GC-MS Analysis of Ethanol Extract of *Taxithelium napalense* (Schwaerg) Broth along with its α-Glucosidase Inhibitory Activity

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Considering the antidiabetic potentiality of the moss *Taxithelium napalense*, the present study was undertaken to explore the chemical constituents that are present in this species. The entire moss *Taxithelium napalense* was extracted by ethanol and the extract obtained was subjected to gas chromatography-mass spectrometry. Sixty nine compounds were identified, which are reported for the first time from this species. From the gas chromatography-mass spectrometry analysis, it was determined that the octadecanoic acid methyl ester was the chief component present in *Taxithelium napalense*. In addition, the total phenolic and flavonoid contents of ethanol extract of *Taxithelium napalense* were found to be 101.43±0.38 mg Q/g and 229.73±3.07 mg GA/g, respectively. Additionally, the IC_{50} of the ethanol extract of *Taxithelium napalense* on α-glucosidase was found to be 34.5 µg/ml, while acarbose value was 29.5 µg/ml. This is the first report on the chemical investigation of the moss *Taxithelium napalense*.

Key words: Moss, chemical constituents, gas chromatography, mass spectroscopy, metabolites

Mosses are non-vascular plants with rhizoids. They are grouped into the division of Bryophyte under the sub-division Musci. About 17 000 species of moss falling in 89 families and ca. 898 genera are distributed across the world and in India, about 1786 moss species are recorded till now[1]. Generally, mosses are habituated in wet environments such as alpine and rainforest ecosystems, in addition, mosses require fresh water to their reproduction[1,2]. Due to their unique habitat, they are scrutinized for biological evaluations because of their nutritional contents[2], for instance, *Sphagnum palustre* moss has renoprotective effects[3] and aromatase inhibitory activity[4].

Taxithelium napalense (Schwaerg) Broth, family *Sematophyllaceae* is an epiphytic moss. *T. napalense* is habituated on twigs and branches of other plants in semi evergreen forests found in palaeotropical region[5]. Earlier, the phytochemical, antioxidant and *in vivo* antidiabetic potential of the ethanol extract of *T. napalense* was reported[6] . In the present study, it was aimed to identify the active metabolites present in this moss using gas chromatography-mass spectrometry (GC-MS) analysis. Additionally, the total flavonoid and phenolic contents along with its α-glucosidase inhibitory activity was also determined.

The specimens of *Taxithelium nepalense* (Schwaerg) Broth was collected from barks of mangrove, *Rhizophora* species from Bhitarkanika island (20°72' N latitude and 86°87' E Longitude) Orissa, India in March 2016. The moss *T. nepalense* was authenticated in the Council of Scientific and Industrial Research (CSIR)-National Botanical Research Institute (NBRI), Lucknow and deposited at Bryophyte Herbarium, CSIR-NBRI, Lucknow, India with an accession number LWG-5/VB-Orissa-2016[1].

The moss specimens collected from the mangrove plants were shade-dried. The dried lichen material was powdered using a blender and about 12 g of moss material was exhaustively extracted thrice with ethanol at room temperature. The mixture was filtered through muslin cloth and evaporated under reduced pressure to obtain ethanol extract (1.21 g, 10.08 % w/w) as a dark greenish solid, which were stored in amber colored vials and preserved at 4° till further use.

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All the chemicals and reagents used in the present study were of analytical grade. *p*-nitrophenyl-α-D-glucopyranoside, rat intestinal acetone powder, sucrose and acarbose, were procured from Sigma-Aldrich (Mumbai, India). Glibenclamide was purchased from Aventis Pharma Ltd. (Mumbai, India).

The phytochemical investigation of ethanol extract of *T. nepalense* was performed on GC-MS equipment (GCMS-QP2010 Plus, Shimadzu, Europe). Experimental parameters of GC-MS system were, column oven temperature: 50°, injection temperature: 250°, injection mode: split; flow control mode: linear velocity; pressure: 29.7 kPa; total flow: 10.9 ml/min; column flow: 0.72 ml/min; linear velocity: 30.7 cm/s; purge flow: 3.0 ml/min; split ratio: 10.0. Oven temperature program was 50° hold time for 2 min, at 200° hold time for 0 min and 280° hold time for 2 min. The GC program was ion-source temperate at 210°, interface temperature at 250°, solvent cut time: 3 min, detector gain: 1.07 kV+0.20 kV and threshold of 1000.

