Study on Elemental Contents, Banda Seed Oil and Antimicrobial Activity of *Terminalia catappa* L. (Banda) Seed in Hinthada Township

Ei Ei Khaing
Hinthada University
eieikhaingchem@gmail.com

Zarchi Oo
Hinthada University
zarchioo198212@gmail.com

Ohn Mar Tin
Hinthada University
ohnmartin209@gmail.com

Abstract

Banda seed collected from Hinthada Township revealed relatively the highest content of K and P on the report of Energy Dispersive X-ray Fluorescence spectrum. Banda seed oil (15.09 g, 30.18 %) was extracted from seed sample by hydro-distillation method. Then Banda seed oil was trans-esterified by base-catalyzed process and assigned by fourier transform infrared(FT IR) and gas chromatography mass spectrometry (GC-MS). It was found that totally ten compounds with the mixture of methyl ester and terpenes contained in Banda seed oil. By direct extraction method, four crude extracts of Terminalia catappa seed were prepared with various solvents: petroleum ether, ethyl acetate, 96 % ethanol and water. Antimicrobial activity of four crude extracts and Banda seed oil were investigated against ten microorganisms by paper disc diffusion method. It was found that all tested seed samples inhibited ten tested microorganisms with the range between 9~27 mm.

Keywords: Terminalia catappa, seed, elements, Banda seed oil, antimicrobial activity

1. Introduction

Terminalia catappa with the family combretaceae [1], namely Indian almond in English, Banda in Myanmar is well known for shaded and ornamental purposes besides its seed within the fruit can be edible when fully ripe. The deciduous tree is large in size [2], 30-35 m in height shown in Figure 1 (a). The fruit shown in Fig. 1(b) is drupe (5-7 cm) long and (3-5.5 cm) broad, red when ripe, containing a single seed. The taste of the *T. catappa* seed shown in Fig. 1(c) is sweetish [3]. It is widely distributed in Myanmar. The seed is commercially used in milk shake, chocolate, cake, ice-cream, biscuit, inks, shampoos, moisturizer and hair oils [4]. The present study focused on the investigation of elemental contents, Banda seed oil extracted from *Terminalia catappa* (Banda) seed and screening of antimicrobial activity.

Figure 1. (a) *T. catappa* plant (b) *T. catappa* fruit (c) *T. catappa* seed

2. Materials and Methods

2.1. Plant Materials

The sample of *T. catappa* seed was collected from Hinthada Township, Ayeyarwady Region in January, 2019. The plant was identified by plant taxonomists at Department of Botany, Hinthada University. The seed sample was dried and powdered for experiments.

2.2. EDXRF Analysis of *T. catappa* Seed

Elemental analysis of *T. catappa* seed was analyzed by EDXRF spectrometer (Shimadzu's EDX-7000/8000) at Monywa University.

2.3. Extraction of Banda Seed Oil by Hydro-distillation Method

The fresh *T. catappa* (Banda) seed (50 g) was distilled with deionized water (400 mL) in Clevenger apparatus for 48 h. After hydro-distillation, floated Banda seed oil was collected with syringe and yield % was calculated.

2.4. Trans-esterification of Banda Seed Oil by Base-catalyzed Process

Banda seed oil was trans-esterified by base-catalyzed process. Methanol (10 mL) and NaOH (0.2 g) were put into conical flask and placed on a magnetic stirrer with hot plate at 60 °C and (10) rpm rotational system to yield sodium methoxide. It was added to the hot seed oil and stirred at 60 °C for 1 h. Then, the mixture was allowed to cool and it was falling into two layers in separating funnel. Upper layer was methylated ester (trans-ester) of Banda seed oil.

2.5. Characterization of Banda Seed Oil by Modern Spectrometric Method

Banda seed oil was characterized by FT IR at Pathein University and GC-MS at National Analytical Laboratory (NAL), Yangon.

2.6. Antimicrobial Activity of Crude Extracts and Banda Seed Oil by Paper Disc Diffusion Method

The powdered sample of *T. catappa* seed (50 g) was extracted with (500 mL) petroleum ether (PE), ethyl acetate (EtOAc), 96 % ethanol (EtOH) and water in separate conical flasks, respectively. The filtrates were evaporated and weighed. The antimicrobial activity of four crude extracts: PE, EtOAc, EtOH and water extracts besides Banda seed oil were determined against ten microorganisms by employing paper disc diffusion method at Department of Chemistry, Hinthada University. This method is simplicity, speed of performance, economy and reproducibility [5]. The ten tested microorganisms from the source of NITE & Kyowa Hakko Co. Ltd. were cultured at Biotechnology and Development Center of Pathein University. The test organisms were incubated in test broth medium and tested in assay medium prepared by glucose (1.0 g), polypeptone (0.2 g), agar (1.6 g) and distilled water (100 mL). After overnight incubation at 27 ˚C, the zones of inhibition diameter including 8 mm discs were measured with digital calipers in millimeter. If clear zones surrounding the paper discs were found to be indicated that it would be the presence of bioactive metabolites which inhibit the growth of test organisms. In the study, blank petri-dish was used as negative control and incubated petri-dish was positive control, and the standard was chloramphenicol.

