

Original article**Biological activity of *Citrus paradisi* peel**Amna Khalid^{1*}, Sara Hayee², Nouman Nasir¹ and Habibullah¹¹ Department of Chemistry, University of the Punjab, Lahore, Pakistan² Department of Zoology, Government Post Graduate College for Women Samanabad, Lahore, Pakistan

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Abstract:

Citrus paradisi (Grapefruit) peel oil has medicinal properties which shows beneficial effects against plant pathogenic fungi **Objective:** The aim of this research was to investigate the antifungal potential of *Citrus paradisi* peel oil against different plant pathogenic fungal strains **Methods:** The essential oil of Grapefruit peel was provided in different concentrations 0.25 to 0.75ml per 100 ml of media to 3 different plant pathogenic fungal strains i.e, *Fusarium oxysporum*, *Fusarium solani* and *Dreschlera tetramera* and its effect on the growth of fungi was recorded **Results:** The studies provided the results compared with the controlled media, which showed the fungal growth was almost completely inhibited at 0.75ml concentration of oil, even the lower concentration was sufficient to retard the growth **Conclusions:** The study provided the blue print for fungicidal spray to cure serious diseases in plants, such as, rice blast, papaya fruit rot, seedling collar rot and wilt diseases.

Key words: *Citrus paradisi*, Grapefruit, Biological activity, essential oil, *Fusarium oxysporum*, *Fusarium solani* and *Dreschlera tetramera*.

Introduction:

The management of industrial and agricultural waste by disposal, recycling or utilization to obtain other useful products has become a paramount need of the modern societies. According to studies if the waste is allowed to accumulate unchecked, the quality of air, soil, oceans and the entire environment will be threatened and even the existence of human and animal life will become a question mark. Improper handling of waste, for example, burning results in the formation of many harmful chemicals that cause many environmental problems [1]. For example, the increase levels of CO₂ by the combustion of fossil fuels and other organic matter has resulted in the greenhouse effect which usually increase the temperature by 2C° in the first era of present century [2]. Chlorofluorocarbons and other chemicals present in excessive amounts have reached the upper atmosphere and caused ozone depletion. This ozone depletion is the basic reason for the environmental pollution and human and animal

life is in danger. In 1970, existence of Tuna fish was alarmed due to the burning of polymers and several other such incidents were reported [3]. Wood, coal, petroleum, natural gas and plants have been the good sources for producing organic chemicals. Chemical literatures indicate that about 200 useful chemicals were obtained from wood by destructive distillation or hydrolysis before World War II. Impact of extractive chemical compounds from durable wood species has also been reported [4]. Similarly, coal has been an important source of fuel, power and organic chemicals. Coal may become more important both as an energy source and as the source of organic chemical feedstock in the 21st century [5]. The demonstrated coal reserves in the world are enough for consumption for over 215 years. Pakistan has recently started an extensive programme for generating electricity from coal based power plants [6]. Fluidised bed combustion technology is an important

technique which is gaining importance around the world in some advanced countries but this is beyond the capacity and expertise of developing countries like Pakistan [7]. From last many years petroleum has been a principle source of energy and organic chemicals. Most of the organic chemicals used these days have been derived from petroleum [8] but the reserves of petroleum are also decreasing day by day. Few countries also have great reservoirs of Natural gas. The main component of natural gas like Sui gas is methane in Pakistan. In addition to methane it also contain sulphur and other impurities. But, natural gas is used as domestic and industrial fuel. Its huge and vast stocks are also decreasing due to its rapid consumption.

From the studies the picture is clear that the dependency on non-renewable sources like petroleum and natural gas for producing chemicals should be avoided because of their continuous use instead the renewable resources like Agriculture crops and forests must be developed as alternative sources. Plants, in fact, are unique pollution free chemical factories of nature. Pakistan being a rich agricultural country has the potential to produce useful chemicals from waste plant materials. Some common waste obtained from different plants are listed below (Table 1)[9].

