



Effect of *Citrus limon* (lemon) Peels and Seeds on Bacteria Isolated From Nose

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ABSTRACT

Citrus peels are the main waste from citrus processing companies. Waste from citrus processing accounts for more than 50% of the total fruit weight. This huge waste must be converted into useful and economical products. Study investigated the antibacterial effects of *Citrus limon* peels(rind) and seeds against bacteria isolated from the noses of students at a higher education institution. Bacteria isolated from nasal swabs were identified by culture and biochemical tests. Sensitivity testing was performed using diffusion in agar wells. Phytochemical tests of lemon peel and seeds were performed by standard methods. Thirty-two bacteria were isolated and identified from the students' nasal samples. *Staphylococcus epidermidis* was the most prevalent organism. The methanolic lemon peel extract was the most active against the isolated bacteria, with minimum inhibitory concentrations of 6.25 mg/ml and 12.5 mg/ml against *S. epidermidis* and *Pseudomonas aeruginosa*, respectively. The qualitative phytochemical tests results of the peels and seeds included alkaloids, flavonoids, tannins, saponins, cardiac glycosides and steroids. Lemon peel extract was active against bacteria isolated from the nose.

Keywords: Nasal samples, lemon peels, seeds, methanol, ethanol, flavonoids

Introduction

Citrus fruits include oranges, lemons, grapefruits, limes, tangerines, tangelos, pomelos, kumquats, and more. (Czech *et al.*, 2020). They contain valuable nutrients that actively stimulate the digestive, immune and cardiovascular systems. They also exhibit antibacterial and anticancer properties. Citrus peels are a good source of limonene, molasses, and pectin. It has been

reported that more secondary metabolites and antioxidant activity are embedded in the peel compared with other parts of the fruit (Rafiq *et al.*, 2018, Manthy and Grohamani, 2001). In addition, citrus peels contain many prenyloxy coumarin compounds and macro- and trace minerals that increase their nutritional and therapeutic value (Genovese *et al.*, 2014). These ingredients present in the peel make them

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suitable as a source of nutraceuticals. Citrus peels are readily available and often discarded as waste. Lemon (*Citrus limon* L.) is a citrus hybrid. *Citrus limon* is one of the plants introduced to Europe by Muslims through Sicily and the Iberian Peninsula (Rahman *et al.*, 2019). A lemon tree can grow up to 10 meters tall, but some are smaller. The branches form an open and thorny crown. Leaves are green, elliptical and glossy. The flowers have a strong fragrance and are white outside with purple stripes on the inside, lemon trees flowers and fruits at the same time (Rahman *et al.*, 2019). Lemons are native to northern India, however, grown on a very large scale in Italy, Spain and Sicily. Lemon peel consists of two layers, the outermost oily skin/rind, often used as a flavoring for cookies, puddings, candies, chocolates (Sheila *et al.*, 2017) and the pith beneath the skin, which is often spongy and bitter. The peel can be used as a source of medicine for humans and livestock. Many studies have explored the possibility of using the peel in food, cosmetic and pharmaceutical industries, targeting valuable nutrients and bioactive ingredients.

Microorganisms are known to cause disease in humans and livestock. Disease outbreaks are constantly increasing due to human population and their impact on the environment. The waste generated from citrus peels, especially lemons, can be a source of antibacterial drugs to fight disease in humans. Lemon has anticancer and antibacterial activities (Kartek *et al.*, 2012). Juice is good for food digestion and constipation (Nidhi, 2016). In addition, it is also used to treat scurvy, body hair, stomach ulcers, gout, gums and urinary disorders (Mohanapriya *et al.*, 2015). However, the peel is underutilized for useful purposes, for instance, could be used for medicinal purposes instead of being discarded as waste. The aim of the study was to determine the effects of lemon peel and seeds on bacteria isolated from the nose.

Materials and Methods

After permission, 20 nasal swab samples were collected from student (male and female) according to the method of Warnke *et al.* (2014). The samples were transported to the Microbiology laboratory. The swab was inoculated in nutrient broth and incubated for 24 h at 37°C. Blood agar and MacConkey agar plates were streaked with the broth culture and incubated for 24 hours at 37°C. Distinct colonies were sub cultured to obtain pure cultures, which were preserved in slants. The isolated bacteria were Gram stained and biochemical tests were performed on the isolates.

Lemon peels and seeds extraction

Lemons used in this study were purchased from Effurun market in Delta State, Nigeria. The fruits were transported to the laboratory, washed and dried. Part of the fruits were taken for identification and voucher UBH-C 362 was raised. The lemon skin/rind and seeds were removed with a sharp knife and dried at room temperature for two weeks. Three solvents (distilled water, methanol and ethanol) were used for the extraction. Soxhlet extraction was performed at 60°C. The dry extract was obtained by heating the extract with a rotary evaporator. The obtained extracts were stored in a clean container at 4°C temperature until needed.

Antibacterial activity of extracts

The antibacterial activity was analyzed using the method described by the Clinical laboratory standard (2016). The overnight inoculum was diluted according to the Macfarland standard and plated on solidified muller hinton plates. Wells were bored on specific areas such that holes were evenly spaced, and extracts diluted at different concentrations were added to the wells and incubated at 37°C for 24-48 h (Sheila, *et al.*, 2017; Adomi, 2022). Standard antibiotics was also tested.

Minimum inhibitory concentrations were studied using standard methods.