The total flavonoid content[7] of the extracts were determined by using aluminium chloride spectrophotometric method, in which $AlCl_3$ forms complex with hydroxyl groups of flavonoids exist in the testing sample. To the extract (1 mg/ml) or standard quercetin solution (3.125, 6.25, 12.5, 25, 50, 100 µg/ml), added 3 ml of methanol, 1 ml of 2 % $AlCl_3$ solution, 200 µl of 1 M potassium acetate and made up to 10 ml with distilled water and incubated for 60 min at room temperature, whereas the blank contained only reagents and the absorbance was noted at 415 nm. Based on the measured absorbance of the test sample, the total flavonoid content was read on the calibration curve and the total flavonoid content was expressed in terms of quercetin equivalent (mg of Q/g of extract).

The total phenolic content[7] was estimated by Folin-Ciocalteu's method. To the extract (1 mg/ml) or standard gallic acid solution (3.125, 6.25, 12.5, 25, 50, 100 µg/ml), added 0.5 ml of Folin-Ciocalteau reagent, 1.5 ml of sodium carbonate (20 %) solution and volume made up to 10 ml with distilled water and incubated at room temperature for 120 min and the absorbance was measured at 750 nm using spectrophotometer against blank (contained only reagents). Based on the measured absorbance of the test sample, the total phenolic content was read on the calibration curve and the total phenolic content was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

The assay of α-glucosidase inhibitory activity was estimated using the method reported by Tatipamula *et al.*[8] in triplicate (n=3). Two microlitres of α-glucosidase from rat intestine acetone powder solution (a stock solution of 1.0 mg/ml in 10 mM phosphate buffer, pH 6.8, diluted 40-fold with phosphate buffer) was mixed with 20 µl of the samples at different concentrations (25, 50, 75 and 100 µg/ml solubilized in dimethyl sulfoxide) to which 100 µl of 50 mM phosphate buffer (pH 6.8) was added in a 96 well microtitre plate and incubated for 5 min at 37°. After incubation, 50 µl of substrate (5 mM of *p*-nitrophenyl-α-D-glucopyranoside prepared in 50 mM of phosphate buffer, pH 6.8) was added and the entire reaction mixture was again incubated for 20 min at 37°. Thereafter the reaction was terminated by adding 50 µl of Na_2CO_3 (1 M) and the final volume was made up to 150 µl. The amount of *p*-nitrophenol released from substrate was noted at 405 nm spectrophotometrically (Spectra MAX plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA). Dimethyl sulfoxide and acarbose were used as control and standard, respectively. Percent enzyme inhibition was calculated using the Eqn., percent inhibition = (C–S)/C×100, where C is the absorbance of the control, S is the absorbance of sample. IC_{50} values of the samples were determined by plotting percent inhibition against concentrations.

After successful extraction of the whole moss material, the dried extract was subjected to GC-MS analysis. The GC-MS spectrum revealed the presence of various chemical components with different retention times (fig. 1), whereas the MS analyzes the compounds eluted at different times to detect its nature and structure of the compounds. The results pertaining to GC-MS analysis of the ethanol extract of *T. nepalense* lead to the identification of a number of compounds. The various compounds present in the entire stressed moss of ethanol extract of *T. nepalense* along with their retention time, molecular formula, molecular weight, peak area (%) and nature of the compound that were detected by the GC-MS were represented in Table 1. A total of 69 compounds were identified by GC-MS analysis (Table 1). In addition, the composition determined for the ethanol extract of *T. nepalense* corresponds to 63.59 % of the entire GC-MS chromatogram.

