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PHARMACOGNOSTICAL STUDY AND PHYTOCHEMICAL SCREENING OF AN UNEXPLORED ORCHID, *BULBOPHYLLUM CRASSIPES* HOOK.F. WITH *IN-VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Abstract

Since ancient times, people have been using therapeutic herbs; in fact, this practice might have been the forerunner of modern medicine. It has been noted that the focus of worldwide research is shifting away from the cultivation or domestication of plant species and toward the discovery of novel medications or active chemicals. Because contemporary synthetic medication is unavailable to the developing world, traditional medicine based on the direct application of medicinal plants is still practiced in many parts of the world due to its low cost. *Bulbophyllum* is the largest genus of the family Orchidaceae. *Bulbophyllum crassipes* Hook.f. is one of the most important epiphytic orchid species amongst the 2199 species (POWO, 2025) of the genus *Bulbophyllum*. It has the special characters of single noded pseudobulb and basal inflorescence. Since ancient times, *Bulbophyllum* orchids have occupied a distinct place in human's life for treatment of a variety of ailments. Owing to insufficient data of this species on the medicinal aspect, there is an immediate need to carry out research on this particular area. In the present communication, pharmacognostic study such as organoleptic characters, macroscopic study, microscopic study, physicochemical analysis, phytochemical screening, and *in vitro* anti-oxidant, and anti-inflammatoy activity of *B. crassipes* are reported. The IC_{50} values for *in-vitro* anti-oxidant activity were reported to be 193.92, 192.12, 204.43, 406.86, 515.52 μgml^{-1}, and 34.01 μgml^{-1} for different extracts (hydroalcohol, methanol, ethyl acetate, petroleum ether, and ascorbic acid, respectively). For anti-inflammatoy activity the IC_{50} of hydroalcohol, methanol, ethyl acetate, and petroleum ether extracts were found as 695.09, 689.65, 728.06, 831.60,854.75 μgml^{-1} respectively, where the IC_{50} of diclofeinac sodium was observed as 167.30 μgml^{-1}.

Introduction

AS ONE of the largest families of angiosperms, the family Orchidaceae holds significant value in ornamental, horticultural, and medicinal fields. Orchids were mentioned in *Charaka Samhita* which was written by Charaka. The *Jeevanti* formulation of Ayurveda has been using *Eulophia dabia, Flickingeria nodosa,* and *Malaxis densiflora,* since ancient times. Orchids like *Crepidium acuminatum*, *Habenaria intermedia*, *Herminium edgeworthii*, and *Malaxis muscifera*, have been used in the Ayurvedic fomulation (*Chyawanprash)* for a long time (Tsering *et al*., 2017). The Ayurvedic fomulation *Astavarga* contains*, Crepidium acuminatum, Habenaria intermedia, Herminium edgeworthii,* and *Microstylis wallichii* orchids. It is found in Sanskrit literature that the leaves of *Vanda roxburghii* had been used in the treatment of rheumatism, fractures, ear infections, and nervous system disorder (Hossain, 2011). China and Japan also described orchids in medicinal use from 3000 to 4000 years ago. The Chinese Emperor *Shen-Nung* described *Bletilla* and *Dendrobium* species in his *Materia Medica*. A large number of *Dendrobium* species are used in traditional Chinese medicine as tonic, astringent, analgesic, and for anti-inflammatory treatment. *Sowa-Rigpa* system of traditional medicine also describes *Cypripedium himalaicum, Dendrobium densiflorum,* and *Gymnadenia conopsea* orchids. The traditional healers of Indian Subcontinent are using a wide range of orchids like *Cymbidium aloifolium, C. giganteum, Dactylorhiza hatagirea, Dendrobium fimbriatum, D. moschatum, D. nobile, Eria muscicola, Eulophia dabia, Liparis odorata, Papilionanthe teres, Phaius tankervilleae, Vanda coerulea, V. cristata,* and *V. tessellata*. Orchids are also being used as useful herbs for the treatment of various diseases in other Asian countries like Malaysia, Mayanmar, Singapore, Thailand, and Vietnam (Pant, 2013).

