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PHYTOCHEMICAL AND ANTI-MICROBIAL POTENTIAL OF *Mallotus mollissimus* AND *Solanum erianthum* EXTRACTS

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Abstract

Mallotus mollissimus (*M. mollissimus*) and *Solanum erianthum* (*S. erianthum*) plants have been reported to possess medicinal properties and have been effectively used by indigenous communities. However, the precise compositional and anti-microbial properties of these plants remain unclear. Hence, this study aims to investigate the qualitative phytochemicals and anti-microbial properties of the extract from *M. mollissimus* and *S. erianthum*. Anti-microbial activities and phytochemical studies were carried out using crude methanolic extract, chloroform fractions and selected chromatography fractions of *M. mollissimus* and *S. erianthum*. Anti-microbial activities targeting Gram-positive and Gram-negative bacteria were performed using the disk diffusion method at 100 mg/mL. *M. mollissimus* have superior anti-microbial activities as compared to *S. erianthum* where *Streptococcus pneumoniae* were inhibited by CE.F3 fraction of *M. mollissimus* with an average inhibition diameter of 7.0 mm ± 0.48. We qualitatively determined the alkaloids, steroids, and cardiac glycosides in both plant extracts using biochemical assays. Interestingly, flavonoids, terpenoids, and tannins were found in *M. mollissimus,* which were correlated to the inhibition of *Streptococcus pneumoniae*. These findings indicate that *M. mollissimus* and *S. erianthum* contains large phytochemicals which give rise to anti-microbial effects.

INTRODUCTION

Anti-microbial resistance (AMR) is an increasing public health problem on a global scale. It was estimated that there are roughly 2,000 daily death cases caused by AMR [1]. The World Health Organization's (WHO) Global Antimicrobial Resistance Surveillance program observed high levels of AMR bacteria of *Escherichia coli, Klebsiella pneumoniae, Salmonella spp., Staphylococcus aureus* and *Streptococcus pneumoniae* [2]. Among the lists of AMR bacteria identified by WHO as a health threat, *Staphylococcus aureus* is among the species that are prevalent in various human diseases such as epidermal infections and septicemia [3]. The antibiotics treatment options for AMR cases have been limited, and AMR mortality is estimated to be up to ten million deaths by the year 2050 [1]. This raising concern has encouraged the search for novel anti-microbial agents.

Plants have been used in medicinal treatment in various forms, such as teas, syrups, or paste, by indigenous communities and ethnic groups [4, 5]. Plants such as *Mallotus* (Euphorbiaceae) and *Solanum* (Solanaceae) genera were used for a wide array of traditional medicinal applications. *M. peltatus* (Geist) leaf extract has been used to treat skin infections, intestinal ailments and trematodic infections [6]. *M. japonicus* and *M. peltatus* leaves extracts have been shown to possess antioxidant, anti-microbial and anti-inflammatory properties [6-8].

Solanaceae is among the family and includes a large number of species that typically grow in tropical and temperate regions. *Solanum erianthum* (*S. erianthum*) is one of the species included in the Solanaceae family, which

originates from the West Indies, Central America and Mexico. Nowadays, it is widespread in the tropics. Being a tropical plant, Asians have used the leaves for the treatment of leucorrhoea, and also as an abortifacient [9]. Therefore, this study reports the phytochemical contents and anti-microbial activities by the leaves of *M. mollissimus* and *S. erianthum*.

MATERIALS AND METHODS

Materials

Methanol, chloroform, silica gel chromatography, Wagner reagent, hydrochloric acid, magnesium tape, sulfuric acid, isopropyl alcohol, acetic anhydride, ammonium hydroxide and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. *M. mollissimus* and *S. erianthum* leaves were collected from Kampung Seri Aman and Kampung Salimandut, Kota Marudu, Sabah. Voucher specimens (BORH 0969 and BORH 0971) were deposited at BORNENSIS, Institute for Tropical Biology and Conservation.

