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Preliminary Phytochemical Profiling Of Leaf Extracts Of *Bulbophyllum Rosemarianum*

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Abstract:
The medicinal orchids are of great importance, as they are known to contain many numbers of phytochemicals which possess various pharmacological activity. Now a day's medications are being developed by incorporating the available natural products, specifically those resulting from medicinal plants. Several studies have reported that phytochemical compounds and molecules extracted from the different species of *Bulbophyllum* are employed in the preparation of plant based medicinesand this genus have attained scientific interest because of its phytochemical components and its efficient biological activities. The present work was carried out to analyze the phytochemicals present in leaf extracts of *Bulbophyllum rosemarianum.* The leaf part of the plant was collected, shade dried and subjected to the Soxhlet technique for the extraction process with three different polar solvents and its results indicated that ethanol was efficient in extracting phytochemicals and qualitative analysis revealed the existence of different phytochemicals like Flavonoids, Terpenoids, Steroids, Coumarins, Tannin, Phenol, Saponins, Steroids, Glycosides and Quinones. Quantitative analysis stated that ethanolic leaf extracts of *Bulbophyllum rosemarianum* exhibited comparable levels of total Polyphenolics, Alkaloids and Flavonoids. Further, isolation of phytochemicals and further studies will pay the way for significant application in medicinal and pharmaceutical industries.
Key Words: *Bulbophyllum rosemarianum,* phytochemicals, Polyphenolics, Alkaloids and Flavonoids.

1. INTRODUCTION

India is endowed with number of Ayurvedic and household formulations made by herbal plantssince ancient time which illustrates the history of bioactive molecules.The people were unaware about the utilization of these compounds having distinct applications. Approximately about 1200 known bioactive compounds serving as component of therapeutic quality, produced by plants are aromatic in nature and used to protect against different parasites. Enhanced knowledge regarding the plant based medicines and undesirable outcomes of modern medications have been motivated the researchers to examine the natural substances from plants which are known to possess medicinal traits (Narang, 2019).

Medications of natural origin, capable of preventing and treating diseases, have gained attention of researchers towards natural substances from which they have initiated the task of isolating, identification, examining structure of compounds and exploring their biological effects (Chaachouay & Zidane, 2024).Kingdom plantae comprising approximately about 25000 species of plants, which are the treasures of nature with therapeutic properties among which, around only 10% have been explored. India, well known for its incredible biodiversity making up about 11.4% of global floral composition and residence for about 47513 plant species, among which 28% are endemic in nature. The most well recognized Western Ghats of south west sections are featured with species richness and exhibit wide range of floral diversity. Among the flowering plants, Orchidaecae is one of the accepted and important family comprising of 25000 species, with over 800

genera. In India, around 1100 species and 163 genera are occurring in different geographical regions and it is estimated that approximately 250 species of various categories of orchids are under threats (Kumar & Singh, 2023).

Bulbophyllum, one of the most prevalent genus in the Orchidaecae family under the subfamily *Epidendroideae* with sub tribe *Bulbophyllinae* are the examplesof medicinal orchids which are in the line of extinction, whose secondary metabolites are used in the conventional medication system in different countries (Sharifi-Rad et al., 2022). Indo-malayan region, well known for its diverse orchid species and It is found that few species belonging to the genus *Bulbophyllum* are more valid in the context of horticulture because of their unique and fascinating attractive flowers but, studies regarding the biological effects and its mechanism are unexplored. (Mustaqim et al., 2025). Data from pharmacological literaturerevealed that various phytoconstituents in some *Bulbophyllum*species have different biological healthpromoting activities such as antimicrobial, anti fungal, antioxidant, anti-inflammatory, anticancer, neuroprotective and no records of toxicological effects have been documented (Zhang et al., 2022).

Orchids, now a day's becoming the most important area of interest for many researchers.because of their colorful and unique structural appearances. Documentation on the orchids application as a therapeutic agent is a major step taken in-order to preserve them because, It is alarming that 1200 plant species is being extinct in every year and this figure is gradually increasing. Our present research is focused on exploration of important bioactive molecules which help to treat the fatal diseases and for the well-being of the future generation continuous efforts on identification of phytochemicals are in progress before the extinct of orchid species (Goswami et al., 2024).

