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Grapes (*Vitis Vitaceae*) - Potent Medicinal Fruit Serves as a Source of Antioxidants and Antibacterial Agent

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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. Grapes are soft fruit crop belongs to the Family of *Vitaceae* and Genus of *Vitis*. Grapes contain excellent source of nutritional values such as vitamins, minerals, proteins and carbohydrate. In this present work, various phytochemical constituents of grapes were identified in different extracts (Ethanol, Acetone and Aqueous). These phytochemicals are used for the treatment of several diseases. The antioxidant property of different extracts of grapes shows better result. The ellagic acid and the natural phenolic antioxidants were also identified. The antimicrobial activity of various grapes extract shows better result against *Enterococcus* and *E.coli* sps. Finally, the grape fruit is a wonderful antioxidant and antimicrobial agent.

KEY WORDS: Antibacterial, Ellagic acid, phytochemicals, Antioxidants.

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, Siddha traditional medicines [2]. But only very few have been studied chemically and pharmacologically for their potential medicinal value. Medicinal plants are important with respect to new drug and pharmacological research development. These medicinal plants are used in the treatment of many infectious diseases. Researchers are turning their attention to natural products to develop better anticancer, antiviral and antibacterial drugs. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [10,2]. In this study traditional human uses of plants, is recognized as an effective way to discover further medicines. From the recent researches, 122 compounds are identified and derived from traditional plants sources which are used in modern medicine. All parts in a plant possess the medicinal values such as leaf, stem, fruits, buds, roots, etc [5]. Now we are ready to use the grape fruits (*vitis vinifera*) as medicinal herbs because it was consumed either as fruit or juice by every individual day by day without knowing their medicinal values. Grapes are considered to be a berry. In the wild species it is 6 mm (0.24) diameter and ripens dark purple to blackish with a pale wax bloom. The wild grape is classified as *vitis vinifera* sub species. Ayurveda has been recognized the medicinal value of grape. Common name: Grape; Type: Tree; Height: 115feet; Water: Medium; Fruit: Edible; Kingdom: Plantae; Order: *Vitales*; Binomial name: *Vitis vinifer* [15].

Grapes are good source of vitamin C and K. They also contain protein, carbohydrates, dietary fiber and minerals [6]. Grape is a one of the most popular fruit and contain large amount of phytochemicals such as phenolic acid, flavonoids, tannins, anthocyannins, cyanidin, ellagic acid, proanthocyanidins which offer health benefits [8]. The anthocyanin present is responsible for the different colours of grape fruit like black, red and purple. Different parts of the plants could be used for a fever, diarrhea and ulcer [7]. The grape fruits must have antioxidant capacity used to treat many various rare diseases. It also serves as an antimicrobial agent because they have many secondary metabolites [11]. The aim of the work is to study the phytochemical analysis in different extracts of grapes (fruit) and to study its effect as anti-oxidant and antimicrobial activities.

2. MATERIALS AND METHODS

2.1. Collection and Extraction of the grape fruit

The fruit of *Vitis vinifera* was collected from erode local market, Tamil Nadu. The fresh plant material is extracted using soxhlet assembly and successively with ethanol, acetone and distilled water. Finally the plant material is macerated with distilled water. The extracted material is concentrated by evaporation.

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2.2. Experimental profile

2.2.1. Ethanol extract of *Vitis vinifera*

About 50gm of fresh fruit was extracted with 250 ml of ethanol by continuous hot percolation using soxhlet apparatus. After completion of extraction, it was filtered and concentrated to fresh mass by vacuum distillation. A dark pink and light yellow colour and waxy residue was obtained. The extract was the stored in a desiccator.

2.2.2 Acetone extract of *Vitis vinifera*

The marc left after ethanol extract was dried and subsequently extracted with 250ml of acetone by continuous hot percolation using soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. A light yellow colour and waxy residue was obtained. The extract was the stored in a desiccator.

2.2.3 Aqueous extract of *Vitis vinifera*

The marc left after acetone extraction was taken and finally macerated with 250ml of distilled water in a narrow mouthed bottle for 3 days. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. A light pink colour and waxy residue was obtained. The extract was the stored in a desiccator.

