



An analysis of the inhibitory effect of various fruit extracts on the Human Pathogenic Bacteria

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Abstract:

Some of the fruits and vegetables that are used in our daily life have proved to be effective in treating the microbial and fungal infections. The current work was done to identify the anti microbial capacity of selected fruit extracts so as to design a more simple and economic means to treat these infections. The selected fruit samples were collected and various extracts such as Water, Ethanol, Chloroform and Methanol samples were made whose efficacy was tested against the selected bacteria Staphylococcus, Edwerdilla, Bacillus and Pseudomonas which are responsible for causing diseases like skin infections, typhoid, throat infections and allergies. The study proved that the samples of Pomegranate peel, Apple peel, Pomegranate seed crude and Tomato peel extracts had inhibitory effect against the test bacteria. This study can be further extended to test the range of organisms against which the samples have the inhibitory property. This effect can be implemented in developing food based therapies in treating infections.

Introduction:

Plant products such as fruits, leaves, stems, seeds, and peels are much of nutritive value. Plants, fruits and their extracts play a major role in preparation of ayurvedic medicines as well as cosmetics. These naturally possess quality of curing manifestations with out any side effects. Especially fruit peels are used in the treatment of skin infections, Urine infections, cancer, throat infections and allergies that are caused by pathogenic bacteria. Fruit peels possess a quality of anti-microbial, anti-inflammatory, anti-bacterial, anti-fungal properties.

PUNICA GRANATUM (pomegranate) provides 16% of an adult's daily vitamin 'c'. It is a good source of vitamin B5 pantothenic acid, Potassium, and natural phenols such as ellagitannis and flavonoids compounds. These fruit contains much potential health Benefits, it may be effective in reducing the heart diseases risk factors, macrophage oxidative status, and foam cell formation. Metabolites of pomegranate juice ellagitannus localize specifically in the prostate gland, colon, and intestinal tissues of mice. This indicates the use of pomegranate juice in treating several diseases.

MALUS DOMESTICA (Apple) is obtained from medium sized tree belonging to the ROSACEACE family. They are rich in vitamin 'c' and beta-carotene. Vitamin c is a powerful natural anti-oxidant. Apples are rich in anti-oxidants, phyto nutrients, flavonoids and poly phenols. However the fruit, contains no saturated fats and cholesterol, but is rich in dietary fiber, which helps prevent the absorption of dietary LDL cholesterol in the gut.

Each of the products from nature has its own healing power if traced accurately, and treated appropriately. The established truth is that nearly all of the fruits have some healing power, which is released on their consumption. Consumption of different types of fruits may cure many common ailments. The peels of pomegranate, apple, tomato, have a very good inhibitory action against human pathogens.

The current study was undertaken to demonstrate the anti-microbial property of fruit extracts on common human pathogens whose investigation showed varied results.

Materials and Methods:

For the isolation of human pathogenic bacteria samples were collected from throat swab, pimples, rashes and urine.

Methods used for the identification of bacteria were Gram's staining and Bio-chemical tests as per Bergey's Manual.

Preparation of fruit extracts:

Fresh fruits of pomegranate, apple, tomato peels were collected and ground to a paste. To the pasted peels different solvents like distilled water, ethanol, chloroform, and methanol are added respectively and centrifuged for 10 minutes at 6000rpm. The supernatants were collected and preserved.

Identification of various compounds in the fruit extracts [qualitative analysis]

The fruit extracts were subjected to qualitative analysis for the identification of compounds following the methodology of HARBONE [1998] and KOKATE [2001]

Test for alkaloids:

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagent is added. Most of the alkaloids are precipitated from neutral or slightly acidic solution by Mayer reagent.

Fig 1: Alkaloid test showing the formation of white yellowish precipitate at the bottom of the tubes.



Test for glycosides

To the solution of the extract in glacial acetic acid 0.5 ml of acetic anhydride and 0.5 ml of chloroform were added. Then concentrated solution was added slowly, formation of reddish brown color at the junction of two layers and the bluish green color in the upper layer would be seen.

Fig 2: Test for glycosides, some tubes showing the formation of reddish brown color at the junction of the two layers.



Test for Terpenoids and Steroids:

4mg of extract was treated with 0.5 ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly. A red violet color is shown for terpenoids and green bluish color for steroids.

Fig 3: Test for terpenoids, one of the tubes showing the color change to green bluish color indicating the presence of steroids.



Test for flavonoids:

4mg of extract was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution 5-6 drops of concentrated HCL was added and red color was observed for flavonoids and orange for flavones.

Fig 4: Test for flavonoids showing the change in the color to orange, indicating the presence of flavones.



Test for tannins:

To 0.5ml of extract 1ml of water and 1-2 drops of ferric chloride solution were added. Blue color was observed for galli tannins and green black for catholic tannins [Iyengar 1995]

Fig 5: Test for tannins, no color change to black or blue indicates the absence of tannins.



Screening of fruit peels of water, chloroform, ethanol and methanol extracts for anti-microbial activity

Muller- hinter agar medium was prepared as per the composition and sterilized. The test organisms Staphylococcus, Pseudomonas, Bacillus, and

Edwerdilla were plated on to the plates. Wells were punched with a sterile borer and were filled with 100ul of water, ethanol, chloroform, and methanol extracts respectively. The plates were incubated and the results were analyzed after 24hrs.

