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Research Article

Phytochemical composition of *Blumea lacera* leaf and its inhibitory effects on the activity of enzymes related to metabolic diseases

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ABSTRACT

Blumea lacera (Bum. f.) DC is an important medicinal plant of Bangladesh having several ethnomedicinal values. To give the scientific basis of ethnomedicinal uses, the present study analyzed phytochemical composition of methanol extract of *B. lacera* leaves (designated as MBLE) and evaluated its inhibitory effects on pancreatic lipase, α-amylase, xanthine oxidase (XO) and angiotensin I-converting enzyme (ACE). Different *in vitro* methods were used to perform enzyme inhibition assays for MBLE and its phytochemical profile was analyzed by GC-MS. In case of all the enzyme inhibition assays, the percentage of inhibition by MBLE was increased with increasing in concentrations. Among these enzymes, MBLE showed strong activity (IC_{50}:13.34±1.05 µg/mL) against ACE as compared with captopril (IC_{50}:8.09±0.74 µg/mL) used as standard reference whereas it exhibited moderate activity against other enzymes. The IC_{50} values of MBLE were found to be 9.87±0.25, 40.17±2.32 and 93.88±5.21 µg/mL for pancreatic lipase, α-amylase and XO, respectively. In addition, some compounds identified in MBLE by GC-MS, were also consistence with these enzyme inhibitory activities. Thus, this study demonstrates the enzyme inhibitory potentials of *B. lacera* leaves for the first time and it might be a potential tool for the treatment of metabolic diseases.

Keywords:
Phytochemical composition, *Blumea lacera*, Leaves, Inhibition, Enzyme, Metabolic diseases

1. INTRODUCTION

Metabolic diseases including obesity, type 2 diabetes, hyperuricemia and hypertension are considered a rising public health threat of this twenty-first century and these diseases form a group of cardiovascular risks factors referred to as metabolic syndrome[1-3]. The prevalence of the metabolic syndrome is increasing to epidemic proportions in developed and developing nations. In most of the countries, 20% to 30% of the adult population is suffered from metabolic syndrome[3-4]. Clinically, different pharmacotherapies are available for treating these metabolic diseases and the drugs used in these therapies, provoke their therapeutic action mainly by inhibiting certain enzymes like α-amylase, pancreatic lipase, angiotensin I-converting enzyme (ACE), xanthine oxidase (XO), etc[5-8]. Over-activities of these enzymes are responsible for excessive production and accumulation of metabolites that predispose to these metabolic diseases[4]. So, inhibitors of these enzymes such as orlistat, acarbose, captopril and allopurinol are used as established drugs to treat obesity, type-2 diabetes, hypertension and gout[6-8]. However, these drugs have some side effects such as hepatic, renal and gastrointestinal tract dysfunction that ultimately limits their clinical uses[9-10]. So, development of effective, safe and inexpensive inhibitors of the enzymes linked to metabolic diseases is the demand of time and in this context, plants extracts and natural products have been well recognized sources of bioactive components with enzymes inhibitory properties[11]. Some studies have

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demonstrated the efficiency of phenolics-rich plant extracts to inhibit relevant enzymes implicated in metabolic diseases[12-13]. Here an important medicinal plant, *Blumea lacera* was taken for investigation in the light of the above facts.

The plant *B. lacera* (Burm.f.) DC. (Family: Asteraceae) is an herbaceous weed locally known as kukursunga and it is grown in uncultivated lands of Bangladesh[14]. Different parts of the plant are used traditionally as antispasmodic, antipyretic, anti-diarrheal, liver tonic, expectorant, diuretic, astringent, cytotoxic and stimulant as well as to treat cholera, bronchitis, fevers and burning sensation[14-16]. The leaf of this plant is edible and it is most used part in the traditional medication system. The juice of leaves is antispasmodic, anthelmintic, astringent, febrifuge, stimulant, and diuretic; and able to cure bronchitis, fevers, and burning sensation[14]. Earlier it was found that methanolic extract of *B. lacera* leaves was quite safe in terms of oral application to animals[17]. Previous findings on the phytochemical profile of *B. lacera* reported the isolation of 21 compounds from different parts of this plant, belonging to natural product classes such as flavonoids, terpene glycosides, phenol glycosides, sterols, essential oils, coniferyl alcohol derivatives, terpenoid ketones and steroidal glycoalkaloids[18-22]. In addition, quantitative analyses also explored the free radical scavenging properties of *B. lacera* leaves along with the high amount of phenolic and flavonoid content[22]. However, so far, there have been no studies on the inhibitory effect of *B. lacera* leaves against enzymes linked to metabolic diseases. Therefore, the objective of this study was aimed to examine the inhibitory activities of methanolic extract of leaves of *B. lacera* against α-amylase, pancreatic lipase, angiotensin I-converting enzyme (ACE) and xanthine oxidase (XO).

