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Antioxidant Activity and Phytochemical Analysis of Leaf Extracts of Pineapple

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ABSTRACT

Traditional medicines are originated from plants that do not form the constituent of routein diet. Beside this most of the medicinal plants have not received much attention / screening. *Ananas comosus* is one such plant traditionally used for treatment of various ailments. The pineapple (*A. comosus*) is the edible member of family Bromeliaceae; Phytochemical screening of the *A. Comosus* leaves extract shows presence of Alkaloids, Flavonoids, Tannins, Phytosterols, Glycosides and Phenols. The present study was designed to evaluate the effect of crude extract of pineapple leaves (PAL) for antioxidant activity. The value of inhibition of 3 extracts of *A. comosus* on 2, 2 Diphenyl-1 picrylhydrazyl (DPPH) assays at a concentration of 99µg/mL was obtained. Result shows highest inhibition which is obtained by hydro alcoholic solution (56.40) and lowest by ethanolic extract (42.86) respectively. Ascorbic acid as positive control shows 96.5% antioxidant activity.

Keywords: Bromeliaceae, Flavonoids, Memory enhancement, Antioxidant, DPPH.

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INTRODUCTION:

The pineapple (*Ananas Comosus Linn.*) is the leading edible member of the family Bromeliaceae which embraces about 2,000 species, mostly epiphytic and many strikingly ornamental. The pineapple plant is a terrestrial herb 2 1/2 to 5 ft. high with a spread of 3 to 4 ft.; a very short, stout stem and a rosette of waxy, strap like leaves, long-pointed, 20 to 72 inch long; usually needle tipped and generally bearing sharp, up curved spines on the margins. The leaves may be all green or variously striped with red, yellow or ivory down the middle or near the margins.[1]

As individual fruits develop from the flowers they join together forming a cone shaped, compound, juicy, fleshy fruit to 12 inch or more in height, with the stem serving as the fibrous but fairly succulent core. The tough, waxy rind, made up of hexagonal units, may be dark-green, yellow, orange-yellow or reddish when the fruit is ripe. The flesh ranges from nearly white to yellow.[2]

It is commonly found in Asia, Africa, India and other tropical & subtropical regions. Primarily this plant is used as an intoxicant and amnesia.[3] The leaves and fruit of pineapple species were rich in alkaloids, including atropine, scopolamine and hyoscyamine.[4-6] The phytoconstituents such as flavonoids, phenols, tannins, glycosides and sterols are found in *A. comosus.*[7]

It is widely use in India, for the treatment of epilepsy, dementia, heart diseases, cough, & inflammation etc[8-9] *A. comosus* was also been used for its anaesthetic orpain- killing properties. Several scientific studies have been reported on antioxidant and phytochemical screening of ethanol and hydro-alcoholic crude extract.[10-12]

MATERIALS AND METHODS:

Collection of leaves:

The leaves were collected from the field of local area and authenticated by Prof. Nawal Kishore Dubey, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, 221005. The collected leaves were washed with water to remove dust and then washed leaves were shade dried for 40 days. After drying the leaves were ground into powder form using a mixer.

Extract preparation: [12]

Soxhlet Extractor: 25 grams of coarse powder of *A. comosus l*eaf was packed in a muslin cloth bag and placed in the body of Soxhlet extractor. Then, 500 mL of Solvent (Ethanol, Methanol, Hydroalcoholic Solution) was poured in the round-bottom flask. The apparatus was then fitted with the help of clamps and stand to support the Soxhlet extractor, round-bottom flask, and condenser. The rubber tube connected to the tap water was attached to the condenser for continuous flow of water. The solvent was heated using the isomantle, which began to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the plant extract. Once the level of solvent reached the siphon, it poured back into the flask and the cycle began again. The process was made to run for a total of 6 h. Finally, the extract (PAL) was collected in the round-bottom flask. Once the process was finished, the ethanol was evaporated using rotary evaporator at 40°C, leaving a small yield of extracted plant material (about 2–3 mL) in the glass-bottom flask. Extract was kept in a porcelain bowl till the remaining ethanol was completely evaporated. The content of extractable matter was calculated in mg/g of air-dried material using digital weighing balance. The extract was stored in the refrigerator till further use.

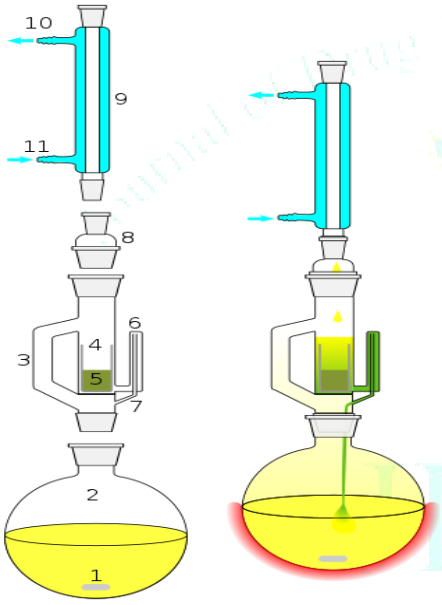

Figure 1: Soxhlet Apparatus

Phytochemical screening:

The phytochemical tests were done for analysing different chemical groups present in the PAL[13, 16]

Test for alkaloids: Took 3 mL of PAL with few drops of 1% HCl and heated on steam bath followed by addition of few drops of Mayer &Wagner's reagent. Turbidity indicates the presence of alkaloids.

Test for flavonoids: To 1 mL of PAL added 1 mL of 10% Lead acetate solution. Yellow precipitate formed, indicates presence of flavonoids.

