GC-MS, ANTIOXIDANT AND ANTIMICROBIAL ANALYSIS OF *CITRUS LEMON* PEEL EXTRACT AGAINST ODOR CAUSING BACTERIA

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Abstract

It is found that waste materials such as peels and seeds obtained after harvesting are rich sources of biologically active phytocomponents containing certain physiological functions. This waste is often discarded or used as food for animals or as a fertilizer. But in developing countries, where herbal medicines are preferred to treat infectious diseases are used for their therapeutic benefits. These are used for the production of effectual, safe, inexpensive, and novel nutraceuticals as they have higher amounts of natural compounds with therapeutic properties. This study was carried out to determine the antimicrobial and antioxidant potential of lemon peel i.e., citrus lemon L. is a good source of molasses, pectin, limonene, and some other secondary metabolites. DPPH assay was used to determine the antioxidant activity of lemon peel, and antibacterial activity was determined using four pathogens. In this study, the Lemon peel extract was evaluated for its antimicrobial activities against organisms that cause odor viz. *S. epidermis* and *Corynebacterium*, and *S. aureus* and *P. aeruginosa*. The results show that Lemon Peel extract has greater potential to scavenge free radicals. Also, it showed greater antimicrobial activity thereby indicating its potent antimicrobial and antibacterial agent and, in the future, can be used for the cosmetic industry.

Keywords: *Citrus lemon L.*, Ethanol seed extract, Antimicrobial activity, Antioxidant activity, GC-MS analysis, DPPH assay.

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1. INTRODUCTION

Medicinal plants are used for therapeutic purposes. Around 80% of the world's population is using herbal medicines to treat diseases (1,2,3) mostly infectious diseases. It is widely used in developing countries. There are many naturally occurring materials having biological actives which can be used for the health of human beings and they also have the potential to produce new drugs. According to World Health Organization (WHO), medicinal plants are used for therapeutic purposes and can be considered pioneering synthetic, semi synthetic chemical drugs (4). According to the statistics, it is widely used in developing countries and less in developed countries. For many years, WHO has advocated traditional medicines as safe remedies for ailments of both microbial and non-microbial origins (WHO, 2015) (5). These medicinal plants hold an important role in the health of individuals and communities. They also have great medicinal value as they have many chemical substances which produce physiological action in the human body. These plants also contain chemical compounds such as alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpene, phenolic compounds, fats, and oils.

Citrus fruit of the family *Rutaceae* is an important medicinal plant, which is mainly used for its alkaloids, which is having potent anticancer and antibacterial activities in its different parts such as leaves, stems, roots, juice, peel, and flower (6,7). The plant is a potent source of vitamin C, and its oil is used in various preparations to reduce skin problems such as skin itching, for skin nourishment, and the pulp left after the extraction of its juice is used for the treatment of pimples, and wrinkles (8), and also can be used to soften the facial skin (9). These facts have inspired the screening of Citrus Lemon L. for its antimicrobial efficacy and antioxidant potential.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The work was done through various well-planned explorations in various range forest areas of North Gujarat like Indroda Park (Gandhinagar district), Danta (Banaskantha district), Ambaji (Banaskantha district), Talod (Sabarkantha district), Vireshwar (Sabarkantha district), Vijayanagar (Sabarkantha district). This field exploration was well organized and covered all the seasons to cover all the ephemeral floral components for the identification of species. The collection of plants was done in different development stages of plants and exhaustive field notes were taken. Taxonomic keys were used to identify different plants which are described in various volumes of Gujarat Flora (Shah, 1978), the E-Flora of Gandhinagar, and the wealth of India (10).

2.2 Extraction and yield quantification

Plant experimental material was prepared using clean dried leaves which are grounded into a fine powder using a mechanical grinder. Further, 20 gm of powder was mixed with 120 ml ethanol. Then it was kept in Soxhlet for 48 hours at room temperature. At 50°C temperature, the obtained extracts were kept in a hot air oven for evaporation. The crude extract was collected and preserved in a refrigerator for experiments. The calculation of percentage yield of crude extract was calculated as:

WC/WP*100;
WC = weight of crude extract, and
WP = powdered plant material weight.

2.3 Antioxidant activity

The DPPH free radicals are organic nitrogen with long life and deep purple color. When this solution is mixed with an anti-oxidant, the color change is observed from purple to yellow due to hydrazine. The antioxidant-reducing ability of DPPH can be evaluated by monitoring the decrease of its absorbance at 525-528 nm. The results are expressed as IC_{50} or as % scavenging of DPPH at a fixed antioxidant concentration for all samples (11).

2.3.1*Preparation of DPPH solution*

By taking 7.89 mg of DPPH, DPPH solution was prepared. dissolving it with 100 ml of 99.5% ethanol, and then, finally keeping it in the dark for 2 hrs.

