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Antibacterial and Antifungal Activities of Leaf Extract of *Morinda elliptica*

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ABSTRACT

This work was designed to evaluate the antimicrobial potential of *Morinda elliptica* by determining the zone of growth inhibition of the leaf extract against selected bacterial and fungal strains. Antifungal and antibacterial properties of the extract at different concentrations (25, 50, 100, 250 and 500 μg/ml) were investigated after successive maceration in four solvents in order of increasing polarity [hexane (180 g), dichloromethane (342 g), ethyl acetate (471 g), and methanol (384)]. The agar disc diffusion method was used against selected human bacteria *Escherichia coli*, *Salmonella typhii*, *Staphylococcus aureus*, and the antifungal activity of the extract against *Aspergillus brasiliences* and *A. flavus*. Zones of growth inhibition of the extract were then compared with the standard antibiotic chloramphenicol (500 μg/ml) for the antibacterial activity, and against nystatin (500 μg/ml) for antifungal activity. The result of the study showed a remarkable bactericidal activity of the plant extract against the test organisms *E. coli* (14.667 $\pm$ 0.577) and *S. typhii* (13.667 $\pm$ 0.577) with a weak activity against the growth of *S. aureus* as compared to standard (21.667 $\pm$ 0.577) at 500 μg/ml. The result of the antifungal activity showed considerable activity of the plant extract against the growth of *A. brasiliences* (11 $\pm$ 0.1000) and weak activity against the growth *A. flavus* at 500 μg/ml. The findings of the study indicated that the leaf extract of *M. elliptica* is a reservoir of bioactive compounds. The compounds can be useful in the development of new pharmaceutical products that can be effective against human pathogenic strains *E. coli* and *S. typhii.* This could serve as a lead for understanding a novel mechanism of action in future research activities.

Keywords: Antimicrobial activity, bacterial strain, fungal strains, *Morinda elliptica,* zone of inhibition

INTRODUCTION

Since their introduction, antibiotics have been of tremendous importance in combating infections caused by bacteria and improving the quality of human well-being. However, over time some commonly used antibiotics have become less effective in combating infections and the health benefits of these antibiotics are under threat. This is because many of them produce toxic reactions that are hazardous to humans, as well as the emergence of drug-resistant species of bacteria (Nayan *et al.*, 2011). The aforementioned limitations, coupled with the increasing cost of synthetic drugs in primary health care has made the traditional system of medicine (herbs and plant-derived products) popular, especially in developing countries. Thus, the interest in natural sources of drug-derived products has become increasingly significant for the discovery of plant materials (natural products) that could combat these drug-resistant species of microorganisms, which can play a significant role in the prevention and treatment of human diseases (Nayan *et al*., 2011). It has been reported that in many developing countries (e.g Nigeria, Cameroon, Sri Lanka, Ghana, Brazil etc.), traditional medicine is one of the basic primary health care systems (Fransworth, 1993; Houghton, 1995; Srikaran & Salika, 2019) providing basic health care needs. The effects of plant extract on bacteria and fungi have been studied by many researchers worldwide (Reddy *et al*., 2001; Prashanth *et al*., 2006; Runyoro *et al*., 2006; Srinivasan *et al*., 2009; Nayan *et al*., 2011; Maria *et al*., 2016) and natural products of higher plants may give a new source of antimicrobial agents with a possibly novel mechanism of action (Vimala *et al*., 2003; Shahidi, 2004).

It is therefore pertinent to reduce the risk associated with using antibiotics and drug-resistant bacteria, to encourage research about the resistance of microorganisms and to develop new drugs, either synthetic or natural to control pathogenic microorganisms (Nayan *et al*., 2011). In an effort to contribute to these leagues of research trends into discovering natural sources of antimicrobial agents, *Morinda elliptica* was selected. *Morinda elliptica* is a shrub or small tree, a genus of the family Rubiaceae (locally called Mengkudu Kecil in Malay) and growing wild in newly developed areas or bushes throughout the Malay Peninsula. It is one of the seven species of *Morinda* found in Malaysia. It is used in traditional medicine in Malaysia in various ways for many health problems and ailments. The leaf is added to rice to boost appetite and taken for headache, cholera, diarrhoea, and particularly a fever. The pounded leaf is applied to the spleen and wounds, and a lotion of leaves is used for haemorrhoids and rubbed on the body after childbirth. It has been reported to contain strong antioxidant activity (using Ferric thiocyanate antioxidant assay and thiobarbituric acid antioxidant assay methods and absorbance below alpha-tecopherol level of 0.30 nm were observed) (Leonjang *et al*., 2015) and anthraquinones (Morimoto, 1998; Ali *et al*., 2000). This work was aimed at exploring the vast medicinal potential of this medicinal plant, and to add to the library of information regarding *M. elliptica.*

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Morinda elliptica* were used for the preparation of the crude extracts. The leaves were collected from uncultivated farm-land in Limbang-Sarawak. It was then identified and authenticated at Faculty of Resource Science and Technology (FRST), Universiti Malaysia Sarawak and given a voucher specimen number of WH/MEL015/03. It was left for 15 days to dry under room temperature.