Name of the waste	Crop
Corn cobs	Maize
Rice Husk	Rice
Rice straw	Rice
Wheat Husk	Wheat
Wheat straw	Wheat
Wheat stalk	Wheat
Bagasse	Sugar Cane
Saw dust	Wood
Cotton straw	Cotton Branches
Peas Hulls	Peas
Fruits skin	Various fruits
Citrus fruit skin	Citrus fruit
Carrot Rind	Carrot

Table 1: Agri-Waste in Pakistan

Citrus paradisi commonly known as grapefruit is a relative newcomer to the citrus clan. The name grape fruit is in international use except in Spanish areas where it is referred as Toronja. The grape fruit tree reaches the height of 15 to 20 ft and can goes upto 45 ft. The fruit is nearly round to oblate or slightly pear shaped 10-15 cm wide with smooth finely dotted peel. Peel could be pale yellow, whitish, nearly pink or even deep red in 11 to 14 segments. Grapefruit seeds are polyembryonic. Grape fruit has many varieties such as, Duncan, Foster, Marsh, Oroblanco, Paradise Navel, Redblush, Star Ruby, Sweetie, Thompson (Pink Marsh) and Triumph. Grapefruit grows in a warm subtropical climate. It is used as a food and Nagarin extracted from its inner peel is used as a bitter in tonic beverages [10] bitter chocolate, ice cream and ices.

"Peel oil obtained from grapefruit peel is basically an essential oil which is more or less a volatile material isolated from an odorous plant by any appropriate physical technique." Sometimes flower oils [11] concentrates and even resinoids are only partially volatile and therefore, referred as essential oils. Essential oils differ from fixed oils which are usually obtained from seeds and stones of the plant. Structurally fixed oils are the glycerides of fatty acid, alkyl groups in natural glycoside may be same or different but the essential oils always have different compounds [12]. Constituents of peel oil are as follows (Table 2):

Components	Percentage (%)
Limonene	90
Waxy fractions(such as, Aldehydes, graniol, cardinene etc)	7-8
Oxygen compounds and Sesquiterpenes (volatile fraction)	2-3
9 coumarins and 22-dihydrostigmasterol	0.88

Table 2: Constituents of peel oil

Essential oils have many uses such as they possess antiseptic, healing, soothing and curing qualities. Generally essential oils are antioxidants [13+14]. They act as food preservatives in food and pickle industry for improving the shelf life of vegetable oils, meat and vegetables, cakes, fruits, butters, jams, jellies, sour and sweet pickles etc. Essential oils extracted from Eucalyptus, pine and violet based investigations of the previous workers revealed that the essential oils exhibit fungicidal and fungi static activities [15].

Methods:

Steam distillation apparatus including condenser, round bottom flask/still, still head, modified dean and stark trap and anhydrous Na_2SO_4 (for water absorption if present in oil). Dean and stark trap was designed from glass ware house of institute of Chemistry, University of the Punjab.

Plant Material

The peel of *Citrus paradisi* was collected from milkshake and juice canteen of University of the Punjab as well as from nearby market. Fresh peel was cleaned and extraction of essential oils was carried out by the process of steam distillation.

Steam Distillation

In the classical method of steam distillation fresh and live steam is introduced in the still from a separate steam generator but in this modified form of steam distillation technique which has been used in this research, steam was generated in the same still and Dean Stark trap separate the oily layer from aqueous layer. Water removal unit was built in to ensure rapid efficient recovery. A known amount of fresh waste material of grapefruit was placed in the still and it was heated on the isomental. Steam containing volatile oil was lead to the Dean Stark trap and allowed to condense to form two distinct layers of oil and water in the lower side tube of the trap. The upper layer of oil was collected by opening the tap while lower aqueous layer was discarded. Anhydrous Na_2SO_4 was added to the oil to absorb water contents if present from the oil.

Physiochemical Properties of Oil

Different physiochemical properties of oil were found out such as boiling point, Refractive index, solubility, viscosity and density. The methods used were standard procedures

Fungal Strains

Three different plant pathogenic fungal strains i.e, *Fusarium oxysporum*, *Fusarium Solani* and *Dreschlera tetramera* were used in this study. These fungal strains cause many serious diseases in plants, such as, rice blast, papaya fruit rot, seedling collar rot and wilt diseases.

Preparation of Media

Malt extract media was prepared by standard established method using malt 2g in 100ml of distilled water per flask. Total 30 flasks were prepared for all fungal species in same manner. All the flasks were autoclaved for 20-25 minutes at 15lb/inch². The contents in each flask were then cooled and treated with chloromycetin by opening the commercially available capsules and thoroughly blending their contents with media.

