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Phytochemical constituents and pharmacological potential of *Solanum khasianum* C.B. Clarke., extracts: Special emphasis on its skin whitening, anti-diabetic, acetylcholinesterase and genotoxic activities

ROKTIM GOGOI[1,2], NEELAV SARMA[1,2], SUDIN KUMAR PANDEY[1,2], AND MOHAN LAL[1,2]✉*

[1]Medicinal Aromatic and Economic Plant Group, Biological Science and Technology Division CSIR-North East Institute of Science and Technology (NEIST), Jorhat, Assam 785006, India
[2]AcSIR - Academy of Scientific & Innovative Research, Ghaziabad, Uttar Pradesh 201002, India

ABSTRACT

This study was designed to evaluate and compare the phytochemical constituents along with the bioactivities of *Solanum khasianum* C.B. Clarke. leaves (SKLME) and berries (SKBME) methanolic extracts. Alkaloids, flavonoids, saponins, steroids, terpenoids, phenols, oxalates and cardiac glycosides were present in SKLME and SKBME. Considerable amounts of phenolic and flavonoids were found in SKLME and SKBME. SKBME showed better antioxidant activity with an IC_{50} value of 20.68 µg/mL vs SKLME (38.30 µg/mL). SKBME and SKLME respectively showed anti-inflammatory activity with IC_{50} of 20.96 and 23.37 µg/mL. Apart from this, the present investigation also reports SKBME skin whitening ability, anti-diabetic potential and anti-cholinesterase activity with IC_{50} values of 33.97, 28.24, and 32.42 µg/mL, respectively. Furthermore, SKLME showed herbicidal property with a total germination rate of 9.06% at a concentration of 80 µg/mL. Bioactivity analysis revealed higher pharmacological importance of the SKBME than SKLME, as well.

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1. Introduction

Medicinal plants are of great use in healing as well as for the treatment of various human and non-human e.g., primates and other animals' diseases due to the presence of useful phytochemical constituents (Nostro et al., 2000). According to the World Health Organization (WHO), 80% of the world's population for the primary health care needs relies on traditional plant origin medicine and one of the reports from WHO recorded that nearly 20,000 medicinal plants exist all over the world (Duraipandiyan et al., 2006). The occurrence of natural products with medicinal properties is due to the tracking of useful herbs with traditional and ethnic importance. Constituents of medicinal plants have been the main source of new pharmaceuticals and healthcare products, including medications for ethnoveterinary medicine. For the maintenance of good and disease-free health, a whole range of plant derivatives, pro-vitamins are now being considered as nutraceuticals and also nowadays used as direct pharmacological compounds. Plant-derived products are also increasingly gaining interest and widely used in the cosmetic industries (Ivanova et al., 2005). One of the ethnic medicinal plants, *Solanum khasianum* (C.B. Clarke) also known as *Solanum aculeatissimum* Jacq., (2n=24) belongs to the family *Solanaceae* and grows wild in Meghalayan hills, Assam, Manipur, Sikkim, West Bengal, Orissa, Arunachal Pradesh and Nilgirishill of India ascending to an altitude of 1600 m. In India, 33 species of *Solanum* are available (Maiti et al., 1965). It is a stout, branched, woody shrub attaining a height of 0.75 to 1.5 m. Stems are spiny, leaves are ovate to lobed bearing spines on both surfaces, the flowers are hermaphrodite, the berries are yellowish when ripe, seeds are small brown in color and abundant. The presence of solasodine in the mature berries has a tremendous commercial possibility and completely

✉Corresponding author: Mohan lal
Tel: 91-8811087277; Fax: +91-376-2370011
E-mail address: drmohanlal80@gmail.com, **doi:10.30495tpr.2021.1917249.1190**

yellowish berries show the presence of alkaloids in maximum quantity (Mann, 1978). The crop *Solanum khasianum* propagated through seeds. They are sown 8-10 cm apart in nursery beds in June. The sown seeds are irrigated daily, after about 4 weeks the plants are ready for transplantation. Under good management, the crop yields upto 60 quintals of dried fruits (http://www.ikisan.com/medicinal-plants-solanum.html). The present cultivation area of the species is more than 4000 ha in Maharashtra, Karnataka, Assam, Tripura, Meghalaya and Manipur state of India. The presence of phytochemical compounds in the plant indicates their medicinal potential which can be determined by phytochemical analysis (Devi et al., 2014). Such knowledge of plants chemical constituents is desirable due to the presence of great economically and medicinally important substances which can be used as drugs (Chetri et al., 2008). Among the phytoconstituents, chlorophyll, proteins and carbohydrates are included in primary and terpenoid, alkaloid, flavonoid, and phenolic compounds are categorized in secondary metabolites. Among the secondary metabolites such as phenolic and flavonoid compounds, they have a number of bioactive compounds e.g., phenolics compound-caffeic, gallic, protocatechuic, 4-hydroxybenzoic, and syringic acids; flavonoid compounds like catechin, epicatechin, epigallocatechin, glausan-3-epicatechin and pro anthocyanidins (Krishnaiah et al., 2007). Recently, much attention has been drawn to natural antioxidants and their association with health benefits (Amous et al., 2001; Gogoi et al., 2018). There are several synthetic antioxidant drugs with potential health risk such as BHA (E-320), BHT (E-321), TBHQ (E-319), propyl gallate (E-310), etc. Plants are the potential source of a large number of antioxidative compounds (Rice-Evans et al., 1997). Therefore, in the search for cost-effective with minimum side effects, the antioxidant properties of the extracts from the leaves and berries of *Solanum khasianum* were investigated in this study. Similarly, inflammation has a close relation with oxidative burst; hence the anti-inflammatory activity of the leaves and berries extracts was also evaluated. Since ancient time, plant products derived from leaves, flowers, fruits, seeds, bark, and roots have been a part of phytomedicines (Criagg and David, 2001). Free radicals are also associated with several diseases such as liver cirrhosis, diabetes, cancer etc. Diabetes and oxidative stress coexists in many cases (Sabu and Kuttan, 2002). As a depigmentation agent in the relevant problems of human skin, tyrosinase is one of the target enzymes. Plant derivatives often show remarkable tyrosinase inhibitory activities. Several synthetic and natural tyrosinase inhibitors recently reviewed in several reports (Kim and Uyama, 2005; Parvez et al., 2007; Chang, 2009; Venditti et al., 2013). Neurodegenerative disorder such as Alzheimer's disease (AD) can be treated by using acetylcholinesterase inhibitors (AChEIs) (Mukherjee et al., 2007; Salemme et al., 2016; Venditti and Bianco, 2020). There were several AChE inhibitors available, but due to bioavailability and possible side effects, there is still great interest for researchers in finding better AChE inhibitors. Diabetes, one of the metabolic disorders, causes millions of death records all over the world each year. Traditionally, medicinal plants and their secondary metabolite e.g., artemisinin, paclitaxel, morphine, codeine, atropine, resveratrol etc. have been reported to be valuable natural remedies to treat human diseases (Akbaeri et al., 2012). Hence, attention is also grabbed by the researchers towards the search for new anti-diabetic drugs (Rasouli et al., 2020).