Phytochemical Test

Phytochemical examination of peels and seeds was determined by standard methods (Trease and Evans, 1985) as described by Okon *et al.* (2015).

Results and Discussion

Tables 1 and 2 show the bacteria isolated from the noses of the students which include *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* sp, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Tables 3, 4 and 5 indicate the antibacterial activities of the peel and lemon seeds with antibiotics and minimum inhibitory concentration. *Staphylococcus aureus* and *Streptococcus* sp were the bacteria most sensitive to lemon peel while the ethanol extract of the same part of the plant had moderate activity. The seed extract was not active against any of the bacteria in this study. The antibiotic levofloxacin produced a zone of inhibition greater than 19 mm for *Streptococcus* sp., comparable to peel extracts. The minimum inhibitory concentrations of the active extracts were 12.5-25mg/ml and 6.25-12.5mg/ml for ethanol and methanol extracts respectively. Table 6 indicates the phytochemical results of *Citrus limon* peel and seed. Plant parts extracted with 3 solvents showed alkaloids, flavonoids, tannins, saponins, cardiac glycosides and steroids. The bacteria isolated in this study matched those isolated from preclinical medical students in

China. Isolated bacteria genera included *Staphylococcus*, *Streptococcus*, *Pseudomonas aeruginosa*, and other members of Enterobacteriaceae. The aim of this study was to determine the efficacy of lemon (*Citrus limon*), peel and seeds against bacteria isolated from the nose. The results of this study indicated that the methanol extract was more active than the ethanol extract and comparable to the standard antibiotic drug used in this study. The aqueous extract of the lemon peel and all extracts obtained from the lemon seeds were inactive against the tested microorganisms. This study is consistent with a previous study of ethanol extract of *Citrus limon* against *E. coli* (Henderson *et al.*, 2018). However, the methanol extract was more active than the ethanol extract in this study, which was also consistent with Sheila *et al.*, (2017) findings. However, acetone extract of lemon peel was more active than sweet orange peel and tangerine peel respectively by other researchers (Okon *et al.*, 2015). Phytochemical tests of lemon peel and seeds revealed the presence of alkaloids, tannins, steroids, cardiac glycosides and other compounds in all extracts, which aligned with Junab *et al.* (2017) study where all phytochemicals were present in their methanol extract. However, this result is in contrast to that reported by Okon *et al.*, (2015) where saponins were absent in their results, which may be due to the solvent used in the extraction process in their study.

Table 1: Biochemical Characteristics of Isolates from Nose

Probable isolate	Gram stain / shape	Indole production	Methyl red test	Voges-proskauer proskauer	Citrate utilization	Urease test	Oxidase test	Motility	Lactose	Mannitol	Glucose	Sucrose	H ₂ S Production	Gas	Coagulase	Catalase test	Mannitol Salt agar test	Hemolysis	Catalase
<i>Escherichia coli</i>	-/rod	+	+	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	+
<i>Pseudomonas aeruginosa</i>	-/rod	-	-	-	+	-	+	+	-	+	-	-	-	-	-	+	-	+	+
<i>Staphylococcus aureus</i>	+/cocci	-	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+
<i>Staphylococcus epidermis</i>	+/cocci	-	-	+	-	+	-	-	+	+	-	+	+	-	-	+	-	-	+
<i>Streptococcus sp</i>	+/cocci	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-

Table 2: The number of each bacterial isolated from nose

Bacteria	No of isolates
<i>Staphylococcus aureus</i>	7
<i>Escherichia coli</i>	5
<i>Streptococcus sp</i>	4
<i>Pseudomonas aeruginosa</i>	6
<i>Staphylococcus epidermidis</i>	10

Table 3: Antibacterial activity of lemon seeds extract at 50 mg/ml and Antibiotics

Diameter of inhibition (mm)	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>
Water	0	0	0	0
Ethanol	0	0	0	0
Methanol	0	0	0	0
Levofloxacin (20 mcg)	15	12	20	17

Table 4: Antibacterial activity of lemon peel (rind) at 50 mg/ml

Diameter of inhibition (mm)	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>Streptococcus sp</i>
Water	0	0	0	0	0
Ethanol	18	15	15	15	17
Methanol	20	17	17	19	20
Levofloxacin (20mcg)	15	12	20	17	20

Table 5: Minimum Inhibitory concentration of Lemon peel (rind) extracts

MIC	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>Enterococcus</i> sp	<i>Streptococcus</i> sp
Ethanol	25	-	12.5	12.5	-	12.5
Methanol	12.5	-	12.5	6.25	-	12.5

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Table 6: Phytochemical extracts of *Citrus limon* aqueous, ethanol and methanol extracts

Test	Peels (rind)			Seeds		
	Ethanol	Methanol	Water	Ethanol	Methanol	Water
Alkaloid	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Steroids	+	+	+	+	+	+

Conclusion

This study aimed to determine the effects of lemon extracts on bacteria isolates from nasal swabs of University students. *Staphylococcus epidermidis* was the most prevalent isolate. Lemon peel extract was more active against isolated bacteria and lemon seed extracts were resistant. The methanol lemon peel extract was more active than the ethanol extract while the aqueous extract was not active against any bacteria. Phytochemicals present in the peel and seed include tannins, alkaloids, saponins, glycosides, and steroids. Lemon peel could be used to fight against bacteria that cause respiratory diseases.

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