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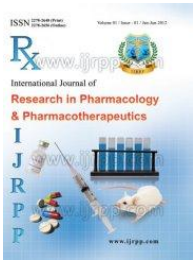

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Phytochemical screening and *in vitro* antioxidant activity of *ananas comosus*

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ABSTRACT

The objective of this study was to determine the phytochemical screening and antioxidant activity of *ananas comosus* L. Different concentration of ethanol and aqueous were used to extract the phenolic compounds. The antioxidant activity of the pineapples was measured using the DPPH radical scavenging assay, hydrogen peroxide scavenging activity, total antioxidant activity. 10% ethanol was chosen as the best solvent for analyzing the antioxidant activity of pineapples. The pineapple was found to contain the highest antioxidant activity. This was also found to have the highest DPPH scavenging activity, total antioxidant and hydrogen peroxide scavenging activity. This different were observed between the ascorbic acid content. The result shows that pineapple and its active constituents may be used in further antioxidant therapy.

Keywords: *Ananas comosus* L, Antioxidant activity, DPPH radical scavenging.

INTRODUCTION

Antioxidant is defined as compounds that protect against oxidation by increasing the oxidative stability of a system Antioxidants can be obtained either synthetically or naturally. Due to the possible hazardous effect of synthetic antioxidants, there is an increasing interest in natural antioxidants such as those in fruits and vegetables. [1]

Antioxidants get their name because they combat oxidation. They are substance that protects other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation [2]

Antioxidants are either hydrophobic. Water soluble or hydrophilic antioxidants are active in the blood plasma while the water insoluble antioxidants protect the cell membranes. An antioxidants is a substance that when present low concentration relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate [3].

Antioxidants are substance that protects the body from free radical damage and are found in nutrients and elements such as vitamins, minerals, amino acids, and enzymes. A free radical is an unstable molecule or group of molecules that contains at least one unpaired electron in its outer orbit. Typically, electrons are paired and negatively charged an inherently stable structure. When an electron is unpaired, another molecule or atom can easily bind with it, causing a chemical reaction. As free radicals react so easily with other compounds, they can cause dramatic changes and damage in the body free radical damage is implicated in everything from the natural process of aging to genetic mutation resulting in cancer [4]

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plant kingdom: approximately 3,000 flavonoid substances have been described. 6 In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers."[5]

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating
Vitamin E. [6]

Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene may work synergistically with vitamin E. 4, 5 A diets that are excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly
Extracted and protected vegetable oils are major sources of vitamin E [7]

The best way to ensure an adequate intake of phytonutrients is to eat a diet rich in a wide variety of fresh fruits and vegetables. Phytonutrient supplements are also now widely available. In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes – glutathione peroxidase, catalase, and superoxide dismutase (SOD) – metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. It has been suggested that an inadequate dietary intake of these trace minerals may compromise the effectiveness of these antioxidant defense mechanisms. [11] Research indicates that consumption and absorption of these important trace minerals may decrease with aging. [11] Intensive agricultural methods have also resulted in significant depletion of these valuable trace minerals in our soils and the foods grown in them. [8]

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer. [14] A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer. [9]

Epidemiological studies have shown that an increase in fruit intake is associated with a reduced morbidity of cardiovascular disease, some types of cancer, and neurodegenerative diseases. (Guo et al., 2003). A possible mechanism for this observation has been attributed to the antioxidant activity in fruits both the phenolic content and ascorbic acid content of fruits are responsible for their antioxidant activity. [10]

Plant phenolics are the largest class of plant secondary metabolites, which serve in plant defense mechanism to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage. The antioxidant activity of phenolics relates to a number of different mechanisms such as free radical scavenging hydrogen donation singlet oxygen quenching metal ion chelation and acting as a substrate for radical such as superoxide and hydroxide. [11]

Like some other dietary supplements, antioxidant supplements may interact with certain medications. For example, vitamin E supplements may increase the risk of bleeding in people who are taking anticoagulant drugs ("blood thinners"). There is conflicting evidence on the effects of taking antioxidant supplements during cancer treatment; some studies suggest that this may be beneficial, but others suggest that it may be harmful. The National Cancer Institute recommends that people who are being treated for cancer, talk with their health care provider before taking supplements. [12]

Pineapple fruit contains large amount of phenolics. Although a number of flavonoids and phenolics have been identified in different pineapple cultivars, little information is available about the antioxidant activity of these phenolics. It has been recently shown that phenolics from edible fruits are effective in vitro antioxidants. [13]

Hence, the present study attempts to investigate the total phytochemical and antioxidant activity of fresh juice from the fruit of *ananas comosus*.

