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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF LEAVES OF ANANAS COMOSUS L. IN DIFFERENT SOLVENT EXTRACTS.

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ABSTRACT

Ananas comosus (L.) Merr. (family Bromeliaceae), commonly named Pineapple, is a herbaceous perennial plant. Plant is known for its folk medicinal utility, besides agricultural utilities such as the fruit for nutritional food. Five fractions of leaves were investigated for their phytochemicals and their antioxidant activity. The extraction ability of different solvents for recovering extractable components vary. Highest yield was for distilled water. Phyto-screening of leaves for secondary metabolites revealed the presence of coumarin, terpenoides, phlobatannin, alkaloids, phenols, saponin, quinone ,cardiacglycoside, steriods and flavanoids**.** Antioxidant activity was determined using 2, 2-diphenyl-1-picryl hydrazine (DPPH) free radical scavenging .The extracts of different fractions exhibited different levels of antioxidant activity. Petroleum ether extract of *A. comosus* recorded the highest DPPH free radical scavenging activity (98.8 %) with IC 50 value 37.7±0.18 at 250µg/ml concentration. The findings of this study indicate that this plant is medicinal with prominent antioxidant property.

KEYWORDS : *Ananas comosus*, Phytochemical analysis, Antioxidant activity

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (1).

Plants in all facet of life have served a valuable starting material for drug development (2). Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques (3). Though the advances in modern medicines are significant, there remains an ever increasing demand for herbal medicines. Effective and potent herbal medicines require evaluation by standard scientific methods so as to be validated for the treatment of diseases. The presents of patent laws have increased the necessity to preserve the claims of these time-tested folk medicines. Thus, it has become imperative to initiate steps to document components and activity of these medicinal plants.

A. comosus is an anti-inflammatory agent, which heals wounds and facilitate healing. It is a mixture of proteolytic enzymes. Helps in improving respiratory health, cure coughs and cold. Bromelain present in all parts of plant interfers the growth of maligant cells and prevent cancer, also improves health and digestion. It help to fight off infections and parasites. Pineapple fruits helps to strengthen bones, improve oral health, boost eye health, improves immune system and increases circulation. Gastro-intestinal complaint had highest use-reports and 3 species of plants, namely *Aegle marmelos* (L.) Corr., *Ananas comosus* (L.) Merr., and *Terminalia chebula* Retz., had the highest fidelity level (FL) of 100%. Leaf extract with milk and sugar candy in rheumatic swellings. Extract of leaf base is taken1teaspoon thrice daily in diarrhea. Use as antirheumatic is a new report. Used for the treatment of dysuria. Cortex is used as alexipharmic, antitussive and antidiarrheal and leaves is used against dyspepsia or antidiarrheal agent(4). Hence, in the present study, we were interested in carrying out phytochemical and *in vitro* antioxidant activity of *A. comosus* using petroleum ether, chloroform, ethyl acetate , methanol and water extracts .

MATERIALS AND METHODS

The fresh leaf of *Ananas comosus* were collected from Kottappuram ,*Thrissur* Kerala, India. Taxonomic identification made with Flora of the Presidency of Madras by JS Gamble (5). The plant name checked with www.theplantlist.org. Leaves of the plant were shade dried for several days.The dried plant material was ground to a course powder and 50 gm of the powdered material was soaked in solvents of increasing polarity starting petroleum ether, chloroform, ethyl acetate, methanol and distilled water (1:5) for 72 hours. The solvent was then removed by rotary evaporation. Each residue was weighed and the yield percentage was determined. The dried extract was stored in refrigerator for further studies.

Phytochemical Screening

The preliminary phytochemical analysis of the plant extracts was carried out to identify the primary and secondary metabolites present in the various alcoholic and aqueous extracts of leaves of *A. comosus* using standard protocol (6) .

Anti-oxidant property screening

The dried plant extracts were re-dissolved in dimethyl sulfoxide to get the solution of 10 mg/10 ml for each extract which was subjected to analysis of *in vitro* anti-oxidant activities.

DPPH Radical scavenging assay

Free radical scavenging activity of the *A. comosus* leaf extracts assessed based on the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (7). The diluted working solutions of the test extracts 50 µg/ml – 250 µg/ml concentration) and 6.34µM solution of DPPH were prepared in methanol, and 100µl test, 100µl DPPH solution and 800 µl of methanol was taken in a test tube and mixed well. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (900 µl) with DPPH solution (6.34 µM, 100 µl) taken as control and methanol as blank. The optical density recorded and % of inhibition calculated using the formula given below.

Percent(%) inhibition of DPPH activity = A-B/A × 100

Where A = optical density of the control and B = optical density of the sample.

Calculation of IC50 concentration

The extract concentration corresponding to 50 percent inhibition (IC_{50}) was calculated from the curve against extract concentration. Each sample was assayed in triplicate for each concentration.

RESULT AND DISCUSSION

Yield of extract

Comparatively, distilled water exhibited higher extraction yield (4.56g) . The extraction ability of different solvents for recovering

extractable components followed the order: distilled water > methanol > chloroform > ethyl acetate > petroleum ether (Table 1).

