# Phytochemical Screening, Antimicrobial Activities and Extraction of Essential Oil from the Peel of *Citrus reticulata* Blanco

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DOI: 10.29322/IJSRP.9.07.2019.p9194
http://dx.doi.org/10.29322/IJSRP.9.07.2019.p9194

***Abstract*-** The famous fruit, *Citrus reticulata* Blanco was selected for chemical analysis and collected from the Pyin Oo Lwin Township, Mandalay Region in Myanmar. The preliminary detection of phytochemical compounds present in the sample was carried out by standard procedures. The sample gave rise to positive results for alkaloid, flavonoid, glycoside, polyphenol, tannin, reducing sugar and terpene respectively. The antimicrobial activities of sample in various solvent systems were determined by agar well diffusion method on six selected microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, and *E. coli*. The main objective of essential oil was extracted from the peel of *Citrus reticulata* Blanco by steam distillation method. The yield of essential was found to be 0.4960% based on the sample weight. Moreover, the extracted essential oil was analyzed by Gas Chromatography Mass (GC-MS) Spectrometry.

## I. INTRODUCTION

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world drugs are of natural product origin. (Kirbag, 2009) Medicinal plant materials have been successfully used for thetreatment of fungal and bacterial infections in humans (Akinyosoye and Oladummoye, 2000), suggesting that some plant materials may also possess antifungal and antibacterial constituents which are useful in controlling plant diseases (Amadioha, 1998).

Antimicrobial screening of plant extracts and phyto-chemicals, then, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for phar-maceutical industry (Ryan, 4th edition). The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* are mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning. Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini, 2003). These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cheng, 2009).

The essential oil of citrus genus belongs to the Rutaceae family (Mozafarian, 1998). The essential oils are aromatic compounds that are widely used in the perfume, pharmaceutical and food industries. Essential oils are mixtures of more than 200 different compounds. These compounds are mainly formed of monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives such as esters, alcohols and aliphatic aldehydes and ketones. Essential oils are generally created by aromatic plants. The specific gravity of essential oils is often less than water and only a small number of essential oils have a higher specific gravity than water. Essential oils are non-miscible with water but can transfer their odors to aqueous layer. These compounds are solved in most of organic solvents such as diethyl ether, hexane and ethyl acetate. Essential oils in the presence of air and heat are evaporated, therefore they might be called volatile oils or ethereal oils (Aberoomand *et al*., 2011; Kamal *et al*., 2011). Essential oils are present in various aromatic plants generally grown in tropical and subtropical countries. They are obtained from various parts of the aromatic plants, including leaves, flowers, fruits, seeds, buds, rhizomes, roots, and barks. Several techniques have been used to obtain essential oils from the plant. They are hydrodistillation, solvent extraction, cold pressing, and supercritical fluid extraction (Billot, 1975; Handa, 2008; Simon, 1990). Nowadays, approximately 3000 essential oils are known, about 300 of which are commercially available. The major constituents of essential oils are terpenes/ terpenoids and aromatic and aliphatic compounds, which are characterized as low-molecular-weight aroma chemicals (Gonzalez-Burgos, 2011; Betts, 2001). In the modern era, essential oils and some of their components have been used in various products such as cosmetics, household cleaning products and air fresheners, hygiene products, agriculture, and food, as well as in medicinal uses. Essential oils are also used in aromatherapy and other para-

medicinal practices (Silva, 2003; Hajhashemi, 2003; Perry, 2003).

**Botanical Description**

Family -Rutaceae
English name - Tangerine
Botanical name - *Citrus reticulata* Blanco
Myanmar name - Pya- lein-hmaw
Part used - Peel

Figure (1) The peel of Pya- lein-hmaw

## II. Materials and Methods

**Sample collection**

The fruits of *Citrus reticulata* Blanco were collected from Pyin Oo Lwin Township, Mandalay Region in Myanmar. The fruits were peel and chopped into small pieces and used throughout the experiment.

