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Antibacterial, Antifungal Activity and Characterization of Citrus Peel and Pulp

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ABSTRACT

Pakistan is big citrus producer of the world so, present research was executed to appraise the phytochemical attributes, antimicrobial and antifungal activities of newly produced *Citrus limon, C. reteculata, C. paradise* and *C. limetta*. In the preliminary examination solvent extracts were assessed for total phenolics, flavonoids, saponins, and terpenoids. Significant presence of bioactive compounds flavonoids, steroids, phenols, saponins, and terpenoids in peel and pulp of the citrus species. The presence of bioactive compounds directed research for antimicrobial potential, and samples of selected citrus species were tested against microbes and showed very promising antimicrobial activity against fungi and bacteria. The dilution method and disk diffusion method were used for antifungal activity and antibacterial activity, respectively. Samples showed a significant reduction in fungal biomass with an enhanced concentration trend in acetone solvent than ethanol. Whereas, both ethanol and acetone extracts showed a zone of inhibition against all bacterial strains at 50 mg/mL concentration. For supporting research study characterization of citrus samples was performed using GC-MS (QP2010 Shimadzu) for identification of different bioactive compounds. Many inter-varietal chemicals were found through mass spectrometry analysis. The presence of bioactive phenolic and flavonoids, aldehydes, and kenotic compounds in selected citrus species, with confirmation of antibacterial and antifungal activity may be concluded that citrus species of Pakistan have great potential for nutraceutical applications.

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INTRODUCTION

Citrus belongs to the family Rutaceae, is the main fruit tree grown and distributed all over the biosphere. Despite the fact that *Citrus sinensis* (sweet orange) is the dominant fruit cultivated and accounts for about 70% of citrus output, along with other citrus species including *Citrus paradise* (grapefruit), *Citrus aurantium*, *Citrus reticulata* (tangerine tree), *Citrus aurantifolia* (lime tree), and *Citrus limom* (lemon tree) (Singh and Raj 2018). Citrus fruits are observed to be a rich source of phyto compounds, and the special citrus smell is the result of essential components like limonoids, which in turn are terpenes (Palanikumar *et al.* 2020). The presence of essential bioactive compounds in citrus shows a climax of antioxidants and antimicrobial activities. Orange peels are of great value for their phytochemical compounds. Carbohydrates, proteins, lipids, terpenoids, fixed oils, phenolic compounds, and alkaloids are analyzed in ethanolic and aqueous extracts of orange peel (Chede 2013). Citrus juice has better antimicrobial activity and includes a high quantity of citric acid, giving it its sharp flavour. They're additionally proper sources of nutrition C, flavanones, and flavones (Kanaze 2009; Kumar *et al.* 2011). The have a look at has demonstrated the occurrence of giant quantity of secondary metabolites which includes tannin, steroids, lowering sugar, proteins and high content of carbohydrates from the aqueous and chloroform extracts and other secondary metabolites from citrus fruit peel wastes of *Citrus limon* and *Citrus sinensis* in which as the ethanolic extract indicated the particular presence of saponin too (Suja *et al.* 2017). Citrus fruits, which belong to the circle of relatives Rutaceae, biosynthesize and acquire in their cells a terrific kind of phytochemicals consisting of alkaloids, terpenoids, flavonoids, saponins, coumarins, tannins, and others. Inside the gift research, the roots of *Citrus paradisi, C. aurantium,* and *C. sinensis* had been selected, and phytochemical screening was carried out regarding antimicrobial and antioxidant activities (Elhag *et al.* 2018). Limonoids appear in large quantities in the juice of citrus species (Arora *et al.* 2018). Citrus peels are a rich source of nutrients and many bioactive compounds, so they are successfully used in tablets or as meal supplements. And it also has antimicrobial activity against many bacterial and fungal species (Fernández *et al.* 2020). Citrus fruit is used for various pharmacological purposes. The citrus fruit possesses anti-cancer, antimicrobial, antioxidant, antiulcer, hypolipidemic, and hepatoprotective ingredients (Aladekoyi *et al.* 2016). Antimicrobial properties of herbs, spices, and their derivatives, including critical oils, extracts, and decoctions, were established three years (Khodja *et al.* 2018). Human beings are in an intimate and dynamic courting with microorganisms found on all the environmentally uncovered surfaces of the body, in addition they cause disease. These microbes viz. viruses, mycoplasma, bacteria, fungi and protozoa colonize the mucosal and dental surfaces within the oral cavity to form multidimensional and structurally prepared multispecies communities termed as biofilms. The biofilm formed on enamel is referred to as dental plaque which is responsible for inflammatory diseases of the periodontium i.e. gingivitis and periodontitis (Manker *et al.* 2017). The latest research carried out on citrus peels found the presence of vital constituents, which can be used in pharmacological or pharmaceutical industries. Antioxidants, antimicrobial, anti-inflammatory, and anti-proliferative properties have been studied in peels of different fruits (Okino and Fleuri, 2016). These days, citrus peel oil has natural antioxidants and antimicrobial properties (Al-Qassabi *et al.* 2018). Until these days, essential oils have been studied ordinarily from the viewpoint of their flavour and fragrance chemistry, which is most effective for flavouring foods, liquids, and other items. Due to multi-functional purposes, essential oils and their components catch the attention of people (Ormancey 2001; Bonesi *et al.* 2018).

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh plants of *Citrus paradise, Citrus limette*, *Citrus limon*, *Citrus reteculata* were collected from the rural area of Gujranwala, Pakistan. For further references, plant specimens were preserved by mounting, drying, and pressing on herbarium sheets and deposited in the Department of Botany, University of Gujrat, Pakistan. Ethnobotanical interviews were conducted by people of different ages via questionnaires and oral communication. The fruits of Citrus *paradise, Citrus limon, Citrus limetta,* and *Citrus reteculata* were collected from the local market. After the collection, the fruit, seed, and pulp were separated. The peel and pulp were dried at room temperature (37 °C) for 4 weeks, peel and pulp became well dried for grinding. After drying, the material was chopped into pieces and coarsely powdered using a pestle and mortar and an electric blender. The powder was dried in an oven at 40 °C for 24hr. And then it will be transferred into an air-tight container with proper labeling for further use in the experiment.

Preparation of plant extract

Aqueous extraction

The dried peels and pulp were added to the distilled water (10 grams of plant powder and 100 mL of water) and mixed in a magnetic stirrer for 1 h, and the extracts were filtered using filter paper. And extracts were kept at 4 °C in a refrigerator until used (Peng *et al.* 2011).

Solvent extraction

The peel and pulp powder of sample plants were extracted with two different solvents, such as acetone and ethanol. Soxhlet apparatus was used for this extraction. About 10 g of peel and pulp powder of each plant was consistently packed into a thimble and extracted with different solvents (200 mL) separately. Each solvent was run in Soxhlet apparatus for 5 h, but not above the boiling point. The extracts were then placed in the rotatory evaporator to evaporate the solvent at 60 °C. After evaporation, extracts were kept in sterile bottles and stored at 4 °C in the refrigerator until use (Salih *et al.* 2003; Hegazy and Ibrahium 2012).

Antibacterial test

To determine the antibacterial activity of citrus species disk diffusion method is used with some modification. Lysogenic broth medium was used to grow all the bacterial species. Filter paper disks were cut out with the help of a punch machine. After that, filter paper disks were soaked in plant extracts, which were prepared in DMSO with 50 mg/ml concentration, and then placed into bacterial culture. After 24hr antibacterial activity was measured with the help of a scale. Bacterial zone of inhibition was measured in mm with the help of a scale (Romulo *et al.* 2018).

