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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF TWO CITRUS BASED FRUITS AGAINST PHYTOPATHOGENS

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Abstract
A considerable interest has been developed over the years in fruits and vegetables due to their potential biological and health promoting effects. The protective effect of value addition fruits along with honey and it has been attributed to their bioactive antioxidant constituents, including vitamins, carotenoids, and polyphenols. The current research showed both pomegranate and V.vinifera juice along with its addition of honey and wine, it gives the significant antibacterial effect had been noticed against both clinical as well phytopathogenic pathogenic organisms. Moreover, this study confirms the anticancer activity of Punica granatum and Vitis viniferafruit juicealong with its two-value addition supplementary products named as Honey and Wine which might be attributed to its phytochemical components most especially flavonoids and quinones so it could be considered as experimental complex.

Introduction

Fruit juice is a popular choice of beverage and the fruit juice market is one that has grown substantially over recent years (Mintel 2009). Indeed, recent research indicates that the market for fruit juice and juice drinks increased by 37% between 1999 and 2004, reaching 2.32 billion pound, with volume increasing by 26% over the same period to around 2.2 billion litres per year (Mintel, 2009). Its rise in popularity may be due in part to an increase in interest in health and nutrition amongst consumers, but also to the fact that the ever-expanding fruit juice market now provides a range of different juice varieties catering for all tastes and needs (Mintel, 2009). Juice is a popular beverage among consumers, seen by many as healthy option, which is also natural.

Objectives

- To investigate the phytochemicals from two various citrus based fruit extracts of *Punica granatum* and *Vitis vinifera* alone as well as with wine and honey
- To study the antimicrobial activities of citrus based fruit juices on phtytopathogenic bacteria.

Classifications of experimental sample -1

a.Punica granatum

Family: Lythraceae
Genus : Punica
Species: granatum

Kingdom:Plantae
Order:Myrtales
Botanical name : *Punica granatum*

b.Vitis vinifera

Family	: Vitaceae
Genus	: Vitis
Species	:vinifera
Kingdom	:Plantae
Order	:Vitales
Botanical name	:*Vitis Vinifera*

Preparation of Experimental Sample

Collect the citrus based current research chosen experimental fruits named such as*Punica granatum* and *Vitus vinifera*. The extract is taken in ethanol, acetone and water. And theextracts are further mixed in honey and wine.

Preliminary Phytochemical Analysis

a.Test for Anthocyanins :1ml of extract was taken and added to 1ml of 2N HCl. The appearance of pink red colour that turns purplish blue after addition of ammonia indicates the presence of anthocyanins.

b.Test for Aminoacids :1 ml of extract was taken to this 2 drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to 2 ml of aqueous filtrate . Appearance of purple colour indicates the presence of aminoacids

c.Test for quinones :1 ml of extract was taken and 1 ml of concentrated H_2SO_4 was added. Formation of red colour indicates the presence of quinones.

d.Test for protein :1 ml of extract was taken and to this few drops of Million"s reagent was added. A white precipitate indicates the presence of proteins.

e.Test for Carbohydrates : To 2 ml of extract, 1 ml of Benedicts reagent was added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-coloured precipitate indicates the presence of sugar.

f.Test for Flavonoids :2 ml of extract and 1 ml of alcoholic solution was added. The freshly prepared FeCl2 solution was added. Blackish red colour was formed

g.Test for Steroids : To 1 ml of extract 1 ml of chloroform and few drops of concentrated sulphuric acid was added along the sides of the test tube. The formation of red chloroform layer indicated the presence of steroids.

Antibacterial Activity

Antibacterial activities of the extracts were studied by disc diffusion method against the isolated strains of plant pathogenic bacteria. Lawns of each organism were prepared on Muller Hilton agar medium. The fruit extract was concentrated by evaporation and was added to sterile filter discs (3mm) and allowed the solvent to evaporate after each addition. The discs were then placed on air dried surface of the medium. The plates were then incubated for 24hrs at 37℃. After incubation the degree of sensitivity were determined by pressuring the zone of inhibition of growth around the discs.

a- Preparation of Pathogenic Bacterial Inoculums

For testing antimicrobial activity, *E. fecalis* and E.coli, Serratia sp., bacterial strains were adjusted equal to 0.5 McFarland standards by adding sterile distilled water. McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that number of microorganisms will be within a given range.