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Porcine pancreatic lipase, α-amylase, xanthine oxidase, acarbose, allopurinol, xanthine, orlistat and captopril were purchased from Merck (Darmstadt, Germany) whereas ACE inhibition kit-WST (100 tests) were collected from Dojindo EU GmbH, Germany. Methanol, dimethyl sulfoxide (DMSO) and other solvents of HPLC grade were purchased from Labscan (Thailand).

2.2. Sample collection and authentication

Leaf of *B. lacera* (Bum. f.) DC was collected from uncultivated lands of Darusha adjacent to Rajshahi Court station area of Rajshahi district (Geographically located at 24°22′26″N, 88°36′04″E) and the collection period of plant materials was January, 2021. This plant was authenticated by Professor Dr. A. H. M. Mahbubur Rahman (a taxonomist), Department of Botany, University of Rajshahi. After authentication it was deposited in the herbarium of Botany department under the specimen record number of 1045. The collected leaves were cleaned and dried at room temperature. Then it was ground into powder by a grinder machine and stored in air tight glass containers for further use.

2.3. Plant extract preparation

The leaf powder (200 g) was soaked in 500 mL methanol for 72 hrs at room temperature. After 72 hrs, the extract was filtered with through a filter paper and concentrated with a rotary evaporator. Then, 3.4 g residue was obtained as methanolic extract of *B. lacera* leaves (designated as MBLE) and it was stored at -80°C for the various analyses.

2.4. Pancreatic lipase inhibition assay

The porcine pancreatic lipase inhibitory activity of MBLE was performed using a reported method[23] with minor modification and p-nitrophenyl palmitate (p-NPP) was used as a substrate. The enzyme under the reaction conditions hydrolyses p-NPP to release p-nitrophenol, a color agent that can be monitored at 410 nm. For inhibition assay, extract and orlistat of different concentrations were prepared in DMSO. Lipase (15 mg) was dissolved in Tris-buffer (50 mM, pH 8) and then it was stirred for 15 min and centrifuged at 2000 rpm for 10 min. The clear supernatant was recovered. In a test tube, 1 mL sample (extract or orlistat) was mixed with 0.5 mL lipase solution. After incubation for 30 min at 37°C, 1 mL substrate p-NPP (3 mM in 2-propanol) was added into it. This reaction mixture was incubated for 2 h at 37°C and finally the absorbance of this mixture was recorded at 410 nm against a blank. The percentage of inhibition was calculated by the following equation:

$$\% \text{ inhibition of lipase} = \left(\frac{A_{control}-A_{sample}}{A_{control}}\right)\times 100$$

where $A_{control}$ and A_{sample} are the absorbance of control and sample, respectively. The control contained all constituents except a test sample. Orlistat was used as a positive control. All the tests were done in triplicate and the mean values were used to draw graph from which the IC_{50} values (μg/mL) were determined.

2.5. Alpha-amylase inhibition assay

The α-amylase inhibitory activities of MBLE and acarbose (used as positive control) were conducted following the protocol described by previously[24] with minor modification. First, 250 μL of sample and 125 μL of α-amylase solution (45 units/mL) in 0.02 M sodium phosphate buffer (pH 6.9) were mixed and incubated at 25°C for 10 min. After incubation, 250 μL of 1% starch

solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) was added to each test tube. The reaction mixtures were further incubated at 25°C for 10 min. Then, the reaction was stopped with 250 µL of 3M HCl and subsequently 150 µL of color developing reagent containing 5 mM I_2 and 5 mM potassium iodide, was added to each test tube. The absorbance of each test tube was measured at 620 nm and the α-amylase inhibitory activity was calculated as follows:

$$\% \text{ inhibition of } \alpha\text{-amylase} = \left(\frac{A_{cont}-A_{samp}}{A_{cont}}\right)\times 100$$

where A_{samp} was defined as absorbance of the sample and A_{cont} was absorbance of the control that included all other reagents and the enzyme with the exception of the test sample.

2.6. Xanthine oxidase (XO) inhibition assay

The XO inhibitory activities of MBLE and allopurinol were determined by the method as described previously[25-27] where formation of uric acid was monitored in a XO system. Here, 4 mM xanthine (substrate) and XO (enzyme) solution (0.16 U/mL) in 0.1M phosphate buffer (pH 7.6) were prepared immediately before use. For starting the assays, a 96-well microplate was taken and the following reaction mixtures were prepared.

Sample: 20 µL sample + 125 µL xanthine solution + 20 µL enzyme solution + 50 µL 0.1 M phosphate buffer

Blank1: 125 µL xanthine solution + 20 µL enzyme solution + 70 µL 0.1 M phosphate buffer

Blank2: 125 µL xanthine solution + 90 µL 0.1 M phosphate buffer

Blank3: 20 µL sample + 125 µL xanthine solution + 70 µL 0.1 M phosphate buffer

The reaction mixture was incubated at 37°C and then absorbance was taken at 295 nm using microplate reader. The percent inhibition ratio (percent) was calculated according to the following equation:

$$\% \text{ inhibition} = 100 - \left(\frac{\text{Sample-Blank3}}{\text{Blank1-Blank2}}\right)\times 100$$

This test was done in triplicate and IC_{50} values (µg/mL) were determined using the graph drawn by the mean values of three replication.