Antifungal test

To make the volume up to 500 ml, 150 mg of plant extract (acetone and ethanol) was dissolved in distilled water and 166 µl of DMSO. To prepare the control solution, 333 µl of distilled water was mixed with 166 µl DMSO. To prepare PDB (potato dextrose broth) medium, in a conical flask, 1ml potato dextrose broth was added and autoclaved at 121 ˚C for 20 min. To avoid the antimicrobial contaminants, antibiotic 250 mg was added to it after autoclaving. Six different concentrations (0, 5, 10, 15, 20 and 25 mg/ml) were prepared by mixing the control solution with the concentration of 100, 80, 60, 40, 20 and 0 µl with stock solution 0, 20, 40, 60, 80 and 100 µl respectively, and to make the volume 1.2 ml, PDB (1 ml) solution added in each. By adding the equal parts of growth medium, three replicates were prepared. In each replicate, 2 µl of mycelia spores of the fungus species were added and then incubated at 27 °C for 3 days. After that, on pre-weighed filter paper, the filtration of fungal mass was done, and to obtain the dry fungal mass, this filter material was dried in an oven at 70 °C. To record the fungal biomass, a similar procedure was applied to all fungal species (Ali *et al.* 2010).

Phytochemical analysis

For saponins identification froth test was used. 1g sample was weighed into a conical flask in and 10 mL of distilled water was added and then boiled for 5 min. After that mixture was filtered, and then in a test tube, 2.5 ml of filtrate was added with 10 ml of distilled water. Then it stands for half an hour. The presence of saponin was indicated by Honeycomb froth (Ogidi *et al.* 2019). For the determination of protein, a 3 ml sample was treated with 4% NAOH, and a few drops of 1% $CuSO_4$ were added. The pink colour indicated the presence of protein (Esmat *et al.,* 2020). Terpenoids were determined by extracting 5 ml of each sample and were mixed in 2 ml of chloroform, and 3 ml of conc. H_2SO_4 was added. The reddish-brown color indicated the presence of terpenoids (Ahmad *et al.* 2020). From the fruit peel and pulp extract, steroids were extracted. 0.5 ml of extract was dissolved in chloroform (3 ml) and then filtered. In the filtrate, concentrated sulphuric acid was added carefully to the test tube. Reddish brown color ring with a slight greenish colouration indicated the presence of steroid (Ananth 2019). Phenols were detected by adding 3-4 drops of $FeCl_2$ solution were added in the plant extract. The appearance of black bluish color indicated the presence of phenols (Dsouza and Bhat, 2018). For the detection of flavonoids were mixed with few drops of NaOH solution. Yellowish color appearance, followed by colorless with dilution of acid, showed the presence of flavonoids (Wani *et al*. 2020). For the determination of carbohydrates, 2 mL of iodine solution was added to the crude extract of plant material. The appearance of purple or dark blue color indicated the presence of the carbohydrate (Topno and Sinha 2018).

RESULTS

Phytochemical analysis

Phytochemical analysis exposed different constituents with different solvents (distilled water, ethanol, and acetone) in different citrus pulps and peels. These results are shown in Tables 1 and 2. Different solvents showed different results. Distilled water extract showed the absence of protein and saponin in both citrus peel and pulp. But it showed the presence of terpenoids in citrus pulp and the absence of terpenoids in citrus peel. And other metabolites like flavonoids, carbohydrates, steroids, and phenols were present in the distilled water extract. In the acetone extract, saponin was absent in the citrus peel and pulp. As compared to acetone, the ethanol extract showed positive results and all metabolites such as flavonoids, proteins, carbohydrates, steroids, phenols, saponin, and terpenoids were present in it.

Table 1. Qualitative analysis of peel of *C.limon*, *C.paradise*, *C.limetta* and *C.reteculata*.

Metabolites	Lemon peel			Grapefruit peel			Musambi peel			Kinow peel		
	Aq	Ace	Eth	Aq	ace	eth	aq	ace	Eth	aq	Ace	Eth
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	BDL	+	+	BDL	+	+	BDL	+	+	BDL	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	-	-	+	-	-	+	-	-	+	-	-	+
Terpenoid	-	+	+	-	+	+	-	+	+	-	+	+

BDL= Below Detection Limit

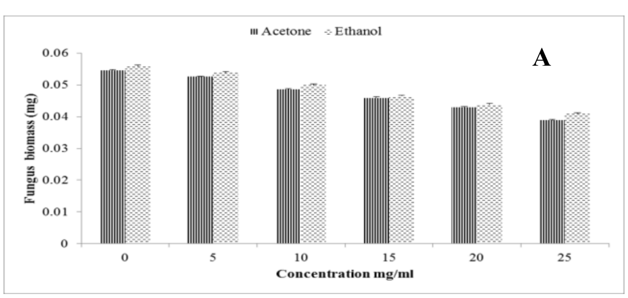
Table 2. Qualitative analysis of pulp of *C. limon*, *C. paradise*, *C. limetta* and *C. reteculata*.

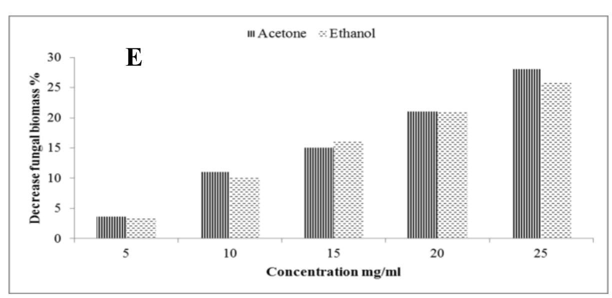
Metabolites	Lemon peel			Grapefruit peel			Musambi peel			Kinow peel		
	Aq	Ace	Eth	Aq	ace	eth	aq	ace	Eth	aq	Ace	Eth
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	BDL	+	+	BDL	+	+	BDL	+	+	BDL	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	-	-	+	-	-	+	-	-	+	-	-	+
Terpenoid	+	+	+	+	+	+	+	+	+	+	+	+

BDL= Below Detection Limit

Antifungal activity analysis

Antifungal activity at concentrations of 5mg/ml to 25mg/ml of both acetonic and ethanolic extract of *Citrus limon, C. limetta, C. reteculata,* and *C. paradise* was analyzed against two fungal strains *Fusarium oxysporum* and *Macrophomina phaseolina.* Acetone extract showed a significant result compared to ethanol extract against both *Fusarium oxysporum* and *Macrophomina phaseolina*. Acetone extracts of *C. paradise* showed significant growth inhibition of fungus biomass as compared to *C. limetta*, *C. limon,* and *C. reteculata* against both strains. Maximum antifungal activity at concentration 25 mg/ml of acetonic extract of *C. paradise* 34 %, *C. limetta* 33 %, *C. reteculata* is 29 %; and *C. limon* is 28%, against *Fusarium oxysporum* was shown. And ethanolic extract showed *C. paradise* 26 %, *C. limetta* 26%, *C. reteculata* is 26 %, and *C. limon* is 26 % decrease in biomass against *Fusarium oxysporum,* and ethanolic extract showed no variation. Maximum antifungal activity of acetonic extract of *C. paradise* 42 %, *C. limon* 41 %, *C. reteculata* is 36 %, and *C. limetta* is 34 % at a concentration of 25 mg/ml against *Macrophomina phaseolina.* And ethanolic extract showed *C. limon* is 37 %, *C. paradise* 35 %, *C. limetta* 30 %, and *C. reteculata* is 30 %, and a decrease in biomass against *Macrophomina phaseolina.* Ethanol extract of *C. limon* showed a 37 % maximum decrease in fungal biomass (Figure 1).





