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Original Article

BERGENIA CILIATA: ISOLATION OF ACTIVE FLAVONOIDS, GC-MS ANALYSIS, ADME STUDY AND INHIBITION ACTIVITY OF OXALATE SYNTHESIZING ENZYMES

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ABSTRACT

Objective: *Bergenia ciliata* (family-Saxifragaceae) is a well-known herb for kidney stone. The main objective of the study was the identification of flavonoids along with ADME profile. Another supportive objective was to check inhibition of enzymes which perform active role in oxalate synthesis.

Methods: The hydromethanolic extract was fractionated by liquid-liquid extraction to obtain ethyl acetate and ethyl ether fractions. The chemical structures of the purified compounds were identified by gas chromatography-mass spectrometry.

Results: A total of 12 volatile chemical compounds belonging to hydrocarbons, esters, alcohols, fatty acids, ketones, etc. were identified and characterized in ethyl acetate fraction through GC-MS analysis Fractions enriched in flavonoids showed glycolate oxidase and lactate dehydrogenase enzyme inhibition with IC_{50} value (µg/ml) 65.76 and 69.84 respectively. The kinetic behaviour of the extracts that inhibit the Glycolate oxidase and Lactate dehydrogenase activity was determined by the Lineweaver-Burk plot. The mode of inhibition of the studied plant extract was type of a non-competitive inhibition. ADMET screening of compounds successfully passed all the parameters of screening.

Conclusion: On the basis of the results, it was found that *Bergenia ciliata* (rhizome) may serve as a novel and rich source of therapeutic compounds and it can be further explored for urolithiasis treatment purposes.

Keywords: Flavonoids, GC-MS, ADME, Glycolate oxidase, Lactate dehydrogenase, Inhibition

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INTRODUCTION

Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Flavonoids help regulate cellular activity and fight off free radicals that cause oxidative stress on your body. In simpler terms, they help your body function more efficiently while protecting it against everyday toxins and stressors. Flavonoids are also powerful antioxidant agents. Flavonoid-rich foods, based on their surprising health effects, are well described as superfoods. These include all plant-origin foods, mainly tea, fruit, vegetables, grains, legumes, nuts, and wine [1]. Flavonoids are important for human health because of their antioxidant, antibacterial, antiviral, antihepatotoxic, antiosteoporotic, antiulcer, immunomodulatory, antiproliferative and apoptotic activity and anti-inflammatory activities and because they act as free radical scavengers as they are potential reducing agents that protect from oxidative damage [2-10]. *Bergenia ciliata* Sternb. (family-Saxifragaceae), a high-value plant of the Sikkim Himalaya has been investigated for antioxidant, antiurolithiac activity and bioactive compounds. However, scientific exploration of *B. ciliata* for phytochemicals and pharmacological properties is in infancy. With this view, the present study was undertaken to investigate *B. ciliata* rhizome ethanolic extract for antiurolithiac activity and bioactive compounds. Glycolate oxidase (GOX, EC 1.1.3.15) the key enzyme involved in oxalate synthesis. It was first associated with the disease primary hyperoxaluria type1 (PH1). The inhibition of GOX activity is a suitable therapeutic strategy for decreasing endogenous oxalate synthesis. The oxidation of Glycolate to glyoxylate is catalyzed by Glycolate oxidase [11] and the reduction of glyoxylate to Glycolate by lactate dehydrogenase (LDH) (EC 1.1.1.27) [12-14]. In normal pathway, alanine glyoxylate aminotransferase (AGT) converts glyoxylate to glycine and so prevents the conversion of glyoxylate to oxalate which further form complex with calcium to lead into the formation of calcium oxalate stones (fig. 1.) [15]. In the case of AGT deficiency, glyoxylate gets directly converted to oxalate by enzymes GOX and LDH. Hence their inhibition proves potential in the management of urolithiasis [16]. GC-MS analysis can identify pure compounds present at less than 1 gm [17]. Simple, cost-effective spectroscopic (UV-Vis, FTIR, GC-MS) methods together or separate can be used for detecting photo components in this sense as well as conventional methods [18-20]. So far reports on the systematic evaluation and scientific investigation of *Bergenia ciliata* or their phytoconstituents as glycolate oxidase and lactate dehydrogenase inhibitors are scare. The mode of interaction of extracted flavonoids (inhibitor) with enzyme, including identifying constituent compounds using GCMS, determining IC50 values using inhibition kinetics analysis and determining inhibitory patterns using Lineweaver-Burk plots and computer analysis using ADME were well described using this approach.

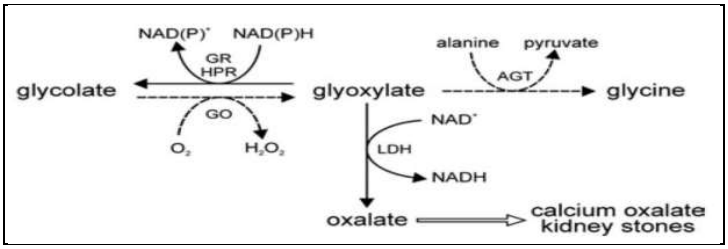


Fig. 1: Pathway associated with oxalate synthesis

MATERIALS AND METHODS

Flavonoid extraction from *Bergenia ciliata* (rhizome)

