

Investigation on Antimicrobial Activities of Essential Oil and Extract from Fresh Peel of *Citrus hystrix* DC. (shauk-nu)

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Abstract

Citrus hystrix DC. locally known as shauk-nu belonging to Rutaceae family has been chosen to investigate some chemical analysis and antimicrobial activity on its fresh fruit peel. Phytochemical investigation reveals the presence of alkaloids, α -amino acids, carbohydrates, flavonoids, glycoside, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. However, cyanogenic glycosides were absent by using the standard methods. In addition, 5.6 % of the essential oil content was extracted from fresh fruit peel of shauk-nu by steam distillation method. The analysis of peel essential oil was assessed based on the chromatographic profile of presence compounds such as 1, 6-Octadiene, α -Myrcene, cis-Linalool oxide, (R) (+)-Citronellal, Terpinen-4-ol, α -Terpineol and Citronellyl formate by Gas Chromatography-Mass Spectrometry (GC-MS). Based on the results of antimicrobial activity, the peel essential oil was able to exhibit the mild antimicrobial activity and also ethyl acetate extract of shauk-nu peel exhibited the strong antimicrobial activity against six species of microorganisms such as *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans* and *E. coli*. The inhibition zone diameter range of peel essential oil and ethyl acetate extract of shauk-nu peel were found to be 15 mm to 20 mm and 46 mm to 60 mm respectively. The ethyl acetate extract was found to possess more potent antimicrobial activity than peel essential oil. Nevertheless, it was investigated that both extract and essential oil were active on all of six species of microorganisms.

Keywords: *Citrus hystrix* DC., Essential oil., GC-MS, Antimicrobial Activity

Introduction

Citrus hystrix DC. is locally known as shauk-nu (Kress *et al.*, 2003). The genus *Citrus* belongs to Rutaceae families. It is also known as kaffir lime, kieffer lime, makrut, or magrood, papeda. It is widely grown worldwide as a backyard shrub. *Citrus hystrix* is a thorny bush, 6 to 35 feet tall, with aromatic and distinctively shaped double leaves. The makrut lime is a rough, bumpy green fruit. The green lime fruit is distinguished by its bumpy exterior and its small size.

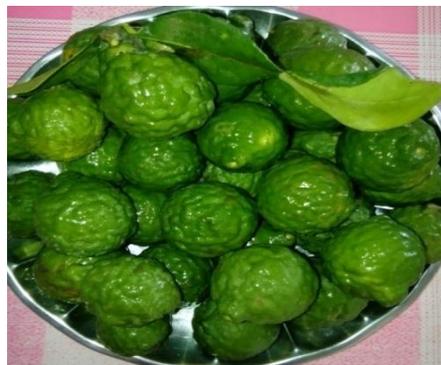


Figure. 1 Photograph of *Citrus hystrix* DC. (shauk-nu fruits)

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The kaffir lime (*Citrus hystrix*) juice and rinds are used in traditional medicine in some Asian countries; the fruit's juice is used in shampoo and is believed to kill head lice Figure 2. The fruit peels essential oil is used in perfumery and it has mainly consisted of monoterpene hydrocarbons (Hongratanaworakit *et al.*, 2007). The compound responsible for the characteristic aroma was identified as citronellal (Ikawati *et al.*, 2017). The fruit peels extract and essential oil has been reported to exhibit strongest antioxidant activity, antibacterial activity (Srisukh *et al.*, 2012), cockroach repellent (Muhammad *et al.*, 2013), hair treatment soap perfumery and cosmetics (Manosroi *et al.*, 1999).



Figure. 2 Photograph showing shauk-nu fruit extract shampoo

Materials and Methods

Sample Collection, Identification and Preparation

The fresh fruits of shauk-nu (*Citrus hystrix* DC.) were collected from Hpa-an Township, Kayin State and identified at the Department of Botany, Hpa-an University. After being washed with water, it were peeled off sharply to collect the fresh fruit peels which further cut into small pieces and tested for the following procedures.

Preliminary Phytochemical Investigation

The preliminary phytochemical investigations were performed in order to determine the different types of chemical constituents present in the fresh peels of shauk-nu according to the standard methods as the procedure describes (Marini-Bettolo *et al.*, 1981).

Extraction of Essential Oil

The fresh peels of shauk-nu (300 g) and distilled water (500 mL) were placed in a (1 L) round bottom flask. The flask was fitted to steam distillation set which was joined to water condenser. When the flask was heated, the condensed oil and water coming out from condenser were collected in the receiver flask. The crude essential oil was obtained. The crude essential oil was extracted with n-hexane in a separating funnel. The n-hexane extract was vaporized by using magnetic stirrer with hot plate. The remaining mixture of water and essential oil was dried over anhydrous sodium sulphate, filtered and to get the purified essential oil which was then weighed and kept in air tight bottle for further studies.

