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Antimicrobial, total phenolic and total flavonoid properties of leaves and seed of *Jatropha curcas, Piper nigrum* L. and *Piper betle* methanolic crude extracts

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ABSTRACT

Aims: Herbal and medicinal plants bioactive compounds of *Jatropha curcas*, *Piper nigrum* and *P. betle* have been shown to possess therapeutic properties. This study investigates the presence and characterization of phytochemical compounds as well as to evaluate the antimicrobial activities of the methanolic crude extract of the leaves and seeds of *J. curcas*, *P. nigrum* L. and *P. betle*.
Methodology and results: The study on antibacterial and antifungal efficacy of the crude extracts of leaves and seeds were carried out using standard disc diffusion method. The crude extracts were found to exhibit an average response of antimicrobial activity with the inhibition zones ranged from 3% to 28% for antibacterial and from 21% to 79% for antifungal activity. Among all extracts, the leaf extract of *P. betle* showed a good antibacterial activity against *Staphylococcus aureus* and excellent antifungal properties against *Aspergillus niger* and *A. flavus.* The phytochemical screening analysis revealed the presence of saponin, tannins, glycosides, terpenoids, reducing sugar, flavonoid and anthraquinones. However, phlobatannins was not present. Total phenolic content (TPC) and total flavonoid content (TFC) were highly detected in the crude extract of *P. betle* and recorded as 13.33 mg of gallic acid equivalents, GAE (mg/ 100 mg sample) and 0.88 mg of RE (mg/100 mg sample), respectively. GC-MS analysis of the bioactive compounds reveals the presence of diethyl phthalate, 2-hexadecen-1-ol (Phytol), hexadecanoic acid, piperine, phenol and other minor compounds.
Conclusion, significance and impact of study: The study suggested that *P. betle* has a potential as a source for antimicrobial agent from plants extracts. Nevertheless, further studies are needed to elucidate their precise mechanism of action.

Keywords: antimicrobial, phenolic, flavonoid, phytochemical screening, *Piper betle*

INTRODUCTION

Bacterial infections have resulted in 17 million deaths worldwide annually, affecting mostly the young and elderly. The morbidity and mortality associated with bacterial infection remained significant, despite the advances in antimicrobial chemotherapy. The situation was made worsened with emergence of bacterial resistance towards available antibiotics. From 1930s to 1960s, twenty classes of antibiotics were marketed but in the last four decade only three new classes of antibiotics have been introduced. Since then, many multiple drug-resistance bacteria emerged and pose a serious threat to human or animal health (McAdam *et al.*, 2012).

Plants represent a key and essential source of active natural products which differ in terms of structural and biological properties and its mechanism of actions (Abreu, *et al*., 2012). Jones in 2011, had pointed out that since ancient times, herbs have been the used in many medicinal therapy until the development of synthetic drugs in the nineteenth century. In Malaysia, a large number of plants have been associated and contributed to human health and well-being (Rezai *et al.*, 2011). Numerous plant products have been regularly used for their therapeutic potential, food or as dietary component and recently have been receiving considerable attention (Ayala-Zavala *et al*., 2011).

Jatropha curcas, *Piper nigrum* and *P. betle* were selected due to their characteristics and common use by the local population and by the small industries that produces spices and materials in the agricultural activity as well as in traditional medicine (Jong *et al.*, 2013). The

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study may in the end increase the value of these plants (Booker *et al.*, 2012). However, further research and evaluations are needed for application purposes. This will in turn help to increase the productivity of plants and determine any side effect for its usage in traditional medicine (Cragg *et al.*, 2012). The plants selected have been reported to be used as natural remedies with potential medicinal purposes for several centuries. The emergence of antibiotic resistance in many bacterial pathogens and the side effects of conventional treatments have increased in the last few decades, therefore, traditional medicine sources have becomes alternatives to overcome this problems (Spellberg *et al.*, 2013).