From the GC-MS analysis, it was observed that this moss species contained mostly aliphatic fatty acids and its derivatives. Therefore, the standard way for cells to synthesize fatty acids is through the fatty acid synthesis cycle as shown in fig. 2. This cycle of eight

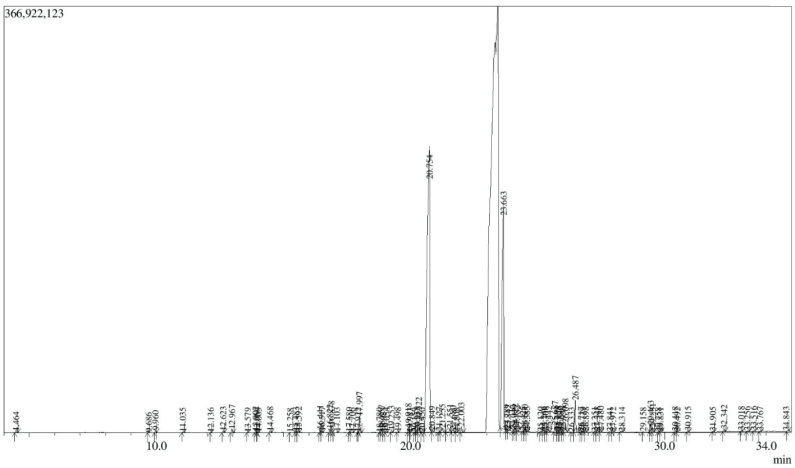

Fig. 1: GC-MS chromatogram of ethanol extract of *T. nepalense*

TABLE 1: COMPOUNDS IDENTIFIED FROM GC-MS ANALYSIS OF THE ETHANOL EXTRACT OF *TAXITHELIUM NAPALENSE*

RT (min)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Nature of the compound
4.464	Hexanal	$C_6H_{12}O$	100.16	0.10	Aliphatic aldehyde
9.686	Nonanal	$C_9H_{18}O$	142.24	0.02	Aliphatic aldehyde
9.960	Octanoic acid	$C_8H_{16}O_2$	144.21	0.15	Aliphatic carboxylic acid
11.035	1-Dodecene	$C_{12}H_{24}$	168.32	0.05	Aliphatic alkene
12.136	2-Decenal	$C_{10}H_{18}O$	154.25	0.03	Aliphatic alkene
12.623	(Z)-2,4-Decadienal	$C_{10}H_{16}O$	152.24	0.13	Aliphatic alkene
12.967	(E,E)-2,4-Decadienal	$C_{10}H_{16}O$	152.24	0.27	Aliphatic alkene
13.579	(E,E)-2-Undecenal	$C_{11}H_{20}O$	168.28	0.02	Aliphatic alkene
13.902	1-Pentadecene	$C_{15}H_{30}$	210.41	0.10	Aliphatic alkene
13.950	2-Decanone	$C_{10}H_{20}O$	156.27	0.01	Aliphatic ketone
14.005	Tetradecane	$C_{14}H_{30}$	198.39	0.03	Aliphatic alkane
14.468	Nonanoic acid	$C_9H_{18}O_2$	158.24	0.13	Aliphatic carboxylic acid
15.258	2-Tridecanone	$C_{13}H_{26}O$	198.35	0.02	Aliphatic ketone
16.441	1-Hexadecene	$C_{16}H_{32}$	224.43	0.19	Aliphatic alkene
16.517	Hexadecane	$C_{16}H_{34}$	226.45	0.10	Aliphatic alkane
16.878	4,5-Dimethoxy benzophenone	$C_{15}H_{14}O_4$	258.27	0.50	Aromatic ketone
17.580	2,4-Di-t-butyl-6-nitro-6-dodecanone	$C_{20}H_{40}NO_3$	342.54	0.05	Aliphatic ketone
17.922	Hexadecanal	$C_{16}H_{32}O$	240.43	0.03	Aliphatic aldehyde
17.997	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.40	1.14	Aliphatic ester
18.780	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.46	0.02	Fatty acid
18.857	16-Methyl-1-octadecene	$C_{19}H_{38}$	265.51	0.26	Aliphatic alkene
18.933	Heneicosane	$C_{21}H_{44}$	296.58	0.11	Aliphatic alkane
19.054	5-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47	0.06	Fatty acid
19.498	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	0.17	Fatty acid
19.918	2-Undecanone	$C_{11}H_{22}O$	170.30	0.07	Aliphatic ketone
19.983	6,10-Dimethyl-9-heptadecanone	$C_{19}H_{38}O$	282.51	0.36	Aliphatic ketone
20.159	10-Methyl-E-11-tridece-1-ol-acetate	$C_{16}H_{30}O_2$	254.41	0.02	Aliphatic ester
20.322	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.41	1.01	Fatty acid
20.450	9-Hexadecenoic acid methyl ester	$C_{16}H_{30}O_2$	268.44	0.05	Aliphatic ester
20.754	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.51	52.09	Aliphatic ester
20.849	3-Hydroxy cyclodecene	$C_{10}H_{18}O$	154.25	0.12	Cyclic hydroxyl compound
21.680	Cyclopropaneoctanoic acid	$C_{11}H_{20}O_2$	184.28	0.28	Fatty acid
22.003	Heptadecanoic acid methyl ester	$C_{18}H_{36}O_2$	248.48	0.50	Aliphatic ester
23.789	Methyl octadec-9-enoate	$C_{19}H_{36}O_2$	297.487	0.11	Aliphatic ester
23.842	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294.48	0.08	Aliphatic ester

