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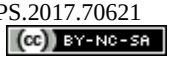
ISSN 2231-3354 

Evaluation of antilipolytic, antioxidant and antibacterial activities of selected ferns

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ABSTRACT

In the present study, three ferns were used (*Adiantum capillus-veneris Cheilanthes albomarginata* and *Asplenium pumilum,*) to identify their functional compounds to reduce obesity. The methanolic fern-extract (MFE) of these ferns was assessed for anti-bacterial activity by measuring inhibition zones against a panel of pathogenic bacterial strains using the agar diffusion method. MFE at a concentration of 25 µg /ml showed marked anti-bacterial activity against all bacterial strains (6mm to 23mm) zone of inhibition and was greatest against *Enterobacter* sp (23 mm). Out of these, the MFE of the *A. veneris* fern extract had the best MIC values of 2.25 µg /ml against *S. aureus* and *Enterobacter sp*. respectively. It had an inhibitory concentration (69.9 %) for 2, 2-diphenylpicryl-1-picryl-hydrazyl (DPPH) scavenging activity. The MFE of the *A. veriens* also possessed good anti-lipolytic activity (76.5%) against a porcine pancreatic lipase (PPL) and cholesterol oxidase inhibition (89%). The hemolytic activity of *A. veneris* was found to be 27.6% at 6.25 µg/mL of MFE. This result showed that the MFE of *A. veriens* is not only a potent source of natural anti-oxidants and anti-bacterial activities but also possesses efficient cholesterol degradation and anti-lipolytic activities, which would be beneficial in body weight management.

INTRODUCTION

Ferns are mostly distributed in high-altitude, mountainous regions. The most common traditional use of ferns is to treat skin problems, wounds, fever, cough and reproductive problems as well as to make insect repellent (Nath *et al*., 2016; Dongmo *et al*., 2003). Common diseases that can be treated with ferns include ulcer and dysentery; in addition, ferns can be used as protective medicine after childbirth. These enthanomedical properties attracted the attention of several scientists who have directed their work toward these medicinal plants and the remedies they offer for various diseases (Reinaldo *et al.,* 2015; Mohan *et al.,* 2012). Natural compounds (alkaloids, flavonoids, tannins and phenolics) rich in antimicrobial properties due to their medicinal importance, they are used in controlling many diseases. Based on these finding, is expected that the screening of phytochemicals from ferns will be beneficial for society. A World Health Organization (WHO) survey found that over 400 million adults are clinically obese and 1.6 billion are overweight (Drew *et al.,* 2007). Orlistat drug approved for long-term use, is currently widely available (Zhi *et al.,* 1994; Hauptman *et al.,* 2000). However, this medication also results in side effects and complications. The efficiency of natural products for the treatment of obesity has been largely unexplored, and such products may be safer and more effective alternative(s) to anti-obesity drugs. Although antinutrients elicit deleterious effects on human health, they have been shown to be of pharmaceutical importance (Birari and Bhutani, 2007; Bouhlali *et al.,* 2016). Other phyto- chemicals like flavonoids, saponins and alkaloids have been identified are reported as biologically active molecules (Nath *et al.,* 2016). Research may confirm that plants are an alternative source of a variety of anti-bacterial and anti-lipolytic therapeutic molecule(s) that can either inhibit pancreatic lipase (PL) or the uptake/ absorption of fat(s) or both.

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In this article we emphasize the potential role of these bioactive compounds extracted from the ferns as anti-bacterial and PL inhibitory molecules.

With this knowledge, in the present study, the MFEs of (*Adiantum capillus-veneris, Cheilanthes albomarginata* and *Asplenium pumilum*) were made and used to assay their biochemical constituents, anti-oxidant/free radical scavenging activity, hemolytic activity, anti-lipolytic activities and MIC besides anti-bacterial activity against a panel of common human pathogenic bacterial strains, The findings could lead to new business ventures in both pharmaceutical and commercial areas as well as contribute to the bioeconomy. Several researchers have reported the anti-oxidant properties and anti bacterial activities of *A. veneris* (Al-Snafi, 2015; Thompson, 1993) but, the novelty of this study is, to assess the effectiveness of MFE of *A. veneris* containing efficient cholesterol degradation and anti-lipolytic potential that may be more beneficial to improve human health and offer major help in reducing body weight. The active principles involved in this plant need to be purified and individually studied for their active anti-bacterial, anti-lipase and anti-cholesterol oxidase biochemical constituents.