Sample Preparation

The liquid extraction method was used to obtain *M. mollissimus* and *S. erianthum* extracts. Briefly, leaves were air dried at room temperature and 500g of leaves soaked in methanol for three days, followed by filtering and concentrating to 10% (v/v) from the initial volume. The concentrated extraction was fractionated to yield chloroform extract (CE) [10]. Upon confirmation of phytochemical and anti-microbial activities, CE extract was further fractionated using methanol:chloroform (1:19) on a silica gel column chromatography (30x3cm i.d) (Sigma-Aldrich, St. Louis, MO, USA). The elution was collected in every 5 mL of elution, with a total of 10 fractions (F1-F10). All fractions were screened for anti-microbial activity and phytochemical components.

Bacterial Strain

Gram-positive (*Bacillus cereus;* ATCC 11778*, Bacillus subtilis;* ATCC 43223*, Staphylococcus aureus;* ATCC 25923*, Streptococcus pneumoniaee;* ATCC 6303) and Gram-negative (*Enterobacter aerogenes;* ATCC 13048*, Escherichia coli;* ATCC 35218*, Proteus vulgaris;* ATCC 6380*, Pseudomonas aeruginosa;* ATCC 9027*, Salmonella typhi;* ATCC 14028) bacteria were used for anti-microbial screening using disk diffusion assay.

Inoculums Preparation and Antibacterial Activity of Plant Extract

Briefly, the tested Gram-positive and Gram-negative bacteria were cultured overnight and prepared at a turbidity of 0.5 McFarland standard. Then, 20 µL of 100 mg/mL extract and positive controls of (amoxicillin and gentamicin) was pipetted on a 5 mm diameter filter disc and left to dry. Then, the filter discs were placed on agar inoculated with strains of Gram-positive or Gram-negative bacteria, respectively. Zones of inhibition (mm ± S.D.) were recorded using a Vernier caliper.

Phytochemical Analysis

M. mollissimus and *S. erianthum* extract and fractions were subject to phytochemical analysis using biochemical assays to confirm the presence of secondary metabolites such as alkaloid, saponins, steroids and tannins [10]; flavonoids [10, 11]; terpenoid and phlobatannins [10, 12]; anthraquinones and cardiac glycosides [10, 13], respectively.

Alkaloids were qualified by mixing 1 mL of extract (10 mg/mL) with a few drops of Dragendroff reagent. The presence of orange colour in the tube indicated the presence of alkaloids [10].

Saponin was detected by mixing 20 mg of extract with 5 mL of water followed by vigorous vertexing. A persistent froth at a depth of 3cm indicated the presence of saponin [10].

The steroid was detected by mixing 1 mL of 20 mg/mL of extract with 1 mL of chloroform. Then, 1 mL of concentrated sulfuric acid were added slowly. Formation of brownish red ring in between two layers of solvents indicated the presence of steroid [10].

The presence of tannin was detected by mixing 1 mL of 10 mg/mL of extract with 1 mL of $FeCl_3$. The formation of black-green indicated the presence of tannin [10].

Willstatter-Sianidin test were used to determine the flavonoid contents. Briefly, concentrated hydrochloric acid were added into tube containing 1 mL of 10 mg/mL of extract followed by 3 cm of magnesium tape. Colour changes from orange to red indicating flavonoids, meanwhile further changes from red to crimson and crimson to magenta validated the flavonoid in the extract [10, 11].

Detection of terpenoid was performed by dissolving 20 mg of extract into 5 mL of chloroform followed by slowly adding 0.5 mL of acetic anhydride and 0.5 mL of sulfuric acid [10, 12].

Phlobatannin was detected by boiling of 1 mL of 10 mg/mL of extract with 2 mL of 1% (v/v) hydrochloric acid.

The deposition of red precipitate indicated the presence of phlobatannin [10, 12].

Anthraquinone was detected by boiling 20 mg of extract in 10% (v/v) of hydrochloric acid for a few mins. Then, chloroform was added into the mixture followed by adding a few drops of 10% ammonium hydroxide. The formation of pink colour indicated the presence of anthraquinone [10, 13].

The detection of cardiac glycoside was performed by mixing 2.5 mL of extract with 1 mL of acetic acid. Then, one drop of $FeCl_3$ was added followed by 1 mL of concentrated sulfuric acid. The formation of brownish ring indicated the presence of cardiac glycoside [10, 13].