3. Results and Discussion

3.1. EDXRF Analysis of *T. catappa* Seed

EDXRF spectrum of Banda seed shown in Figure 2, had relatively the highest content of potassium (0.535 %) and phosphorous (0.464 %) whereas minor component of calcium (0.180 %), sulphur (0.124 %), iron (0.008 %), zinc (0.003 %), hafnium (0.003 %), copper (0.002 %), manganese (0.001 %), rubidium (0.001 %) and nickel (0.001 %) were observed. Among these elements potassium peak was also the most prominent and so it showed potassium was the highest content of this plant.

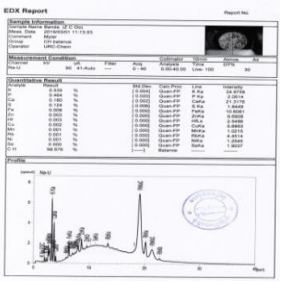

Figure 2. EDXRF spectrum of *T. catappa* seed

3.2. Extraction and Trans-esterification of Banda Seed Oil from *T. catappa* Seed

By hydro-distillation method, (15.09 g, 30.18 %) pale yellow Banda seed oil was yielded from *T. catappa* seed. Then, (10.06 g, 20.12 %) trans-esterified seed oil was obtained by base-catalyzed process and identified by GC MS.

3.3. Characterization of Banda Seed Oil

According to FT IR spectrum (Figure 3) of Banda seed oil, absorption bands at 2924 cm^{-1} and 2854 cm^{-1} showed C-H stretching of aliphatic hydrocarbon due to symmetric and asymmetric of alkane group. The stretching vibration of C=O for ester was exhibited at 1745 cm^{-1}. Bending vibration of C-H of alkane group showed at 1460 cm^{-1}. By GC-MS analysis, Banda seed oil could be deduced as follow with their respective retention time (RT). Banda seed oil was found to contain totally mixture of ten compounds such as 3-Carene (RT: 3.448 min), D- Limonene (RT: 4.008 min), ß-Bisabolene (RT: 10.122 min), Methyl tetradecanoate (RT: 12.542 min), Pentadecanoic acid, Methyl ester (RT: 13.654 min), Hexadecanoic acid, Methyl ester (RT: 15.411 min), 9- Octadecenoic acid, Methyl ester, (E)- (RT: 18.820 min), Methyl stearate (RT: 19.112 min), Eicosanoic acid, Methyl ester (RT: 22.422 min) and Docosanoic acid, Methyl ester (RT: 25.762 min) expressed in Figures 4(a), (b), (c), (d), (e), (f), (g), (h), (i), (j). From the results, Banda seed oil was assigned as the mixture of methyl esters and terpenes.