MATERIAL AND METHODS

Collection of ferns

The selected ferns (*Adiantum capillus-veneris, Cheilanthes albomarginata* and *Asplenium pumilum*) were collected from Himalayan region. The collection was on the basis of ethno-medicinal properties in the literature (Singh, 2003). The fern specimens were identified and certified in H.P.U botany department Shimla.

Preparation of fern tissue extracts

Fresh fern/plant materials (whole plant) were washed under running tap water, air dried and then homogenized to a fine powder. The powered preparations were stored in airtight glass vials. For preparing tissue extracts, 1.0 g of air-dried powder was placed in 10 ml of methanol in a conical flask and plugged with cotton. The same was kept on a rotary shaker at 200 rpm for 48 h. The extract was filtered through Whatmann filter paper No.1 (Sigma-Aldrich) 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 residue was dissolved in 0.05 M phosphate buffer saline (PBS), pH 7.2 to reach a final concentration (20mg / 5µl) and was sterilized by filtration (0.22 µm Millipore filter). The filtered and sterilized preparations of MFEs obtained were stored in a freezer at −70°C in airtight vials for further studies.

Bacterial and fungal growth medium

The appropriate amount of Muller Hinton (MH) medium, MH broth and potato dextrose agar (PDA) medium were purchased 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 with a sterile cork borer. The plates with wells were used for the anti-bacterial and anti-fungal studies.

Test microorganisms

Twelve cultures of different bacterial strains (*Staphylococcus aureus, Klebssiella pneumonia, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella flexerni, Escherichia coli, Staphylococcus citreus, Enterobacter* sp., *Salmonella typhimurium, Streptococcus mutans, Proteus vulgaris, Salmonalle epidermis*) and two fungal strains *Aspergillus niger*, and *Fusarium oxysporum*) were maintained at 4^{o}C on agar slants of MH and PDA media. Active cultures for experiments were prepared by transferring a loopful of microorganisms from the stock culture to eppendorf tubes aseptically using sterile loop which contained 1 ml of MH broth.

Assay for antimicrobial activity by well diffusion method

Anti-bacterial activities of the MFE preparations were tested using the well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using the streak plate method. Wells were made on the agar surface with a 6 mm cork borer. The MFE was poured into each 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 around the well (in mm) including the well diameter were recorded. The observations were taken in three different directions in all three replicates and the average values ± standard error of the mean (SEM) were tabulated.

Anti-bacterial activity assay

A total of 12 bacterial strains (*Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella flexerni, Escherichia coli, Staphylococcus citreus, Enterobacter* sp., *Salmonella typhimurium, Streptococcus mutans, Proteus vulgaris, Salmonella epidermidis* were employed to determine the antimicrobial activities of the MFE. The antibacterial activities of the MFE preparation were tested using the well-diffusion method.

The Petri plates containing MH agar were inoculated with different selected strains of bacteria using the 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 ± 0.5 ºC for 24 h 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.

Antifungal activity assay

Two fungal strains (*Aspergillus niger* and *Fusarium oxysporum)* were employed to determine the antifungal activities of the MFE. The petriplates containing PDA were inoculated with

two selected strains of fungal using the surface spreading method. The uniform diameter (0.5 cm) wells were created in the PDA 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 ºC for 24 h for fungal growth and zone of clearance to appear.