All these extracts were used for the identification of active constituents by following tests.

2.3 Preliminary studies of *Vitis vinifera* fruit extract

2.3.1. ALKALOIDS

a) Mayer's test: To a few ml of filtrate, two drops of Mayer's reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate indicates the presence of alkaloids.

b) Wagner's test: To a few ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate indicates test as positive.

c) Dragendroff's test: To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent reddish brown precipitate indicates positive test.

2.3.2. AMINO ACIDS: The extract is dissolve in 10ml of distilled water and filtered through Whatmann filter paper 1.0 and the filtrate was subjected to test for amino acids.

a) Ninhydrin test: Two drop of Ninhydrin solution is added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

2.3.3. CARBOHYDRATES

a) Molish's test: To 2 ml of filtrate, two drops of alcoholic solution of 1 – Napthol was added. The mixture was shaken well and 1 ml of concentrated Sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

b) Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

2.3.4. OILS AND FATS

a) Spot test: A small quantity of extract is pressed between the two filter papers. Oils stain on the paper indicates the presence of fixed oils.

b) Saponification test: A few drops of 0.5N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixer is heated on a water bath for 2hours formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.3.5. PHENOLIC COMPOUNDS AND TANNINS

a) Ferric chloride test: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

b) Lead acetate test: A small quantity of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

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2.3.6. PHYTOSTEROLS AND TRITERPENOIDS

a) Libermann – burchard's test: 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. Red, pink or violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

2.3.7. PROTEIN: The extract is dissolve in 10ml of distilled water and filtered through Whatmann filter paper 1.0 and the filtrate was subjected to test for protein.

a) Millon's test: To 2ml of filtrate few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

b) Biuret test: 2ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1ml of ethanol is added, followed by excess of potassium hydroxide pellets Pink colour layer indicates the presence of proteins.

2.3.8. SAPONINS

a) Foam or Froth test: A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

2.4. Estimation of Ash Content and Moisture Content

2.4.1. Estimation of Ash content: Empty silica crucial was cleaned well and heated over a Bunsen flame to red hot. Cooled in a desiccator and weighed. Heating, cooling and weighing were repeated to obtain constant weight. About 4.0g of sample was taken in two different crucibles. The crucibles were heated over a Bunsen burner until the sample turned ash. Heating, cooling and weighing were repeated until constant weight were got.

2.4.2. Estimation of Moisture content: An empty crucible was cleaned well and heated to red hot over a bunsen burner. Cooled it in a desiccator and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. 4.0g of sample was taken and heated in an oven at 100^{o}c for 6 hrs. Cooled in adesiccators and again weighed. This procedure was repeated until constant weight was obtained.

2.5. Test for biochemical components

2.5.1. Estimation of protein (Lowry's et al 1951)

In a series of test tubes, pipette out 0.2 ml to 1.0 ml of the working standard BSA solution corresponding to 20 µg to 200 µg values. Then take 1.0 and 2.0 ml of the extracts in another two test tubes. Make up the volumes to 1 ml in all test tubes. A tube with 1 ml of water serves as the blank. Add 5 ml of alkaline copper reagent to each tube including the blank. Mix well and incubate at room temperature in the dark for 30 mins. The blue colour developed was read at 660 nm in UV-Visible Spectrophotometer.

2.5.2. Estimation of phosphorous (Fiske subbarow's method)

In a series of test tubes, pipette out 0.2 ml to 1.0 ml of the working standard solution corresponding to 1.6 µg to 8.0 µg values. Then take 1.0 and 2.0 ml of the extracts in another two test tubes. The volume of all the test tubes is made up to 9 ml with distilled water. 9 ml of distilled water is taken in a blank. 1 ml of Ammonium Molybdate and 0.4 ml of ANSA was added to all the test tubes. Mixed well and incubate at room temperature at 10 minutes. The blue colour developed was read at 680nm.

2.5.3. Estimation of magnesium (Titan yellow method)

In a series of test tubes, pipette out 0.2 ml to 1.0 ml of the working standard magnesium solution corresponding to 10 µg to 50 µg values. Then take 1.0 and 2.0 ml of the extracts in another two 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 titan yellow and 1 ml of NAOH was added to all the test tubes. The red colour was developed immediately read at 540nm.

