Direct Research Journal of Biology and Biotechnology
Vol. 8(3), Pp. 19-23, November 2022
ISSN 2734-2158
DOI: https://doi.org/10.26765/DRJBB698217430
Article Number: DRJBB698217430

https://directresearchpublisher.org/drjbb

Full-Length Research Paper

Study on Antibacterial and Antifungal Potentials of Root Extract of *M. elliptica*

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Received 9 August 2022; Accepted 29 September 2022; Published 30 November 2022;

ABSTRACT: The study was designed to assess the antimicrobial activity of the root extract of *Morinda Elliptica* after successive maceration in four solvents (hexane, dichloromethane (DCM), ethyl acetate and methanol) by determining the zone of growth inhibition against selected human pathogenic bacteria, *Escherichia coli*, *Salmonella typhii, Staphylococcus aureus* and fungi, *Aspergillus Brasiliensis and Aspergillus Flavus.* Agar disc diffusion method was used in the determination of the antimicrobial potentials. The mean values of zones of growth inhibition of the various extracts were then compared with standard antibiotic (chloramphenicol), antifungal drug (nystatin) and dimethyl thiosulphate (DMSO) using a statistical software SPSS 22 and the result was expressed at 95 % level of confidence. A remarkable antibacterial was observed against all the bacterial strains at 500 µg/ml. The result of the antifungal potential also shows a considerable zone of growth inhibition in ethyl acetate and methanol extracts against *A. niger* and in DCM against *A. flavus* at 1000 µg/ml. The findings in this study indicates the extract contains active phytochemicals that are potential sources for the discovery of bioactive antibacterial agents that could contribute in the search for antimicrobial agentsin the fight against drug resistant bacteria species from natural sources.

Keywords: Antimicrobial activity, *Morindaelliptica,* bacterial strain, fungal strains, inhibition

INTRODUCTION

In recent years' research on medicinal plants have attracted a lot of attention globally and researchers have reported quite a number of promising findings especially on antimicrobial potentials of these plants. These findings demonstrated the promising potentials of medicinal plants that are being used in different systems of medicine in treating human diseases especially microbial infections. Some plants that are rich in secondary metabolites have been found in vivo to contain antimicrobial properties (Dahanukar et al., 2000; Boyan et al., 2005; Nayan et al., 2011), and clinical microbiologist becomes interested in plant extracts exhibiting medicinal potentials. Most of the antimicrobial phytochemicals from plants will find their way into the arsenals of antimicrobial drugs (Nayan et al., 2011) and this will boost the fight against human pathogenic microbes especially the drug resistant species. Some species of fungi like *chalaraparadoxa* which are responsible for black rot of post-harvest fruits also affect human health, likewise the chemical fungicides (Maria et al., 2015), thus the search for effective natural sources by researchers is necessary. Medicines derived from herbs have been the basis of treatment and cure for various diseases in traditional methods (Prashanth et al., 2006). Several species are being used by different ethnic groups to meet basic health needs ranging from minor infections to other ailments like dysentery, asthma, diabetes, malaria and other indicators (Dahanukar et al., 2000; Prashanth et al., 2006; Runyoro et al., 2006).

In the past few decades, dramatic increases have been reported about microbial resistance to antimicrobial agents (Chopra et al., 1996; Baquero, 1997) and this has led to repeated use of antibiotics with resulting insufficient control of diseases (NCID, 2002). This prompt researchers to explore natural sources like medicinal plants, with the view of finding natural agents that will be effective against the drug resistant microorganism, or understanding their mechanism of actions, and tremendous achievement has been recorded. Also searching for new lead compounds to be developed as drugs or as templates for analogue synthesis, and the evaluation of traditional medicine and herbal medicinal products, are the two basic reasons for the advancement of work on medicinal plants. This could also be partly justified by the fact that natural product inspired molecules represented about 80 % of drugs that had been put into the market (Raphael, 2011; Ntie-Kang et al., 2013). This prompted the choice of *M. elliptica.*

In Peninsular Malaysia, *Morinda* comprises nine species (Wong, 1984) which includes *Morindaelliptica*, a shrub or small tree. It is a genus of the Rubiaceae family (locally called Menkudukacil in Malay) and growing wild in newly developed areas or in bushes throughout the Malay Peninsula. It is used in traditional medicine in Malaysia (Vimala et al., 2003) and has been reported to contain strong antioxidant activity (Leonjang et al., 2015) and anthraquinones (Vimala et al., 2003; Ali et al., 2015).