Preparation of Plant Extract

The dried leaves of *M. elliptica* were ground into powdered form using a laboratory grinder machine. Serial extraction was done with four different solvent systems (n-hexane, dichloromethane, ethyl acetate, and methanol). About 100 g of the powdered sample was extracted using the cold soaking method with hexane. The sample was soaked in the hexane with the ratio of 1:3 in 5 L Erlenmeyer flasks at room temperature for 5 days. The resulting hexane solution was then filtered using Whatman filter paper No. 4 and the residue was then re-extracted (washing) with fresh hexane and filtered. Both extracts were combined and evaporated with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure below 50 ^{o}C to obtain the hexane crude extract. The residue was re-extracted using a similar procedure with dichloromethane, followed by ethyl acetate and methanol to obtain respective crude extracts. Stock solutions of the extracts (5 mg/ml) were prepared by dissolving a known amount of the dry extract in 98% methanol. The working solution of each extract (1, 10, 50, 100 and 500 mg/ml) was prepared from the stock solution using suitable dilution.

Microorganisms

In this study, bacteria strains *Escherichia coli, Salmonella typhii, Staphylococcus aureus* and fungal strains *Aspergillus brasiliences* and *A. flavus* were selected. The bacterial and fungal strains were obtained from UNIMAS, and were used for antimicrobial activities. The stock cultures of the bacteria and fungi were incubated at 37 ^{o}C for 24 h on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, and were stored at 4 ^{o}C. Plates containing Mueller-Hinton agar (MHA) were used to grow the bacterial strains at 37 ^{o}C and the fungal strains were grown in PDA media at 27 ^{o}C. The stock cultures were then kept at 4 ^{o}C (Boyan *et al*., 2008).

Antimicrobial Activity (Determination of Zone of Growth Inhibition)

Morinda elliptica leaves extracts were examined for antimicrobial activities. Antibacterial activities of the leaf extract was determined against three pathogenic bacterial strains *E. coli*, *S. typhii* and *S. aureus*, and the antifungal activity was determined against two fungal strains *A. brasiliences* and *A. flavus*, using agar disk diffusion method as reported by previous studies (Boyan *et al*., 2008; Prashanth *et al*., 2006; Apu *et al*., 2010). The extract was dissolved using dimethyl sulfoxide (DMSO) and sterilised then stored at 4 ºC prior to use. The zones of inhibition of the pure strains of the bacteria and fungi were compared against standard antibiotics. The extracts were then screened for their antimicrobial activity against the bacterial and fungal strains. A set of five dilutions were prepared for antimicrobial activity (25, 50, 100, 250 and 500, µg/ml) of the leaves extract of *M. elliptica,* and standard drugs (chloramphenicol and nystatin for antibacterial and antifungal, respectively) were prepared in distilled water. Sterile plates containing Mueller-Hinton agar were seeded with indicator bacterial strains and control experiment using chloramphenicol and nystatin (for antibacterial and antifungal respectively) as standard drugs and were kept on bench for 3 h at 37 ºC. They were then incubated for 18 to 24 h for bacterial strains and 48 to 96 h for fungal strains at 37 ºC in an incubator. The zones of growth inhibition around the disks were measured in mm. The antimicrobial activity of the test organisms on the plant extracts was determined by measuring the size of the inhibitory zones (this include the diameter of the disk) on the surface of the agar around the disk. The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition was calculated.

Statistical Analysis

From the data, the mean values for the zones of growth inhibition of the plant extracts were calculated using statistical software 22 and the result was expressed at a 95% level of confidence ($p<0.05$). The values of ≥9 mm were considered active against the microorganism for antibacterial activity while the values ≥15 mm were considered active for antifungal activity (Prashanth *et al.,* 2006).

RESULTS AND DISCUSSION

Over the years, there has been a tremendous increase in the report of antimicrobial properties of medicinal plants by researchers worldwide. This has contributed enormously to the understanding and discovery of natural agents that could be effective in combating microorganism in human health delivery. In this study, the leaf extracts of *Morinda elliptica* showed significant bactericidal effects against the test bacterial strains *Escherichia coli, Salmonella typhii* and *Staphylococcus aureus,* as shown in Table 1 below.