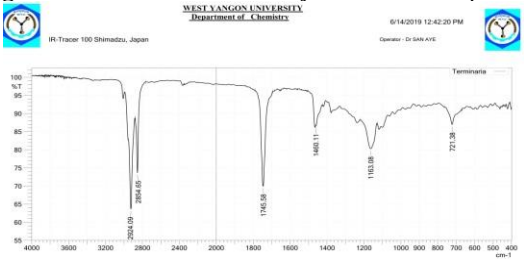

Figure 3. FT IR spectrum of Banda seed oil

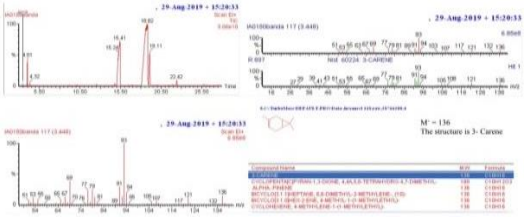

Figure 4(a). GC-MS spectrum of compound 1 from Banda seed oil

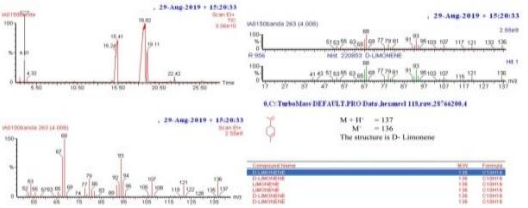

Figure 4(b). GC-MS spectrum of compound 2 from Banda seed oil

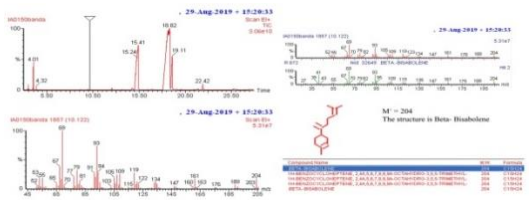

Figure 4(c). GC-MS spectrum of compound 3 from Banda seed oil

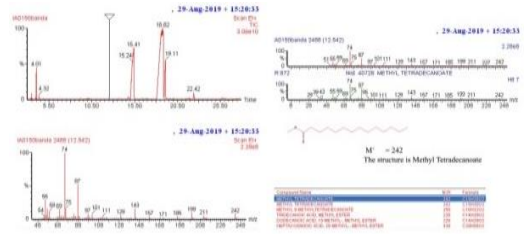

Figure 4(d). GC-MS spectrum of compound 4 from Banda seed oil

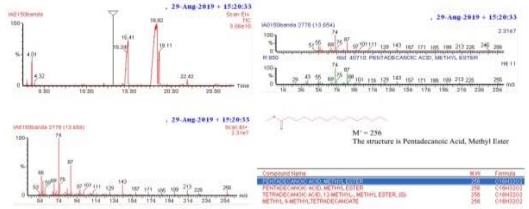

Figure 4(e). GC-MS spectrum of compound 5 from Banda seed oil

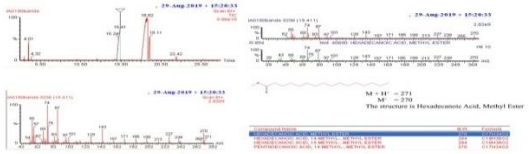

Figure 4(f). GC-MS spectrum of compound 6 from Banda seed oil

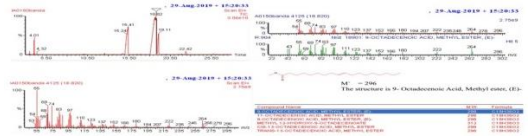

Figure 4(g). GC-MS spectrum of compound 7 from Banda seed oil

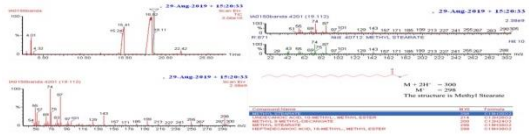

Figure 4(h). GC-MS spectrum of compound 8 from Banda seed oil

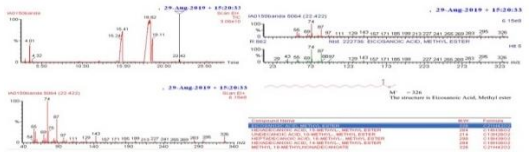

Figure 4(i). GC-MS spectrum of compound 9 from Banda seed oil

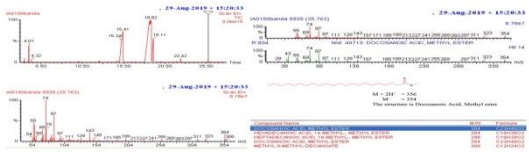

Figure 4(j). GC-MS spectrum of compound 10 from Banda seed oil

3.4. Antimicrobial Activity of Four Crude Extracts and Banda Seed Oil

The dried seed powder was extracted by various solvents and the yield % of petroleum ether extract (32.86 %), ethyl acetate extract (44.82 %), 96 % ethanol extract (10.30 %) and water extract (12.46 %) were obtained. In this study, antimicrobial activity of four crude extracts and Banda seed oil obtained from *T. catappa* seed were investigated on ten species of microorganisms: *Agrobacterium tumefaciens* NITE09678, *Aspergillus parasiticus* IFO5123, *Bacillus subtilis* IFO90571, *Candida albicans* NITE09542, *Micrococcus luteus* NITE83297, *Salmonella typhi* AHU9743, *Escherichia coli* AHU5436, *Saccharomyces cerevisae* NITE52847, *Pseudomonas fluorescens* IFO94307 and *Staphylococcus aureus* AHU8465 by paper disc diffusion method. It is based on the zone diameter in (mm) of paper disc. The larger the zone diameter is the more activity on the tested microorganisms. The microorganisms species used in the test were isolated from plant disease, diarrhea, food poisoning, GI tract infection and abscess in (skin, mouth and nose). From the results given in Figure 5 and Table 1, it was observed that all four crude extracts and Banda seed oil obtained from *T. catappa* seed exhibited inhibition zone diameters between 9~27 mm against all tested microorganisms. Out of the all tested plant samples, PE, EtOAc and 96 % EtOH extracts of *T. catappa* seed showed the most significant antimicrobial activity against ten tested microorganisms ranging the inhibition zone diameter 9~27 mm. Banda seed oil showed medium activity (9~19 mm) in comparison with standard(Sd), chloramphenicol (18~34 mm). In addition, 96 % EtOH extract (27 mm) showed the highest activity against *Candida albicans*. Thus, it may be effectively used as active remedy for the treatment of their related diseases, fungal infection.