Though various attempts have been carried out in pharmacognostical studies and phytochemical screening in different orchid species (Hoque *et al*., 2021, 2024; Kumari and Pathak, 2021, 2025a,b; Paul *et al*., 2022; Rahman *et al*., 2023; Sharma and Pathak, 2024). There is currently no available information on phytochemical screening, pharmacognostic analysis, or identifying the anti-inflammatory and antioxidant qualities of the presently studied species. During the present study, anti-inflammatory and antioxidant activity are evaluated using various fractionated extracts (Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydro alcohol). The leaves and pseudobulbs of *Bulbophyllum crassipes* Hook.f were used in the whole study.

Material and Methods

The plants of the *Bulbophyllum crassipes* were collected from the campus of North Bengal University, West Bengal,

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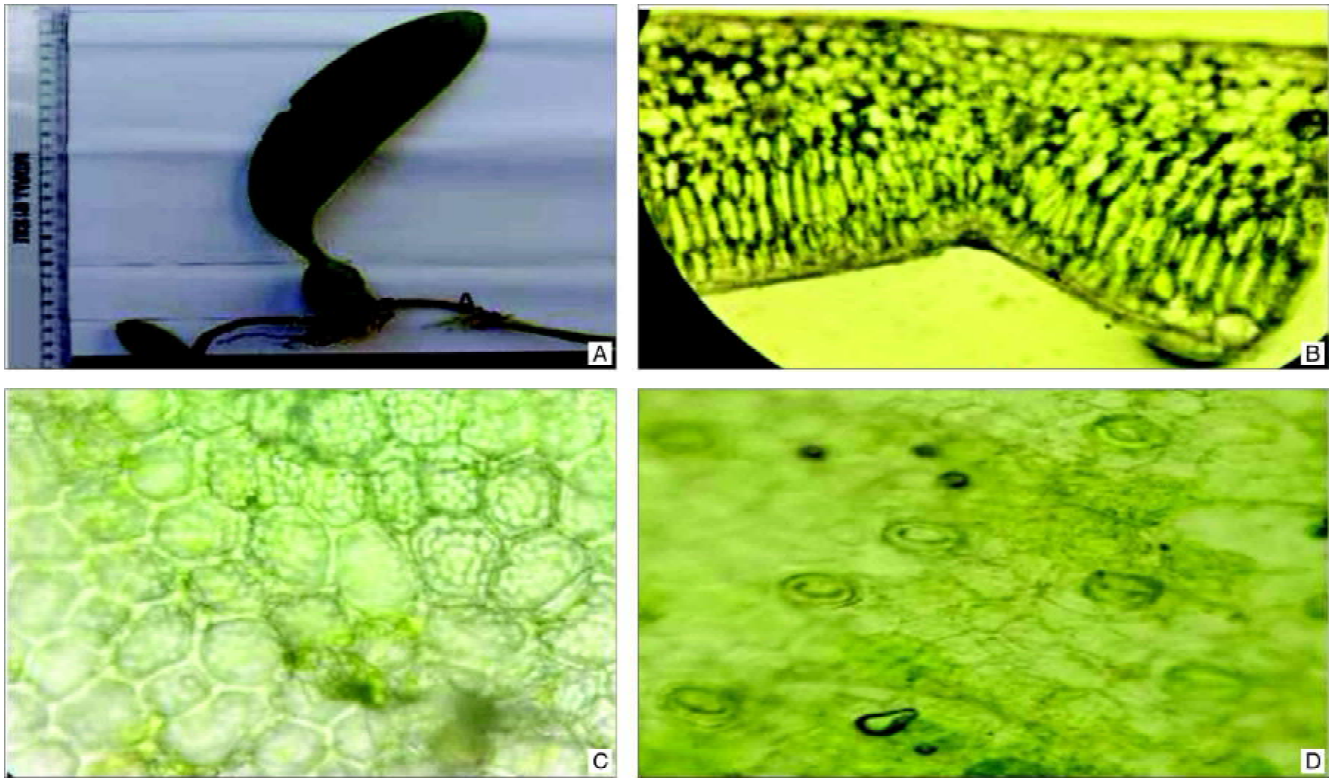

Fig. 1A-D. *Bulbophyllum crassipes* Hook.f.: A, Whole Plant; B, T.S. of leaf; C, The absence of stomata, Upper epidermis; D, The presence of stomata, lower epidermis.

