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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF LEAF, STEM AND FRUIT OF ANANAS COMOSUS

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ABSTRACT

The present study was aimed to evaluate the phytochemicals and antioxidant activity of leaf, stem and fruit of *Ananas comosus*. The leaf, stem and fruit of plant were used for preparation of extracts by grinding process using sodium acetate buffer. Phytochemical and biochemical analysis of crude extracts showed the presence of saponin, terpenoid, flavonoid, aminoacid, protein, phytosterol, carbohydrate, alkaloid, cardiac glycosides but the extract showed negative for tannin, Phenols. The crude extracts were further subjected to purified fractions and those extracts were used for anti - oxidant assay by 2, 2 - diphenyl - 1 - picrylhydrazl (DPPH) method. The crude extract of stem (70.2%) showed significant activity than crude extracts at leaf, fruit and purified fractions.

KEYWORDS: *Ananas comosus*, phytochemical, biochemical, antioxidant activity.

INTRODUCTION

Ananas comosus belongs to the Bromeliaceae family, which contains 50 genera and about 2,500 known speices. This plant is used in folk remedies for digestive disorder and diuretic property. Juice of ripe fruit regarded also as antiscorbutic, chalogogic, diaphoretic, refrigerant

and useful in jaundice. It contains the proteolytic enzyme called bromelain, which is used as a meat tenderizing agent and for medicinal purposes. Phytochemicals are naturally occurring in plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. The secondary metabolites such as alkaloid, flavanoid, phenol, tannins, steroids etc were present in *Ananus comosus.* (Kalaiselvi, *et al*., 2012). The aqueous extract of *Ananas Comosus* mainly shows a positive response to the pharmacologically active compounds like alkaloids, flavonoids, glycosides, phytosterols, saponins and triterpenoids (Sampath Kumar, 2012). The ethanolic extract of *Ananas comosus* peel was screened for invitro antoxidant activity. It showed high antiradical activity compared with the standard butylatedhydroxyl toluene (BH7) and Vitamin C (Kalaiselvi *et al*., 2012). The results obtained *Ananas comosus* extracts have interesting pharmacological active compounds and used in ethanomedicine for the treatment of some infections and ailments.

MATERIALS AND METHODS

Collection of Plant Material

The *Ananas comosus* is a tropical plant with edible multiple fruit. The leaf, stem and fruit *of Ananas comosus* were collected from Kattakada in Trivandrum district, Kerala. The study of biochemical analysis, phytochemical analysis and anti-oxidant assay were performed.

Preparation of plant extract

Extraction of crude extracts of leaf, stem and fruit

The leaf, stem and fruit were taken and cut it in to pieces and grinded with 0.1M Sodium acetate buffer (PH-7). The juice was filtered with the help of cheese cloth and centrifuged at 8000 rpm for 10 minutes. The Sodium Benzoate was added at a concentration of 1 gm/kg for storage. The filtrate was used as crude extracts of leaf, stem and fruit (Anannya Mohapatra, *et al,* 2013).

Biochemical analysis

Estimation of carbohydrate (Anthrone Method)

Carbohydrate estimation was carried out by Anthrone method followed by Hansen and Moller (1975). 0.2 to 1ml of working standard glucose was taken and 0.1 to 0.3 ml of crude leaf, stem and fruit extracts was added in separately to a series of test tubes. It was made up to 1ml in all test tubes by adding distilled water. 4 ml of anthrone reagent was added to all test

tubes. It was followed by incubating 8 minutes in a boiling water bath. Test tubes were cooled rapidly and the green color developed was red at 630nm. Using a standard graph the amount of total carbohydrate present in the sample solution was calculated.

Estimation of protein (Lowry's method)

Protein estimation was carried out by the method of Lowry's (1951). Different aliquots of standard bovine serum albumin (BSA) (0.2 to 1 ml) were taken in each test tube. 0.2 ml of crude leaf, stem and fruit extracts were taken in separate tubes. The volume was made up to 1 ml with distilled water. 1 ml of reagent D{(mixture of reagent A-48ml, B- 1ml and C- 1ml). Reagent A: Na2Co3-2%, "NaOH- 0.1M, Reagent B: NaK- 1%, Reagent C: CuS04- 0.5%} as added to each tube mixed thoroughly and allowed to stand for 10 minutes at room temperature. Then 0.3 ml of reagent E (Folin's phenol) was added and the tubes were mixed. The tubes were incubated for 30 minutes at room temperature. The absorbance was measured at 660 run.