In organic farming, plant products can be used as a useful weed controller (Tworkoski, 2002). The present work also addresses the seed germination and early seedling growth of *Vigna radiata* concerning the effects of *Solanum khasianum* leaves and berries extracts. Three closely related species, namely *S. melongena*, *S. nigrum* and *S. myriacanthus* were also evaluated for their phytochemical compositions. Among them, *S. melongena* and *S. nigrum* were abundant in protein and alkaloids, whereas, tanins and alkaloids were abundant in *S. myriacanthus*. Few of the other phytochemical constituents such as saponins and, flavonoids were also present in more or less quantity (Ashrafudoulla et al., 2016). To date, there is no scientific report available in the public domain of *Solanum khasianum* leaves and berries extract, as the plant is abundant and still unexplored. Hence, we tried to make a base for its further study towards human welfare.

2. Experimental

2.1. Sample collection

The plant samples, leaves and berries of *Solanum khasianum* were collected from the experimental farm of CSIR-NEIST, Jorhat (26.7378°N, 94.1570°E). It has an average altitude/elevation of 116 m (381 feet) from the sea level. The plants were identified by the taxonomist cum breeder (Dr. Mohan Lal) of MAEP of CSIR-NEIST, Jorhat. The plant species were identified from its hirsute foliage, straight, patent prickles, deeply lobed leaves, unarmed flowering/fruiting calyces, acuminate corolla lobes as per botanical description suggested by Babu and Hepper, (1979) as well as the corresponding fruits, which are mottled mix of whitish and dark greens that resemble a watermelon. Mature fruits are yellow in color and 2-4 cm in diameter and identified with the help of herbarium. The voucher plant specimen was deposited in the institute herbarium with a number of RRLSK-1072. Leaves and berries were washed, freeze-dried in a lyophilizer (Borg Scientific, Chennai, India) and homogenized into powder and stored in airtight container for analysis.

2.2. Chemicals used in the study

Chemicals and reagents used are HPLC grade methanol (CH_3OH), ethanol (CH_3CH_2OH) ascorbic acid ($C_6H_8O_6$), ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), potassium ferricyanide ($K_3Fe(CN)_6$), tris-buffer ($C_4H_{11}NO_3$), aluminum chloride ($AlCl_3$), silica-

gel were acquired from HiMedia Laboratories Pvt.Ltd. (Dindori, Nashik, India). Trichloro acetic acid ($C_2HCl_3O_2$), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), boric acid (H_3BO_3), oxalic acid ($C_2H_2O_4$), *n*-butanol (C_4H_9OH), ethylacetate ($C_4H_8O_2$), chloroform ($CHCl_3$), potassium iodide (KI), mercuric chloride ($HgCl_2$), glacial acetic acid (CH_3COOH), sodium hydroxide (NaOH), acetylcholinesterase (AChE) ($C_{17}H_{16}NO_2$), galanthamine hydrobromide ($C_{17}H_{22}BrNO_3$), acetylcholine chloride ($C_7H_{16}ClNO_2$), acetylthiocholine iodide ($C_7H_{16}INO_2$) were from Sigma-Aldrich Company (Steinheim, Germany). All other unlabelled chemicals involving sodium diclofenac-100 mg (Ranbaxy Laboratories Mohali, India) and fresh egg albumin were acquired from local market of Jorhat Assam and are of analytical grade.

2.3. Extract preparation

Fifty grams of freeze-dried lyophilized powdered leaves and berries samples were extracted with methanol (99%) in the ratio of 1:6 (powdered sample: solvent) by subjecting it to maceration at room temperature for three days and filtered with Whatman's filter paper. The residue from the filtration was extracted again twice using the same procedure to ensure that all the bioactive components extracted completely. The filtrates obtained were combined and then air-dried. A total of 5 g of the air-dried extract was finally obtained and subjected to chemical test as per the methods mentioned below for the detection of various phytochemicals (Kumar et al., 2013; Devi et al., 2014). All the experiments were carried out in triplicates to reduce the experimental error.

2.4. Phytochemicals screening

2.4.1. Detection of flavonoids

One milliliter of the plant extracts was taken in a test tube and a few drops of dilute sodium hydroxide (NaOH) solutions were added. An intense yellow color appeared in the test tube which became colorless on the addition of a few drops of dilute acid that indicated the presence of flavonoids.

2.4.2. Detection of phenol (Ferric chloride test)

Five milliliters of the extracts were dissolved in 2 mL of distilled water. To this, a few drops of ferric chloride ($FeCl_3$, 10%) solution was added. Dark green color indicates the presence of phenolic compounds.

2.4.3. Detection of terpenoids (Salkowski's test)

Five milliliters of the extracts were dissolved in 2 mL of chloroform ($CHCl_3$) and then 3 mL of concentrated sulphuric acid (H_2SO_4) was added to the solution. The formation of a reddish-brown colored interface showed the presence of terpenoid.

2.4.4. Detection of steroids

One mL of the plant extracts was taken in a test tube and dissolved with 10 mL chloroform ($CHCl_3$) and then an equal volume of concentrated sulphuric acid (H_2SO_4) was added to the test tube by sides. The upper layer in the test tube turned in red and sulphuric acid layer showed yellow color with green fluorescence that indicates the presence of steroids.

2.4.5. Detection of oxalates

Four milliliters of the extracts were added to 2 mL of acetic acid (CH_3COOH) containing 1 drop of ferric chloride ($FeCl_3$). Thereafter, 2mL of sulphuric acid (H_2SO_4) was added. Brown ring at interface indicated the presence of oxalates.

2.4.6. Detection of cardiac glycosides (Keller killiani test)

One milliliter portions of the extracts were dissolved in 1 mL of glacial acetic acid and cooled. After cooling, 2-3 drops of ferric chloride ($FeCl_3$) were added. To this solution, 2 mL of concentrated sulfuric acid (H_2SO_4) was added carefully along the walls of the test tube. The appearance of the reddish-brown color at the junction of two layers indicates the presence of glycosides.

2.4.7. Detection of saponin (Froth test)

The extracts were diluted with distilled water to 20 mL which were further shaken in a graduated cylinder for 15 minutes. The formation of a 1 cm layer of foam represented the presence of saponin.