India. The prepared herbarium was sent to The Botanical Survey of India for the identification and authentication purpose with specimen No. NBU/PT/06-2021 dated 01-04-2021. The plant was identified and authenticated by Scientist-E, Mr. K Karthigeyan, Botanical Survey of India, Central National Herbarium, Howrah, India with reference No. CNH/Tech.II/2021/13. The collected plants were washed with tap water to remove sand *etc*. and was allowed to dry. The leaves and pseudobulbs were separated in different trays. Then these were sliced into small pieces and allowed to shade dry. After the drying, these were coarse powdered by mechanical grinder. The coarse powder of both parts was extracted by successive cold maceration process using different solvents like Petroleum ether, Chloroform, Ethyl acetate, Methanol, and Hydro alcohol (cf. Hoque *et al*., 2021).

The macroscopic studies were performed by using organoleptic evaluation methods. The arrangement, base, colour, size, shape, apex, margin, venation, taste, and odour of leaves and pseudobulbs were observed, by following the quality method (Arora *et al*., 2023). Evaluations were conducted on the following: Swelling Index, Loss on Drying, Alcohol Soluble Extractive Value, Water Soluble Extractive Value, and extractive value in various solvents, and phytochemical screening (Tatiya *et al*., 2012). Antioxidant and anti-inflammatoy activity was performed by DPPH and egg albumin coagulation methods, respectively (Rahman and Huda, 2021; Robustelli *et al*., 2019).

Macroscopic Evaluation

The macroscopic study is associated with the visual examination of physical properties such as arrangement, size, shape, colour, odour, taste, appearance, margin, apex, base, surface, texture, venation, and presence of petiole (Anjum *et al*., 2024)

Microscopic Evaluation

The microscopic study was performed by ZEISS phase contrast microscope at 10X and 40X magnifications. The transverse section (T.S.) of leaf was examined at 10X, 20X, and 40X objective lens magnifications. Stomata were searched at lower and upper epidermis (Liu *et al*., 2022).

Swelling Index

The swelling index was conducted as per World Health Organization quality control methods for herbal materials (Pandiyan and Ilango, 2022; WHO, 2011).

Loss on Drying (*LOD*)

Three grams of ground sample was dried at 105°C until constant weight was obtained to determine loss on drying (WHO, 2011).

Alcohol Soluble Extractive Value

Five grams of the coarse powder of both leaf and pseudobulbs was weighed accurately and placed in conical flask with a glass stopper separately in 100 ml of the methanol (Rafi *et al*., 2020).

Water Soluble Extractive Value

Five grams of the coarse powder of both leaf and pseudobulb was weighed accurately and placed in conical flask with a glass stopper separately in 100 mL of the water (Rafi *et al*., 2020).

Extractive Value in Different Solvents by Successive Extraction

According to polarity, petroleum ether, chloroform, methanol and, hydro alcohol were used as solvents. The extraction was carried out by maceration procedure. The yield value of petroleum ether extract was very poor whereas it increased gradually with the higher polarity.

Preliminary Phytochemical Screening the Leaf and Pseudobulb Extracts

Test for carbohydrates

(A) Molisch's Test: The extract was treated with 1% alcoholic α-naphthol solution in presence of concentrated sulphuric acid. The violet colour appeared due to presence of carbohydrates.

(B) Fehling's Test: The solution of extract was treated with equal volume of Fehling's A and Fehling's B solution and heated for sometimes. The presence of brick red colour precipitate indicated the presence of carbohydrates (Sonam *et al*., 2017).

Test for Alkaloids

(A) Dragendorff's reagent test: The solution of extract was treated with a few drops of Dragendorff's reagent. Appearance of orange precipitate indicates the presence of alkaloids.