2.3.2 *DPPH assay procedure*

The DPPH solution of 1,000µl was added to 100 µl of buffer solution i.e., Tris-HCl (pH 7.4) in a test tube. Another testing sample solution was added and mixed quickly. Then at room temperature, the solution was kept for 30 min. The absorbance of the solution was noted at 517 nm. A mixed solution with 1,200 µl of ethanol and 200 µl 0f Tris-HCl was used as a blank. The inhibition ratio was obtained using the following equation:

$$\text{Inhibition ratio (\%)} = (A1 - A2) \times 100/A1,$$

Where A1 is the absorbance of the addition of ethanol instead of the testing sample and A2 is the absorbance of the testing sample solution.

2.4 Calculation of IC_{50}

The IC50 of each sample was calculated according to the following process: The graph of inhibition ratios *(y)* was plotted against the sample concentration *(x)* at all six points, and the respective regression line *(Y = AX + B)* was drawn. It is not necessary that the regression line should pass through the origin. As the inhibition curve was not completely straight, but slightly curved, the IC50 value can be calculated using the interpolation method by joining the two points around 50% inhibition with a straight line as follows:

Two points enclosing a 50% inhibition ratio were selected, and a regression line (*Y = AX + B)* was drawn. The regression line doesn't need to pass through the origin.

X (sample concentration) was to be calculated by substituting the value of
Y with 50 in the regression equation of
Y = AX + B (12).

2.5 Antimicrobial activity

The antimicrobial activities can be determined using the disc diffusion method by the well diffusion method. Fresh cultures microorganisms *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermis,* and *Corynebacterium sp.* Which were grown for 24 hrs. were used and diluted 10^{-1} with 0.85% NaCl i.e., sterile physiological saline solution. 100 µl of tested microorganisms containing 2.0 x 10^6cfu/ml (colony forming units/milliliter) for bacteria were inoculated on the surface of Nutrient Agar (HiMedia) plates. Then 100 µl of extract were dropped in well sterile conditions and incubated at $=37^0 \pm 2^0$C for 24 hrs. After incubation, the inhibition zone diameter was measured in millimeters on the plates. This process was repeated 3 times (13). As a negative control, sterile distilled water was used.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of *Citrus Lemon L.* was determined using both dilution methods (14). Bacterial species *S. epidermis* and *Corynebacterium sp. w*ere grown in 20 ml of Nutrients both for 24 hrs. For the determination of MIC and MBC, the dilution method was used. Briefly, a plant extract at different concentrations was taken after serial dilution of 500 mg/ml, 250 mg/ml, 125 mg/ml, 100 mg/ml, and 50 mg/ml in test tubes using micropipette (1000 µl) of *S. epidermis* and *Corynebacterium Sp.* culture, 0.1 McFarland standards was inoculated into test tubes containing 1 mL of the various concentrations of plant extract sample in nutrient broth. The tubes were incubated at 37^0C for 24 hrs. and thereafter observations were done for the growth and turbidity. Subsequently, 100 µl of broth from each test tube not showing growth was inoculated into a nutrient agar plate. Then, the agar plates were examined for growth or turbidity using unaided eyes. The lowest concentration i.e., highest dilution of extracts that produces no visible growth i.e., no turbidity in the initial 24 hours when it was compared with control tubes was considered as initial MIC. The dilution which shows no turbidity was incubated further for 24 hrs. at 37^0C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 hrs. was considered the final MIC.

MBC i.e., Minimum Bacterial Concentration value was determined by sub-culturing the test dilution that showed no visible turbidity on freshly prepared respective agar media. Further, the plates were incubated for 24 hrs. at 37^0C. The highest dilution that yielded no

single bacterial colony on the nutrient agar plates was taken as MBC.

2.7 Gas Chromatography- Mass Spectroscopy Analysis (GC–MS):

GC-MC analysis of *Citrus Lemon L.* extract proceeded for Gas Chromatography-mass spectrometry (GCMS) characterization analysis to identify different chemical substances within a test sample. The phycocompound's analysis was carried out by GC-MS technique at SAIF IIT Bombay.

GC-MS analysis was carried out on the Agilent GC7890A model with a column EB5, MS model which was used was AccuTof mass from jeol. As a carrier, Helium gas was used at a flow rate of 1 ml/min. The results were compared by using the NIST library search program, (15)

3. RESULTS AND DISCUSSION

3.1 Extraction and yield quantification

The final quantity of *Citrus Lemon L*peel extract was found to be 29.53% with solvent i.e., ethanol, after Soxhlet.

