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Phytochemical and Bioactivity Analysis of Several Methanolic Extracts of Nine Bryophytes Species

Ebru KOCADAĞ KOCAZORBAZ[1], Kerem TOK*[1], Hichem MOULAHOUM[1], Rabia Nur ÜN[1]

Abstract

Bryophytes are a class of organisms found all over the globe except the sea. They can grow on different surfaces and are known for their fragrant aromas and strongly hot and bitter taste. Bryophytes have been used in traditional Chinese medicine for the treatment of many pathological conditions. In the current study, we describe the bioactivities present in methanolic extracts obtained from 9 species of bryophytes. Plant samples were dried and extracted in a water/methanol solution which was explored for flavonoid and phenolic content. Afterward, the extracts were analyzed for their potential bioactivities including DPP4 inhibition, metal chelation, antioxidant, and antiglycation activities. Results indicate that the methanolic extracts of each species showed high effectiveness for different bioactivities. The current findings suggest these bryophytes as a promising source of therapeutics against oxidative stress, hypertension, and diabetes.

Keywords: Bryophytes, bioactive molecules, diabetes, antiglycation, DPP-4

1. INTRODUCTION

Bryophytes can be defined as an irregular group that includes three kinds of non-vascular land plants such as the hornworts, liverworts, and mosses. This division contains 1036 genera and about 18,409 species. The most important factor for these plants is the humid environment needed to live. Bryophytes are sometimes completely buried underwater, found on wet floors, humid environments, on soil and rocks, and tree trunks. The antioxidant activity of mosses has been reported to be higher than that of many vegetables. In research, the high antioxidant effect of moss has shown great importance in the preparation of drugs and cosmetic products. On the other hand, moss species have been widely used to treat some diseases owing to their high phenolic content [1]. Bryophytes have been used to treat various diseases such as anti-leukemic activity, wound healing, heart disease, nervous prostration, diarrhea, cold and fever, microbial infectious, angina [2-4]. They are still using for antimicrobial agents, source of antibiotics, used for infections and swellings, growth of hairs, skin ailments, antipyretic, antidote, urinary difficulties [5-7].

Diabetes mellitus (DM) is a multifactorial chronic metabolic disorder that is characterized by deteriorated carbohydrate metabolism. In addition to the metabolic problems of diabetes, long-term exposure to deteriorated metabolites leads to the

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development of microvascular and macrovascular complications including nephropathy, neuropathy, retinopathy, and atherosclerosis. There are two types of diabetes. These are type 1 (insulin-dependent, IDDM) and type 2 (non-insulin-dependent, NIDDM) diabetes mellitus [8]. According to the International Diabetes Federation, there are 463 million diabetics in the world, and it is believed that this number will increase to 700 million in 2045. The number of people exposed to this disease is increasing daily, which makes prevention approaches and new treatment development a necessity. One of the diabetes outcomes is the formation of advanced glycation end products (AGEs) under hyperglycemic conditions. Non-enzymatic glycation occurs between glucose and the amine terminal side of proteins which results in Schiff-base intermediates and eventually forms more stable Amadori compounds that later form AGEs. Accumulation of AGEs causes functional changes in tissue proteins [9]. These AGEs can cause various important diabetic complications such as rheumatoid arthritis, neurodegenerative diseases, cataract, Alzheimer's disease, etc. [10]. For this reason, preventive approaches against AGE formation are of high interest. Hypertension is amongst the most widespread chronic diseases and is a risk factor for coronary heart diseases, congestive heart failure, stroke, and kidney diseases. Hypertension and diabetes are often associated and are risk factors for each other. Hypertension can be treated with antihypertensive medicines and changes in lifestyle [11].

α-glucosidase and α-amylase are enzymes responsible for the hydrolysis of disaccharides, trisaccharides, oligosaccharides, and starch. These enzymes are important for the regulation of blood glucose levels in diabetic patients. Inhibition of these enzymes decreases carbohydrate digestion and delays glucose absorption. The strategy of inhibiting these target enzymes is a key point in obesity and type 2 diabetes treatments [12].