Bergenia ciliata (rhizome) was procured from Yogesh pharma Pvt. Ltd., Nanded (MS), India. The plant material was washed thoroughly with water to remove dust and dried under the shade at room temperature for 5 d. The dried parts were ground using blender to obtain the course powder and kept in an air-tight container till further use. Extraction of flavonoids was done as per method reported by Subramnian S and Nagarjan S [21]. Hundred grams of finely powdered sample were soxhlet extracted with 80% hot methanol (500 ml) on a water bath for 24 h and filtered. The filtrate obtained was re-extracted successively with petroleum ether (fraction I), ethyl ether (fraction II), and ethyl acetate (fraction III) using separating funnel. Fraction of petroleum ether was discarded due to being rich in fatty substances. The fractions of (ethyl ether-fraction II) and (ethyl acetate-fraction III) were further analysed for free and bound flavonoids, respectively. Ethyl acetate fraction of the sample was refluxed for the hydrolysis using 7% H_2SO_4 for 2 h (for removal of bounded sugars) and again filtrate was extracted in separating funnel with ethyl acetate. Ethyl acetate extract thus obtained was washed with distilled water to neutrality. Ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in rota vapour and weighed.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the ethanolic extract was carried out using a Agilent 7890 A gas chromatogram equipped and coupled to a mass detector 5975 MSD spectrometer with DB 5 MS and 30m × 0.25 µm DF of capillary column. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were at all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed from 60 °C (hold for 2 min) to 320 °C at a rate of 3 °C/min. The crude sample was diluted with an appropriate solvent (1/10, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 30-600 amu. The percentage composition of the crude extract constituents was expressed as a percentage by peak area. The identification and characterization of chemical compounds in ethanolic crude extract were based on GC retention time. AMDIS and NIST Version-Year 2011 was used MS data library and comparing the spectrum obtained through GC-MS compounds present in the plant's sample was identified.

Glycolate oxidase enzyme inhibition assay

Glycolate oxidase enzyme inhibition activity was performed in cuvette with a 1-cm light path using a UV-spectrophotometer. Each assay contained 200µM potassium phosphate (pH 7.0), 1 mg of bovine serum albumin, 3µM EDTA, 0.1µM DCIP, enzyme and water to a volume of 3 ml and a solution of test plant extracts in DMSO was incubated at room temperature for 15 min. The reaction was started by the addition of 2µM Sodium glycolate was then followed by measure of the decrease in absorbance at 600 nm. The inhibitory activity of each test compound was indicated by their IC50 values calculated using a linear regression curve. The percent inhibition of enzyme activity was calculated using standard formula [22].

$$\text{Percent of Inhibition (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

Where control represents reaction mixture as described above excluding test compounds instead contain DMSO only whereas sample represents reaction mixture same as described in method.

Lactate dehydrogenase enzyme inhibition assay

Lactate dehydrogenase activity was assayed at pH 7.4 by measuring the decrease in absorbance at 340 nm associated with NADH oxidation. Assay mixture contained 0.3 mmol NADH, 2.0 mmol pyruvate and 100 µL enzyme in volume of 3.0 ml. The reaction was started by the addition of enzyme. Lactate dehydrogenase inhibitory activity of test plant extracts was monitored spectrophotometrically following the absorbance at 340 nm under aerobic condition. The reaction mixture containing 0.3 mmol NADH, 100 µL enzymes in volume of 3.0 ml and a solution of test plant extracts in DMSO was incubated at room temperature for 15 min. The reaction was started by addition of 2 mmol pyruvate and l-lactate formation was then followed by measure of decrease in absorbance at 340 nm. The inhibitory activity of each test compound was indicated by their IC50 values calculated using linear regression curve. The percent inhibition of enzyme activity was calculated using standard formula [22].

$$\text{Percent of Inhibition (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

Where control represents reaction mixture as described above excluding test compounds instead contain DMSO only whereas sample represents reaction mixture same as described in method.

ADMET predictions

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analyses constitute the pharmacokinetics of a drug molecule [23]. In this study, prediction and significant descriptors of drug-likeness such as mutagenicity, toxicological dosage level and pharmacologically relevant properties of the compounds were predicted using Swissadme (http://www.swissadme.ch) and admetSAR (lmmd.ecust.edu.cn: 8000) servers.