In this study, the work include the identification of crude bioactive metabolites derived from *J. curcas*, *P. nigrum* L. and *P. betle* and assessment of the potential uses including the impact and response towards the growth of microbes. The determination of these plants as antibacterial agents would be beneficial to local planters as well as to increase their market value.

MATERIALS AND METHODS

Samples preparation

The fresh leaves and seeds of *J. curcas*, *P. nigrum* and *P. betle* were collected from the local farmers around Kuching and Kota Samarahan, Sarawak. The plant age for all the collected samples was approximately 2 years. The leaves and seeds were collected, cleaned and dried in a 50 °C incubator for five days to a constant weight. Subsequently, the dried samples were ground into powder form. The powder form samples were then stored in an airtight container at room temperature for further analysis (Kim and Verpoorte, 2010; Ajayi *et al*., 2011). The powdered form samples were weighed at 150 g for seed and 50 g for leaves, and transferred into the conical flask before being soaked with analytical grade methanol and is left for seven days. The mixture was then filtered using Whatman No.1 filter paper. The process was repeated for at least three times. The filtrate was then extracted and concentrated using rotary evaporator (BUCHI R-210). The formula for measuring the estimated percentage of yield from the extraction was according to Equation 1:

$$\%\ \text{yield extract of plant} = \frac{\text{Crude extract}}{\text{Pulverized sample}} \times 100\ \% \quad (1)$$

Antibacterial and antifungal properties

The antibacterial activity of the crude extract was determined via agar disc diffusion method (Bakht *et al.*, 2013). The bacterial isolates; *Streptococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and yeast like fungus, *Candida albicans*, were first grown on a blood agar for 18-24 h. Meanwhile, the fungi *A. flavus, A. niger, Trichoderma harzianum, Phytophthora capsici* and *Fusarium solani,* were selected to test for antifungal activity of the methanolic crude extracts.

The standard disc diffusion method was undertaken in this study and agar well diffusion method was done for comparison of the results obtained. A 6.0 mm^2 disc was prepared from sterile Whatman filter paper No.1. Approximately, 20 µL of the test solution was dispensed onto each disc. A triplicate set of the analysis were performed for each of the crude extracts (Upadhya *et al.*, 2010).

The concentrations of the methanolic crude extracts for antibacterial analysis used were determined to be 0.5 mg/mL, 1.0 mg/mL, 2.5 mg/mL, 5.0 mg/mL and 10.0 mg/mL. For the study of antifungal properties, a higher concentration was used, 5.0 mg/mL, 10.0 mg/mL and 15.0 mg/mL. The antibiotics penicillin and ampicillin were used as positive controls for the antibacterial test, whereas miconazole was used as positive control for the antifungal test. The negative controls were set up in parallel using 2% dimethyl sulfoxide (DMSO) that replaces the methanolic crude extracts. The plates were incubated for 24 h at 37 °C (for bacteria) and 48 h at 25 °C (for fungus) (Baris *et al.*, 2006). The appearance of zones of inhibition was later determined for all plates. The percentage of inhibition for the antibacterial and antifungal analysis was measured based on the Equation 2 (Avinash *et al.*, 2015):

$$\%\ \text{of inhibition} = \frac{\text{Inhibition by extract (mm)}}{\text{Inhibition by standard drugs (mm)}} \times 100\ \% \quad (2)$$

Phytochemical screening

The crude extracts were also screened for the presence of secondary metabolites (Savithramma *et al.*, 2011). The standard procedure to identify the constituents was done as described by Singh *et al.* (2012). The methanolic crude extracts were tested for the presence of tannins, saponin, phlobatannin, glycosides, anthraquinones, terpenoids, flavonoids and phenolic.