2.7. Angiotensin 1-converting enzyme (ACE) inhibition assay

The ACE inhibition assay kit was used to determine the percentage (%) of ACE by MBLE and captopril. The colorimetric detection system of ACE inhibition assay kit determines the amount of 3-Hyroxybutylic acid (3HB) generated from 3-Hydroxybutylyl-Gly-Gly-Gly with the enzyme method[28-29]. Enzyme working solution and indicator working solution were prepared according to the instruction of kit. 20 µL of sample solution in DMSO was added to sample well of a microplate whereas 20 and 40 µL of deionized water were added to well of blank-1 and blank-2 well, respectively. Then, 20 µL of substrate buffer was added to each well and after addition of substrate buffer, 20 µL of enzyme working solution was added to sample and blank-1 using a multi-channel pipette for minimizing the well-to well time lag. Microplate was incubated at 37°C for 1 hour. Finally, 200 µL of indicator working solution was added to each well. Then the absorbance was taken at 450 nm after 10 min incubation at room temperature. Percentage (%) inhibition of ACE was calculated by the following equation:

$$\text{Percentage (\%) inhibition of ACE} = \left(\frac{A_{blank-1}-A_{sample}}{A_{blank-1}-A_{blank-2}}\right)\times 100$$

IC_{50} values (µg/mL) for ACE were determined by the same way as described in the above assays.

2.8. GC-MS analysis of MBLE

The bioactive compounds of MBLE were investigated through GC-MS (Gas chromatography-mass spectrometry) with electron impact ionization (EI) method on gas chromatography (GC-17A, Shimadzu Corporation, Kyoto, Japan), coupled with a mass spectrometer (GC-MSTQ 8040, Shimadzu Corporation, Kyoto, Japan). This instrument was equipped with a Rxi-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane) and AOC-20i+s (autosampler) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness. The injector temperature was maintained at 270°C and 2 µL of the samples was injected neat, with a split ratio of 1:10. Here helium was used as carrier gas at flow rate of 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. The constituents were identified after comparison with those available in the computer library (NIST 08-S) attached to the GC-MS instrument.

2.9. Statistical analysis

All values were expressed as mean±SD (Standard Deviation). The data were expressed as mean±standard deviation (n=3), and the significance of differences was compared using one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test at $p<0.05$ using SPSS statistical software of 16 version. The letters (a, b) used in the tables indicate the significance of the differences according to Duncan's multiple range test.

3. RESULTS AND DISCUSSION

The percentage (%) inhibitory activity of MBLE against pancreatic lipase, α-amylase, XO and ACE as function of concentration caused IC_{50} values that ultimately presented the inhibitory potential of MBLE against the corresponding enzyme (Table 1 & 2).

Table 1. Percentage inhibition and IC_{50} values of MBLE and corresponding standard inhibitor against pancreatic lipase and α-amylase.

Samples	Lipase			α-Amylase		
	Test concentration (µg/mL)	Percentage inhibition	IC_{50} (µg/mL)	Test concentration (µg/mL)	Percentage inhibition	IC_{50} (µg/mL)
MBLE	1	8.3 ± 1.04	9.87 ± 0.25[a]	5	9.2 ± 1.41	40.17 ± 2.32[a]
	2	14.4 ± 1.09		10	24.8 ± 0.70	
	4	27.5 ± 0.61		20	41.8 ± 1.25	
	8	49.3 ± 1.87		40	49.7 ± 1.51	
	16	71.8 ± 1.58		80	75.3 ± 1.52	
Orlistat	0.25	21.2 ± 1.06	1.41 ± 0.1[b]	-	-	-
	0.5	38.0 ± 1.01		-	-	
	1	46.5 ± 0.45		-	-	
	2	67.2 ± 1.22		-	-	
	4	85.5 ± 0.46		-	-	
Acarbose	-	-	-	5	14.8 ± 2.57	15.08 ± 0.85[b]
	-	-		10	27.1 ± 1.15	
	-	-		20	54.8 ± 1.31	
	-	-		40	68.6 ± 0.77	
	-	-		80	88.5 ± 1.50	

Results are means±standard deviations (SD) of triplicate determinations. Different superscripts letters (a, b) for a given value within a column representing IC_{50}, are significantly different from each other (Duncan's significant difference multiple range post-hoc test, $P<0.05$).
IC_{50}: Concentration of extract that inhibited enzyme activity by 50%.

Table 2. Percentage inhibition and IC_{50} values of MBLE and corresponding standard inhibitor against xanthine oxidase (XO) and angiotensin converting enzyme (ACE).

