Antimicrobial activity and bioactive evaluation of *Plectranthus amboinicus* essential oil

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Abstract

Plectranthus amboinicus or locally known as *bangun-bangun*, is an indigenous vegetable which can be freshly eaten. However, the plant is unpopular among local people and being neglected. It has been reported to be traditionally used for medicine to cure common illnesses such as cough, stomachache, headache and skin infection. Based on the potential, a study was conducted to bioprospect the antimicrobial activity and phytochemical compounds of the essential oil. *Plectranthus amboinicus* essential oil was tested against nine (9) microorganisms i.e. *Staphylococus epidermis*, *S. aureus*, *Serratia marcencens*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, methicillin-resistant S. *aureus* (MRSA), *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Candida tropicalis*. It was found that all microorganisms were susceptible to *P. amboinicus* essential oil except *P. aeruginosa.* The oil showed the highest inhibition zones against *E. coli*, *S. aureus* and *C. tropicalis* as compared to commercial antibiotic, streptomycin and nystatin. The minimum inhibitory concentration (MIC) of the oil for *E.coli* and *C. tropicalis* were 780 ug/ml and 62.5 x 10^2 ug/ml, respectively. Phytochemical analysis using gas chromatography-mass spectrophotometer (GC-MS) showed that camphor, carvacrol and 3-carene were the major components that may contribute to the antimicrobial activity.

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Introduction

Plectranthus is a large genus, with more than 300 species from the family of Lamiaceae. It has a rich diversity of ethnobotanical and medicinal uses. Several species of the genus possess interesting medicinal properties such as the extract of *P. barbatus* is used for the treatment of stomachache and as a pugitive, nausea and gastritis and intestinal spasms in Brazil. *Plectranthus caninus*, *P. laxiflorus* and *P. barbatus* are used in the treatment of teeth and gum disorders. It is also reported that *P. amboinicus* and *P. barbatus* are used to treat a wide range of diseases such as for the treatment of digestive system, skin conditions and allergies, infections and fever, genito-urinary conditions, pain, respiratory conditions and muscular-skeletal conditions (Lukhoba 2006).

Plectranthus amboinicus or locally known as *bangun-bangun, bebangun, sedingin or hati-hati hijau*, is an indigenous vegetable which can be freshly eaten. The leaf of *P. amboinicus* has many medicinal uses, especially for the treatment of common illnesses such as of cough, stomachache, headache, skin infection, asthma and urinary conditions (Siti Fuziah 2012). The plant extracts especially the volatile essential oils from the leaves have been reported to possess antioxidant, antibacterial, antimicrobial, anti-inflammatory and fungitoxic activities (Lukhoba 2006; Gurgel et al 2009; Murthy 2009; Bhatt & Negi 2012) but due to the geographical region and variety, the activity and composition of essential oils may be vary (Murthy 2009). Therefore, it is important to access the local *P. amboinicus* to screen the potential biological activity especially antimicrobial properties and volatiles components of the plant.

Materials and Methods

1. *Microorganisms*

The microbial strains tested were *Staphylococus aureus*, *Staphylococus epidermidis*, methicillin-resistant *Staphylococus aureus (MRSA), Serratia marcescens, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Bacillus subtilis, Candida albicans* and *Candida tropicalis.*

2. *Raw materials*

2.1 *Plant material*

The whole plants of *P. amboinicus* were collected from nursery of Strategic Resource Centre, MARDI Headquarters, Serdang.

2.2 *Bangun-bangun* essential oil (BEO)

The essential oil (volatile fraction) was isolated by using hydro distillation technique using a Clevenger apparatus. The essential oil was stored in clean clear glass vials after removing water with anhydrous sodium sulphate.