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The procedure for the estimation of total phenolic compounds was based on the protocol by Sharma *et al.* (2011) by using the Folin-Ciocalteau reagent. Approximately, 200 µL of the methanolic crude extract was added into 10 mL of 1:10 Folin-Ciocalteau reagent. The reaction mixture was mixed and incubated for 5 min before the addition of 7 mL of 0.115 mg/mL Na_2CO_3. The reaction mixture was further incubated for 2 h before measuring the absorbance at 765 nm. Gallic acid was used as the standard and the result was expressed as the Gallic Acid Equivalents (GAE) per gram of dried weight samples.

The total flavonoid was determined based on the method as described by Oskoueian *et al.* (2011) that uses the aluminium chloride ($AlCl_3$) colorimetric method. Approximately, 250 µL of the methanolic crude extract was mixed with 1.25 mL of ddH_20 and added with 75 µL of a 5% (w/v) solution of $NONO_3$. After 6 min of incubation, 150 µL of $AlCl_3$ solution was mixed thoroughly with the

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reaction mixture and incubated for another 5 min with an addition of 0.5 mL of 1M NaOH. After the incubation period, the optical density (OD) of the reaction mixture was measured at 510 nm. Rutin was used as the reference, which was expressed as Rutin Equivalent (RE) per gram of dried weight samples.

Gas chromatography mass spectrometry (GCMS) analysis of bioactive compound

The methanolic crude extracts bioactive compound of leaves and seed of *J. curcas*, *P. nigrum* L. and leaves of *P. betle* were quantitatively measured by Gas Chromatography Mass Spectrometry (GCMS) (Hossain and Rahman, 2011; Namuli *et al.*, 2011). Approximately, 6 µL of the methanolic crude extracts were analysed on BPX-5 SGE ultra-low-bleed 5% phenyl polydimethylsiloxane capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Splitless injection was performed with a purge time of 1 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The column temperature was maintained at 50 °C for 3 min, then programmed at 5 °C/ min to 80 °C and at 10 °C/ min to 340 °C. The inlet and detector temperatures were 250 °C and 340 °C, respectively, and the solvent delay was set at 4 min. The identification of the bioactive components or compounds was done by matching the peak obtained with the database in National Institute of Standard and Technology (NIST) library based on the values of the retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area %).

RESULTS AND DISCUSSION

Yield of extraction process

The ground leaves and seed of *J. curcas*, *P. nigrum* and leaves of *P. betle* were extracted using methanol as solvent to extract the compounds. The filtrate for each sample was combined, dried using rotary evaporator and weighed. Methanol was used as solvent because it is a solvent that has the highest polarity compared to other solvents, resulting in the liberation of greater amount of bioactive compound. (Sasidharan *et al.*, 2011; Yadav and Agarwala, 2011). Table 1 shows the percentage of yield with the highest yield of bioactive compound were obtained from *P. betle* leaves (9.11%), followed by *J. curcas* leaves (5.68%) and *P. nigrum* (2.88%). These findings indicated that the best samples to be used that will produce a high yield of bioactive compound are the leaves samples.

Antibacterial and antifungal properties

The antibacterial properties of the methanolic crude extract samples were done using the disc diffusion technique and analysed after performing triplicate sets. The results obtained confirmed that not all of the methanolic crude extracts shows evidence of antibacterial properties (Figure 1). The highest rate of inhibition was led by the methanolic crude extract of *P. betle* leaves followed by *P. nigrum* L. leaves, *J. curcas* leaves and *P. nigrum* L. seed. Meanwhile, no inhibition was observed from the extract of *J. curcas* seed. The concentrations of the crude extracts significantly affect the size of zone of inhibition. A few trial runs before the exact tests were done to determine the most suitable methanolic crude extracts concentration to be applied.

Table 1: The percentage of crude extract yield obtained after solvent extraction.