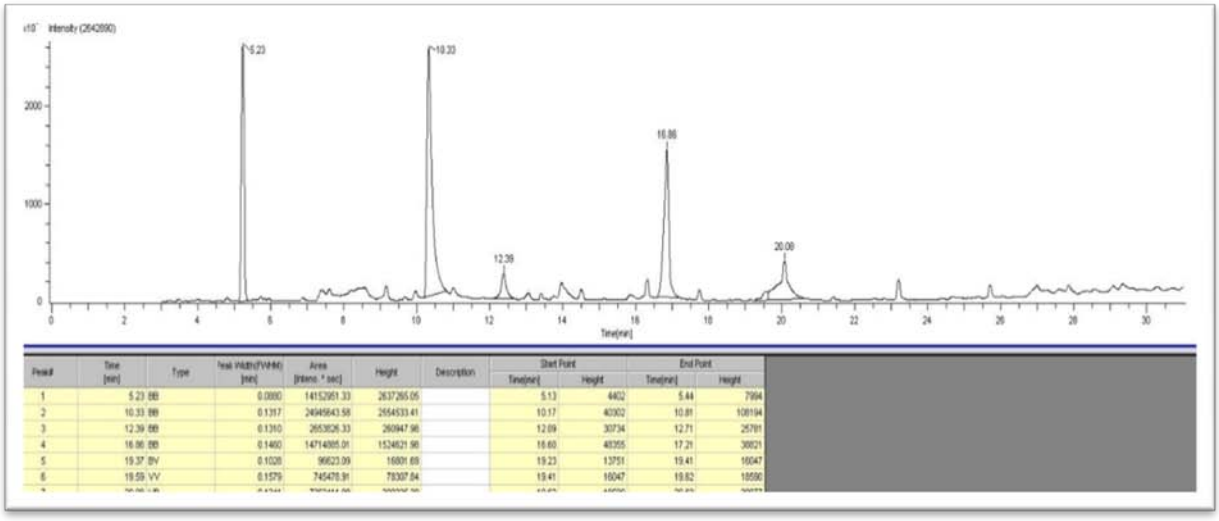
RESULTS AND DISCUSSION

Flavonoid extraction from *Bergenia ciliata* (rhizome)

The ethyl acetate fraction of *Bergenia ciliata* (rhizome) showed many peaks in the chromatogram (fig. 2a), however, only twelve compounds could be identified and characterized (fig. 2b). Plants contain so many phytoconstituents, many of which are biologically active compounds and are responsible for pharmacological activities [24]. The bioactive secondary metabolites have been shown to reduce the risk and progression of diseases such as cancer, renal disorders, etc., through various biological mechanisms. A total of 12 volatile chemical compounds belonging to hydrocarbons, esters, alcohols, fatty acids, ketones, etc. were identified and characterized in ethyl acetate fraction through GC-MS analysis (table 1).

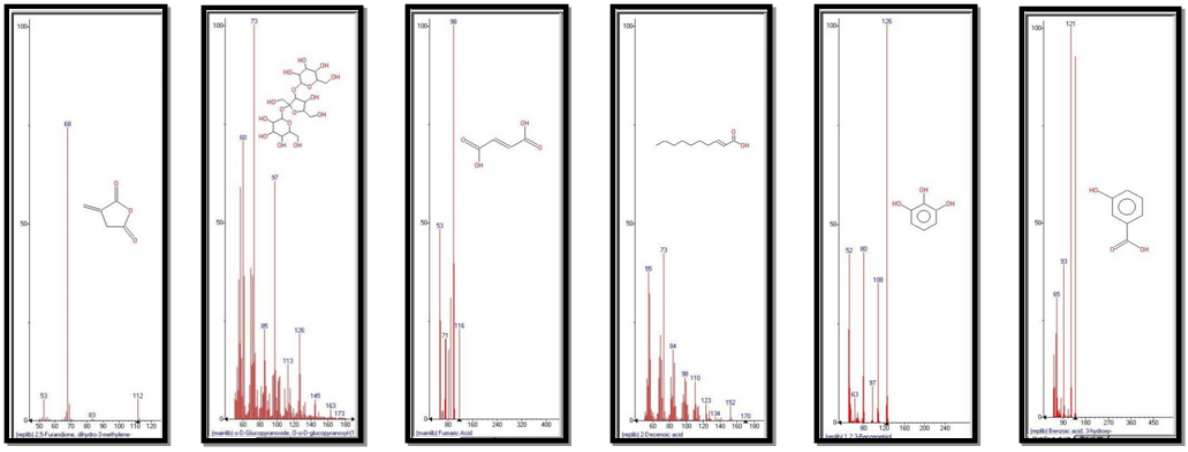
Glycolate oxidase and lactate dehydrogenase inhibition and mode of inhibition *Lineweaver–Burk plots*

The experimental evidence indicates that ethyl acetate fraction of *Bergenia ciliata* showed a good activity profile for inhibition of glycolate oxidase and lactate dehydrogenase as indicated by IC50 (µM) (fig. 3; table 2). To determine the mode of inhibition by active compounds from the plants, Lineweaver–Burk plot analysis was performed [25]. This kinetics study was carried out in the absence and presence of active compounds with varying concentrations of substrate. The initial velocity was expressed as the absorbance decrease at 340 nm for lactate dehydrogenase and 600 nm for glycolate oxidase per 10 s in the assay. In case of glycolate oxidase and lactate dehydrogenase inhibition, *B. ciliata* (ethyl acetate fraction) were found to be non-competitive inhibitors as Km values were constant while Vmax consequently decreased with increased inhibitor concentration. These finding suggest that inhibition of glycolate oxidase and lactate dehydrogenase activity, leading to retardation of oxalate synthesis, is one of the mechanism through which the plant tested in this study could be exhibiting their antiurolithiac effect. Modulation of glycolate oxidase and lactate dehydrogenase activity by compounds in these extracts would thus eventually lead to a lowering of oxalate content. Presence of glycolate oxidase and lactate dehydrogenase inhibitor blocks the normal pathway of conversion of glyoxylate to oxalate which further form a complex with calcium to lead into the formation of calcium oxalate stones. Thus, oxalate synthesis can be blocked in hyperoxaluria conditions with glycolate oxidase and lactate dehydrogenase inhibitors. This glycolate oxidase and lactate dehydrogenase inhibition could occur in a concentration-dependent or independent manner depending upon the bioactive compounds. Inhibition of glycolate oxidase and lactate dehydrogenase may occur due to these phytoconstituents. This is the first report as per author's knowledge.