3.2 Antioxidant activity

Because of the electron donation capacity, the antioxidant effect on DPPH (Chen et al., 2020). Radical scavenging is very important to inhibit the harmful effect of free radicals.Using DPPH assay, the presence of phenolic and flavonoid compounds in plant extract can also be detected (16) and it is also the popular mechanism for the study of the antioxidant property of plant extracts.

Our result at different concentrations (50, 100, 250, 500µg/ml) revealed significant antioxidant activity in extract and positive control, and the values were noted higher with mounting concentration. For different concentrations, the DPPH activity value was able to reduce DPPH radicals with percentages of 73.53%, 61.92%, 59.48%, and 57.33% for 500 µg/ml, 250 µg/ml,100 µg/ml, and 50 µg/ml respectively. The extract showed IC_{50} values of 22.193µg/ml (**Table 1**). For free radical scavenging, the presence of phenol is important. Plant extracts that have DPPH free radical scavenging properties and antioxidants could donate hydrogen in lipid peroxidase or hydro peroxidase free radicals which are the key propagators of the autoxidation of the lipid chain. Non-radicals that disrupt lipid peroxidation chain reactions are also produced by them (17)

Table 1: %DPPH and IC50 value

Concentration (µg/ml)	%DPPH	Absorbance
50	79.85%	0.272
100	80.51%	0.263
250	81.55%	0.249
500	83.25%	0.226

3.3 Antimicrobial activity

The antimicrobial activities of *Citrus Lemon L.* peel extract were studied by the absence or presence of zone diameter and inhibition zone. The results are in Table 2. According to Table 1, the ethanol extract has a stronger and broader spectrum of antimicrobial activities against 4 bacterial strains; *Staphylococcus epidermis Staphylococcus aureus* ATCC 6538, *Corynebacterium sp.*, and *Pseudomonas aeruginosa* MTCC 168.

Table 2: Antimicrobial activity of *Citrus Lemon L* peel extract

Sr. No	Pathogen	Zone 1 (in mm)	Zone 2 (in mm)	Zone 3 (in mm)	Mean
1	*Staphylococcus epidermis*	16	15	16	15.66
2	*Staphylococcus aureus* ATCC 6538	13	13	12	12.66
3	*Pseudomonas aeruginosa* MTCC 168	0	0	0	0
4	*Corynebacteriumsp*	18	13	17	16

3.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Extract

Sr. No.	Citrus Lemon L. Extract Concentration (mg/ml)	Microorganisms	
		S. epidermis	***Corynebacterium***
1	500	No Growth	No Growth
2	250	No Growth	No Growth
3	125	140 cfu/ml; No inhibition	Lawn growth & No Inhibition

a. GC-MS analysis

By common method, using chemicals, the phytochemical components have been analyzed in our lab. The GC-MS analysis of *Citrus Lemon L.* peel ethanolic extract showed 14 compounds such as Thymol, Diethyl phthalate, Phthalic acid, ethyl pentyl ester,2,4-dimethoxy-3-methylpropiophenone, N-hexadecenoic acid,5α-pregnane-16-en-20-one,9-octadecenoic acid, methyl ester, Oleic acid,6,9,12,15-docosatetraenoic acid, methyl ester, Olean-12-ene-3,28-diol, {3b}-,9,12,15-octadecatrienoic acid,2-{(trimethylsilyl)oxy}-1{{(trimethylsilyl)oxy}methyl}ethyl ester,{z,z,z}, Heptacosane, Acetate,{6-(acetyloxy)-5,5,8a-trimethyl-2-methyleneperhydro-1-naphthalenyl}methyl ester, 2-5-cyclohexadine-1,4-dione,2-methyl-5-(1-methyl ethyl) (Figure 1). (18) also found Thymol and other volatile componds in *Citrus Lemon L.* extract.

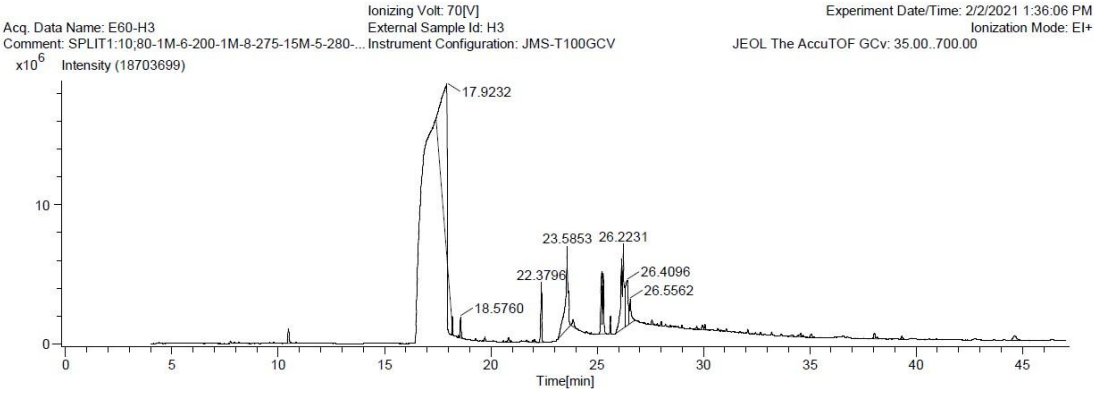

Figure 1 GC-MS of *Citrus Lemon L.* peel extract

Table 4: Phytochemical obtained from GC-MS output.