There are approved various reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, etc., and free radicals in our body [13]. These factors affect serious diseases and systems such as the inflammatory and cardiovascular system, diabetes, stroke, and cancer diseases [14]. Currently, there are many useable synthetic molecules demonstrating important antioxidant activities. Yet, the problem in using these molecules has been linked with some levels of harm to the body [15]. Hence, the investigation of natural antioxidative molecules from living sources has been important.

In this study, methanolic extracts of 9 types of mosses (*T. barbuloides, B. stricta, L. sciuroides, F. antipyretica, M. polymorpha, A. californica, C. conicum, B. pomiformis, G. lisae)* were evaluated for selected bioactivities such as α-glucosidase and Dipeptidyl peptidase 4 (DPP-4) inhibitory activities, anti-glycation activity, antioxidant and metal-chelating abilities. This study aims to propose these bryophytes as a source of natural bioactive compounds and their potential in medical approaches.

2. MATERIAL AND METHODS

2.1. Extraction of phenolics and flavonoids

The plant samples were identified by Prof. Dr. Adnan ERDAG, and the voucher numbers were defined [16]. All plant samples have been dried and pulverized. *A. californica* (1.13 g), *B. pomiformis* (7.45 g), *B. stricta* (7.28 g), *C. conicum* (4.98 g), *F. antipyretica var. gracilis* (4.29 g), *G. lisae* (2.80 g), *L. sciuroides* (4.90 g), *M. polymorpha* (10.29 g), and *T. barbuloides* (2.06 g) were boiled with methanol/water (1:1) in soxhlet for 4-6 hours. The aqueous extract was filtered and evaporated at 45^{o}C using Heidolph Laborota 4000. The samples were lyophilized and stored at 4°C for the upcoming tests.

Table 1 Type, localization, and identification year of the various bryophytes used in the current work

Plants name	**Localization**	**Identification Year**
Antitrichia californica Sull. (Leucodontaceae)	Aydın, Turkey	03.02.1998
Bartramia pomiformis Hedw. (Bartramiaceae)	Aydın, Turkey	10.03.1998
Bartramia stricta Brid. (Bartramiaceae)	Cine, Aydın, Turkey	14.04.2000

Conocephalum conicum (L.) Underw. (Conocephalaceae)	Bozdogan, Aydın, Turkey	04.11.1999
Fontinalis antipyretica Hedw. var. *gracilis* (Lindb.) Schimp. (Fontinalaceae)	Besparmak dagı, Cavdar koyu, Aydın, Turkey	05.03.1999
Grimmia lisae De Not. (Grimmiaceae)	Cavdar koyu, Aydın, Turkey	27.01.1998
Leucodon sciuroides (Hedw.) Schwägr. (Leucodontaceae)	Cine, Aydın, Turkey	09.04.2000
Marchantia polymorpha L. (Marchantiaceae)	Cine, Aydın, Turkey	14.04.2000
Timmiella barbuloides (Brid.) Mönk. (Pottiaceae)	Cine, Aydın, Turkey	14.04.2000

2.2. Total phenolic content of extracts

The total phenolic content of samples was analyzed by the Folin-Ciocaltaue method with slight modifications [17]. Standards (Gallic acid, range between 0.05-0.5 mg/mL) and plant extracts were prepared in methanol. Test tubes containing 0.5 mL sample with different dilutions, 2.5 mL Folin reagent (10% in water), and sodium carbonate (20% in water) were vigorously mixed and kept in dark for an hour. The color change was measured at 750 nm and results were given as gallic acid equivalent (µg/mL GAE).

2.3. Total flavonoid content

The total flavonoid content of the extracts was analyzed according to Arvouet-Grand et al. method [18]. Standards (Quercetin, 5.0-100 µg/mL) and extracts were prepared in methanol. A reaction mixture consisting of a 1.0 mL sample of different dilutions and 1 mL $AlCl_3$ (2.0% in water). The tubes were incubated at 25°C for 10 min. The absorbance was read at 405 nm and results were given as quercetin equivalent (µg/mL QE).