Medicinal orchids are composed of metabolically active compounds such as flavonoids, phenanthrenes, terpenoids,alkaloids, moscatilin, denbinobin, erianin, dendrochrysanene, fimbriatoneand cirrohopetalanthrin and bibenzyl derivatives, which are found in leaves, roots, pseudobulbs, and flowers have demonstrated significant anticancer activities.but, there are insufficient reports on these compounds and their pharmacological properties(Li et al., 2022).It is essential to record and evaluate the traditional medicines from orchids, which have been used for centuries to medicate diseases. The study of the medicinal orchid's cytotoxic capability served as a benchmark for the chemical identification of the active ingredient having anticancer properties (Joshi et al., 2020). In this regard, it is essential to analyse the phytochemical properties of different species of orchids, Keeping this in view, research has been conducted to record the medicinal aspects of *Bulbophyllum rosemarianum.*

2. Materials and Methods

1. Collection and Processing of the Plant Samples.

Green fresh leaves of the *Bulbophyllum rosemarianum* plant were collected from the several growing areas of Ponnam pete taluk of Kodagu District, Karnataka. The leaves were washed with sterile water to remove associated soil debris, then it is pat dried and allowed for drying in shade, to prevent photolysis and thermal degradation. The dried leaves were chopped into small pieces and grind into coarse powder form in a mechanical grinder. The dried powered samples were then kept in an airtight clean container and stored at 4^0 C for further use.

2. Preparation of Plant Extracts.

Extraction process were carried out using three different polar solvent in soxhlet apparatus which is connected at the top of collecting flask below which an reflux condenser is present. Powdered leaf samples and solvents were added and heated to a temperature lower than boiling point of solvent. The obtained crude extracts were further evaporated and residues were preserved in air tight containers and kept at lower temperature of about (4^OC) for further use (Tzanova et al., 2020).

Table 1:

Sl. No	Parameter	Soxhlet Extraction
1	Sample Size (g)	25 gm
2	Extraction Solvents	Methanol, Ethanol and Water
3	Solvent Volume	300ml
4	Temperature	Methanol(60^0C), Ethanol(75^0C) and Water(95^0C)
5	Time	6 Cycles of 3Hrs

Conditions used in Soxhlet Extraction

3. Qualitative phytochemical analysis

Qualitative tests were performed by using fresh, powdered samples, following standard procedures to identify the constituents as described.

1. Detection of flavonoids (Ghoshal et al., 2022)

Ferric chloride test: 1ml of Extracts were taken and with few drops of Ferric chloride solution were added, appearance of blackish color indicate the presence of flavonoids.

Alkaline reagent test: 1ml of extracts, on addition of few drops of sodium hydroxide solution, intense yellow colour will be observed and it becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test: 1ml of extracts were taken and few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids

2. Detection of Terpenoids (Dauda et al., 2020)

Salkowski test: For 2ml of extract, add 2ml of chloroform and Concentrated sulfuric acid along the sides of test tube, shaken well and allowed to stand for some time, the presenceof terpenoids were confirmed by the appearance of reddish brown colour.

3. Detection of steroids (Sukumar et al., 2020)

Libermann-Burchard test: 1ml of extract was taken and 1ml of acetic anhydride and concentrated sulphuric acid was added along the sides of the test tube, appearance of blue green colour indicatesthepresenceofsteroids.

4. Detection of Coumarins (Ghoshal et al., 2022)

To 1ml of extract, 1ml of 10% Na OH solution were added, formation of yellow colour indicates the presence of Coumarins.

5. Detection of tannins (Bhawna et al., 2023)

1ml of extract mixed with 1ml of potassium dichromate, formation of precipitate showed the presence of tannins.

6. Detection of phenols(Dauda et al., 2020)

To the plant extract, add few drops of 10% $Fecl_3$ solution and appearance of blue or green colour indicates presence of phenols.

7. Detection of saponins(Bhawna et al., 2023)

Frothing test: 0.5 ml of plant extract mixed with 5 ml of distilled water, frothing persistence indicated the presence of saponins .

8. Detection of anthocyanins (Godlewska et al., 2023)

Add few ml of plant extract, 10% NaOH was added and observed for the appearance of blue colour which is positive test for anthocyanins.

9. Detection of glycosides (Ghoshal et al., 2022)

Keller Killiani Test – 1ml of plant extract , mixed with few drops of glacial acetic acid and $FeCl_3$ solution and on addition of Conc.H_2SO_4 along the sides of test tube, observe for the formation of lower reddish brown layer and upper acetic acid layer appears to be bluish green in colour indicates presence of glycosides.

10. Detection of quinines (Dauda et al., 2020)

On addition of 1ml of con.H_2SO_4 to 1ml of extract, formation of red colour indicates the presence of Quinones.