No	Name of Compound
1.	Thymol
2.	Diethyl Phthalate
3.	Diethyl Phthalate
4.	Phenol, 2-ethoxy-4-[2-propenyl]-
5.	Phthalic acid, ethyl 2-pentyl ester
6.	Hexadecanoic acid, methyl ester
7	Hexadecanoic acid, ethyl ester
8	2H-1-Benzopyran-2-one, 5,7-dimethoxy-
9	9,12-Octadecadienoic acid [Z,Z]-, methyl ester
10	9-Octadecenoic acid [Z]-, methyl ester
11	9,12-Octadecadienoic acid, ethyl ester
12	Ethyl Oleate
13.	9,12-Octadecadienoic acid [Z,Z]-
14.	Octadecanoic acid, ethyl ester

Table 5: Chemical structural details retrieved from PubChem database

No.	Name of Compound	PubChem ID	Mol. Formula	Mol. Weight	CAS ID	SMILE Structure
1.	Thymol	6989	$C_{10}H_{14}O$	150	89-83-8	CC1=CC(=C(C=C1)C(C)C)O
2.	Diethyl Phthalate	6781	$C_{12}H_{14}O_4$	222	84-66-2	CCOC(=O)C1=CC=CC=C1C(=O)OCC
3.	Diethyl Phthalate	6781	$C_{12}H_{14}O_4$	222	84-66-2	CCOC(=O)C1=CC=CC=C1C(=O)OCC
4.	Phenol, 2-ethoxy-4-[2-propenyl]-	601250	$C_{11}H_{14}O_2$	178	1755-54-0	CCOC1=C(C=CC(=C1)CC=C)O
5.	Phthalic acid, ethyl 2-pentyl ester		$C_{15}H_{20}O_4$	264	315474	
6.	Hexadecanoic acid, methyl ester	8181	$C_{17}H_{34}O_2$	270	112-39-0	CCCCCCCCCCCCCCCC(=O)OC
7	Hexadecanoic acid, ethyl ester	12366	$C_{18}H_{36}O_2$	284	628-97-7	CCCCCCCCCCCCCCCC(=O)OCC
8	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	2775	$C_{11}H_{10}O_4$	206	487-06-9	COC1=CC2=C(C=CC(=O)O2)C(=C1)OC
9	9,12-Octadecadienoic acid [Z,Z]-, methyl ester	5284421	$C_{19}H_{34}O_2$	294	112-63-0	CCCCC/C=C\C/C=C\CCCCCCCC(=O)OC
10	9-Octadecenoic acid [Z]-, methyl ester	5364509	$C_{19}H_{36}O_2$	296	112-62-9	CCCCCCCC/C=C\CCCCCCCC(=O)OC
11	9,12-	11001	$C_{20}H_{36}O_2$	308	7919	CCCCCC=CCC=CCCCCCCCC(

	Octadecadienoic acid, ethyl ester				-08-1	=O)OCC
12	Ethyl Oleate	536369	$C_{20}H_{38}O_2$	310	111-62-6	CCCCCCCC/C=C\CCCCCCCC(=O)OCC
13.	9,12-Octadecadienoic acid [Z,Z]-	5280450	$C_{18}H_{32}O_2$	280	60-33-3	CCCCC/C=C\C/C=C\CCCCCCCC(=O)O
14.	Octadecanoic acid, ethyl ester	8122	$C_{20}H_{40}O_2$	312	111-61-5	CCCCCCCCCCCCCCCCCC(=O)OCC

4. Conclusion

Lemon Peel known as citrus lemon L. is widely used in many Ayurvedic herbal formulations and traditional Indian cooking. The fact that *Citrus Lemon L.* exhibits a broad spectrum of antibacterial activity and comparable efficacy to synthetic antioxidants shows the medicinal value of *Citrus Lemon L.* as a potential source of drug development as an effective and safe antibacterial drug. Since *Citrus Lemon L.* has considerable antibacterial and antioxidant properties and considering its nutritional value, prevention of health issues using this should be promoted. Due to its antibacterial and anti-oxidant activity against skin flora and the presence of bioactive compounds in it, it can be used in many cosmetic products.

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