2.5. Antioxidative activity

2.5.1. DPPH free radical scavenging assay

Free radical scavenging activity of *Solanum khasianum* leaves and berries extracts was determined spectrophotometrically. The changes in color (from deep-blue to light-yellow) were measured at 517 nm. The radical scavenging activity of extracts was measured by the standard method (Blois, 1958; Gogoi et al., 2020). Various concentrations of both the extracts (5, 10, 15, 20, 25, 30 µg/mL) were prepared in respective solvents and 1 mL of DPPH solution (0.2 mM) was then added. The mixture was shaken and incubated for 30 min at 37 ºC in dark. The absorbance was finally measured at 517 nm with a UV/Vis spectrophotometer. The percentage inhibition activity was calculated by the following (Eqn. 1):

DPPH scavenging effects (%) = [(Acontrol – Asample) / Acontrol]×100 (Eqn. 1)

Where Acontrol is the absorbance of DPPH radical without the sample (control) and Asample is the absorbance of DPPH radical with the oil sample of various concentrations. IC_{50} values (µg/mL) were determined from a plotted graph of scavenging activity against the concentrations of the extracts, where IC_{50} is

defined as the total amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

2.5.2. Reducing power

The reducing antioxidant power of *Solanum khasianum* leaves and berries extracts were determined by the previously published standard methods (Oyaizu, 1986; Gogoi et al., 2020). Various concentrations of leaves and berries extracts ranging from 5 to 30 µg/mL were prepared in 0.2 M phosphate buffer (pH-6.6) followed by the addition of 1.25 mL of potassium ferricyanide ($K_3Fe(CN)_6$, 1.1%). The mixture was incubated at 50 ºC for 20 minutes. Then, 1.25 mL of acetic acid (10%) was added to the mixture which was then centrifuged at 3000 rpm. 1.5 mL of the solution from the upper layer was shaken with 1.5 mL of distilled water and 0.3 mL of $FeCl_3$ (0.1%). The absorbance of the reaction mixtures was recorded at 700 nm using UV/Vis against a blank. The increased absorbance of the reaction mixture indicates an increase in the relevant reducing power.

2.5.3. Total phenolic content estimation

Total phenolic compounds of *Solanum khasianum* leaves and berries extracts were determined by Folin-Ciocalteu method (Folin and Ciocalteu, 1927). Standard gallic acid was prepared in different concentrations ranging from 20 to 160 µg/mL and mixed with 2.5 mL of Folin and Ciocalteu phenol reagent (1:9; FC:methanol) for 2 min. Then, 2 mL of sodium carbonate (Na_2CO_3, 7.5%) was mixed and incubated at room temperature for 2 h. The same protocol was followed for *Solanum khasianum* leaves and berries extracts. Spectrophotometric analysis was finally done at 760 nm.

2.5.4. Total flavonoids content estimation

The aluminum chloride spectrophotometric method was used for flavonoids determination (Sultana et al., 2009). 0.5 mL of the extracts from the leaves and berries were separately diluted with 2 mL of respective solvent and mixed with 0.15 mL of $NaNO_2$ (5%). 0.15 mL of aluminum chloride ($AlCl_3$, 10%) was then added with to reaction mixture. After 6 minutes, 1 mL of NaOH (1.0 M) and 1.2 mL of double distilled water were added and mixed well. Thereafter, absorbance was measured at 510 nm in a spectrophotometer. A standard solution of quercetin (20-160 µg/mL) was used for the construction of calibration curve.

2.6. *In vitro* anti-inflammatory activity, protein (egg albumin) denaturation assay

This assay was performed by the standard protocol (Sangita et al., 2012). The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of drug with varying concentrations (5, 10, 15, 20, 25 and 30 µg/mL). A similar volume of double-distilled water served as the control. In the next step, the mixtures were incubated at 37±2 ºC in a BOD incubator for 15 minutes and then heated at 70 ºC for five minutes in a hot water bath. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Sodium diclofenac at the concentrations of 5, 15, 20, 25 and 30 µg/mL was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula (Eqn. 2).

$$\text{Inhibition (\%)} = (Vc - Vt / Vc) \times 100 \quad \text{(Eqn. 2)}$$

Where, V_t = absorbance of the test sample, V_c = absorbance of control. Each experiment was done in triplicate and the average was taken.

2.7. Tyrosinase inhibitory activity assay

Tyrosinase inhibitory activity was assayed using a modified dopachrome method (Sarikurkcu et al., 2018). 125 µL portions of sample solutions of SKLME and SKBME were mixed with 200 µL of tyrosinase solution with 400 µL of phosphate buffer (pH 6.8) in test tubes and incubated at 25 ºC for 15 min. Then, L-DOPA 200 µL was added to start the reaction. Similarly, a blank was prepared by adding sample solutions and other reagents without the enzyme. After an incubation period of 10 min at 25 ºC, the absorbance of sample and blank were read at 492 nm. The absorbance of the blank was subtracted from the absorbance of the sample and tyrosinase inhibitory activity was compared with standard kojic acid (Eqn. 3).

$$I\ (\%) = (AC - AS / AC) \times 100 \quad \text{(Eqn. 3)}$$

Where I (%) is the percentage of inhibition, Ac is the absorbance of the control and As is the absorbance of the test sample.

2.8. Anti-diabetic activity assay

The antidiabetic activity of SKLME and SKBME was analyzed using a reliable standard methodology (Xiao et al., 2006). Accordingly, the reaction mixture was prepared using 100 µL of extract/standard solution over the concentration range of 5-30 µg/mL, 900 µL of sodium phosphate buffer (0.01 M, pH 6.9) and NaCl (0.006 M) containing α-amylase solution (0.5 mg/mL) or distilled water (blank). The reaction mixture was then incubated at 37 ºC for 10 minutes. After that, 500 µL of a starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9) with NaCl (0.006 M) was added to each tube. The reaction mixture was again incubated for 10 min at 37 ºC. The reaction was then stopped with 1 mL of HCl (10%), followed by the addition of 300 µL of the working indicator iodine potassium iodide solution. Finally, the reaction mixture was diluted by adding 10 mL of distilled water. The absorbance was measured and read at 620 nm by spectrophotometer (Eppendorf). α-Amylase activity inhibitory activity of the SKLME and SKBME were evaluated using the following formula (Eqn.4).

I (%) = (Abss – Absb ÷ Absc – Abso) × 100 (Eqn. 4)

Where I (%) - is the inhibition percentage; Absc was for 100% enzyme activity with only solvent and the enzyme; Abso was for 0% enzyme activity with solvent in absence of the enzyme; Abss – test sample with tyrosinase; Absb – a blank as a test sample without tyrosinase.