11. Detection of alkaloids (Ghoshal et al., 2022)

Mayer's Test: 1ml of Extracts mixed with few drops of mayers reagent, the presence of alkaloids is confirmed by the appearance of white creamy precipitate.

Hagers Test: Few drops of Hagers reagent added to 1ml of **e**xtracts and looked for formation of yellow colour precipitate, indicate the presence of Alkaloids.

4. Determination of Total Polyphenolic content in different extracts

Folin-Ciocalteu colorimetric method whose principle is based on oxidation-reduction reaction in which FC reagent oxidizes phenol or phenolic-hydroxy groups to reduce heteropoly acid (phosphomolybdate-phosphotungstate) intoa blue colour molybdenum-tungsten complex, whose intensity can be measured at a wavelength of 765 nm, was used to estimate the total Polyphenolic content in plant extracts.

Various concentrations(1, 2, 4, 8 and 16 µg/ ml) of Quercitin solutions in methanol were prepared, 1 ml of each concentration were added and 5 ml of 10% FC reagent followed by 4 ml 7% $NaHCO_3$ were added to get a total volume of 10 ml. As a result the blue colour mixture was formed which was shaken well and incubated for 30 minutes at 40°C in a water bath. Then, the absorbance was measured at an wavelength of about 765 nm against blank.

The average absorbance values obtained at different concentrations of quercitin were used to plot the calibration curve and sample extracts were prepared at various concentrations of the (2.5µl, 5.0µl, 7.5µl, 10µl) and to this 2.5ml of 7.5% $NaHCO_3$ and 2.5ml of FC reagent was added followed by incubation at 45^0C for 45 min . The absorbance was read at 765nm . Based on the absorbance of various Quercitin concentrations (Std. 0.01mg/ml) the standard curve was plotted and the total phenolic content of the sample was estimated from the standard calibration curve and expressed as mg Quercitin equivalent (Quer)/g by using the following equation (Sari et al., 2023)

$$T = \frac{C\ X\ V}{M}$$

Where, T = Total Polyphenolic content in mg/g of sample

C = Concentration of quercitin in mg/ml

V = Volume of the extract solution in ml (1ml)

M = Extract weight in grams (0.01g)

5. Alkaloids separation and its estimation in three different Extracts

An small amount of extract residue, dissolved in 2N HCL and followed by filtration, 1 ml of filtrate was poured to separatory funnel and washed for three times with 10 ml of chloroform. By adding required amount of 0.1 N NaOH, pH of the solution was adjusted to neutral and then BCG solution and phosphate buffer of 5 mlwere added. The mixture complex was extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking andthe extract was collected in avolumetric flask (10 ml) and diluted with chloroform.

Different aliquot (0.4, 0.6, 0.8, 1 and 1.2 ml) of standard Atropinesolution were transferred to different separatory funnels, to which 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was added and the mixture was shaken with

extract with 1, 2, 3, and 4 ml of chloroform, then the extracts were collected in 10 ml volumetric flask and diluted to adjust solution with chloroform.The optical density of the complex was read at an wavelength of about 470 nm
The total alkaloids content in all three extractswere calculated by using linear equation obtained from the standard calibration curve of Atropineand results were expressed as atropine equivalents (mg AE/g dry weight)(Akter et al., 2019).

6. Determination of the Total Flavonoid Content in three different extracts

Aluminum chloride (AlCl3) methodusing quercitin as a standard is used to estimate the total flavonoid content in the leaf extractsin which 0.25 ml of extracts was added to 1.25ml DDW followed by 75 µl of 5% NaNO2 and 10% of AlCl3 (0.15 ml) wasadded after 5 min at room temperature (RT), Followed by the incubation for 6 min at RT, the reaction mixture was treated with 0.5 ml of 1 mM NaOH. Finally, the reaction mixture was diluted with 275 µl of DDW and it was Further, incubated for 20 minutes at RT and the absorbance was measured at 510 nm. All tests were performed in triplet. By using the standard calibration curve of quercitin the flavonoid content was calculated(Middha et al., 2013).

3.RESULTS

Soxhlet extraction - Yield of Extracts in different solvent

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. It will give an idea about the ex-tractability of the plant studied under different conditions.
The % yield was obtained using dry weight, from the equation

Percentage yield of extract (g/100 g) = $\frac{\mathbf{W1 \ X \ 100}}{\mathbf{W2}}$

Where, W_1 is the extract weight
W_2 is the weight of dried leaf powder.

