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Qualitative Analysis using GC-MS and Maceration of Antifungal and Antioxidant Potential in an Indigenous Plant from Borneo

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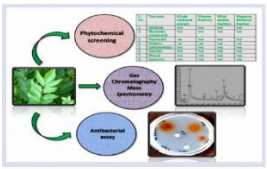
GRAPHICAL ABSTRACT



Identification of bioactive compounds by screening and anlyse using GC-MS

ABSTRACT

The unnamed indigenous plant from Borneo were used in this project is known to have medicinal properties by natives for treatment of certain illness. The compounds were extracted by maceration method and further analysis of bioactive compounds (antioxidant and antifungal) by gas chromatography coupled with mass spectrometry (GC-MS, Agilent 7890 GC with 5977A MSD). The molecular weight and structure of the compounds of test material were ascertained by interpretation of the mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST). In this work, twenty three phytochemicals were analysed by GC-MS technique. Nine bioactive compounds were positively identified which are phytol, phthalic acid, 2-thiobarbituric acid, 2,5-bis(1,1-dimethylethyl)phenol, alpha-cholestan-2-one, 2,5- dihydroxybenzoic acid, 2,4- di-tert-butylphenol, dibutyl phthalate and neophytadiene. Phytol and gentisic acid are known to have antioxidant properties while dibutyl phthalate is known as an antifungal agent. This analysis revealed that contains unnamed plant extract mainly dibutyl phthalate. Therefore, it should be concluded that this plant is a potential candidate as a phytopharmaceutical plant.

Keywords: Borneo, indigenous plant, GC-MS, maceration, antifungal, antioxidant

1. INTRODUCTION

Medicinal plants are used as a medical resource whether in cosmetic and pharmaceutical. The medicinal plant usually known as herbs or plants that have potential to treat diseases. Most plants can be found in tropical forest that are being used to treat diseases. The traditional Chinese medicine is known to be existed since 5000 years ago [1]. Additionally, this traditional medicine continues being developed and modified with technologies which means their medicine have be known due to the effectiveness to treat diseases and known as one of modern medicines.

The parts of medicinal plants that may be used such as seeds, roots, leaves, fruit, barks, flowers or analyze using the whole plant. Flower buds with presence of quercetin, quercitrin and bergenin that play important roles in inflammation and microbial. However, every part has different of active compounds that useful as medicinal agents.

There is much research done by using maceration and mostly this method were effective for extraction of phenolic compound. Some phytochemical like flavonoids was found to be lowest in maceration method. Jovanović et al, 2017 evaluated the extraction efficiency of polyphenols from Serpylli herbal using various extraction processes such as maceration, heat assisted extraction and ultrasonic-assisted extraction which shows the content of total polyphenols in ultrasonic-assisted extraction produced the highest total flavonoids yield and there is no significant difference were found between maceration and heat assisted extraction.

Ethanol is usually use in traditional medicine but in form of natural ethanol and it is environmentally use compare to other organic solvent. Ethanol is also cheap and easy to get in form of wine to make a traditional medicine. Furthermore, ethanol also was used in many studies perform as a good solvent in extraction and less toxic then methanol even based on the polarity show methanol is more polar than ethanol but it is unsuitable for extraction in certain kind of studies as it may lead not better result to form natural product in pharmaceutical field.

Gas-chromatography coupled mass spectrometry (GC-MS) is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds [3]. The qualitative analysis of the extracts from the plant is to identify bioactive compounds with screening from GC-MS result and determine the preliminary and major compound that based on their biology properties. In this study, a qualitative analysis is obtained from extract plant of Ocimum sanctum shows ten compounds were identified in hydroalcoholic extract by GC-MS [4]. There are various of compounds potential as an antifungal and antioxidant properties. The present study was aimed to identify bioactive compounds that present in leaves extract using maceration method with qualitative screening of bioactive compounds and to discover predominance of bioactive that potent of antifungal and antioxidant by GC-MS.

2. EXPERIMENTAL

2.1. Collection of plant material

Plant sample (unnamed plant) was collected from East of Malaysia, Sarawak province. The sample were identified and prepared by Sarawak Biodiversity Centre. There samples are in the form of fresh sample.

2.2 Extraction method

In the method called Folk method, 0.5 g of whole or coarsely powdered crude drug is placed in a container with 50 ml of different type of solvents like ethanol. All samples is allowed to stand at room temperature for 7 days. Then, samples will be filtered using filter paper and concentrate using vacuum rotary evaporator until form viscous paste. Triplicate for this method.