24.040	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	308.51	0.18	Aliphatic ester
24.122	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.52	0.40	Aliphatic ester
24.425	9-Cis,11-trans-octadecadienoate-1-docosene	$C_{18}H_{32}O_2$	280.45	0.07	Fatty acid
25.246	Behenic alcohol	$C_{22}H_{46}O$	326.61	0.08	Aliphatic hydroxyl compound
25.442	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.44	0.17	Fatty acid
25.828	9,12,15-Octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	292.46	0.11	Aliphatic ester
25.890	6,9,12-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.44	0.11	Fatty acid
25.996	Oxiraneoctanoic acid 3-octyl-methyl ester	$C_{19}H_{36}O_3$	312.49	0.11	Aliphatic ester
26.098	Cyclopropaneoctanoic acid methyl ester	$C_{12}H_{22}O_2$	198.31	0.94	Aliphatic ester
26.333	8-Hexadecenal	$C_{16}H_{30}O$	238.42	0.04	Aliphatic aldehyde
26.717	(9E,12E)-9,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	298.90	0.07	Chlorinated aliphatic ketone
26.779	4,4',6,6'-Tetra-tert-butyl-2,2'-biphenyldiol	$C_{28}H_{42}O_2$	410.64	0.05	Biphenolic compound
26.898	2-Butyl-5-methyl-3-(2-methylprop-2-enyl) cyclohexanone	$C_{15}H_{26}O$	222.37	0.06	Cyclic ketone
27.251	9-Octadecenoic acid methyl ester	$C_9H_{36}O_2$	296.50	0.05	Aliphatic ester
27.348	(Z,Z)-6,9-cis-3,4-epoxynonadecadiene	$C_{19}H_{34}O$	278.48	0.11	Aliphatic alkene
27.480	1-Docosene	$C_{22}H_{44}$	308.59	0.12	Aliphatic alkene
27.841	1-(2,2-Dimethylcyclopentyl) ethanone	$C_9H_{16}O$	140.23	0.06	Cyclic ketone
27.972	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.57	0.07	Fatty acid
28.314	7,10-Hexadecadienoic acid	$C_{16}H_{28}O_2$	252.40	0.10	Fatty acid
29.158	9-Heptadecanol	$C_{17}H_{36}O$	256.47	0.02	Aliphatic hydroxyl compound
29.463	Docosanoic acid	$C_{22}H_{44}O_2$	340.59	0.97	Fatty acid
29.531	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166.13	0.40	Aromatic acid
29.758	9,11-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	0.04	Fatty acid
29.831	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47	0.04	Aliphatic acid
30.418	1-Hexadecanol	$C_{16}H_{34}O$	242.45	0.10	Aliphatic hydroxyl compound
30.492	Octadecane	$C_{18}H_{38}$	254.50	0.03	Aliphatic alkane
30.915	Tricosanoic acid	$C_{23}H_{46}O_2$	354.62	0.10	Aliphatic acid
31.905	Pentatriacontane	$C_{35}H_{72}$	492.96	0.04	Aliphatic alkane
32.342	Tetracosanoic acid	$C_{24}H_{48}O_2$	368.65	0.22	Fatty acid
33.018	Geranylgeraniol	$C_{20}H_{34}O$	290.49	0.08	Aliphatic hydroxyl compound
33.256	1-Heptacosanol	$C_{27}H_{56}O$	396.74	0.08	Aliphatic hydroxyl compound
33.516	Squalene	$C_{30}H_{50}$	410.73	0.09	Aliphatic alkane
33.767	Pentacosanoic acid methyl ester	$C_{26}H_{52}O_2$	396.70	0.06	Aliphatic ester
34.843	Heptacosane	$C_{27}H_{56}$	380.75	0.04	Aliphatic alkane