GC-MS Analysis of Essential Oil

The chemical constituents of essential oils from fresh fruit peel of shauk-nu were detected at the Department of Chemistry, West Yangon University Research Center applying GC-MS (Trace 1300ISQ QD).

Preparation of Ethyl Acetate Extract

The fresh peels of shauk-nu (100 g) were extracted with ethyl acetate (300 mL) for 12 hours by using successive solvent extraction method and filtered. The filtrate was evaporated under reduced pressure by means of a rotatory evaporator to give the respective ethyl acetate extract.

Screening on Antimicrobial Activity of Essential Oils and Ethyl Acetate Extract of Fresh Peels of Shauk-nu (*Citrus hystrix* DC.)

In vitro antimicrobial activity on essential oil and ethyl acetate extract of fresh peels of shauk-nu against *Bacillus subtilis* (N.C.T.C-8236), *Staphylococcus aureus* (N.C.P.C-6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus* (N.C.I.B-8982), *Candida albicans* and *Escherichia coli* (N.C.I.B-8134) were screened by Agar Well Diffusion method at Pharmaceutical Research Department (PRD), Yangon.

Results and Discussion

Preliminary Phytochemical Investigations

From the experiments, alkaloids, α -amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, steroids, tannins and terpenoids were observed and also the absent of cyanogenic glycosides in fresh peels of shauk-nu.

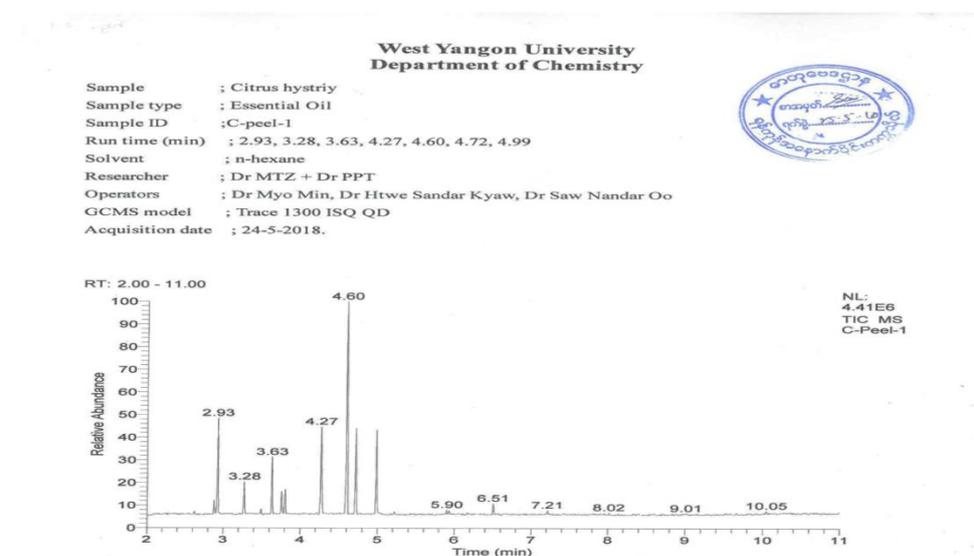
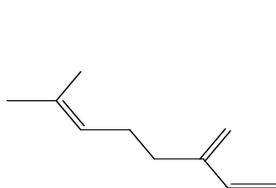