Inoculation

The media was thus ensured against bacterial contamination and inoculated with the selected strains of the fungi i.e, *Fusarium oxysporum*, *Fusarium Solani* and *Dreschlera tetramera*. Different known volumes of the essential oils ranging from 0.25 to 0.75ml were then introduced in different flasks. Each flask was numbered to identify the amounts of its ingredients. The observations were followed for 2 weeks and compared with the growth pattern of reference or blank flasks. After 2 weeks, the growth patterns occurring in each set of flasks were recorded in terms of the weight of the fungus. For this purpose, the fungus was filtered from each flask on a pre-weighed filter paper and kept in oven at 80 C⁰ for 24 hours (to dry the fungus and filter paper). The mean weight of each fungus was calculated.

Results:

Physiochemical Properties of Oil

Physiochemical properties of oil were determined using standard established procedures and recorded as follows (Table 3).

Physical property	Result
Boiling point	195-200C ⁰
Refractive index(Abbbes refractometer)	1.7
Solubility	Soluble in petroleum ether, acetone, n-hexane, xylene Insoluble in water and methanol
Density	0.91g/ml
Viscosity (Ostwald's viscometer)	0.298 poise

Table 3: Physiochemical Properties of Oil

Biological Activity

After 2 weeks, recorded data was studied and it was found that the comparison of flasks with blank flask showed remarkable antifungal activity against selected strains, so this grapefruit peel oil showed remarkable antifungal effect and can be used as an antimycotic agent in the form of inhalers, ointments and in medical soaps after pharmacological and toxicological testing. The results thus obtained are given in the form of tables and graphs as follows (Table 4-6; Figure 1-3):

Vol. Of Oil (ml)	Weight of fungus (gm)	Results
0.0	0.32	Normal growth
0.25	0.13	+ve antifungal
0.50	0.10	+ve antifungal
0.75	0.02	+ve antifungal

Table 4: Results for *Fusarium oxysporum* Fungus Strain

Vol. Of Oil (ml)	Weight of fungus (gm)	Results
0.0	0.30	Normal growth
0.25	0.21	+ve antifungal
0.50	0.06	+ve antifungal
0.75	0.05	+ve antifungal

Table 5: Results for *Fusarium solani* Fungus Strain

Vol. Of Oil (ml)	Weight of fungus (gm)	Results
0.0	0.35	Normal growth
0.25	0.29	+ve antifungal
0.50	0.21	+ve antifungal
0.75	0.15	+ve antifungal