Anti-oxidant activity

Free radical scavenging activity

The 2, 2-diphenylpicryl- 1-picryl-hydrazyl (DPPH) stable free radical method is an easy, rapid and sensitive way to analyze the antioxidant activity of a specific compound or plant extract (Koleva, 2002). The method described by (Zhu, 2005) was used to test for free radical scavenging activity. Specifically 100 mM of stock solution of DPPH (4 mg/10ml) was diluted to 1: 10. Then 0.5 ml of this solution was added at different concentrations of MFE. An equal amount of distilled water was also added to make the final volume 1.0 ml. After 30 min. of incubation, the absorbance was measured at 517 nm against a blank on the UV-visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH. The calibration curve was prepared by using ascorbic acid as standard. The DPPH radical scavenging capacity was estimated from the difference in absorbance for the sample and blank and expressed as percentage of DPPH scavenging. The degree of discoloration of violet color of DPPH radical, as it gets reduced, indicates the radical scavenging potential of the antioxidant.

DPPH radical-scavenging activities of the fractions were determined *in vitro*. Given that light-sensitive nature of DPPH, the solution was prepared in a vial wrapped with an aluminum foil. The mixture was shaken vigorously on a vortex mixer followed by incubation for 1 h in a water bath set at 37°C, after which the A_{517} values of the reaction mixture was recorded. A reference profile was calibrated using appropriate concentrations of ascorbic acid.

$$\%\ \text{Scavenging of AFE} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by anti-oxidants through the donation of hydrogen to form the stable DPPH molecule.

Human red blood cell (RBC) hemolysis test

RBCs in the human blood (Blood group 'Rh+) taken in 10% citrate phosphate dextrose buffer (pH 7.3) were harvested by centrifugation (1,000 g for 5 min at 4°C). The packed RBCs were washed 5 times with phosphate buffered saline (PBS; 0.05 M, pH 7.3) and were used to 0.4% (v/v) suspension in PBS. RBCs suspension (100 µl) was transferred to each of the wells of a 96-wells micro-titer plate and mixed with (100 µl) of appropriately diluted MFE followed by incubation at 37°C for 1 h. The supernatant (100 µl) was transferred to new wells and A_{414} were measured to monitor RBC lysis. RBCs in PBS alone acted as a negative control and RBCs lysed with Triton X-100 (0.1%, v/v) were used to measure 100% lysis (a positive control). The percentage of hemolysis was determined as followed:

$$\text{Hemolysis}(\%) = \frac{A_{414,control} - A_{414,sample}}{A_{414control}} \times 100$$

***MIC value of MFE of Adiantum capillus-veneris* selected bacterial strains**

The MIC assay was performed in a 96-well micro-titer plate; 12 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 1st well 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 added to the 100 µl of the broth taken 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. This way a range of 2-fold serial dilutions was thus 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. Upon 24 h incubation of the micro-titer plate at 37°C, visual inspection for bacterial growth was made and the MIC of the MFE for each of the test bacteria were recorded and expressed in µg/ml of MFE.

Assay for cholesterol oxidase activity

A previously reported colorimetric method was used to assay the cholesterol oxidase (Richmond, 1976) 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 enzymes 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 ± 1ºC.

Anti-lipase assay

To 2.9 ml of tris-HCl buffer (0.1 M, pH 8.5) was added 80 µl of MFE of *A. veneris*. Then reaction mixture was incubated at 37°C in a water bath for 10 min in order to remove the turbidity, after which 80 µl of the substrate (p-NPP, 20 mM) along with 20 µl of PPL was added to it. The reaction mixture was re-incubated at 55°C in 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 A_{410} (Perkin Elmer UV/VIS spectrophotometer Lambda 12) after allowing the tubes to reach room temperature. A standard curve of p-NP was plotted at the selected concentration (10-100 µg/ml) vs. observed A_{410} values.