Plant sample	Dry weight (After grinding)	Crude extract (g)	Percentage of yield
J. curcas seed	150 g (dry in 600 mL of CH_3OH)	6.56 ± 0.05	4.37%
J. curcas leaves	50 g (dry in 200 mL of CH_3OH)	2.84 ± 0.05	5.68%
P. nigrum L. seed	150 g (dry in 600 mL of CH_3OH)	2.59 ± 0.05	1.73%
P. nigrum L. leaves	50 g (dry in 200 mL of CH_3OH)	1.44 ± 0.05	2.88%
P. betle seed	-	-	-
P. betle leaves	50 g (dry in 200 mL of CH_3OH)	4.56 ± 0.05	9.11%

Triplicate test experiment of the crude extracts against the bacteria showed that the *P. betle* exhibit more susceptible zone of diameters at the concentration 10.0 mg/mL as compared to other crude extracts used in these experiments. Diameter for zone of inhibition by itself is of no direct significance. However, provided that the precise conditions of the test are followed, the zone of inhibition, zone size can be related to minimum inhibitory concentrations by regressions lines from ratios for antibiotic and each bacterial species.

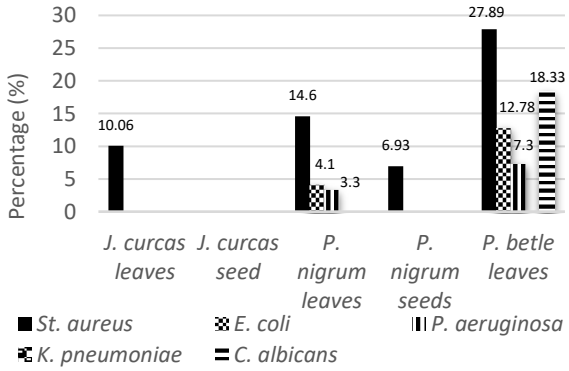

Figure 1: Effects of methanolic crude extracts leaves and seed of *J. curcas*, *P. nigrum* L. and leaves of *P. betle*

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against bacteria. Concentration of sample was 10 mg/mL. Values were presented as mean ± standard deviation of triplicate independent analyses.

The analysis of the methanolic crude extracts against fungal samples were done and when the concentrations were reduced to 5.0 mg/mL, *P. betle* methanolic crude extract significantly indicate a sign of susceptible reaction even when the size of inhibition zone were reduced against both *A. flavus* and *A. niger* (Figure 2). In general, the extract from *Piper* sp. mainly the *P. betle* demonstrated the ability and prospective to develop as inhibitor against *A. flavus*. This result provides an overview of the antifungal capability of the methanolic crude extracts from *Piper* sp. to be further developed as antifungal agent in the near future.

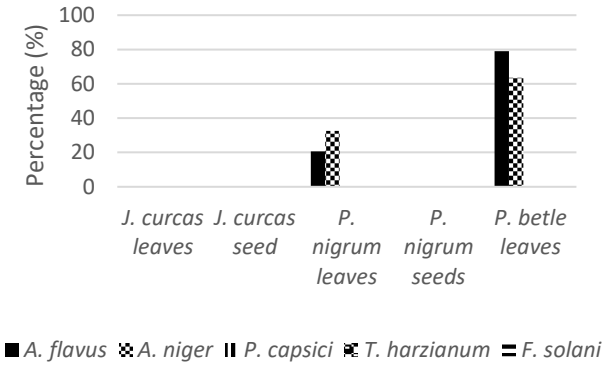

Figure 2: Effects of methanolic crude extracts leaves and seed of *J. curcas*, *P. nigrum* L. and leaves of *P. betle* against fungi. Concentration of sample was 15 mg/mL. Values were presented as mean ± standard deviation of triplicate independent analyses.

Piper betle exhibits higher diameter for the zone of inhibition towards the antimicrobial activity especially for the reaction against the *S. aureus* (Gram negative bacteria) and moderate activity against *E. coli*, *P. aeruginosa* and *C. albicans*. The antifungal susceptibility testing exhibited by *P. betle* leaves extract showed a moderate activity against *A. flavus* and *A. niger* at higher concentration of up to 15 mg/mL of crude extract. However, the crude extract did not show any antifungal activity against *P. capsici*, *T. harzianum*, and *F. solani*. This finding indicated that *P. betle* contain certain compounds that exhibit antimicrobial properties.