(B) Wagner reagent test: A few drops of Wagner's reagent were added with the extract solution. In presence of alkaloids, brown to reddish brown colour precipitates were observed.

(C) Hager's reagent test: A few drops of saturated picric acid solution were added with the solution of extract. The appearance of crystalline precipitate represents the presence of alkaloids (Harwoko and Warsinah, 2020).

Test for Glycosides

(A) Kellers kiliani Test: The solution of extract was added with acetic acid. Then ferric chloride solution was traced with it. Then a few drops of concentrated sulphuric acid were added at the side of test tube. A reddish brown colour turns blue to green at the junction.

(B) Legal Test: Pyridine and sodium nitropruside were added in the solution of extract. Then it was made alkaline with the solution of sodium hydroxide. Appearance of pink to red colour indicates the presence of cardiac glycosides.

(C) Raymonds Test: Methanolic sodium hydroxide was added with solution of extract. Formation of violet colour indicates the presence of cardiac glycosides (Li *et al*., 2020).

Test for Flavonoids

(A) Shinoda Test: The magnesium turnings were added into the solution of extract. Then concentrated sulphuric acid was added. The appearance of red colour represents the presence of flavonoids.

(B) Alkali Test: Small amount of 10 % sodium hydroxide solution was added into the solution of extract and allowed to stand for some times. The yellow colour solution turns to colourless, in presence of flavonoids.

(C) Acid Test: A few drops of concentrated sulphuric acid were added into the test solution. A yellow orange colour indicates the presence of flavonoids (Kumar *et al*., 2020).

Test for phenolic compounds

(A) Lead Acetate Test: Small amount of 10 % Lead acetate was added into the test solution. White precipitate indicates the presence of poly phenols.

(B) Acetic acid Test: Small amount of acetic acid was added with the test solution. Formation of transient red colour indicates the presence of polyphenols.

(C) Iodine Test: A few drops of dilute iodine solution were added with test solution. Yellow colour appears in the presence of polyphenols (Suleria *et al*., 2020).

Test for Tannins

A few drops of 5% ferric chloride solution were added with the test solution. Formation of blue to black colour indicates the presence of tannins (Hayat *et al*., 2020).

Test for Saponin

Small amount of test solution was diluted with water and shaken vigorously. Formation of permanent foam indicates the presence of saponin (Mohlakoana, 2020).

Test for Mucilage

Small amount of test solution was diluted with water and ruthenium red solution was added with test solution. Formation of pink colour indicates the presence of mucilage (Cowley *et al.,* 2020)

In Vitro Antioxidant Activity

The measurement of *in vitro* anti-oxidant activity was performed so as to evaluate the radical scavenging activity of different extracts using the DPPH method (Mensor *et al*., 2001). The different extract (hydroalcohol, methanol, ethyl acetate, and petroleum ether) solution of pseudobulbs were treated with 1 ml of DPPH (0.3 mm) solution. The solutions (50 µl, 100 µl, 200 µl, 300 µl, and 400µl) were stored in a dark place for 30 min. Then absorbance was measured at 517 nm against the blank. Each test was repeated thrice and the mean absorbance was recorded. The percentage of DPPH scavenging activity was calculated by using following equation (Ashraf *et al*., 2021).

Percentage inhibition (%) = $[(A_c - A_t)/ A_c] \times 100$

Where, A_c = Absorbance of Control

A_t= Absorbance of Test

In Vitro Anti-inflammatory Activity

This *in vitro* assay carried out was based on the method (Jajo *et al*., 2024; Madhuranga and Samarakoon, 2023). The different extract (hydroalcohol, methanol, ethyl acetate, and petroleum ether) solution of pseudobulbs (50µl, 100µl, 200µl, 300µl, 400µl) and fresh 0.2 ml egg albumin were mixed with 2.8 ml of phosphate buffer (pH 6.4) and incubated in an incubator at 37°C for 15 min. Then these were heated at 70°C on water bath for 5 min and the reaction mixtures were allowed to cool down at room temperature for 15 min. The absorbance was measured at 660 nm against the blank. Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was calculated by using following equation (Lakshmi *et al*., 2020).