2.3. *Antibacterial and antifungal activity assay*

The antibacterial activity of the BEO was studied against various bacteria: *Staphyloccous aureus, Staphylococcus epidermidis*, methicillin-resistant *Staphyloccous aureus (MRSA), Serratia marcescens, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* and *Bacillus subtilis.* The fungal used in this study were *Candida albicans* and *Candida tropicalis.* The test was carried out by placing 6mm diameter of paper disc containing antibiotic onto a microbial inoculated plate. The microbial cultures were standardized to 0.5 McFarland standard turbidity, which was equivalent to 10^8 cfu/ml. Nystatin was used as positive control for fungi and streptomycin, was used as positive control for gram-negative and gram-positive bacteria. Bacteria were incubated at 30-37°C for 16-24 hours while fungi were incubated for 24-48 hours or until sufficient growth has occurred. The inhibition zone was measured after incubation hour. Sterile distilled water was used as a negative standard.

3. *GC-MS analysis*

The BEO was subjected to GC-MS analysis, using a Shimadzu GC-MS QP2010 with DB-5 capillary column (30 m × 0.25 mm I.D × 0.25 μm film thickness). 1 μl of BEO was injected in to the column at the flow rate 1 ml/minute. The injector was operated at 250°C and the oven temperature was programmed as follows: 40°C for 3 minutes, then gradually increased to 150°C for 4 minutes, and then held at 280°C for 10 minutes. The identification of components was based on comparison of mass spectra with those of Wiley and NIST library.

Results and Discussion

Essential oil from *P. amboinicus* was obtained by using modified Clevenger apparatus and hydro distillation technique. The yield of oil was 1ml / 570g of the fresh leaves. The technique used in the oil extraction was modernized separation process for temperature or heat sensitive materials, which are insoluble in water like oils, resins and hydrocarbons and etc.

Essential oil of *Plectranthus amboinicus* (BEO) was tested against bacterial and fungal pathogens. Eight (8) species of gram-positive and gram-negative bacterial pathogens, and 2 species of fungal pathogens were screened for antimicrobial activity.

Antibacterial activity

The significant results of the antibacterial activities have been clearly presented in Table 1 and Figure 1. The BEO was effective against both gram-positive bacteria and gram-negative bacteria. Out of eight bacteria, *E. coli* was the most sensitive organism to BEO with the highest mean zone of inhibition (22.0 ± 0.58mm), followed by *S. aureus* (17.0 ± 0.00mm), which is less equal to the standard antibiotic disc, Streptomycin. BEO also showed moderate activity against *S. epidermis*, *S. marcencens*, *P. vulgaris*, MRSA and *B. subtilis*, and no inhibition against *P. aeruginosa*. BEO had high antibacterial activity against *S. aureus* (17mm), which is higher than that obtained for standard antibiotic, Streptomycin 12mm.

Our findings are in accordance with previous findings, which reported that *P. amboinicus* essential oil has inhibitory effect against *E. coli* and *S. aureus* (Manjamalai 2012). Manjamalai (2012) reported that the essential oil is susceptible to *P. aeruginosa*, but our finding show the other way around. Table 1 also shows the minimum inhibitory concentration (MIC) of the essential oil against the bacterial and fungal pathogens. MIC is the lowest concentration inhibiting visible growth of test organisms. *Escherichia coli* had the lowest MIC value (780 ug/ml).

Table 1. Inhibition zone and minimum inhibitory concentration of BEO against selected bacterial and fungal pathogens

Microorganisms	Inhibition zone[a] (mm)		Minimum inhibitory concentration, MIC (ug/ml)	
	BEO	Commercial antibiotics	BEO	Commercial antibiotics
Bacillus subtilis	12.0 ± 0.00	34	nd	nd
Candida albicans	20.0 ± 0.58	23	12.5×10^3	30.5
Candida tropicalis	17.0 ± 1.73	20	62.5×10^2	30.5
Escherichia coli	22.0 ± 0.58	23	78×10^1	97.7
Methicillin-resistant *S. aureus*	12.0 ± 0.00	23	62.5×10^2	30.5
Proteus vulgaris	14.7 ± 0.33	28	62.5×10^2	97.7
Pseudomonas aeruginosa	-	12	nd	nd
Serratia marcencens	10.0 ± 0.00	24	nd	nd
Staphylococcus aureus	17.0 ± 0.00	12	12.5×10^3	25×10^3
Staphylococcus epidermidis	11.6 ± 0.33	26	25×10^3	24.4

*, mean of triplicate ± standard error of mean (SEM);
-, no inhibition;
nd, not determined.