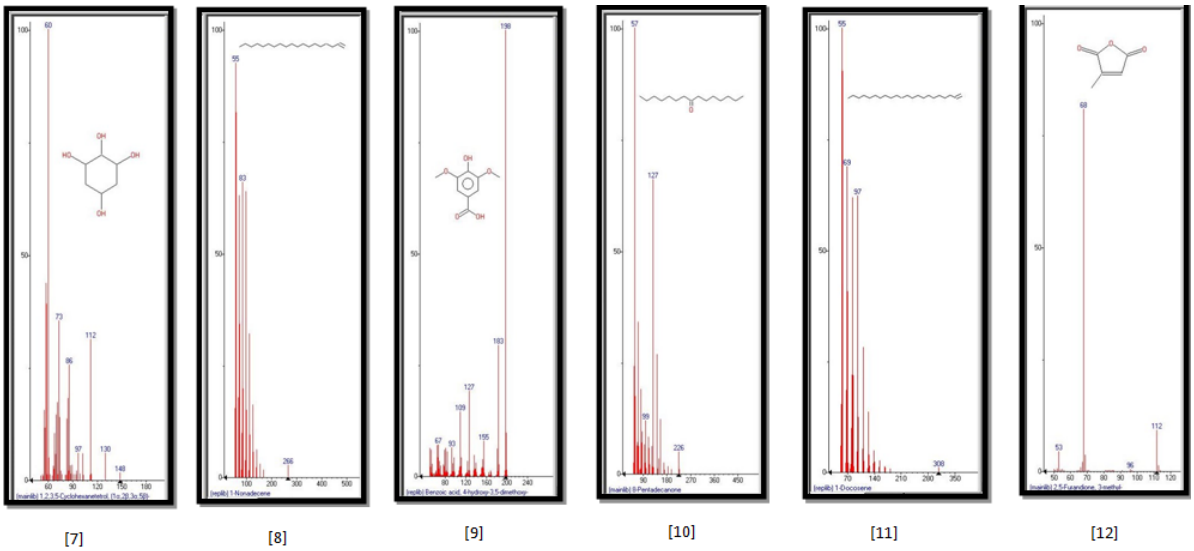
Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	5.23	BB	0.0880	1415295 1.33	2637265.05		5.13	4402	5.44	7994
2	10.33	BB	0.1317	2494564 3.58	2554533.41		10.17	40302	10.81	108194
3	12.39	BB	0.1310	2653826.33	260947.98		12.09	30734	12.71	25791
4	16.86	BB	0.1460	14714085.01	1524621.98		16.60	48355	17.21	38821
5	19.37	BV	0.1028	96623.09	16801.69		19.23	13751	19.41	16047
6	19.59	VV	0.1579	745478.91	78307.84		19.41	16047	19.82	18590

Fig. 2a: Total ion chromatogram (GC-MS) of *Bergenia ciliata* (rhizome) (ethyl acetate fraction)



[1] [2] [3] [4] [5] [6]

[1]: 2,5-Furandione, dihydro-3-methylene-[2]: α-D-Glucopyranoside,O-α-D-glucopyranosyl-(fwdarw 3)-β-D-fructofuranosyl, [3]: Fumaric acid, [4]: 2-decenoic acid, [5]: 1,2,3-Benzenetriol, [6]: Benzoic acid, 3-hydroxy-



[7] [8] [9] [10] [11] [12]

[7]: 1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β), [8]: Nonadecane, [9]: Benzoic acid, 4-hydroxy-3,5-dimethoxy-, [10]: 8-pentadecanone, [11]: Docosene, [12]: 2,5- Furandione,3-methyl-

Fig. 2b: Ion chromatograms (GC-MS) of identified compounds from *Bergenia ciliata* (rhizome) (ethyl acetate fraction)

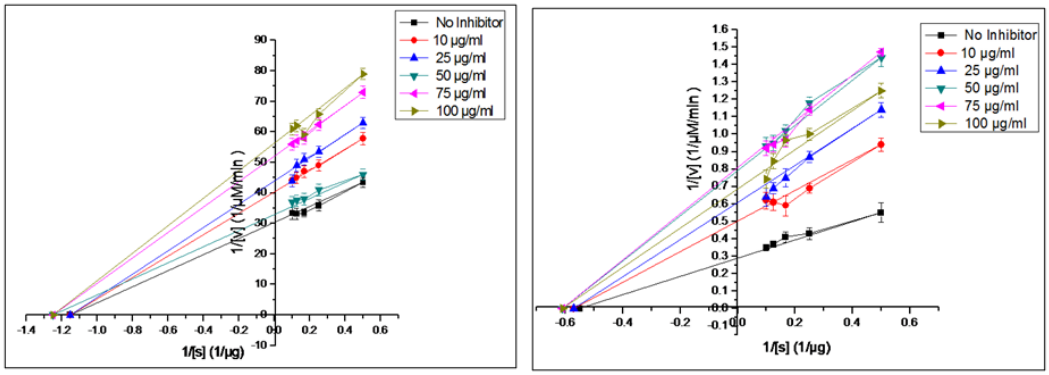
Table 1: Bioactivity of phytocomponents identified in the ethyl acetate fraction of *Bergenia ciliata*