Estimation of ammo acid (Ninhydrin Method)

Estimation of amino acid was carried out by Ninhydrin method followed by Moor and Stein (1948). Different aliquots of standard leucine solution from 0.2 to 1 0 ml were pipetted out and 0.2 to 0.4 ml of crude leaf, stem and fruit extracts were taken in separate tubes. Then all the tubes weremade up to 1ml using distilled water and 2 ml ninhydrin was added to all the test tubes and heated for- 15 minutes in boiling water bath and cooled to room temperature. 3 ml of 50% ethanol was added to all the test tubes, and read the intensity of the purple color against a reagent blank in a calorimeter at 570 nm.

Phytochemical analysis

Phytochemical tests were carried out using the crude extracts from leaf, stem and fruit of *Ananascomosus.* Using standard procedures to identify the constituents as described by Sofowara and Horborne, 1998.

Test for Tannin

Approximately 2ml of the plant extract was dissolved in 5 ml of distilled water and added with a few drops of 10% ferric chloride. A blue-black, green or blue-green precipitate indicted the presence of tannins.

Test for Saponin

Froth test

1 ml of each extract was taken and added 1 ml of distilled water in each test tube and left undisturbed for 20 minutes. Persistent froth was observed.

Test for Terpenoid

1 ml of each extract was mixed with 1 ml of chloroform and 1 ml of concentrated H_2S0_4. After addition of H2SO4 the sample was heated for 2 minutes, greenish colour was observed.

Test for flavonoid

Lead acetate test

1 ml of each extract was treated with few drops of lead acetate solution. Yellow colour precipitate was observed.

Test for amino acid and protein

(a). Ninhydrin test

1 ml of each extract was treated with few drops of Ninhydrin reagent and boiled for few minutes. Violet colour appeared suggesting the presence of amino acid and proteins.

(b). Millon's test

1 ml of each extract was treated with 2 ml of millon's reagent a white precipitate was observed.

Test for phenolic compound

(a). Ferric chloride test

1 ml of each extract was treated with few drops of ferric chloride solution. Bluish black colour was observed.

Test for phytosterols

(a) Salkowskis test

1 ml of each extract was mixed with 1 ml chloroform and filtered. A few drops of concentrated H_2S0_4 was allowed to it shaked well. After few minutes golden yellow colour was observed.

(b) Libermann burchard's test

1 ml of each extract was dissolved in 1 ml of chloroform and few drops of concentrated H_2S0_4

were added by sides of test tube. Upper layer was red and H_2S0_4Iayer was yellow with green fluorescence showed the formation of steroids.

Test for carbohydrate

(a) Fehling's test

1 ml of each extract was treated with few drops of Fehling's solution. A brick red precipitate appeared at the bottom of the test tube indicate the presence of reducing sugars.

(b). Benedict's test

1 ml of each extract was treated with 2 ml of Benedict's reagent and boiled. A reddish brown precipitate formed which indicates the presence of carbohydrate.

Test for alkaloid

(a). Wagner's test

Dissolve 1.2g Iodine and lg Kl in 20 ml water, and make up with water to 100ml. A brown precipitate in acidic solution would suggest the presence of alkaloid.

(b). Dragendroffs test

1 ml of each extract was treated with few drops of Dragendroffs reagent. Orange brown precipitate was formed.

Test for Cardiac Glycosides

(a) Keller-Killiani test

5 ml of extract was treated with 2 ml glacial acetic acid containing few drops of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2S0_4 was observed for the formation of brown ring.

Test for Iodine

1 ml of each extract was treated with 2 ml of iodine solution. Dark blue or purple colour was observed the presence of carbohydrates. Purification of crude extracts of leaf, stem and fruit of *Ananas comosus*

Antioxidant activity

DPPH Radical assay

The free radical scavenging activity of the crude extracts of leaf stem, fruit and purified fractions was measured with stable 1,1 diphenyl-2-picrylhydrazyl radical spectro

photometrically. 0.002gm of DPPH is dissolved in 100 ml methanol was used as free radical. 1 ml of different extracts and 1 ml of DPPH were mixed in clean and labeled test tube separately. Control was taken and incubated in dark for 30 minutes. The optical density was measured at 517 nm using UV-V spectrophotometer. The degree of stable DPPH decolorization to DPPH (reduced form of DPPH) yellow indicated the scavenging efficiency of extract. The scavenging activity of extract against the stable DPPH was calculated using the following equation. (Krings and Berger., 2001).

Scavenging activity = (Control - Sample / Control) x **100%**

RESULT

Biochemical analysis

Biochemical anlaysis of crude extracts of leaf, stem and fruit of *Ananas comosus* revealed the presence of various biochemcial constituents such as carbohydrate, protein and amino acid at various concerntrations. The protein showed higher amount in crude stem extract (4.2mg) and the carbohydrate and amino acid showed higher amount in crude leaf extract (0.78 mg and 0.29mg). The results were recorded in tabel no-1.