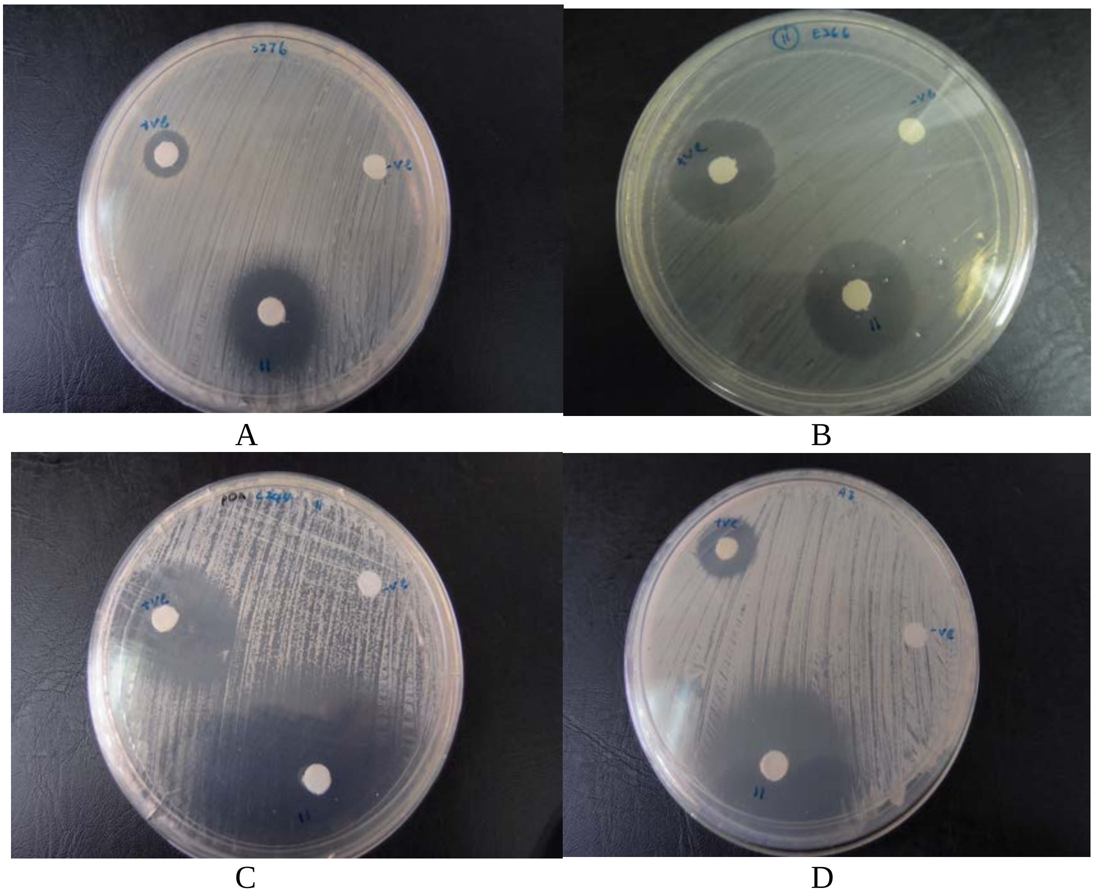

Figure 1. Inhibition zones of BEO against *S. aureus* (A), *E. coli* (B), *C. albicans* (C) and *C. tropicalis* (D). Each plate divided into three compartments; positive standard, negative standard and BEO.

Antifungal activity

Antifungal properties were screened onto 2 species of fungal pathogens, *Candida albicans* and *Candida tropicalis*. The result of antifungal activities of BEO was presented in Table 1 and Figure 1. Our finding showed that BEO has promising antifungal activities against *Candida* spp. The results shows that *C. albican* and *C. tropicalis* are susceptible to BEO with mean zone of inhibition of BEO against *C. albicans* were recorded at 20.0 ± 0.58mm and *C. tropicalis*, 17.0 ± 1.73mm, which is more or less equal to positive control, nystatin. The MIC value value showed that the growth of *C. tropicalis* could be inhibited as low as 62.5 x 10^2 ug/ml. The results indicate that the BEO is having a significant antifungal activity.