Figure. 3 GC-MS spectrum of essential oil from shauk-nu peel

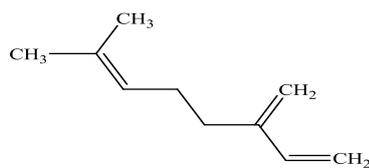


1,6-Octadiene

MW 136

Formula $C_{10}H_{16}$

Run time (min) 2.92

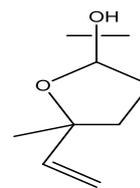


α -Myrcene

MW 136

Formula $C_{10}H_{16}$

Run time (min) 3.28



Cis-linalool oxide

MW 170

Formula $C_{10}H_{18}O_2$

Run time (min) 3.63

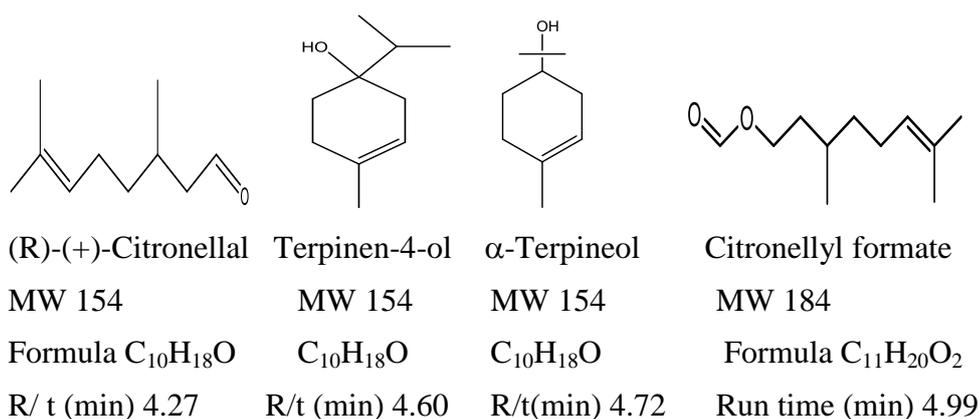


Figure. 4 Structure of some chemical constituents isolated from shauk-nu peel essential oil (*Citrus hystrix* DC.)

Investigation on GC-MS Spectrum of Essential Oil

The essential oil (5.6 %) was yielded from fresh fruit peels of shauk-nu extracted by the steam distillation method. Based on the test results of GC-MS (Gas Chromatography-Mass Spectrometry), seven constituents were identified. In detail, the essential oil from shauk-nu peel showed the highest percentage in the compound terpinen-4-ol (100%), α -myrcene (50%), citronellal (50%), 1,6-octadiene, *cis*-linalool oxide, α -Terpineol and citronellyl formate respectively.

Screening on Antimicrobial Activity of *Citrus hystrix* DC.

The comparison of inhibition zone diameters for essential oil and ethyl acetate extract of fresh fruit peels of shauk-nu were illustrated in Figure 5, 6, 7 and Table 1.

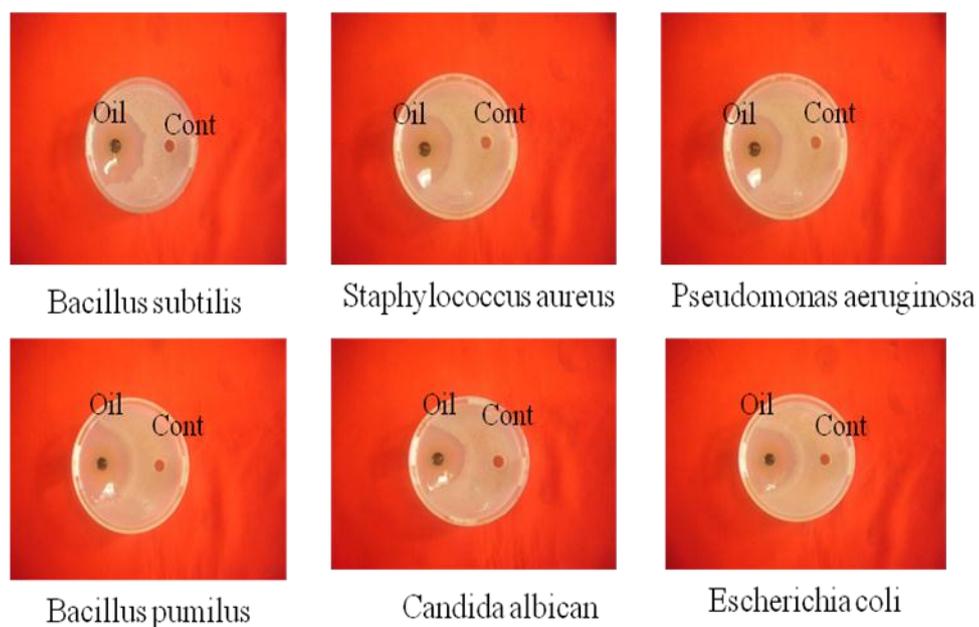


Figure. 5 Antimicrobial activity on essential oil of shauk-nu against six microorganisms by agar well diffusion method