The yield of extracts is different in different solvent based on the composition of phytoconstituentsand polarity of the solvent. The basic parameters influencing the quality of an extract are plant part used as starting material,solvent used for extraction and extraction procedure.
Our Findings revealed that maximum percent yield was obtained when leaves of Bulbophyllum *rosemarianum* was extracted by the Soxhlet extraction with ethanol, (35.2%); followed by Methanol (17.6%) and Aqueous (16.0%)
Our results Shows that ethanol was efficient in extracting phytochemicals compare to other solvents

Table 2: Results of Soxhlet extraction

Name of the plant	Plant part used	Solvent used for extraction	% yeild	colour
Bulbophyllum rosemarianum	Leaves	1.Methanol	17.6%	Green
		2. Ethanol	35.2%	Dark green
		3. Water	16.0%	brown

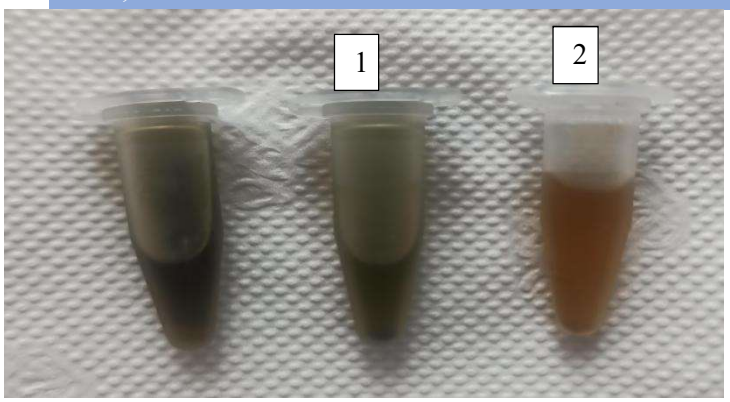


Fig 1: Colour of the different extracts of leaf of *B. rosemarianum*

Qualitative phytochemical analysis

The data in table shows results of screening of three different extracts of leaves of Bulbophyllum *rosemarianum* based on phytochemical tests which detected the presence of various bioactive compounds which might be responsible for their medicinal attributes.

Table 3: Results of

Chemical test	Leaf extract		
	Methanolic	Ethanolic	Aqueous
1. Test for Flavonoid			
Ferric chloride test	**Absent (-)**	**Absent (-)**	**Present (+)**
Alkaline Reagent test	**Present (+)**	**Present (+)**	**Present (+)**
Lead acetate test	**Present (+)**	**Present (+)**	**Present (+)**
2. Test for Terpenoids			
Salkowski test	**Present (+)**	**Present (+)**	**Present (+)**
3. Test for Steroids			
Liebermann Burchard Test	**Present (+)**	**Present (+)**	**Absent (-)**
4.Test for Coumarins	**Present (+)**	**Present (+)**	**Absent (-)**
5.Test for Tannins	**Absent (-)**	**Present (+)**	**Absent (-)**
6.Test for Phenols	**Absent (-)**	**Absent (-)**	**Present (+)**
7.Test for Saponins	**Absent (-)**	**Present (+)**	**Present (+)**
8.Test for Anthocyanins	**Absent (-)**	**Absent (-)**	**Absent (-)**
9.Test for Glycosides	**Present (+)**	**Present (+)**	**Present (+)**
10.Test for Quinones	**Present (+)**	**Present (+)**	**Present (+)**
11.Test for Alkaloids	**Absent (-)**	**Absent (-)**	**Absent (-)**