Bioactive evaluation

Bioactive evaluation of BEO is conducted by using GC-MS to obtain the quantitative analysis of the essential oil. Table 2 shows the results of GC-MS analysis with 95% equal comparison with the Wiley and NIST library. There are more than 19 compounds were detected presence in BEO as presented in Figure 2. Major constituents with peak number 5 (3-carene, 20.8%), 6 (alpha-terpinene, 6.04%), 7 (o-cymene, 5.06%), 9 (gamma-terpinene, 8.94%), 10 (camphor, 17.96%) and 12 (carvacrol, 19.3%) were the most abundance monoterpenoid compounds. The major compounds are 3-carene, carvacrol and camphor accounting for 58.03% of the total oil compositions. 3-carene has been identified the most abundant component in the BEO and has been proved to produce sensitizing potential and allergic effect, but it also reported that no sensitization reaction was produced in concentration of 10% in petrolatum (Opdyke 1973).

Table 2. GC-MS report of *P. amboinicus* essential oil

Peak No	Retention time	Area	Area %	Name of compound
1	9.163	193277	0.74	
2	9.932	209205	0.8	
3	11.479	274005	1.05	
4	11.727	131601	0.5	
5	**12.599**	**5412653**	**20.78**	**3-carene**
6	**13.065**	**1576689**	**6.04**	**alpha-terpinene**
7	**13.535**	**1319160**	**5.06**	**o-cymene**
8	13.666	280730	1.08	
9	**15.069**	**2334600**	**8.94**	**gamma-terpinene**
10	**19.357**	**4686669**	**17.96**	**camphor**
11	22.895	193977	0.74	
12	**25.391**	**5035742**	**19.29**	**carvacrol**
13	27.902	222859	0.85	
14	29.51	760236	2.91	
15	29.857	1098196	4.21	
16	30.779	219387	0.84	
17	31.855	624479	2.39	
18	32.537	280490	1.07	
19	34.272	1806896	0.69	

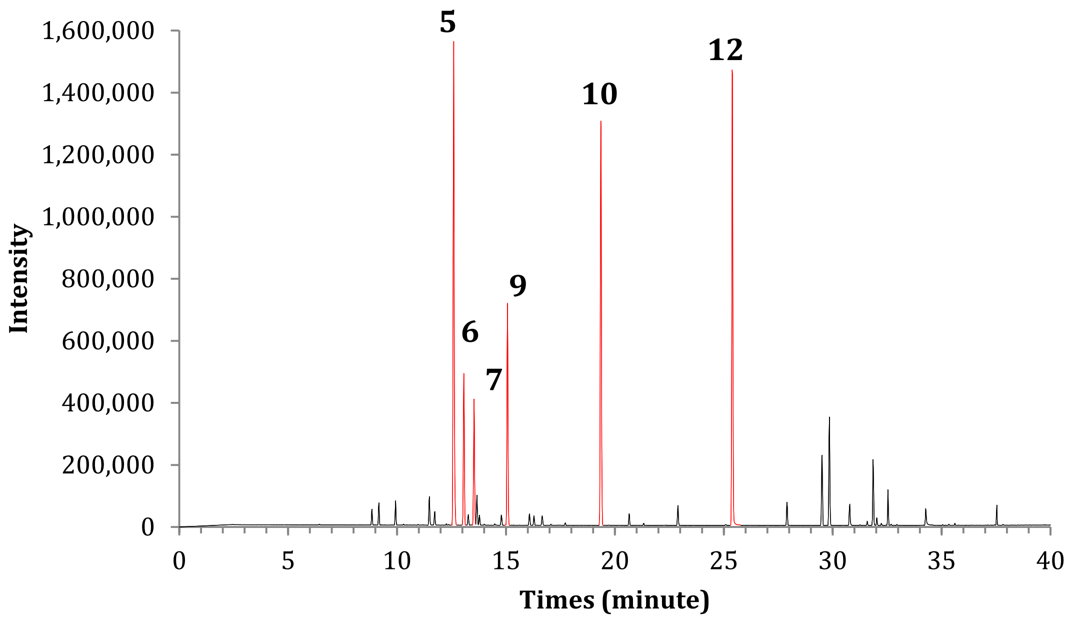

Figure 2. Total ion chromatogram profile of BEO using gas chromatography-mass spectrometer (GC-MS).