2.9. Acetylcholinesterase activity assay

Acetylcholinesterase activity of SKLME and SKBME were analyzed using a standard modified methodology (Bhavya et al., 2018). Acetylcholinesterase (AChE) with concentration of 0.04 U/mL and acetylthiocholineiodide (ATCI, 75 mM) were dissolved in phosphate buffer (0.1 M) with pH 8.5. 5'-dithiobis-(2-nitrobenzoic acid) (DTNB 0.01 M) was prepared in 10 mL of phosphate buffer (0.1 M) having pH 7.0 consisting of 15 mg sodium bicarbonate ($NaHCO_3$). Assessment of AChE inhibition was performed by the spectrophotometric method of Ellman (1960). Fifty microlitre of inhibitor solution (extracts/standard cholinesterase inhibitor drug galanthamine hydrobromide) and 0.5 mL of AChE were mixed in a test tube and set at 25 ºC in an incubator. DTNB solution (100 µL) and 2.4 mL of buffer at pH 7.0 were added to the test tube and incubated at 25 ºC for 5 minutes. There reaction was again started by adding 40 µL ATCI and the medium was further incubated at 25 ºC for 20 minutes. After that, the absorbance of the reaction mixture was measured at 412 nm using the UV/Vis spectrophotometer (Eppendorf, Germany). Methanol was taken as reaction blank, methanol in place of standard or sample with other reagents was considered as control. The percentage of inhibition towards cholinesterase activity was calculated as follows (Eqn. 5).

Inhibition% = (absorbance of control - absorbance of sample / absorbance of control) × 100 (Eqn. 5)

2.10. Seed germination assay

This assay was performed by using the standard protocol with slight modification (Paudel et al., 2008). Seeds of *Vigna radiata* L. (purchased from Sahib Seed Limited, Karnal, India) were air-dried with moisture content below 12%, and for the assay imbibed in tap water for a period of 24 h. Three layers of filter paper were stacked in all Petri plates and moistened by 3 mL of tap water. Then, 1 mL of each extract was poured on the filter paper and allowed to diffuse by covering the plates. Each of the treatment combinations had three replications. Twenty seeds were kept in each plate maintaining equidistance. Plates were incubated at 25±1 ºC. The Petri plates were examined for germination regularly.

2.11. Test for genotoxicity (*Allium cepa* assay)

Onion bulbs (*Allium cepa*, L.) were obtained from the vegetable market at Jorhat (Assam), India. They were cleaned and the dried outer scales were carefully removed leaving the ring of root primordial intact. These were used for the bioassay according to a standard procedure (Gogoi et al., 2020). For the root growth inhibition, three onion bulbs were placed on distilled water treated by each of the *Solanum khasianum* leaves and berries extracts in a concentration of 1 µg/mL. Distilled water and ethyl methanesulfonate (EMS) were considered as the negative and positive mutative agent. The base of each of the bulbs was suspended on the leaves and berries extracts inside 100 mL beakers for 72 h. Tested extracts and EMS were changed daily. At the end of the exposure period, the length of the roots of onion bulbs with the best growth was measured (in cm) with a ruler at each treatment. From the weighted averages for each extract and the control, the percentage root growth inhibition concerning negative control was determined for each extract (Gogoi et al., 2020). The effect of each sample on the morphology of growing roots was also examined. For the evaluation of induction of chromosomal aberration, 3 onion bulbs were suspended on each of the leaves and berries extract and both the positive and negative control for 72 h. At the end of 72 h, root tips from these bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v). These were hydrolyzed in HCL (1 N) at 60 ºC for five minutes after which they were washed in distilled water. Two root tips were then squashed on each slide, stained with acetocarmine for 10 minutes and cover slips carefully lowered on to exclude air bubble. Three slides were prepared for each concentration and the control (at 500 cells per slide) was analyzed at×1000 magnification for induction of chromosomal aberration. The mitotic index was calculated as the number of dividing cells per 500 observed cells (Gogoi et al., 2020). The frequency of aberrant cells percentage was calculated based on the numbers of aberrant cells per total cells scored at each of the *Solanum khasianum* leaves and berries extracts (Gogoi et al., 2020).

2.12. Statistical analysis

Statistical analysis was done using Microsoft Office Excel for the standard deviation (SD) and IC_{50} values were calculated using Graph Pad Prism 7.04 version software which is a very powerful combination of biostatistics, curve fitting and scientific graphing. Each of the experiments was performed in triplicate to reduce error. A difference was considered statistically significant when $p \leq 0.05$.

3. Results and Discussion

3.1. Phytochemical screening of *S. khasianum* leaves and berries extract

Results of the preliminary phytochemical screening are summarized in Table 1. As seen, many of the phytochemicals such as alkaloid, flavonoid, steroid, terpenoid, phenol, oxalate and cardiac glycoside were found to be present in both the SKBME and SKLME. Among them, SKBME extract showed the presence

Table 1
Phytochemical screening of leaves and berries methanol extracts of *Solanum khasianum*.

Constituents	Test performed	Extracts	
		SKLME	SKBME
Alkaloids	Mayer's test	+ve	+ve
Flavonoids	Ammonia reduction test	+ve	+ve
Saponins	Froth test	-ve	+ve
Steroids	Salkowski's test	+ve	-ve
Terpenoids	Salkowski's test	+ve	-ve
Phenols	Ferricchloride test	+ve	+ve
Oxalates	Acetic acid test	+ve	+ve
Cardiac glycosides	Keller-Killiani test	+ve	+ve

SKLME: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract; **+ve**: present; **-ve**: absent.

of saponins. Previously, a study of three *solanum* species such as *Solanum torvum*, *Solanum trilobatum* and *Solanum xanthocarpum* also showed the presence of phytochemicals like alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and phenolic compounds (Sundari et al., 2013). A research study by Mahato et al. (1980) has shown the presence of glycoalkaloids, khasianine, solamargine and solasonine in ethanol extracts of *Solanum khasianum* berries. However, there is no such report regarding *Solanum khasianum* leaves and berries methanol extracts, which makes our present investigation first of its kind. Alkaloids are also used as an anaesthetic agent (Herouart et al., 1988). Terpenoids exhibit various important pharmacological activities including anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities (Mahato and Sen, 1997) and both the useful constituents were found to be present in *Solanum khasianum* SKBME and SKLME. Alkaloids are also known for their AChE inhibitory properties. One of such plant species, *Solenanthus lanatus* was used for the isolation of new pyrrolizidine alkaloid, 7-*O*-angeloylechinatine *N*-oxide with three known compounds of the same class (3′-*O*-acetylheliosupine *N*-oxide, heliosupine *N*-oxide, and heliosupine), and all these alkaloid compounds showed inhibition activity against the AChE, with IC_{50} of 0.53-0.60 mM (Benamar et al., 2016a). Among the alkaloids, 7-*O*-angeloyllycopsamine *N*-oxide, echimidine *N*-oxide, echimidineand 7-*O*-angeloylretronecine, were isolated from the whole plant ethanolic extract of *Echiumcon fusum* Coincy, and they were also reported to show moderate AChE inhibitory activity with IC_{50} of 0.276-0.769 mM (Benamar et al., 2016b). Phenolic and flavonoid compounds were found to serve as active antioxidant and anti-inflammatory products (Del-Rio et al., 1997; Salah et al., 1995; Okwu, 2004) which were found to be present in *Solanum khasianum* leaves as well as berries extracts. Few of the other constituents present in *Solanum khasianum* berries extracts including saponin produce an inhibitory effect on inflammation (Just et al., 1998). Steroids were reported to have antibacterial properties (Raquel, 2007), and glycosides are a potent antihypertensive agent (Nyarko et al., 1990). The present investigation tested that bioactive constituents are the basis of therapeutic potential as well as physiological activity of the plant.

