ORIGINAL ARTICLE

Medicinal Properties Screening of *Mallotus paniculatus* Extract

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ABSTRACT

Introduction: Traditionally, *Mallotus paniculatus* (Balik Angin) plant is used in the treatment of various diseases in rural areas such as remedy after childbirth, wound healing and fever. In this present study, four medicinal properties of the plant were investigated which included antibacterial, antifungal, anticancer and antioxidant activities. **Materials and Methods**: Potential medicinal compounds were extracted from the plant leaves by sonication with 3 different solvents namely ethanol, ethyl acetate and hexane respectively. The antibacterial and antifungal properties were determined using disc diffusion agar and broth dilution assay, the antioxidant activity by DPPH scavenging assay and the anticancer effect by MTT assay. **Results**: From the screening of the medicinal properties, *M. paniculatus* leave extracts were shown to possess antibacterial, antioxidant and anticancer properties but not antifungal properties. Ethanolic and ethyl acetate extracts of the leave were active against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) but not gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The antioxidant activity of the ethanolic crude extract was high; with IC_{50} of 30 µg/ml comparable with the positive controls; ascorbic acid and butylated hydroxytoluene (BHT). Both ethanolic and ethyl acetate extracts were cytotoxic against breast cancer (MCF7), colon cancer (HT-29), cervix cancer (Hela) cell lines. **Conclusion**: *M. paniculatus* leave extract has many potential medicinal values for further studies.

KEYWORDS: *Mallotus paniculatus*, antibacterial, anticancer, antioxidant, sonication

INTRODUCTION

Natural products are very useful[1] and economically essential[2] as it is relatively cost-effective[3] than modern drugs[3] and it has been used in traditional medicine.[4] Undoubtedly, scientific investigation on the traditional medicinal plants has greatly helped the natural products to be marketable commercially and has taken a secondary role in drug discovery and drug development.[5] The increasing demand for natural products in maintaining health[6] and treating diseases[7] encourages many researchers globally to investigate traditional medicinal plants extensively.[8]

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In Malaysia, there are many traditional medicinal plants, including *petai* (*Parkia speciosa*), *pegaga* (*Centella asiatica*), *kunyit* (*Curcuma domestica*), *asam gelugor* (*Garcinia atroviridis*) and papaya fruit (*Carica papaya*).[9] Scientific studies showed that *P. speciosa* and *C. asiatica* possess anti-diabetics, anti-oxidant as well as anti-hypertensive activities[10] while *C. domestica* demonstrated anti-inflammatory, anti-tumour, cardio-protective and neuro-protective properties.[9] *G. atroviridis* was found to possess antibacterial, anti-nematodes, anti-tumour activities, and *C. papaya* has antioxidant property.[11]

Another traditional medicinal plant in Malaysia that is used to treat diseases is *Mallotus paniculatus*.[12] In Malaysia, the local name of this tree is '*Balik angin*'. Often it is utilized as a remedy after childbirth, for wound healing and fever treatment. It belongs to Euphorbiaceae family which is a major group of angiosperm (flowering plant) commonly found in southern Asia including Malaysia and north-eastern

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Figure 1: 'Balik angin' trees were found at Jalan Kepong, Selayang

MATERIALS AND METHODS

Collection of plant material

The plant material used in this experiment was collected from an area in Selayang, Malaysia. The species was identified and authenticated by a certified officer from the National Herbarium of Forest Research Institution Malaysia (FRIM) (PID 321017-20). The leaves were dried, grounded to become powder and stored for extraction. Only plant leaves were used in the present study as most traditional medicinal practices used this part of the plant in the treatment modality.

Preparation of the Extracts

Plant crude extracts were prepared by extracting the powdered leaves with ethanol, hexane and ethyl acetate individually in an ultrasonic bath (JAC Ultrasonic 4020, Korea) in a ratio of 1:10 (w/v) (g/ml) at a temperature of 40°C for 30 min with amplitude 24 kHz. The decoction was filtered using the Whatman paper and the resulting filtrate was dried at 50°C for 4-7 hours. Various solvents were used in this study to obtain compounds of different polarities.

Bacterial and Fungal Cultures

The bacteria and fungi used in the present study were *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442) as gram negative species and *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 23857) as gram positive species; and fungi: *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 24433), *Aspergillus niger*, respectively.