Statistical analysis

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

RESULTS

Anti-bacterial activities of MFEs

When tested for anti-bacterial activities against 12 pathogenic bacteria using the well diffusion technique, the MFE preparation of *A. veneris* showed excellent zone of inhibition of 23.6 mm, 23.0 mm and 20 mm against *Enterobacter* sp., *E. coli* (Gram negative bacterium) and *S. aureus* (Gram positive bacterium), respectively (Table.1). As for the MFE of *C. albomarginata*, the highest bactericidal activity was recorded against *E. coli* (15 mm) as presented in Table. 2. As for the MFE of *A. pumilum* marked activity was demonstrated against *Shigella flexneri* (6.7 mm). The Student's t-test analysis of the data revealed that the MFE of *A. veneris* ($p < 0.05$) showed highly significant anti-bacterial activity against the tested pathogens. The anti-bacterial activity of MFE of *A. veneris* against *S. aureus, S.mutans, S. epidermidis, Enterobacter* sp., *Salmonella paratyphi, Pseudomonas aureginosa, Salmonella typhimurium* were highly significant ($p < 0.05$) as compared to the MFEs of *C. albomarginata* and of *A. pumilum*

Table 1: Zone of inhibition in bacterial culture.

Test organism	*Adiantum capillus-veneris*	*Chelianthes albomarginata*	*Asplenium pumilinum*
	Zone of inhibition on MH-medium (mm ± SEM) [at 70 µl of bacterial culture and 18 mg/ml AFE]		
Gram negative Bacteria			
Salmonella typhimurium	11.5± 0.44 (ac)**	11.1± 0.53 (bc)**	7.5± 0.40
Proteus vulgaris	1.1± 0.22 (ab)**	5.0± 0.18 (bc)*	4.2± 0.28 (ca)**
Pseudomonas aerugenosa	9.4± 0.30	8.5± 0.40	7.8± 0.32
Shigellaflexneri	3.2± 0.26 (ab)**	5.9± 0.22 (ac)**	6.4± 0.46(ca)**
Salmonella paratyphi	8.2± 0.29 (ab)** (ac)**	7.1± 0.22 (bc)**	6.2± 0.28
Klebsiellapneumoniae	6.5± 0.40(ac)*	7.0± 0.57	5.4±0.36
S. mutans	18.0± 0.57 (ab)*	12.6± 0.83(bc)*	8.1± 0.50
E. coli	23.0± 0.57(ac)*	25.0± 0.57 (bc)*	21.4± 0.69
*Enterobacter*sp	23.6± 0.71 (ab)*	20.5± 0.40	19.5± 0.70
Gram positive bacteria			
S. epidermidis	15.7± 0.70 (ab)** (ac)*	6.6± 0.83 (bc)*	13.1± 0.64
S. citreus	9.3± 0.70	6.4± 0.7	7.1± 0.59
S. aureus	20.1± 0.50 (ab)** (ac)*	14.0± 0.54	15.8± 0.95

a: *Adiantum capillus veneris* b: *Chelianthes albomarginata* c: *Asplenium pumilinum*. *Indicates significant value at $p < 0.05$. **Indicates highly significant value at $p < 0.001$.

Table 2: Efficiency of MFE against selected pathogenic bacterial strains.

Adiantum capillus-veneris		*Chelianthes albomarginata*		*Asplenium pumilinum*	
Zone of inhibition (mm)					
Pseudomonas aeruginosa	9.4	*Salmonella typhimurium*	11.1	*Shigella flexneri*	6.7
Salmonella paratyphi	8.2	*Proteus vulgaris*	5.0		
S. citrus	9.3	*Klebsiella pneumoniae*	7.0		
*Enterobacter*sp.	23.6	*E. coli*	15.0		
S. epidermidis	15.7				
S. mutans	18.0				
S. aureus	20.1				

Table 3: Zone of inhibition of MFEs against selected fungal strains.

Test organism	*Adiantumcapillus-veneris*	*Chelianthes albomarginata*	*Asplenium pumilinum*
	Zone of inhibition on PDA-medium (mm ± SEM) at 85 µl of fungal culture and AFE of 18mg/ml concentration		
Aspergillus niger	18.5± 0.70 (ac)**	17.5± 0.40 (bc)**	10.7± 0.61
Fusarium orzospora	7.5± 0.66	6.8± 0.50	9.1± 0.64 (ca)*

a: *Adiantum capillus veneris* b: *Chelianthes albomarginata* c: *Asplenium pumilinum*. * Indicates significant value at $p < 0.05$. **Indicates highly significant value at $p < 0.001$.