3.2. Antioxidant activity determination and total phenolic and flavonoid contents

3.2.1. DPPH free radical scavenging activity, reducing power determination and total phenolic/flavonoid content

Free radical scavenging activity and reducing power of *Solanum khasianum* leaves and berries methanolic extracts are summarized in Table 2 and Table 3. As shown, IC_{50} values of leaves and berries methanolic extract (38.30 and 20.68 µg/mL, respectively) accounted for lower antioxidative potential than standard ascorbic acid (20.63µg/mL); hence berries ethanolic extract was the most effective DPPH free radical scavenger. Similarly, reducing power assay also revealed that the standard ascorbic acid has the maximum reducing capacity (0.650±0.019 au) better than berries (0.632±0.032 au) and leaves methanolic extract (0.628±0.003 au). Phytochemical analysis showed berries methanol extract as a good solvent system, keeping it in view it can be said that due to the strong capacity of phytochemical extraction, both the methanolic extracts possess considerable antioxidative potential. The total phenolic content (TPC) and flavonoids content (TFC) of *Solanum khasianum* leaves and berries methanol extracts were summarized in Table 4. The amount of TPC and TFC of *Solanum khasianum* were 1.18 and 22.417 mg/mL for leaf methanol extract as well as 1.198±0.032 and 26.05±0.044 mg/mL for berry methanolic extract , respectively. There were few reports available that ethanolic fruit extract of *S. torvum* constituted 5.15 gGAE/100 g of total phenolics, ethylacetate fruit extract of *S. americanum* a synonym of *S. nigrum* constitutes

Table 2
DPPH free radical scavenging activities of *Solanum khasianum* leaves and berries methanol extracts.

Sample concentration (µg/mL)	AA % of inhibition ± SD IC_{50}=20.63 µg/mL	SKLME extracts % of inhibition ± SD IC_{50}=38.30 µg/mL	SKBME extracts % of inhibition IC_{50}=20.68 µg/mL
5	9.70±0.342	2.12±0.108	12.15±0.0121
10	16.85±0.078	5.14±0.811	18.06±0.675
15	29.67±0.003	12.45±0.013	32.11±0.211
20	47.16±0.123	21.06±0.245	48.05±0.432
25	63.09±0.308	28.55±0.338	60.12±0.786
30	79.36±0.004	39.89±0.120	78.04±0.886

SKLME: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract; **AA**: ascorbic acid; **SD**: standard deviation; **IC_{50}**: 50% inhibition; **µg**: microgram; **mL**: milliliter.

Table 3
Reducing power determination of *Solanum khasianum* leaves and berries methanol extracts.

Sample concentration (µg/mL)	Control absorbance (nm)±(SD)	Ascorbic acid absorbance (nm)±(SD)	SKLME (nm)±(SD)	SKBME (nm)±SD
0	0.405±0.089	-	-	
5		0.465±0.027	0.414±0.396	0.420±0.021
10		0.493±0.003	0.427±0.048	0.451±0.004
15		0.540±0.071	0.442±0.001	0.497±0.012
20		0.588±0.044	0.518±0.030	0.530±0.001
25		0.612±0.038	0.560±0.087	0.588±0.046
30		0.650±0.019	0.628±0.003	0.632±0.032

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **nm**: nanometer; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

Table 4
Total phenolic (TPC) and flavonoid contents (TFC) of *Solanum khasinum* leaves and berries methanol extracts.

Standard /*Solanum khasianum* leaf extracts	TPC (mg/mL)±SD	TFC (mg/mL)±SD
Gallic acid	1.09±0.005	-
Quercitin	-	0.956±0.013
SKLME	1.180±0.001	22.417±0.290
SKBME	1.198±0.032	26.05±0.044

mg: milligram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

9.37 gQE/100 g of total flavonoids (Fidrianny et al., 2015), but there is no such report available about the species *S. khasianum*.

There is not any record on *Solanum khasianum* antioxidant activities, but *Solanum nigrum* methanolic extract was reported to have antioxidant properties (Prakash et al., 2009). Phenols and flavonoids are among the important dietary components and plant secondary metabolites with strong antioxidant activity (Martinez et al., 2000; Schimid, 2006). Additionally, *S. khasianum* berries methanolic extract showed the highest TPC and TFC contents. Strong antioxidant activity of the extracts in the present investigation could be positively correlated with these compounds. An earlier report on *Solanum nigrum* leaf extracts showed the presence of considerable quantity of phenolics compounds (Alam et al., 2012), but there is no such evidence available of *Solanum khasianum* leaves or berries extract.

3.3. *In vitro* anti-inflammatory activity

Results of the *in vitro* anti-inflammatory activity of the *Solanum khasianum* leaves and berries methanol extracts, administered at the dose ranging from 5, 10, 15, 20, 25 to 30 µg/mL are presented in Table 5. Here, the strongest activity was shown by the standard sodium diclofenac (IC_{50}=18.88 µg/mL) followed by berries (20.96 µg/mL) and leaves methanolic extract (23.37 µg/mL). Although, *Solanum khasianum* berries ethanolic extract was reported to have considerable anti-inflammatory properties, *S. khasianum* berries produced significant reductions in the fresh carrageenan-induced acute inflammation of the rat hind paw. The activity was found to be dose-dependent and the extracts exhibited better activity during 3-4 h than standard sodium diclofenac. The previous result reported that for the ethanolic extract at concentration of 200 mg/kg, the mercury displaced was 0.63±0.03 in 4 hours, while for ethanolic extract at the concentration of 300 mg/kg, the mercury displaced was 0.59±0.03 and for ethanolic extract at the concentration of 400 mg/kg, the mercury displaced was 0.54±0.02 which indicates the inhibition of 34%, 38%, 43%, respectively (Jarald et al., 2008). Another species of the same genus, *Solanum nigrum,* also possess anti-inflammatory activities. According to the report, compound 1[α]14D-27.5 (c0.50, MeOH) ($C_{51}H_{82}O_{26}$) exhibited significant inhibition on NO production with an IC_{50} value of 9.7 µM, and some other compounds identified in methanol (70%) extract of the green fruits of *S. nigrum* exhibited significant inhibition effects on the LPS-induced IL-6 and IL-1 β production (Yihai et al., 2017). Another report by Ravi et al. (2009), showed that the methanolic extract of *Solanum nigrum* decreased edema induced in hind paw rat and reported to be a good anti-inflammatory agent at the dose of 375 mg/kg of *S. nigrum*. *Solanum corymbiflorum* was also previously reported to reduce the crotonoil-induced ear edema and myeloperoxide activity with a maximum inhibition of 87±3% and 45±7% respectively in the dose of 1 mg/ear of male Swiss mice (Piana et al., 2016). *Solanum xanthocarpum* dried fruit water extracts were also reported to possess 66.41% of anti-inflammatory activity in 500 mg/kg of hind paw edema rats (Anwikar and Bhitre, 2010). To date, there is no report available regarding the anti-inflammatory activity of *S. khasianum* leaves and berries methanol extracts. *S. khasianum* leaves and berries methanolic extracts both showed the presence of alkaloids, flavanoids and there are numerous reports regarding the anti-inflammatory potential of alkaloids and flavanoids (Barbosa-Filho et al., 2006; Pan et al., 2010). In the present investigation, the considerable anti-inflammatory activity SKLME and SKBME could be positively co-related with the presence of alkaloids and flavaonoids. There is no report regarding the anti-inflammatory activity of *S. khasianum*, therefore the present investigation tries to provide a base for the future search and preparation of natural origin inflammation inhibitory drug.