MATERIALS AND METHODS

Collection of plant materials

The pineapple fruit was obtained from a local supermarket of Mayiladuthurai, Nagai district, Tamilnadu, south India. The mature (150-180 days from fruit set to yellow colour) and medium size of classification (500-1000 mg) of pineapple was used. The pineapple is oval to cylindrical shape.

Preparation of fruit juice

About 1 kg of pineapple fruit was taken. The peel of the fruit and the pulps were collected. Those pulps were mashed by grinding method and the fruit juice was extracted.

Preparation of fruit extract

About 50 ml of fresh fruit juice of pineapple fruit exhaustively extracted with 50 ml of 25% aqueous, ethanol the resultant extract was boiled in a water bath until a syrupy consistency was obtained.

Preliminary phytochemical screening

Phytochemical analysis of all extracts was carried out by standard procedures. [24]

ANTIOXIDANT SCREENING

DPPH radical scavenging activities

DPPH radical scavenging activity was evaluated by Shimada, et al., 1992 [14] Briefly, a 2 ml aliquot of DPPH (2,2-diphenylpicrylhydrazyl) 1 ml methanol solution (25 g/ml) was added to 0.5 ml sample solution at different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

Total antioxidant capacity

With 1 ml of extract of different concentrations was treated with 1ml of reagent solution (0.6mm sulphuric acid, 28mm sodium phosphate and 4mm ammonium molybdate) in eppendeff tube. Capped tubes were incubated in the thermal block at 95 C for 90 mins. After cooling to room temperature, the absorbance was measured at 695nm against the blank. The activity was compared with ascorbic acid as standard. [15]

Hydrogen peroxide scavenging activity

1 ml of sample is mixed with 3 ml of phosphate buffer 1 ml of H_2O_2 and incubated for 10 mins at 37^oC after incubation the absorbance value of the reaction mixture was recorded at 230 nm. ascorbic acid used as standard. [16]

RESULTS AND DICUSSION

The present investigation was carried out to screen the phytochemical and antioxidant activity of *Ananas comosus.*

Table 1: phytochemical screening of *Ananas comosus*

S.No	Name of the test	Aqueous	Ethanol
1	Steroids	+	+
2	Phenols	+	+

3	Tannins	+	+
4	Flavonoid	+	+
5	Alkaloids	+	+
6	Glycosides	+	-
7	Terpenoids	-	+
8	Saponins	+	+
9	Quinine	+	+
10	Coumarins	-	+
11	Phlobatannins	-	-
12	Carbohydrate	+	+
13	Anthraquinones	-	-

(+) = Indicates presence
(-) = Indicates absence

Medicinal plants contain many antioxidants such as vitamins (A, C, E, K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanins, catechins, isocatechins), polyphenols (ellagic acid, gallic acid, tannins), saponins, enzymes and minerals (selenium, copper, manganese, zinc, chromium, iodine, etc.). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumor, and hepatoprotective properties. The source of natural antioxidants may be all or any part of plants such as fruits, vegetables, nuts, seeds, leaves, roots, barks, peels, plant, etc. [17]

The qualitative phytochemical analysis carried out in ethanol and aqueous extracts revealed the presence of carbohydrates phenolic compounds, flavanoids, steroids, tannins, saponins, alkaloids, and quinines.

In the ethanol extract of *a. comosus* the results were positive for eight compounds (carbohydrates, phenols, tannin, flavanoids, saponins, steroids, alkaloids, quinines). The *a. comosus* aqueous extract shows the positive results are ten compounds (carbohydrates, phenols, tannins, flavanoids, saponins, terpenoids, steroids, alkaloids, coumeriens, quinines (table 1).

The phytochemical analysis has shown the presence of potent phytochemical substances such as tannins, flavanoids, alkaloids, phenols, glycosides, terpenoids, saponins, carbohydrates and steroids [18]

Tannin containing drugs is used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering, and also medically used as healing agents in inflammation, leucorrhoea, gonorrhea, burns and piles. Besides anti-inflammatory they also have antiviral, antibacterial, [19]

Saponin is used as mild detergent in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in the human diet for controlling cholesterol and for weight loss.

Several studies have been carried out and results generated indicate *A.comosus* has useful phytochemical applications. However, these results are yet to be amalgamated and critically compared so as to chart the way forward as to whether *A.comosus* acceptance as a phytochemical supplement. The purpose the present studies is to highlight some relevant contributions regarding a. comosus phyto medical applications that have been reported in recent times.

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity 18 Phytochemicals could act as an antioxidant and anti-inflammatory. It plays a vital role in detoxification of harmful and deleterious chemicals of the body. The phytochemical tests were carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones and is presented [20].