Table No 1: Biochemical analysis of crude extracts of leaf, stem and fruit of *Ananas Comosus.*

S/No	Constituents	Amount (mg/ml)		
		Crude leaf extract	Crude stem extract	Crude fruit extract
1	Carbohydrate	0.78	0.66	0.56
2	Protein	3.4	4.2	3.8
3.	Amino acid	0.29	0.24	0.28

Phytochemical analysis

The phytochemical analysis of crude extracts of leaf, stem and fruit of *ananas comosus* were performed. The crude extracts showed the presence of phytochmical constituents such as saponin, terpenoid, flavonoid, amino acid, protein, phytosterol, carbohydrate, cardiac glycosides and alkaloid. The results were evaluated by the change in colour was recorded in table no-2.

Table: 1: Phytochemical Screening of crude extracts of leaf, stem and fruit of *ananas comosus.*

Sl. No	Test	Leaf extract	Stem extract	Fruit extract
1	Tannin	-	-	-
2	Saponin	-	+	-
3	Terpenoid	+	-	+
4	Flavanoid 1.Lead acetate test	+	+	+
5	Amino acid and protein 1. Ninhydrin test 2. Millon's test	 + +	 + +	 + +
	Phenolic compound 1. Ferric chloride	 -	 -	 -
7	Phytosterol 1. Salkowski's test 2. Libermann Burchard's test	 + +	 + +	 + +
8	Carbohydrate 1. Fehling's test 2. Benedict's test	 - +	 - +	 - -
9	Alkaloid 1. Wagner's test 2. Dragendroff's test	 + +	 + +	 + +
10	Cardiac glycosides 1. Keller – Killiani test	 -	 +	 +
11	Iodine test	-	-	-

'+' indicates positive and '-' indicates negative

Antioxidant activity of crude extracts and purified fractions of *Ananas comosus*

In vitro evaluation of antioxidant activity by DPPH Assay Crude (leaf, stem and fruit) extracts and purified fractions of eluent I of stem extract, eluent I of fruit extract and eluent II of leaf extract of *Ananas comosus* used for antioxidant activity. The scavenging activity of crude extract of stem (70.2%) showed significant activity than crude extracts of leaf, fruit and purified fractions. The results were recorded in table no -3 figure1.

Table 3: DPPH free radical scavenging activity of crude extracts and prufiifed fraction of *Ananas comosus.*

Sample extracts (100µg/µl)	Optical Density t 517 nm		Scavenging Activity (%)
	Control	Sample	
Crude leaf extract	1.542	0.594	61.4%
Crude stem extract	1.542	0.457	70.2%
Crude fruit extract	1.542	0.626	59.4%
Eluent II of leaf extract	1.542	0.819	46.8%
Eluent I of stem extract	1.542	0.722	53.1%
Eluent I of fruit extract	1.542	0.661	57.1%

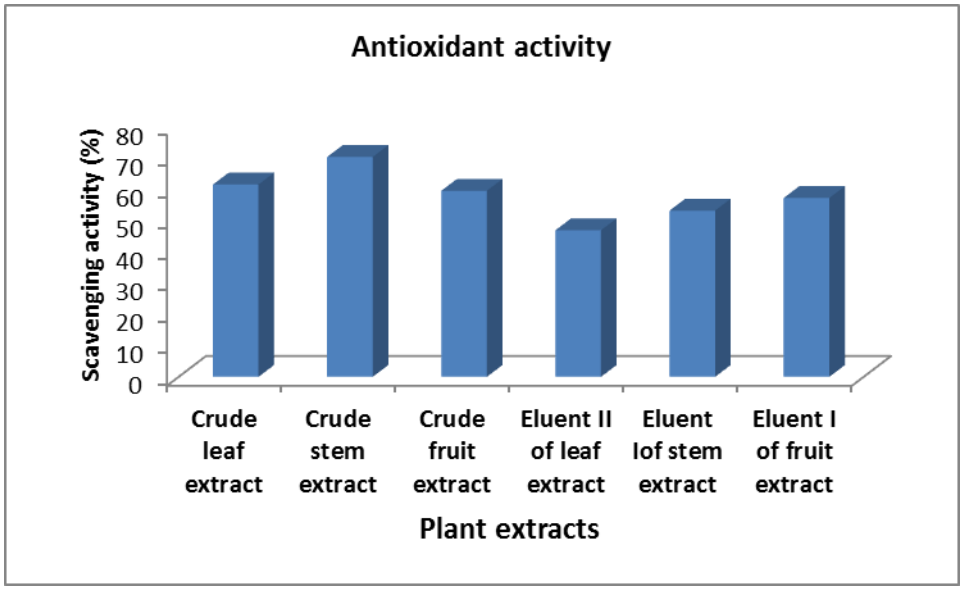

Figure No: 1: DPPH free radical scavenging activity of crude extracts and purified fractions of *Ananas comosus.*