Table 5
In vitro anti-inflammtory activities of *Solanum khasianum* leaves and berries methanol extracts.

Concentration (µg/mL)	%of inhibition ± SD		
	Sodium diclofenac IC_{50}=18.88 µg/mL	SKLME IC_{50} = 23.37 µg/mL	SKBME IC_{50} = 20.96 µg/mL
5	11.25±0.090	6.93±0.002	14.76±0.045
10	28.90±0.112	10.09±0.034	22.32±0.023
15	42.06±0.065	18.90±0.673	30.86±0.022
20	59.00±0.099	47.07±0.043	49.10±0.001
25	65.20±0.004	53.13±0.111	60.64±0.089
30	73.01±0.106	69.40±0.301	72.38±0.032

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

3.4. Skin whitening activity of *S. khasinum* leaves and berries extracts

Tyrosinase inhibitory activity of *S. khasinum* leaves and berries extracts were evaluated using a standard methodology (Sarikurkcu et al., 2018). The maximum percentage of inhibitory activity was shown by SKBME 44.85±0.0001%, followed by kojic acid 28.60±0.0732% and SKLME 23.90±0.0404% (Table 6).

Table 6
Tyrosinase inhibitory activity of *Solanum khasianuam* leaves and berries methanolic extract.

Concentration (µg/mL)	Kojic acid % of inhibition IC_{50} = 53.37 µg/mL	SKLME % of inhibition IC_{50} = 61.12 µg/mL	SKBME % of inhibition IC_{50} = 33.97 µg/mL
5	02.49±0.0432	02.08±0.0001	04.67±0.0404
10	04.68±0.0002	04.32±0.0121	09.24±0.0022
15	07.94±0.0181	07.42±0.0452	16.28±0.0121
20	12.40±0.0004	11.90±0.0003	26.11±0.0024
25	20.34±0.0004	18.22±0.0331	35.42±0.0004
30	28.60±0.0732	23.90±0.0404	44.85±0.0001

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

The skin whitening ability was confirmed from the IC_{50} values of all the tested samples and standards. SKLME stands to have the lowest skin whitening ability with an IC_{50} of 61.12 µg/mL followed by standard kojic acid (IC_{50} 53.37 µg/mL) and the highest skin whitening property was shown by SKBME with an IC_{50} of 33.97 µg/mL. The present investigation relates to a skin whitening agent, skin care cosmetic agent, anti-aging agent that contains at least one of the skin whitening factors. A number of potent tyrosinase inhibitors are of synthetic, semi-synthetic and natural origins but the commonly used chemical skin whiteners possess toxic effects due to the presence of mercury, mercury chloride or ammoniated mercury (Jennifer et al., 2012). In the present investigation, skin whitening ability of *S. khasianum* berries methanolic extracts could be due to the presence of skin whitening alkaloid compounds. Additionally, there is no record regarding the skin whitening potential of the species. However, there are some other plant species such as *Morus spp*., *Isatis costata*, *Balanophora fungosa*, *Willughbeia coriacea* Wall., *Phyllanthus urinaria* etc., with proven skin whitening ability (Jennifer et al., 2012). Among the aforesaid species, *Morus* species has many polyphenols such as moracin (M-6,3′-di-*O*-glucopyranoside) which showed 4.5-fold high ertyrosianse inhibition than kojic acid (Lee et al., 2002). Similarly, nor atocarpetin showed 10.4-fold higher inhibition (Ryu et al., 2008). *Isatis costata* composes of 6 oxindole alkaloids constinones A and B; isatinones A and B; indirubin and trisindoline. These all compounds exhibited pronounced tyrosinase inhibitory activity in comparison to standard L-mimosine (Ahmad et al., 2010). *Balanophora fungosa* 50% ethanolic extract with compounds 1-*O*-(*E*)-caffeoyl-3-*O*-galloyl-4,6-(S)-HHDP-β-d-glucopyranose and 1-*O*-(*E*)-caffeoyl-3,4,6-tri-*O*-galloyl-β-d-glucopyranose prevented melanin pigmentation in a three-dimensional cultured human skin model (Ogi et al., 2011). *Willughbeia coriacea* bark, aerial, root and root of *Phyllanthus urinaria* showed potent tyrosinase inhibitory activity of more than 40% using L-tyrosinase as a substrate (Arung et al., 2011). One of the tyrosinase inhibitor tiliroside from raspberry inhibited 34.5% of intracellular mushroom tyrosinase activity and 54.1% inhibition in melanin production (Lu et al., 2009). Dihydro morin and artocarpetin isolated from *Actocarpus heterophyllus* wood were also reported for their tyrosinase inhibitory potential (Arung et al., 2006; Zheng et al., 2008). Chloroporin from heart wood of *Chlorophora excels* exhibited 14.8-fold tyrosinase inhibition (Kuniyoshi et al., 2003). Few of the other secondary metabolites like the novel flavanone, dalenin was found to be 52 and 495 times more effective tyrosinase inhibitor than hydroquinone and kojic acid, respectively (Chiari et al., 2011).

