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Characterisation of Phytochemical Constituents, Antioxidant and Anti Bacterial Properties of Red Strawberry against *Klebsiella* and *Streptococcus*

[1]Mr. G. Karthikeyan, [2]Ms. M. Eureka, [3]Ms. J. Amalsiya Bernath

[1]Assistant Professor, Department of Biochemistry, Kongu Arts and Science College (Autonomous), Erode

[2,3]II - M.Sc Biochemistry, Kongu Arts and Science College (Autonomous), Erode

ABSTRACT

In plant kingdom, medicinal plants are main important resource for a variety of drug like emetics, anti-cancer and antimicrobials. Medicinal herbs are highly cultured in India, which includes more than 2000 species are present. Strawberry is soft fruit crop belongs to the family *rasacea* and genus *fragaria* and it contain excellent source of vitamins, potassium. Phytochemical constituents are identified in different extract (ethanol, acetone and aqueous). Secondary metabolites are used for the treatment of several diseases. Strawberry is the one of the best natural sources of antioxidants. Total antioxidant capacity was identified in all the extracts. Antibacterial activity of *fragaria x ananassa* was evaluated against and *Klebsiella* and *Streptococcus.* From this study, concluded that the strawberry fruit have potent medicinal value.

1. INTRODUCTION

India has a rich culture of medicinal herbs and spices which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurveda, unani, siddha traditional medicines [2]. A plant makes many chemical compounds for biological function and including defence against insects, fungi and herbivorous mammals. Plants have a great importance in our lives because they fulfil our basic needs for food, shelter, clothing, fuel, ornamentals, flavouring and medicine [8]. Plant-derived materials or products with therapeutic properties are known as herbal medicines, they may contain processed or raw ingredients from one or more plants that are beneficial for human health [20].

People use herbs to treat different diseases because they are cheap and effective, but doctors are often reluctant to prescribe them because of knowledge deficiency, real concerns [12]. In this study, traditional uses of plant are recognized as an effective way to discover further medicines. There are many parts in plants such as leaf, stem, fruits, buds, roots, etc., now we are ready to use the fruits of strawberry *(fragaria x ananassa*) as medicinal herbs (Common name: Strawberry; Kingdom: plantae; Type: plant; Height: 10 - 12 inches (~25-30cm); Bloom time: February; Fruit: Edible; Order: Rosales; Subfamily: Rosacea; Genus: Fragaria; Species: *Fragaria x ananassa*) [9].

Strawberry *(fragariax ananassa duch*) is a soft fruit crop. Strawberries are unique with highly desirable taste, flavour, and excellent source of vitamins, potassium, fibre and sugars. As compared to other berry fruits, strawberries contain a higher percentage of vitamin C, Phenolics and flavonoids. Strawberry fruits are characteristics aroma bright red colour, juicy texture and sweetness [17]. It is consumed in large quantities, either fresh or in such prepared foods as preserves, fruit juice, ice creams and milk shakes. Nutritional and health aspects of fruit, they present alkaloids, flavonoids, carbohydrate, terpenoids, phenols makes the antioxidant potential as a well as protect some diseases as cancer or heart disorders.

The present investigation is aimed to characterize the medicinal effect of strawberry fruits using phytochemical analysis, antioxidant property and antibacterial activity [5].

2. MATERIALS AND METHODS

The present study deals with the study of phytochemical constituents, antioxidant capacity and antibacterial activity of *fragaria x ananassa fruit*.

2.1. Collection of Fruit

The *Fragaria x ananassa* (Strawberry) fruit was collected from erode local market and stored in refrigerator.

Figure 2.1: Image of sample used

2.2. Preparation Of Extracts (1:10 ratio)

Take 10 grams of freshly collected fruit sample and cut in to small pieces. Then grained with 100 ml selected solvents like Distilled water, Acetone and Ethanol. The contents were filtered through Whatmann filter paper and stored the filtrate in a respective conical flask with lid. This is used for the further analysis.

2.3. Phytochemical Analysis

2.3.1. Test for Alkaloids: To a few drops of extract, 2 drops of Mayer's reagent is added by the side of the test tube. A green coloured precipitate confirms the test as positive.