Phytochemical properties

The phytochemical compounds that occur naturally in plants have a very wide function and responsible for the colour, physiology functions or even the organoleptic properties of the plant (Maffei, 2010). Plants have provided an inspiration for natural drug discovery especially for treatment in medicine that has created a large contribution to human health and their well-being (Giampieri *et al.*, 2012).

Preliminary screening process was done and revealed that the entire methanolic crude extract samples contain saponin, flavonoids, and anthraquinones. Interestingly, the methanolic crude extract sample of *P. betle* contains all of the compounds that have been screened for this experiment. Indirectly, this shows that the *P. betle* contain high levels of phytochemicals (Bajpai *et al.*, 2010). Obasi *et al.* (2011) reported that the phytochemical screening from *J. curcas* stem bark extract reveal the presence of saponin, steroids, tannins, glycosides, alkaloids and flavonoids.

Among the crude extracts, *P. betle* contains high phytochemical compounds such as tannins, saponin, trepenoid and other. Cushnie and Lamb (2005) also mentioned that the phytochemical present in the leaves extract were high but low antimicrobial activity were observed, which might due to the fact that different phytochemicals exert their effect differently.

Total phenolic and flavanoid content estimation

Determinations of total phenolic content were expressed in milligrams of gallic acid equivalents (GAE) using the Folin-Ciocalteau reagent and gallic acid as a standard. The total phenolic estimation was done and from several repetitions of experiments conducted, it was found that the rate of presence of total phenolic content (TPC) was highest recorded as in mg/100 mg from the sample of *P. betle*.

As shown in the Figure 3, the *P. betle* expressed the highest amount of phenolic content as of the Gallic Acid Equivalent (GAE). Therefore, there is a significant relationship between the high amount of phenolic compound and the antioxidant activity, thus, emphasizing on the fact that *P. betle* have major medicinal effect and had been used as traditional medicine (Palombo, 2011).

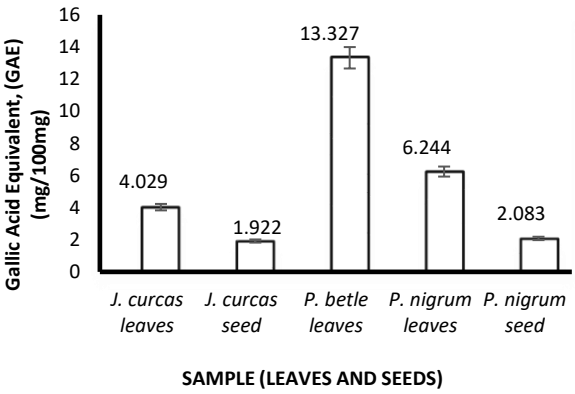

Figure 3: Total phenolic content (TPC) of methanolic crude extracts leaves and seed of *J. curcas*, *P. nigrum* L. and leaves of *P. betle*. Values were presented as mean ± standard deviation of triplicate independent analyses.

Absorbance for the total flavonoid (TF) content were done according to *Rutin standard* (y = 9.26 x) at 510 nm. The concentration of flavonoids was the highest in the leaf

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extract of *P. betle* recorded as in mg/100 mg, followed by *P. nigrum* leaves and seeds.

However, the low rate of flavonoid levels is derived from extracts of *J. curcas* leaves and seeds. This studied also shows that the TPC and TFC were correlated with the results obtained from antimicrobial activity. It give a clear picture that crude extract of *P. betle* possess a compound that related with antibacterial and antifungal inhibition activity compared to other crude extract studied in this investigation.