2.4. Antioxidant activity

The evaluation of the anti-oxidant effects of the different plant extracts was determined by the CUPRAC method [19]. A mixture of $CuCl_2$ (25 µL, 10 mM), 7.5 mM neocuproine (prepared in EtOH), ammonium acetate buffer (1.0 M, pH 7.0) is deposited into a 96-well plate. 50 µL of the sample was added to the reaction mixture and incubated for 30 min at 37°C. The same procedure was applied for the standard curve using ascorbic acid (20-100 µmol/mL). The color changes were estimated at 450 nm and the antioxidant activity was calculated as mM ascorbic acid equivalent (AAE)/ µg phenolics.

2.5. Metal chelation efficiency

The samples were evaluated for their metal chelation efficiency via the Cu^{+2} chelation method [20]. The copper solution includes 10 mM hydroxylamine hydrochloride buffer (pH 5.0, 10 mM KCl). The reaction consisted in mixing 50 µL of different sample dilutions, 50 µL of copper solution (0.25 mM), and 25 µL of murexide (1.0 mM). The formed color was read separately at 462 and 530 nm after 3 min incubation at 25°C. EDTA was also evaluated as a control molecule. Metal chelation efficiency was calculated according to the following formula: Metal chelation efficiency (%) = $[1 - ((A_{sample} - A_{blank})/A_{control})] \times 100$.

2.6. DPP4 inhibition activity

The DPP4 inhibition activity of extracts was analyzed via the method described before [21]. Briefly, equal volumes (10 µL) of the enzyme and the plant extracts were pre-incubated in a 96-well plate at 37°C for 15 minutes. Then, 90 µL of 0.1 M Tris-HCl (pH 8.0) and 100 µL of the substrate (Gly-Pro-pNA, 2.0 mM) were added into the wells and incubated at the same conditions. Diprotin A was used as a positive reference control for DPP4 inhibition. The vivid yellow color of the end-product was analyzed at 405 nm and the results were given as inhibition percentage/µg phenolics. The inhibition percentage of samples was calculated with the following equation: % Inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.7. α-Glucosidase Inhibition activity

The evaluation of α-glucosidase inhibition of plant extracts was determined according to Matsui et al. [22] with slight modifications. The first part of the reaction mixture contained 100 µL of 50 mM sodium phosphate buffer (pH 6.8), 50 µL of the enzyme (prepared from yeast), and 25 µL of plant samples or control drug, and pre-incubated for 15 min at 37°C. 75 µL of 4-Nitrophenyl α-D-glucopyranoside (4-NPGP, 2.0 mM) was added to start the reaction and the change in absorbance was followed at 405 nm with a thermo-scientific microplate reader. Acarbose was used as a positive control for α-Glucosidase inhibition. Results were presented as inhibition percentage/µg phenolics. The inhibition percentage of samples was calculated according to the following equation: % Inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.8. Antiglycation activity

The in vitro antiglycation model system was established with BSA (10 mg/mL in sodium phosphate buffer, pH 7.4) and 500 mM D-glucose (in the same buffer) [23]. Briefly, reaction tubes containing BSA (1.0 mL), D-glucose solution (1.0 mL), 0.9 mL buffer, and 100 µL sample (dissolved in ddH_2O), and tubes were incubated at 60°C for 2 hours. Fluorescence of the advanced glycation end products (AGEs) was measured with an excitation wavelength of 370 nm and an emission wavelength of 440 nm. Aminoguanidine was also evaluated as a control molecule and. Antiglycation activity was calculated with the following formula: %inhibition = $[(A_{control} - A_{sample})/A_{control})] \times 100$.