**Preliminary Phytochemical Constituents of Peel of *Citrus reticulata* Blanco**

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the peel of ***Citrus reticulata*** Blanco. (Harborne, 1993)

**Test for Alkaloid**

The dried powder of sample (2 g) was boiled with 1% hydrochloric acid (10 mL) for about 10 minutes, allowed to cool and filter. The filtrate was divided into two portions and tested with Dragendroff's reagent. The red precipitate was obtained, that indicated the presence of alkaloid.

**Test for Flavonoid**

(0.5 mL) of ethanol extract, 3-10 drops of concentrated hydrochloric acid and a small piece of magnesium was added in a test tube. The solution was boiled for few minutes. The appearance of pink color indicates the presence of flavonoid.

**Test for Glycoside**

The dried sample (2 g) was boiled with distilled water for about 10 minutes, allowed to cool and filter. The filtrate was tested with 10 % lead acetate solution. The formation of white precipitate indicates the presence of glycoside.

**Test for Polyphenol**

The ethanol extract of sample (10 mL) was treated with (1 mL) of 1% potassium ferrocyanate and (1 mL) of 1 % ferric chloride solution. The appearance of greenish blue color indicates the presence of polyphenol.

**Test for Tannin**

The dried sample (2 g) was boiled with distilled water for about 10 minutes, allowed to cool and filter. The filtrate was tested with a few drops of 10 % ferric chloride solution. If a bluish black color is produced, which disappeared on addition of a few drops of dilute sulphuric acid solution followed by the formation of a cream precipitate the presence of tannin.

**Test for Reducing Sugar**

(5 mL) of Benedict's solution was added to (0.5 mL) of aqueous extract of sample and boiled for 5 minutes. On cooling down, brick red precipitates indicate the presence of reducing sugar.

**Test for Terpene**

A mixture of (2 mL) of concentrated sulphiric acid, (1 mL) of acetic anhydride and (2.5 mL) of chloroform was added to ethanol extract of sample. Formation of brown color indicates the presence of terpene.

**Antimicrobial Activities of Crude Extracts of Peel of *Citrus reticulata* Blanco**

The antimicrobial activities of crude extract sample of the peel of useful fruits were examined by using agar well diffusion method on six selected microogranisms, at Central Research and Development Centre (CRDC), Insein, Yangon. (Magaldi, 2004) (Valgas, 2007)

In this case, the nutrient agar medium was prepared and the study of antimicrobial activities was performed as below. Firstly, nutrient agar was boiled and then 20-25 mL of it was poured into the test tube, plugged with cotton wool and sterilized at 121°C for 15 minutes in an autoclave. After sterilizing, the tubes were cooled down to 30-35°C, the agar in the tube was poured into the sterilized Petri-dish and 0.1-0.2 mL of test organisms were also added into the dish. These were allowed to set the agar for 2-3 hours. After the agar was set, 7 mm petals agar well were made by the help of sterilized agar well cutter. After that, about 0.2 mL of sample was introduced into the agar well and incubated at 37°C for 24-28 hours. The inhibition zone appeared around the agar well indicates the presence of antimicrobial activity.

**Extraction of Essential Oil by Steam Distillation**

The extraction of essential oil was done by steam distillation method at Department of Chemistry, University of Mandalay. The yield percent of extraction oil was determined. (Pratt, 2013) The apparatus was used, 2 L of distilled water was poured into the still body and perforated cone was set over it, 430 g of the sample was placed on the perforated cone of the still. It was heated carefully without decomposition of oil. The time taken was five hours per day.

After heating for five hours, a mixture of volatile oil and stream was came out passed into the condenser. The oil

collecting on the surface of the water was separated by using petroleum ether and separating funnel .And then petroleum ether in essential oil was allowed to evaporate. The filtrate was stored for the use of next chemical composition determination. The dehydrated oil obtained by passing through the anhydrous sodium sulphate preserves for best quality. The above experiment was carried out for three times, each time using 430 g of sample.

Figure (2) Steam distillation apparatus

**Determination of Chemical compositions by GC-MS**

The peel of *Citrus reticulata* Blanco was extracted with steam distillation and analyzed by GC-MS using pet-ether as solvent for identification of different compounds, at the Department of Chemistry, University Research Center (URC), Mandalay.

