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RESEARCH ARTICLE

Phytochemical Screening, GC Analysis and Antibacterial Activity of *Citrus limon* Peel Extract and Essential Oil

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Abstract

Lemon (*Citrus limon*) is the most commonly grown tree fruit in the world. The fresh lemons were collected from local market of Kathmandu, Nepal. Hexane and methanol extracts of plant material i.e. lemon peel were screened for the analysis of presence of phytochemicals as well as their antibacterial activity. Methanol extract of lemon peel showed the maximum positive phytochemical test with the presence of alkaloids, flavonoid, polyphenols, terpenoids, glycosides, saponin, tannins etc. Essential oil was obtained by steam distillation from fresh peels of lemon using Clevenger apparatus and analyzed by gas chromatography (GC). Twenty six (26) chemical components were identified in the essential oil of lemon peel. Lemon peel essential oil indicated the presence of Pinene (β) (15.46 %), Limonene (28.94 %), Terpinene (γ) (8.64), Terpinen-4-ol (3.29 %), Neral (4.20 %), Geranial (5.28 %) as major components. The lemon peel essential oil was found to be potent antibacterial agents against the *Bacillus subtilis* (21 mm).

Keywords: Antibacterial activity, Essential oil, GC, Lemon peel, Phyto constituents,

Introduction

Bio deterioration in assorted domain including food security, health care, and environment as well as in the protection of cultural heritage can be reduced. This can be achieved by the ability to control growth and colonization of bacteria [1]. The management of disease and infections are remarkably flourishing due to the static and cidal effects of antibiotics [2]. However, the overuse of antibiotics stimulates the genetic potential of bacteria to adapt the drugs through modification [3]. Thus, microbial-antibiotic resistance has increased that create a possible threat in drug industries. Consequently, efficient, environmentally friendly and economical alternatives from natural sources such as plant derived products are being studied [4, 5]. Hence, the antibacterial activity of citrus has been a vital attraction as auspicious source of pharmaceutical agents [6].

Citrus are well known for their therapeutic, functional and prophylactic activities [7]. According to World Health Organization (WHO) 'The plants with therapeutic substance or of chemo pharmaceuticals semi-synthetic new drugs are esteemed as medicinal' [8]. The medicinal plants precede the preface of antibiotics and other contemporary medications that discourage the harmful approaches regarding medicinal practice [9]. *Citrus limon* as medicinal plant shows anticancer and antibacterial activities due to presence of alkaloid. Furthermore, it can be used for treatment of skin problems, weight loss, indigestion, constipation etc [10].

Citrus peels are usually atrophied in industries. Since, the juice of citrus is less half of the fruit weight, they are regarded as worthy byproduct of citrus processing industries [11]. Citrus peel contain abundant amount of active phytochemicals like limonin, nomilin,

octanol, cineole and naringin that are responsible for its bitter taste [12]. The constituents like flavanones and many polymethylated flavones which are unusual in other plants are the richest component of citrus peels [13]. Hence, it can be efficiently use as drug derivatives or as food supplements [14].

Citrus limon of Rutaceae family are storehouse of vitamin C, alkaloids, flavonoids and essential oils which holds antimicrobial and anticancer property [15]. Phytochemical possess some disease preventive properties like antioxidant and protect cell against free radical damage e.g. polyphenols, carotenoids etc. [16]. Essential oils consequent to their better diffusibility and mode of contact are efficacious in controlling biofilm culture [17]. Lemon peels contains innumerable oil bearing glands that hedge eloquent amount of citrus oil [18]. Citrus essential oil are composed of terpenes, aliphatic sesquiterpenes, oxygenated derivatives, flavonoids (flavone, flavonol, flavanone) [19]. Therefore, they are used as antimicrobial, anti-diabetic, antioxidant, insect repellent, larvicidial, anti-hepatotoxic factor [20]. Hence, in this paper the potential of lemon peel essential oil (clevenger apparatus) analyzed by gas chromatography (GC) and its extract (Soxhlet extraction solvent methanol and hexane) phytochemicals on standard micro-organism strains were evaluated using Agar well diffusion method.

