ANTI-LIPOLYTIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF*CHELIANTHESALBOMARGINATA* AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT:The aim of the present study is to evaluate the therapeutic properties of selected fern,*Chelianthusalbomarginata*,and to identify its functional compounds. The methanolic fern-extract (MFE) of these ferns was assessed for anti-bacterial activities by measuring inhibition zones against a panel of pathogenic bacterial strains using an agar diffusion method. MFE at a concentration of 25 µg/ml showed marked anti-bacterial activity against all bacterial strains (6-23mm zone of inhibition) and was maximum against *Enterobacter* sp. (23 mm). In addition, the MFE of *C. Albomarginata*had obtained the best MIC values of 2.25µg/ml against *S. aureus* and *Enterobacter* sp., respectively. The MFE also possessed good anti-lipolytic activity (66.5%) against porcine pancreatic lipase (PPL), and cholesterol oxidase inhibition (79%). This result showed that MFE of *C. albomarginata*,under optimal concentration, is not only a potent source of natural anti-oxidants and anti-bacterial agents but also possesses efficient cholesterol degradation and anti-lipolytic activities, that can be beneficial in body weight management.

ABSTRAK:Tujuan kajian ini adalahbagi menilai ciri-ciri terapeutik paku-pakis terpilih, *Chelianthus albomarginata* dan bagi mengenal pasti fungsi sebatian. Aktiviti antibakteria pada ekstrak metanol pakis (MFE) ini telah dinilai dengan mengukur zon perencatan terhadap panel strain bakteria patogenik mengguna kaedah penyebaran agar. Pada kepekatan 25 µg/ml, MFE menunjukkan aktiviti antibakteria yang paling ketara terhadap semua antigen bakteria (zon perencatan 6-23mm) dan maksimum terhadap *Enterobacter* sp (23 mm). Di samping itu, MFE *C. albomarginata* mempunyai nilai MIC terbaik 2.25µg/ml terhadap *S. aureus* dan *Enterobacter* sp., masing-masing. MFE juga menunjukkan aktiviti antilipolitik yang baik (66.5%) terhadap enzim lipase pankreas babi (PPL) dan kolesterol perencatan oksidase (79%). Keputusan ini menunjukkan MFE *C. albomarginata* pada kepekatan optimum bukan sahaja sumber semula jadi antioksidamujarab dan antibakteria,malah turut mempunyai degradasi kolesterol berkesan dan aktiviti antilipolitik, iaitu bermanfaat bagi pengurusan berat badan.

KEYWORDS:*Chelianthesalbomarginata; phytochemicals; anti-oxidant; antibacterial; antilipolytic*

1. INTRODUCTION

Chelianthesalbomarginata is a member of *Adiantaceae* family, which can grow up to 60 cm in length. The ferns are naturally rich in phytochemicals and many types of

valuable alkaloids, tannins, and flavonoids. Ferns offer us bioactive molecules that may serve as safer therapeutics to combat existing new world diseases. The medicinal value of ferns has been known to many for more than 2000 years [1]. The screening of medicinally important fern extracts for antibacterial activity may be beneficial for humans and animal diseases [1]. People use traditional herbs to treat a variety of diseases including bacterial infections, but nowadays bacterial strains resistant most of the antibiotics and drugs [2] which cause approximately one-half of all death in world. Plants are the only common sources of potentially valuable new drugs. This provokes us to assess ferns as a source of potential chemotherapeutic agents for antimicrobial and anti-lipolytic activity based on their ethnomedicinal properties. To date, no reports exist on the anti-lipolytic activity of ferns. The most common traditional use of ferns is to treat skin problems, wounds, fever, cough, reproductive problems, and also insect repellent [3]. The common diseases that could be treated by ferns include, ulcers (used fern stem juice), dysentery, and as protective medicine after childbirth [4].

With this knowledge, in the present study, the MFE of *C. albomarginata* was made and used to assay its biochemical constituents. Other activities like anti-bacterial, anti-lypolytic and MIC activity against a panel of common human pathogenic bacterial strains. This could lead to new business ventures in pharmaceutical area as well as in commercial area and contribute to the bioeconomy. The anti-oxidant properties and anti-bacterial activities of *A.veriens* has been reported by several researchers [5] but here, the novelty of this study is to assess the effectiveness of MFE of *C. albomarginata*containing efficient cholesterol degradation and anti-lipolytic potential.

2. MATERIALS AND METHODS

2.1 Collection of Ferns

*Chelianthesalbomarginata*ferns were collected from the Kuantan region and near the UMP area was done on the basis of ethno medicinal properties mentioned in the literature [6].