Qualitative analysis of different extracts of leaf of *B.rosemarianum*

Quantitative analysis

1. Total Polyphenolic content

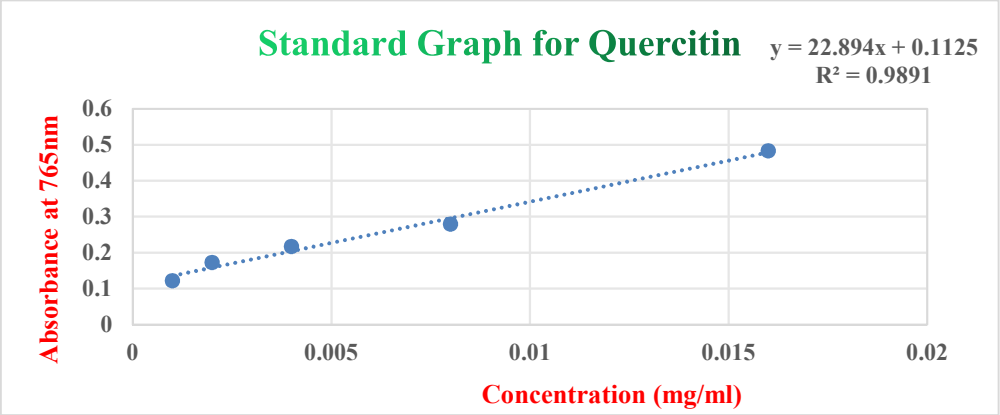


Figure 2: Standard caliberation curve for quercitin

The concentration of total polyphenolics in the extracts were determined from the standard calibration graph and the results are expressed in terms of Quercitin of the extract which was calculated by linear equation obtained from the standard calibration curve of Quercitin.($y = 22.894\ X + 0.1125$, $R^2 = 0.9891$)

Where Y is the absorbance of the sample and X represents the amount of quercitin in µg/ml.

Volume of extract taken	TPC (mg/g) (Quercitin equivalent)		
	Ethanolic extract	Methanolic extract	Aqueous extract
2.5 µl	0.2381	0.1114	0.1638
5µl	0.7142	0.7753	0.4433
7.5 µl	1.1903	1.3344	0.7797
10 µl	1.9634	1.8324	0.9413

Table 4: The total Polyphenolic content in the extract are expressed in terms of quercitin equivalent (mg quercitin /g extract)

Name of the Extract	Total polyphenolic mg Quer /g extract
Ethanoilc extract	4.1059±0.73
Methanolic extract	4.0534± 0.74
Aqueous extract	2.3281± 0.34

Table 5: **The results were expressed as mean (± sd)

Data clearly shows, both ethanolic and methanolic leaf extracts of *Bulbophyllum rosemarianum* exhibited comparable levels of total polyphenolics.