Percentage inhibition (%) = $[(A_c - A_t)/ A_c] \times 100$

Where, A_c = Absorbance of Control

A_t= Absorbance of Test

Results and Discussion

Presently, pharmacognostic study such as organoleptic characters, macroscopic study, microscopic study, physicochemical analysis, phytochemical screening, and *in vitro* anti-oxidant, and anti-inflammatoy activity were studied in *B. crassipes.* The results are briefly described as follows:

Macroscopic Evaluation

During the present investigation, visual examination of physical properties such as arrangement, size, shape, colour, odour, taste, appearance, margin, apex, base, surface, texture, venation, and presence of petiole were made (Table 1).

Table 1. Macroscopic evaluation.

Characters	Observations	
	Leaf	Pseudobulb
Arrangement	Solitary	Solitary
Size	(9-21 cm × 2-3.5)cm	(3.5-5cm × 3-4.5cm)
Shape	Oblong	Ovate
Colour	Green	Green
Odour	Characteristic	Characteristic
Taste	Pungent	Pungent
Appearance	Smooth	Smooth but furrows when dried
Margin	Entire	Smooth
Apex	Obtuse	Obtuse
Base	Symmetrical	Symmetrical
Surface	Smooth	Smooth
Texture	Smooth	Smooth
Venation	Parallel	—
Petiole	Sessile	—

Microscopic Evaluation

Vascular bundles were present. At the periphery, the vascular bundles were smaller but were larger at the centre. The outer layer of epidermis was made up single layer parenchyma cells with sunken stomata (Fig. 1A-D). The epidermis was covered with waxy cuticle. The presence of mucilage cells is a unique characteristic feature for authentication and standardization. Stomata were present on abaxial surface but were absent on adaxial surface.

Swelling Index

The swelling index of pseudobulbs was higher than the leaves. Swelling index of the pseudobulbs and leaves are indicated in Table 2. The swelling properties are enhanced due to the presence of mucilage.

Loss on Drying (*LOD*)

The loss on drying of pseudobulbs was found to be higher than in the leaves. The loss on drying (LOD) is an important parameter for crude drug evaluation (Table 2).

Table 2. Physicochemical parameters of leaf and pseudobulb.

Parameter	Observations for Leaf	Pseudobulb
Swelling index	0.8 ±0.03 ml	1.6 ±0.19 ml
Loss on drying	7±0.05%	12±0.13 %
Alcohol soluble extractive value	8±0.07 %	7.2 ±0.11 %
Water soluble extractive value	19.2 ±0.08 %	67.60 ±0.02%

Alcohol Soluble Extractive Value and Water Soluble Extractive Value

The water soluble extractive values were observed as more than the extractive values of alcohol soluble. Extractive values play an important role in pharmacognostical study (Table 2). The extracts contained a lot of primary and secondary metabolites.

Extractive Value in Different Solvents by Successive Extraction

According to polarity petroleum ether, chloroform, methanol and water were used as solvent. The extraction was carried out by maceration procedure. The yield value of petroleum ether extract was very poor whereas gradually increased with the higher polarity (Table 3).

Table 3. Extractive value in different solvents of leaf and pseudobulb.

Solvent extract	Percentage of yield (w/w) in Leaf	Pseudobulb
Petroleum ether	0.91	1.19
Chloroform	5.98	4.87
Methanol	7.12	12.72
Water	17.32	23.29

Preliminary Phytochemical Screening of the Leaf Extracts

Phytochemical screening test is the most effective qualitative test for primary and secondary metabolites of natural sources. Alkaloids, glycosides, tannins, reducing sugar, and phenols were detected in chloroform, methanolic, and water extract of the leaf (Fig. 2A-H). The percentage of alkaloids was found to be higher in chloroform extract than that in the water extract. The presence of saponins was prominently found in the water extract. The '-' indicates absence of phytochemicals in test whereas '+', '++' and '+++' indicates its poor, medium, and high quantity, respectively (Table 4). The phytochemical test of leaf extracts reported a variety of metabolites and this information may be used for further researches.