Anti-fungal activities of MFEs

The anti-fungal activities of the MFEs against 2 fungal strains were tested using the well diffusion technique. An average zone of inhibition against *Aspergillus niger* (18.5 mm) and *Fusarium orzospora* (9.1 mm), respectively the MFE of both *A. veneris* and *C. albomarginata* at 18 mg/ ml ($p < 0.05$) showed significant activity against the *A. niger* in comparison to that of *A. pumilum.* However, the MFE of *A. pumilum* was better ($p < 0.05$) in comparison to other two MFEs for anti-fungal activity against *F. orzospora* (Table. 3).

Anti-oxidant assays

Free radical scavenging activity (DPPH activity)

The 2, 2-diphenylpicryl-1-picryl-hydrazyl (DPPH) radical scavenging activities in all the MFEs were measured according to the method. The radical scavenging activity was measured as a decrease (%) in the absorbance of DPPH or increase (%) in activity; hence, best scavenging (%) activity (Table. 4) was observed in MFE of *A. veneris* (69.9 %). By statistical analysis, it was observed that all three selected MFEs had significant values for the DPPH assay.

MIC as a measure of anti-bacterial activity of MFE

The MIC values of MFE of *A. veneris*, were recorded against selected common pathogenic bacteria (Table. 1). The MFE of *A. veneris* had the most superior MIC values against *S. aureus* and *Enterobacter sp*. (2.25 µg/ml; Table. 5).

Table 4: Scavenging activity of AFEs in DPPH assay.

Alcoholic fern extract	A_{517}		DPPH scavenging activity (%)
	Control	Test Mean ± SEM	
Adiantum capillus-veneris	0.971	0.292 ± 0.10	69.9
Chelianthes albomarginata	0.969	0.355 ± 0.09	63.4
Asplenium pumilinum	0.966	0.477 ± 0.07	50.9

Table 5: MIC of MFE of *Adiantum capillus-veneris*, against a panel of common pathogenic bacterial strains.

Microorganism	Tested concentration of MLE (mg/mL								MIC (mg/mL)
	18.0	9.0	4.5	2.25	1.12	0.56	0.28	0.14	
S. typhi	-	-	+	+	+	+	+	+	18.0
S. paratyphi	-	-	-	+	+	+	+	+	4.50
E. coli	-	-	-	-	+	+	+	+	2.25
S. citreus	-	-	-	-	+	+	+	+	2.25
Enterobacter sp.	-	-	-	-	+	+	+	+	2.25
Klebsiella pneumonia	-	-	-	-	-	-	+	+	0.56
Proteus vulgaris	-	-	-	-	+	+	+	+	2.25
S. mutans	+	+	+	+	+	+	+	+	18.0
S. epidermidis	-	-	-	-	-	+	+	+	1.12
Shigella flexneri	-	-	-	-	-	-	-	+	0.28
S. aureus	-	-	-	+	+	+	+	+	4.50
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	18.0

Hemolytic activity

The hemolytic assay of MFE was done using human blood of group 'O' Rh+. The hemolytic activity of *A. veneris* was found to be 27.6% at 6.25 µg/mL of MFE. The other two ferns *C. albomarginata* and *A. pumilum* were found to exhibit no hemolytic activity.

Anti-lipolytic assay and cholesterol oxidase activity of MFE

The lipase activity was assayed by the method of Winkler and Stuckmann (1979) measuring the micromoles of p-nitrophenol released from p-nitrophenyl palmitate. The MFE of *A. veneris* was found to possess 76.5% anti-lipolytic activity against PPL and 89.0% cholesterol oxidase activity.