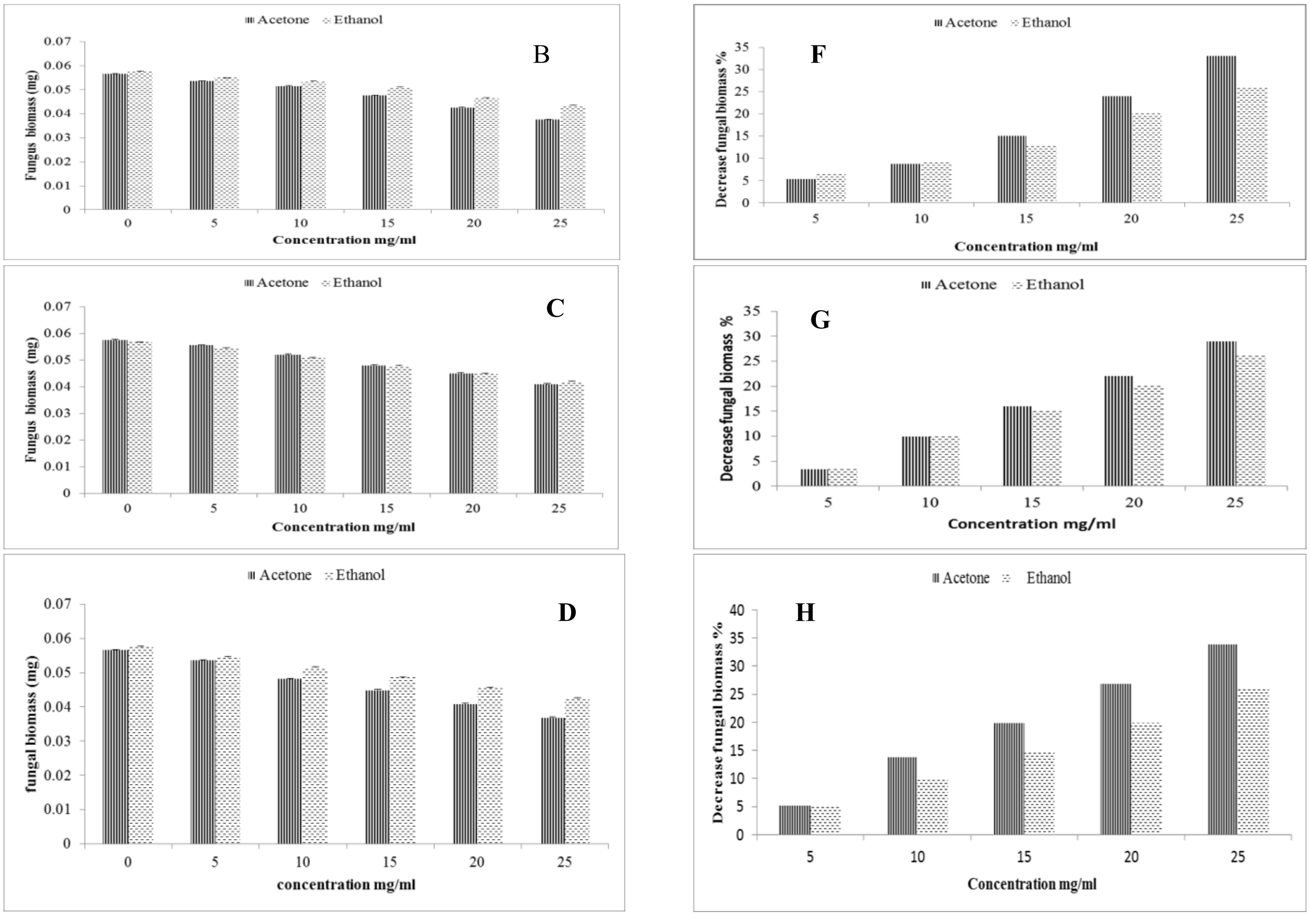


Figure1. Effect of different concentrations of acetonic and ethanolic extract of (A) *Citrus limon*, (B) *C. limetta*, (C) *C. reteculata*, (D) *C. paradise* (Peel) Against *Fusarium oxysporum* and percentage reduction in fungal biomass to different concentrations of ethanolic and acetonic Extract of (E) *Citrus limon*, (F) *C. limetta*, (G) *C. reteculata*, (H) *C. paradise* over control.

Antibacterial activity

Antibacterial activity at concentrations 0mg/ml to 50mg/ml of both acetonic and ethanolic extract of *Citrus limon, C. limetta, C. reteculata,* and *C. paradise* species was analyzed against two bacterial strains *Ralstonia solanacearum* and *Erwinia carotovora.* The 0mg/ml acted as a control at which the citrus species did not show any zone of inhibition against both stains. At concentration 50mg/ml acetonic extract of *C. limon* and *C. limetta* showed a large zone of inhibition against *Ralstonia solanacearum* of diameter 40 mm and 33mm, as compared to the ethanolic extract of *C. limon* and *C. limetta,* which showed a zone of inhibition against *Ralstonia solanacearum* of diameter 25mm and 25mm. But ethanolic extract of *C. paradise* and *C. reteculata* showed a large zone of inhibition against *Ralstonia solanacearum* of diameter 30 mm and 31mm, as compared to acetonic extract of *C. paradise* and *C. reteculata,* which showed a zone of inhibition against *Ralstonia solanacearum* of diameter 25mm and 22mm.

At concentration 50mg/ml acetonic extract of *C. limon, C. paradise,* and *C. limetta* showed large zones of inhibition against *Erwinia carotovora* of diameter 30 mm, 31mm, and 25mm, as compared to the ethanolic extract of *C. limon, C. paradise,* and *C. limetta,* which showed zone of inhibition against *Erwinia carotovora* of diameter 25 mm, 25 mm, and 20 mm. But the alcoholic extract of *C. reteculata* showed a large zone of inhibition against *Erwinia carotovora* of diameter 33mm as compared to the acetonic extract of *C. reteculata,* which showed a zone of inhibition against *Erwinia carotovora* of diameter 27mm (Figures 2, 3, and 4).

