012).

Table.1: Secondary metabolites of different solvent of leaf extract of *Calanthe masuca*

S.NO	Secondary metabolites chemical compound (mg/g)	Aqueous extract	Methanol extract	Petroleum Ether extract
1	Alkaloids	27.36±0.03	54.40 ± 0.33	21.46±0.03
2	Flavonoids	28.83±0.24	73.20 ±0.62	24.35±0.32
3	Phenol	15.52±0.09	31.50 ±0.22	11.21±0.23
4	Tannins	12.24±0.02	24.60 ±0.02	11.78±0.02
5	Saponin	19.75±0.01	35.00 ±0.05	12.45±0.28
6	Steroids	37.22±0.32	60.95 ±0.57	9.31±0.01
7	Terpenoids	08.14±0.17	14.00 ±0.26	7.08±0.02

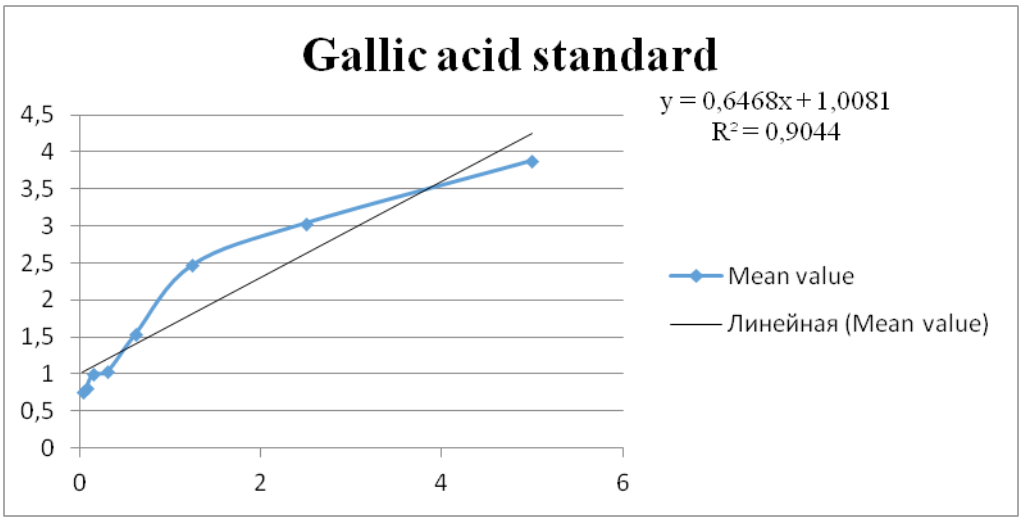

Figure 1 Standard calibrate on curve of Gallic acid

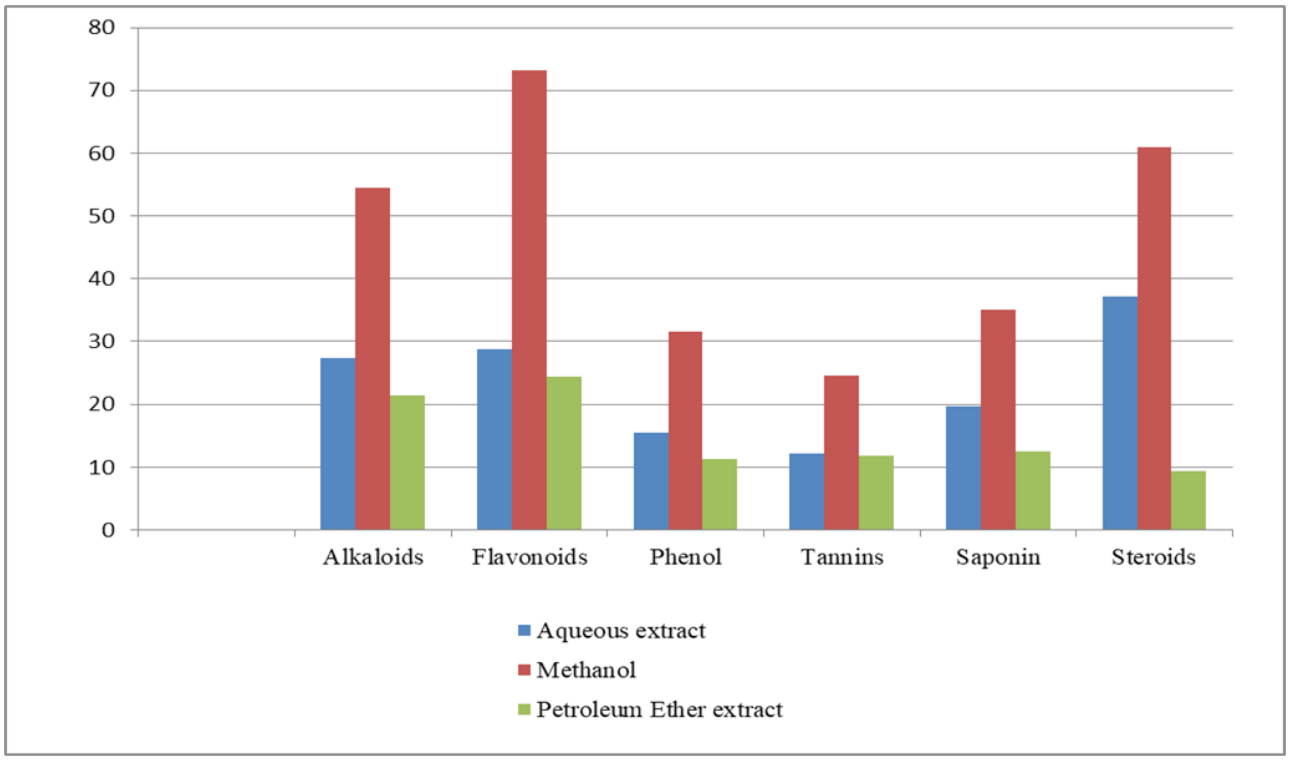

Figure 2 Quantitative phytochemical analysis of *Calanthe masuca* leaf extracts

Calanthe triplicata plants have reported that the calibration graphs showed that strong positive linear correlation there are close to +1. These graphs indicate that as the value of concentration increases, values for absorbance also increase. Total phenol, alkaloid, flavonoid and tannin contents in ethyl acetate extract were found to be 29.43 mg of GAE, 65.34 mg of AE, 90.24 mg of QE and 82.92 mg of GAE/ g of extract respectively. Petroleum ether, chloroform and methanolic extracts were made known less content of phenol, alkaloid, flavonoid and tannin. (Mythyili *et al*., 2014; Edern *et al*., 2016). Singh *et al*, have reported that the total phenols and total flavonoids content of methanol extract of *Dendrobium denudans* are found to be 129.02±0.865 mg GAE/g and 108.333±1.155 mg CAT/g respectively.

The present investigation shows significant variation in the contents like flavonoids, steroids, alkaloids, saponin, phenol, tannin and terpenoid content (Fig; 2). These variations are due to many environmental factors such as

climate changes, altitude, rainfall, etc. The phytochemical constituent is a source to use in drugs and improve therapeutic medicinal values. The phytochemical analysis of the leaf has a profitable interest in both research institutes and pharmaceutical companies to develop new modern drugs for the treatment of various diseases.

CONCLUSIONS

The present study concluded that the traditional use of the leaf of *Calanthe masuca* has a rich source of naturally occurring bioactive secondary compounds. This plant is a endangered medicinal therapeutic orchids and it is grown in evergreen forest terrestrial herbal plants. There are extracted efficiently with methanol, aqueous and petroleum ether solvent. The methanol extracts showed a higher potency of secondary metabolites such as phenols, flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids. The result indicates that the high quantity of secondary metabolites in the methanol extracts and it is traditionally cures various diseases. In this investigation the active compound may separate and serve for the production of sized the drugs for various therapies. Finally it is used to future pharmaceutical studies.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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