Materials and methods

Materials

Solvents like methanol and hexane (Thermo Fischer Scientific India Pvt. Ltd. Mumbai) were of Analytical lab grade. Distilled water (Ultra Super TM, Marech Pvt. Ltd. Lalitpur, Nepal), Soxhlet (Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai, India), Rotavapour (Bulchi Labourtechnic Aq. Flawil, Swiss), Clevenger Apparatus, were used in the experiment.

Methods

Extraction of plant peel

Citrus limon was taken from local market of Kathmandu. The collected Citrus fruit was washed by fresh water to remove other contamination like dust, soil. The lemon peels was peeled off carefully with help of sharp razor blade and cut into smaller pieces. 50 g of powdered form lemon peel was taken and crude extract was extracted by Soxhlet method [21]. Two different solvents (250 mL), namely n-Hexane and methanol were used at room temperature and extract was concentrated by rotary evaporator under reduced pressure, maintaining temperature lower than the boiling point of respective solvents used. These extracts were stored in the refrigerator.

Phytochemical screening

Preliminary phytochemical screening of hexane and methanol extracts of *Citrus limon* peels were done using the standard procedure put forward by Ciulei I [22, 23].

Extraction of essential oil

Steam distillation was the most popular method for the heat sensitive oils [24]. In this method Clevenger apparatus was used where the small piece of lemon peel were placed in the R.B. flask and two third of distilled water was added and fixed with a condenser and a receiver to pass steam for 2 hours. The distillate was collected and the oil was separated by using separating funnel.

GC analysis of extracted essential oil of Lemon peel

GC analysis was performed for discerning of the constituents present in essential oil of *Citrus limon* by perceptible elucidation comparing their retention indices and mass spectra with reference spectra using a GC-MS thermofocus GC-DSQ II equipped with 2B-5 fused silica capillary tubes column (30m×0.25 mmμ 0.25μm). The volume of the injection at the flow of 1 mL/min and a split ratio of 1:50 was 1μL using helium as carrier gas. The initial oven temperature raised to 60°C was maintained for 5 min, increased to 310°C at the rate of 10μ/min and finally kept for 1 min. Interface temperature 245°C, ion source temperature 280°C electron impact ionization (EII) 70 eV were some other required conditions for GC analysis. Spectra were scrutinized in the full scan mode over the range of 30 to 550 AMU and retention indices (RI) of all constituent were determined by the Kovals method for the analysis of component present in essential oil.

Antibacterial assay

Liquid LB media (Luria Broth) containing 10 mg/L and NaOH (5N) 200μL was used in culturing Microbial strain: *Escherichia coli* (Gram negative) ATCC 25922 and *Bacillus subtilis* (Gram positive) ATCC 6051 for 24 hours. 100μL culture broth of each strain was plated on LB- agar plate for 15 min of incubation at 37°C. After that, the extract and essential oil of lemon peel were accordingly filled with positive control antibiotic (chloramphenicol 1 mg/mL) and negative control solvent with respective solvent extract (hexane and methanol) used during extraction process were kept on LB- agar plate and incubated overnight at 37°C. Next day, the antimicrobial activities of the extract and essential oil against *E. coli* and *B. subtilis* microbial strain was investigated by Agar well diffusion method [25].

Results and discussion

The phytochemical screening of *Citrus limon* different extract are present in Table 1.