Samples	Xanthine oxidase (XO)			Angiotensin converting enzyme (ACE)		
	Test concentration (µg/mL)	Percentage inhibition	IC_{50} (µg/mL)	Test concentration (µg/mL)	Percentage inhibition	IC_{50} (µg/mL)
MBLE	5	6.5 ± 0.88	93.88 ± 5.21[a]	2	16.0 ± 0.75	13.34 ± 1.05[a]
	10	15.0 ± 1.02		4	28.8 ± 1.03	
	25	28.5 ± 1.58		8	45.3 ± 1.24	
	50	43.2 ± 1.38		16	67.0 ± 1.37	
	100	58.3 ± 1.70		32	81.3 ± 1.84	
Allopurinol	5	13.1 ± 0.84	39.40 ± 3.86[b]	-	-	-
	10	24.5 ± 1.78		-	-	
	25	45.2 ± 1.69		-	-	
	50	70.5 ± 1.18		-	-	
	100	89.3 ± 1.39		-	-	
Captopril	-	-	-	2	14.8 ± 2.57	8.09 ± 0.74[b]
	-	-		4	27.1 ± 1.15	
	-	-		8	49.8 ± 1.31	
	-	-		16	68.6 ± 0.77	
	-	-		32	88.5 ± 1.50	

Results are means±standard deviations (SD) of triplicate determinations. Different superscripts letters (a, b) for a given value within a column representing IC_{50}, are significantly different from each other (Duncan's significant difference multiple range post-hoc test, $P<0.05$).
IC_{50}: concentration of extract that inhibited enzyme activity by 50%.

3.1. Pancreatic lipase inhibitory activity

Generally, IC_{50} values indicate the inhibitory potential of corresponding test samples and smaller values of IC_{50} corresponds to higher anti-lipase activity. In lipase inhibitory assay, the IC_{50} value was 9.87±0.25 µg/mL for MBLE whereas it was 1.41±0.1 µg/mL for orlistat used as positive control (Table 1). The anti-lipase activity of orlistat is higher than MBLE and previous report also showed similar type of inhibition efficacy for methanolic extract of *Cornus alba* fruits where orlistat showed better activity with lower IC_{50} value (13.4±3.6) in respect to extract (22.6±3.0)[30]. In case of both MBLE and orlistat, the percentage of inhibition was increased with increasing the concentration and MBLE exhibited moderate activity at various concentration levels as compared with orlistat (Table 1). The moderate pancreatic lipase inhibitory activity of MBLE suggests that it may be useful to slow down the rate of formation, absorption and accumulation of fatty acids from dietary fats digestion, which can be an important approach for controlling obesity[31].

3.2. α-Amylase inhibitory activity

As a digestive enzyme, α-amylase play vital role in the digestion and absorption of dietary carbohydrates and because of this role, inhibition of α-amylase was considered as one of the strategies in the control of post prandial hyperglycemia associated with type 2 diabetes[32]. In this study, MBLE showed moderate inhibition of α-amylase and its IC_{50} value was found to be 40.17±2.32 µg/mL (Table 1) whereas acarbose (a reference inhibitor) had a stronger inhibitory activity against α-amylase (IC_{50}:15.08±0.85 µg/mL). The percentage of inhibition of MBLE against α-amylase was also gradually increased with increasing of test concentration (Table 1). This increasing pattern in percentage inhibition with concentration was also analogous with previous report where methanolic extract of *Paronychia argentea* and *Aloe vera* showed inhibitory activity against α-amylase[33].

3.3. XO inhibitory activity

In this study, MBLE was evaluated for xanthine oxidase inhibition activity. The results of xanthine oxidase inhibition activity of MBLE and standard allopurinol are given in Table 2. Results showed that significant xanthine oxidase inhibition was shown by MBLE with IC_{50} of 93.88±5.21 µg/mL (Table 2). Here, allopurinol, a reference XO inhibitor with IC_{50} of 39.40± 3.86 µg/mL, had a more potent inhibitory effect than MBLE. In this study, MBLE elicited a dose dependent inhibition of xanthine oxidase enzyme activity (Table 2). Through the inhibition of XO, and consequently, slowing of uric acid formation, MBLE may be beneficial for mitigating hyperuricemia and inflammation resulted from excessive XO activity[34]. The XO inhibitory activity was first time reported for MBLE prepared from leaves of *B. lacera*.

3.4. ACE inhibitory activity

Type-2 diabetes mellitus is responsible for damaging the small blood vessels in human body thereby causing the walls of the blood vessels to stiffen. This leads to develop hypertension which is a risk factor of cardiovascular diseases. Angiotensin converting enzyme (ACE) catalyzes the formation of angiotensin II (a physiologically potent vasoconstrictor) by cleavage of angiotensin I and so inhibition of ACE has been considered as one of the appropriate therapeutic approaches for regulating the blood pressure[35]. In this investigation, MBLE inhibited ACE (IC_{50}:13.34±1.05 µg/mL) as much as captopril, a standard ACE inhibitor with IC_{50} of 8.09± 0.74 µg/mL (Table 2). The inhibition of ACE activity by MBLE revealed that leaves of *B. lacera* could possess the ability to control blood pressure by reducing the production of angiotensin II.