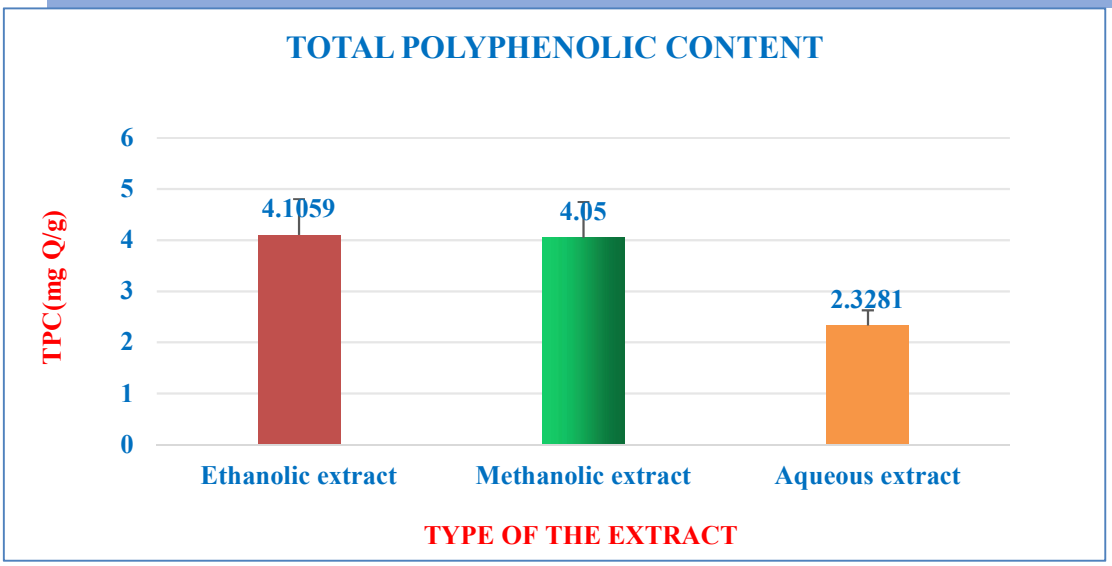


Figure 3: Comparision of total polyphenolics content in different extracts

2. Total Alkaloids

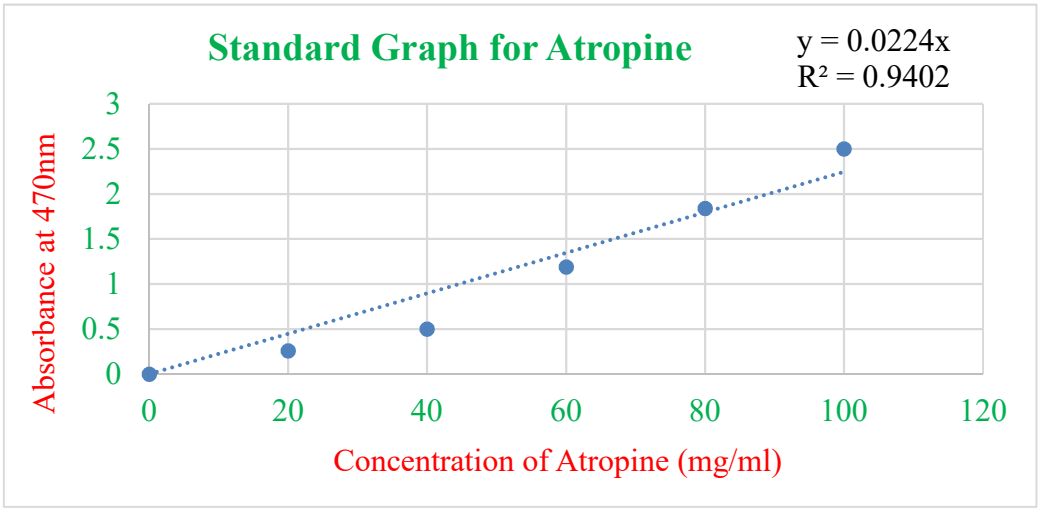


Figure 4: Standard calibration curve for Atropine

The concentration of total alkaloids in the test sample was calculated by using the linear equation obtained from the standard calibration curve of Atropine (y = 0.0224 X, R^2 = 0.989) and the results are expressed as Atropine equivalents (mg AE/g dry weight)

Name of the Extract	Total Alkaloid mg AE /g dry weight
Ethanoilc extract	1.88+0.24 mg/gm dry weight
Methanolic extract	1.74+0.07 mg/gm dry weight
Aqueous extract	0.32 ±0.31 mg/gm dry weight

Table 6 : **The results were expressed as mean (± sd)

Data clearly shows, both Ethanolic and Methanolic leaf extracts of *Bulbophyllum rosemarianum* exhibited comparable levels of total Alkaloids