2.5.4 Separation of amino acids by Ascending paper chromatography

The chromatography paper is cut carefully to convenient size (40×24cm). Draw a line with pencil across the sheet about 5cm away from one end. Marked a number of points at travel of 3cm, applied a small volume (25µl) of each amino acid as a separate small spot using a capillary tube. Stream of hot air from a hair dryer facilitates fast drying of spot. The spot should be as small as possible for better resolution.

Similarly spot unknown (*Vitisvenifera fruit* sample) mixture of amino acids. After spotting the paper was folded in the form of cylinder and lengthwise edges were stitched together. The paper was placed upright with the spots at the bottom in a large petridish and closed the chamber airtight. Care was taken to see the paper and as the solvent ascends the amino acids travel with

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different mobility. Note the solvent front and dry the chromatogram free of solvent at room temperature by hanging with clips with the starting end downward. Spray the chromatogram with the Ninhydrin reagent and dry the paper for about 5min at room temperature followed by 100ºC. Similarly spot unknown mixture of amino acids. After spotting, the paper placed in an oven for 2-3 minutes. Amino acids appear as purple spots, hydroxyl proline and proline gives yellow colored spots. The amino acids present in the unknown mixture are then mixed and then identified by comparing the Rf values with that of authentic amino acids, co-chromatographed.

2.6 Antibacterial activity (bewer et al 1966)

At 45ºc, 20 ml of sterilized culture media is poured into the petri plates. After the agar is solidified, the testing organisms (100 µl) are spread over it therough swab method. The test organisms are the *Staphylococcus aurous, E. coli* and *pseudomonas species*. Then add the different extracts (100 µl) to the respective wells in the petriplate. These petriplates are incubated at 37ºC for 24 hours at inverted position. After the incubation period, the zone of inhibition was determined. The result was obtained by measuring the zone in mm/ diameter.

2.7. Total antioxidant capacity

In a series of test tubes, pipette out 0.2 ml to 1.0 ml of the working standard ascorbic solution corresponding to 10 µg to 50 µg values. Then take 1.0 ml of the extracts in another test tube. 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 and 1 ml of ammonium molybdate and 1ml of sulfuric 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

2.8. Test for ellagic acid

- **Procedure 1:** A 5g portion of homogenized fruit was weighted into a Rotary vapor flask and 5ml of water containing 80mg of ascorbic acid, 20ml of methanol, and 5ml of concentrated HCL was added. The mixture was refluxed for 5 hours at 90°C.
- **Procedure 2:** The extraction of phenolic compound was carried out with three subsequent extractions with 15ml of methanol/HCL 0.01 N (9:1) on 10g of homogenized fruits. Seeds and skin are not separated, whereas elderberry stalks were removed. Each time, after acidified methanol addition, the sample was vortexes for 2min and then centrifuged at 3500g for 10min at 10ºC.

  Flavones were detected an aglycones after acidic hydrolysis, performed according to the method applied by ageel et al. On *Vitis venifera* fruit extract, but reducing the volume of the reaction mixture. Precisely, 100microml of methanol extract were diluted with 200 microml of water and 50 ml of 1.7 M HCL and vortexes for 2 min. tubes were then placed in a boiling water bath for 30min and allowed to react. Once the hydrolysis was completed, the sample was cooled under running water and 150 ml of MEOH was added, in order to obtain a final volume of 500 microml of the hydrolyze. Finally, the presence of pink colour indicates the presence of Ellagic acid.

3. RESULT AND DISCUSSION

In the present study, evaluates the phytochemicals, biochemical analysis and achieve the antibacterial and antioxidant activity of the *Vitis venifera.*

3.1. Phytochemical screening of *Vitis venifera fruit*

In this study, the phytochemical constituents like carbohydrates, protein, amino acids, alkaloids, tannins, phenol and terpenoids were analyzed. The above phytochemical constituents were highly present in the ethanol, acetone and aqueous extracts *Vitis vinifera* fruit sample. This was shown in the table 3.1.