2.2Preparation of Fern Tissue Extracts

Fresh fern/plant material (whole plant) were washed under running tap water, air dried and then homogenized to fine powder. The powered preparations were stored in airtight glass vials. For preparing tissue extracts, 1.0g of air-dried powder was placed in 10 ml of methanol in a conical flask, plugged with cotton and the same was kept on a rotary shaker at 200 rpm for 48 h. The extract was filtered through Whatman filter paper No.1 and the filtrate was centrifuged at 10,000 rpm at 4^{o}C for 15 min. The supernatant was collected and completely evaporated at room temperature. The left over MFE (methanolic fern extract) residue was dissolved in PBS (0.05 M phosphate buffer saline, pH 7.2) to reach a final concentration and was rendered sterilized by filtration (0.22 μm Millipore filter).The filter-sterilized preparation of MFE obtained was kept at -70^{o}Cin airtight vials for storage.

2.3 Bacterial Growth Medium

An appropriate amount of Muller Hinton medium (MH) and MH broth were bought from Hi-Media, mixed with distilled water, and then sterilized in an autoclave. The sterilized media were poured into petri dishes. The solidified plates were bored out with a sterile cork borer. The plates with wells were used for the antibacterial studies.

2.4 Assay for Antimicrobial Activity by Well Diffusion Method

Antibacterial activities of the MFE preparations were tested using a Well-diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using a streak plate method. Wells were made on the agar surface with 6 mm cork borer. The MFE were poured into the well using a sterile auto pipette. The plates were incubated at 37ºC for 24 h for the bacterial growth to appear. The plates were observed for zone clearance around the wells. The zones of inhibition(s) around the well (in mm), including the well diameter, were recorded. The observations were taken in all 3 replicates and the average values ±SEM were tabulated.

A total of 12 bacterial strains that included *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella paratyphi, Shigellaflexerni, Escherichia coli, Staphylococcus citreus, Enterobacter* sp.*, Salmonella typhimurium, Streptococcus mutans, Proteus vulgaris, and Salmonella epidermis* were employed to determine the antimicrobial activities of the MFE .The antibacterial activities of the MFE preparation were tested using a Well-diffusion method. The Petri plates containing Muller-Hinton (MH) agar were inoculated with different selected strains of bacteria using a surface spreading method. The uniform diameter (0.5 cm) wells were created in the MH-agar plates with a sterile borer. The MFE was poured at different concentrations into each of the wells using an auto-pipette. The plates were incubated thereafter at 36.5 ± 0.5ºC for 24 h in order for bacterial growth and zone of clearance to appear. Values less than 8 mm for the tested fern extracts were considered as not active against microorganisms [12].

2.5 MIC of MFE of *Chelianthes albomarginata* Selected Bacterial Strains

The MIC assay was performed in a 96-well micro-titer plate. For the MIC assay, twelve wells in each of the rows of micro-titer plate were used; out of which last two wells were taken as control (no MFE was added). Each of the 10 wells received 100 µl of the MH broth; except the 1stwell that received 200 µl of broth containing 500 µg/ml of the MFE. From the 1st well, 100 µl of the MH-broth containing MFE was withdrawn with a sterile tip, and the same was transferred to the 100 µl of the broth in the 2nd well. Contents were mixed 4 times, then 100 µl of MH-broth was withdrawn from 2nd well and was added to the 3rd well. In this way, a range of 2-fold serial dilutions was prepared. The MH broth in each of the wells was inoculated with 2 µl of the pure bacterial culture and the content was mixed by 10 clockwise and 10 anti-clockwise rotations on a flat surface. The micro-titer plate was incubated at 37^{o}C for 24 h. Thereafter, the observations for growth of bacteria were visually made and MIC of MFE for each of the test bacteria were recorded and expressed as µg/ml of MFE.

2.6 Assay for Cholesterol Oxidase Activity

A previously reported [12] colorimetric method was used to assay the cholesterol oxidase activity in the MFE. Approximately diluted commercial grade bacterial cholesterol oxidase (Sigma Chemical Co., Saint Louis, USA) was employed to calibrate a reference profile using cholesterol as a substrate. One unit (U) of cholesterol oxidase activity was defined as the amount of enzyme capable of converting 1.0 µmole of cholesterol to 4-cholesten-3-one per minute at pH 7.5± 0.1 and at a temperature of 37 ± 1 ºC.