***Bergenia ciliata* (Ethyl acetate fraction)**	
Name of the compound	**Biological Activity****
Fumaric acid	Acidifier, Acidulant, Arachidonic acid-inhibitor, Increase aromatic amino acid Decarboxylase activity, Inhibit production of uric acid, Urinary-Acidulant, Urine-acidifier.
α-D-Glucopyranoside, O-α-D-glucopyranosyl-(fwdarw 3)-β-D-fructofuranosyl	Aldehyde-oxidase-inhibitor, Anticancer, Antidote, Antiretinitic, Antitumor, Catechol-O-Methyl-Transferase-Inhibitor, Increase Osteocalcin, Inhibit Production of Tumor Necrosis Factor, Inhibit production of uric acid, Lower oxalate, Ionic Channel Opener, NADH-Oxidase Inhibitor, Nitric Oxide Synthase Inhibitor, Occuloirritant, Occulotensive, Odontolytic
2-decenoic acid	Acidifier, Acidulant, Arachidonic acid-inhibitor, Increased aromatic amino acid Decarboxylase activity, Inhibit production of uric acid, Urinary-Acidulant, Urine-acidifier.
Benzoic acid, 3-hydroxy-	17-beta-hydroxysteroid dehydrogenase-inhibitor, Aryl-Hydrocarbon-Hydroxylase-Inhibitor, Testosterone-Hydroxylase-Inducer, Acidifier, Acidulant, Arachidonic acid-inhibitor, Increase aromatic amino acid Decarboxylase activity, Inhibit production of uric acid, Urinary-Acidulant, Urine-acidifier.
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	Acidifier, Acidulant, Arachidonic acid-inhibitor, Increased aromatic amino acid Decarboxylase activity, Inhibit production of uric acid, Urinary-Acidulant, Urine-acidifier, 17-beta-hydroxysteroid dehydrogenase-inhibitor, Aryl-Hydrocarbon-Hydroxylase-Inhibitor, Testosterone-Hydroxylase-Inducer.
2,5-Furandione,3-methyl-	Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Methyl-Donar, Methyl-Guanidine-inhibitor.

(**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database)

Table 2: Vmax and Km of *B. ciliata* (ethyl acetate extracts) for glycolate oxidase (GOX) and lactate dehydrogenase (LDH) inhibition

Samples	Concentrations (µg/ml)	V_{max} (µg/min)	K_m (µg/ml)	Type of Inhibition	IC_{50} value (µg/ml)
B. ciliata (gox)	10	0.025	0.86	Non-competitive	65.76
	25	0.025	0.86		
	50	0.029	0.8		
	75	0.0196	0.8		
	100	0.0181	0.8		
B. ciliata (ldh)	10	3.33	1.75	Non-competitive	69.84
	25	1.66	1.75		
	50	1.25	1.63		
	75	1.25	1.63		
	100	1.42	1.63		



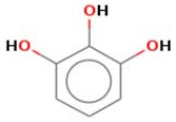
[a] Ethyl acetate fractions of *B.ciliata* [b] Ethyl acetate fractions of *B.ciliata*

Fig. 3: Lineweaver-Burk plot for enzyme Glycolate oxidase and Lactate dehydrogenase

ADMET predictions

The potential ADME profiles of the compounds as predicted using the admetSAR server, while the distribution profile of the compounds as obtained from the admetSAR server is shown in (table 11). Computational study for the prediction of the relevant properties influencing bioactivity of the lead compounds was performed. The ADME properties of the compounds were evaluated, and the selected properties are linked to metabolism and cell permeation.

Compound 1: 1,2,3-Benzenetriol



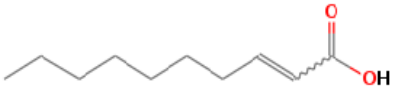
ADMET predicted profile---Classification

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB-	0.6478
Human Intestinal Absorption	HIA+	0.9642
Caco-2 Permeability	Caco2+	0.7355
P-glycoprotein Substrate	Non-substrate	0.6749
P-glycoprotein Inhibitor	Non-inhibitor	0.9691
	Non-inhibitor	0.9916
Renal Organic Cation Transporter	Non-inhibitor	0.9289
Distribution		
Subcellular localization	Mitochondria	0.6807
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8234
CYP450 2D6 Substrate	Non-substrate	0.9031
CYP450 3A4 Substrate	Non-substrate	0.7441
CYP450 1A2 Inhibitor	Non-inhibitor	0.7436
CYP450 2C9 Inhibitor	Non-inhibitor	0.8271
CYP450 2D6 Inhibitor	Non-inhibitor	0.9494
CYP450 2C19 Inhibitor	Non-inhibitor	0.9397
CYP450 3A4 Inhibitor	Non-inhibitor	0.8682
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.6899
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9526
	Non-inhibitor	0.9299
AMES Toxicity	AMES toxic	0.7459
Carcinogens	Non-carcinogens	0.8816
Fish Toxicity	High FHMT	0.6928
Tetrahymena Pyriformis Toxicity	High TPT	0.8900
Honey Bee Toxicity	High HBT	0.6927
Biodegradation	Ready biodegradable	0.6003
Acute Oral Toxicity	III	0.8089
Carcinogenicity (Three-class)	Non-required	0.5339

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-0.4107	LogS
Caco-2 Permeability	0.5771	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	2.2344	LD50, mol/kg
Fish Toxicity	1.1417	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	0.5649	pIGC50, ug/l