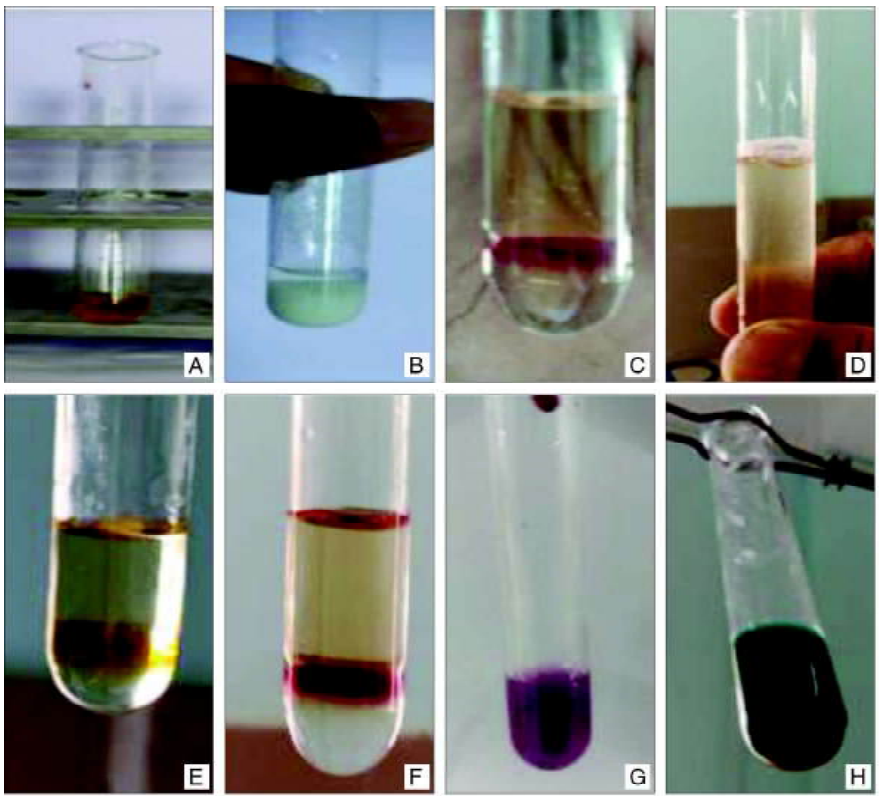

Fig. 2. A, Dragendorff's test; B, Mayer's test; C, Molisch test; D, Foam test; E, Keller-Kiliani test; F, Salkowski test; G, Ruthenium red test; H, Fehling test.

Preliminary Phytochemical Screening of the Pseudobulbs Extracts

The qualitative phytochemical screening represents the primary and secondary metabolites. A simple chemical test may play an important role to detect pharmaceutical constituents. In the present work, alkaloids, glycosides, tannins, and saponins were successfully screened. The '-' indicates the absence of the phytochemical in test whereas '+', '++' and '+++' indicate poor, medium and high quantity, respectively in pseudobulbs (Table 5). Saponins and tannins are present in methanol and water extracts. These phytochemical tests will be useful further in future prospect.

In Vitro Antioxidant Activity

This assay describes the reduction power of various concentrations of extract and standard (ascorbic acid) which are responsible for reduced $DPPH_2$. The absorbance was detected at 517 nm due to occurrence of