3.5. Anti-diabetic activity of *S. khasianum* leaves and berries methanolic extract

Results of the anti-diabetic activity of *S. khasinum* leaves and berries methanol extracts were presented in Table 7. The highest percentage of inhibition was shown by SKBME 52.47±0.0352%, followed by SKLME 41.65±0.0682%, and acarbose 32.55±0.09%. The IC_{50} value for SKBME was 28.24 µg/mL, SKLME: 39.10 µg/mL and acarbose: 48.34 µg/mL (Table 7). The genus *Solanum* comprises about one thousand five hundred species among which most of the members have been extensively used as traditional medicine or food. According to literature, there were total of eight *Solanum* species reported to have anti-diabetic properties. These eight species are *S. anguivi* (including saponin with high ability to attenuate the hyperglycaemia, hyperlipidaemia at 20-100 mg/kg dose in induced diabetic rat), *S. torvum* (involving methyl caffeate with anti-diabetic at 10, 20 and 40 mg/kg in streptozotocin induced diabetic rat), *S. lycocarpum* (having blood glucose lowering activity in alloxan induced diabetic rat), *S. melongena* (with ability to inhibit starch hydrolyzing enzymes like α-amylse, α-glucosidase in dose dependent manner), *S. xanthocarpum* (representing anti hyperglycaemic activity and improved serum enzymes and lipid profile in alloxan induced diabetic rat), *S. trilobatum* (capable of lowering hyperglycaemia and free radical formation in alloxan induced diabetic rats), *S. nigrum* (possessing significant blood glucose lowering effect in oral glucose tolerance test) and *S. pubescens* (decreasing the elevated blood glucose and lipid parameters in alloxan induced diabetic rats) (Kandimalla et al., 2015). But till date, there has been no report regarding the anti-diabetic potential of *S. khasianum* extracts. The compound responsible for the anti-diabetic potential of those eight *Solanum* species is not explored except *S. trovum* in which the observed anti-diabetic effects are due to the presence of methyl caffeate.

Table7
Anti-diabetic activity of *Solanum khasianum* leaves and berries methanolic extract.

Concentration (µg/mL)	Acarbose % of inhibition ± SD IC_{50} = 48.34 µg/mL	SKLME % of inhibition ± SD IC_{50} = 39.10 µg/mL	SKBME % of inhibition ± SD IC_{50} = 28.24 µg/mL
5	04.26±0.0460	09.18±0.0382	10.85±0.0602
10	07.11±0.0234	11.82±0.0462	16.76±0.0144
15	10.47±0.0642	17.90±0.0384	28.14±0.0942
20	16.50±0.0422	22.48±0.0001	34.30±0.0121
25	22.94±0.0364	30.90±0.0424	46.19±0.0044
30	32.55±0.0980	41.65±0.0682	52.47±0.0352

µg: microgram; **mL**: milliliter; SD: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

3.6. Anti-cholinesterase activity of *S. khasianum* leaves and berries methanol extract

AChE activity of SKLME and SKBME were evaluated using a standard modified method (Bhavya et al., 2018). The maximum percentage of inhibition was

exhibited by SKBME 14.38±0.0002% followed by standard galanthamine 11.29±0.0444% and SKLME 8.48±0.0710%. The 50% inhibition concentration of SKBME was 32.42 µg/mL, standard galanthamine: 40.11 µg/mL and SKLME: 45.75 µg/mL (Table 8). For the species *S. khasianum*, this is

Table 8
Anti-cholinesterase activity of *Solanum khasinum* leaves and berries methanolic extract.

Concentration (µg/mL)	Galanthamine % of inhibition ± SD IC_{50} = 40.11 µg/mL	SKLME % of inhibition ± SD IC_{50} = 45.75 µg/mL	SKBME % of inhibition ± SD IC_{50} = 32.42 µg/mL
1.5	01.64±0.0425	0.02±0.0642	02.05±0.0620
3	02.88±0.0488	1.30±0.0846	03.81±0.0043
4.5	04.12±0.0302	2.84±0.0725	05.22±0.0011
6	06.67±0.0517	4.11±0.0980	07.19±0.0354
7.5	08.14±0.0001	6.70±0.0542	10.20±0.0438
9	11.29±0.0444	8.48±0.0710	14.38±0.0002

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

the first report regarding the anti-cholinesterase activity. Among the family Solanaceae, *Solanum nigrum* L., leaf extract ATChI hydrolyzed 82 $pmols^{-1}g^{-1}$fr.wt was previously reported for its cholinesterase inhibitory activities and , while the activity for other parts like stem, branch and root was not detected. *Datura innoxia* Mill., young plant leaf 2005, branch 705, root 408; old plant leaf 1263, branch 100, root 408 $pmols^{-1}g^{-1}$fr.wt of ATChI hydrolyzed showed anti-cholinesterase activity. In the same study, *Physalis minima* L. young plants leaf: 1716, branch: 1481, root: 163 $pmols^{-1}g^{-1}$fr.wt of ATChI hydrolyzed were also reported for their AChE inhibitory activities (Gupta and Gupta, 1997). Cholinesterase inhibitory activity of *S. khasianum* leaves and berries methanolic extracts in the present investigation could be directly correlated with the bioactive secondary metabolites. Some ChE inhibitor factor might be present in the methanolic extracts. As plants and animals have co-evolved and ChEs are known for their neurotoxic potential, there might be a relationship between animals eating habits and the presence or absence of anti-cholinesterase agent in plants (Gupta and Gupta, 1997). Detailed chemical profiling and individual component bioactivity analysis is required in this aspect to fully validate the findings.

3.7. Effect of extracts on seed germination and seedling length of *Vigna radiata* seed

The effect of different concentrations of leaves and berries methanolic extracts of *Solanum khasianum* was evaluated after 24 h of the treatment (see Table 9 and Table 10). The obtained data revealed that the germination of seeds and the length of seedlings significantly decreased with increasing concentration of methanol extracts growth rate by *Vigna radiata* seedlings. The observation for inhibition of germination, seedling growth was more prominent at the methanolic extract of leaves with the highest concentration as compared to the methanolic extract of the berries. Leaves and berries methanolic extract respectively showed 0.28±0.109 cm and 0.94±0.061 cm of growth at a concentration of 80 µg/mL. These findings might be due to the presence of allelochemicals as the chemicals produced by animals and plants as secondary metabolites located in certain specialized organs of the plant, like juglone, sorgoleone, artemisin, escopoletin etc. exerting a detrimental physiological effect on individuals of another species when released into the environment in *Solanum khasianum* leaves and berries extracts. Shreds of evidence imply that many phenolic compounds e.g., gallic acid, caffeic acid, chlorogenic acid, etc. are allelopathic to germinating seeds and also reported to affect plant fundamental processes such as photosynthesis, chlorophyll production and plant water relations (Horsley, 1977; Einhelling and Ramussen, 1979; Rice, 1979), protein synthesis (Dank and Flethcher, 1975), respiration (Van et al., 1971; Koeppe, 1972; Demos et al., 1975), and cell membrane permeability (Glass and Dunlap, 1974). The presence of such phenolic compounds may be affecting the germination of *Vigna radiata* seeds. From this study, it can be suggested that a high dose of methanolic extracts is found to be perfect to apply as herbicides but further studies are still required in this aspect to fully validate the findings.

3.8. Genotoxic effect

The genotoxicity study of *Solanum khasianum* leaves and berries extracts revealed a negligible effect on onion root tips growth and cell division (*Allium cepa* assay) (Table 11, Table 12, Table 13 and Fig. 1), and was confirmed by chromosomal aberration test. The presence of chromosome bridge, stickiness, multipolarity, and breakage were considered for the detection of genotoxic potential (Fig. 1). *Solanum khasianum* leaves, berries extract and negative control (untreated onion) showed similar mitotic index of 17.56, 18.30 and 19.32% and 4.18% by EMS treated onion root.