2.3.2. Test for Amino Acids: To a few drops of extract, few drop of Ninhydrin solution was added in a test tube. A characteristic blue colour indicates the presence of amino acids.

2.3.3. Test for Carbohydrates: To a few drops of extract, 2 ml of molish's reagent is added. The mixture is shaken well and 2 ml of Conc. H_2SO_4 is added slowly along the sides of the test tube and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.

2.3.4. Test for Tannins: To a few ml of extract, few drops of 1% Lead acetate is added. The mixture is shaken well. A yellowish precipitate indicates the presence of tannins.

2.3.5. Test for Phenols: To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow colour appears which indicates the presence of Phenols.

2.3.6. Test for phytosterols: *The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Appearance of Red, pink or violet colour at the junction of the liquids indicates the presence of phytosterols.*

2.3.7. Test for Proteins: To a few ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of protein.

2.3.8. Test for Saponins: To a few ml of extract, 20 ml of distilled water is added in the test tube and the test tube is continuously shaken for 10 minutes. The foam formed confirms the presence of saponins.

2.3.9. Test for Terpenoids: To 2 ml of extract, 2 ml of acetic anhydride and Conc. H_2SO_4 is added. Formation of blue, green rings indicate the presence of terpenoids.

2.3.10. Test for Steroids: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of Conc. H_2SO_4. Formation of violet to blue green colour indicates the presence of Steroids.

2.4. Antibacterial Activity (Bewer *et al* 1966)

Agar well diffusion method was used to evaluate the antibacterial activity of extracts against test microorganism. Nutrient agar medium (pH 7.0) was prepared and autoclaved. It was allowed to cool up to 45°C. Then it was seeded aseptically with 500 µl of freshly prepared inoculum (106 colony forming unit, CFU) and immediately mixed. For inoculum preparation, the colonies of bacteria such as *Klebsiella* and *Streptococcus* were suspended in nutrient broth and turbidimetrically adjusted. 25 ml of seeded nutrient agar media was transferred into each Petri plate and solidify. The organisms were spreaded in different petri plates. Four wells were made in each plate. Test solution of 50 µl was poured into each respective well. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth was measured in millimetre (mm). Experiment was done in triplicate and mean value of zone inhibition was calculated with standard error.

2.5. Test for Total Antioxidant Capacity

Pipetted out 1.0 ml of *fragaria x ananassa fruit* sample extracts in a respective test tubes. The volume of all the test tubes is made up to 3 ml with distilled water. 3 ml of distilled water is taken in a blank. 1 ml sodium phosphate, 1 ml of

ammonium molybdate and 1ml of sulphuric acid was added to all the test tubes. The blue colour was developed immediately read at 540 nm. The amount of total antioxidant is expressed as mg/g of fresh weight. Experiment was done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

In this study, the phytochemical constituents like alkaloids, amino acids, carbohydrates, tannin, phenols, phytosterols, protein, saponins, steroids and terpenoids were analysed. The above phytochemical constituents were equally present in all the extracts of *fragaria x ananassa* fruit sample.

Table 3.1: Phytochemical screening of different extracts of *fragaria x ananassa*

S. No	Phytochemicals	Extracts		
		Aqueous	Acetone	Ethanol
1	Alkaloid	+	+	+
2	Amino Acid	-	-	+
3	Carbohydrates	+	+	+
4	Tannins	-	+	-
5	Phenols	+	+	-
6	*Phytosterols*	+	+	+
7	Proteins	+	-	+
8	Saponins	-	-	-
9	Terpenoids	-	-	-
10	Steroids	-	-	-