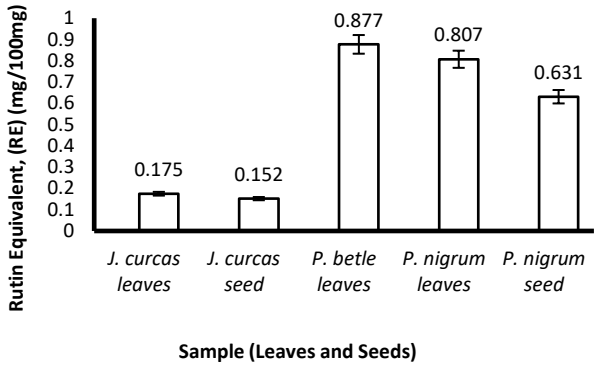

Figure 4: Total flavonoid content (TFC) of methanolic crude extracts leaves and seed of *J. curcas*, *P. nigrum* L. and leaves of *P. betle*. Values were presented as mean ± standard deviation of triplicate independent analyses.

Gas chromatography mass spectrometry (GC-MS) analysis of bioactive compounds

Gas Chromatography (GC-MS) analysis was undertaken to find out the bioactive compounds present in the methanolic crude extract of *J. curcas* leave and seed, *P. nigrum* leave and seed and *P. betle*. From this analysis, the active values are their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) as presented in Figure 5 which indicates the existence of several bioactive compounds from the crude extracts and comparing them with the mass spectra of identified compounds in the database.

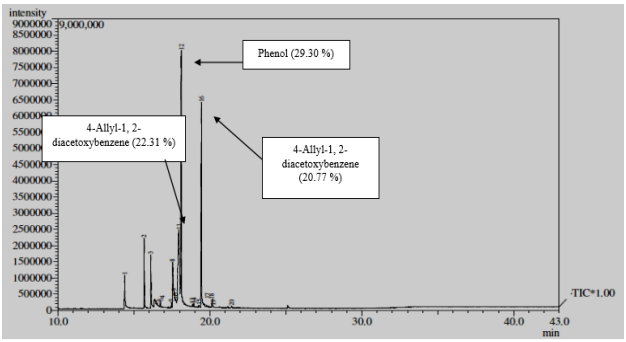

Figure 5: Gas Chromatography Mass Spectrometry (GC-MS) chromatogram for methanol extract of *P. betle* leaves crude extract.

The extract of *P. betle* leaves resulted in the presence of Phenol (29.30%) and 4-Allyl-1, 2-diacetoxybenzene (43.08%) (Figure 5). Phenolic compound is the main phytochemical compound established in most plants that possess antimicrobial and antioxidant properties and is frequently attributed to health benefits (Ignat *et al.*, 2011). Meanwhile for *P. nigrum*, the identified compound of this extract are 1, 2-Propanediol (10.43%), Piperazine-1-yl (11.07%) and N-Ethyl-N-Furfurylaniline (14.94%) for leaves sample but for the seed of *P. nigrum*, the compounds are Piperine at 26.10% and Caryophyllene at 23.44%; the highest among the number of 20 compounds detected (Table 2).

The extract of *J. curcas* leaves show that the 2-Hexadecen-1-ol and Gamma-sitosterol are the main compounds identified which consist of 12.06% and 18.94% of the peak area of the chromatogram (Table 2). Gas chromatograms results obtained revealed that *P. betle* contain high amount of phenolic-based compounds which is an indicator for this plants to be studied further and to be developed as ailments or new drug in medicine.

Table 2: The list of major bioactive compounds analysed with GC-MS from methanolic crude extracts.

Plants	Part	Methanolic crude extract main compound	Area (%)
J. curcas	Leaves	β-sitosterol @ Stigmast-5-en-3-ol	18.94
		Phytol hexadecen-1-ol	12.06
	Seed	9-octadecenoic acid @ methyl ester	36.67
		9,12-octadecadienoic acid @ Linoleic acid	23.73
P. nigrum L.	Leaves	Morpholine @ N-Ethyl-N-furfurylaniline	14.94
		2,3-Diphenylcyclopropyl	11.67
	Seed	Piperidine @ Piperine	26.10
		Caryophyllene	23.44
P. betle	Leaves	Phenol	29.32
		Benzoic acid	22.38