3.5. Chemical composition analysis by GC-MS

Qualitative analysis of chemical compounds is effective in proving the bioactivities of plant extracts and in this regard, gas chromatogram combined with mass spectroscopy is considered as an important tool. It provides the qualitative information about the chemical constituents of plant extract. In this study, eighteen compounds were identified from MBLE through GC-MS analysis (Figure 1). The name of chemical components, molecular weight and molecular formula were

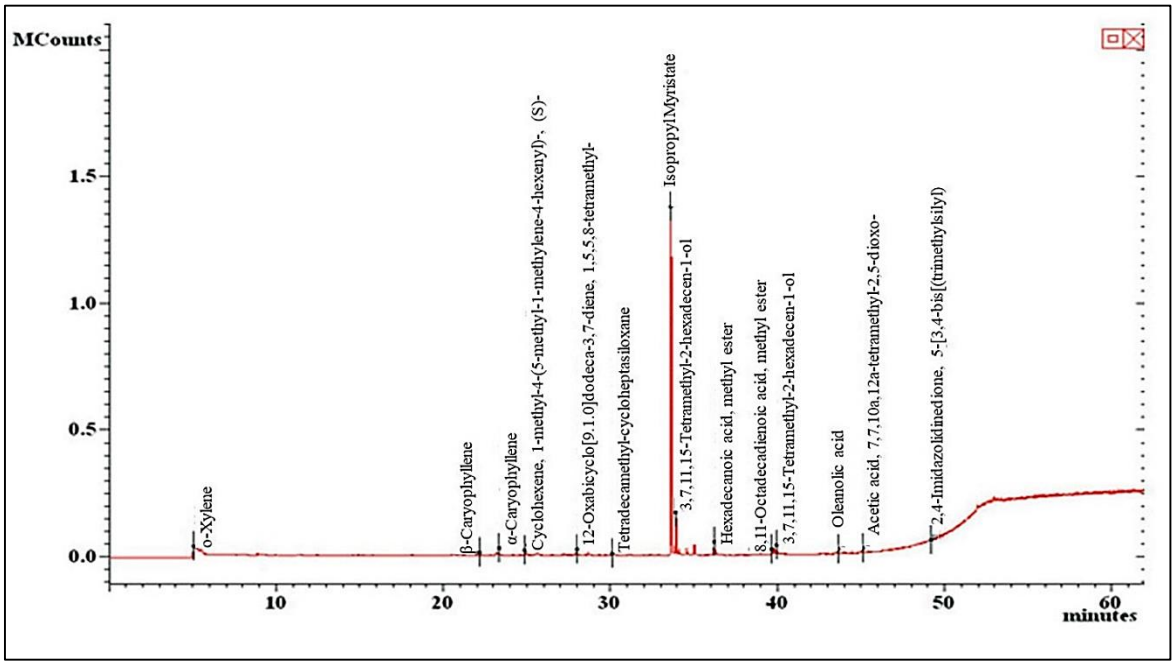

Figure 1. GC-MS chromatogram of MBLE.

Table 3. Chemical composition of MBLE analysed by GC-MS.

Sl.	RT	Compound Name	Area (%)	MW	MF
1	5.04	p-Xylene	1.23	106.16	C_8H_{10}
2	5.10	o-Xylene	1.85	106.16	C_8H_{10}
3	22.21	β-Caryophyllene	1.68	204.35	$C_{15}H_{24}$
4	23.18	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	0.77	204.35	$C_{15}H_{24}$
5	23.33	α-Caryophyllene	1.27	204.35	$C_{15}H_{24}$
6	24.90	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	1.33	204.35	$C_{15}H_{24}$
7	27.19	Caryophyllene oxide	1.39	220.35	$C_{15}H_{24}O$
8	27.99	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	1.09	220.35	$C_{15}H_{24}O$
9	30.10	Tetradecamethyl-cycloheptasiloxane	0.36	519.07	$C_{14}H_{42}O_7Si_7$
10	33.64	Isopropyl Myristate	68.07	270.45	$C_{17}H_{34}O_2$
11	33.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.38	296.53	$C_{20}H_{40}O$
12	34.10	6,10,14-trimethyl-2-Pentadecanone	1.18	268.47	$C_{18}H_{36}O$
13	36.21	Hexadecanoic acid, methyl ester	4.04	270.45	$C_{17}H_{34}O_2$
14	39.68	8,11-Octadecadienoic acid, methyl ester	1.41	294.47	$C_{19}H_{34}O_2$
15	39.79	Methyl (Z)-5,11,14,17-eicosatetraenoate	0.97	318.25	$C_{21}H_{34}O_2$
16	43.62	Oleanolic acid	2.40	456.36	$C_{30}H_{48}O_3$
17	45.10	Acetic acid, 7,7,10a,12a-tetramethyl-2,5-dioxo-1,2,3,4,4a,4b,5,7,8,9, 10,10a,10b,11,12,12a-hexadecahydro-1-azachrysen-8-yl ester	0.97	387.51	$C_{23}H_{33}NO_4$
18	49.19	5-[3,4-bis[(trimethylsilyl)oxy]phenyl]-3-methyl-5-phenyl-1-(trimethylsilyl)-2,4-Imidazolidinedione	0.05	514.83	$C_{25}H_{38}N_2O_4Si_3$