Compound 2: 2-decenoic acid



ADMET predicted profile---Classification

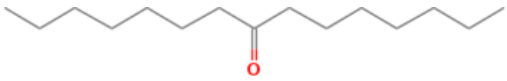
Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9638
Human Intestinal Absorption	HIA+	0.9908
Caco-2 Permeability	Caco2+	0.8326
P-glycoprotein Substrate	Non-substrate	0.6523
P-glycoprotein Inhibitor	Non-inhibitor	0.9689
	Non-inhibitor	0.7699

Model	Result	Probability
Renal Organic Cation Transporter	Non-inhibitor	0.9061
Distribution		
Subcellular localization	Plasma membrane	0.6894
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.7322
CYP450 2D6 Substrate	Non-substrate	0.9059
CYP450 3A4 Substrate	Non-substrate	0.7043
CYP450 1A2 Inhibitor	Inhibitor	0.7152
CYP450 2C9 Inhibitor	Non-inhibitor	0.8948
CYP450 2D6 Inhibitor	Non-inhibitor	0.9474
CYP450 2C19 Inhibitor	Non-inhibitor	0.9459
CYP450 3A4 Inhibitor	Non-inhibitor	0.9608
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.8882
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8983
	Non-inhibitor	0.9369
AMES Toxicity	Non AMES toxic	0.9737
Carcinogens	Non-carcinogens	0.5263
Fish Toxicity	High FHMT	0.9784
Tetrahymena Pyriformis Toxicity	High TPT	0.9996
Honey Bee Toxicity	High HBT	0.7359
Biodegradation	Ready biodegradable	0.7618
Acute Oral Toxicity	III	0.8593
Carcinogenicity (Three-class)	Non-required	0.6623

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-3.5855	LogS
Caco-2 Permeability	1.3217	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.9685	LD50, mol/kg
Fish Toxicity	0.8959	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	0.7180	pIGC50, ug/l

Compound 3: 8-pentadecanone



ADMET predicted profile---Classification

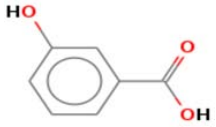
Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9882
Human Intestinal Absorption	HIA+	0.9955
Caco-2 Permeability	Caco2+	0.8766
P-glycoprotein Substrate	Non-substrate	0.6680
P-glycoprotein Inhibitor	Non-inhibitor	0.8320
	Non-inhibitor	0.7768
Renal Organic Cation Transporter	Non-inhibitor	0.8727
Distribution		
Subcellular localization	Mitochondria	0.4585
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8589
CYP450 2D6 Substrate	Non-substrate	0.8439
CYP450 3A4 Substrate	Non-substrate	0.6531
CYP450 1A2 Inhibitor	Inhibitor	0.6890
CYP450 2C9 Inhibitor	Non-inhibitor	0.9433
CYP450 2D6 Inhibitor	Non-inhibitor	0.9502
CYP450 2C19 Inhibitor	Non-inhibitor	0.9645
CYP450 3A4 Inhibitor	Non-inhibitor	0.9815
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.8752
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8043
	Non-inhibitor	0.7659

Model	Result	Probability
AMES Toxicity	Non AMES toxic	0.9859
Carcinogens	Carcinogens	0.6310
Fish Toxicity	High FHMT	0.7423
Tetrahymena Pyriformis Toxicity	High TPT	0.8910
Honey Bee Toxicity	High HBT	0.7254
Biodegradation	Ready biodegradable	0.8731
Acute Oral Toxicity	III	0.8455
Carcinogenicity (Three-class)	Non-required	0.7622

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-2.2537	LogS
Caco-2 Permeability	1.3709	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.5870	LD50, mol/kg
Fish Toxicity	0.8094	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	0.5873	pIGC50, ug/l

Compound 4: Benzoic acid, 3-hydroxy-



ADMET predicted profile---Classification

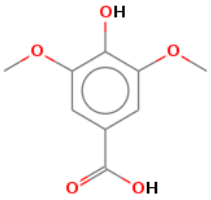
Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.5320
Human Intestinal Absorption	HIA+	0.9872
Caco-2 Permeability	Caco2+	0.8937
P-glycoprotein Substrate	Non-substrate	0.7493
P-glycoprotein Inhibitor	Non-inhibitor	0.9890
	Non-inhibitor	0.9927
Renal Organic Cation Transporter	Non-inhibitor	0.9078
Distribution		
Subcellular localization	Mitochondria	0.9063
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8115
CYP450 2D6 Substrate	Non-substrate	0.9377
CYP450 3A4 Substrate	Non-substrate	0.7652
CYP450 1A2 Inhibitor	Non-inhibitor	0.9752
CYP450 2C9 Inhibitor	Non-inhibitor	0.9697
CYP450 2D6 Inhibitor	Non-inhibitor	0.9827
CYP450 2C19 Inhibitor	Non-inhibitor	0.9651
CYP450 3A4 Inhibitor	Non-inhibitor	0.9493
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9554
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9617
	Non-inhibitor	0.9771
AMES Toxicity	Non AMES toxic	0.9826
Carcinogens	Non-carcinogens	0.8226
Fish Toxicity	High FHMT	0.7616
Tetrahymena Pyriformis Toxicity	Low TPT	0.8365
Honey Bee Toxicity	High HBT	0.7797
Biodegradation	Ready biodegradable	0.8413
Acute Oral Toxicity	III	0.5472
Carcinogenicity (Three-class)	Non-required	0.6300

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-1.3479	LogS
Caco-2 Permeability	1.1511	LogPapp, cm/s