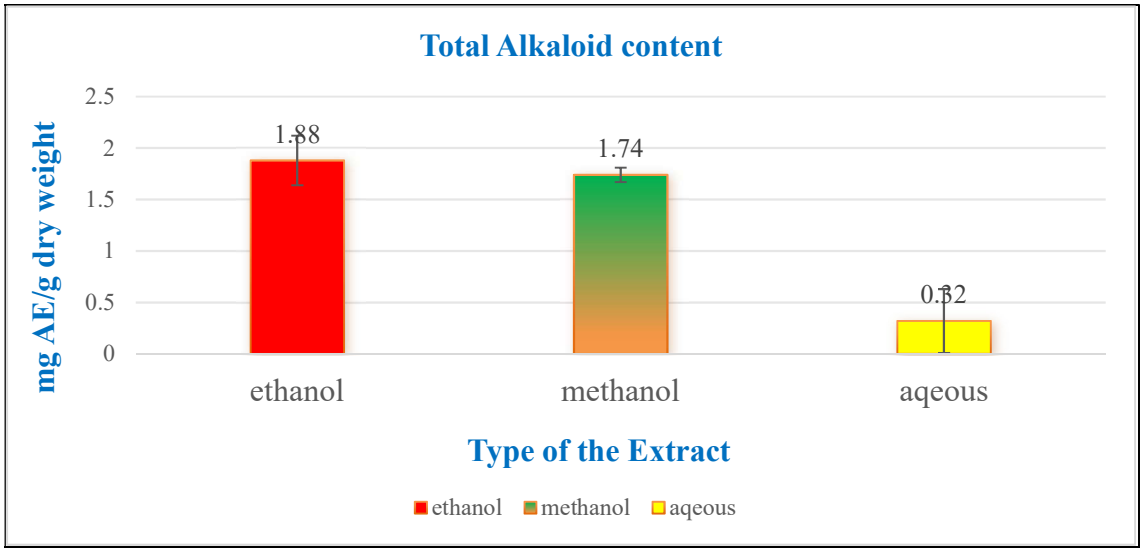


Figure 4: Comparison of total Alkaloids content in different extracts

3. Total Flavonoid content

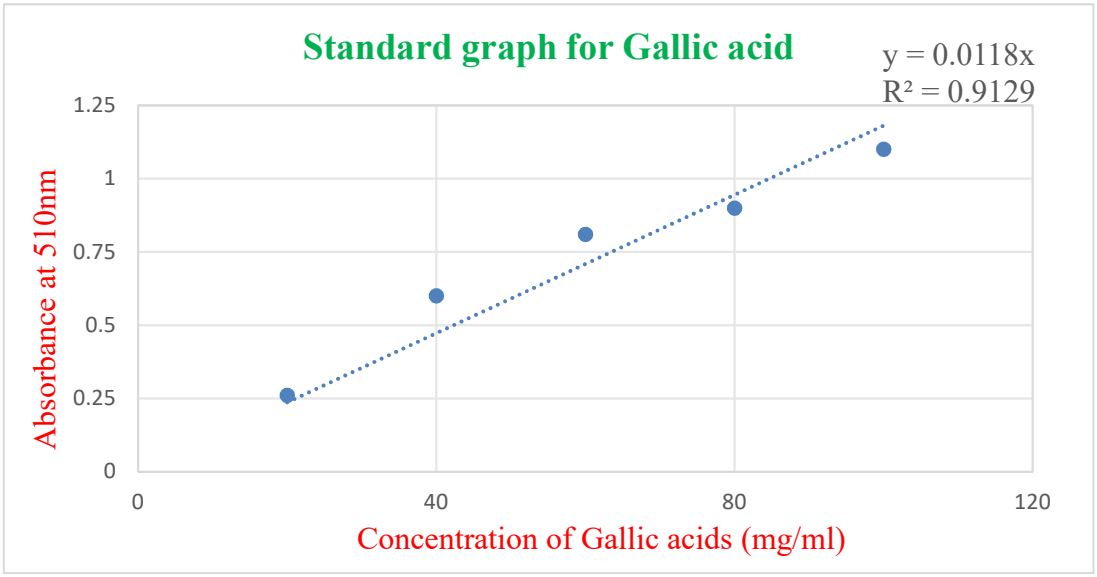


Figure 6: Standard calibration curve for Gallic acid

The total Flavonoid content in the extracts were calculated by using linear equation obtained from the standard calibration curve of standard Gallic acid (y = 0.0118 X, R^2 = 0.9129) and the results are expressed as Gallic acid equivalents (GAE mg/gm dry weight)

Name of the Extract	Total Flavonoid GAE mg/gm dry weight
Ethanoilc extract	29.61± 4.86 GAE mg/gm dry weight
Methanolic extract	22.98± 2.26 GAE mg/gm dry weight
Aqueous extract	17.72±1.17 GAE mg/gm dry weight

Table 7:**The results are expressed as mean (± sd)

Data clearly shows, both ethanolic and methanolic leaf extracts of *Bulbophyllum rosemarianum* exhibited comparable levels of total Flavonoids.