Abbreviation: MF, molecular formula; MW, molecular weight; RT, retention time.

shown in Table 3. The most prevailing compounds present in MBLE were o-xylene (1.23%); β-caryophyllene (1.68%); α-caryophyllene (1.27%); caryophyllene oxide (1.39%); isopropyl myristate (68.07%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.38%); hexadecanoic acid, methyl ester (4.04%); 8,11-Octadecadienoic acid, methyl ester (1.41%) and oleanolic acid (2.40%) (Figure 2).

The presence of some identified compounds proves the validity of enzyme inhibitory potentials of MBLE. The significant xanthine oxidase inhibition exercised by MBLE may be explained by the existence of sesquiterpene hydrocarbons like β-caryophyllene, α-caryophyllene and its oxides (Figure 2). Our results are in good agreement with those obtained for the essential oil of *Marrubium peregrinum* L.[36] where the β-caryophyllene and caryophyllene oxide of this essential oil exhibited free scavenging and xanthine oxidase inhibition properties.

In this study, MBLE showed stronger angiotensin converting enzyme (ACE) inhibitory activity as compared with captopril (used as a standard inhibitor) and it contained oleanolic acid as confirmed by GC-MS analysis (Table 3). Previously it was reported that oleanolic acid as a secondary metabolites of *Radix rehmanniae*, showed strong ACE inhibition[37]. So, oleanolic acid is responsible for inhibition of ACE by MBLE. In addition, moderate inhibition of α-amylase and pancreatic lipase by MBLE was also found here and according to the previous reports, this observed inhibitory property may be due to the presence of hexadecanoic acid methyl ester, 8,11-octa-decadienoic acid methyl ester and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Figure 2) in MBLE[38-40]. Thus, some of the identified compounds may play their key role in the inhibition of enzymes by MBLE.

4. CONCLUSION

The data of this study suggest that methanolic extract of *B. lacera* leaves (MBLE) had the ability to exert a prominent xanthine oxidase (XO) and angiotensin converting enzyme (ACE) inhibitory activity. It also exhibited moderate inhibitory properties against of α-amylase and pancreatic lipase. Furthermore, the presence of certain compounds in *B. lacera* leaves identified by GC-MS, were also consistence with this enzyme inhibitory properties. However, further studies on humans and experimental animals will be useful in order to demonstrate possible therapeutic relevance of *B. lacera* leaf extracts.

Conflicts of interest
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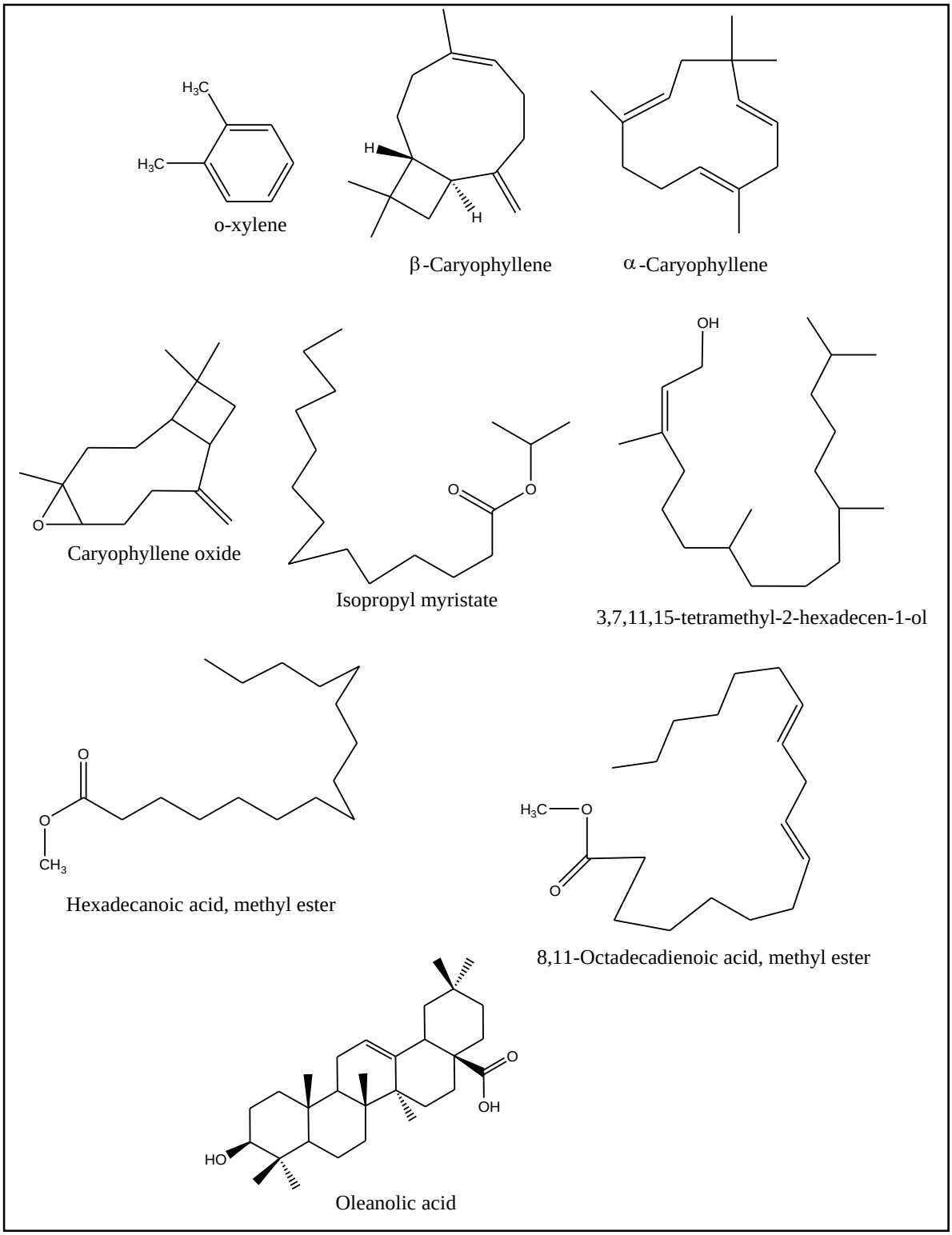