***Table 1**: Phytochemical screening of Citrus limon peel extracts*

S.N.	Test of phytochemicals	Hexane extract	Methanol extract
1.	Alkaloid test		
	Mayer's Test	-	-
	Wagner's Test	-	+
	Dragondorf's Test	+	+
2.	Flavonoid Test		
	Shinoda's Test	+	+
	Shibata's Test	-	-
3.	Polyphenols Test	+	+
4.	Terpenoids Test	-	-
5.	Glycosides Test	+	+
6.	Saponin Test	+	+
7.	Tannins Test	+	+
8.	Quinine Test	+	+
9.	Fehling's Test	-	-

(+) indicates presence and (-) indicates absence

Methanol extract of lemon possessed more phytochemicals than the hexane extract. In this study, methanol extract of lemon peel showed the maximum phytochemical screening with the presence of alkaloids, flavonoid, polyphenols, terpenoids, glycosides, saponin, tannins etc. and the hexane extract showed absence of most of the phytochemicals. Since, the phytochemicals present in *Citrus limon* were more exposed by the polar compound than the non-polar one. The difference in yield could be explained by the excellent activity of methanol to dissolve bioactive compound and higher heating stability which enables retention of volatile compounds. Flavonoid, tannins, polyphenols were dominant group of bioactive compounds that act as primary anti-oxidants or free radical scavengers [26]. Glycosides were naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia [27]. The presence of tannin and phenolic compound specified that the *Citrus limon* peels had potential antimicrobial properties [28].

GC analysis of extracted oil of lemon peel

Twenty six (26) chemical components were identified in the essential oil of Lemon peel were listed in Table 2. Lemon peel essential oil indicated the presence of β-Pinene (15.46 %), limonene (28.94 %), terpinene (γ) (8.64%), terpinen-4-ol (3.29 %), neral (4.20 %), geranial (5.28 %) as major components. However, Kaskoos reported limonene (29.52 %), β-pinene (23.89 %), citronellal (11.53 %) and thymol (9.79 %) as the major constituents of essential oil of lemon peels [29]. Limonene was found in the highest amount which is responsible for the familiar aroma of citrus. Therefore, it is used in perfume for its excellent fragrance. It is also used as active solvent ingredient in several applications like adhesive stain removers, cleaner of strippers due to its environmentally friendly nature [30] The chemical constituents reside to hydrocarbon and oxygenated category namely monoterpene, sesquiterpenes, alcohol, aldehydes monoterpenoids and sesquiterpenoids [31]. Linalool, β-pinene and other components were super intended for the exhibition of antimicrobial activity [32]. Lemon peels was good source of essential oils and were found very useful in number of cleaning products and in food recipes due to their pleasant aroma and powerful solvent properties [33].

Antibacterial activity

The result of antibacterial activity of *Citrus limon* peel hexane and methanol extract and its essential oil in two bacterial strain that is Gram positive *Bacillus subitilis* and Gram negative *Escherichia coli* with standard positive control antibiotic chloramphenicol (Figure 1, 2, 3) with zone of inhibition 25 mm were discussed.

***Table 2**: Chemical components identified in lemon peel essential oil*

SN	Chemical Constituent	Area (%)
1	Thujene <alpha->	0.54
2	Pinene <alpha->	3.18
3	Sabinene	2.86
4	Pinene <beta->	15.46
5	Myrcene	1.99
6	Terpinene <alpha->	0.83
7	Cymene <para->	2.69
8	Limonene	28.94
9	Ocimene <(E)-, beta->	0.67
10	Terpinene <gamma->	8.64
11	Terpinolene	1.44
12	Linalool	1.65
13	Terpinen-4-ol	3.29
14	Terpineol <alpha->	5.37
15	Decanal<n->	0.79
16	2,6-Octadien-1-ol	1.96
17	Neral	4.20
18	Geraniol	2.29
19	Geranial	5.28
20	Linalool isobutyrate	0.68
21	Caryophyllene <(E)->	1.30
22	trans-alpha-Bergamotene	1.29
23	Germacrene D	0.42
24	Farnesene <(E,E)-, alpha->	1.73
25	Bisabolene	1.83
26	Germacrene B	0.67

The methanol extract of lemon peel was slightly susceptible towards the Gram positive (*B. subtilis*) bacteria with the same diameter of zone of inhibition (8 mm) which was also shown by the negative control methanol solvent (Figure 1 a, b). But, the Gram negative (*E. coli*) was resistance to the methanol extract of lemon peel with 6 mm diameter of zone of inhibition which was less than the diameter of zone of inhibition (9 mm) shown by the negative control itself.