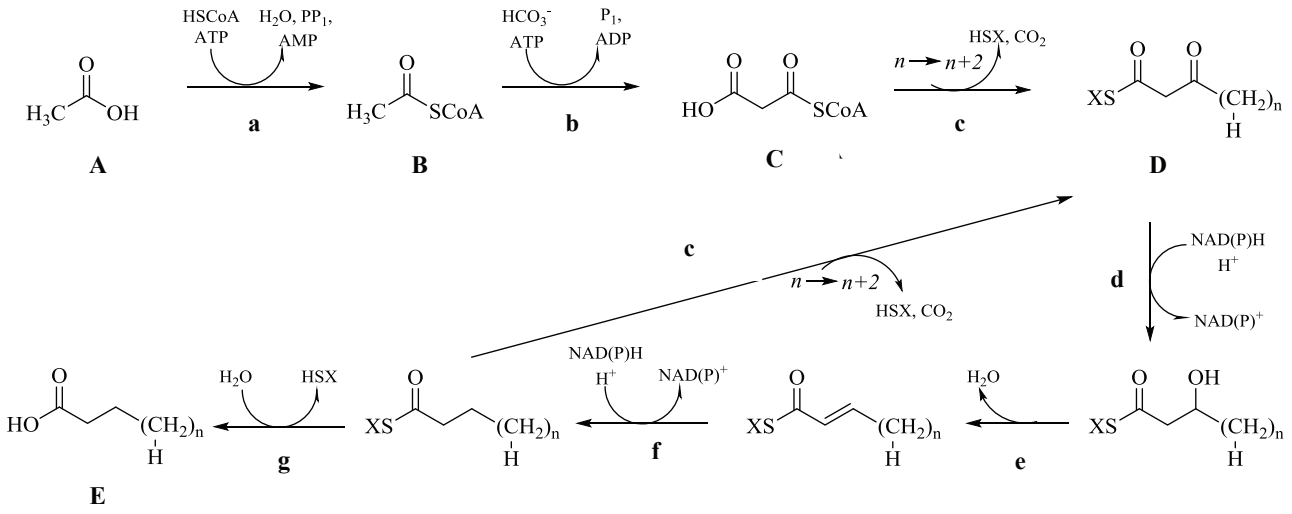

Fig. 2: Biosynthetic pathway of fatty acid

A: acetic acid; B: acetyl Co-A; C: malonyl Co-A; D: malonyl Co-A derivative; E: fatty acid. (a) Acetyl Co-A synthase; (b) acetyl Co-A carboxylase; (c) ketoacyl synthase and acyltransferase; (d) ketoacyl reductase; (e) hydroxyacyl dehydratase; (f) enoyl reductase; (g) thioesterase

TABLE 2: α-GLUCOSIDASE INHIBITORY ACTIVITY OF TNEE

COMPOUND	Inhibition (%)*				IC_{50} values (µg/ml)
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
TNEE	44.94±6.40	58.02±5.06	65.27±4.65	72.43±5.38	34.5
Acarbose	46.11±4.74	68.25±6.47	76.06±5.78	85.79±5.24	29.5

TNEE is *Taxithelium napalense* ethanol extract *Mean±SD values (n=3)

