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Pancratium verecundum: A Comparative Analysis of Leaf and Bulb Extracts

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ABSTRACT

Leaf and bulb extracts of *Pancratium verecundum* were explored for their phytochemical, antioxidant and cytotoxic activities. Four solvent extract for each plant part were prepared these were namely hexane, chloroform, acetone and methanol using soxhlet extraction method. Phytochemical analysis revealed the presence of flavonoids in both leaves and bulbs, whereas bulb extracts also consisted of saponin, terpenoids and terpenins. Cytotoxic activity of acetone extract was maximum in both the parts with 74% in bulb and 63% in leaf extracts. Leaf exhibited good antioxidant activity in acetone and methanol (79 & 81%) whereas antioxidant activity of chloroform and acetone extract (83 and 76% respectively) was recorded in bulbs.

INTRODUCTION

Pancratium verecundum belongs to family Amaryllidaceae, which is endowed by a number of important medicinal plants [1]. Alkaloids of the family have been explored for antimicrobial, anti-inflammatory and anticancer potential[2,3]. Family is also rich source of medicinally important molecule like Galantamine which is being used in Alzheimer's disease as potent inhibitor of acetylcholinesterase[4]. Amongst the parts used in studies are bulb and leaves in case of *Pancratium illyrycum*[5].

Pancratium verecundum is exclusively confined to India and is known for fleshy bulbs and fragrant flowers[6]. Same has not been explored for its medicinal usage. Keeping in view of the medicinal potential of Pancratium genus, scarcely explored species of i.e., *Pancratium verecundum* was selected for exploration. As leaf and bulb has shown activity in other species so same parts were explored for their antioxidant and cytotoxic activities. Phytochemical analysis was also conducted.

MATERIALS AND METHODS

Collection and processing of medicinal plants

Pancratium verecundum leaves and bulbs were collected from the medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar. Samples were dried in shade and made into fine powder using mechanical grinder.

Extraction of plant sample: Successive Solvent extraction was done by the standard method of soxhlet extraction technique for the preparation of crude extracts[7]. Four extracts namely hexane, chloroform, acetone and methanol were prepared for each sample. Semisolid extracts were stored in tight screw cap vials till further use.

Phytochemical analysis

Phytochemical analysis was conducted using standard protocols[8,9]. A brief account of the different tests conducted is as follows:

Phlobotannin: 1 ml of aqueous 1% HCl was added to 1 ml of sample followed by boiling. A red precipitate is indicative of presence of phlobotanins.

*Alkaloids***:** 2 ml of 1% aqueous HCl was added to 1 ml of solvent extract. Thereafter it was heated for few minutes. 2 drops of dragondroff reagent was added to the solution. Reddish brown precipitate with turbidity depicts alkaloid's presence.

*Flavonoids***:** To 5 ml of extract, 1 ml of 10% NaOH solution was added. From the side of the beaker 2 drops of concentrated HCl was added. Yellow colour turning to colourless is an indication of presence of flavonoids.

*Anthraquinone***:** To 1 ml of extract, 2 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed. Pink colour shows the presence of anthraquinones.

*Saponins***:** About 2 ml of 1% sodium bicarbonate was added to 1 ml of extract and shaken. Lather like formation persistent for some time is indicative of presence of Saponins.

*Steroids***:** 100 µl extract *was* taken in a test tube and 400 µl of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Brown ring at the boundary of mixture shows the presence of steroids. (N.B. Test tube was kept in ice as exothermic reaction occurs.)

*Glycosides***:** 100 µl extract was taken in a test tube and 400 µl of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Blue-Green colour showed the presence of glycosides.

Tannin: 1gm of sample added with 100ml of distilled water, boiled and cooled, and then filtered. 1% ferric chloride was added dropwise to the filtrate. Green black precipitate shows the presence of tannin.

Determination of total phenolic content

The total phenolic contents of the plant were determined by Folin's ciocalteu method[10]. Extracted samples of both the parts (100µl) were mixed with 750µl of Folin Ciocalteu reagent (10 fold dilution with distilled water) and incubated at 25ºC for 5 min. Then 750µl of sodium carbonate (60 g/L) solution was added to the mixture. Following 90 min incubation at 25ºC, absorbance was measured at 725 nm using UV-visible spectrophotometer. The total phenolic content was measured using a standard curve of gallic acid at 0.02 – 0.1 mg/mL concentrations. Total phenolic content was calculated for each sample and expressed as milligrams of gallic acid equivalent per 100 mL of sample.

Brine shrimp lethality test:

Brine shrimp (*Artemia salina*) eggs were incubated for 48hrs in 8% saline to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts was prepared at a concentration of 10mg/ml. All the extracts were evaluated at doses 25, 50,100 and 200microgram/ml. For each dose level, three replicates were done.