Figure 2. The chemical structure of prevailing compounds identified in MBLE by GC-MS.

REFERENCES

1. Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;20(2):12.
2. Galeno DML, Carvalho RP, Boleti AP, Lima AS, O de Almeida PD, Pacheco CC, et.al. Extract from *Eugenia punicifolia* is an antioxidant and inhibits enzymes related to metabolic syndrome. Appl Biochem Biotechnol. 2014;172:311-24.
3. Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, et al. The metabolic syndrome. Endocr Rev. 2008;29 (7):777-822.
4. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol. 2008;28:629-36.
5. Viner RM, Hsia Y, Tomsic T, Wong ICK. Efficacy and safety of anti-obesity drugs in children and adolescents: systematic review and meta-analysis. Obes Rev. 2010;11:593-602.
6. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today. 2007;12(19-20):879-89.
7. Tucci SA, Boyland EJ, Halford JC. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of

obesity: a review of current and emerging therapeutic agents. Diabetes Metab Syndr Obes. 2010;3:125-43.
8. Emmerson BT. The management of gout. N Engl J Med. 1996; 334(7):445-51.
9. Khoo BP, Leow LH. A review of inpatients with adverse drug reactions to allopurinol. Singapore Med J. 2000;41(4):156-60.
10. Vyssoulis GP, Karpanou EA, Papavassiliou MV, Belegrinos DA, Giannakopoulou AE, Toutouzas PK. Side effects of antihypertensive treatment with ACE inhibitors. Am J Hypertens. 2001;14 (S1):114A-15A.
11. Cicero AF, Colletti A. Role of phytochemicals in the management of metabolic syndrome. Phytomedicine. 2016;23(11): 1134-44.
12. Costamagna MS, Zampini IC, Alberto MR, Cuello S, Torres S, Pérez J, et al. Polyphenols-rich fraction from *Geoffroea décorticans* fruits flour affects key enzymes involved in metabolic syndrome, oxidative stress and inflammatory process. Food Chem. 2016;190:392-402.
13. Villiger A, Sala F, Suter A, Butterweck V. *In vitro* inhibitory potential of *Cynara scolymus*, *Silybum marianum*, *Taraxacum officinale*, and *Peumus bolduson* key enzymes relevant to metabolic syndrome. Phytomedicine. 2015;22:138-44.
14. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Dhaka: Asiatic Society of Bangladesh; 2003.
15. Singh UP, Parthasarathy R. Comparative antidiarrheal activity of ethanolic extract of root of *Blumea lacera* var lacera and *Blumea eriantha* DC on experimental animals. J Pharm Biomed Sci. 2012;17:1-4.
16. Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. J Nat Med. 2014;68:246-52.
17. Hossen MA, Reza ASMA, Ahmed AMA, Islam MK, Jahan I, Hossain R, et al. Pretreatment of *Blumea lacera* leaves ameliorate acute ulcer and oxidative stress in ethanol-induced Long-Evan rat: A combined experimental and chemico-biological interaction. Biomed Pharmacother. 2021;135:111211.
18. Agarwal R, Singh R, Siddiqui IR, Singh J. Triterpenoid and prenylated phenol glycosides from *Blumea lacera*. Phytochemistry. 1995;38:935-38.
19. Ragasa Y, Wong J, Rideout JA. Monoterpene glycoside and flavonoids from *Blumea lacera*. J Nat Med. 2007;61:474-75.
20. Akter R, Uddin SJ, Tiralongo J, Grice ID, Tiralongo E. A new cytotoxic steroidal glycoalkaloid from the methanol extract of *Blumera lacera* leaves. J Pharm Pharma Sci. 2015;18:616-33.
21. Akter R, Uddin SJ, Tiralongo J, Grice ID, Tiralongo E. A new cytotoxic diterpenoid glycoside from the leaves of *Blumea lacera* and its effects on apoptosis and cell cycle. Nat Prod Res. 2016;30:2688-93.