2.9. Statistics

Bioactivity analyses were performed in triplicates and results are presented as mean ± SD. Statistical analysis was performed using Student's *t* test (GraphPad Software, San Diego, CA). $p<0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

Natural and plant-based remedies have seen a great interest in the recent years with the introduction of functional food stuff. They are very abundant and cheap to procure. However, several plant sources have not been explored for biomedical potential. Indeed, the phytochemistry data of bryophytes in the literature show a large number of bioactive compounds such as aromatic polyphenols, organic acids, acetogenins, phenylquinones, and terpenoids in their content that exhibit valuable potential bioactivities. However, scientific reports about these materials have been sporadic and scarce. For this reason, we aimed to explore a group of 9 bryophytes for their flavonoid and polyphenol content as well as measuring various disease-related activities in order to demonstrate the biomedical and therapeutic potential of these species.

3.1. Flavonoid and phenolic contents

Bryophytes are often associated with a distinctive smell that suggests the presence of aromatic molecules in their constitution such as phenolic compounds. One of the strategies to prevent or treat modern diseases is the use of flavonoids. Flavonoids are a major constituent of plant secondary metabolites with thousands of known structures that is both important to the physiology of plants but also possess a large medicinal application. They are found in vascular plants as well as bryophytes [24]. However, vascular plants have been receiving a great focus on their potential as flavonoid resources leading to bryophytes being less studied and only some sporadic reports can be found [25]. The lack of studies is mainly accredited to their small size which makes it is hard to collect in large enough amounts for chemical experiments such as flavonoids and phenolic compounds research [26]. Total flavonoid values provide insights on active substances with higher inhibitory activities present in plants. In the literature, the amount of flavonoids in plants has been reported between 95 µg/g to 25 mg/g for spermatophyte species while it is higher than 50 mg/g for pteridophytes [27-30]. Our results demonstrated total flavonoid content closer to spermatophytes values which

concur with previous studies on other bryophytes describing the same range of amounts [25].

Table 2 Total flavonoid and phenol amounts determined in different bryophytes species

Types of Bryophytes	Flavonoid content (µg QE/g plant)	Phenolic content (µg GAE/g plant)
T. barbuloides	34.8 ± 1.2	92.7 ± 1
B. stricta	28.6 ± 0.7	88.7 ± 1.6
L. sciuroides	19.1 ± 1.3	274 ± 5.7
F. antipyretica	41.1 ± 1.4	543 ± 6.2
M. polymorpha	37.8 ± 0.8	189 ± 3.3
A. californica	46.7 ± 1.1	223.7 ± 1.9
C. conicum	121.8 ± 3.9	222.7 ± 2.1
B. pomiformis	106.8 ± 2.8	223 ± 2.7
G. lisae	11.6 ± 0.5	96.4 ± 0.9

Total phenolic content was explored to determine the active substances found in the different bryophytes methanolic extracts. The results were calculated and given as µg gallic acid equivalent/g plant (Table 2). The maximum values were seen in *F. antipyretica*, *L. sciuroides*, *A. californica*, *C. conicum*, and *B. pomiformis* reaching 543 ± 6.2, 274 ± 5.7, 223.7 ± 1.9, 222.7 ± 2.1, and 223 ± 2.7 µg GAE/g plant, respectively. Aslanbaba et al. [31] investigated the phenolic properties of plants belonging to the bryophytes family obtained from methanolic extraction. According to the results, the phenolic contents were found in *T. tamariscinum* 1075.15 µg gallic acid equivalent/g plant, *P. riparioides* 784.25 µg gallic acid equivalent/g plant. It has been shown in a recent study that phenolic extracts from *T. tamariscinum* and *P. riparioides* were associated with antioxidant activity providing a basis for potential application in medicine, cosmetics, and the food industry [31]. The results provide great evidence that *F. antipyretica*, *L. sciuroides*, *A. californica*, *C. conicum*, and *B. pomiformis* extracts have potential antioxidative activity. Phenolic molecules in these species consider providing significant antioxidant activity.