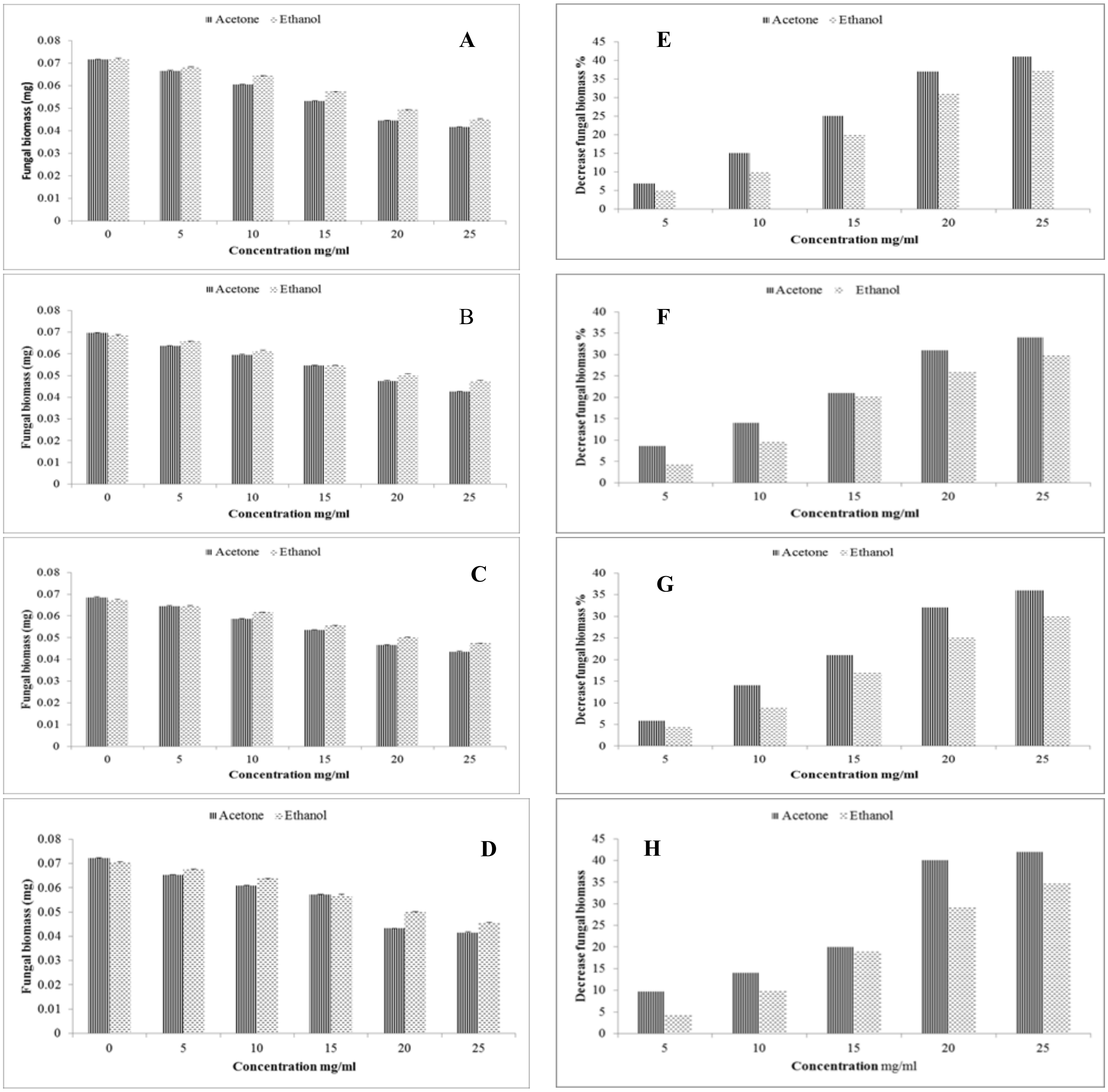


Figure 2. Effect of different concentration of acetonic and ethanolic extract of (A) *Citrus limon*, (B) *C. limetta*, (C) *C. reteculata*, (D) *C. paradise* (Peel) against a *Macrophomina phaseolin* and percentage reduction in fungal biomass to different concentrations of ethanolic and acetonic extract of (E) *Citrus limon*, (F) *C. limetta*, (G) *C. reteculata*, (H) *C. paradise* over control.

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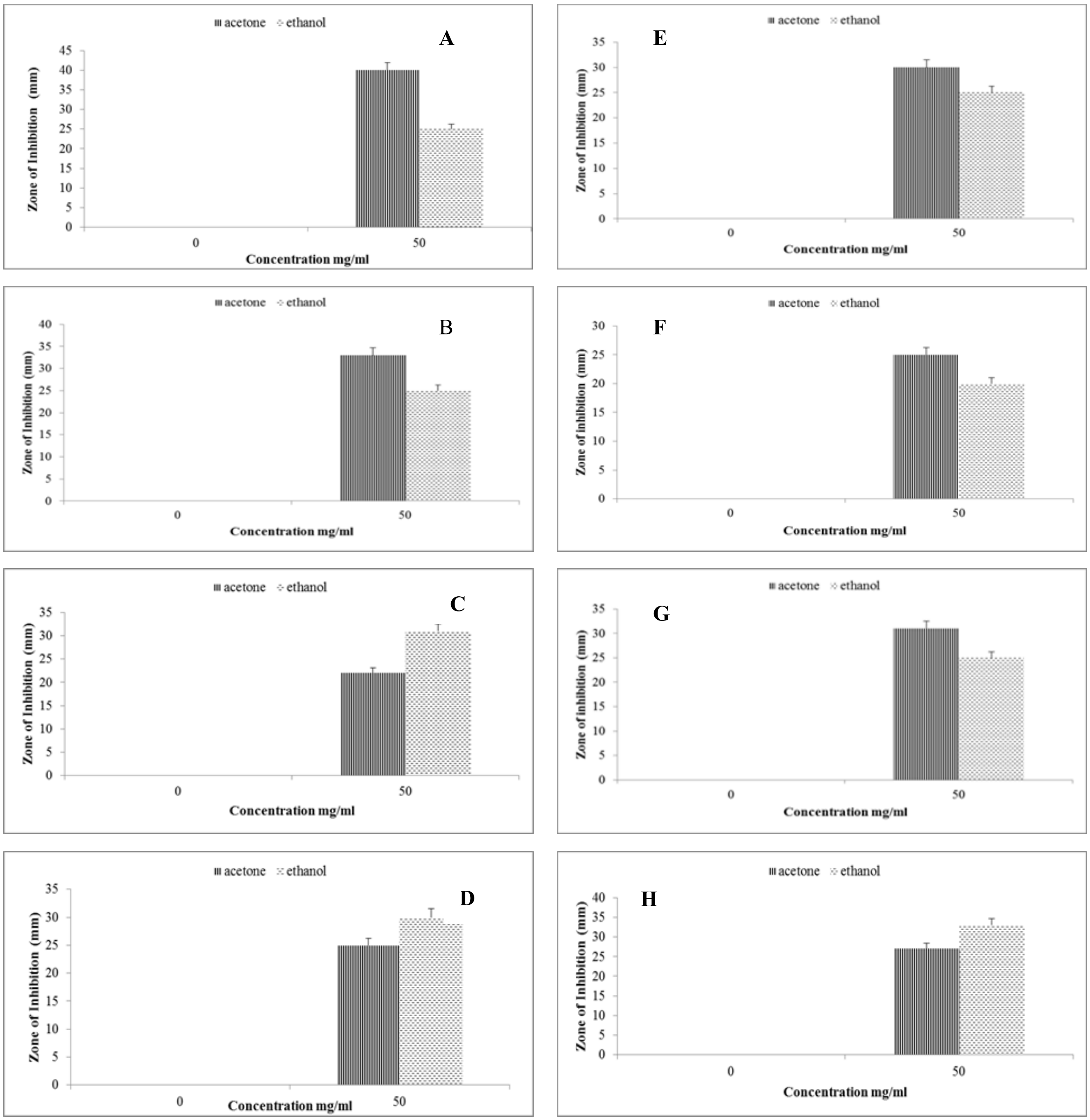

Figure 3. Effect of different concentrations of ethanolic and acetonic extract of (A) *Citrus limon*, (B) *C. limetta*, (C) *C. reteculata*, (D) *C. paradise* (Peel) against *Ralstonia solanacearum* and (E) *Citrus limon*, (F) *C. limetta*, (G) *C. reteculata*, (H) *C. paradise* against *Erwinia carotovora*.

