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EFFECTS OF ANTIMICROBIAL ACTIVITY OF *VITIS VINIFERA* (RED GRAPE) AND *CAMELLIA SINENSIS* (GREEN TEA) EXTRACTS

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ABSTRACT

Food and nutrients play vital role in normal functioning of the body. The samples selected for this study were Ethanolic extract of Red grape seed, Ethanolic extract of green tea leaves, Commercially available grape seed extract / Isotretinoin tablet, green tea extract, Vitamin A and E tablets. The Nutraceutical effects of grape seed extract and green tea extract was analyzed. The antimicrobial activity of all the above samples were studied by disc diffusion method against *Pseudomonas spp.*, *Flavobacterium spp.*, *Aeromonas spp.*, *Enterobacteriaceae*, *E. coli*, *Lactobacillus spp.*, and *Staphylococcus spp.* The antibiotic tetracycline disc used as a standard. All samples had been found to contain potent antimicrobial activity. The antioxidant activity was measured by DPPH method and compared with vitamin A and vitamin C. Both the prepared and commercially available extracts obtained more antioxidant activity than vitamin A & C. Thus the results showed that the grape seed extract and green tea leaves has antioxidant and antimicrobial activity.

INTRODUCTION: Foods and nutrients play vital role in normal functioning of the body. Worldwide acceptance of this fact formed a recognition link between “nutrition” and “health” and the concept of “nutraceuticals” was evolved.

Nutraceutical can be defined as, “a food (or part of a food) that provides medical or health benefits including the prevention and /or treatment of a disease.”¹ Nutraceuticals provide physiological benefits or reduce the risk of chronic disease, above and beyond their basic nutritional functions².

Recently green tea, which is the traditional drink of Japan and China, has been recognized as healthful. Catechins, which are polyphenol chemical compounds found in abundance in green tea, possess physiological

effects including antioxidative and bactericidal action as well as antitumor activity³.

The present study covers the nutraceutical effect that the health benefits of phytochemicals from black grape seed extract and green tea leaves extract.

MATERIALS AND METHODS

Collection of Samples: The tablet of green tea leaves extract and grape seed extract marketed by Parry nutraceuticals. For the preparation of ethanolic extracts grape seeds are collected from the local area and green tea leaves are collected from the estate Moonar.

A. Specifications of Parry's Green Tea leaf Extract

Polyphenols – 98%, catechins – 75%

Product Name: Green tea Leaf Extract power

Source: *Camellia Sinensis*

Carriers used: Dried Buds

Parry's Green Tea Leaf extract powder in a parameters specification description.

Light brown color powder with astringent taste and characteristic odor. Loss on drying – 3.0%, Particle size – 100% and Solubility in water – 95%.

◆ Identification Positive for catechins by HPLC

Epigallo catechin gallate by HPLC – 45%, Total polyphenol by UV method – 85%, Total catechins by HPLC method – 60%, Caffeine – 2%

◆ Microbiological:

Total Plate count – Nil

Yeast and mould – Nil

E. Coli, *Salmonella Staphylococcus aureus*, *Shigella* – Negative

B. Specification of Red Grape seed extract:
Isotretinoin extract- 20mg and Grape seed extract - 25mg

C. Preparation of ethanol solvent extract:

Soxhlet Method: For preparation of Extracts. Successive solvents extraction method was followed using soxhlet apparatus. The dried and powdered leaves of green tea leaves, grapes seed (45gm) were packed in the body of soxhlet extractor. The concentrated compounds after dryness were collected and used for various phyto chemical analyses.

D. Grape seed extract/ isotretinoin (Tablet) in ethanol and water (25mg / ml).

E. Green tea leaves extract (Tablet) in ethanol and water (35mg / ml).

Screening of phytochemical compounds: The various solvent extracts of the coarse powder of leaves of

green tea leaves and Grape seed extracts were subjected to chemical tests for the identification of various active constituents, using the methodology. The following major pharmaceutically valuable phytochemical compounds were analysed.

Detection of alkaloids: A few drop of dilute hydrochloric acid treated with 1ml of various extracts, filtered and was treated with Mayer's reagent. The formation of cream precipitate confirmed the presence of alkaloids.

Detection of flavonoids: To five ml each of the various extracts were separately dissolved with 1ml of alcohol (Stock Solution) then one ml each of stock alcoholic solution was added. With a few drops neutral ferric chloride solution. Formation of blackish red color indicated the presence of flavonoids.

