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**Phytochemical Screening and Antibacterial Activity of Cultivated Medicinal Plants *Citrus paradisi***

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**Abstract** Fruits are the main fruit trees grown throughout the world and are well-appreciated for their refreshing juice and health benefits. This study of *Citrus paradisi* (peels) of hexane, acetone and methanol extracts was carried out to show phytochemical screening and antibacterial activity. Plants and plant products are continuously being explored in medicine against the increasing number of antibiotic resistant organisms. The antibacterial activity of plant under study has been done by using agar well diffusion method against bacteria. Phytochemical analysis showed the presence of terpenoids, alkaloids, flavonoids, tannins, reducing sugars and glycosides. *Citrus paradisi* extracts at a concentration of 100 mg/ml was determined by agar well diffusion method against pathogenic microorganisms; (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*). The crude extract of *Citrus paradisi* was very active against *Bacillus subtilis* strains (IZ = 25 mm zone of inhibition). This study has shown that grape peels contain some antibiotic principles which may be explored for use in the treatment of certain diseases.

**Keywords** *Citrus paradisi*, Methanol, Phytochemical, Antibacterial, Agar

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**Introduction**

The most important processed citrus fruits product is orange juice. Orange juice can be presented in different forms. The major types of orange juice are the freshly squeezed orange juice and frozen concentrated orange juice [1-2]. *Citrus* is the largest genus belong to the family Rutaceae and is the most traded horticultural Citrus fruits, are one of the main fruit tree crops grown throughout the world. Although sweet orange (*Citrus*) is the major fruit in this group accounting for about 70% of citrus output. The group also encompasses small citrus fruits such as tangerine tree (*Citrus reticulata*), grapefruit tree (*Citrus vitis*), lime tree (*Citrus aurantifolia*) and lemon tree (*Citrus limonum*) [3]. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi or viruses. Some plants have been investigated scientifically for antimicrobial activity and a large numbers of plant products have been shown to inhibit the growth of pathogenic microorganism.

The increasing problems of antibiotic drug resistance by pathogenic organisms in the past few decades and recently have led to the continuous exploration of natural plant products for new antibiotic agents [4-7]. Many of these products are produced in plants as secondary metabolites and often used in plants for defense against microbial attack [7].

The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as flavonoids, terpenoids, tannins, alkaloids that are present in these plants [8]. The plant is used as an ingredient in local medicine for several ailments.



Some plants are known as medicinal because they contain active substances that cause certain reaction, from relating to the cure of disease on the human organism [9]. Citrus is considered an important fruit in world production because its great value for human diet. Citrus is member of *Rutaceae* family from sub-tropical origin and is known for its semi-sweet taste.

Grapefruit contains many flavonoid glycosides, naringenin, quercetin, kaempferol, hesperetin and apigenin being the most abundant among their aglycones antibacterial effect of essential oils from *Citrus para-disi* peels from Turkey has been reported [10].

## Materials and Methods

### Plant material

Identification: [11] (Broun and Massey, 1929), [12] (Braun *et al*, 1991), which authenticated by the Herbarium at the Botany Department, Faculty of Science, were consulted for their identification. Voucher specimens of the plant material were deposited at the Botany. Traditional method of preparing the herbal preparations, The plant used in this study was collected from Albaha area (paneda ) market. The plant material was carefully examined for phytochemical screening Table (1) and then subjected to antibacterial activity screening Table (2).

**Table 1:** Phytochemical screening of different extracts

Phytochemical	Hexane	Actone	Methanol
Terpenes	+	+	+
Saponins	-	-	-
Flavonoids	-	+	+
Glycosides	-	-	-
Reducing sugar	-	+	+
Tannins	-	+	+
Alkalioids	-	+	+

+ present , - absent

**Table 2:** Extracts of *Citrus paradisi* against four standard bacterial organisms

Family / Botanical and Vernacular Name	Part used	Yield %	Solvent used	Test organism used MDIZ (mm)			
				Bacteria			
				Sa	Bs	Ec	Ps
Rutaceae / <i>Citrus</i>		1.50	Hexane	16	16	15	14
<i>paradisi</i>	Internal fruit	2.08	Acetone	13	18	18	-
Grape fruit	peels	6.00	Methanol	18	25	14	17

Values are in Inhibition Zones Diameter (mm)

M.D.I.Z = Main Diameter of growth inhibition Zone in mm

> or = 18 very active , 14-17 active , 0 < 14 inactive

Standard bacteria:

Sa: *Staphylococcus aureus*,

Bs: *Bacillus subtilis*;

Ec: *Escherichia coli*,

Ps: *Pseudomonas aeruginosa*

Bacteria used	Code No.
<i>Bacillus subtilis</i>	NCTC 8236
<i>Staphylococcus aureus</i>	ATCC 25923
<i>Escherichia coli</i>	ATCC 25922
<i>Pseudomonas aeruginosa</i>	ATCC 27853

### Preparation of the crude extracts

Each of the coarsely powdered plant material (50 g) exhaustively extracted for week with hexane, acetone and methanol-water (80:20) respectively in Soxhlet apparatus. The extracts were filtered and evaporated under reduced



pressure using rotary evaporator. The extracted plant material after extraction with each solvent was then air-dried, repacked in the Soxhlet. Each residue was weighed and the yield percentage was determined Table (2).