Mammalian Cell culture

The cell lines involved in this study were breast cancer (MCF-7, ATCC No: HTB-22), colon cancer (HT-29, ATCC No: HTB-38) and cervical cancer (Hela, ATCC No: CCL-2), while normal cell (Vero, ATCC No: CCL-81) was used as the negative control. These cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) at 37°C in an incubator supplied with 5% CO_2 until confluent. The confluent cells were trypsinized and cell count was conducted under microscope using a haemocytometer for cytotoxic assay.

Agar Disc Diffusion Assay

Antibacterial and antifungal activities of the plant leave extracts were tested by agar disc diffusion method with few modifications.[17] To conduct the assay, the bacteria and fungi were first plated on Luria Bertani (LB) agar and potato dextrose agar, separately and incubated at 37°C for 18-24 hours and 48 hours, respectively. These bacterial and fungal cultures were then transferred into freshly prepared LB and potato dextrose broth respectively and adjusted to 0.5 McFarland turbidity standards (1.5 X 10^8 cells/ml at a wavelength of 600 nm and 540 nm,

respectively) by diluting them with sterile distilled water. The adjusted inoculums of 100 µl were then plated on the Mueller Hinton agar plates before 6 mm round-shaped filter paper impregnated with leaves extracts (5 mg per disc) were placed on the plates. Antibiotic, streptomycin (100 µg) was used as the positive control while disc impregnated with 10% dimethyl sulfoxide (DMSO) served as the negative control. For this assay, 0.5 g/ml extracts were prepared by dissolving the dried filtrate in 10% DMSO. The test was determined by the size of the zone of inhibition which indicated the sensitivity of the microorganism to the extract.

Broth Dilution Assay

For broth dilution assay, bacterial suspension culture was prepared by adjusting it to 0.5 McFarland turbidity standards at 600 nm wavelength. Then, 100 µL of the culture was placed into a 96-well microplate. This was followed by adding 5 mg/mL of plant extract into the microplate. A serial dilution of the extracts was made and the plate was incubated overnight. The optical density (OD) of overnight culture was measured to determine half maximal inhibitory concentration (IC_{50}) of the plant extracts. All determinations were performed in triplicate readings.

Antioxidant activity by 2,2'-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay

For this assay, the sample solution at 1 mg/ml was prepared by mixing the crude extracts with ethanol and was diluted to a dilution series.[18] This was followed by mixing them (100 µL) with 3.9 mL of methanolic DPPH in a 15 mL test tube, individually. The mixtures were then allowed to stand for 30 minutes at room temperature in the dark. At the same time, a control containing 100 µL of ethanol and methanolic DPPH was prepared and was also left in the dark. After that, the absorbance (OD) of the control and the sample were measured at 517 nm by using UV spectrophotometer. All determinations were performed in triplicate readings. The DPPH radical scavenging activity of the tested samples was calculated according to equation shown below.[19] A radical scavenging activity versus concentration of the sample was plotted and the concentration of sample required for 50% activity were determined and represented as IC_{50} value.

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{(\text{OD control} - \text{OD sample})}{\text{OD control}}\right] \times 100\ \%$$

Cytotoxic activity by MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay is a colorimetric assay for assessing cell metabolic activity. The assay was carried out by seeding a known number of cells (5×10^3 cell/well in 100 µl of DMEM supplemented with serum) into a 96-well plate and incubated for 24 hours before adding plant extract with different dilutions. MTT at 0.5 mg/ml were added into the wells after 24 hours treatment and DMSO was later transferred to each well to solubilize the MTT formazan formed. The control (5×10^3 cell/well in 100 µl medium with serum) was carried out at the same time without plant extract treatment. Absorbance at 540 nm of each well was recorded using microplate reader. The results were analysed in triplicate and the cytotoxic effect of the extract was evaluated based on the percentage of cell viability using the following equation 2;[20]

$$\text{Cell Viability (\%)} = \frac{\text{OD sample}}{\text{OD of control}} \times 100\ \%$$

RESULT AND DISCUSSION

Antibacterial and antifungal activities

i. Disc diffusion assay

Table I shows that ethanolic and ethyl acetate extracts possess antibacterial activity against *S. aureus* and *B. subtilis* with inhibition zone, 14 mm and 11 mm, respectively. However, none of the plant extracts showed inhibition against *E. coli* and *P. aeruginosa* growth. These results indicated that gram positive bacteria are more susceptible towards the plant extracts compared to the gram negative bacteria. The different effect of these extracts may be due to cell wall structure of gram negative and gram positive bacteria. Although the bacterial cell wall of gram positive compose of a thicker peptidoglycans layer compared to the gram negative bacteria, the latter has an outer membrane which function as an extra layer of protection to the organism.[21] Hence, it may cause the gram negative bacteria to be less susceptible towards the plant extracts.