Previous studies reported and *vitis vinifera* fruitcontains alkaloid, flavonoid, saponins, triterpenes in ethanol and aqueous extract (Wael Abdel-Mageed et al 2013) fruits contains sterols, phenolic compounds and fixed fats in ethanol extract (Atul Kabra et al 2013)

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Table 3.1: Phytochemical screening of *Vitis venifera* fruit

Tests	Ethanol	Acetone	Aqueous
Alkaloid			
1. Wager's Test	+	+	+
2. Dragendorff's Test	+	+	+
3. Mayer' Test	+	+	+
Amino Acid			
Ninhydrin Test	+	+	+
Carbohydrates			
1. Molish'Test	+	+	+
2. Benedict's Test	+	+	+
Fixed Oils and Fats			
1. Spot Test	-	-	-
2.Saponification Test	-	-	-
Tannin			
Ferric Chloride Test	+	+	+
Phenol			
1.Ferric chloride Test	+	+	+
2. Lead Acetate Test	+	+	+
Phyto sterols			
1. Libermann Test	+	+	+
2. Burchard's Test	+	+	+
Protein			
1. Million's Test	+	+	+
2. Biuret's Test	+	+	+
Saponins	-	-	-

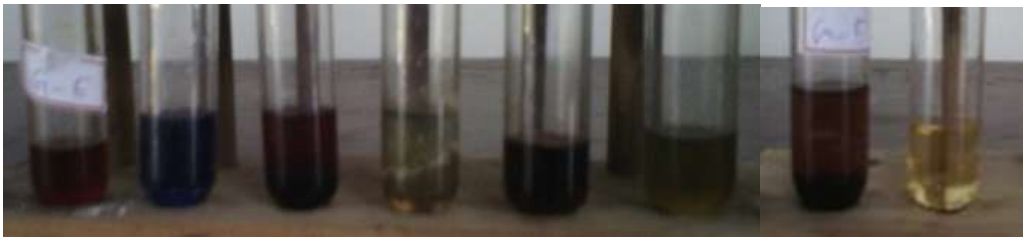

Figure 3.1: Ethanol extract of *Vitis venifera*

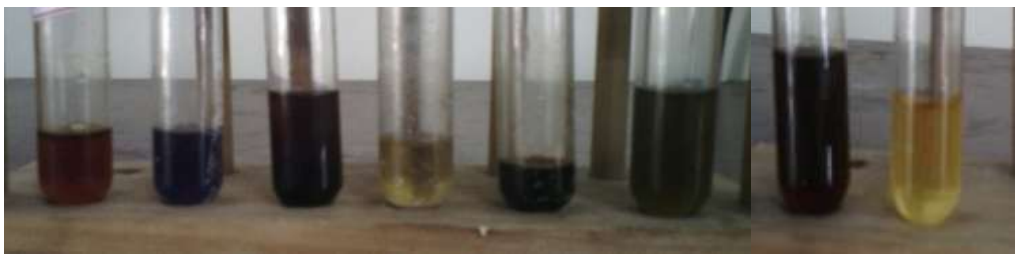

Figure 3.2: Acetone extract of *Vitis venifera*

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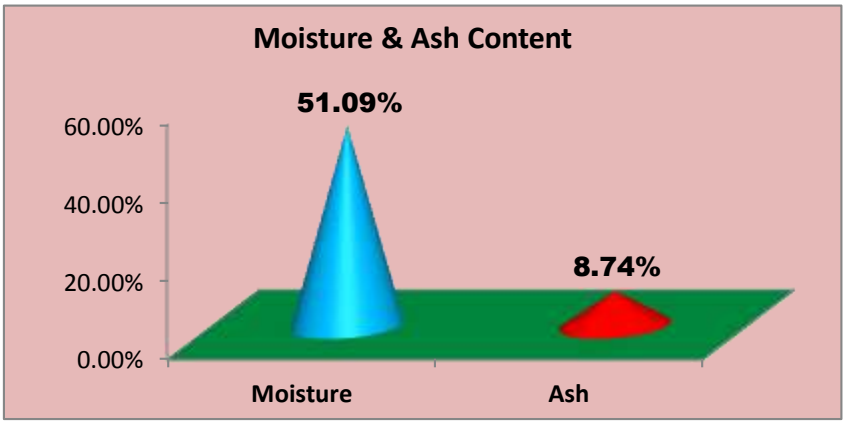
Figure 3.3: Aqueous extract of *Vitis venifera*

3.2. Ash & Moisture content of *Vitis venifera*

The present study showed that the moisture and ash content of *Vitis venifera fruit* represented as in %, this was shown in the following Table.