Table 6: Results for *Dreschlera tetramera* Fungus Strain

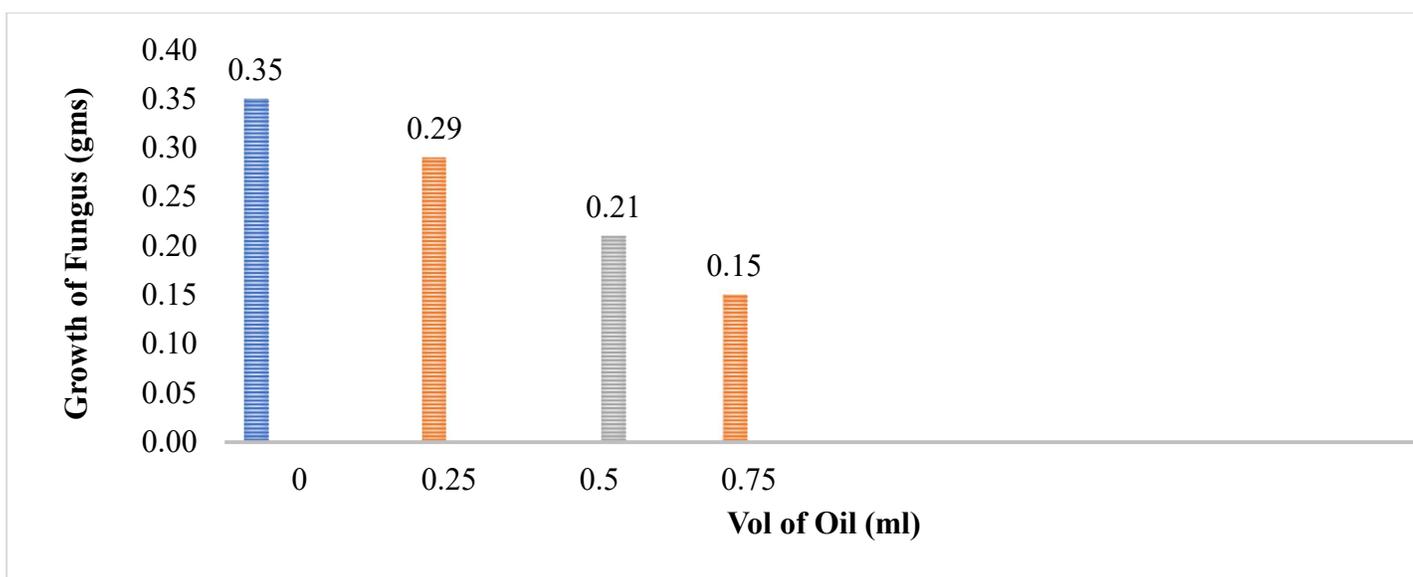


Figure 1: Growth Pattern of *Fusarium oxysporum*

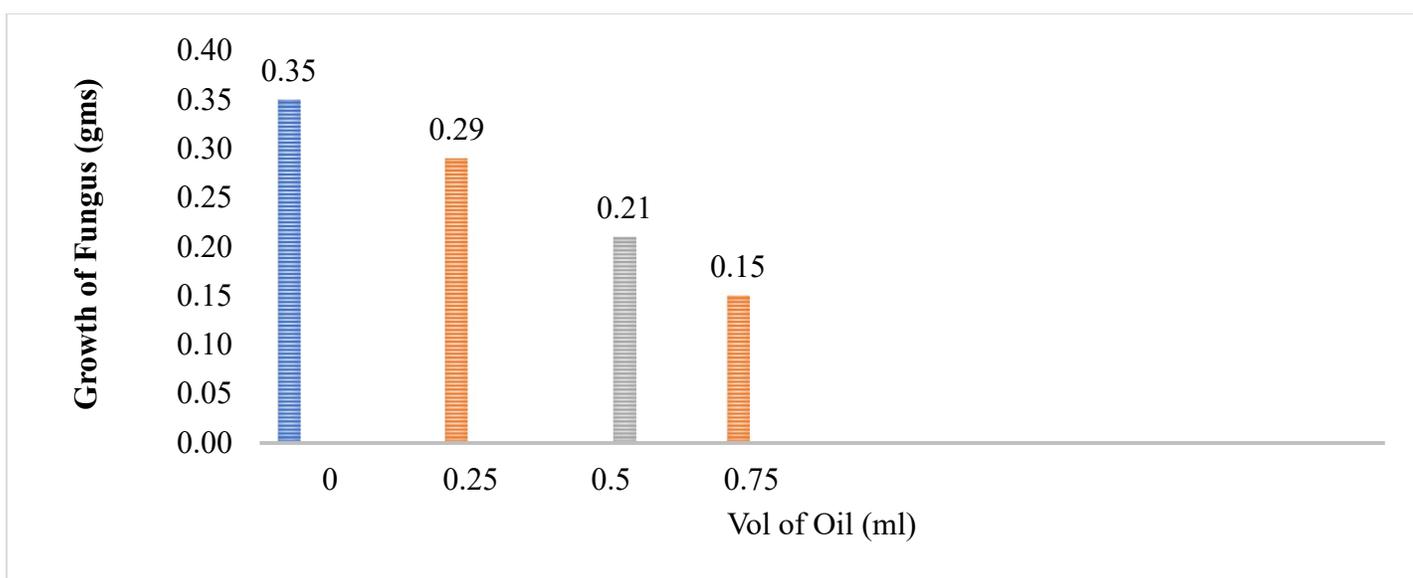


Figure 2: Growth Pattern of *Fusarium solani*

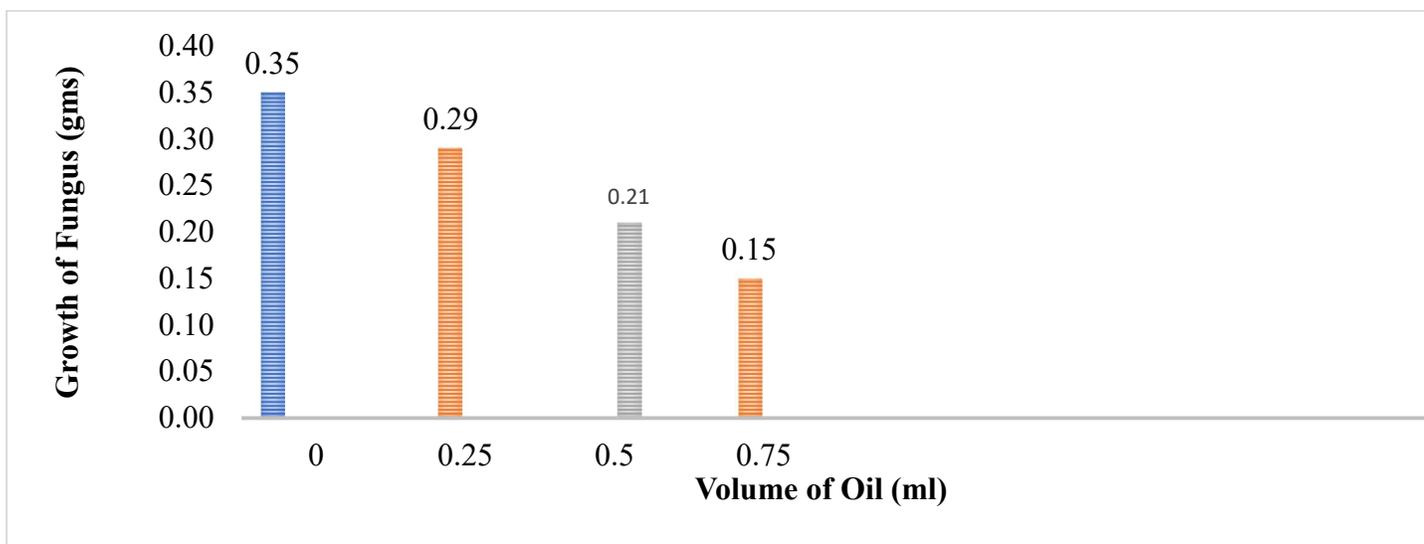


Figure 3: Growth Pattern of *Dreschlera tetramera*

Discussion:

Citrus fruit constitutes a large part of fruit cultivation in Pakistan. Several species of these fruits are cultivated in Pakistan. Some common species include oranges, lemons, grapefruit and lime. According to statistical data citrus fruits production in Pakistan has been continuously increasing. Citrus fruits are extensively used in various industries like tobacco, paint, canning and more commonly in the manufacturing of bakery items. Citrus fruit is also widely processed in squashes, beverages, jams and juices etc. Most of these industries use pulp and juices while peel is discarded as a waste. Computed on the basis that about 300 gram of peel is yielded from a kilogram of the fresh fruit. So, this waste material is used to extract essential oil by different methods [16] including steam distillation, expression, enfleurage and maceration. Grapefruit peel oil found to possess remarkable antimicrobial activity. The oil also possesses antiseptic, healing, soothing, curing and rubifacial qualities. The anthelmintic properties of certain volatile oils specially worm seed oil, are well known [17]. The essential oils are generally antioxidants [18-20]. They act as food preservatives in food industry. The essential oils extracted from garlic and lemon has been reported to lower the cholesterol and triglycerides level in blood stream [21]. Grapefruit