2.3 GC-MS analysis

GC-MS analysis were performed using a Agilent 7890A system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-5MS, fused silica capillary column (30 m, 0.25 mm I , 0.25 um df, composed of 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 1 ul was employed (Splitless) injector temperature 300°C; ion-source temperature 280°C. The oven temperature was programmed from 50°C. Total GC running time was 43.10 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

2.4 Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was ascertained.

3. RESULTS AND DISCUSSION

3.1. Gas chromatography – Mass spectrometry (GC-MS) analysis

The chromatogram generated from GC-MS that showed the presence of bioactive compounds were analyzed by comparing their retention time and identification by their mass spectra. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure 1. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the maceration method of the unnamed plant had shown the presence of lots of bioactive compounds which strength contribute to the medicinal bioactive of that plant. The GC-MS analysis was performed of that unnamed plant was extracted using maceration method and ethanol as a mobile phase. From GC-MS analysis, the retention time is adjusted at 43.10 min and the column semi-polar is used to identify polar compounds of ethanolic extract plant. The polar compounds is expected to stay longer in polar column due to same polarity and the peak at 30 min still considered as peak for bioactive compounds in this study.

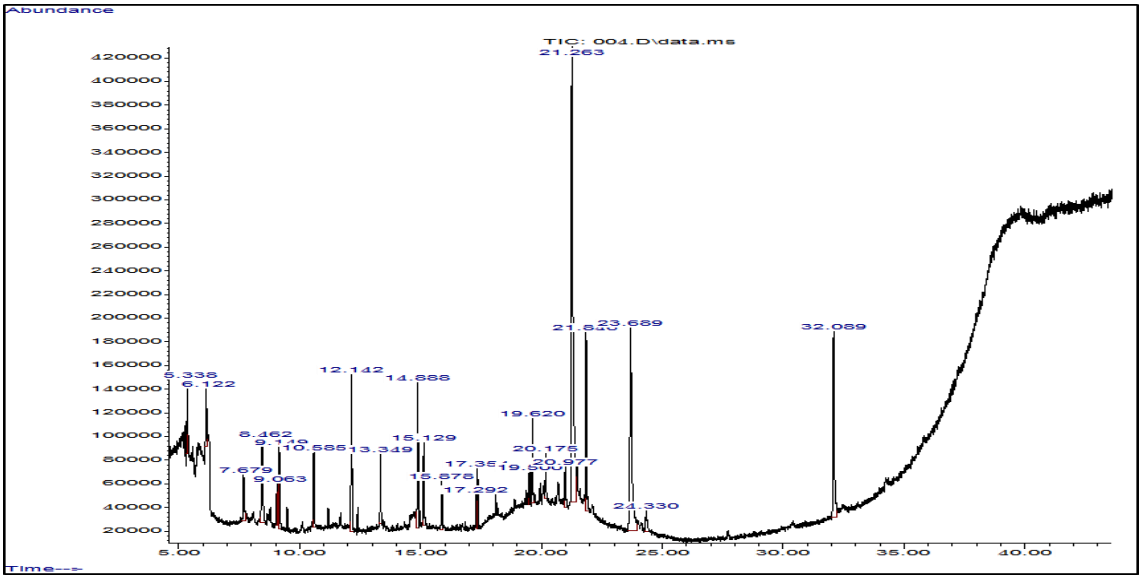


Figure 1. Chromatogram of unnamed plant by GC-MS

3.2. Selected bioactive compounds from extract unnamed plant

The selected bioactive compounds from GC-MS analysis in Table 1. Name of compounds, retention time, peak area and molecular weight were obtained from the NIST library. The highest peak area from this result showed major content in extract plant which is dibutyl phthalate. The selected bioactive compounds of unnamed plant are revealed from GC-MS analysis. These compounds were compared with other studies with the same solvent used in the extraction method which is ethanolic. Such bioactive compounds detected in the GC-MS analysis are Phytol (14.05), Phthalic acid (8.83), 2-Thiobarbituric acid (2.13), 2,5-bis(1,1-dimethylethyl)phenol (6.50), Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro (6.34), 2,5-dihydroxybenzoic acid (1.91), 2,4-Di-tert-butylphenol (2.64), Dibutyl phthalate (28.10), Neophytadiene (2.32) as listed in Table 1.