2.7 Anti-lipase Assay

To 2.9 ml of Tris-HCl buffer (0.1 M, pH 8.5), 80 µl of MFE of *C.albomarginata* was added. The reaction mixture was then incubated at 37^{o}C in a water bath for 10 min to remove the turbidity then 80 µl of the substrate (*p*-NPP, 20 mM) was added along with 20

µl of PPL. The reaction mixture was re-incubated at 55°Cin a water-bath for 10 min. The reaction was stopped by chilling at -40°C. The amount of *p*-nitrophenol (*p*-NP) released was measured at A410 (Perkin Elmer UV/VIS Spectrophotometer Lambda 12) after bringing the tubes to room temperature. A standard curve of *p*-NP was plotted at the selected concentration (10-100 µg/ml) vs. observed A410 values.

2.8 Statistical Analysis

The results were expressed as the mean ± Standard error of the mean (SEM) for each group. Statistical difference was evaluated using Student's t-test. Results were observed to be highly significant at $p<0.05$ and $p < 0.001$.

3. RESULTS

3.1 Anti-microbial Activity of MFE

The MFE of *C. albomarginata*showed excellent activity against *S. aureus* with the average zone of inhibition of 24±0.5 mm, and *S. epidermidis* (22±0.5 mm), followed by marked antibacterial activities against *Salmonella paratyphi*, *Salmonella typhi*, and *Enterobacter* sp. with zone of inhibition of 23.0±2.9, 23.0±1.5, and 22.0±1.5 mm respectively (Fig. 1A-D). The least antibacterial activity of MFE was recorded against *Salmonella typhimurium* (18.0±1.0 mm) in Table 1.

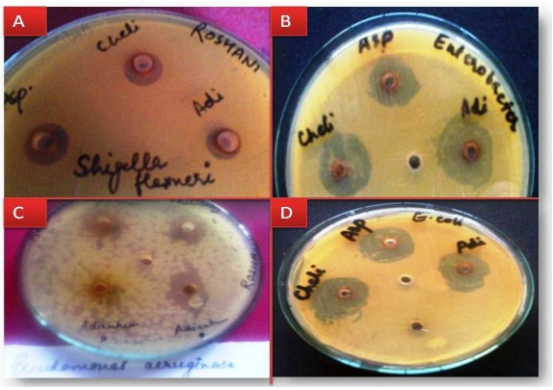

Fig.1: Anti-bacterial activity of MFE of *C.albomarginata*against selected microorganisms. Inhibition zones for (A) *Shigella flexneri;* (B)*Enterobacter* sp.*;* (C) *Pseudomonas aeruginosa*;(D) *E.coli.*

Table1: Anti-bacterial activity of MFE of *C.albomarginata* against selected pathogenic bacteria

Organism	Inhibition zone (mean value in mm±SD)	Organism	Inhibition zone (mean value in mm±SD)
Pseudomonas aeruginosa	20.0± 1.0	*Enterobacter sp*	22.0± 1.5
Shigellaflexneri	23.0± 2.7	*Klebssiella pneumoniae*	23.0± 1.9
Escherichia coli	20.0± 0.5	*Staphylococcus aureus*	24.0± 0.5
Salmonella typhimurium	18.0± 1.0	*Streptococcus mutans*	22.0± 1.0
Salmonella paratyphi	23.0± 2.9	*Staphylococcus citreus*	22.0± 1.0
Salmonella typhi	23.0± 1.5	*Staphylococcus epidermidis*	22.0± 0.5

3.2 MIC as a Measure of Antibacterial Activity of MFE

The MIC values of MFE of*C. albomarginata*were recorded against selected common pathogenic bacteria (Table 2). The MLE of*C. Albomarginata*had the best MIC values against *S. aureus* and *Enterobacter* sp. (2.25 mg/ml; Table 2).

Table2: MIC of MFE of *C. albomarginata* againsta panel of common pathogenic strains

Organism	MIC (mg/ml)	Organism	MIC (mg/ml)
Pseudomonas aeruginosa	4.50	*Enterobacter sp*	2.25
Shigellaflexneri	4.50	*Klebssiella pneumoniae*	4.50
Escherichia coli	4.50	*Staphylococcus aureus*	2.25
Salmonella typhimurium	4.50	*Streptococcus mutans*	4.50
Salmonella paratyphi	4.50	*Staphylococcus citreus*	4.50
Salmonella typhi	4.50	*Staphylococcus epidermidis*	4.50

3.3 Anti-lipolytic Assay and Cholesterol Oxidase Activity of MFE

Lipase activity was assayed by the method [13] by measuring the micromoles of *p*-nitrophenol released from pnitrophenyl palmitate. The MFE of*C.Albomarginata*was found to possess 48.5% anti-lipolytic activity against PPL and 66.0% cholesterol oxidase activity.