The results also demonstrated that the ethanolic extract possessed a better antibacterial activity compared to the other extracts. The hexane extract did not show any inhibition zone against all the bacteria. This may be due to the different chemical

constituents isolated in the extracts depending on the polarity of each solvent used as extractant.[22] Previous study found that *M. paniculatus* methanol extract consists of flavonoids and pentacyclic triterpenoids.[23] Flavonoids are hydroxylated phenolic substances in the form of C6-C3 unit linked to an aromatic ring. They are known to be effective antimicrobial substances against a wide array of microorganisms. This activity could be due to their ability to form complex with extracellular and soluble proteins through nonspecific forces such as hydrogen bonding, hydrophobic effects and covalent bonding. They are also able to bind with bacterial cell walls, microbial adhesins, enzymes, and cell envelope transport proteins. This may result in the loss of vital metabolites from the cells and becomes responsible for the death of the cells.[24]

Table I : Inhibition zone of *Mallotus paniculatus* leave extracts with different solvents towards various bacteria

	Inhibition zone (mm)			
		Type of solvents		
Bacteria	Strepto-mycin	Ethanol	Ethyl acetate	Hexane
Staphylococcus aureus	22	14 ± 0.5	11 ± 0.5	-
Bacillus subtilis	20	14 ± 0.6	11 ± 0.9	-
Escherichia coli	32	-	-	-
Pseudomonas aeruginosa	25	-	-	-

Triterpenoids that were found in *M. paniculatus* extract were sterols; friedelin, epifriedelinol, 29-nor-21αH-hopane-3, 22-dione, and steroids; ß-itosterol, stigmasterol.[14] Pentacyclic triterpenoids in *Limnophila indica* Linn have been shown to be toxic toward microorganism.[25] However, no study has been conducted yet on triterpenoids in *M. paniculatus* for the same bioactivity.

Regarding the antifungal activity however all the crude extracts did not show any inhibition zone against these fungi species; *Candida albicans*, *Candida glabrata*, and *Aspergillus niger*. This could be due to resistance of the fungi towards the extract.

Broth dilution

For broth dilution result, MIC was reported based on the turbidity of cultured cell where the lowest concentration of the extract prevented visible growth of the organism.[26] IC_{50} was extrapolated from the graph that was plotted between bacterial growth inhibition percentage versus concentration of sample.[12]

In Table II, both extracts showed inhibitory effect against the *S. aureus* and *B. subtilis* with concentrations ranged from 200 µg/mL to 1500 µg/mL. However, the ethanolic extract had a better MIC (200µg/mL) and IC_{50} (157 and 175±1 µg/mL) compared to the ethyl acetate extract (700; 650±0.58 µg/mL and 1500; 1400±0.6 µg/mL) against *S. aureus* and *B. subtilis*. Considering that the ethanolic extract was a crude extract, the antibacterial activity was somewhat effective as the MIC and IC_{50} values of the extract were not far from the positive control with MIC and IC_{50} of 100; 90±0.09 µg/mL and 70 ; 50±1.1 µg/mL) for both *S. aureus* and *B. subtilis*, respectively.

Table II: Antimicrobial activity of Mallotus paniculatus extract against Staphylococcus aureus and Bacillus subtilis based on the mean inhibitory concentration (MIC) and half maximal inhibitory concentration (IC_{50})

Test microorganism	Strepto-mycin (µg/mL)		Type of extract solvents			
			Ethanol (µg/mL)		Ethylacetate (µg/mL)	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
Staphylococcus aureus	100	90± 0.09	200	157± 0.58	700	650± 0.58
Bacillus subtilis	70	50± 1.1	200	175± 1	1500	1400 ±0.6

Antioxidant activity

The results shown in Figures 2a-2c below represent the half maximal inhibitory concentration (IC_{50}) of three different extracts to reduce the absorbance of DPPH by 50%. Low IC_{50} indicated the extract was more effective in combating oxidative stress. The IC_{50} of ethanol, ethyl acetate and hexane were recorded as 30, 810 and 750 µg/mL, respectively (Figures 2a-2c). Based on these results, the ethanolic extract exhibited the best antioxidant activity. Interestingly, the antioxidant activity of ethanolic extract was comparable with the positive control, ascorbic acid and butylated hydroxytoluene (BHT) that recorded as 19.2 µg/mL and 30 µg/mL, respectively. It may be possible that the ethanolic extract contained a lot of antioxidant compounds compared to the ethyl acetate and hexane extracts.