After 24hrs number of live larvae were counted and compared with the experimental control and percentage of inhibition was calculated by comparing the number of surviving larvae in treated samples with the controls.

Antioxidant activity:

To detect antioxidant activity, qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay was carried out. The plates were first air dried and then the chromatograms were sprayed with 0.2% 2, 2, diphenyl-2-picryl-hydrazyl in methanol as an indicator [11]. The presence of antioxidant compounds was detected by yellow spots against a purple background on the TLC plates sprayed with 0.2%DPPH in methanol. About 5µl of each sample was loaded on the TLC sheet and the chromatograms are developed in following solvent systems:

- Ethyl acetate/methanol/water (40:5.4:4)[EMW] (polar neutral);
- Chloroform/ ethyl acetate/ formic acid(5:4:1)[CEF](intermediate polarity/acidic);
- Benzene/ethanol/ammonium hydroxide (90:10:1)[BEA](Non-polar/basic)[12]

Quantitative analysis using radical scavenging DPPH assay

DPPH radical scavenging assay: - The radical scavenging activity of different extracts against DPPH was determined spectrophotometrically by the method of *Sanchez et al*[13]. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 50µl of 1mM DPPH solution and 100 µl of various concentrations of plant extracts (31.25, 62.5, 125, 250, 500 and 1000µg/ml) were prepared in methanol. A test tube containing only100µl of methanol and 50 µl of DPPH solution was taken as control. Then the tubes were incubated in dark for 30 min at room temperature. The

yellow colour chromophore formed was measured at 517nm using Multiplate Reader. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.

$$\% \text{ of inhibition } (\%I) = \frac{A(\text{control})-A(\text{sample})}{A(\text{control})} \text{ X } 100$$

Where $A_{control}$ is absorbance of control and A_{sample} is absorbance of sample.

Ascorbic acid was used as standard antioxidant and IC50 of solvent extracts was compared with the standard.

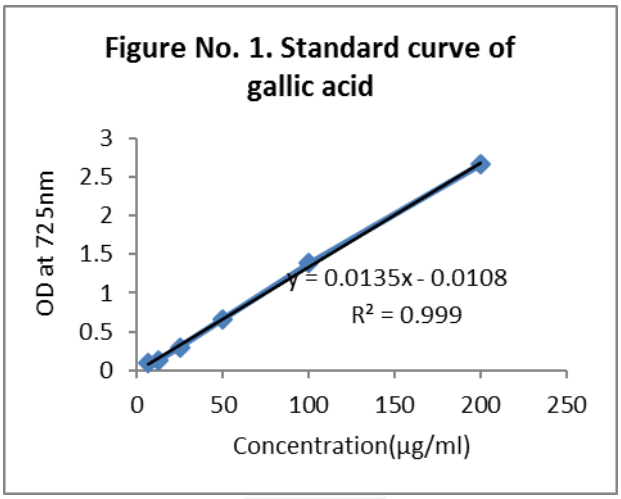
RESULTS AND DISCUSSIONS

Phytochemical analysis- The phytochemical analysis of the leaves and bulbs of *Pancratium verecundum* revealed that only flavonoids were present in leaf extracts (hexane, acetone, and methanol) of *Pancratium verecundum* whereas bulb extracts consisted of a number of phytochemicals like Terpenoids, saponins, triterpenes and proteins were present in Hexane and chloroform extracts, while flavonoids, saponins and proteins were present in acetone and methanol extracts(Table 1). Presence of flavonoids in leaf extracts indicate the possibility of antioxidant properties in leaf extracts[14]. Besides phytochemical analysis, protein was also found in the bulb extract, this is possible as bulb and corms are the storehouses of nutrients, besides the bulbs are used as famine food[15], hence presence of protein indicates the nutrition value of bulbs which was absent in leaf extracts.

Table No. 1: Phytochemical analysis of leaf and bulb extracts.

Phytochemical analysis	Hexane extracts		Chloroform extracts		Acetone extracts		Methanol extracts	
	leaf	bulb	leaf	bulb	leaf	bulb	leaf	bulb
ALKALOIDS	-	-	-	-	-	-	-	-
TERPENOIDS	-	+	-	+	-	-	-	-
GLYCOSIDES	-	-	-	-	-	-	-	-
TANNINS	-	-	-	-	-	-	-	-
FLAVONOIDS	+	-	-	-	+	+	+	+
SAPONINS	-	+	-	+	-	+	-	+
ANTHRAQUINONE	-	-	-	-	-	-	-	-
TRITERPENES	-	+	-	+	-	-	-	-
PROTEIN	-	+	-	+	-	+	-	+

Total phenolic content: Total phenolics content of leaf and bulb extracts of *Pancratium verecundum* was compared. Gallic acid was taken as standard and standard curve(Fig 1) was drawn in order to estimate Milimolar(MM) concentration of GAE (Gallic acid equivalent) of each extract(hexane, chloroform, acetone and methanol). Then GAE±SD was calculated from mean value. Comparative analysis of total phenolic content estimation between leaf and bulb extracts of *Pancratium verecundum* revealed that Acetone extracts of leaf was rich in total phenolics content (52.92) whereas amongst solvent extracts of bulb methanolic extract was the best(59.84GAE).