Model	Value	Unit
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.3983	LD50, mol/kg
Fish Toxicity	2.2036	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	-0.8949	pIGC50, ug/l

Compound 5: Benzoic acid, 4-hydroxy-3,5-dimethoxy



ADMET predicted profile---Classification

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.5861
Human Intestinal Absorption	HIA+	0.9165
Caco-2 Permeability	Caco2+	0.7124
P-glycoprotein Substrate	Non-substrate	0.6033
P-glycoprotein Inhibitor	Non-inhibitor	0.9199
	Non-inhibitor	0.8879
Renal Organic Cation Transporter	Non-inhibitor	0.9136
Distribution		
Subcellular localization	Mitochondria	0.8825
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8213
CYP450 2D6 Substrate	Non-substrate	0.8899
CYP450 3A4 Substrate	Non-substrate	0.6258
CYP450 1A2 Inhibitor	Non-inhibitor	0.9052
CYP450 2C9 Inhibitor	Non-inhibitor	0.9316
CYP450 2D6 Inhibitor	Non-inhibitor	0.9445
CYP450 2C19 Inhibitor	Non-inhibitor	0.8579
CYP450 3A4 Inhibitor	Non-inhibitor	0.9538
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.8767
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9858
	Non-inhibitor	0.9664
AMES Toxicity	Non AMES toxic	0.9342
Carcinogens	Non-carcinogens	0.8809
Fish Toxicity	High FHMT	0.8272
Tetrahymena Pyriformis Toxicity	High TPT	0.9067
Honey Bee Toxicity	High HBT	0.7522
Biodegradation	Ready biodegradable	0.7199
Acute Oral Toxicity	II	0.4765
Carcinogenicity (Three-class)	Non-required	0.7159

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-2.1167	LogS
Caco-2 Permeability	0.7627	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	2.5353	LD50, mol/kg
Fish Toxicity	1.6288	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	0.4613	pIGC50, ug/l

Compound 6: Docosene

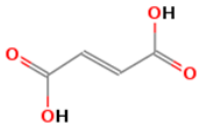
ADMET predicted profile---Classification

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9763
Human Intestinal Absorption	HIA+	0.9928
Caco-2 Permeability	Caco2+	0.7989
P-glycoprotein Substrate	Non-substrate	0.6522
P-glycoprotein Inhibitor	Non-inhibitor	0.7775
	Non-inhibitor	0.5881
Renal Organic Cation Transporter	Non-inhibitor	0.8619
Distribution		
Subcellular localization	Lysosome	0.4578
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8261
CYP450 2D6 Substrate	Non-substrate	0.8048
CYP450 3A4 Substrate	Non-substrate	0.7278
CYP450 1A2 Inhibitor	Inhibitor	0.5418
CYP450 2C9 Inhibitor	Non-inhibitor	0.9157
CYP450 2D6 Inhibitor	Non-inhibitor	0.9426
CYP450 2C19 Inhibitor	Non-inhibitor	0.9190
CYP450 3A4 Inhibitor	Non-inhibitor	0.9832
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.6838
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.7812
	Non-inhibitor	0.8777
AMES Toxicity	Non AMES toxic	0.9904
Carcinogens	Carcinogens	0.6000
Fish Toxicity	High FHMT	0.9954
Tetrahymena Pyriformis Toxicity	High TPT	0.9981
Honey Bee Toxicity	High HBT	0.7751
Biodegradation	Ready biodegradable	0.5000
Acute Oral Toxicity	III	0.6572
Carcinogenicity (Three-class)	Non-required	0.5494

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-4.9876	LogS
Caco-2 Permeability	1.3776	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.3452	LD50, mol/kg
Fish Toxicity	-1.1807	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	1.4804	pIGC50, ug/l

Compound 7: fumaric acid



ADMET predicted profile---Classification

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9017
Human Intestinal Absorption	HIA+	0.8740
Caco-2 Permeability	Caco2-	0.6728
P-glycoprotein Substrate	Non-substrate	0.8006
P-glycoprotein Inhibitor	Non-inhibitor	0.9850
	Non-inhibitor	0.9808
Renal Organic Cation Transporter	Non-inhibitor	0.9583
Distribution		

Model	Result	Probability
Subcellular localization	Mitochondria	0.7863
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8262
CYP450 2D6 Substrate	Non-substrate	0.9397
CYP450 3A4 Substrate	Non-substrate	0.8039
CYP450 1A2 Inhibitor	Non-inhibitor	0.9659
CYP450 2C9 Inhibitor	Non-inhibitor	0.9490
CYP450 2D6 Inhibitor	Non-inhibitor	0.9606
CYP450 2C19 Inhibitor	Non-inhibitor	0.9773
CYP450 3A4 Inhibitor	Non-inhibitor	0.9554
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9899
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9836
	Non-inhibitor	0.9891
AMES Toxicity	Non AMES toxic	0.9132
Carcinogens	Non-carcinogens	0.5130
Fish Toxicity	High FHMT	0.8398
Tetrahymena Pyriformis Toxicity	Low TPT	0.9808
Honey Bee Toxicity	High HBT	0.7308
Biodegradation	Ready biodegradable	0.7561
Acute Oral Toxicity	III	0.7762
Carcinogenicity (Three-class)	Non-required	0.7191

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-0.3321	LogS
Caco-2 Permeability	0.4098	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.6871	LD50, mol/kg
Fish Toxicity	0.9694	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	-0.6339	pIGC50, ug/l