4. DISCUSSION

Here we propose a sensitive and rapid method for screening the antimicrobial activity of *C. albomarginata* ferns. Antibacterial activity obtained in this study with methanolic solvent was used for extraction. *C. albomarginata*represents a rich natural source of anti-microbial agents. Plants are used medicinally in different countries and are important sources of many potent and powerful drugs. A wide range of medicinal plants (or their parts) are used to extract raw drugs and they possess varied medicinal properties [7,8]. In the present investigation, the methanolic plant extract of *C. albomarginata*was formulated and studied for its anti-microbial activity against 12 common potentially pathogenic bacteria. The MFE of *C. albomarginata*showed excellent activity against *S. aureus* with the average zone of inhibition of 24.0±0.5 mm (around 25mm). The results of this experiment revealed that the MFE of *C. albomarginata*is an effective antimicrobial agent for *S. aureus* [9] and reported that extracts of fern inhibited *S. aureus, S epidermidis, Salmonellatyphi*including *V. cholerae*. In the present study, the MIC values for MLE were determined by micro-dilution method. The MFE of *C. albomarginata*had the best MIC values against *S. aureus* and *Enterobacter*(2.25 µg/ml, respectively). A similar finding was reported for *Bordetella pertussis* [9]. The aqueous extracts of *Pterisvitatta* inhibited cariogenic streptococci, including *S. mutans*. The anti-bacterial activity against other harmful mouth flora has also been reported in patented literature [15]. A potentially valuable anti-cariogenic effect of ferns was suggested by inhibition of the synthesis of insoluble glucans by *S. mutans.* This includes flavonols, mainly quercetin, kaempferol, myricetin, and their glycosides. *C.albomarginata* has been shown to have a wide range of beneficial physiological and pharmacological effects. The use of ferns is clearly still a long way from clinical application, but there are promising leads in the dental context [10]. The concept of being able to exploit an antimicrobial agent which is a new chemical entity

found in an abundantly available and renewable source is indeed an important achievement. The cholesterol degradation (cholesterol oxidase), as well as antioxidant activity, of MFE of ferns was studied by employing in vitro assay systems. The MFE of *C. albomarginata*showed the best MIC values against *S. aureus* and *Enterobacter* sp. (2.25 mg/ml, respectively). There are some contradictions over precisely which bacterial species are inhibited by ferns, as previously [11] *S. typhimurium* and *Campylobacter jejuni* have been reported to be both resistant and susceptible to methanolic extract of *C. albomarginata*thesedifferences in observations might be seen because of bacterial strain variation. Our study also provided a strong evidence of the ability of the MFE of *C. albomarginata*to inhibit the activity of mammalian lipase (PPL) in vitro. The MFE showed (66%) anti-lipolytic activity against PPL as well as cholesterol oxidase inhibition of 66%. The present study thus indeed provided strong experimental evidence that the MFE of *C. albomarginata* is not only a potent source of natural anti-oxidants and anti-bacterial activities but also possesses efficient cholesterol degradation and anti-lipolytic potential.

5. CONCLUSION

MFE has potential to inhibit the growth of many common bacterial pathogens. There are still scanty studies on anti-bacterial properties of *C. albomarginata* or their biochemical constituents. It may be suggested that the plants/tea extracts may possess effective antimicrobial activities that may be explored in the management of common bacterial infectious diseases. *C. albomarginata*may represent a new source of antimicrobial phyto-chemicals with stable biological activity that can extend a scientific base for the use of tea in modern medicine. The need for the screening of other known or unknown medicinal plants becomes more compelling because of indiscriminate/irrational use of potent antibiotics, many bacteria/microorganisms have developed genetic modification to overcome bactericidal/bacteriostatic effects of commonly used antibiotics. Most of the diseases against which the lycophytes are said to have curative properties are caused by both Gram-positive and Gram-negative bacteria. The discovery of novel phyto-constituents that are likely to have least toxicity and/or undesired side-effects is a much desired event. Finally, it could be concluded that the MFE of *C. albomarginata*not only possessed strong to moderate anti-bacterial activities against 12 common pathogenic and opportunistic bacteria but also markedly effected the degradation of cholesterol in vitro as well as appeared to be an effective anti-lipolytic preparation. MFE of *C. albomarginata*appeared to be a potent source of natural anti-oxidants and anti-bacterial activities besides possessing efficient cholesterol degradation and anti-lipolytic potential that might be beneficial to improve human health. The active principles involved in this plant need to be purified and individually studied for their active antibacterial, anti-lipase and anti-cholesterol oxidase biochemical constituents.

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