Table 3.2: Moisture and Ash content of *Vitis venifera* fruit

S. No	Moisture & Ash	Percentage of Moisture & Ash
1	Moisture	51.09 %
2	Ash	8.74 %



Grape 3.1: Comparison of Moisture and Ash content of *Vitis vinifera*

Figure 3.4: Moisture Content of *vitis vinifera*

Figure 3.5: Ash Content of *vitis vinifera*

Previous studies reported the values of ash and moisture content as % of rosacea and vitaceae species (Mounior et al 2013).

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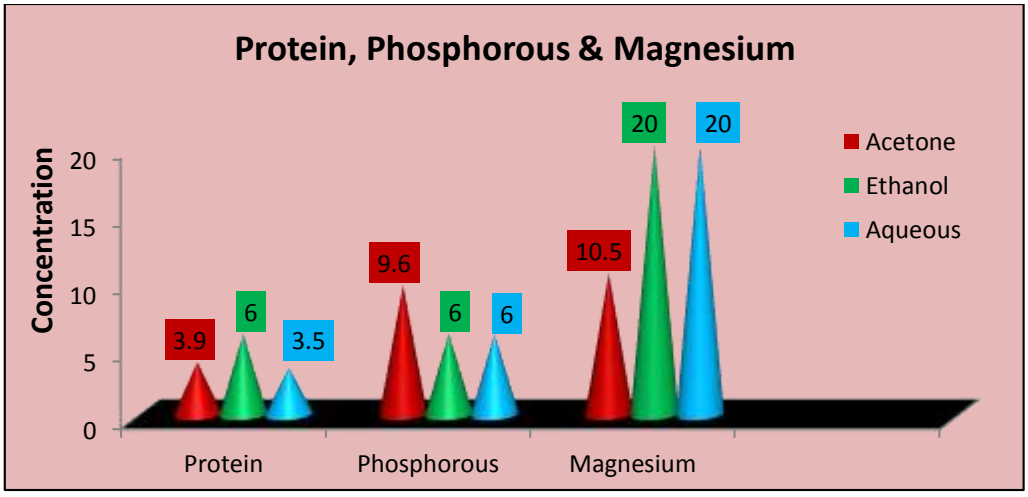
3.3. Biochemical constituents of *Vitis venifera*

In this study, the biochemical constituents like Protein, Magnesium and Phosphorous were analyzed. The above biochemical constituents were considerably present in the ethanol, acetone and aqueous extracts of *Vitis venifera* fruit sample. The amount of those constituents present in the fruits as follows,

Table 3.3: Various biochemical constituents in grape fruit

Constituents	Extracts of *Vitis venifera*		
	Acetone	Ethanol	Aqueous
Protein	3.9 mg	6.0 mg	3.5 mg
Phosphorous	9.6 mg	6.0 mg	6.0 mg
Magnesium	10.5 mg	20.0 mg	20.0 mg



Graph 3.2: Biochemical parameters of Grape extract

Figure 3.6: Protein content in Acetone, Ethanol and Aqueous extract of Grape

Figure 3.7: Phosphorous content in Acetone, Ethanol and Aqueous extract of Grape

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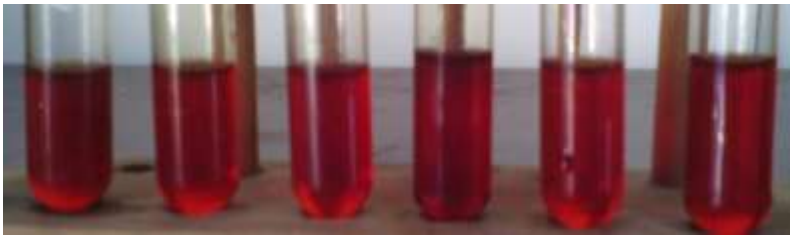