Based on this study, carvacrol showed the lower composition (19.3%) as compared to study by Murthy et al (2009) where carvacrol is major (70%) component. Grace et al (2011) and Selvakumar (2012) reported much lower carvacrol composition (13-14%). The related species of *Plectranthus* synonyms *Coleus amboinicus* has the range of carvacrol component between 58 – 65% (Murthy et al 2009). The difference of the compositions could be due to geographical region, plant variety, age of plant, extraction and drying techniques (Murthy et al 2009). Therefore, the compositions of chemical components vary as the studied plant was planted in Malaysia, while others planted in India and Brazil.

The presence of carvacrol and camphor could contribute to the antimicrobial activity of BEO as plant extract and essential oils has long term being recognized to possess antimicrobial activity (Tajkarimi 2010). The high inhibitory effect of *P. amboinicus* is likely due to its high content of carvacrol and camphor, which efficient antimicrobial known (Shunying et al 2005). In addition, the component in lower composition also could contribute to antimicrobial activity such as alpha-terpinene, gamma-terpinene and o-cymene.

Conclusion

The essential oil of *Plectranthus amboinicus* showed a significant antimicrobial activity. It is strongly suggest that the antimicrobial activity may be because of the presence of major monoterpenoid compounds, carvacrol and camphor. Detailed study on pharmacological activities (such as antiflammatory study and etc.) on this plant is needed for future health care product formulations as Rice at al (2011) has indicated that the economic potential of *Plectranthus* in various sectors.

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References

Bhatt, P. and Negi, P. S. (2012). Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus ambionicus*). *Food and Nutrition Sciences 3:* 146-152

Gurgel, A. P. A. D., da Silva, J. G., Grangiero, A. R. S., Danielli, Oliveira, D. C., Lima, C. M. P., da Silva, A. C. P., Oliveira, R. A. G. and Souza, I. A. (2009). *In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae). *Journal of Ethnopharmacology 125*: 361-363

Lukhoba, C. W., Simmonds, M. S. J. and Paton, A. J. (2006). Plectranthus: A review of ethnobotanical uses. *Journal of Ethnopharmacology 103:* 1-24

Manjamalai, A., Alexander, T. and Berlin Grace, V. M. (2012). Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. *International Journal of Pharmacy and Pharmaceutical Sciences 4 (3):* 205 – 211

Murthy, P. S., Ramalakshmi, K. and Srinivas, P. (2009). Fungitoxic activity if Indian borage (*Plectranthus amboinicus*) volatiles. *Food Chemistry 114:* 1014 – 1018

Opdyke, D. L. J. (1973). Monograph of fragrance raw materials : 3-carene. *Food and Cosmetics Toxicology 11 (6)*: 1053-1054

Rice, L. J., Brits, G. J., Potgieter, G. J. and Van Staden, J. (2011). *Plectranthus* : A plant for the future?. *South African Journal of Botany 77*: 947-959

Roshan, P. Naveen, M. Naheed, W. Nitin, U. and Sudarshan, S. (2010). Phyto-Physicochemical investigation of leaves *Plectranthus amboinicus* (Lour.) Spreng. *Pharmacognosy Journal 2(13)* : 536-542

Selvakumar, P., Edhaya Naveena, B. and D Prakash, S. (2012). Studies on the antidandruff activity on the essential oil of *Coleus amboinicus* and *Eucalyptus globulus*. *Asian Pacific Journal of Tropical Biomedicine* : 715-719

Shunying, Z., Yang, Y., Huaidong, Y., Guolin, Z. 2010. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *Journal of Ethnopharmacology 96* : 151-158.

Siti Fuziah, Y. (2012). *Ulaman di sekeliling kita*. Ar-risalah Product. Selangor

Tajkarimi, M. M., Ibrahim, S.A. and Cliver, D. O. 2010. Antimicrobial herb and spice compounds in food. *Food Control 21* : 1199-1218.