## III. Results and Discussion

**Preliminary Phytochemical Screening of the Peel of *Citrus reticulata* Blanco**

**Table (1) Results of Phytochemical Screening of the Peel of *Citrus reticulata* Blanco**

| No. | Test | Reagent | Observation | Bark |
|---|---|---|---|---|
| 1. | Alkaloid | 1 % HCl, Dragendroff's | Red ppt | + |
| 2. | Flavonoid | Conc: HCl, Mg turning | Pink color solution | + |
| 3. | Glycoside | 10 % lead acetate | White ppt | + |
| 4. | Polyphenol | 1 % $FeCl_3$ and 1 % $K_3[Fe(CN)_6]$ | Greenish blue color solution | + |
| 5 | Tannin | 1% gelatin solution | Cream ppt | |
| 6. | Reducing Sugar | Benedict's solution | Brick red ppt | + |
| 7. | Terpene | Acetic anhydride, $CHCl_3$, conc: $H_2SO_4$ | Brown ppt | + |

(+) = presence (-) = absence ppt = precipitate

According to above table, the sample contained alkaloid, flavonoid, glycoside, , polyphenol, tannin, reducing sugar and terpene compounds respectively.

**Determination of Antimicrobial Activities of Crude Extracts of the Peel of *Citrus reticulata* Blanco**

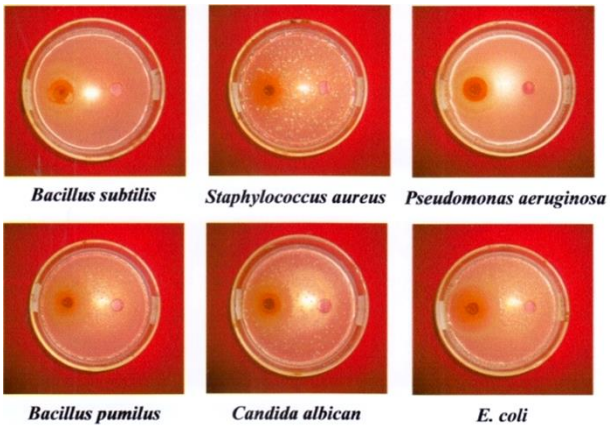


Figure (3) Antimicrobial Activities of *Citrus reticulata* Blanco

**Table (2) The Results of Antimicrobial Activities of Crude Extract Sample of the Peel of *Citrus reticulata* Blanco**

| | | Inhibition Zone | | | | | |
|---|---|---|---|---|---|---|---|
| **Samples** | **Sol-vent** | **I** | **II** | **III** | **IV** | **V** | **VI** |
| Peel of *Citrus reticulata* Blanco | EtOH | 16mm (++) | 19mm (++) | 17mm (++) | 12mm (+) | 15mm (++) | 38mm (+++) |
| Control | EtOH | – | – | – | – | – | – |

Agar well
~ 10 mm Organism
10 mm ~ 14 mm (+) = low activity
15 mm ~ 19 mm (++) = medium activity
20 mm ~ above (+++) = high activity

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *E. coli*

According to this table, ethanol extract of useful peel are activity on all microorganisms. Moreover, ethanol extract of sample gives rise to medium activity on four organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans.* The highest activities was found on *E. Coli.*

**Extraction of Essential Oil by Steam Distillation Method**

**Table (3) The Yield (%) of Essential Oil by Steam Distillation Method**

| No. of Experiment | Weight of Sample (g) | Weight of extracted essential oil | % of essential oil |
|---|---|---|---|
| 1 | 430 | 2.1550 | 0.4960 |
| 2 | 430 | 2.1550 | 0.4960 |

| | | | |
|---|---|---|---|
| 3 | 430 | 2.1550 | 0.4960 |

According to this table, the average yield percent of essential oil is 0.4960% based on the weight of peel of *Citrus reticulate* Blanco.

### Identification of Components by GCMS

Identification of the extracted oil components was based using a National Institute of Standards and Technology (NIST) mass spectral library according to the reference mass spectra from published sources, and retention indices (RI). The essential oil from the peel of *Citrus reticulate* Blanco by GC-MS analysis showed the presence of some compounds, shows in Table.