3.2. Metal chelating activity

Metal chelating activity is very important for determining the antioxidant capacity which holds metals that cause lipid peroxidation. For this reason, chelating compounds bind transition metals in the organism resulting in preventing radical formation. Thus, one of their function is preventing the damage caused by free radicals [32]. In this study, metal chelating capacity was evaluated using the CUPRAC method. Metal chelating properties of extracts were compared according to the quantities of their phenol compounds. According to the results of the study, the metal chelating activity of nine bryophytes was given in Table 3 The highest metal-chelating effect was observed in *T. barbuloides*, *B. stricta*, *L. sciuroides*, *M. polymorpha* compared to the reference molecule EDTA (2.19 %/µg phenolic), while *C. conicum*, and *A. californica* were less effective than the reference molecule. Molecules in the organism can be affected by reactive oxygen species which increases during metal abundance. Metal chelating properties of phenolic compounds are able to prevent some various damages related to these metals. Moreover, phenolics with metal chelating ability facilitate mineral bioavailability.

Table 3 Biological activities of the different bryophytes extracts

Types of bryophytes	DPP-4 inhibitory (%/µg phenol)	Anti-glycation (%/µg phenol)	Metal chelating (%/µg phenol)	Antioxidant activity (mM AAE/ µg phenol)	α-Glucosidase inhibitory (IC_{50}:µg /mL phenol)
T. barbuloides	$0.44 \pm 2x10^{-2}$ ^{c}	N.D.	7.94 ± 0.9 ^{c}	27.70 ± 2.2	N.D.
B. stricta	$0.68 \pm 6x10^{-2}$ ^{c}	$0.22 \pm 1x10^{-3}$ ^{c}	3.95 ± 0.3 ^{c}	18.8 ± 0.8	114.1 ± 5.7 ^{c}
L. sciuroides	$0.15 \pm 3x10^{-3}$ ^{c}	N.D.	3.76 ± 0.4 ^{c}	25.56 ± 1.6	713.3 ± 3.9 ^{c}
F. antipyretica	$0.04 \pm 1x10^{-3}$ ^{c}	0.74 ± 0.1 ^{c}	$0.29 \pm 3x10^{-3}$ ^{c}	7.76 ± 0.4	N.D.
M. polymorpha	$1.0 \pm 1x10^{-2}$ ^{c}	N.D.	3.44 ± 0.2 ^{c}	11.08 ± 1	63.5 ± 2.8 ^{c}
A. californica	$1.21 \pm 7x10^{-2}$ ^{c}	$0.84 \pm 4x10^{-2}$ ^{c}	1.96 ± 0.3 ^{ns}	8.11 ± 0.3	161.3 ± 3.1 ^{c}
C. conicum	$0.06 \pm 2x10^{-2}$ ^{c}	$0.07 \pm 2x10^{-2}$ ^{c}	2.08 ± 0.2 ^{ns}	12.7 ± 0.7	62.66 ± 2.4 ^{c}
B. pomiformis	$0.09 \pm 3x10^{-2}$ ^{c}	N.D.	0.55 ± 0.1 ^{c}	1.26 ± 0.2	N.D.
G. lisae	N.D.	N.D.	1.51 ± 0.1 ^{b}	8.09 ± 0.6	123.8 ± 3.9 ^{c}

N.D.: Not determined.
ns: non-significant, [b]p<0.01, and [c]p<0.001 vs. different control molecules. Diprotin A was used as a control for DPP-4 inhibitory activity; aminoguanidine for antiglycation activity; EDTA for metal chelating activity; Ascorbic acid for antioxidant activity; and acarbose for α-Glucosidase inhibitory activity.

3.3. DPP-4 inhibitory activity

DPP-4 is a serine protease that cleaves N-terminal dipeptides from polypeptides. Its inhibitors not only stimulate insulin pancreatic β-cells but also helps in the regeneration and differentiation of β-cells. Incretin hormones such as GLP-1 and GIP are releasing in response to meal ingestion. The problem is both these hormones have a short half-life due to their rapid degradation by DPP-4. Inhibition of DPP-4 is necessary to maintain the endogenous inactive form of GLP-1 and its longer half-life.