Detection of phenols: To One ml of various extracts were dissolved in 5ml of alcohol and treated separately with a few drops of neutral ferric chloride solution. The change in color indicated the presence of phenols.

Defection of protein and free Amino acids: To 5ml of each of various extracts were dissolved in 5ml of water separately and were subjected to Ninhydrin test purple or pink color indicate the presence of protein.

Detection of Saponins: To One ml each of the various extracts were separately mixed with 20ml of distilled water and then agitated in a graduated cylinder for 15min. The formation of foam indicated the presence of saponins.

Detection of phytosteroids and terpenoids: To five ml each of various extracts were dissolved in 5ml of chloroform separately. Then they were subjected to Liebermann – Burchard test. Formation of brown ring at the junction of the two layers and the upper layer turned green indicated the presence of phytosteroids and terpenoidal sapogenins.

Detection of tannins: To five ml each of the various extracts were dissolved in minimum amount of water separately and filtered and added a few drops of aqueous basic lead acetate solution. Formation of reddish brown precipitate indicated the presence of tannins.

Detection of sugars: To five ml each of the various extracts were dissolved separately in distilled water, filtered and then subjected to Fehling's test. The formation of reddish brown color indicated the presence of sugars.

Detection of glycosides: Another portion of the extracts was hydrolysed with hydrochloric acid for few hours on the water bath and the hydrolysate was subjected to Legal's test to detect the presence of different glycosides. The appearance of pink to red color showed the presence of glycosides.

Antioxidant The free radical scavenging capacity was analyzed by DPPH assay which is a radical generating substance of free radical scavenging abilities of various antioxidants.

Procedure: Take 4ml of 0.1mm DPPH was taken at various concentrations (9mg, 17.5mg) were added vitamin A and vitamin C (9mg, 17.5mg) were also taken and to this 4ml of DPPH was added. All the tubes were incubated at room temperature in dark for 1 hour. After incubation to all the tubes 1ml of distilled water was added. The color changed from deep violet to pale yellow color. The reagent without the extract was used as the blank. The absorption was measured colorimetrically at 517 nm.

Antibacterial assay: Antibacterial assay was done by Kirby Bauer testing. The test organisms grow in a smooth "Lown of confluent" on the petriplate except in a clear zone around the antibiotic discs, which inhibited the growth of the organisms and indicate the susceptibility of the organisms.

Concentration Used: (Ethanol / Water):

| Samples | Concentration (mg/ml) |
|-----------------|-----------------------|
| GTE (T)/GTE(S) | 17.5mg/ml |
| | 4.25 mg/ml |
| GSE(T) / GSE(S) | 17.5mg/ml |
| | 4.25 mg/ml |

Screening of anti-bacterial activity: For antibacterial activity commercial antibiotic tetracycline was used. The standards of antibiotic disc were taken from the container using the forceps and placed gently on the surface of the inoculated petriplates. The extracts of various solvents were taken in separate containers. Sterile disc that were soaked in various solvent extracts

were then placed in the inoculated plates. The petridishes were then at 37°C for 24hrs in an inverted position. The inhibition zone of microbes was observed the results were tabulated and compared.

Concentration Used: (Ethanol / Water):

| Samples | Concentration (mg/ml) |
|-----------------|-----------------------|
| GTE (T)/GTE(S) | 17.5mg/ml |
| | 4.25 mg/ml |
| GSE(T) / GSE(S) | 17.5mg/ml |
| | 4.25 mg/ml |

Anti-mutagenic study by Ame's test:

RESULTS AND DISCUSSION: Figure 1a and b shows the results of the inhibitory efficiency of grape seed extract, green tea leaf extract against various pathogens. The highest zone of inhibition is exhibited by ethanolic extract of green tea extract on *Aeromonas* (Fish pathogens) and *Enterobacter* (human pathogens).

Grape seed extract showed maximum inhibitory activity against *E. coli* moderately inhibitory against (fish pathogens) *Aeromonas*, *Flavobacterium*, *Edverchila Tarda* and (Human pathogens) *Enterobacter* and *Staphylococcus*.

In the case of grape seed extract among the three concentrations used 17.5mg/ml showed maximum inhibitory zone (15mm) *Aeromonas* and *Flavo bacterium*.

In the case of green tea leaf extract among the three concentrations used 35mg/ml showed maximum inhibitory zone (19 mm) *Aeromonas* and *Enterobacter*.