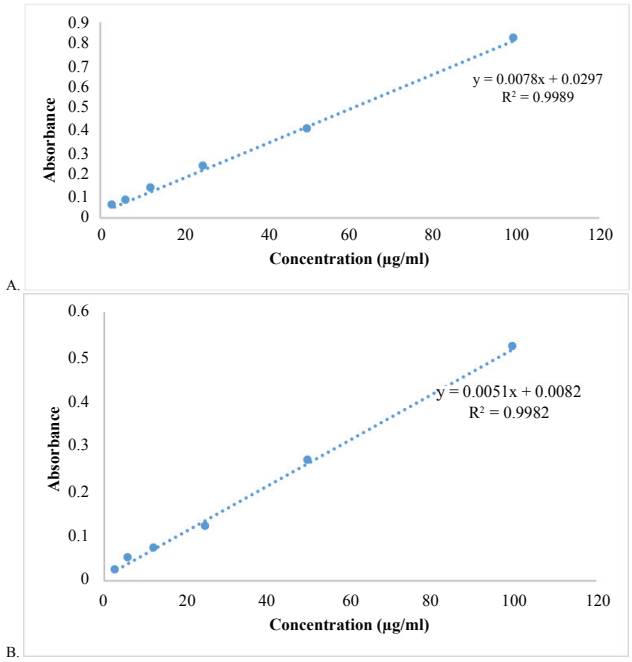

Fig. 3: Standard quercetin and standard gallic acid curves (A) Total flavonoid content of standard quercetin and (B) total phenolic content of standard gallic acid, R^2, n=3

enzymes (acyl-CoA synthase, acyl-CoA carboxylase, acyltransferase, ketoacyl synthase, ketoacyl reductase, hydroxyacyl dehydratase, enoyl reductase, and thioesterase) and acyl carrier protein) was initiated with acetic acid, CoA, and ATP by using acyl-CoA synthase as catalyst to make acetyl-CoA (fig. 2). By using another ATP and bicarbonate ion catalyzed by acyl-CoA carboxylase, which yields malonyl-CoA (fig. 2). The obtained malonyl-CoA was added to an acyl chain by catalysis with ketoacyl synthase and acyltransferase, which were usually activated with acyl carrier protein, to make an acyl chain two methylene groups longer (fig. 2). Additionally, reduction, dehydration, and reduction with ketoacyl synthase, hydroxyacyl dehydratase, and enoyl reductase catalysis, respectively, leads to a saturated and unhydroxylated acyl chain activated with acyl carrier protein (fig. 2). If the chain is of appropriate length, it was attacked by thioesterase to release acyl carrier protein, yielding the finished fatty acid (fig. 2). The various fatty acids derived by this pathway was subjected to further reduction, dehydration, and reduction reactions in the cells to yield respective derivatives namely aliphatic aldehydes, aliphatic hydroxyl, aliphatic ketones and aliphatic esters.

The results of total flavonoid content[7] was expressed in terms of mg of Q/g of extract by using standard calibration line Eqn., y = 0.0078x+0.0297 R^2=0.9989 (fig. 3A), similarly, the total phenolic content were expressed in terms of mg of GA/g of extract by using standard calibration line Eqn., y = 0.0051x+0.0082 R^2=0.9982 (fig. 3B). The ethanol extract of *T. nepalense* contained more total phenolic content than total flavonoid content. The total phenolic content[7] was found to be 229.73±3.07 mg GA/g, whereas the total flavonoid content was 101.43±0.38 mg Q/g. In addition, the GC-MS analysis also depicted the presence of higher amounts of phenolic compounds in ethanol extract of *T. nepalense.*

The *in vitro* antidiabetic activity was assessed by α-glucosidase inhibitory assay[8] using acarbose as standard and *p*-nitrophenyl-α-D-glucopyranoside as a substrate. From the inhibitory assay, it was estimated that the ethanol extract of *T. nepalense* exhibited significant inhibition of α-glucosidase enzyme with IC_{50} values of 34.5 µg/ml, whereas acarbose with 29.5 µg/ml (Table 2). The results of α-glucosidase inhibitory assay proved the antidiabetic potentiality of *T. nepalense.*

To conclude, this is the first report on chemical components from stressed moss *T. nepalense.* This study confirmed the presence of various compounds in the moss *T. nepalense* and also justified the use of this moss as a remedy to diabetes in traditional medicine. This research helped to predict the active metabolites present in the *T. nepalense*, in addition, it also helped in establishing the chemical composition of this moss as well. From these results, it could be concluded that *T. nepalense* contained various bioactive metabolites. Additionally, the ethanol extract has more total phenolic content than total flavonoid content. However, the isolation of individual secondary metabolites and investigating the biological activity possessed by these would give impetus for further research on the antidiabetic potential of this plant.

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Conflicts of interest:

The authors declare that there is no conflict of interest.

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