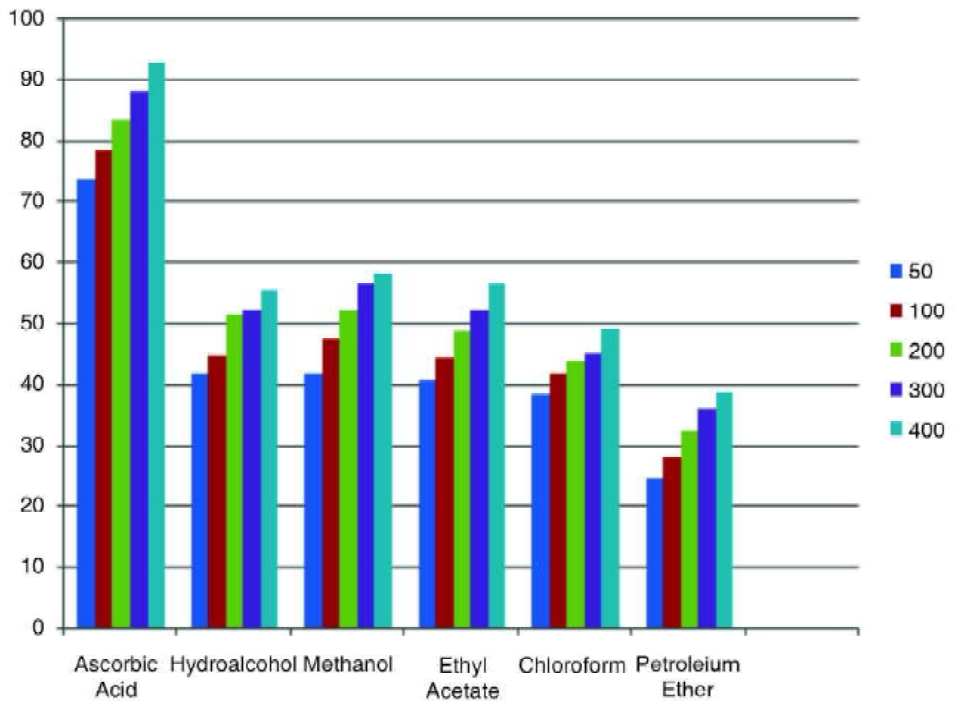

Fig. 3. *In vitro* anti-oxidant activity of pseudobulb.

decolourization. Fig. 3 represents the graph between the percentage of radical scavenging activity and concentrations which showed a constant increment in scavenging property of pseudobulb extract and the standard (ascorbic acid). The IC_{50} values were reported to be 193.92 μgml^{-1}, 192.12 μgml^{-1}, 204.43 μgml^{-1}, 406.86 μgml^{-1}, 515.52 μgml^{-1}, and 34.01 μgml^{-1} for different extracts (hydroalcohol, methanol, ethyl acetate, and petroleum ether) and ascorbic acid, respectively.

In Vitro Anti-inflammatory Activity

This assay is evaluated by *in vitro* anti-inflammatory effect of different extract (hydroalcohol, methanol, ethyl acetate, and petroleum ether) solutions of pseudobulb against denaturation of egg albumin. The absorbance was detected at 660 nm of different extract solutions of pseudobulb and diclofenac sodium respectively. The results are summarized in Fig. 4. The IC_{50} of hydroalcohol, methanol, ethyl acetate, and petroleum ether extracts were 695.09, 689.65, 728.06, 831.60, 854.75 μgml^{-1} respectively, where the IC_{50} of diclofenac sodium was found as 167.30

Table 4. Preliminary phytochemical screening in leaf extracts of *B. crassipes*.

Test/Reagent	Petroleum Ether extract	Chloroform extract	Methanol extract	Aqueous extract
Carbohydrates/ Molisch's Test	-	++	+++	+++
Carbohydrates/ Fehling's Test	-	++	+++	+++
Alkaloids/ Dragendorff's reagent	-	++	++	++
Alkaloids/ Wagner reagent	-	+++	+++	+++
Alkaloids/ Hager's reagent	-	+++	+++	+++
Glycosides/ Kellers kiliani Test	-	++	+++	+++
Glycosides / Legal Test	-	++	+++	+++
Glycosides/ Raymonds Test	-	++	+++	+++
Flavonoids/ Sinoda Test	-	-	-	-
Flavonoids /Alkali Test	-	-	-	-
Flavonoids /Acid Test	-	-	-	-
Phenolic compounds/ Lead Acetate Test	-	-	++	+++
Phenolic compounds / Acetic acid Test	-	-	++	+++
Phenolic compounds / Iodine Test	-	-	++	+++
Tannins	-	++	+++	++
Saponins	-	-	++	+++
Mucilage	-	-	-	+

'+', '++' and '+++' indicates poor, medium and high quantity, respectively and '-' indicates absence of phytochemicals.