Table 1. Selected bioactive compounds of unnamed plant

No.	RT	Name of compound	Peak Area %	Molecular formula	MW
1.	23.689	Phytol	14.05	$C_{20}H_{40}O$	296.5310
2.	32.089	Phthalic acid, di(2-propylpentyl)ester	8.83	$C_{24}H_{38}O_4$	390.5561
3.	10.585	2-Thiobarbituric acid, S-trimethylsilyl-, bis(trimethylsilyl) ether	2.13	$C_{13}H_{28}N_2O_2SSi_3$	360.695
4.	15.123	Phenol, 2,5-bis(1,1-dimethylethyl)	6.50	$C_{14}H_{22}O$	206.3239
5.	21.846	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro	6.34	$C_{18}H_{18}N_4O$	306.362
6.	17.354	2,5-dihydroxybenzoic acid (Gentisic acid)	1.91	$C_5H_{11}NO_2$	117.1463
7.	15.129	2,4-Di-tert-butylphenol	2.64	$C_{14}H_{22}O$	206.3239
8.	21.263	Dibutyl phthalate	28.10	$C_{16}H_{22}O_4$	278.3435
9.	19.620	Neophytadiene	2.32	$C_{20}H_{38}$	278.5157

From this analysis, dibutyl phthalate is considered as major compounds in this extract plant with 28.10% based on peak area but this bioactive compound still not been classified on their biological activities. However, there are eight bioactive compounds from this preliminary result also were considered as potential act as an antifungal and antioxidant

properties. The bioactive compounds were screened based on their groups and GC-MS analysis is the best technique to identify the constituents of volatile compounds, long chain, branched chain hydrocarbon, alcohol acids, ester and others. ethanolic extract from this study showed presence of terpene, phenol, aldehyde, alkaloids, phenolic compounds.

3.3. Antifungal and antioxidant properties from preliminary compounds

The biological properties were identified from selected bioactive compounds based on an antioxidant or antifungal agent in Table 2. The potential as antifungal and antioxidant agents was referred to another study showing that they are positive toward the biological test such as DPPH for antioxidant assay while against fungi species using disk diffusion method act as antifungal agents.

Table 2. Antifungal and antioxidant properties of bioactive compounds

No.	Name of compound	Biological Activity	Reference
1.	Phytol	Antioxidant	Velmurugan, et. al. 2017 [10]
2.	Phthalic acid, di(2-propylpentyl)ester	Antioxidant	Velmurugan, et. al. 2017 [10]
3.	2-Thiobarbituric acid, S-trimethylsilyl-, bis(trimethylsilyl) ether	Antioxidant	Ghani MA, et al. 2017 [4]
4.	Phenol, 2,5-bis(1,1-dimethylethyl)	Antifungal	Yuan Wang, et al. 2018 [11]
5.	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro	Antifungal	Ajayi GO, et al. 2011 [2]
6.	2,5-dihydroxybenzoic acid (Gentisic acid)	Antioxidant	Rico M, et al. 2013 [6]
7.	2,4-Di-tert-butylphenol	Antifungal and antioxidant	Varsha KK, et al. 2015 [9]
8.	Dibutyl phthalate	Antifungal	Ahsan T, et al. 2017 [1]
9.	Neophytadiene	Antioxidant	Simoh S, et al. 2018 [8]

Antifungal and antioxidant properties in extract plant which from preliminary compound that positive act as a biological agent. Then, each of compounds were identified whether act as an antifungal or antioxidant properties. There are five compounds potential as an antioxidant agents which are Phytol, Phthalic acid, di(2-propylpentyl)ester, 2-Thiobarbituric acid, S-trimethylsilyl-, bis(trimethylsilyl) ether, 2,5-dihydroxybenzoic acid (Gentisic acid), and Neophytadiene. Then, other compounds have potential as an antifungal such as Phenol, 2,5-bis(1,1-dimethylethyl), Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro and Dibutyl phthalate have potential as an antifungal.

3.4. Selected major potential bioactive compounds from extract of unnamed plant

The selected potential bioactive compounds is shown in Table 3. The compounds were identified by referring another source such as drugbank, USP, NIH and other sources which are showed the compounds have been clarified as a medicinal agent while Table 2 showed that compounds can be considered as an antifungal or antioxidant agent with compared the compounds in another research.

Table 3. Major potential bioactive compounds

No.	Name of compounds	Biological activity
1.	Phytol	Antioxidant
2.	2,5-dihydroxybenzoic acid (Gentisic acid)	Antioxidant
3.	Dibutyl phthalate	Antifungal

One of major potential compound from the extract plant is Phytol which is positively as an antioxidant agent. Rukhsana et al, state that phytol compounds known as antioxidant properties. The presence of phytol in the leaves of *Kirganelia reticulata* aerial parts, which was found to be effective in different stages of arthritis [5]. However, phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes [5]. Potential of Phytol compound showed that have variety potential as a medicinal agent. Gentisic acid or known as a 2,5-dihydroxybenzoic acid is phenol compound that potential act as antioxidant agent. The gentisic acid is most active phenolic compound among natural phenolic compounds [6]. In their study, quercetin, catechin, rutin and gentisic acid were identified in the extracts of P. major L. and B. aurea S., confirming their phytomedicinal potentials as natural sources of well-known antioxidant compounds [7].