22. Hossen MA, Reza ASMA, Amin MB, Nasrin MS, Khan TA, Rajib MHR, et al. Bioactive metabolites of *Blumea lacera* attenuate anxiety and depression in rodents and computer-aided model. Food Sci Nutr. 2011;9(7):3836-51.
23. Maqsood M, Ahmed D, Atique I, Malik W. Lipase inhibitory activity of *Lagenaria siceraria* fruit as a strategy to treat obesity. Asian Pac J Trop Med. 2017;10(3):305-10.
24. Wang Y, Huang S, Shao S, Qian L, Xu P. Studies on bioactivities of tea (*Camellia sinensis* L.) fruit peel extracts: Antioxidant activity and inhibitory potential against α-glucosidase and α-amylase *in vitro*. Ind Crops Prod. 2012;37(1):520-26.
25. Sweeney AP, Wyllie SG, Shalliker RA, Markham JL. Xanthine oxidase inhibitory activity of selected Australian native plants. J Ethnopharmacol. 2001;75(2-3):273-7.
26. Chiang HC, Lo YJ, Lu FJ. Xanthine oxidase inhibitors from the leaves of *Alsophila spinulosa* (Hook) Tryon. J Enzyme Inhib. 1994;8(1):61-71.
27. Kong LD, Abliz Z, Zhou CX, Li LJ, Cheng CH, Tan RX. Glycosides and xanthine oxidase inhibitors from *Conyza bonariensis*. Phytochemistry. 2001;58(4):645-51.
28. Lam LH, Shimamura T, Sakaguchi K, Noguchi K, Ishiyama M, Fujimura Y, et al. Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyric acid. Anal Biochem. 2007;364(2):104-11.
29. Lam LH, Shimamura T, Manabe S, Ishiyama M, Ukeda H. Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyrate with water-soluble tetrazolium salt. Anal Sci. 2008;24(8):1057-60.
30. Świerczewska A, Matthias TB, Monika FM, Czerwińska E. *In vitro* α-amylase and pancreatic lipase inhibitory activity of *Cornus mas* L. and *Cornus alba* L. fruit extracts. J Food Drug Anal. 2019;27(1):249-58.
31. Zhang A, Deng Z, Ramdath DD, Tang Y, Chen PX, Liu R, et al. Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory effects on a-glucosidase and pancreatic lipase. Food Chem. 2015;172:862-72.
32. Pradeep PM, Sreerama YN. Impact of processing on the phenolic profiles of small millets: evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia. Food Chem. 2015;169:455-63.
33. Soud RSA, Hamdan II, Afifi FU. Alpha amylase inhibitory activity of some plant extracts with hyperglycemic activitv. Sci Pharm. 2004;72:25-33.
34. Vazquez-Mellado J, Alvarez-Hernandez E, Burgos-Vargas R. Primary prevention in rheumatology: The importance of hyperuricemia. Best Pract Res Clin Rheumatol. 2004;18(2):111-24.
35. Ma TK, Kam KK, Yan BP, Lam YY. Renin-angiotensin-aldosterone system blockade for cardiovascular diseases: current status. Br J Pharmacol. 2010;160(6):1273-92.
36. Kaurinovic B, Vlaisavljevic S, Popovic M, Vastag D, Djurendic-Brenesel M. Antioxidant properties of *Marrubium peregrinum* L. (Lamiaceae) essential oil. Molecules. 2010;15(9):5943-55.
37. Chao C, Hsu J, Chen M, Shih Y, Lee C, Wu M, et al. Anti-hypertensive effects of *Radix Rehmanniae* and its active ingredients. Nat Prod Res. 2020;34(11):1547-52.
38. Maqsood M, Ahmed D, Atique I, Malik W. Lipase inhibitory activity of *Lagenaria siceraria* fruit as a strategy to treat obesity. Asian Pac J Trop Med. 2017;10(3):305-10.
39. Ismail GA, Gheda SF, Abo-shady AM, Abdel-karim OH. *In vitro* potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. Food Sci Technol. 2020;40(3):681-91.
40. Iftikhar H, Ahmed D, Qamar MT. Study of Phytochemicals of *Melilotus indicus* and α-amylase and lipase Inhibitory Activities of Its Methanolic Extract and Fractions in Different Solvents. ChemistrySelect. 2019;4:7679-85.