Many studies show that flavonoids and tannins in plants are good antioxidants. *M. paniculatus* contain

apigenin, fridelin, quercetin, quercitrin, myricetin, and kaempferol-3-O-l-rhamnosyl which are flavonoid.[23] Flavonoids consist of phenolic groups that mainly have redox properties which are responsible as reducing agents, hydrogen donors, singlet oxygen quenchers as well as having metallic chelating potential. Flavonoids consist of 15-carbon flavan structure which is arranged in three aromatic rings ($C_6C_3C_6$) commonly denoted as A, B and C. Several classes of flavonoids give the different level of saturation of the C ring.

Individual compound within substitution pattern of the A and B rings may affect the phenoxyl radical stability and the antioxidant properties of the substances.[27] The scavenging activity of natural compounds depends on the location and number of free –OH groups on the flavonoid skeleton.[28] Multiple hydroxyl groups on the flavonoids contribute to more effective antioxidants than those with only one. Some previous studies reported that the presence of the ortho-3,4-dihydroxy structure increases the antioxidative activity.[29]

In addition, the interactions of flavonoids with metal ions can contribute to chelate formation. The potential coordination spaces in some structure of flavonoids can chelate many ions of metals and form different complexes. These metal-flavonoid complexes possess stronger free radical scavenging properties than free flavonoids.[30] They play important role in protection against oxidative stress. This also can be crucial in the prevention of radical generation, which damage target biomolecules.

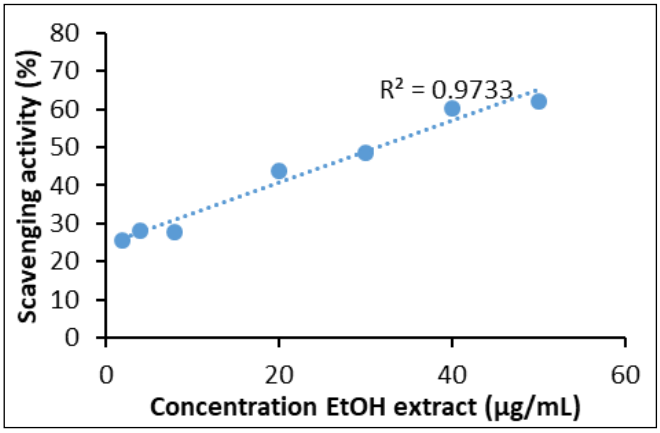

Figure 2a. DPPH scavenging activity of ethanolic Mallotus paniculatus extract

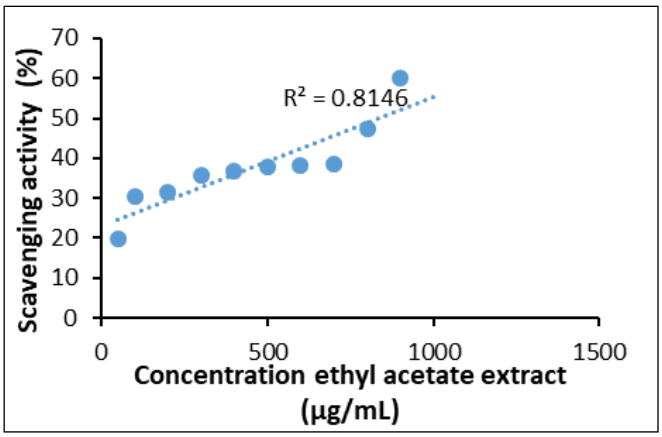

Figure 2b. DPPH scavenging activity of ethyl acetate Mallotus paniculatus extract