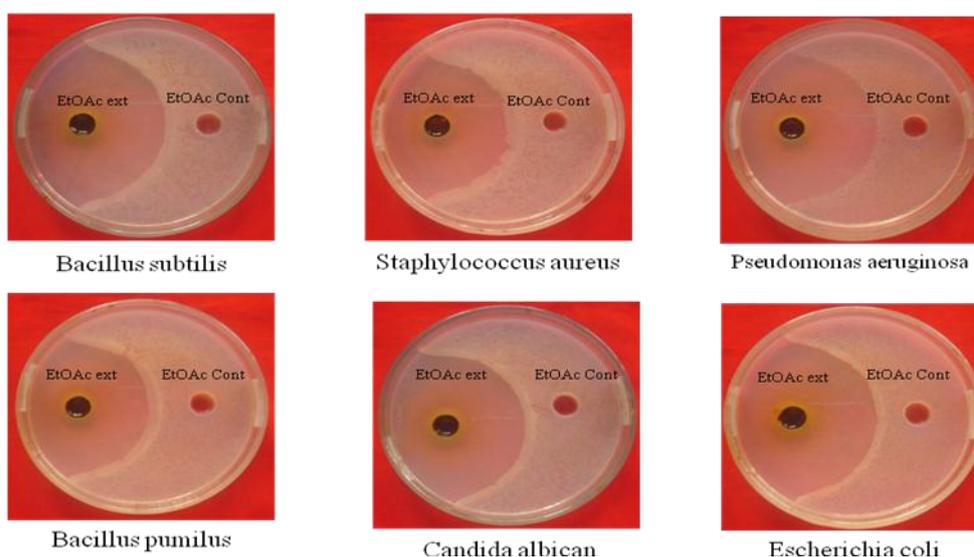


Figure. 6 Antimicrobial activity on EtOAc extract of shauk-nu against six microorganisms by agar well diffusion method

Table. 1 Antimicrobial Screening of Essential oil and EtOAc Extract from Shauk-nu Peel by Agar Well Diffusion Method

Samples	Organisms and inhibition zone diameters (mm)					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. pumilus</i>	<i>C. albicans</i>	<i>E. coli</i>
Essential Oil	18 mm (++)	18 mm (++)	20 mm (++)	15 mm (++)	15 mm (++)	15 mm (++)
EtOAc extract	46 mm (+++)	55 mm (+++)	60 mm (+++)	50 mm (+++)	53 mm (+++)	48 mm (+++)

Agar Well- 10mm

Organisms

10mm ~14m (+)

(1) *Bacillus subtilis* (N.C.T.C-8236)

15mm ~19mm (++)

(2) *Staphylococcus aureus* (N.C.P.C-6371)

20 above (+++)

(3) *Pseudomonas aeruginosa* (6749)

(4) *Bacillus pumilus* (N.C.I.B-8982)

(5) *Candida albicans*

(6) *Escherichia coli* (N.C.I.B-8134)

In accord with the table 1 and figure 5, 6, 7, it was investigated that the essential oil of shauk-nu peels was able to exhibit the mild antimicrobial activity and ethyl acetate extract was exhibited to possess the strong antimicrobial activity against six species of microorganisms such as *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans* and *E. coli*. The inhibition zone diameter range of essential oil between 15 mm to 20 mm and ethyl acetate extract between 46 mm to 60 mm were investigated. The ethyl acetate extract was found to possess more potent antimicrobial activity than essential oil of shauk-nu peels. Nevertheless, it was investigated that both extract and essential oil were active on all of six microorganisms.

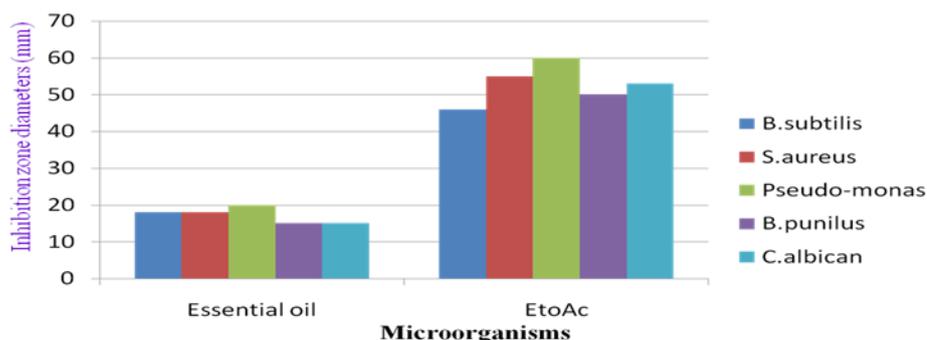


Figure. 7 A bar graph of inhibition zone diameters of essential oil and EtOAc extract from fresh fruit peels of shauk-nu

Conclusion

From the finding, the major compounds found from the essential oil of shauk-nu peel are 1, 6-Octadiene, α -Myrcene, cis-Linalool oxide, (R) (+)-Citronellal, Terpinen-4-ol, α -Terpineol and Citronellyl formate by Gas Chromatography-Mass Spectrometry (GC-MS). These compounds provide distinctive aroma. Based on the results of antimicrobial activity, the inhibition zone diameter range of peel essential oil and ethyl acetate extract of shauk-nu peel were found to be 15 mm to 20 mm and 46 mm to 60 mm respectively. It was investigated that both extract and essential oil were active on all of the six species of microorganisms.

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