GC-MS analysis revealed from extract plant that the Dibutyl phthalate compound is mainly compound in this study. The dibutyl phthalate is considered mainly to be responsible for antifungal properties. Dibutyl phthalate is the main constituent and it is responsible for its repellent property. Dibutyl phthalate was found this compound potential as an antifungal due to against *R. solani* [1].

3.5. Comparison between natural and synthetic compounds

The natural compounds and synthetic compounds were compared in Table 4. Natural compounds are extracted from the plant while synthetic compounds were synthesized from a chemical. Similarities for both compounds are biological activities. However, natural compounds still considered as an important medicinal agent rather than synthetic compounds although the price is low for synthetic compounds because it is usually used in industrial.

Table 4 Comparison between natural and synthetic compounds

No.	Name of compounds	Biological Activities	Ref.	Synthesis Compounds
1.	Phytol	Antioxidant	(Velmurugan, et al. 2017) [10]	Isophytol or Trans Phytol For synthetic forms of Vitamin K1 and vitamin E, convert to phytanic acid to gut fermentation of ingested plant material
2.	Gentisic acid	Antioxidant	(Rico M, et al. 2013) [6]	Synthetic antioxidant is butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)
3.	Dibutyl Phthalate	Antifungal	(Ahsan T, et al. 2017) [1]	Synthesis of phthalic acid dibutyl for plasticizer

The result show three compound as an antifungal and antioxidant agent and compared with synthetic compound that used in many fields included food, pharmaceutical, product and cosmetic. The synthetic compounds were made from chemical synthesis and not from natural plant for industrial uses and reduce the cost without concern on human health. Synthetic products also have weaknesses that cause harm to human health such as BHA and BHT product if use exceed amount can cause toxicity and dietary effect on blood in progress. Gentisic acid is known as most of active phenolic compounds even the content of the compound not major compared to other compounds but known as natural antioxidant compounds which the compounds showed positive as an antioxidant. Dibutyl phthalate also known as antifungal that found from Streptomyces strain against fungi and it is from phthalate which naturally as bioactive compounds against disease found in plant and fungi. Dibutyl phthalate show effective antifungal when act as binding site to MIPs which known as good candidates for extraction of bioactive compounds in that extract plant.

Natural compounds are better than synthetic compounds even though they are not cheap as synthetic compounds, but they are good for human consumption. The identified major compounds possess some important biological potentials for future drug development. There is growing awareness in correlating bioactive compounds with their biological activities.

3.6. Selected potential bioactive compounds in structure

The structure of bioactive compounds from the extract plant was recognized for phytol, phthalic acid, dibutyl phthalate, neophytadiene, gentisic acid, indazol-4-one, 2,4-di-tert-butylphenol, 2-thiobarbituric acid, and 2,5-bis(1,1-dimethyl)phenol. The structure of compounds is found in functional groups such as diterpene, sesquiterpene, and aromatic compound. Figure 2-4 show some of bioactive molecules.

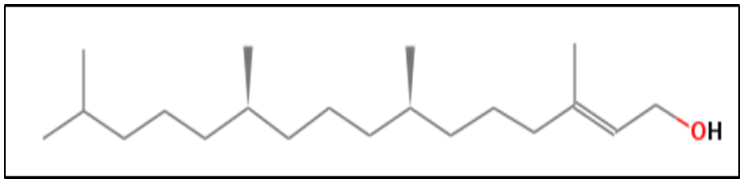


Figure 2. Phytol structure (Velmurugan, et. al. 2017)

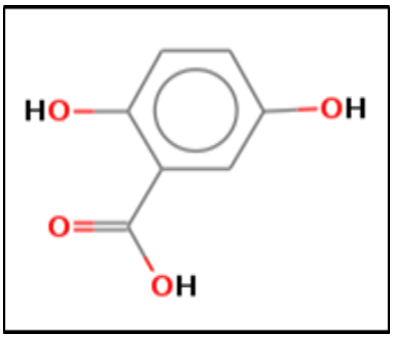


Figure 3. 2,5-dihydroxybenzoic acid (Gentisic acid) structure (Rico M, et al. 2013)