Figure 3.8: Magnesium content in Acetone, Ethanol and Aqueous extract of Grape

Previous studies reported the *Vitis venifera* fruit contains carbohydrates, protein, total sugar and lipids in ethanol and aqueous extract (Wael Abdel-Mageed et al 2013) and studies *vitis venifera* fruit contains carbohydrates, protein in ethanol extract (Atul Kabra et al 2013).

3.4. Separation of Amino Acids by ascending paper chromatography in *vitis venifera*

In this study, the Aromatic amino acid like aspartic acid and glycine were analyzed. The above Aromatic amino acids were highly present.

Table: 3.4: Rf values of Standard Amino acids and Amino acid in *Vitis venifera*

Amino Acid	Distance travelled by the solute (cm)	Distance travelled by the solvent (cm)	Rf value
A	0.7	7.0	0.1
B	0.9	7.0	0.12
Unknown	0.9	7.0	0.12

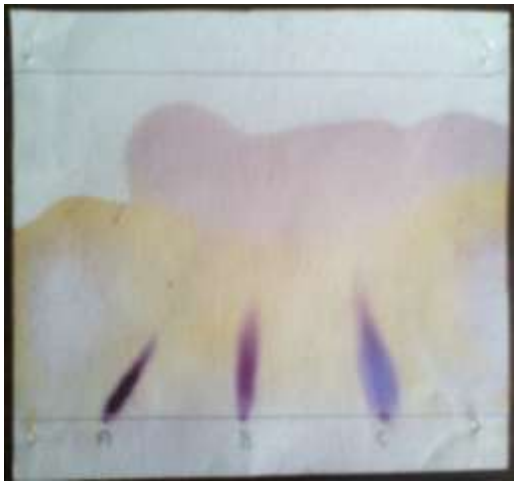

Figure 3.9: Chromatogram indicates the presence of Amino acid in *Vitis vinifera sample*

The Rf value of unknown Amino acid is close to that of sample B, so as we conclude the Amino acid present in *Vitis vinifera* is an Glycine. Previous studies reported the *vitis venifera* contains Linoleic acid, 2,5-Octadecadiynoic acid separated by Gas chromatography/mass spectroscopy (Sherif H. Abd-Alrahman et al 2013).

3.5. Antibacterial Activity of *Vitis venifera*

The extracts of *Vitis venifera* (fruit) had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against *E. coli* and *Enterococcus sps by* Gel diffusion method. The extracts showed enormous activity against 2 bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm).

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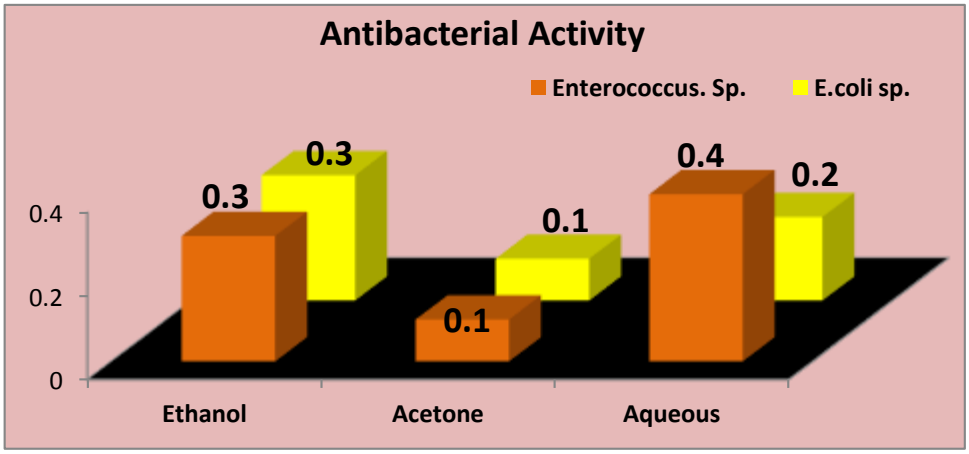
Table: 3.5: Level of antibacterial Activity of *Vitis venifera* extracts