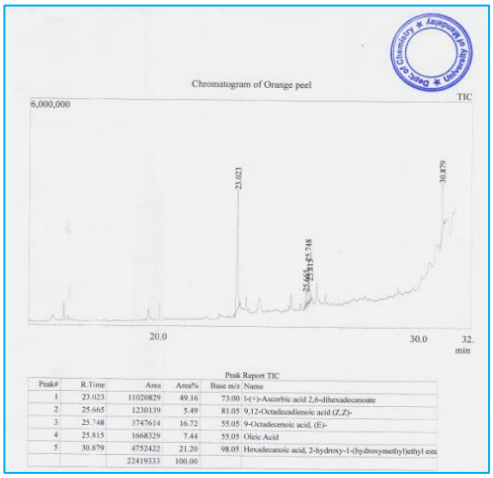


Figure (4) Total ion chromatogram of extracted essential oil

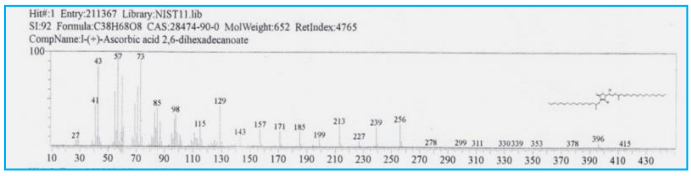


Figure (5) EI mass spectrum of Ascorbic acid 2,6-dihexadecanoae

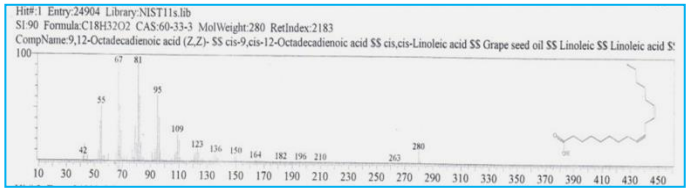


Figure (6) EI mass spectrum of 9, 12-Octadecadienoic acid

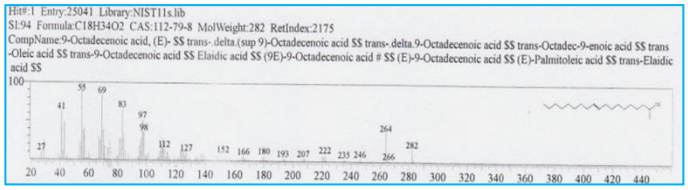


Figure (7) EI mass spectrum of trans-9-Octadecenoic acid

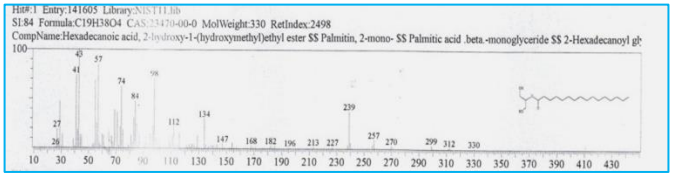


Figure (8) EI mass spectrum of Hexadecanoic acid

This is the total ion chromatogram for essential oil extract of the peel of *Citrus reticulate* Blanco by GC-MS using steam distillation. In this spectrum, X-axis represents time (min) and Y-axis represents percent(%).

According to this experimental data, the extracted essential oil comprised the high level of Ascorbic acid 2, 6-dihexadecanoate is comparison with other.

## IV. Conclusion

In this research work, the peel of *Citrus reticulate* Blanco were collected from Pyin Oo Lwin Township in Mandalay Region. According to phytochemical screening which gave .positive tests for alkaloid, flavonoid, glycoside, polyphenol, tannin, reducing sugar and terpene compounds respectively. The antimicrobial activities of various solvent systems were tested by agar well diffusion method on six selected organisms. The ethanol extract of useful plants are activities on all microorganisms. Moreover, ethanol extract of sample gives rise to medium activity on four microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.* The highest activities on *E.coil*. In addition, the essential oil was isolated by steam distillation method. The yield percent of essential oil was found to be 0.4960% based in crude sample. The results obtained in this study showed that possess essential oil in the peel of *Citrus reticulate* Blanco and that their oil compositions were quantitatively different. Ascorbic acid 2, 6-dihexadecanoate was found to be the highest constituent 23.023% in peel extract. It can be used for the purpose of medicinal and beneficial to man-kind.

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