Table 2: DPPH activity of *Ananas comosus*

S. No	Concentration	DPPH (%)		
		Aqueous	Ethanol	Standard
1	50	32.2±1.2	43.3±0.8	20.6±0.8
2	100	40.6±1.7	54.3±0.3	28.9±0.3
3	150	49.4±2.3	65.4±0.7	32.7±1.2
4	200	58.9±2.1	73.6±0.4	40±2.3

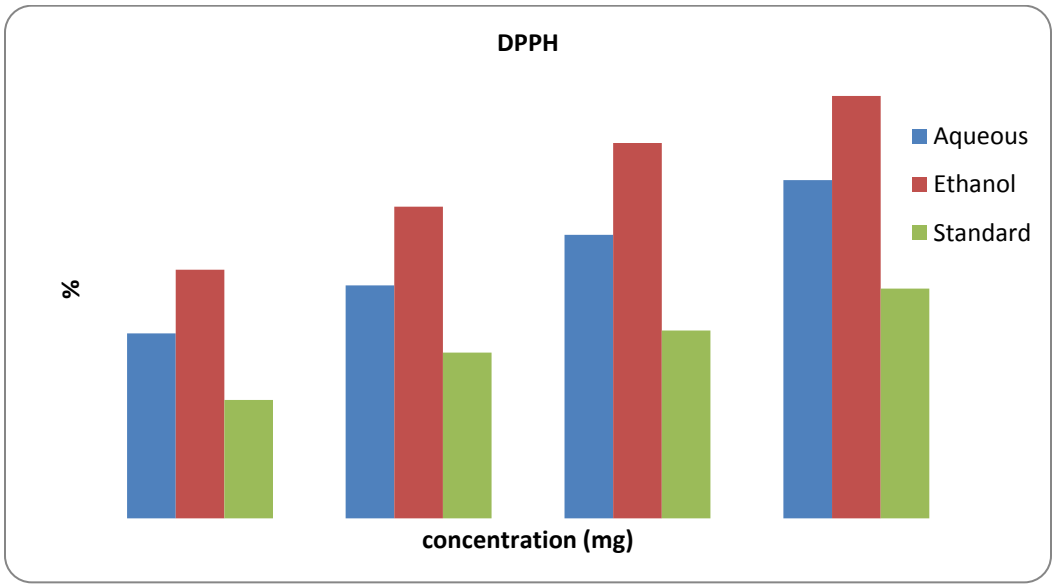

Figure 1: DPPH activity of *ananas comosus*

Figure 1 and table 2 shows the DPPH activity *of a.comosus* in comparison with ascorbic acid standard. It indicates the level of DPPH activity of *a. comosus* which is increased in methanolic extract when compared to aqueous extract.

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity.

DPPH is a stable free radical compound with a characteristic absorption at a wavelength of 517 nm. Antioxidants upon interaction with DPPH either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The colour of the reaction mixture changes from purple to red resulting in an absorbance decrease. The degree of discolouration indicates the scavenging potential of the antioxidants [21].

Table .3: Hydrogen peroxide scavenging activity of *Ananas comosus*

S.No	concentration	H_20_2		
		Aquous	Ethanol	Standard
1	50	18.8±0.65	27.8±1.6	20.6±0.8
2	100	21.2±0.5	35.2±0.68	28.9±0.3
3	150	24.9±0.5	41.4±0.96	32.7±1.2
4	200	26.9±0.4	46.5±1.6	40±2.3

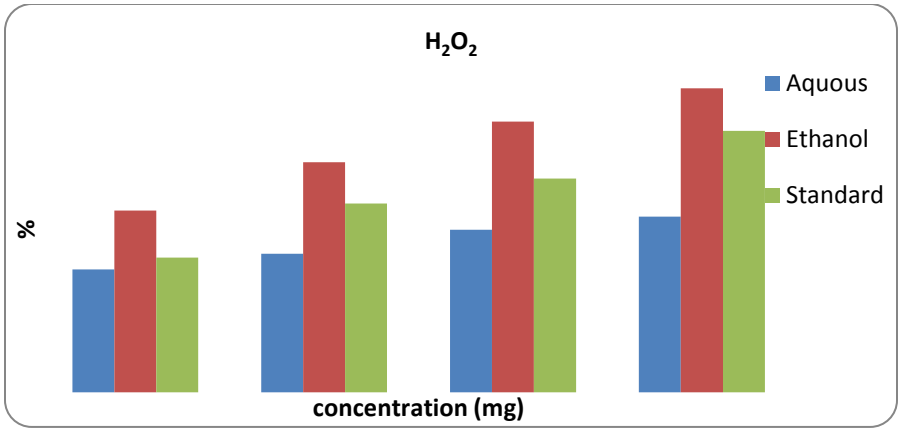

Figure: 2 Hydrogen peroxide scavenging activity of *Ananas comosus*

Figure 2 and table 3 shows the hydrogen peroxide scavenging activity of *A.comosus* in comparison with Ascorbic acid standard. It also shows the hydrogen peroxide scavenging activity of *A.comosus* which is increased ethanolic extract when compared to aqueous extract.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with and Fe2+possibly Cu2+ ions to form hydroxyl radicals and this may be the origin of many of its oxide effects. From the results, it appeared that the H2O2 scavenging activity of the plant extract is significant compared to that of the standard ascorbic acid. Scavenging of H2O2 by extract may be attributed to their phenolics, which can donate electrons to H2O2, thus neutralizing it to water. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method where they are compared with that of ascorbic acid as standard [22].