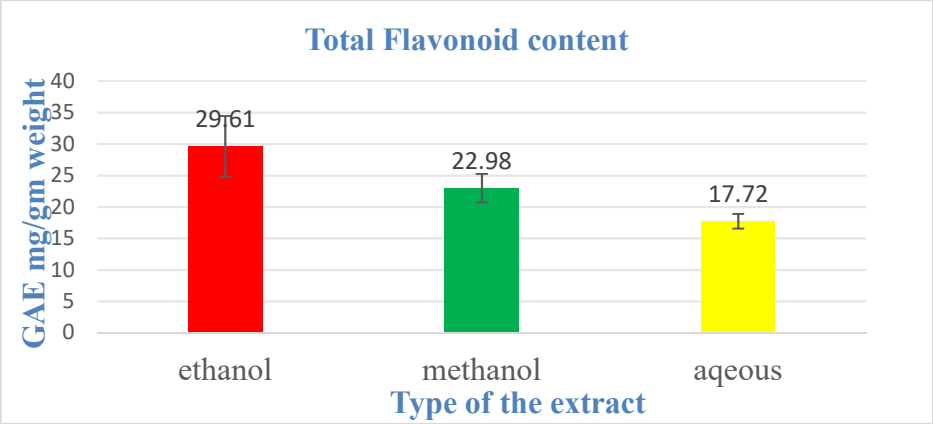


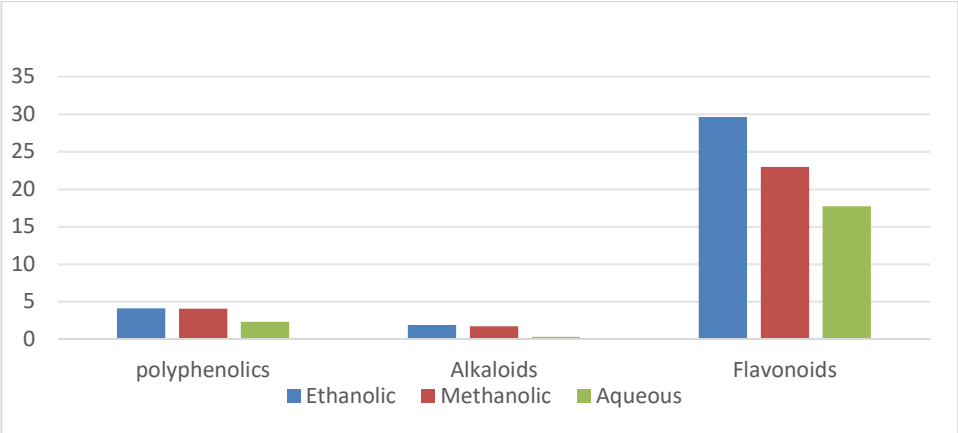
Figure 7: Comparison of total Flavonoids content in different extracts

Table 8: Total polyphenolics, Alkaloids and Flavonoids of different solvent of leaf extract of *Bulbophyllum rosemarianum*

Figure 8 :

Sl. No	Secondary metabolites chemical compound (mg/g)	Ethanolic Extract	Methanolic Extract	Aqueous Extract
1	Total Polyphenolics	4.1059±0.73	4.0534± 0.74	2.3281± 0.34
2	Total Alkaloids	1.88±0.24	1.74± 0.07	0.32 ±0.31
3	Total Flavonoids	29.61± 4.86	22.98± 2.26	17.72±1.17

Quantitative phytochemical analysis of*Bulbophyllum* leaf extracts



4. Discussion

India has an valid record of utilizing different Phytochemicals and natural medicines to combat different ailments. Globally, plants are regarded as fundamental source of raw materials for the synthesis of traditional and contemporary medications. Research on medicinally important orchid species contributes to the validation of orchid plant ability to treat illnesses and it is likely a better option to create affordable and efficient medications using the raw materials that are available. Historically, plants have been used as an excellent sources of medications and existence of bioactive

secondary metabolites is facilitating the invention of effective therapeutics. Phytochemicals are non nutritive molecules produced by plants which have protective or disease preventive properties. The therapeutic efficacy of most of the orchid species is yet to be investigated scientifically by considering their usage in traditional medicines as a remedy to treat many infections.

The extraction method used in extraction process is Soxhlet, an appropriate method for isolation of various compounds including those which have low temperature evaporation point and this method is considered to have advantageous properties and high recovery compared to other extraction methods, especially for crude extract from plant materials. The highest recovery in material extraction was found in the leaf Ethanol extract. This indicated that Ethanol could extract the highest numbers or concentration of phytochemicals in the leaf compared to other solvents in other plant organs.

The preliminary phytochemical screening analysis, useful in detection of the phytochemicals which are leading to the drug discovery and advancement in the field of medicine. However, a different number of phytochemicals have been discovered in orchids butnature still has many more. The therapeutic properties of orchids arise from the biologically active molecules present in them. Hence, the phytochemical contents were assessed qualitatively and Quantitativel in the *Bulbophyllum rosemarianum* leafextracts.

The phytochemical screening in the current study, showed the presence of Flavonoids, Terpenoids, Steroids, Coumarins, Glycosides and Quinones in methanolic leaf extracts and The Ethanolic leaf extracts showed the presence of Flavonoids, Terpenoids, Steroids, Coumarins, Tannins, Saponins, Glycosides and Quinones. The Aqueous leaf extracts showed the presence of Flavonoids, Terpenoids, Phenols, Saponins, Glycosides and Quinones. Quantitative analysis demonstrated that **ethanolic extract has** the highest levels of Polyphenolics, total alkaloids and Flavonoids. Allover findings suggested that ethanol is preferable solvent for phytochemical extraction from the leaf extracts compared to methanol and water.

5. Conclusion

In present scenario, researchers have made an intense effort in identifying novel bioactive compounds and their biological activity. The development of unique drugs has always been focused on natural compounds and their derivatives. Orchids are a rich source of bioactive substances with therapeutic value, which can influence metabolic activities and exhibit useful properties like an antioxidant effect, inhibition of receptor functions, induction or inhibition of enzymes and gene expression

Our present research indicates that ethanolic leaf extracts of *Bulbophyllum rosemarianum* have good source of potential phytochemicals which can be an beneficial medications with unique biological characteristics. In the future, this plant may be utilized to create synthetically superior medicinal compounds, which would increase the likelihood of creating even more inventive treatments. Although encouraging, further studies are ongoing by which this can be made a reality.

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