Test for saponin: 2 mL of PAL was shaken vigorously with 5 mL of distilled water and heated on water bath; stable foam was formed, indicates the presence of saponin.

Test for tannins: 2 mL of PAL was taken with 2mL of distilled water and stirred followed by addition of few drops of Ferric chloride solution, green precipitate appeared, showed presence of tannins.

Test for steroids: Dissolved 2mL of PAL into Chloroform and added 2mL of concentrated Sulphuric acid to the mixture, red colour developed indicates presence of steroids.

Test for terpenoids: Take 1 mL of PAL followed by addition of 0.5 mL of acetic anhydride and then few drops of concentrated Sulphuric acid. Appearance of Bluish green precipitate confirmed presence of terpenoids.

Test for phlobatannins: 2 mL of PAL was hydrolysed using 1% HCl and mixture was boiled for few minutes. Deposition of red precipitate indicates the presence of phlobatannins.

Test for glycosides: Dissolved 2 mL of PAL into Chloroform and add 2 mL of Acetic acid into the mixture followed by heating than cooled. Added few drops of Sulphuric acid colour changed from blue to green confirmed presence of glycosides.

Test for amino acids: Took 1 mL of PAL and treated with few drops of Ninhydrin reagent, development of purple colour confirmed presence of amino acids.

Antioxidant activity:

DPPH free radical scavenging: The DPPH assay was done according to the method reported by Brand –Williams Method[17] using Ascorbic acid as standard. Samples were prepared using PAL (10mg/mL). The sample solutions (33µL) were added with 1 mL DPPH solution. The mixture was vortexed vigorously for 1 minute and kept in a dark for 20 minutes at 27^{0}C, absorbance of all samples were taken at 517 nm. The percentage inhibition was calculated using the formula,

% Inhibition =Abs control – Abs extract / Abs control × 100.

RESULT AND DISCUSSION:

Ananas Comosus Leaf Extract (PAL):

Various extract were obtained using Methanol, Ethanol and Hydro-alcoholic Solution. The percentage yields are presented in table 1.

Table 1: Extraction yield of PAL

Solvent	Extraction Yield (g)	Yield (%)
Methanol	7.04	7.04
Ethanol	7.36	7.36
Hydro-alcoholic Solution	4.48	4.48

Highest % Yield was obtained in ethanol followed by methanol and hydro alcoholic solution because ethanol can extract polar as well as non-polar components.

Phytochemical screening:

Phytochemical screening shows presence of flavonoids, tannins, alkaloids and glycosides. Different phytochemical constituents which are present in samples are known to be biologically active compounds and they are responsible for different activities such as antimicrobial, antioxidant, antifungal, anticancer and antidiabetic activities. Tannins, glycosides, flavonoids, and glycosides have hypoglycaemic and anti-inflammatory activities.[13, 15]

Table 2: Phytochemical analysis of PAL

Component	Result
Alkaloid	++
Tannins	++
Saponins	_
Flavonoids	++++
Steroids	_
Terpenoids	_
Glycosides	++
Amino acid	-

+ = Presence; - = Absence.

Antioxidant activity:

DPPH radical scavenging: The photometric evaluation of the antioxidant activity of PAL shows good antioxidant capacity. The value of inhibition of 3 PALon DPPH assay at a concentration of 99µg/mL was obtained. Result shows highest inhibition which is obtained by hydro alcoholic solution (56.40) and lowest by ethanolic extract (42.86) respectively. Ascorbic acid as positive control shows 96.5% antioxidant activity. Results show that the polar extracts have highest antioxidant activity.

Table 3: DPPH free radical scavenging activity of PLE

Solvent	% Inhibition
Ascorbic Acid	*96.52*
Methanol	*47.52*
Ethanol	*42.86*
Hydro-alcoholic Solution	*56.40*

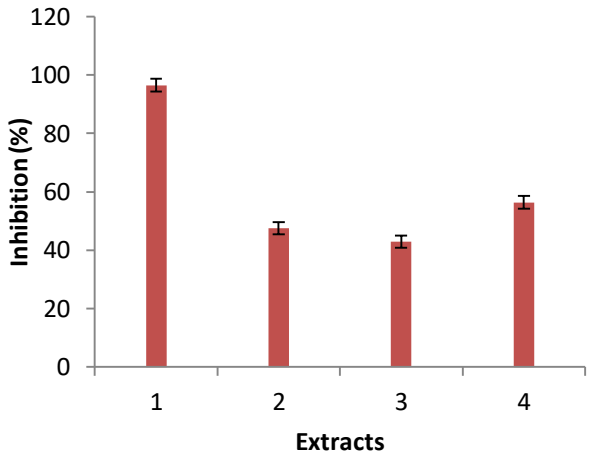

Figure2: DPPH free radical scavenging activity of PAL such as Ascorbic Acid (1), Methanol (2), Ethanol (3) and Hydro-alcoholic Solution. Each bar shows mean ± SD (n = 3)

CONCLUSION:

High antioxidant activity was observed in hydro-alcoholic extractsof *A. comosus.* When compared to other extracts. The PAL shows the presence of secondary metabolites such as Alkaloids, Glycosides, Phlobatannins, Tannins and Flavonoids. The bioactive compounds from *A. comosus* serve as good phototherapeutic agent. The value of inhibition of 3 PAL on DPPH assay at a concentration of 99µg/mL was obtained. Result shows highest inhibition which is obtained by hydro alcoholic solution (56.40) and lowest by ethanolic extract (42.86) respectively. Present study shows that pineapple leaf extract can be used as antioxidant as well as memory enhancer.

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