Exploring medicinal phytochemical compounds using extracts by GC-MS analysis are of great interest to researchers especially in the field of medicine because most of the drug-producing companies depend on the part of plants for the production of pharmaceutical products (Velmurugan *et al.*, 2011). Tongpoothorn and co-workers, (2012) analysed the bioactive components in *J. curcas* leaves using GC-MS analysis with supercritical fluid CO_2 extraction and found that the components contains multiple compounds with anti-tumour, anti-viral and antimicrobial activities. Periyanayagam *et al.* (2011) analysed the aqueous and ethanol extract of *P. betle* leaves to detect the phytochemical present in it and revealed the presence of six compounds which relate to an activity of antibacterial, antifungal, antioxidant, anti-cancerous and anti-inflammatory.

CONCLUSION

The identified compounds have antimicrobial activity with

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highly significant quantity of phenolic compounds found in the crude extract leaves of *P. betle* in terms of Gallic Acid Equivalent (GAE) followed by the leaf of *P. nigrum*. The lowest phenolic content was found in the crude extract of *J. curcas* and *P. nigrum* seed. *P. betle* leaves contained various bioactive compounds and therefore it could be recommended as a plant of phytopharmaceutical importance. The information obtained would help us to identify the potential of applying *P. betle* and *P. nigrum* as biological agents for the development of new medicine that will benefit mankind. Further investigations on *P. betle* crude extract with phytopharmaceutical analysis by methods such as the toxicology analysis, drug screening, structure elucidation and HPLC methods is recommended for future studies.

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REFERENCES

Abreu, A. C., McBain, A. J. and Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Reports* **29(9), 1007-1021.**

Ajayi, I. A., Ajibade, O. and Oderinde, R. A. (2011). Preliminary phytochemical analysis of some plant seeds. *Research Journal of Chemical Sciences* **1(3), 58-62.**

Avinash, K. S., Ashwini, H. S., Ramesh Babu, H. N., and Krishnamurthy, Y. L. (2015). Antimicrobial potential of crude extract of *Curvularia lunata*, an endophytic fungi isolated from *Cymbopogon caesius*. *Journal of Mycology* **Article ID 185821.** DOI: 10.1155/2015/185821.

Ayala-Zavala, J. F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W. and González-Aguilar, G. A. (2011). Agro-industrial potential of exotic fruit by-products as a source of food additives. *Food Research International* **44(7), 1866-1874.**

Bajpai, V., Sharma, D., Kumar, B. and Madhusudanan, K. P. (2010). Profiling of *Piper betle* Linn. cultivars by direct analysis in real time mass spectrometric technique. *Biomedical Chromatography* **24(12), 1283-1286.**

Bakht, J., Muhammad, T., Ali, H., Islam, A. and Shafi, M. (2013). Effect of different solvent extracted sample of *Allium sativum* (Linn) on bacteria and fungi. *African Journal of Biotechnology* **10(31), 5910-5915.**

Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M. and Ozbek, T. (2006). Biological activities of the essential oil and methanol extract of *Achilea Biebersteinni* Afan (Asteraceae)**.** *Turkish Journal of Biology* **30, 65-73.**

Booker, A., Johnston, D. and Heinrich, M. (2012). Value chains of herbal medicines: Research needs and key challenges in the context of Ethnopharmacology. *Journal of Ethnopharmacology* **140(3), 624-633.**

Cushnie, T. P. and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* **26(5), 343-356.**

Cragg, G. M., Katz, F., Newman, D. J. and Rosenthal, J. (2012). The impact of the United Nations Convention on biological diversity of natural products research. *Natural Product Reports* **29(12), 1407-1423.**

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., and Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition* **28(1), 9-19.**

Hossain, M. A. and Rahman, S. M. M. (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Research International* **44, 672-676.**

Ignat, I., Volf, I. and Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables**.** *Food Chemistry* **126(4), 1821-1835.**