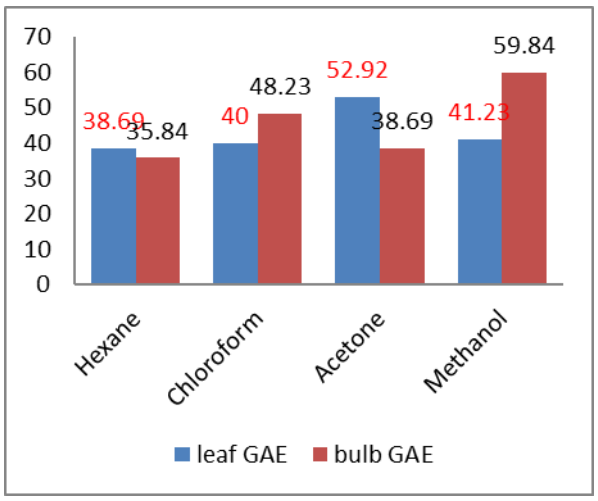


Figure No. 2: Phenolic content of extracts

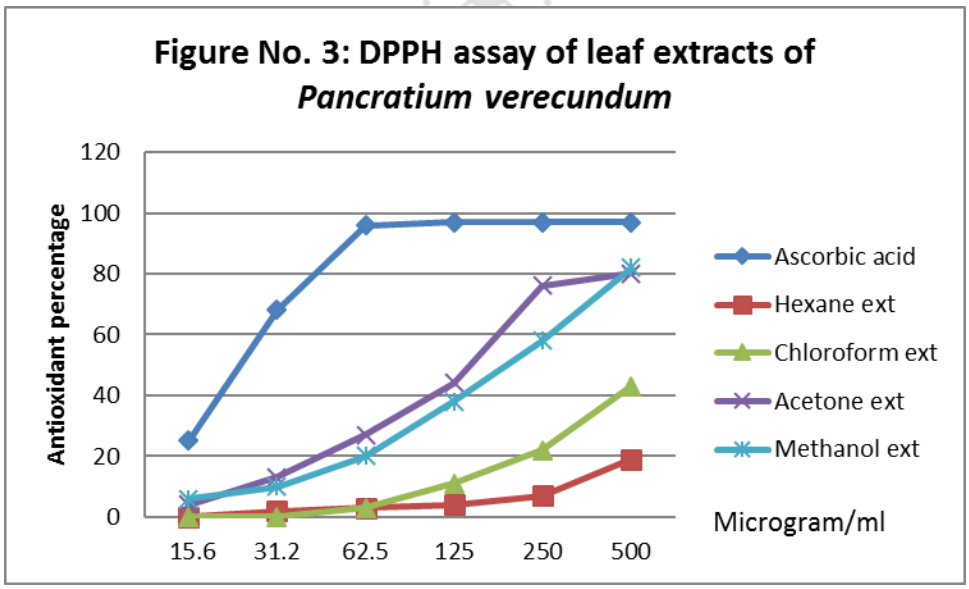
Antioxidant activity- Qualitative

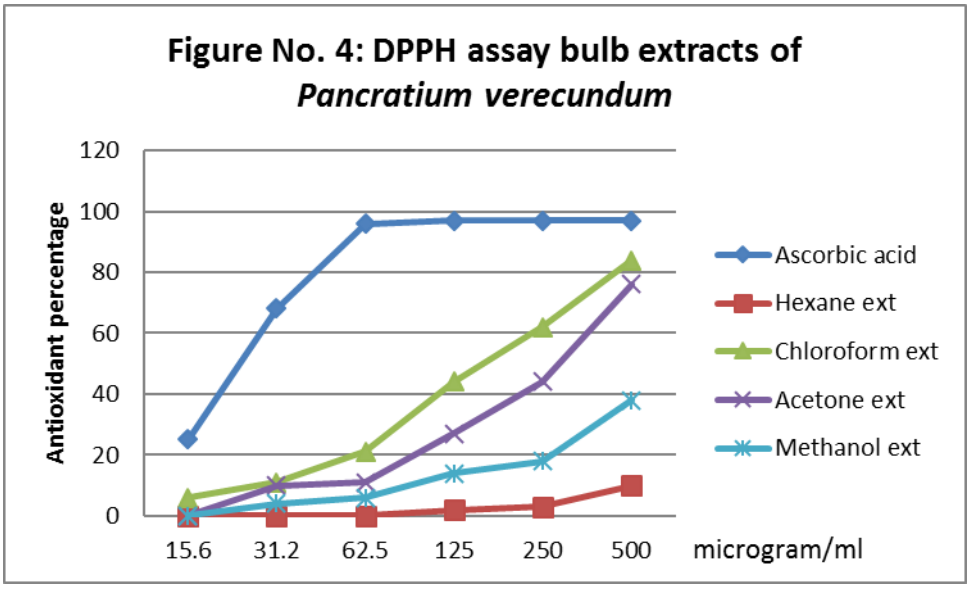
TLC-based antioxidant assay of leaf and bulb extracts of *Pancratium verecundum*: TLC-based antioxidant activities were done in leaf and bulb extracts of *Pancratium verecundum*. Result revealed that, BEA(benzene, ethanol and ammonium hydroxide) was considered as