Table.1 Percentage yield (gm) of leaf of *A. comosus*

Petroleum ether	Chloroform	Ethyl acetate	Methanol	Distilled water
0.78%	1.24%	0.94%	4.38%	4.56%

Preliminary phytochemical screening

Phytochemical screening results (Table 2) of the leaf extracts in petroleum ether showed the presence of sugar, protein, cardiac glycoside, flavanoids, coumarin, steroids and phenols. Chloroform extract contained sugar ,protein ,quinine ,alkaloid ,coumarin and phenol . Metabolites present in ethyl acetate are sugar ,protein , quinone, alkaloid and phenol. Sugar, carbohydrate, ketose ,protein ,quinone,cardiacglycoside,coumarin ,alkaloid and phenol are present in methanol extract. Metabolites like carbohydrate, sugar, ketose, protein, quinone, cardiac glycoside ,saponin, flavanoid and phenol are present in aqueous extract .

Table 2. Qualitative Analysis of the phytochemicals of leaves of *A. comosus*

Primary /Secondary Metabolites	Name of the Test	Petroleum Ether	Chloroform	Ethyl acetate	Methanol	Distilled Water
Carbohydrates	Molisch's Test	_	_	_	+	+
Aldehyde	Fehling Test	_	_	_	_	_
Starch	Iodine Test	_	_	_	_	_
Sugar	Benedict's Test	+	+	+	+	+
Ketose	Seliwanoff's test	_	_	_	+	+
Proteins	Lowry's Method	+	+	+	+	+
Aminoacid	Ninhydrin test	_	_	_	_	_
Fats	Filter paper Test	_	_	_	_	-
Quinone	H2SO4 Test	_	+	+	+	+
Cardiac glycoside	Kellar –Killani test	+	_	_	+	+
Steroids	Salkowski test	+	_	_	_	_
Flavonoids	Fluorescent Test	+	_	_	_	+
Phenols	Folin Test	+	+	+	+	+
Saponins	Foam Test	_	_	_	_	+
Alkaloids	Mayers-Test	_	+	+	+	_
Tannin	Iron salt test	_	_	_	_	_
Phlobatannin	HCL Test	_	_	_	_	_
Terpenoids	Salkowski test	_	_	_	_	_
Acid	NaHCO3 Test	_	_	_	_	_
Coumarin	FeCl3 Test	+	+	_	+	_
Resin	HCl Test	_	_	_	_	_

+ indicate the presence of constituents and-indicate the absence of constituents.

In-vitro* Anti-oxidant property screening of leaves of *A. comosus

DPPH free radical scavenging assay.

The free radical scavenging activity of different extracts of leaves of *A. comosus* was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazyl resulting in decrease in absorbance. Petroleum ether extract showed maximum activity of 98.8% at 250µg/ml (Table 3). All the five extracts exhibited very good DPPH free radical scavenging activity.

Table. 3: Percentage Inhibition of DPPH free radical by different leaf extracts of *A. comosus* at 517nm Values are presented as mean ± standard deviation (n=3).

Sl. No	Concentration (µg/ml)	Percentage of inhibition of DPPH free radical				
		Petroleum ether	Chloroform	Ethyl acetate	Methanol	Distilled water
1.	50	55.8±1.5	65.4±0.71	52.96±1.6	52.9±0.645	56.36±3.7
2.	100	60.2±0.27	73.86±3.2	72±0.95	55.96±1.26	74.96±5.7
3.	150	75.73±0.65	85.5±0.55	81±1.57	61.16±1.05	85.9±2.05
4.	200	94.8±0.49	92.3±2.0	91±1.4	62.9±1.4	92.7±0.83
5.	250	98.8±0.75	97.9±0.49	97.63±1.9	75.96±4.6	97.7±2.01

IC_{50} indicate the potency of scavenging activity. The antioxidant potential obtained through 1,1- Diphenyl-2-picryl Hydrazyl method shows the petroleum ether leaf extract exhibit the lowest IC_{50} value (37.7±0.18). Lower the IC_{50} value indicates the high antioxidant potential and property. The standard ascorbic acid used as reference compound in this assay has IC50 value 16.742µg/ml which is comparable with IC50 value of petroleum ether extract. The different solvents with their respective IC_{50} values are depicted in the Table.4

Table. 4: Comparison of IC_{50} Values of different leaf extracts of *A. comosus*. Values are presented as mean ± standard deviation (n=3).

Sl.No.	Solvents	Ic50 (µg/ml)
1	Petroleum ether	37.7±0.18
2	Chloroform	42.67±2.55
3	Ethyl acetate	47.88±1.63
4	Methanol	47.79±1.89
5	Distilled water	44.48±2.01

CONCLUSION

Phytochemical studies portray the presence of several biologically active secondary metabolites such as coumarin, terpenoides, phlobatannin, alkaloids, phenols, saponin, quinone ,cardiacglycoside, steriods and flavanoids in the leaves of *A. comosus* for the first time. The petroleum ether extract of *A. comosus* leaf exhibited significant DPPH free radical scavenging activity of 98.8 % at 250µg/ml concentration with IC 50 value 37.7±0.18.On top of that, these natural antioxidants can have potential advantages among various diseases with oxidative stress. However, further studies by *in vivo* models are still needed to confirm this property.

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