peel oil has immense antifungal effects. One used strain in this study is *Fusarium oxysporum* [22] which causes diseases such as fusarium yellow on asparagus wilt on China aster and banana. Similarly, *Fusarium solani* is known to induce a variety of diseases on many different hosts. Mostly this strain causes disease in Papaya fruit. Sudden death syndrome (SDS) is a fungal disease of soyabeans caused by *Fusarium solani* [23]. Another strain *Dreschlera tetramera* used in this research is plant pathogenic causes diseases in plants specially rice [24]. This disease in rice crop is known as rice blast. Studies have showed that the bioactivity and chemical composition of commercial essential oils have established the authenticity of commercial essential oils against different bacterial strains [25]. In short Grapefruit peel oil is found to have great antifungal effect against these plant pathogenic fungal strains. Due to this remarkable antimicrobial effect its chemical composition [26] has also been determined using GC-MS.

Conclusions:

This study revealed that the use of *Citrus paradisi* peel oil in different concentrations ranging 0.25 to 0.75ml against plant pathogenic fungi inhibited the growth of fungi and 0.75ml almost completely inhibited the fungus growth. Finally, it

is concluded that the essential oil extract of *C. paradisi* peel possess outstanding antifungal effect. It has also been investigated through this study that essential oils can be extracted in greater quantities using modified steam distillation technique incorporating Dean and Stark trap.

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