best mobile phase solvent for separation of hexane, chloroform, acetone and methanol extract for leaves of *Pancdsratium verecundum* when compared to CEF(chloroform, ethyl acetate, formic acid) and EMW(ethyl acetate, methanol, water). However, only 5 antioxidants bands were observed in leaf extracts, whereas streak was obtained in bulb extracts suggesting a large number of molecules could not be separated as were of very close molecular weights.

Quantitative antioxidant activity: DPPH free radical scavenging assay

DPPH free radical scavenging assay was used to investigate the antioxidant potential of leaf and bulb extracts as free radical scavengers of hydrogen donors. The reduction of DPPH radical was determined by decrease in its absorbance induced by antioxidants at 517nm. Acetone and methanol extracts of leaves of *Pancratium verecundum* exhibited significant activities (79.66% and 81.603% respectively). Whereas, chloroform and acetone extracts of bulb of *Pancratium verecundum* expressed better antioxidant activities (83.67% and 75.90% respectively). Thus, it could be derived that molecular profile of leaf and bulb extracts are quite variable in comparison to each other.





Cytotoxic activity of leaf and bulb extracts of *Pancratium verecundum*: Comparative analysis of cytotoxic activities of *Pancratium verecundum* in leaf and bulb extracts revealed that Acetone extract of leaf was most active at a higher dose of 200 microgram/ml (74.0%) followed by Chloroform extract of bulb showing higher 63.4 % of inhibition at the same dose.(Fig. 5). As there is a direct correlation of cytotoxic activity against brine shrimp assay with that of anti-inflammatory activity and anticancer activity[16], hence a good activity of leaf and bulb has indeed provided a valuable lead for further exploratory work.

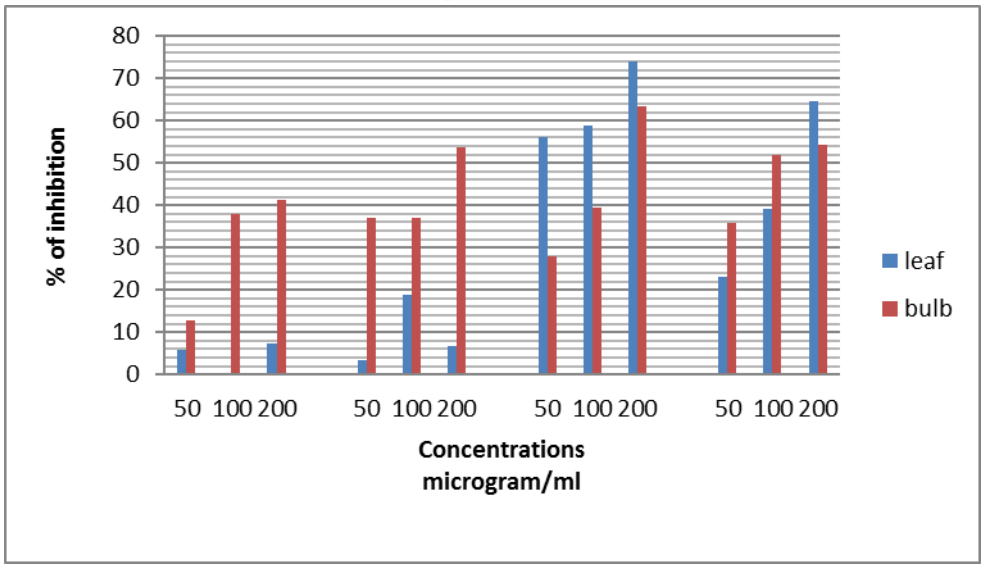


Figure No. 5: Comparative analysis of cytotoxic activities of leaf and bulb extracts of *Pancratium verecundum.*

CONCLUSION

On comparative analysis it was found that both the parts leaves as well as bulb were endowed with biological activity. Leaf extracts were almost at par with the bulb extracts.

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