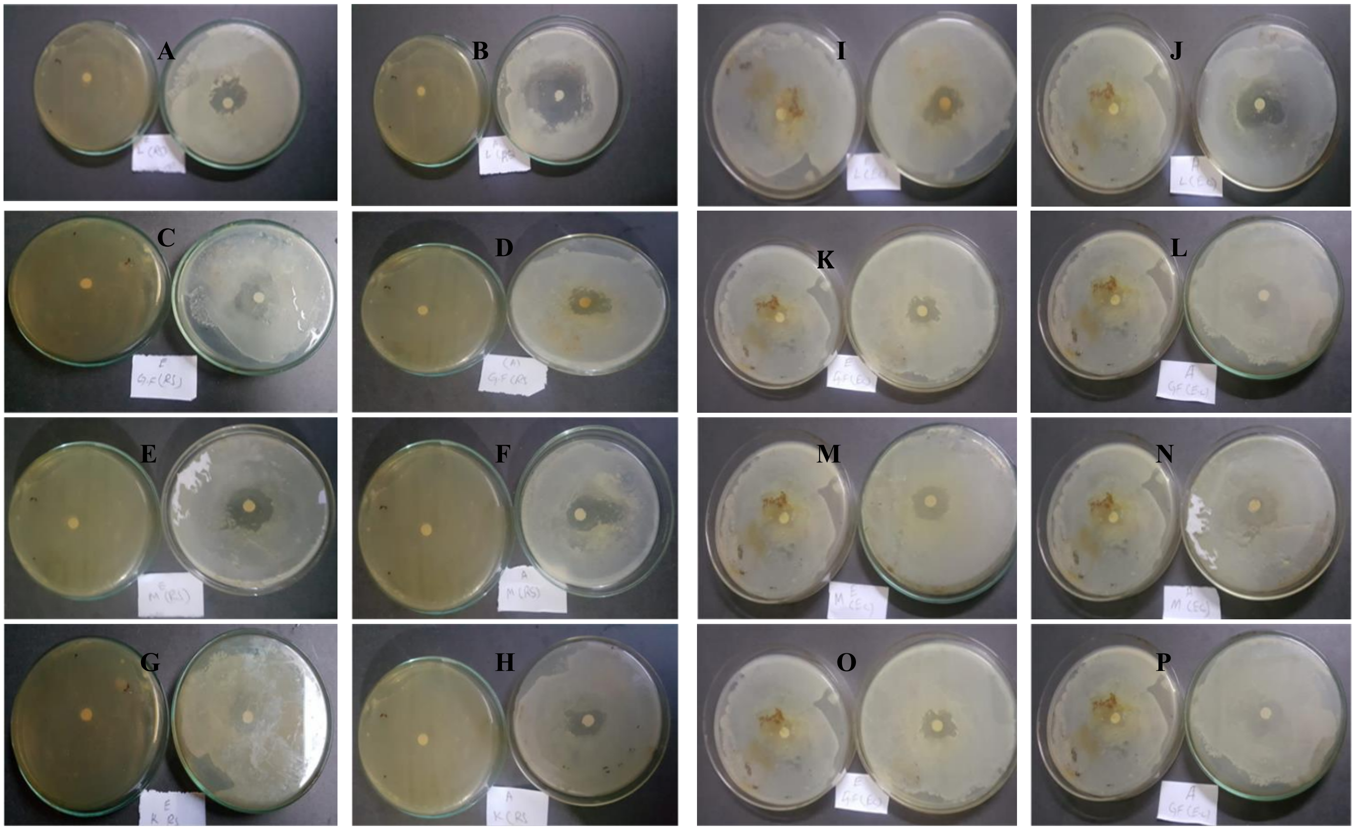

Figure 4. Effect of different concentrations of ethanol and acetone extract of (A,B) *Citrus limon*, (C,D) *C. paradise* (E,F) *C. limetta* (G,H) *C. reteculata* (Peel) against *Ralstonia solanacearum* and effect of different concentration of ethanol and acetone extract of (I.J) *Citrus limon* (K,L) *C. paradise* (M,N) *C. limetta* (O,P) *C. reteculata* (Peel) against *Erwinia carotovora*.

Gas Chromatography Mass Spectrometery Analysis

From the GC-MS analysis of acetone fraction, different bioactive compounds were identified in peel and pulp of *Citrus limon, C. limetta, C. reteculata,* and *C. paradise*. The table is the representation of aceton extract from the peel and pulp of *Citrus limon, C. limetta, C. reteculata,* and *C. paradis*. The constituents of the extract were recorded for retention time (RT), concentration of component (%), and molecular formula. The acetone fraction of *C. limon* peel contains fractions of Furfural (0.08 %), D-limonene (0.31%), linalool (0.01%), Levoglucosenone (0.02%), cis-9-Hexadecenal (1.33%). Out of these 5 compounds, cis-9-Hexadecenal showed 1.33% high peak value. In the acetone fraction of *C. limon* pulp Furfural (0.01%), Cyclohexanone (1.20%), gamma.-Terpinene (0.01%), Levoglucosenone (0.04%), Nonanal (0.09%), Linoleic acid ethyl ester (0.32%), Oleic Acid (3.39%) were analyzed. Out of these, Oleic acid showed (3.39%) high peak value.

In the acetone fraction of peel of *C. paradise,* Furfural (0.11%), n-Decanoic acid (0.06%), Linoelaidic acid (1.10%), soauraptene (1.14%), Octadecanoic acid (2.04%), Cyclohexanone (2.35%), Oleic Acid (3.07%) were analyzed. Out of these 7 compounds, Oleic Acid showed 3.07 % high peak value. Acetone fractions of *C.paradise* contain fractions of Furfural (0.02%), Levoglucosenone (0.03%), n-Hexadecanoic acid (6.97%), and cis-9-Hexadecenal (14.52%). Out of these 4 compounds, cis-9-Hexadecenal showed 14.52% high peak value.

Compounds identified in the acetone fraction of *C. limetta* peel were documented as Levoglucosenone (0.06%), 2-Decenal, (E) (0.01%), 2-Undecenal (0.02%), n-Decanoic acid (0.10%), cis-9-Hexadecenal (23.88%), and Erucic acid (3.12%). Out of these 6 compounds, Erucic acid showed (3.12%) high peak value. The compounds identified from acetone fraction of *C. limetta* pulp were Linalool (0.01%), Furfural (0.02%), trans-Linalool oxide (furanoid) (0.02%), Levoglucosenone (0.11%), Linoleic acid ethyl ester (0.40%), 9,12-Octadecadienoic acid (Z,Z) (3.18%). Out of these 6 compounds, Linoleic acid ethyl ester showed 0.40% high peak value.

The acetone fraction of peel of *C. reteculata* contains constituents of Prenol (0.04%), Acetic acid, methyl ester (0.13%), Furfural (2.84%), Cyclohexanone (2.66%), 1, 2-Cyclopentanedione (0.27%), Isomaltol (0.15%), Levoglucosenone (0.22%), Oleic Acid (0.81%), Octadecanoic acid (0.44%). Out of these 9 compounds, Furfural showed 2.84% high peak value. The following compounds were analyzed in the acetone fraction of *C. reteculata:* gamma-Terpinene (0.76%), Furfural (1.41%), D-Limonene (0.06%), Terpinen-4-ol (0.23%), Carvenone (0.10%), n-Decanoic acid (0.05%), and 2-Nonanone (0.08%). Out of these 7 compounds, 2-Nonanone showed a 0.08 % high peak value (Table 1, 2, 3, 4).

Table 3. Composition of citrus peel and pulp of different citrus species.

	Compounds	*Citrus limon*	*Citrus paradise*	*Citrus limetta*	*Citrus reteculata*	Molecular Formula
		Percent Composition of Essential Compounds				
1	Furfural	0.08	0.11	BDL	2.84	$C_5H_4O_2$
2	D-limonene	0.31	BDL	BDL	BDL	$C_{10}H_{16}$
3	Linalool	0.01	BDL	BDL	BDL	$C_{10}H_{18}O$
4	Levoglucosenone	0.02	BDL	0.06	0.22	$C_6H_6O_3$
5	cis-9-Hexadecenal	1.33	BDL	23.88	BDL	$C_{16}H_{30}O$
6	Cyclohexanone	BDL	2.35	BDL	2.66	$C_6H_{10}O$
7	n-Decanoic acid	BDL	0.06	BDL	BDL	$C_{10}H_{20}O_2$
8	Linoelaidic acid	BDL	1.10	BDL	BDL	$C_{18}H_{32}O_2$
9	Oleic Acid	BDL	3.07	BDL	0.81	$C_{18}H_{34}O_2$
10	Octadecanoic acid	BDL	2.04	BDL	0.44	$C_{18}H_{36}O_2$
11	Soauraptene	BDL	1.14	BDL	0.44	$C_{15}H_{16}O_4$
12	2-Decenal, (E)-	BDL	BDL	0.01	BDL	$C_{10}H_{18}O$
13	2-Undecenal	BDL	BDL	0.02	BDL	$C_{11}H_{20}O$
14	n-Decanoic acid	BDL	BDL	0.10	BDL	$C_{10}H_{20}O_2$
15	Erucic acid	BDL	BDL	3.12	BDL	$C_{22}H_{42}O_2$
16	Prenol	BDL	BDL	BDL	0.04	$C_5H_{10}O$
17	Acetic acid, methyl ester	BDL	BDL	BDL	0.13	$C_3H_6O_2$
18	1,2-Cyclopentanedione	BDL	BDL	BDL	0.27	$C_5H_6O_2$
19	Isomaltol	BDL	BDL	BDL	0.15	$C_6H_6O_3$

Table 4. GC-MS Analysis of pulp of *Citrus limon, Citrus paradise, Citrus limetta* and *Citrus reteculata*.