The hexane extract of lemon peel showed significant antibacterial activity towards Gram positive *B. subtilis* bacteria with 15 mm zone of inhibition (ZOI) higher than that of negative control hexane solvent (13 mm ZOI) used (Figure 2 a, b). However, it was slightly susceptible towards the Gram negative *E. coli* bacteria with zone of inhibition was same with that of the negative control solvent (12 mm). Hence, the hexane extract of lemon peel showed the antibacterial properties.

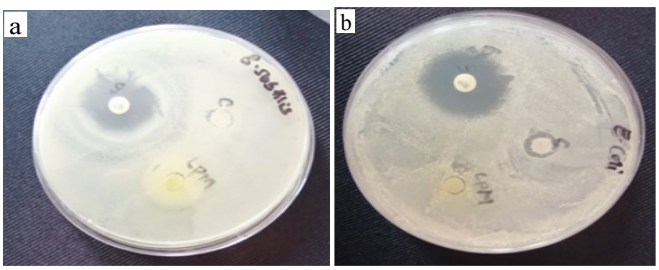

***Figure 1**: Antibacterial activity of lemon peel methanol extracts (a) B. subtilis and (b)E. coli*

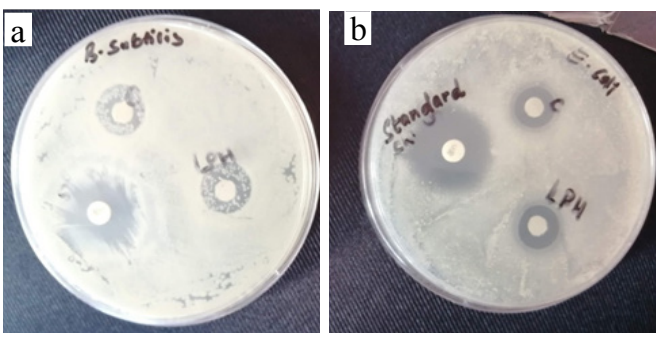

***Figure 2:** Antibacterial activity of lemon peel Hexane extracts* (a) *B. subtilis* (b) *E. coli*

The lemon peel essential oil was highly susceptible with Gram positive *B. subtilis* bacteria with 21 mm zone of inhibition which is close to the 25 mm ZOI shown by the positive control antibiotic chloramphenicol (Figrue 3 a, b). However it showed 13 mm ZOI against gram negative *E. coli* strain. It is reported that monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives exhibited antimicrobial activities [34, 35]. Hence lemon peel essential oil exhibited antibacterial activity.

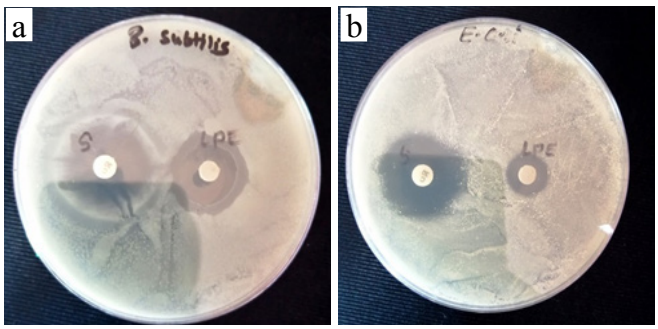

***Figure 3**: Antibacterial activity of lemon peel essential oil against (a) B. subtilis and (b) E. coli*

Conclusion

The phytochemical screening of methanol and hexane extracts of lemon peel indicated the presence of major phytochemicals like alkaloids, flavonoids, polyphenols, and terpenoids. The antibacterial activity of essential oil and extracted hexane and methane extract showed effective activity toward Gram positive *B. subtilis* than the Gram negative *E. coli*. Limonene, and β-pinene were identified as major constituents in essential oil *via* gas chromatography (GC). The study revealed that essential oil contains pharmacologically active substances. Therefore, it can be a source for the drug development against several infectious diseases.

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