The aim of this work is to explore the antimicrobial potential of *M. elliptica* to further boost the medicinal potential of the plant. Furthermore, the study will serve as a baseline for the discovery of novel phytochemicals that could be effective against infections and drug resistant microorganism.

MATERIALS AND METHODS

Plant materials

Fresh root of *M. elliptica* was used for the preparation of the crude extracts, the root was collected from an uncultivated farm land in Limbang-Sarawak and was identified and authenticated in Faculty of Resource Science and Technology, Universiti Malaysia Sarawak by Prof. Dr. Zaini B. Assim and was given a voucher room specimen number WH/MER015/05, it was dried under temperature.

Preparation of plant extract

The freshly dried root of *M. elliptica* was grounded into powdered forms using laboratory grinder machine (FGR-350, Quest Scientific), serial extraction was done with four different solvent systems (n-hexane, dichloromethane (J.T. Baker), ethyl acetate and methanol (Merck KGaA). 100 g of the powdered sample was weighed into an Erlenmeyer flask and each solvent (three times the weight of the extracts) was added successively, the solution was covered and shaken at time interval of an hour and then allow to stand for 7 days at room temperature. The mixture was then filtered using Whatman filter paper No.4 and the solvent was evaporated using a rotary evaporator (Heidolph Laborato 400, Germany) under reduced pressure below 50°C. The stock solution of the extracts (5 mg/ml) as prepared by dissolving known amount of the dry extract in 98% methanol. Working solution of each extract (1, 10, 50, 100, 500 and 1000 ppm) was prepared from the stock solution using suitable dilution.

Test microorganism

Bacterial strains *Escherichia coli* (*E-coli*), *Salmonella typhii*(*S. typhii*), *Staphylococcus aureus* (*S. aureus*) and fungal strains *Aspergillusbrasiliences*(*A. brasiliences*) *and Aspergillusflavus* (*A.* flavus) were selected for the study. The bacterial and fungal strains were obtained from the microbiology laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak and were used for the antimicrobial activities. The stock cultures of the bacteria and fungi were incubated at 37°C for 24 hours on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India) respectively, and were stored at 4 °C. Plates containing Mueller-Hinton agar (MHA) were used to grow the bacterial strains at 37°C and the fungal strains were grown in PDA media at 27°C. The stock cultures were then kept at 4℃.

Antimicrobial activity (Determination of zone of growth inhibition)

Antimicrobial activities were examined for *M. elliptica*root extracts. Antibacterial activity of the root extract of the plant 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. niger and A. flavus,* using agar disk diffusion method as reported by various authors (Prashanth et al., 2006; Morimoto et al., 1998; Boyan et al., 2005). The extract was dissolved using dimethyl sulfoxide (DMSO) and sterilized by filtration and stored at 4 °C until use. Standard antibiotics were used for comparison of the zone of inhibition of the pure strains of the bacteria and

Table 1: Mean values of the antibacterial activity of root extract of *M. elliptica* in different solvents at 500 µg/ml.

Bacteria spp	**Diameter of zone of growth inhibition in mm**					
	Concentration in µg/ml					
	Chloramphenicol	**DMSO**	**Hexane**	**DCM**	**Ethyl acatate**	**Methanol**
E-coli	21	2	14*	12*	14*	13*
S. typhii	21	2	7	11*	12*	11*
S. aureus	21	2	13*	9*	9*	13*

*= significant activity observed Result is Mean $\pm$ SD. N = 3

Table 2: Mean values of the antifungal activity of the root extract of *M. elliptica* against *Aspergillus brasiliences* at different concentrations

	Diameter of zone of growth inhibition in mm							
Solvent	**Concentration in µg/ml**							
	Control	**Nystatin**	**25**	**50**	**100**	**250**	**500**	**1000**
Hexane	34	4	34	40	32	28	25	16*
DCM	31	4	28	33	31	28	25	16*
EA	31	5	32	33	31	28	25	14*
Methanol	34	4	30	34	42	25	22	15*

*= significant activity was observed; Result is Mean $\pm$ SD. N = 3

fungi. The extracts were then screen for their antimicrobial activity against the bacterial and fungal strains. Set of four dilutions for antibacterial activity (50, 100, 250, 500 µg/ml) and five dilutions for antifungal activity (50, 100, 250, 500 and 1000 µg/ml) of the root extract of *M. elliptica*and standard drugs (chloramphenicol and nystatin for antibacterial and antifungal respectively) were prepared. 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 were kept for 3 hours at 37 ^{o}C. They were then incubated for 18 to 24 hrs for bacterial strains and 48 to 96 hours for fungal strains at 37^{o}C, and the zones of growth inhibition around the disks were measured in mm. The antimicrobial activity of the test organisms on the plant extracts were 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, and the values <9 mm were considered as not active against the microorganism for antibacterial activity and <16 mm were considered as active for antifungal activity. 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 a statistical software SPSS 22 and the result was expressed at 95 % level of confidence (Nayan et al., 2011; Apu et al., 2010).