Table 4: Total antioxidant capacity of *ananas comosus*

S.no	Concentration	TAC (%)		
		Aqueous	Ethanol	Standard
1	50	19.15±0.6	22.7±1.7	20.6±0.8
2	100	24.6±3.8	28.5±0.9	28.9±0.3
3	150	33.4±0.6	34.8±0.5	32.7±1.2
4	200	41.7±1.2	44.3±1.5	40±2.3

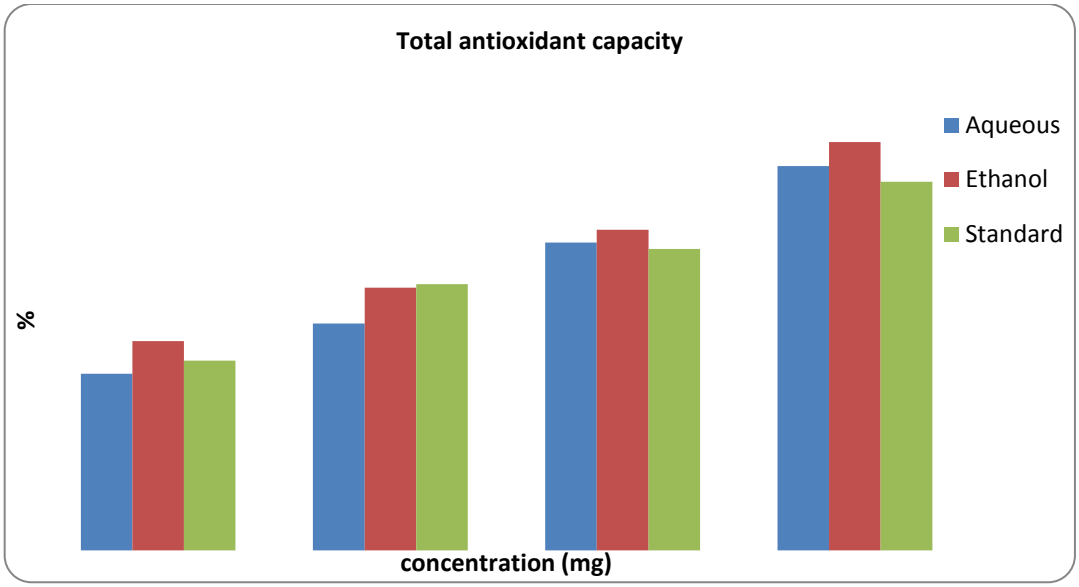

Figure: 3 Total antioxidant capacity of *Ananas comosus*

Figure 3 and table 4 indicates the total antioxidant activity of *A.comosus* in comparison with standard ascorbic acid. It shows that the total antioxidant activity of *A.comosus* which is increased in ethanolic extract when compared to aqueous extract.

The phosphosmolybdenum method used for total antioxidant activity is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximum absorption at 695 nm. The antioxidant activity was determined by the regression equation of calibration curve and expressed as ascorbic acid equivalents (AAE). Among both extracts of the plants, the ethanolic extract of *ananas comosus* showed the highest antioxidant activity. The extracts of *ananas comosus* were found to possess considerable antioxidant capacity. The antioxidant activity of plant extracts may be due to their phenolics and flavonoid contents. [23]

SUMMARY AND CONCLUTION

From the study it can be summarized that all the *ananas comosus* fruits are sources of various nutrients and can be helpful in fighting against viral diseases, inflammations, arthritis etc.,

The following investigations were done in this study

1. Screening of extracts for different types of phytoconstituents indicates the presence of steroids, phenol, tannins, flovonoids, alkaloids, saponins, carbohydrates and the absence of phiobatanins, antraquinones in aqueous and ethanolic extracts. Glycosides was absent in ethanolic extract. Terpenoids, coumarins were absent in aqueous extract.
2. The Determination of Total antioxidant activity, DPPH scavenging activity and Hydrogen peroxide radical scavenging activity gives the Ananas *comosus* fruit contains a high amount of antioxidant activity in ethanolic extract when compared to aqueous and standard.
3. It can be concluded that all the ananas *comosus* fruits contain different type of phytochemicals and higher activity of antioxidant. Most of the biologically active phytochemicals were present in the ethanolic extract of ananas comosus fruit juice.

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