S. No	Extracts	Zone of Inhibition (mm in diameter)	
		Enterococcus. Sp.	*E.coli sp.*
1	Ethanol	0.3 ± 0.2	0.3 ± 0.2
2	Acetone	0.1 ± 0.3	0.1 ± 0.1
3	Aqueous	0.4 ± 0.1	0.2 ± 0.1



Graph 3.3: Antibacterial Activity of *Vitis venifera* extracts

Figure 3.10: Shows the Antibacterial activity of *Vitis vinifera* extracts

From the result, we observed that the zone of inhibition of *Enterococcus sps* is higher in aqueous extract whereas the zone of inhibition of *Escherichia coli* is Ethanolic extract than others.

3.6. Total Antioxidant Capacity of *Vitis vinifera*

In this study, the Total antioxidant capacity was analyzed. The antioxidant capacity was highly present in the acetone extract of *Vitis vinifera* fruit sample.

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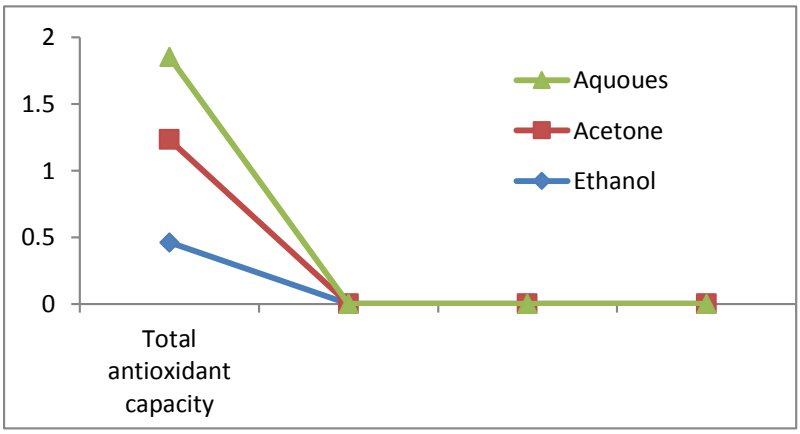

Graph 3.4: Total Antioxidant Capacity of *Vitis vinifera*

Figure 3.11: Total antioxidant capacity of different extracts of *Vitis vinifera*

3.7. Identification of Ellagic acid in *Vitis vinifera*

The identification of Ellagic acid in the grape fruit is the formation of pink colour in the reaction mixture. This is confirmed by the same procedure is done in banana also. But there is no any colour change in the banana sample so the Ellagic acid is absent.

Procedure 1:

Procedure 2:

Figure 3.12 & 3.13: Presence of Ellagic acid in *Vitis vinifera*

Figure 3.14 : Absence of Ellagic acid in Banana

4. CONCLUSION

Different solvent extract namely ethanol, acetone, aqueous extract were prepared from *vitis vinifera* 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 other solvent extracts. Bio-macromolecules like protein, phosphorous and magnesium were

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estimated quantitatively. This study confirmed the medicinal importance of *vitis vinifera* fruits. All the extracts of *Vitis vinifera* have antibacterial activity against selected microorganisms. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms.

This study has confirmed that the antibacterial activity of *Vitis vinifera* fruits extract against certain microorganisms. Results of this study showed that have found for that ethanol, acetone, aqueous extract of *vitis vinifera* fruit sample was quite adequate inhibiting the growth of *E.coli* and *Enterococcus*. This study also evaluated the Total antioxidant capacity of *Vitis vinifera* fruit extracts. Result of this showed that have high level of antioxidant capacity in aqueous extract.

From this study we identified the Ellagic acid in *Vitis vinifera*. Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. The anti-proliferative and antioxidant properties of ellagic acid have prompted research into its potential health benefits. So the consumption of grape leads to prevent many diseases like cancer because they have Ellagic acid which possesses the antioxidant, anti-mutagen and anti-cancer properties.

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