DISCUSSION

The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth *et al*., 1988). In the present study the crude extracts of leaf, stem and fruit of *Ananas comosus* revealed the concentration of carbohydrate, protein and amino acid. The concentration of protein showed higher amount in crude extract of stem (4.2mg) and the concentration of carbohydrate and amino acid showed higher amount in crude extract of leaf (0.78mg and 0.29mg). Gautm, *et al*., (2010) reported that the protein concentration (bromelain) of crude extracts of stem and fruit were determined by Lowry's method. Protein concentrations of stem bromelain were found to be 0.7 mg/ml and 0.08 mg/ml.

In the present study, crude extracts of leaf, stem and fruit of *Ananas comosus* showed the presence of phytochemical constituents such as Terpenoids, flavanoids, amino acid, protein, phytosterols, carbohydrate, alkaloids, saponins, and cardiac glycosides. Praveen *et al*., (2014) reported that the carbohydrate, saponins, flavanoids, tannins, glycosides and terpenoids are present in the *Ananas comosus* fruit.

In this study revealed that the crude extracts and purified fractions of *Ananas comosus* have the antioxidant properties. It could be observed that the crude extract of stem had powerful

antioxidant activity (70.2%) compared with the other extracts. Anynda Yuris (2014) also mentioned antioxidant activity of the *ananas comosus* was measured using the 2, 2-diphenyl-1-picryhydrzyl (DPPH) radical scavenging assay the highest DPPH scavenging activity (25.2±0.5 mg AA/100g fruits).

CONCLUSION

The oxidants produced in the body can damage most cell structures through their chain reactions. This may cause many human disease like cancer, Alzheimer's disease, cardiac, reperfusion abnormalities, kidney disease and fibrosis etc., Antioxidants are the molecules can slow down the chain reaction of the oxidants by stabilizing them and able to reduce the damaging effects of them. The present study confirms the antioxidant activity of *Ananas comosus* and the extracts have different compounds in them may be responsible for their activity in reduction of the oxidants.

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REFERENCES

2. Manokaran Kalaiselvi, Ganesan Ravikismar, Chandrasekar Uma: Invitro free radical scavenging activity of *Ananas comosus* (L.) *International journal of pharmacy and pharmaceutical sciences*, 2012; 4(2): 0975-1491.
3. Sampath Kumar, N. Phytochemical investigation and the evaluation of hypolipidemic activity of an aqueous extract of Ananas Comosus (L) Merr. *Journal of biochemistry and biotechnology*, 2012; 3(2): 61-64.
4. Anannya Mohapatra V, Madhava Rao, Mala Ranjan: Comparative study of the increased production and characterization of bromelain from the peel, pulp and stem *p\mapp\e(Ananas comosus).* International journal of advancements in research and technology, 2013; 2(8): 2278-7763.
5. Hansen, J., and Moller, I. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Anal. Biochem*, 1975; 68(1): 87- 94.
6. Lowry, O.H., N.J., Rosbrough, A.L., Farr, R.J., Randall. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.,* 1951; *193:* 265.
7. Saxena L, Iyer B K, Ananathanarayan L: Three phase partitioning as a novel method for purification of ragi bifunctional amylase/ protease inhibitor. Process biochemistry, 2007;

42: 491-495.

8. Farnsworth, N. R. Biodiversity. *National Academy Press Washington*, 1988; 83-97.
9. Gautam, S., S., Mishra, S., K., Dash, V., Amit, K., Goyal, Rath, G. Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant. *Thai journal pharmaceutical and sciences*, 2010; 34(2): 67-76.
10. Praveen, N., C., Raajesh, A., Manish Madan, Vishwajit, Rampratap Chaursia, Neel, V., Hiremath, Akanksha Manmohan Sharma. In vitro evaluation of antibacterial efficacy of pineapple extract (Bromelain) on periodontal pathogens. *Journal of International Oral Health*, 2014; 6(5): 96-98.
11. Anynda Yuris, Lee-Fong Siow. A comparative study of the antioxidant properties of three pineapple varieties. *Journal of food studies*, 2014; 3(1): 40-56.