	Compound	*Citrus limon*		*Citrus paradise*		*Citrus limetta*		*Citrus reteculata*	
		Peak area%	Molecular formula	Peak area%	Molecular formula	Peak area%	Molecular formula	Peak area%	Molecular formula
1	Furfural	0.01	$C_5H_4O_2$	0.02	$C_5H_4O_2$	0.02	$C_5H_4O_2$	1.41	$C_5H_4O_2$
2	Cyclohexanone	1.20	$C_6H_{10}O$	BDL		BDL		BDL	
3	Gamma.-Terpinene	0.01	$C_{10}H_{16}$	BDL		BDL		0.76	$C_{10}H_{16}$
4	Levoglucosenone	0.04	$C_6H_6O_3$	0.03	$C_6H_6O_3$	0.11	$C_6H_6O_3$	BDL	
5	Nonanal	0.09	$C_9H_{18}O$	BDL		BDL		BDL	

6	Linoleic acid ethyl ester	0.32	$C_{20}H_{36}O_2$	BDL		0.40	$C_{20}H_{36}O_2$	BDL	
7	Oleic Acid	3.39	$C_{18}H_{34}O_2$	BDL		BDL		BDL	
8	n-Hexadecanoic acid	BDL		6.97	$C_{16}H_{32}O_2$	BDL		BDL	
9	cis-9-Hexadecenal	BDL		14.52	$C_{16}H_{30}O$	BDL		BDL	
10	trans-Linalool oxide (furanoid)	BDL		BDL		0.02	$C_{10}H_{18}O_2$	BDL	
11	Linalool	BDL		BDL		0.01	$C_{10}H_{18}O$	BDL	
12	9,12-Octadecadienoic acid (Z,Z)-	BDL		BDL		3.18	$C_{18}H_{32}O_2$	BDL	
13	D-Limonene	BDL		BDL		BDL		0.06	$C_{10}H_{16}$
14	Terpinen-4-ol	BDL		BDL		BDL		0.23	$C_{10}H_{18}O$
15	Carvenone	BDL		BDL		BDL		0.10	$C_{10}H_{16}O$
16	n-Decanoic acid	BDL		BDL		BDL		0.05	$C_{10}H_{20}O_2$
17	2-Nonanone	BDL		BDL		BDL		0.08	$C_9H_{18}O$

BDL= Below Detection Limit

DISCUSSION

In phytochemical analysis *C. limon*, *C .limetta*, *C. paradise,* and *C. reteculata* protein, saponin, terpenoids, flavonoids, carbohydrates, steroids, and phenols are identified in aqueous, acetone, and ethanol extraction. These results showed resemblance with (Mamta and Parminder 2017; Suja *et al.* 2017) work in which protein, saponin, terpenoid sterioids, and flavonoids are analyzed in *C. sinensis* and *C. limon* in aqueous, acetone, and ethanol extraction.

Antifungal activity of acetonic extract of *C. limon* against *F. oxysporum* growth indicated the reduction in fungal biomass from 3.6 to 28% as compared to the control. At 25mg/ml concentration, a maximum 28% decrease in biomass of fungi was noted. It was observed that acetone showed better results at all levels against the biomass of *F. oxysporum*. Our results showed resemblance with (Viuda *et al.* 2008) where *C. limon* showed the reduction in mycelia growth 26% against the *Aspergillus niger* at concentration 0.47%. Acetone extract of *C. limon* showed significant results and a great reduction in biomass of *M. phaseolina* from 6.9 to 41% as compared to control. Maximum reduction of 41% was seen at a concentration 25 mg/ml of acetone extract of *C. limon*. Our results showed resemblance with (Viuda *et al.* 2008) where at concentration 0.71% *C. limon* showed the reduction in mycelia growth 40% against the *Aspergillus niger.*

Acetone extract of *C. limetta* showed a significant result and a great reduction in biomass of fungi from 5.3-33%. Maximum reduction 33% was seen at a concentration 25 mg/ml of acetone extract of *C. limetta.* According to (Viuda *et al.* 2008) *C. limon* at concentration 0.47% showed 26% reduction in mycelia growth against the *Aspergillus niger.* Acetonic extract of *C. limetta* showed a reduction in biomass of *M. phaseolina* with different concentrations ranging from 5 to 25 mg/ml. Minimum 8.6% antifungal activity at 5 mg/ml and maximum 34% activity was seen at 25 mg/ml conc. Aceonic extract showed better antifungal activity as compared to ethanolic extract. Our results were comparable to Viuda *et al.* (2008) where *C. sinesis* at the concentration 0.27% showed the 29.5% reduction in mycelia growth of *Aspergillus niger.*

Acetone extract of *C. reteculata* showed a significant reduction of fungus biomass with different concentrations ranging from 5 to 25 mg/ml. Minimum 3.4% antifungal activity is seen at the 5 mg/ml concentration, and maximum 29% antifungal activity is seen at 25 mg/ml concentration. Our results showed the resemblance with the (Chutia 2009) where *C. reteculata* reduced the 42% growth of *Fusarium oxysporum* at 0.2 ml/100 ml. Acetone extraction of *C. reteculta* showed a reduction in fungal biomass compared to control. Maximum 36% reduction at 25 mg/ml concentration. Acetone extract showed a better result as compared to ethanol extraction. In literature *C. reteculata* at concentrations (0.27%, 0.47%, and 0.71%) showed the reduction in mycelia growth (55.5%, 62.8% and 64.8%) against *Aspergillus flavus* (Viuda *et al.* 2008).

Acetone extraction of *C. paradise* showed a reduction in fungal biomass compared to control. Maximum 34% reduction at concentration 25mg/ml. Acetone extract showed a better result as compared to ethanol extraction. No results were found for *C. paradise* against this fungus. Our results showed similarity with (Viuda *et al.* 2008) where grapefruit showed a 34.5% reduction in growth against *Penicillium verrucosum.* The antifungal activity of acetonic extract of *C. paradise* against the growth of *M. phaseolina* showed a decrease in fungal biomass from 9.7-42% compared to control. A maximum 42% decrease in fungal biomass was noted at 25 mg/ml concentration. It was observed that acetone showed good results against the biomass of *M. phaseolina*. Our results showed the resemblance with (Viuda *et al.* 2008) where *C. paradise* at the concentration (0.27% and 0.47%) showed the reduction in mycelia growth (35.5% and 41.2%) against the *Penicillium chrysogenum.*

Acetonic extract of *C. limon* showed a large zone of inhibition 40mm, as compared to ethanolic extract 25mm. Our results showed the resemblance with the (Kumar *et al.* 2011) where the acetonic extract showed zone of inhibition (12mm) as compared to the ethanolic extract (11mm) against *Escherichia coli.* Against the *E. carotovora* at the concentration of 50mg/ml acetonic extract of *C. limon* showed a large zone of inhibition 30mm as compared to the ethanolic extraction, which showed a 25mm zone of inhibition. Our results showed the resemblance to the work of Kumar *et al.* 2011), where acetone extract showed a large zone of inhibition (10mm) as compared to ethanol extract (9mm) against *Staphylococcus aureus.*

The Ethanol extract of *C. paradise* showed a large zone of inhibition 30mm as compared to the acetone extract, which was 25mm. Our results showed resemblance with the studies of (Cvetnic *et al.* 2004) where the ethanol extract of grapefruit showed a large 15mm zone of inhibition against the *Streptococcus faecalis.* Acetonic extraction of *C. paradise* showed a large zone of inhibition 31mm, as compared to the ethanolic extract of *C. paradise,* which showed a 25mm zone of inhibition against the *E. carotovora*. Our results showed resemblance with (Cventic *et al.* 2004) where ethanolic extracts of grapefruit showed the lower antibacterial activity against *Bacillus subtilis* (14mm).