RESULTS AND DISCUSSION

The extracts of *M. mollissimus* and *S. erianthum* demonstrated similar anti-microbial activity against Gram-positive and Gram-negative bacteria. Methanol extract (ME) of *M. mollissimus* exhibits anti-microbial activity (mm ± S.D.) against Gram-positive of *Staphylococcus aureus* (6.5 ± 0.82), *Streptococcus pneumoniae* (7.3 ± 0.75); and Gram-negative of *Pseudomonas aeruginosa* (8.0 ± 0) (Table 1). The CE exhibits anti-microbial activity (mm ± S.D.) against Gram-positive *Bacillus cereus* (9.5 ± 0.71) *Staphylococcus aureus* (6.5 ± 0.82), *Streptococcus pneumoniae* (7.3 ± 0.75); and Gram-negative of *Pseudomonas aeruginosa* (8.0 ± 0). Further fractionation of CE was performed, and we observed that only CE.F2 and CE.F2 exhibited anti-microbial activity. Other fractions did not exhibit anti-microbial activity. CE.F2 possesses anti-microbial activity (mm ± S.D.) against *Staphylococcus aureus* (7.5 ± 0) and *Streptococcus pneumoniae* (7.0 ± 0.58). CE.F3 show anti-microbial activity (mm ± S.D.) against *Bacillus cereus* (8.0 ±0), *Staphylococcus aureus* (7.0 ± 0.48), *Streptococcus pneumoniae* (7.1 ± 0.58) and *Pseudomonas aeruginosa* (7.0 ± 0.48).

We observed that methanol extract (ME) of *S. erianthum* shows anti-microbial activity (mm ± S.D.) against *Staphylococcus aureus* (6.9 ± 0.25) and *Pseudomonas aeruginosa* (8.3 ± 0.50). The CE extract of *S. erianthum* shows a broader anti-microbial activity compared to *M. mollissimus*. CE extract shows anti-microbial activity (mm ± S.D.) against *Bacillus cereus* (7.5 ± 0.71), *Staphylococcus aureus* (7.3 ± 0.61), *Streptococcus pneumoniae* (6.9 ± 0.58), and *Pseudomonas aeruginosa* (13.3 ± 0.5). Further fractionation and anti-microbial testing of CE shows that three fractions (CE.F1, CE.F2, and CE.F9) exhibit anti-microbial properties. CE.F1 showed growth inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 7.0 ± 0 and 7.9 ± 0.25 inhibition zones (mm ± S.D.), respectively. CE.F2 demonstrated inhibition activities (mm ± S.D.) against *Bacillus cereus* (8.0 ± 1.41) , *Staphylococcus aureus* (7.3 ± 0.61), *Streptococcus pneumoniae* (6.9 ± 0.58), and *Pseudomonas aeruginosa* (13.3 ± 0.50). Finally, CE.F9 only showed anti-microbial activity against *Staphylococcus aureus*, with an inhibition zone of 7.0 mm ± 0. Plants contain huge and diverse metabolites such as aromatic amino acids, branch chain amino acids, chlorogenic acid, flavonoids and phenylpropanoids [14]. Hence, after fractionation into multiple fractions, the present study observed a wide and diverse anti-microbial activity in selected fractions that contains potential anti-microbial agents as supported by the phytochemical profiles.

Phytochemical analysis shows that *M. mollissimus* and *S. erianthum* have different phytochemical profiles, which constitutes the anti-microbial activity of extracts against various Gram-positive and Gram-negative bacteria (Table 2). *M. mollissimus* contains alkaloid, flavonoids, steroids, terpenoid, tannins and cardiac glycosides. Meanwhile, saponins, phlobatannins and anthraquinones were absent. The phytochemical analysis *of S. erianthum* shows that alkaloid, saponins, steroids and cardiac glycosides were present and flavonoids, terpenoid, tannins, phlobatannins and anthraquinones were absent.