Table 5. Preliminary phytochemical screening in pseudobulbs extract of *B. crassipes*.

Test/Reagent	Petroleum Ether extract	Chloroform extract	Methanol extract	Aqueous extract
Carbohydrates/ Molisch's Test	-	+++	+++	+++
Carbohydrates/ Fehling's Test	-	++	+++	+++
Alkaloids/ Dragendorff's reagent	-	++	++	++
Alkaloids/ Wagner reagent	-	+++	++	+++
Alkaloids/ Hager's reagent	-	+++	+++	+++
Glycosides/ Kellers kiliani Test	-	++	+++	+++
Glycosides / Legal Test	-	++	+++	+++
Glycosides/ Raymonds Test	-	++	+++	+++
Flavonoids/ Sinoda Test	-	-	-	-
Flavonoids /Alkali Test	-	-	-	-
Flavonoids /Acid Test	-	-	-	-
Phenolic compounds/ Lead Acetate Test	-	-	++	+++
Phenolic compounds / Acetic acid Test	-	-	++	+++
Phenolic compounds / Iodine Test	-	-	++	+++
Tannins	-	+++	+++	++
Saponins	-	-	++	+++
Mucilage	-	-	-	+++

'+', '++' and '+++' indicates poor, medium and high quantity, respectively and '-' indicates absence of phytochemicals.

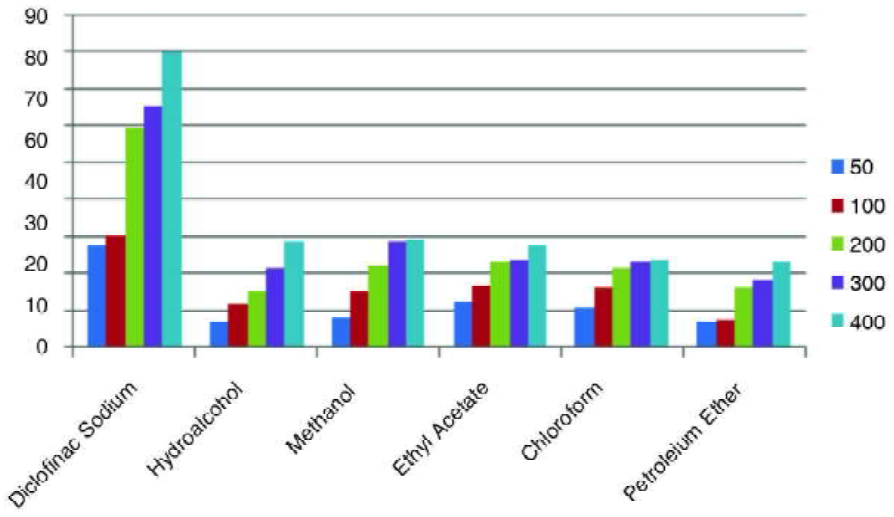

Fig. 4. *In vitro* anti-inflammatory activity of pseudobulb.

µgml^{-1}. The effect was may be due to the presence of flavonoids, phenolics, alkaloids, and tannins, which are all known for their anti-inflammatory activities.

Conclusion

Traditional knowledge of the past and present ethnomedicine is an integral part for the development of newer drug compounds. The present research on pharmacognostical and phytochemical screening in *B. crassipes* was successfully conducted. The detected alkaloids, glycosides, tannins, saponins, and mucilage may play an important role in further research. Further, the strong antioxidant and anti-inflammatory activities indicate positive therapeutic potential in the species. Experimental and clinical studies may be conducted further in the detected mucilage and also isolation and structure elucidation of detected alkaloids, glycosides, tannins and saponins need to be carried out.

Acknowledgements

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