Figure 3.1: Phytochemical screening of Aqueous extract

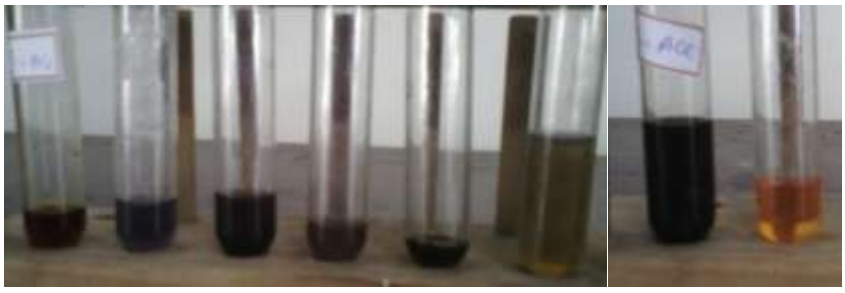

Figure 3.2: Phytochemical screening of Acetone extract

Figure 3.3: Phytochemical screening of Ethanol extract

3.2. Antibacterial Activity of *fragaria x ananassa*

3.2.1 Sub culturing of microorganism

The *Klebsiella* and *Streptococcus* organisms are sub cultured on a nutrient broth and incubated at 37ºC for 24 hours. The grown culture stored at 4º C for further studies

3.2.2. Zone of Inhibition

The extracts of the *fragaria x ananassa* had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against *Klebsiella* and *Streptococcus* organisms. The extracts showed enormous activity against the two bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm). Experiment was done in triplicate. The results were shown in the Table 3.2 and Figure 3.4.

Table 3.2: Antibacterial Activity of *fragaria x ananassa*

S. No	Extracts	Zone of Inhibition (mm in diameter)	
		Klebsiella Sp.	***Streptococcus***
1	Aqueous	9.0± 0.3	6.0± 0.2
2	Acetone	5.0 ± 0.3	3.0± 0.1
3	Ethanol	2.0± 0.1	1.0± 0.1

Figure 3.4: Antibacterial Activity of *fragaria x ananassa*

3.3. Total Antioxidant Capacity of *fragaria x ananassa*

In this study, the Total antioxidant capacity was analysed. The antioxidant capacity was highly present in the acetone extract of *fragaria x ananassa* fruit sample.

Table 3.2: Percentage of total antioxidant activities

Extracts	Total Antioxidant capacity (%)
Water	36 ± 3.0
Acetone	52 ± 2.8
Ethanol	42 ± 2.0

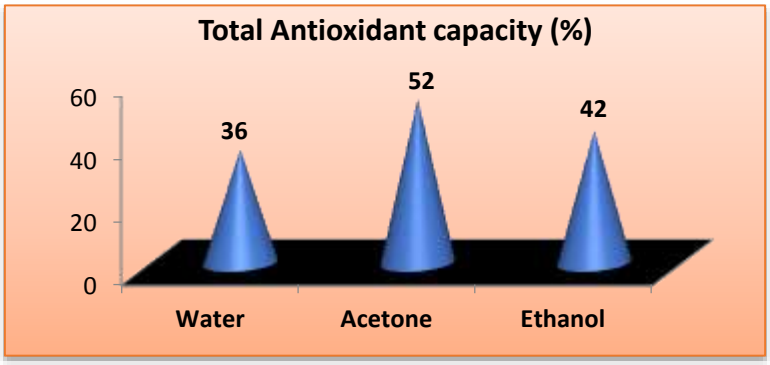

Figure 3.5: Shows the Total Antioxidant capacity

Figure 3.6: Indicates the presence of Total Antioxidants

4. CONCLUSION

The present study was carried out to determine the presence of bioactive constituents in fruit sample of *fragaria x ananassa.* In this study, we also examined the antibacterial activity of *fragaria x ananassa* fruit. Different solvent extract namely ethanol, acetone, aqueous extract were prepared from *fragaria x ananassa* fruit and screened for its phytoconstituents. The Aqueous and Acetone extract was found to be the best source of various phytochemicals (Proteins and Amino acids) when compared with ethanol extracts. This study has confirmed that the antibacterial activity of *fragaria x ananassa* extract against certain microorganisms. Results of this study showed that have found for that ethanol, acetone, aqueous extract of *fragaria x ananassa* fruit sample was quite adequate inhibiting the growth of *Streptococcus* & *Klebsiella* species. Total antioxidant capacities of fragaria x ananassa fruit extract were analysed. High level of Total antioxidant capacity was found in Acetone extract of *fragaria x ananassa* fruit. The results provide evidence that fruit sample of *fragaria x ananassa* extracts might indeed be used as a potential source of effective natural antimicrobial and antioxidant agents in pharmaceutical and food industries.

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