Acetone extraction of *C. limetta* showed a large zone of inhibition 33mm, as compared to ethanol extract of *C. limetta,* which showed a 25mm zone of inhibition against the *R. solanacearum*. According to (Hasija *et al.* 2015), *C. limetta* showed the 13mm maximum zone of inhibition against *Candida albicnas* as compared to *C. sinensis,* which showed the 12.33mm against *Candida albicnas.* Acetonic extract of *C. limetta* showed a large zone of inhibition 25mm, as compared to the ethanolic extract, which a 20mm zone of inhibition at the concentration of 50mg/ml against *E. carotovora*. Our results are harmonious with those of Rehab *et al.* (2018), where *C. sinensis* showed a 25mm zone of inhibition against *Pseudomonas aerogenes.*

In this experiment, ethanolic extraction of *C. reteculata* showed a large zone of inhibition 31mm, as compared to the acetonic extraction, which showed a 22mm zone of inhibition against the *R. solanacearum* at the concentration of 50mg/ml. Our results showed the resemblance with (Suja *et al.* 2017) where ethanolic extract of *C. sinensis* showed a 10mm large zone of inhibition against the *E. coli* as compared to *C. limon,* which showed a 5mm small zone of inhibition against *E. coli.* Acetonic extract of *C. reteculata* showed a 27mm zone of inhibition against *E. carotovora* and which was small compared to the ethanol extract, 33mm. DMSO was used as a control solution in the experiment, and it does not show any zone of inhibition. Our results found to resemble Rehab *et al.* (2018), where *C. sinensis* showed a 27mm zone of inhibition against *Pseudomonas aerogenes. C. limon* peel contains fractions of Furfural (0.08 %), D-limonene (0.31%), linalool (0.01%), Levoglucosenone (0.02%), cis-9-Hexadecenal (1.33%). In the acetone fraction of *C. limon* pulp Furfural (0.01%), Cyclohexanone (1.20%), gamma.-Terpinene (0.01%), Levoglucosenone (0.04%), Nonanal (0.09%), Linoleic acid ethyl ester (0.32%), Oleic Acid (3.39%) were analysed.

In the acetone fraction of peel of *C. paradise,* Furfural (0.11%), n-Decanoic acid (0.06%), Linoelaidic acid (1.10%), soauraptene (1.14%), Octadecanoic acid (2.04%), Cyclohexanone (2.35%), Oleic Acid (3.07%) were analysed. Acetone fractions of *C. paradise* contain fractions of Furfural (0.02%), Levoglucosenone (0.03%), n-Hexadecanoic acid (6.97%), and cis-9-Hexadecenal (14.52%). Compounds identified in the acetone fraction of *C. limetta* peel were documented as Levoglucosenone (0.06%), 2-Decenal, (E)- (0.01%), 2-Undecenal (0.02%), n-Decanoic acid (0.10%), cis-9-Hexadecenal (23.88%), and Erucic acid (3.12%). The compounds identified from acetone fraction of *C. limetta* pulp were Linalool (0.01%), Furfural (0.02%), trans-Linalool oxide (furanoid) (0.02%), Levoglucosenone (0.11%), Linoleic acid ethyl ester (0.40%), 9,12-Octadecadienoic acid (Z,Z) (3.18%).

The acetone fraction of peel of *C. reteculata* contains constituents of Prenol (0.04%), Acetic acid, methyl ester (0.13%), Furfural (2.84%), Cyclohexanone (2.66%), 1, 2-Cyclopentanedione (0.27%), Isomaltol (0.15%), Levoglucosenone (0.22%), Oleic Acid (0.81%), Octadecanoic acid (0.44%). The following compounds were analyzed in the acetone fraction of *C. reteculata:* gamma-Terpinene (0.76%), Furfural (1.41%), D-Limonene (0.06%), Terpinen-4-ol (0.23%), Carvenone (0.10%), n-Decanoic acid (0.05%), and 2-Nonanone (0.08%). Furfural, D-limonene, linalool, Decanal compounds were reported by Mondello *et al.* (2004) and Fattah *et al.* (2015) in *Citrus sinensis.* But Levoglucosenone, Oleic Acid, Octadecanoic acid and Nonanal were analysed in acetone fraction of peel and pulp of *Citrus limon, C. limetta, C. reteculata* and *C. paradise.*

CONCLUSION

Orange belongs to the family Rutaceae and the genus Citrus, one of the most widely cultivated crops around the world due to its dietary advantages. Citrus fruits have high nutritional, medicinal properties and are found widely in the tropics. The peel of citrus is rich in flavonoids, glycosides, coumarins, β and γ- sitosterol, glycosides, and volatile oils. In the present study, ethno botanical survey, phytochemical analysis, antibacterial and antifungal activities of *Citrus limon*, *C. reteculata*, *C. paradise,* and *C. limetta* were investigated. Quantitative analyses were untaken using the GC-MS assay. To check the bioactive compounds of *C .limon*, *C. reteculata*, *C. paradise* and *C. limetta* different qualitative tests were performed and flavonoid, proteins, carbohydrates, steroids, phenols, saponin, terpenoids are detected in peel and pulp with water and organic solvent extract, and ethanol extracts showed good results related to acetone and water extract. From the GC-MS analysis of acetone fraction, different bioactive compounds were identified. In peel of *C. limon* 5 compounds were analyzed. Out of these 5 components, cis-9-Hexadecenal showed 1.33% high peak value. In the pulp of *C. limon,* 7 components were analyzed. Out of these 7 components, Oleic Acid showed 3.39% high peak value. In the peel of *C. reteculata,* 9 compounds are identified. Out of these 9 compounds, Furfural Showed 2.84% high peak value. In pulp of *C. reteculata* 7 components were analysed, out of these 7 compounds, Furfural showed 1.41% high peak value. In the peel of *C. paradise,* 7 compounds were identified. Out of these 7 components, Cyclohexanone showed 2.35% high peak value. In the pulp of *C. paradise,* 4 components were analysed. Out of these 4 compoundscis-9-Hexadecenal showed 14.52% high peak value. In peel of *C. limetta* 6 compounds were identified. Out of these 6 compounds, cis-9-Hexadecenal showed 23.88% high peak value. In pulp of *C. limetta,* 6 compounds were identified. Out of these 6 components, 9,12-Octadecadienoic acid (Z,Z)- showed 3.18% high peak value.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES

Ahmad, G., M.H. Masoodi, N. Tabassum and R.A. Mir. 2020. Phytochemical Analysis and Anti-inflammatory Activity of Various Extracts Obtained from Floral Spikes of *Prunella vulgaris* L. Jordan Journal of Pharmaceutical Sciences, 13(1).

Aladekoyi, G., S.V. Omosuli, O.O. Orungbemi and A.T. Adesuyi. 2016. Evaluation of antimicrobial activity of oil extracted from three different citrus seeds (*Citrus limon, Citrus aurantifolia, and Citrus aurantium*). International Journal of Scientific Research and Engineering Studies, 3(3), 16-20.