Table 1 showed that the Grape seed extract obtained from alcohol extraction by Soxhlet method has got the greatest antioxidant activity 87%. Green tea leaf extract is also having greater antioxidant activity 49% when compared with vitamin A and Vitamin C.

Table 2 showed that the antimutagenic effect higher in grape seed extract than the green tea leaves extract.

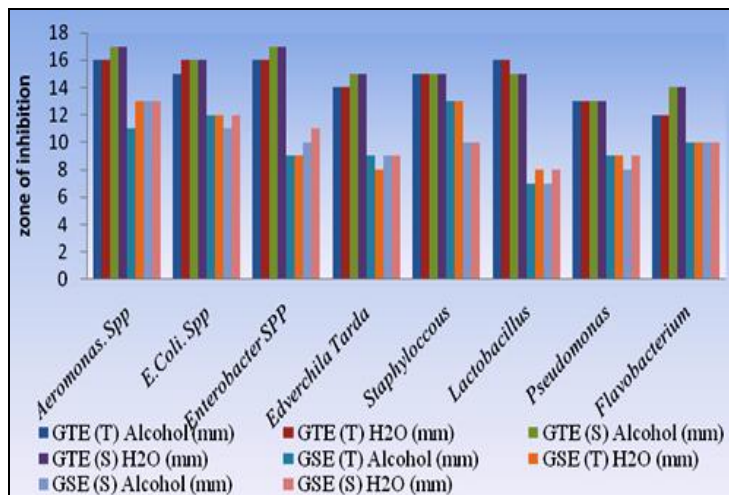


FIGURE 1B: ANTIBACTERIAL ACTIVITY OF SELECTED PLANT EXTRACTS (4.25mg/ml) GTE(T) Green tea extract tablet: GTE(S) Green tea extract skin : GSE(T) Grape seed extract tablet: GSE(S) Grape seed extract skin

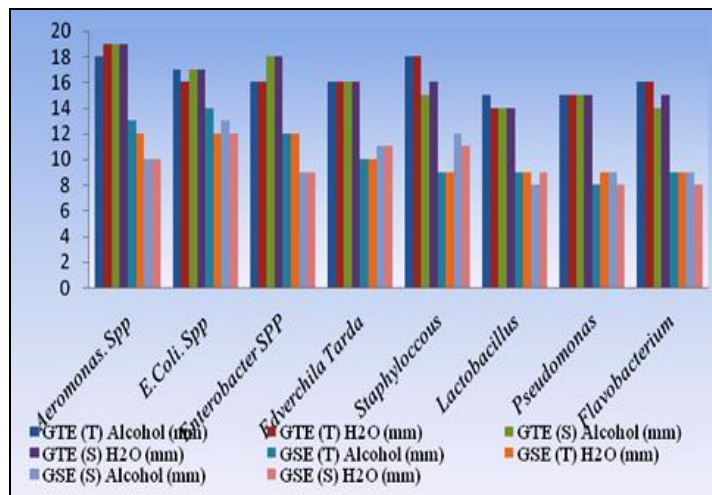


FIGURE 1A: ANTIBACTERIAL ACTIVITY OF SELECTED PLANT EXTRACTS (17.25mg) GTE(T) Green tea extract tablet :GTE(S) Green tea extract skin : GSE(T) Grape seed extract tablet :GSE(S) Grape seed extract skin

TABLE 1:

| S. No. | Concentration (mg/ml) | Ethanol Fraction | | | | | |
|----------------|-----------------------|------------------|---------------|-------------|-------------|-------------|-------------|
| | | Vit A (%) | Vitamin C (%) | GSE (T) (%) | GSE (S) (%) | GSE (T) (%) | GSE (S) (%) |
| 1 | 4.25 | 37% | 24% | 45% | 69% | 17% | 22% |
| 2 | 17.5 | 39% | 24% | 49% | 75% | 29% | 23% |
| Water Fraction | | | | | | | |
| 1 | 4.25 | 35% | 37% | 49% | 85% | 49% | 17% |
| 2 | 17.5 | 41% | 47% | 50% | 87% | 27% | 34% |

TABLE 2:

| S. No. | Samples | No. of Colonies |
|--------|---------------------------------------|-----------------|
| 1 | Sodium azide | 202 |
| 2 | Without Sodium azide | 160 |
| 3 | Grape seed extract + Sodium azide | 56 |
| 4 | Green Tea Leaf extract + Sodium azide | 89 |

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