Compound 8: Nonadecane

ADMET predicted profile---Classification

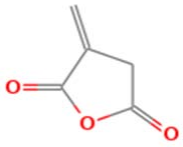
Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9821
Human Intestinal Absorption	HIA+	0.9921
Caco-2 Permeability	Caco2+	0.8284
P-glycoprotein Substrate	Non-substrate	0.6915
P-glycoprotein Inhibitor	Non-inhibitor	0.8985
	Non-inhibitor	0.7267
Renal Organic Cation Transporter	Non-inhibitor	0.8780
Distribution		
Subcellular localization	Lysosome	0.5981
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8480
CYP450 2D6 Substrate	Non-substrate	0.7762
CYP450 3A4 Substrate	Non-substrate	0.7237
CYP450 1A2 Inhibitor	Non-inhibitor	0.6175
CYP450 2C9 Inhibitor	Non-inhibitor	0.9349
CYP450 2D6 Inhibitor	Non-inhibitor	0.9373
CYP450 2C19 Inhibitor	Non-inhibitor	0.9540
CYP450 3A4 Inhibitor	Non-inhibitor	0.9877
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.8149
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8620
	Non-inhibitor	0.8109
AMES Toxicity	Non AMES toxic	0.9965
Carcinogens	Carcinogens	0.6420

Model	Result	Probability
Fish Toxicity	High FHMT	0.9374
Tetrahymena Pyriformis Toxicity	High TPT	0.9947
Honey Bee Toxicity	High HBT	0.7485
Biodegradation	Ready biodegradable	0.7561
Acute Oral Toxicity	III	0.6143
Carcinogenicity (Three-class)	Non-required	0.6328

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-5.1776	LogS
Caco-2 Permeability	1.3807	LogPapp, cm/s
Distribution		
Metabolism		
Excretion		
Toxicity		
Rat Acute Toxicity	1.3444	LD50, mol/kg
Fish Toxicity	-0.7109	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	0.3450	pIGC50, ug/l

Compound 9: 2,5-Furandione, dihydro-3-methylene



ADMET predicted profile---Classification

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9627
Human Intestinal Absorption	HIA+	0.9833
Caco-2 Permeability	Caco2+	0.5452
P-glycoprotein Substrate	Non-substrate	0.7919
P-glycoprotein Inhibitor	Non-inhibitor	0.6266
	Non-inhibitor	0.9951
Renal Organic Cation Transporter	Non-inhibitor	0.8772
Distribution		
Subcellular localization	Mitochondria	0.6548
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8815
CYP450 2D6 Substrate	Non-substrate	0.8935
CYP450 3A4 Substrate	Non-substrate	0.7178
CYP450 1A2 Inhibitor	Non-inhibitor	0.8434
CYP450 2C9 Inhibitor	Non-inhibitor	0.9264
CYP450 2D6 Inhibitor	Non-inhibitor	0.9524
CYP450 2C19 Inhibitor	Non-inhibitor	0.8061
CYP450 3A4 Inhibitor	Non-inhibitor	0.9487
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9099
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9172
	Non-inhibitor	0.9875
AMES Toxicity	Non AMES toxic	0.8025
Carcinogens	Non-carcinogens	0.8480
Fish Toxicity	High FHMT	0.9233
Tetrahymena Pyriformis Toxicity	Low TPT	0.7182
Honey Bee Toxicity	High HBT	0.8293
Biodegradation	Ready biodegradable	0.7452
Acute Oral Toxicity	III	0.7565
Carcinogenicity (Three-class)	Non-required	0.5918

ADMET predicted profile---Regression

Model	Value	Unit
Absorption		
Aqueous solubility	-0.6464	LogS
Caco-2 Permeability	0.9549	LogPapp, cm/s
Distribution		
Metabolism		

Model	Value	Unit
Excretion		
Toxicity		
Rat Acute Toxicity	1.9632	LD50, mol/kg
Fish Toxicity	-0.0057	pLC50, mg/l
Tetrahymena Pyriformis Toxicity	-0.4252	pIGC50, ug/l

Previous study showed that phytochemical constituents of *B. ciliata* are gallic acid, bergenin, (+)-afzelechin [26], 11-O-galloyl bergenin [27], paashaanolactone [26], β-Sitosterol [28] and β-Sitosterol-*D*-glucoside [29]. Pharmacological properties have demonstrated that the root is well known in traditional medicine for protection against diarrhea; cough, in uric acid diathesis and in pulmonary infections [28]; coughs and colds, hemorrhoids, asthma and urinary problems [30]. The juice of *B. ciliata* leaves is used as drops to relieve earaches [30]. Currently, Various Ayurvedic classical drugs such as Pashanabhedadi kwath, Pashanabhedadi ghrit, Pashanabhedadi Churan etc. are prepared from Pashanbhed rhizome.

CONCLUSION

We have extracted, purified flavonoids by the liquid-liquid extraction method. Finally, ethyl acetate fractions were collected and characterized by GC-MS analysis. These fractions are used to perform glycolate oxidase and lactate dehydrogenase inhibition study. Flavonoids fraction of *B. ciliata* showed good enzyme inhibitory activity and ADME profile. Lineviewer-Burk plot and mode of inhibition confirmed the potential of it's as glycolate oxidase and lactate dehydrogenase inhibitors. However, further dose adjustment and molecular mechanism study need to perform for better understanding.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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