Flavonoids were found in photosynthesis cells, which occur widely in the plant kingdom [15]. It is interesting that we found flavonoids in *M. mollissimus*, and not in *S. erianthum*. The presence of flavonoids in ME of *M. mollissimus* correlated to the anti-microbial activity against *Streptococcus pneumonia*, which was not observed in *S. erianthum*. Flavonoids have been shown to contain antibacterial properties in other studies [16]. Flavonoids are bacteriostatic compounds that reduce colony viability by inducing bacterial aggregate formation via various potential mechanisms of action [17]. Flavonoids such as quercetin act by inhibiting the nucleic acid synthesis mechanism through binding to the GyrB subunit of *E. coli* DNA gyrase and inhibiting the ATPase activity [18, 19].

Terpenoids and tannins were also present only in *M. mollissimus* and not in *S. erianthum*. Terpenoids are among the largest and diverse plant metabolites which play a role in metabolic functions and ecological interactions [20]. Plant terpenoids have been demonstrated to confer a wide range anti-microbial activity [21]. Tannins have been reported to have soothing, skin regeneration, anti-inflammatory and potential anti-microbial properties [22, 23]. Bioflim formation of bacteria has been reported to be associates with the raise of anti-microbial resistence [24]. Tannin has been shown to inhibits biofilm formations of *Staphylococcus aureus* through an immunodominant staphylococcal antigen A (IsaA)-dependent manner [25]. IsaA is a lytic transglycosylase which can cleave the polysaccharide backbone of the peptidoglycan layer [26]. The changes of peptidoglycan layer due to cleavage can release kinase signaling molecules which leads to the modulation of biofilm formation mechanism [27]. However, the work elucidation this exact mode of mechanism is under way.

Table 1. Average inhibition diameter (mm) of *M. Mollissimus* and *S. Erianthum* extracts against Gram-positive and Gram-negative bacteria

	M. mollissimus					***S. erianthum***				
Bacteria	**ME**	**CE**	**CE.F1**	**CE.F2**	**CE.F3**	**ME**	**CE**	**CE.F1**	**CE.F2**	**CE.F9**
Gram-positive										
Bacillus cereus	0	9.5 ± 0.71	0	0	8.0 ± 0.00	0	7.5 ± 0.71	0	8.0 ± 1.41	0
Bacillus subtilis	0	0	0	0	0	0	0	0	0	0
Staphylococcus aureus	6.5 ± 0.82	7.5 ± 1.11	0	7.5 ± 0.00	7.0 ± 0.48	6.9 ± 0.25	7.0 ± 0.61	7.0 ± 0	7.3 ± 0.61	7.0 ± 0
Streptococcus pneumoniae	7.3 ± 0.75	8.5 ± 0.84	0	7.0 ± 0.41	7.1 ± 0.58	0	7.1 ± 0.58	0	6.9 ± 0.58	0
Gram-negative										
Enterobacter aerogenes	0	0	0	0	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	8.0 ± 0	9.0 ± 0	0	0	7.0 ± 0.48	8.3 ± 0.50	7.1 ± 0.63	7.9 ± 0.25	13.3 ± 0.50	0
Salmonella typhi	0	0	0	0	0	0	0	0	0	0

Table 2. Phytochemical analysis of *M. Mollissimus* and *S. Erianthum*

Species	***M. mollissimus***				***S. erianthum***				
Extracts/ Fractions	**ME**	**CE**	**CE.F2**	**CE.F3**	**ME**	**CE**	**CE.F1**	**CE.F2**	**CE.F9**
Alkaloid	-	-	-	+	-	-	-	-	+
Flavonoids	+	+	-	-	-	-	-	-	-
Saponins	-	-	-	-	+	+	-	-	+
Steroids	+	-	-	-	+	+	+	+	+
Terpenoid	+	+	-	-	-	-	-	-	-
Tannins	+	+	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	+	+	+	+

Note: plus (+) sign indicates the presence and minus (-) signifies absence

CONCLUSION

Raise of anti-microbial resistance bacteria is a concerning event. We have shown that *M. mollissimus* and *S. erianthum* extracts contains anti-microbial activity against multiple Gram-positive and Gram-negative bacteria. The extracts contain several potential phytochemicals that are correlated with anti-microbial activity. The anti-microbial activity of *M. mollissimus* extracts was superior to *S. erianthum* extracts because it has anti-microbial activity against Streptococcus pneumoniae.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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