Results and Discussion:

Isolation of pathogenic bacteria from collected samples:

The collected samples like Skin sample, Pimples, Urine were used to isolate causative bacteria which are pathogenic. Nutrient agar medium was used as a nutritional source and spread plate method was used for initial isolation of organisms.

Identification of Bacteria:

Based on the results of Gram’s staining and Biochemical tests the isolated organisms were found to be Staphylococcus, Edwerdilla, Bacillus and Pseudomonas whose sensitivity to the extracts was later tested in the study.

Identification of the compounds:

Table 1: Showing the results of compound identification:

Compounds		Alkaloids	Glds	Tpds	Flds	Stds	Tannis
Apple extracts	Water	-VE	-VE	+VE	-VE	-VE	-VE
	Ethanol	+VE	+VE	-VE	-VE	-VE	-VE
	Chloroform	+VE	+VE	+VE	-VE	+VE	+VE
	Methanol	+VE	+VE	+VE	-VE	-VE	+VE
Pomegranate seed extract	Water	-VE	+VE	-VE	+VE	-VE	-VE
	Ethanol	-VE	+VE	+VE	+VE	+VE	-VE
	Chloroform	+VE	+VE	-VE	+VE	+VE	-VE
	Methanol	+VE	+VE	+VE	+VE	-VE	+VE
Pomegranate peel extract	Water	+VE	-VE	+VE	-VE	-VE	-VE
	Ethanol	-VE	+VE	+VE	+VE	+VE	+VE
	Chloroform	+VE	-VE	-VE	+VE	-VE	+VE
	Methanol	+VE	-VE	+VE	+VE	+VE	+VE
Tomato extract	Water	-VE	-VE	-VE	-VE	-VE	-VE
	Ethanol	-VE	+VE	-VE	-VE	+VE	+VE
	Chloroform	+VE	+VE	-VE	-VE	-VE	+VE
	Methanol	-VE	-VE	+VE	-VE	-VE	-VE

Glds-Glycosides; Tpds-Terpinoids;
Flds-Flavonoids; Stds-Steriods;

Table 2 showing the Mean diameter of zones of inhibition

		Staphalococcus	Edwerdilla	Pseudomonas	Bacillus
Apple	Water	0.45cm	—	-	-
	Ethanol	1.225cm	0.80cm	-	0.635cm
	Chloroform	-	1.025cm	-	-
	Methanol	0.95cm	1.5cm	-	0.40cm
Pomegranate seed	Water	0.85cm	1.63cm	1.60cm	0.95cm
	Ethanol	1.20cm	0.45cm	0.30cm	0.35cm
	Chloroform	1.10cm	0.33cm	1.15cm	0.62cm
	Methanol	0.32cm	1.43cm	0.44cm	0.11cm
Pomegranate peel	Water	1.625cm	1.60cm	1.50cm	2.50cm
	Ethanol	1.55cm	1.50cm	2.075cm	2.225cm
	Chloroform	0.75cm	-	0.70cm	-
	Methanol	1.65cm	1.875cm	1.25cm	2.05cm
Tomato peel extracts	Water	0.40cm	—	—	—
	Ethanol	—	0.43cm	0.35cm	0.45cm
	Chloroform	0.525cm	1.15cm	0.43cm	0.75cm
	Methanol	0.375cm	--	--	0.3cm

Fig 6: The figure shows the zones of inhibition for the pomegranate extracts against the Staphylococcus species

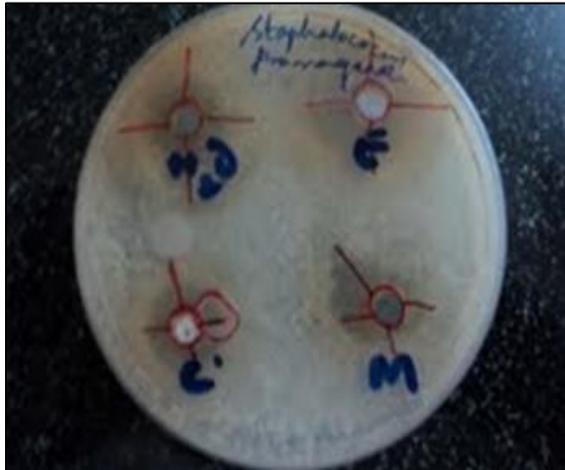


Fig 7: The zones of inhibition observed for apple extracts against Edwerdilla



Inference: From the above results it can be concluded that highest inhibitory action was seen for the aqueous extract of pomegranate peel Extract towards the organism Bacillus. Chloroform extracts were found to be less inhibitory for all the organisms tested.

Conclusion:

The current work was focused to study the medicinal values of abundantly available fruits so as to use them in the field of herbal treatments. The test pathogenic bacteria Staphylococcus, Edwerdilla, Bacillus and Pseudomonas are the most common human pathogens and are responsible for causing various skin infections, allergies, Urinary tract infections etc. All these pathogens showed considerable susceptibility to the tested fruit extracts. Among the tested samples pomegranate extract shows maximum antibacterial activity on staphylococcus 2.0cm and minimum anti microbial activity was shown by apple chloroform extract 0.9cm on edwerdilla. These results indicate that the naturally available fruit extracts can be employed as the best sources for the treatment of infections caused by the pathogenic bacteria.

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