Table 1. Mean values of the zone of growth inhibition of extracts of *M. elliptica* at 500 µg/ml of leaves extract and chloramphenicol

Microorganism	Treatment	Crude extracts Diameter of zone of inhibition (mm)			
		Hexane	Dichloromethane	Ethyl acetate	Methanol
Escherichia coli	Chloramphenicol	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577
	DMSO	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001
	Extract	14.667 ± 0.577*	11.667 ± 0.577*	11.001 ± 0.00*	10.667 ± 0.577*
Salmonella typhii	Chloramphenicol	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577
	DMSO	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001
	Extract	13.667 ± 0.577*	11.333 ± 1.155*	11.333 ± 1.155*	8.667 ± 1.155
Staphylococcus aureus	Chloramphenicol	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577	21.667 ± 0.577
	DMSO	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001	2.025 ± 0.001
	Extract	11.333 ± 1.155*	10.667 ± 1.155*	10.667 ± 1.155*	10.333 ± 1.155*

Note: '*' indicates significance between treatments, $p<0.05$

There was a remarkable zone of growth inhibition of the leaf extracts against the test bacterial strains. However, moderate activity was observed against *E. coli* with a zone of growth inhibition of 14.667 ± 0.577 mm in hexane and 13.667 ± 0.577 mm against *S. typhii* in the hexane extract as compared to the standard drug chloramphenicol, which exhibited a zone of growth inhibition of 21.667 ± 0.577 mm (Table 1). The findings are congruent to the studies reported on the effects of medicinal plants on *E. coli* and *S. aureus* by some authors (Daljit & Jasleen, 1999; Nayan & Shukla, 2011; Gayathri *et al*., 2016; Sandeep & Abhilasha, 2018; Sinulingga *et al*., 2018).

Table 2. Mean values of the zones of growth inhibition of leaves extracts of *M. elliptica* leaf against *A. brasiliences* at different concentrations

Solvent system	Zone of inhibition (mm) Concentration in µg/ml				
	25	50	100	250	500
Nystatin	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000
DMSO control	30 ± 0.840	30 ± 0.840	30 ± 0.840	30 ± 0.840	30 ± 0.840
Hexane	30 ± 0.1155	29 ± 0.0764	24 ± 0.4160	20 ± 0.0764	14 ± 0.1000*
Dichloromethane	32 ± 0.100	30 ± 0.257	27 ± 0.840	21 ± 0.416	14 ± 0.840*
Ethyl acetate	33 ± 0.4160	30 ± 0.4160	27 ± 0.1000	21 ± 0.8400	13 ± 0.0764*
Methanol	31 ± 0.1155	28 ± 0.2570	26 ± 0.1155	18 ± 0.4160	11 ± 0.1000*

Note: '*' indicates significance between treatments, $p<0.05$

Table 2 shows the antifungal activity of the leaves extract of *M. elliptica* observed in all the extracts against *A. brasiliences* with the mean value of zone of growth inhibition ranging from 11 ± 0.1000 to 14 ± 0.840 mm. The results are in line with the findings by Apu *et al*. (2010). However little activity was observed against *A. flavus* in all extracts except for ethyl acetate, where a significant zone of growth inhibition was observed to be 15 ± 0.416 mm at 500 µg/mL as compared to the standard drug nystatin (Table 3).

Table 3. Mean values of the zones of growth inhibition of leaves extracts of *M. elliptica* leaf against *A. flavus* at different concentration

Solvent system	Zone of inhibition (mm) Concentration (µg/ml)				
	25	50	100	250	500
Nystatin	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000	4 ± 0.1000
DMSO control	30 ± 0.840	30 ± 0.840	30 ± 0.840	30 ± 0.840	30 ± 0.840
Hexane	40 ± 0.1155	36 ± 0.416	33 ± 0.840	28 ± 0.416	20 ± 0.416
Dichloromethane	38 ± 0.416	37 ± 0.1155	32 ± 0.229	27 ± 0.1258	25 ± 0.229
Ethyl acetate	32 ± 0.1155	27 ± 0.229	22 ± 0.416	19 ± 0.1155	15 ± 0.416*
Methanol	35 ± 0.840	31 ± 0.840	27 ± 0.416	24 ± 0.840	19 ± 0.1258

Note: '*' indicates significance between treatments, $p<0.05$

The results in Table 2 exhibit an increase of activity with the increase of the concentration of the leaf extract. The greater activity was observed at the highest concentration of the plant extract, at 500 µg/ml. The increase of activity indicated that the leaf extract of *M. elliptica* contains phytochemicals, which are potential sources of antimicrobial agents. The result is congruent to the findings by West *et al*. (2012) who observed a concentration dependent decreases in cell growth in all the organisms tested, suggesting that the phytochemicals possess antibacterial properties. The antimicrobial property could be due to the presence of secondary metabolites in the leaves as reported by Ismail *et al*. (2000).

CONCLUSION

The results obtained from this study revealed that the leaf extract of *M. elliptica* contains potential antimicrobial agents. This shows that the plant can be a valuable natural source for the treatment and discovery of novel phytochemicals that could be effective against antimicrobial infections and drug-resistant microorganisms.

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