Jones, A. W. (2011). Early drug discovery and the rise of pharmaceutical chemistry. *Drug Testing and Analysis* **3(6), 337-344.**

Jong, W. W., Tan, P. J., Kamarulzaman, F. A., Mejin, M., Lim, D., Ang, I. and Lee, H. B. (2013). Photodynamic activity of plant extracts from Sarawak, Borneo**.** *Chemistry and Biodiversity* **10(8), 1475-1486.**

Kim, H. K. and Verpoorte, R. (2010). Sample preparation for plant metabolomics. *Phytochemical Analysis* **21(1), 4-13.**

Maffei, M. E. (2010). Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany* **76(4), 612-631.**

McAdam, A. J., Hooper, D. C., DeMaria, A., Limbago, B. M., O'Brien, T. F. and McCaughey, B. (2012). Antibiotic resistance: How serious is the problem, and what can be done? *Clinical chemistry* **58(8), 1182-1186.**

Namuli, A., Abdullah, N., Sieo, C. C., Zuhainis S. W and Oskoueian E. (2011). Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn.extract. *Journal of Medicinal Plants Research* **5(16), 3982-3990.**

Obasi, N. L., Ejikeme, M. P. and Egbuonu, C. A. (2011). Antimicrobial and phytochemical activity of methanolic extract and its fractions of *Jatropha curcas* Linn. (Eurphorbiaceae) stem bark. *African Journal of Pure and Applied Chemistry* **5(5), 92-96.**

Oskoueian, E., Abdullah, N., Ahmad, S., Saad, W. Z., Omar, A. R. and Ho, Y. W. (2011). Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. *International Journal of Molecular Sciences* **12(9), 5955-5970.**

Palombo E. A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of

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oral diseases. *Evidence-Based Complementary and Alternattive Medicine* **Article ID 680354.** DOI:10.1093/ecam/nep067.

Periyanayagam, K., Mubeen, M., Saleem, M. M. and Basha, S. S. (2011). Phytochemical studies and GC/MS analysis on the isolated essential oil from the leaves of *Piper betle* L. var. sirugamani1 (SGM1). *Journal of Pharmacy Research* **4(7), 2411-2413.**

Rezai, G., Mohamed, Z. and Shamsudin, M. N. (2011). Malaysian consumer's perceptive towards purchasing organically produce vegetable. *In*: 2nd International Conference on Business and Economic Research **20(20), 1774-1783.**

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. and Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines* **8(1), 1–10.**

Savithramma, N., Rao, M. L. and Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research* **8(3), 579-584.**

Sharma, G. N., Susheel, K. D., Nitin, S. and Jyotsana, S. (2011). Phytochemical screening and estimation of total phenolic content in *Aegle marmelos* seeds. *International Journal of Pharmaceutical and Clinical Research* **3(2), 27-29.**

Singh, D., Kumar, T. R. S., Gupta, V. K. and Chaturvedi, P. (2012). Antimicrobial activity of some promising plant oils, molecules and formulations. *Indian Journal of Experimental Biology* **50(10), 714-717.**

Spellberg, B., Bartlett, J. G. and Gilbert, D. N. (2013). The future of antibiotics and resistance. *New England Journal of Medicine* **368(4), 299-302.**

Tongpoothorn, W., Chanthai, S., Sriuttha, M., Saosang, K. and Ruangviriyachai, C. (2012). Bioactive properties and chemical constituents of methanolic extract and its fractions from *Jatropha curcas* oil. *Industrial Crops and Products* **36(1), 437-444.**

Upadhyay, R. K., Dwivedi, P. and Ahmad, S. (2010). Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian Journal of Medical Sciences* **2(3), 152-158.**

Velmurugan, P., Kamaraj, M. and Prema, D. (2011). Phytochemical constituents of *Cadaba trifoliata* root extract. *International Journal of Phytomedicine* **2(4). 379-384.**

Yadav, R. N. S. and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology* **3(12), 10-14.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538