Ali, I., F.G. Khan, K.A. Suri, B.D. Gupta, N.K., Satti, P. Dutt, and I.A. Khan. 2010. In vitro antifungal activity of hydroxychavicol isolated from *Piper betle* L. Annals of Clinical Microbiology and Antimicrobials, 9(1), 7.

Al-Qassabi, J.S.A., A.M. Weli and M.A. Hossain. 2018. Comparison of Total Phenols Content and Antioxidant Potential of Peel Extracts of Local and Imported Lemons Samples. Sustainable Chemistry and Pharmacy, 8, 71-75.

Ananth, V. 2019. Pharmacognostical and preliminary phytochemical profile of the leaf extracts of *Embelia ribes* Burm. F. J. Pharmacognosy and Phytochemistry, 8(1), 1861-18.

Arora, M. and P. Kaur. 2013. Phytochemical screening of orange peel and pulp. International Journal of Research in Engineering and Technology, 2, 12.

Arora, S., P. Mohanpuria and G.S. Sidhu. 2018. Citrus limonoids: Mechanism, function, and its metabolic engineering for human health. Fruits,73(3).

Bonesi, M., M.R. Loizzo, M. Leporini, M.C. Tenuta, N.G. Passalacqua and R. Tundis. 2018. Comparative evaluation of petitgrassin oils from six citrus species alone and in combination as potential functional anti-radicals and antioxidant agents. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 152(5), 986-993.

Chede, P.S. 2013. Phytochemical Analysis of *Citrus sinensis* peel. International Journal of Pharma and Bio Sciences, 4(1), 339-343.

Chutia, M., P.D. Bhuyan, M.G. Pathak, T.C. Sarma and P. Boruah. 2009. Antifungal Activity and Chemical Composition of *Citrus reticulata* Blanco essential oil against phytopathogens from NorthEast India. LWT-Food Science and Technology, 42(3), 777-780.

Cvetnic, Z.D.E.N.K.A. and S.A.N.D.A. Vladimir-Knezevic. 2004. Antimicrobial activity of grapefruit seed and pulp ethanolic extract. Acta Pharm, 54(3), 243-250.

Dsouza, M.R. and V. Bhat. 2018. Evaluation of pharmacological activities of seed and pericarp of *Litchi chinensis* Sonn. Journal Homepage, 6(1).

Elhag, N.H.O., R.S.M. Abadi and S.M.H. Ayoub. 2018. Phytochemical screening and assessment of antioxidant and antimicrobial activities of the root extracts of three Sudanese Citrus species.

Esmat, A.U., S. Mittapally and S. Begum. 2020. GC-MS analysis of bioactive compounds and phytochemical evaluation of the ethanolic extract of *Gomphrena globosa* L. Flowers. Journal of Drug Delivery and Therapeutics, 10(2), 53-58.

Fernández Fernández, A.M., E. Dellacassa, A. Medrano Fernandez and M.D. Del Castillo. 2020. Citrus waste recovery for sustainable nutrition and health. Food wastes and by products: Nutraceutical and Health Potential, 193-222.

Hegazy, A.E. and M.I. Ibrahium. 2012. Antioxidant activities of orange peel extracts. World Applied Sciences Journal, 18(5), 684-688.

Intekhab, J., N.U. Siddiqui and M. Aslam. 2008. Flavonoid from the roots of *Citrus sinensis*. Bioscience, Biotechnology Research Asia, 5, 443-446.

Kanaze, F.I., A. Termentzi, C. Gabrieli, I. Niopas, M. Georgarakis and E. Kokkalou. 2009. The phytochemical analysis and antioxidant activity assessment of orange peel (*Citrus sinensis*) cultivated in Greece–Crete indicates a new commercial source of hesperidin. Biomedical Chromatography, 23(3), 239-249.

Khodja, N.K., L. Boulekbache, F. Chegdani, K. Dahmani, F. Bennis and K. Madani. 2018. Chemical composition and antioxidant activity of phenolic compounds and essential oils from *Calamintha nepeta* L. Journal of Complementary and Integrative Medicine, 15(4),2017-0080.

Kumar, K.A., M. Narayani, A. Subanthini and M. Jayakumar. 2011. Antimicrobial activity and phytochemical analysis of citrus fruit peels-utilization of fruit waste. International Journal of Engineering Science and Technology, 3(6), 5414-5421.

Mankar, C.D., M.U. Shah, Y.S. Doshi, M. Bajaj, V. Kevadia and R. Vinod. 2017. Evaluation of antimicrobial activity of orange peel extract against oral biofilm forming organisms: an in vitro microbial study and scanning electron microscopic assessment. International Journal of Basic & Clinical Pharmacology, 5(5), 1917-1923.

Mondello, L., A. Casilli, P.Q. Tranchida, P. Dugo and G. Dugo. 2005. Comprehensive two-dimensional GC for the analysis of Citrus essential oils. Flavour and Fragrance Journal, 20, 136-140.

Ogidi, O.I., D.G. George and N.G. Esie. 2019. Ethnopharmacological properties of *Vernonia amygdalina* (Bitter Leave) medicinal plant. Journal of Medicinal Plants, 7(2), 175-181.

Okino Delgado, C.H. and L.F. Fleuri. 2016. Orange and mango by-products: agro-industrial waste as source of bioactive compounds and botanical versus commercial description—a review. Food Reviews International, 32(1), 1-14.

Ormancey, X. 2001. Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites, 157, 30-40.

Palanikumar, P.,D. Wahjuningrum, P. Abinaya, M.M. Babu and T. Citarasu. 2020. Usage of plant natural products for prevention and control of white feces syndrome (WFS) in Pacific white leg shrimp

Litopenaeus vannamei farming in India. Aquaculture International, 28(1), 113-125.

Peng, X.M., Y.F. Hu and C.W. Jin. 2011. Solubilities of imidazolium-based ionic liquids in aqueous salt solutions at 298.15 K. The Journal of Chemical Thermodynamics, 43(8), 1174-1177.

Rehab M.A. El-Desoukey., S.B.S. Areej and F Alhowamil. Heelah. 2018. "The Phytochemical and antimicrobial effect of *Citrus sinensis* (Orange) peel powder extracts on some animal pathogens as eco-friendly". EC Microbiology, 14,6, 312-318.

Romulo, A., E.A. Zuhud, J. Rondevaldova and L. Kokoska. 2018. Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. Pharmaceutical Biology, 56(1), 287-293.

Salih, H.A. and A.M. Abass. 2003. Study of the fruit peels of *Citrus sinensis* and *Punica granatum.* Journal of Babylon University, (9)3, 243-342.

Singh, P.K. and J.D. Raj. 2018. Extraction of oil from lemon peels and some nutritional characteristics of peels and oil of lemon peels. The Allahabad Farmer, 74(2).

Suja, D., G. Bupesh, R. Nivya, V. Mohan, P. Ramasamy, N.S. Muthiah and K. Prabu. 2017. Phytochemical screening, antioxidant, antibacterial activities of *Citrus limon* and *Citrus sinensis* peel extracts. International Journal of Pharmacognosy and Chinese Medicine, 1(2), 1-7.

Topno, S.C. and M.R. Sinha. 2018. Phytochemical analysis of floral extract of three medicinal plants used by local inhabitants of West Singhbhum district, Jharkhand (India) to cure skin diseases. Journal of Medicinal Plants,6(1), 27-30.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez. 2008. Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control, 19(12), 1130-1138.

Wani, N.A., W.I. Khanday and S. Tirumale. 2020. Phytochemical analysis and evaluation of antibacterial activity of different extracts of soil-isolated fungus *Chaetomium cupreum.* Journal of Natural Science, Biology and Medicine, 11(1), 72.

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