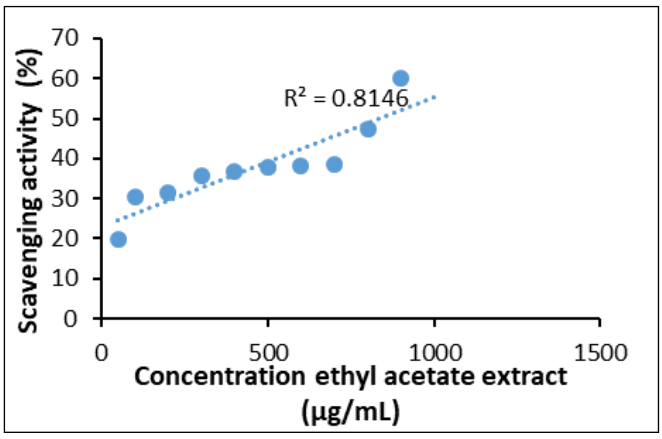

Figure 2c. DPPH scavenging activity of hexane *Mallotus paniculatus* extract

Cytotoxic activity

The activity was determined based on the growth inhibition of cancer cells exerted by the extracts. IC_{50} values of each extract were estimated based of this inhibition (Table III). The results showed the ethanolic extract demonstrated the most cytotoxic effect against McF-7 (230 ±3.1 µg/mL) and HeLa (280 ±1.2 µg/mL) compared to the ethyl acetate and hexane extracts (700 ±.2.5 µg/mL; 1100 ±2.32 µg/mL and 400 ±1.5 µg/mL; 700 ±3.4 µg/mL, respectively). Meanwhile, the ethyl acetate extract (250 ±0.9) showed the most significant effect against HT-29 compared to the other two extracts (300 ±1.94 µg/mL and 1500 ±2.23 µg/mL). When all of these extract were tested on normal cells, Vero cells; they all showed very little effect.

The cytotoxic effect demonstrated by *M. paniculatus* in this study is possibly explained by the presence of flavonoids and other bioactive compounds in the plant.[23] Flavonoids were also claimed to have anti□proliferative and apoptosis□promoting effects

Table III: Anticancer activity of Mallotus paniculatus leave extract against various cancer cells based on IC_{50} values

Cell lines	IC_{50} (µg/mL)*		
	Types of extract solvents		
	Ethanol	Ethyl acetate	Hexane
Breast cancer (McF-7)	230 ± 3.1	700 ± 2.5	400 ± 1.5
Colon cancer (HT-29)	1500 ± 2.23	250 ± 0.9	300 ± 1.94
Cervical cancer (HeLa)	280 ± 1.2	1100 ± 2.32	700 ± 3.4
Kidney epithelial normal cell (Vero)	>1000 ± 1.5	>1000 ± 3.5	>1000 ± 0.8

** Low IC_{50} value indicated the extract was more potent in inhibiting the growth of cancer cells*

against cancer cells apart from the antibacterial and antioxidant activities mentioned above.[31]

They also have the ability to block cell cycle followed by apoptosis.[32] They have recently been used for the treatment of prostate, pancreatic, breast, cervical, and ovarian cancers.[33] From previous studies, flavonoids have been shown to induce cell cycle arrest in G2 and mobile phase of cell cycle, inhibited heat-shock protein, tyrosine kinase and Ras protein as well as downregulated oestrogen receptor-binding capacity. [34] As most pathogenesis of cancer involve genetic abnormalities that result in p53-mutated proteins, downregulation of these proteins by flavonoid intake also affect cancer growth.[34]

Interestingly, other Mallotus species such as *M. apelta* was found to have a strong cytotoxic effect against cancer cells. Benzopyran, 6-[10-oxo-30(R)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran, that was present in the plant was cytotoxic against two human cancer cell lines, human hepatocellular carcinoma and rhabdosarcoma.[35] While another benzopyrans, Malloapelta B detected in the same plant, was shown to be cytotoxic against three cancer cell lines KB (human epidermoid carcinoma), FL (fibrillary sarcoma of the uterus), and Hep-2 (human hepatocellular carcinoma) cells.[35]

CONCLUSION

The current study reveals that *M. paniculatus* extracts possess several potential medicinal activities. Based on the antibacterial results, the ethanolic and ethyl acetate extracts show inhibitory effect against the gram positive bacteria. Meanwhile, for antioxidant property, the ethanolic extract exhibits strong free radical scavenging activity, comparable with the positive control. Interestingly, the ethanol and ethyl acetate extracts record a promising cytotoxic activity against three cancer cell lines; McF-7, HeLa, and HT-29. These indicate that this plant contains significant compounds with potential medicinal properties.

CONFLICT OF INTEREST

Nothing to declare

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Not applicable

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