Table 9

Seedling lengths of *Vigna radiata* treated (24 h) with *Solanum khasianum* leaves and berries methanol extracts.

***Solanum khasianum* extracts/tap water**	**Concentrations (µg/mL)**						
	0	**2.5**	**5**	**10**	**20**	**40**	**80**
	Seedling lengths (cm±SD)						
Control	3.3±0.019						
SKLME	-	2.19±0.013	2.04±0.002	1.96±0.098	1.70±0.034	0.93±0.002	0.28±0.109
SKBME	-	2.16±0.144	2.10±0.043	1.95±0.540	1.84±0.032	1.40±0.142	0.94±0.061

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

Table 10

Seed germinations of *Vigna radiata* treated (24 h) with *Solanum khasianum* leaves and berries methanol extracts.

Sample/ Control	**Concentrations (µg/mL)**						
	0	**2.5**	**5**	**10**	**20**	**40**	**80**
	Seed germination (%±SD)						
Control	100±0.00						
SKLME	-	95.07±0.002	81.98±0.023	77.06±0.104	32.00±0.40	18.94±0.202	09.06±0.174
SKBME	-	98.21±0.012	83.05±0.322	78.24±0.441	45.11±0.564	34.32±0.111	18.88±0.087

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

Table 11

Root growth test for *Solanum khasianum* leaves and berries methanol extracts.

Sample	**Root lengths (cm±SD)**
Distilled water	6.700±0.184
SKLME	6.523±0.244
SKBME	6.611±0.638
Ethyl methanesulfonate	0.871±0.061

µg: microgram; **mL**: milliliter; **SD**: standard deviation; **SKLME**: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

Table 12

Mitotic index and percentage (%) of prophase, metaphase, anaphase and telophase in tap water, *Solanum khasianum* leaves and berries methanol extracts, EMS treated *Allium cepa* root mitotic cell division.

Sample	**Mitotic index (%)**	**Prophase (%)**	**Metaphase (%)**	**Anaphase (%)**	**Telophase (%)**
Distilled water (-ve control)	19.32	48.26	36.04	8.02	7.05
SKLME	17.56	52	31.14	5.73	6.25
SKBME	18.3	48.39	29.66	10.04	11.91
Ethyl methanesulfonate (+ve control)	4.18	91.2	8.8	NA	NA

SKLME: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract.

Table 13

Chromosome aberration test for *Solanum khasianum* leaves and berries methanol extracts.

Sample	**Concentration (µg/mL)**	**Time hour**	**Bridge**	**Stickiness**	**Multipolarity**	**Breakage**	**Totala berration (%)**
Distilled water (-ve control)	0	72 h	2	2	1	0	1
SKLME	1 µg/mL	72 h	3	2	0	2	1.4
SKBME	1 µg/mL	72 h	3	3	1	3	2
Ethyl metyhanesulfonate (+ve control)	1 µg/mL	72 h	28	20	4	6	11.6

SKLME: *S. khasianum* leaf methanol extract; **SKBME**: *S. khasianum* berry methanol extract; **h**: hour.

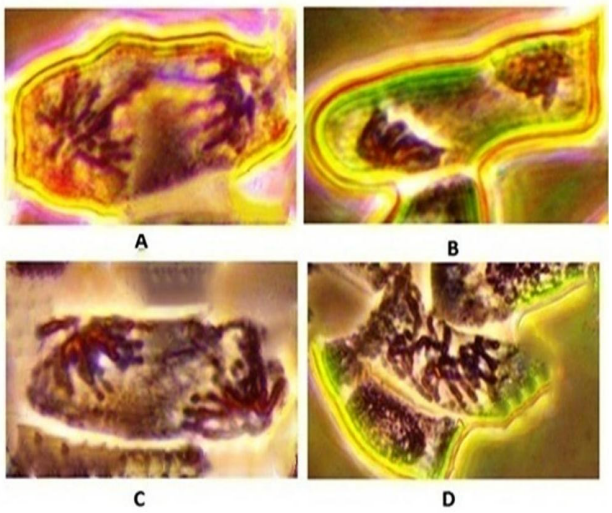

Fig.1. Chromosome aberrations for *Solanum khasianum* leaves and berries methanol extracts genotoxicity study (**A**=chromosome bridge, **B**=stickiness, **C**=multi polarity, and **D**=chromosome breakage).

Similarly in the chromosome aberration test negative control, *Solanum khasianum* leaves and berries methanol extracts were found in the range of 1.0%, 1.4% and 2.0%, respectively. On the other hand, EMS treated onion exhibited a maximum percent of aberration that is 11.6%. These findings revealed the negligible genotoxic potential of *Solanum khasianum* leaf and berries methanol extracts at a concentration of 1 µg/mL. From the phytochemical stand point and the constituents present in both the SKBME and SKLME, it can be stated that at the concentration of 1 µg/mL, they are not genotoxic. But there are lots of investigations that have revealed that many plants used for food and traditional medicines have mutagenic, cytotoxic and genotoxic impacts (Askin and Aslantrurk, 2007). Therefore, toxicological evaluations of all medicinal plants are important to ascertain their safety level. Genotoxic substances are known to be potential mutagenic or carcinogenic agents. The exposure of cells to genotoxic substances damages chromosomes or forms micronuclei (Kramer et al., 1990). Based on the aforementioned reports in the literature, it is worthwhile to study the genotoxicity of *S. khasianum* leaves and berries methanolic extracts, which have not been yet documented. This paves the path towards the development of safe, easily available and cost-effective pharmacologically important drugs.

4. Concluding remarks

From the results of phytochemical analysis, antioxidative, anti-inflammatory, anti-tyrosinase, anti-diabetic, anti-cholinesterase activities and seed germination study, it can be concluded that *Solanum khasianum* leaves and berries produce alkaloid, flavonoid, saponin, steroid, terpenoid, phenol, oxalate and cardiac glycosides with strong antioxidative, anti-inflammatory, skin whitening, anti-diabetic and neurodegenerative inhibitory potential. Considering all the experiments carried in the present study, it was revealed that *Solanum khasianum* leaves and berries could be a source of future or developing drugs against many chronic diseases and a new, easily available, cost-effective source for natural remedies. *Solanum khasianum* leaf methanolic extracts showed the potential of their application as herbicides in the agricultural field. Additionally, both the extracts showed negligible genotoxicity. This plant is still unexplored but abundant in the North-Eastern region of India with immense potential for commercial cultivation as it has great scope in the pharmaceutical sector. The CSIR-NEIST, MAEP scientist's team is trying to develop high solasodine and high fruits yielding varieties of *Solanum khasianum*. They have collected more than 254 germplasms across the Eastern Himalayan, so far. Therefore, deep analysis with advanced techniques should be implemented for isolation and screening of its bioactive phytoconstituents.

Conflict of interest

The authors declare that there is no conflict of interest.

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