DISCUSSION

Phytochemicals like flavonoids, alkaloids and saponins have been reported as biologically active molecules that have been identified in many pharmacological studies (Wang *et al*., 2016; Butnariu *et al*., 2016: Schopke and Hiller, 1990; Muhammad *et al.,* 2011) Phytochemicals exhibit promising role(s) in some cardiovascular treatments (Sagara *et al.,* 2004) and as strong anti-inflammatory and anti- oxidant agents (Just *et al.,* 1998; Putnoky *et al.,* 2013). They have also been demonstrated to suppress reactive oxygen species and free radicals (Huong *et al.,* 1998; Sofidiya *et al.,* 2006; Ogbunugafor *et al.,* 2011). In the present study, phytochemicals extracted from ferns have been demonstrated to have great anti-oxidant activities (DPPH and hemolytic activity) *A. veneris* showed results superior to two other ferns. A similar finding was reported in *C. officinalis* flowers (Butnariu *et al.,* 2012). *A. veneris*, *C. albomarginata* and *A. pumilum* show significant anti-microbial activities. Out of these, *A. veneris* shows highest antibacterial activity against 12 pathogenic bacteria (zone of inhibition measuring 23.6 mm) and antifungal activity against two fungal strains (zone of inhibition measuring 18.5 mm). A similar finding was reported to analyzed the antimicrobial activity (Helsper *et al.,* 1993) are also considered that phytochemicals as anti-tumor agents (Butler, 1989), anti-microbial agents (Ayres *et al.,* 1997), anti-helmintic agents (Niezen *et al.,* 1995; Molan *et al.,* 2002; Lasisi *et al.,* 2003) and anti-fungal (Bagiu *et al.,* 2012). According to these authors, the antimicrobial activities of these plants are not lower than those of any other plant groups. In a more recent study, (Al-Snafi, 2015; Singh, 2008) has observed that some species of the genus *Adiantum* exhibited a higher antimicrobial activity than the commercial antimicrobial agents, gentamicin and ketoconazole. According to Rashed *et al.,* 2014 extract of *Bauhinia racemosa* highly active against *C. albicans*, suggesting its possible use in the treatment of fungal infections. The MIC values for MFE were determined by micro-dilution method. The MFE of *A. veneris* had the greatest MIC values against *S. aureus* and *Enterobacter* (1.52 and 6.25 µg/ml, respectively). It is noteworthy that *A. veneris* has been shown to possess remarkable MIC values against *S. aureus* and *Enterobacter* (1.52 and 6.25 µg/ml) than *C. albomarginata* (4.50 and 9.0 µg/ml), respectively. A similar finding was reported for *Dryopteris erythrosora* (6.25µg/ml) (Lee, 2008). The hemolytic activity, PPL inhibition, cholesterol degradation (cholesterol oxidase) and anti-oxidant activity of MFE of fern were studied by employing *in vitro* assay systems. Our study also provided evidence for the ability of the MFE of *A. veneris* to inhibit the activity of mammalian lipase (PPL) in vitro. The MLE showed 76.5% anti-lipolytic activity against PPL but also inhibition of cholesterol oxidase inhibition (79%). The present study thus in deed provided strong experimental evidences that the MFE of *A. veneris* is not only a promising source of compounds with not only potential natural anti-oxidant and anti-bacterial activities but also efficient cholesterol degradation and anti-lipolytic potential that might be beneficial in weight management and obesity-related diseases. Against this background, in search of safer anti-obesity therapeutic, attempts have been made to explore and identify potent pancreatic lipase (PL) inhibitory agents. Many bioactive phytochemicals from ferns have been screened for their anti-obesity potential, which may lead the desirable discovery of novel phyto-constituents that are likely to have tolerable toxicity and undesired side-effects.

CONCLUSION

In general, it could be concluded that methanolic extracts of *A. veneris* showed superior anti-oxidant and anti-lipolytic activities compared with two other ferns (*C. albomarginata* and *A.*

pumilum). Furthermore, the results of the present study may contribute to a reduction in the application of synthetic drugs against drugs, which in turn increases the opportunity for natural control drugs. *A. veneris* is a promising source of natural compounds with not only potential anti-oxidants and anti-bacterial activities but also efficient cholesterol- degradation and anti-lipolytic properties that might be beneficial in weight management and obesity-related diseases. It may be envisioned that further studies on the tested samples (plants) which lead the discovery of novel phyto-constituents with tolerable toxicity.

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