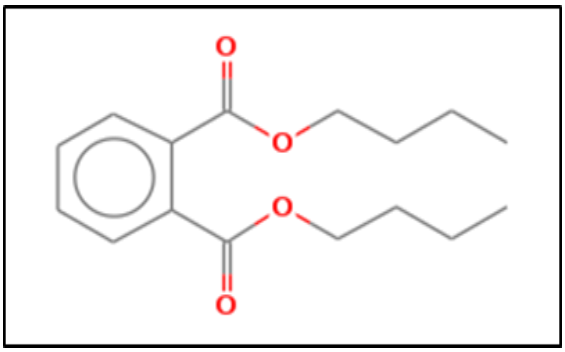


Figure 4. Dibutyl phthalate structure (Ahsan T, et al. 2017)

These structure of bioactive compounds that selected from GC-MS analysis which are possibilities act as an agent which are nine bioactive compounds from total compounds that have been identified from the maceration technique of the unnamed plant. Most of compound that present in this analysis are aromatic compounds. There are several compounds consist noncyclic compounds such as phytol and neophytadiene. Hence, the compounds were extracted also known as hydrophobic compounds which soluble in ethanol.

In spite of the advantage of modern high drug discovery and screening techniques, traditional medicinal knowledge has also given clues to the discovery of valuable drugs. There is growing awareness in correlating the phytochemical compounds with their biological activities. The macerate leaf extract obtained from unnamed plant were subjected to qualitative analysis by GC-MS method which confirmed the presence of bioactive compounds which are responsible for pharmacological activities.

4. CONCLUSION

Investigation of benzophenone in the dried fruits of *P. macrocarpa* were done with extraction method using cold ethanol. Compound 2,6,4'-trihydroxy-4-methoxybenzophenone **(1)** was successfully isolated from the fractionation and purification process from the crude extract. The structure of isolated compound was identified and elucidated by spectroscopic techniques of Infrared (FTIR) spectroscopy and Nuclear Magnetic Resonance (NMR). The quantification of 2,6,4'-trihydroxy-4-methoxybenzophenone **(1)** using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) was done for fruits of *P. macrocarpa* from different harvesting periods of January, March, April, June, July, September and November. The benzophenone content showed the highest in March by 0.69% followed by April, June, January, November, September and the lowest month is July with 0.43% w/w. This quantification shows the standardization of marker compound from *P. macrocarpa* fruits but the concentration of compounds in the fruits are different from different harvesting periods.

REFERENCES

[1] Ahsan, T., Chen, J., Zhao, X., Irfan, M., & Wu, Y. (2017). Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express, 7(1), 54-59.

[2] Ajayi, G., O. Olagunju, J., Ademuyiwa, O., & Martins, O. (2011). Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of Plumbago zeylanica, 1(5), 1-5.

[3] Devendran G, et al. (2013). Qualitative phytochemical screening and GC-MS analysis of Ocimum sanctum L. leaves. Asian J. Plant Sci. Res, 1(4), 44-48.

[4] Ghani, M. A., Barril, C., Bedgood, D. R., & Prenzler, P. D. (2017). Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. Food Chemistry, 230, 195-207.

[5] Nanadagopalan V, et al. (2015). GC-MS analysis of biomolecules on the leaves extract of Sterculia urens Roxb. . Journal of Pharmacognosy and Phytochemistry, 3(6), 193-196.

[6] Rico M, et al. (2013). Screening of the antioxidant properties of crude extracts of six selected plant species from the Canary Islands (Spain). Journal of Applied Botany and Food Quality, 86(1), 217 - 220.

[7] Silva, et al. (2002). Structure-antioxidant activity relationship of flavonoids: A reexamination. Free Radic. Res, 36(1), 1219-1227.

[8] Simoh S, et al. (2018). Comparative Analysis of Metabolites and Antioxidant Potentials from Different Plant Parts of Curcuma aeruginosa Roxb. Sains Malaysiana, 47(12), 3031-3041.

[9] Varsha KK, et al. (2015). 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp. Int J Food Microbiol, 15(211), 44-50.

[10] Velmurugan, et al. (2017). GC-MS Analysis of Bioactive Compounds on Ethanolic Leaf Extract of Phyllodium pulchellum L. Desv. International Journal of Pharmacognosy and Phytochemical Research, 9(1), 114-118.

[11] Yuan Wang, et aa. (2018). Antifungal mechanism of cinnamaldehyde and citral combination against Penicillium expansum based on FT-IR fingerprint, plasma membrane, oxidative stress and volatile profile. The Royal Society of Chemistry, 1(1), 1-7.