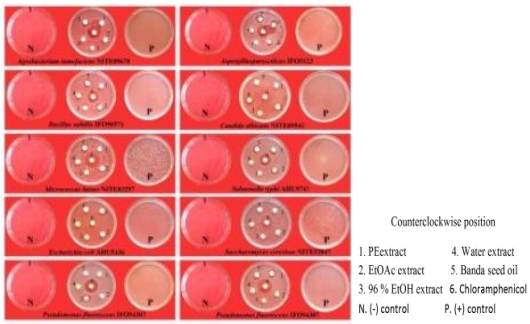

Figure 5. Inhibition zones diameters of test samples against ten microorganisms

Table 1. Inhibition zone diameters of test samples from *T. catappa* seed

Micro-organisms	Inhibition Zone Diameters (mm) of Test Samples					
	1	2	3	4	5	Sd
A. tumefaciens NITE09678	10	9	11	10	15	26
A.parasciticus IFO5123	18	15	10	12	19	26
B.subtilis IFO90571	12	17	12	14	16	24
C.albicans NITE09542	12	15	27	16	9	34
M.luteus NITE83297	11	10	10	10	13	30
S.typhi AHU9743	16	16	11	14	16	26
E.coli AHU5436	10	10	10	10	10	18
S.cerevisae NITE52847	10	12	16	9	11	26
P.fluorescens IFO94307	10	10	9	9	9	28
S.*aureus* AHU8465	16	14	12	11	16	30

Paper disc diameter = 8 mm

Test Samples

1 = PE extract 2 = EtOAc extract
3 = EtOH extract 4 = Water extract
5= Banda seed oil Sd. = Chloramphenicol

4. Conclusion

From Energy Dispersive X-ray florescence (EDXRF) spectrum, the sample of *T. catappa* (Banda) seed had relatively the highest content of potassium and phosphorous whereas minor component of calcium, sulphur, iron, zinc, hafnium, copper, manganese, rubidium and nickel were observed. Banda seed oil (30.18 %) was obtained from the *T. catappa* seed and tested antimicrobial activity. According to FT IR and GC-MS analysis, it was found that totally ten mixture of methyl esters and terpenes compounds contained in Banda seed oil. Screening of antimicrobial activity of PE extract, EtOAc extract, 96 % EtOH extract, water extract and Banda seed oil from seed sample was also investigated by paper disc diffusion method against ten tested microorganisms. It was observed that all extracts and Banda seed oil from *T. catappa* seed exhibited inhibition zone diameters between 9-27 mm against all tested microorganisms. Based on the findings of present study it is concluded that *T. catappa* seed possess nutrient elements as well as significant antimicrobial activity. Thus, Banda seed oil may be used as main materials for the traditional medicine formulation for the treatment against typhoid, food poisoning, diarrhea, gastrointestinal tract infection and abscess in (skin, mouth and nose). The result of this study is an encouragement for further research that it will be the physicochemical analyses for unsaturated fatty acid composition of the essential oil.

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References

[1]R. Sen, A. C. Halder and D.C. Pal, "Botany and Ethnobotany of Indian Almond", *J. of Eco. and Taxonomic Botany*, vol. 10, 1987, pp. 239-240.

[2] Chitmanat, C.K., Tongdonmuan, P. and Khanom, P., *Antiparasitic, and Antifungal Activities Derived From a T. catappa Solution Against Some Pathogens*, Acta Horticulturae, 2005, p. 197.

[3] E.O. Omeje, G.B. Okide, C.O. Esimone and U. Ajali, "Kinetics of Autoxidation of an Oil Extract from *Terminalia catapp*", *Indian Journal of Pharmaceutical Science*, vol. 70, 2008, pp. 260-262.

[4] C.C. Lin, Y.F. Hsu, and T.C. Lin, "Antioxidant and Free Radical Scavenging Effects of the Tannins of *T. catappa*", *Anticancer Res*., vol.21, 2001, p.224.

[5] Ando, K., *Sampling, Isolation, Cultivation and Preservation of Microorganisms*, *Biological Resource Center*, National Institute of Technology and Evaluation (NITE), Japan, 2014.