Most synthetic inhibitors are well tolerated, adverse side effects are known such as mild infections and headaches [33, 34]. Therefore, studies have been focused on discovering the natural DPP-4 inhibitors as alternative treatments for NIDDM [35]. Mosses are rich in phenolic compounds [36]. Many studies have shown the presence of DPP-4 inhibitory activity in many phenolic-rich extracts [37-40]. Also, medicinal plants with DPP-4 inhibitory activity such as *O. europaea* [41], *C. australis* [42], *V. unguiculata, U. lobata, S. china, F. cretica, C. quinoa* [43] play an important role in the management of NIDDM by delaying the development of disease, complications and correcting metabolic abnormalities.

The phenolic extracts of different bryophytes were analyzed for their DPP4 inhibition activity. Results were evaluated according to the positive control molecule Diprotin A. The highest DPP-4 inhibitory activity was observed for *M. polymorpha* and *A. californica extracts* with $1 \pm 1\times10^{-2}$ %/µg phenol and $1{,}21 \pm 7\times10^{-2}$ %/µg phenol compared to 3.60%/µg phenol demonstrated by the control molecule (Table 3). The IC_{50} value of *M. polymorpha* was found as 42,7 µg/mL and 93,25 µg/mL for *A. californica* extracts (Figure 1). Studies on anti-diabetic activity from bryophytes are very limited [36]. This is the first report on the DPP-4 inhibitory activity of the *M. polymorpha* and *A. californica*. In this experiment, the results showed that the M. polymorpha and A. californica contained potential inhibitors for this enzyme. Our results indicate that the phenolic content of bryophytes has an impact on DPP-4 inhibition. Hence, finding a novel and natural molecule that plays a role in DPP-4 inhibition would cure or lower the risks of diabetes mellitus.

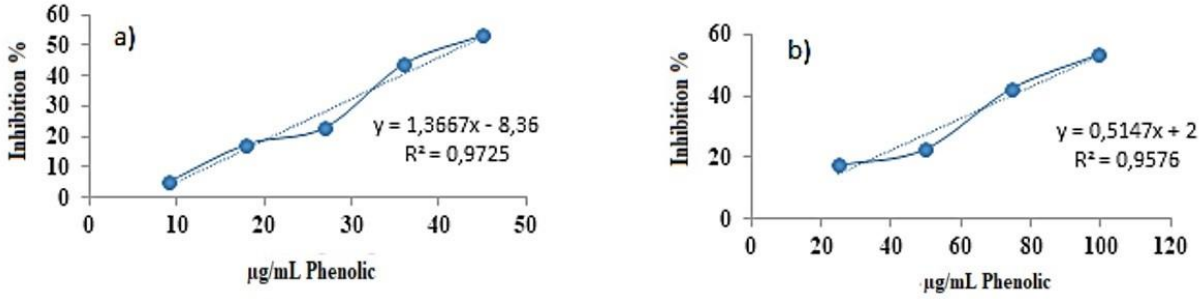

Figure 1 IC_{50} for DPP4 inhibitory activity of a) *M. polymorpha* and b) *A. californica*

3.4. Glucosidase inhibitory activity

The inhibition of polysaccharide hydrolysis enzymes such as α-glucosidase and α-amylase in the digestive system delays glucose absorption [44]. Due to the storage conditions and side effects of synthetic original inhibitors, interest has been increased in the search for finding new and natural antidiabetic drugs or other alternatives. Therefore, there is growing interest in α-glucosidase inhibition to develop novel pharmacological agents [45]. The effect of nine different bryophytes extracts on α-glucosidase activity was investigated. As shown in Table 3, *M. polymorpha* (IC_{50} = 63.5 µg/mL phenol) and *C. conicum* (IC_{50} = 62.66 µg/mL phenol) plants have

shown (Figure 2) the most effective α-glucosidase inhibitory activity compared to reference molecule acarbose (IC_{50} = 1.39 ± 0.23 mg/mL). Tran et al. investigated α-glucosidase inhibitory activity of n-hexane, chloroform, ethyl acetate, and ethanol extracts of the liverwort. They have found the ethyl acetate, ethanol, and n-hexane fractions of IC_{50} values 84.25, 361.40, and 11.89 µg/mL, respectively [46]. Our results show that extract of *M. polymorpha* and *C. conicum* has better α-glucosidase inhibitory activity compared to others. Pant et al. have shown that miscellaneous medicinal plants and related species belonging to bryophytes (*Asterella*, *Marchantia*), pteridophytes (*Adiantum*, *Oleandra*, and *Tectaria*), and flowering plants (*Hedychium*, *Rhus*, *Rubus*, *Smilax*, and *Sonchus*). The highest percentage inhibition of α - glucosidase found (81.13 ± 1.36) was showed from *R. chinensis* extracts [47]. This study suggests that *M. polymorpha* and *C. conicum* are promising sources of active compounds that can prevent the development of type 2 diabetes.