### Phytochemical Analysis

Phytochemicals are the main constituents of any plant sample, which are responsible for secondary metabolites also, table (1). The other works of these phytochemical are flavouring, colors etc [13].

#### Test for Alkaloids

0.2 g of extracts was shaken with 1 % HCl for two minutes. The mixture was filtered and drops of Dragendorff's reagent added. Formation of a precipitate indicated the presence of alkaloids.

#### Test for Saponins

0.2 g of extracts was shaken with 5 ml of distilled water in a test tube and heated. Frothing which persists on warming was taken as evidence for the presence of saponins.

#### Test for Flavonoids

0.2 ml of sample was added in 0.2ml of NaOH. And then add 1-2 drops of HCl, yellow to colorless showed the positive result.

#### Test for tannins

0.2 g of extracts was stirred with distilled water and filtered. Ferric chloride was added to the filtrate. A blue black, green or blue-green precipitate was taken as an evidence for the presence of tannins.

#### Test for reducing sugars

0.2 g of the extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for two minutes. An orange precipitate on boiling with the Fehling's solution indicated the presence of reducing sugars.

#### Test for Glycosides

Extract was hydrolyzed with HCl solution and neutralized with NaOH solution. Few drops of Fehling's solution were added, red precipitate showed the presence of glycosides.

#### Test for terpenoids (Salkowski test)

To 0.25 g of each of the crude and solvent fractions of *Rhamnus prinoides* leaves, 2 ml of chloroform was added. Then, 3 ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids [14].

### Antimicrobial Activity

The antibacterial activity was determined by the agar well diffusion method against different strains of bacteria. Each test bacterium was spread onto sterile Muller-Hinton Agar (Hi-Media). A 6 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 0.1 ml of the plant extract. Sterile dimethyl sulfoxide (DMSO) served as negative.

#### Bacterial Organisms

The standard organisms were obtained from the national collection of type culture (NCTC), Colindale, England and American type culture collection (ATCC), Rock Ville, Land, USA. Table (2).

### Results and Discussion

The percentage yields of methanol 80% in water (6.00 %) was much higher than those of acetone (2.08 %) and hexane (1.50 %) there for methanol is the best solvent, Table (2). In the present study in vitro evaluation was progressed for *Citrus paradisi* against four standard bacterial organisms *Staphyococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The results in (Table 2) showed that methanolic extract (80 % in water) of *Citrus paradisi* peels have the largest zones of inhibition with (25 mm) against *Bacillus subtilis* tested of methanolic extract, while hexane extract showed active (14-17 mm) inhibition zone towards all positive and negative organisms (Fig 1). Actone extracts have no activity toward *Pseudomonas aeruginosa* organisms, thus showed same activity with abroad spectrum against *Escherichia coli* strains (18 mm) and *Bacillus subtilis* (25 mm) zone of inhibition (Fig 1). So



methanol extract was proved highly active against all organisms of inhibition zone (14-18 mm), which consider a good solvent of all.

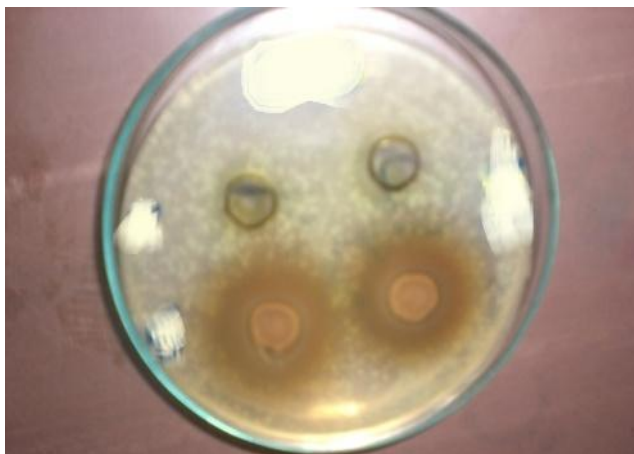


Figure 1

Development of new antibacterial principles to substitute with in efficient ones is a major weapon to combat the problem. Although the nature and number of active antibacterial principles involved in each extract of the present study are not clear, the broad spectra of activity of several plant extracts especially against antimicrobial organism.

### Conclusion

*Citrus paradisi* peels contains some useful potential antibiotic principles that are inhibitory to a broad spectrum of clinical isolates. Thus, it may be considered as a natural source of antimicrobials for therapeutic purposes. The result of Phytochemical analysis are responsible for the identification of components which are responsible for antimicrobial activity of plant, thus these traditional species can be used as a potential source of drugs against various diseases. antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of lemon fruit peels.

### Recommendation

A number of compounds, representing a variety of chemical compounds isolated from the various plant species, may provide interesting leads for further pharmacological considerations.

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