RESULTS

The results of this study show that *M. elliptica* root extract contains active antimicrobial agents that are effective against the test organisms. Table 1 displays the mean values of zone of growth inhibition of *M. elliptica* root extract against bacterial strains in mm. The results show that the root extract is effective against the test organisms at 500 µg/ml, with the exception of *S. typhii*, which did not show significant activity in the hexane extract. In comparison to the standard drug nystatin, the results in (Table 2) show significant activity of zone of growth inhibition against *Aspergillus niger* at 1000 µg g/ml in all extracts. The results in (Table 3) did not show any significant activity against *Aspergillus flavus* in any of the extracts when compared to the standard drug nystatin.

DISCUSSION

The results of this study show that the root extract of *M. elliptica* contains antimicrobial properties. Except for the result observed in the hexane extract against *S. typhii,* a remarkable antibacterial activity of the root extract of *M. elliptica*was observed against the test organism (bacterial strains, *E-coli*, *S. typhii* and *S. aureus*) in the other extracts as compared to the standard drug chloramphenicol (Table 1). The mean zone of growth

Table 3: Mean values of the antifungal activity of the root extract of *M. elliptica* against *Aspergillus flavus* at different concentrations

	Diameter of zone of growth inhibition in mm							
Solvent	**Concentration in µg/ml**							
	Control	**Nystatin**	**25**	**50**	**100**	**250**	**500**	**1000**
Hexane	44	4	44	40	41	438	34	24
DCM	46	5	45	44	45	42	36	18
EA	47	5	47	46	46	39	33	21
Methanol	45	5	43	42	42	37	36	24

Result is Mean ± SD. N = 3

inhibition observed was between 12 -14 against *E-coli*, 11 -12 against *S. typhii* and 9-13 against *S. aureu*s at a concentration of 500 µg/ml. The results exhibit a concentration dependant increment which is an indication that the root extract contains active antibacterial activity. The result obtained in this study is congruent to the findings of Ali *et al*. (2011); Rula and Talal, (2010). Similar antibacterial activity of medicinal plants in hexane and methanol extract against *E-coli* and *S. aureus* were reported by other authors (Nayan et al., 2011; Daljit and Jasleen, 1999; Srinivasan et al., 2009; Mohamed et al., 2010; Opawale et al., 2011). The root extract also exhibited antifungal property against the test fungal strain *A. brasiliences* in all the extracts at 1000 µg/ml (Table 2), the mean vales of the zone of growth inhibition observed is between 14 -16 mm as compared to the standard drug nystatin. These findings were congruent with past studies reported by other researchers on the effects of medicinal plants against *A. brasiliences* (Apu et al., 2010; Alzoreky MS. and Nakahara, 2003; Biruhalem., 201; Haniyeh et al., 2010]. The result in (Table 3) however show the root extract of *M. elliptica* did not exhibit reasonable activity against *A. flavus*as compared to the standard drug nystatin at all concentrations, this might be because of the plant part used or the concentration of the extract used may be little to exhibit remarkable activity. It has been reported that plant has continued to be a rich source of therapeutic drugs and the active principles of many drugs are found in plants or are produced as secondary metabolites (Boyan et al., 2005; Runyoro et al., 2006; Daljit and Jasleen, 1999). Therefore, the findings in this study show the root extract of *M. elliptica* has the potential to be used as a valuable source of natural antimicrobial agents.

Conclusion

The result obtained from this study revealed the root extract of *M. elliptica* contains potential antimicrobial agents, especially against the bacterial strains *E-coli*, *S. typhii* and *S. aureus* and the fungal strain *A. brasiliences.* this shows 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 microorganism.

Acknowledgement

The authors are grateful to Unimas for the grant provided to support this research work (grant number F07 (DPP19)/1187/2014(19)).

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