b- Assessment of Antimicrobial Activity

Antimicrobial activity of three fruit juices was tested by well-diffusion assay. Inoculums of each of bacterial strains were suspended in 5 ml of Brain Heart infusion broth and incubated at 37°C for 24 h. After incubation, 100 µl of inoculums was spread on sterile Muller-Hinton agar plates. Wells of 8 mm size were made with sterile borer into Muller-Hinton agar plates containing the bacterial inoculums and the lower portion was sealed with a little molten agar medium. 100 µl volume of the fruit extract was poured into each well of inoculated plates. The plates thus prepared were left at room temperature for 10 min allowing the diffusion of the extracts into the agar. After incubation for 24 h at 37°C, the plates were observed. Antibacterial activity present on the plates was indicated by an inhibition zone surrounding the well containing the fruit juice. The zone of inhibition was measured by measuring scale in millimeter. Data collected was coded, computerized, and analyzed using statistical package for social sciences (SPSS version 9.0)

Result

Table-1:-Preliminary Phytochemical Estimation Of Experimental Sample *Punica granatum* and *Vitis vinifera* extracts in Ethanol, Acetone and Water

Test for anthocyanins	**Punica granatum extract**	**Vitis vinifera extract**
Ethanol	-	-
Acetone	+	-
Water	+	-
Test for quinones		
Ethanol	+	-
Acetone	+	-
Water	+	-
Test for carbohydrates		
Ethanol	+	+

Acetone	+	+
Water	+	+
Test for amino acids		
Ethanol	+	+
Acetone	+	+
Water	+	+
Test for flavonoids		
Ethanol	+	-
Acetone	+	-
Water	+	-

Antibacterial spectrum of *Punica Granatum* extract against Plant Pathogenic Bacterial (PPB) organisms

Name of microorganism	Name of solvent	Zone of inhibition in average
PPO1	**Ethanol**	0.45 ±0.51
	Acetone	0.31±0.35
	Distilled Water	0.62±0.15*
PPO2	**Ethanol**	0.2±0.17
	Acetone	0.35±0.29
	Distilled water	0.1±1.4^{is}
PPO3	**Ethanol**	0.2±0.75
	Acetone	0.14±0.06
	Distilled Water	0.80±1.13**
PPB1	**Ethanol**	0.64±75*
	Acetone	0.37±0.14
	Distilled water	0.25±0.11
PPB2	**Ethanol**	0.46±0.16**
	Acetone	0.46±0.21
	Distilled water	0.34±0.24
PPB3	**Ethanol**	0.40±0.11
	Acetone	0.65±0.15*
	Water	0.28±0.20

***- Denotes statistically significant at P< 0.05% level**
****- Stands for P<0.005% Level of Highly significant**

plant phytopathogenic organism from bamboo (PPB) and Orchid (PPO)Antibacterial activity of *Vitis vinifera* extract against host Plant Leaf Pathogenic Bacterial organisms

Name of microorganism	Name of solvent	Zone of inhibition in average
PPO1	**Ethanol**	0.1±0.06
	Acetone	0.1±0.04
	Distilled Water	0.3±0.1
PPO2	**Ethanol**	0.7±0.08*
	Acetone	0.3±0.33
	Distilled water	0.1±0.038
PPO3	**Ethanol**	0.3±0.16
	Acetone	0.2±0.14
	Distilled Water	0.2±0.11
PPB1	**Ethanol**	0.61±0.07*
	Acetone	0.28±0.14
	Distilled water	0.3±0.10
PPB2	**Ethanol**	0.14 ± 0.06^{IS}
	Acetone	0.81±0.0.08*
	Distilled water	0.51±0.1.44*
PPB3	**Ethanol**	0.15±0.0.06
	Acetone	0.41±0.0.09
	Water	$0.29\pm0.0.06^{IS}$

***- Denotes statistically significant at P< 0.05% level**
****- Stands for P<0.005% Level of Highly significant**
Plant phytopathogenic organism from bamboo (PPB) and Orchid (PPO)

Conclusion

A considerable interest has been developed over the years in fruits and vegetables due to their potential biological and health promoting effects. The protective effect of value addition fruits along with honey and it has been attributed to their bioactive antioxidant constituents, including vitamins, carotenoids, and polyphenols. The current research showed both pomegranate and *V.vinifera* juice along with its addition of honey and wine, it gives the significant antibacterial effect had been noticed against both clinical as well phytopathogenic pathogenic organisms. The juice has also been observed as a great antimicrobial source against the phytopathogen. Further studies must be done so as to improve the natural use of fruit juices against various plant and animal diseases. The fruit juices being good source of nutrients, minerals must be included in one's diet so that it can prevent various lifestyle diseases.