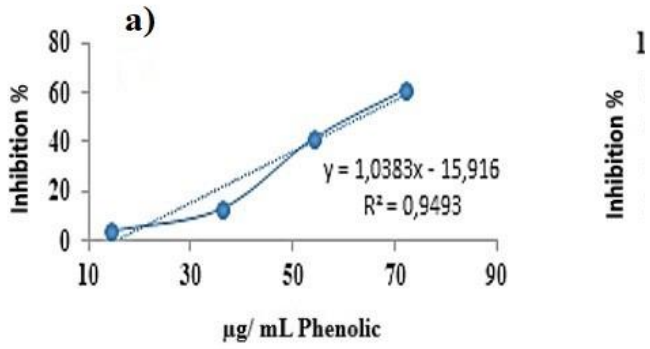

Figure 2 IC_{50} for α-glucosidase inhibitory activity of a) *M. polymorpha* and b) *C. conicum*

3.5. Antiglycation activity

Advanced glycation end products (AGEs) are formed non-enzymatically under long-term chronic hyperglycemic conditions and affect protein structures and functions. Diabetic complications, age-dependent diseases, Alzheimer's disease, rheumatoid arthritis, cancer, neurodegenerative diseases, cataract, and many others are associated with aggregates formed by AGEs [48]. Thus, the prevention of these aggregate formations is of great importance. It is very considerable to select a target for inhibiting glycation at distinctive stages such as Schiff base, advanced glycation end-products (AGE), and protein aggregation. It has been shown that liverworts (bryophytes) possess antidiabetic activities through the study of three species (*P. striatus, P. epiphylla, and B. oshimensis*) collected from Eastern Himalaya [49]. Brown algae extracts were shown to possess antiglycation effects in bovine serum albumin glycation models with fructose, glyoxal, and methylglyoxal. The antiglycative effect was associated with the presence of phenolic compounds in the extract (dieckol, phlorofucofuroeckol-A, and quercetin) [50]. Our results show that the *F. antipyretica* (0.74 ± 0.1%/µg phenol) and *A. californica* ($0.84 \pm 4x10^{-2}$ %/µg phenol) were very effective on antiglycation activity compared to control molecule Aminoguanidine (0.27 %/µg phenolic), and the *B. stricta* was similar to that of the control molecule. *C. conicum* antiglycation activity was insufficient.

4. CONCLUSION

In this study, different pharmacological activities (antiglycation, α-glucosidase and DPP-4 inhibition, antioxidant, and metal chelating ability) associated with diabetes were investigated in nine types of bryophytes methanolic extracts. To the best of our knowledge, this is the first report describing the bioactivity of these nine bryophytes and their potential as antidiabetics, antioxidants, and phenolic reservoirs. Bryophytes are poorly investigated compared to vascular plants which are exploited commercially. The production of active compounds obtained from bryophyte species can have a tremendous impact on commercial and medical applications especially in the fields of food, medicine, and pharmacy.

Declarations

Author's Contributions

1st Author performed the experiment and results analysis.

1st, 2nd, and 3rd Authors drafted and wrote the manuscript.

2nd and 3rd Author assisted in the analytical analysis, supervised the experiment's progress, results interpretation, and helped in manuscript preparation.

4th Author assisted in preparing the samples.

Funding

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Ethics

There are no ethical issues after the publication of this manuscript.

Consent for publication

All authors read and approved the current version for publication

Conflict of interest

There is no conflict of interest to be declared.

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