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Evaluation of the antioxidative potential of the berries of *Solanum khasianum* Clarke using *in-vitro*, *ex-vivo*, and *in-silico* studies

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Abstract

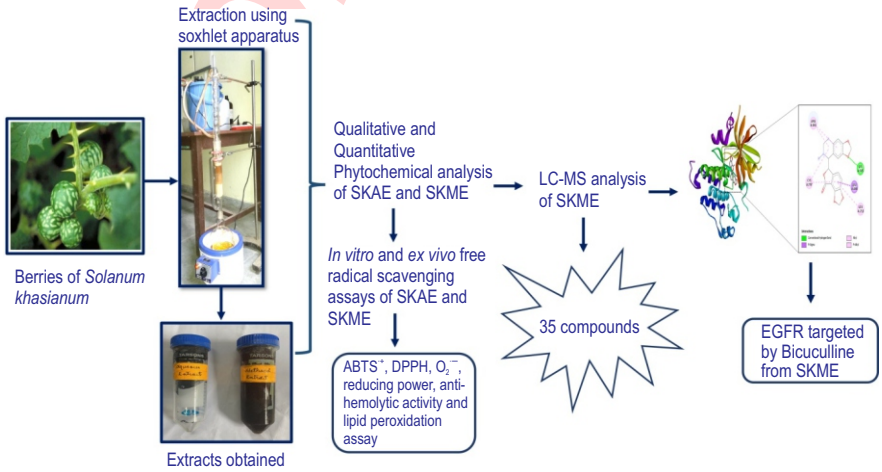
Aim: To determine the phyto-chemical content and antioxidative potential of *Solanum khasianum*.

Methodology: The antioxidative activity of *S. khasianum* extracts was evaluated by assessing their ability to scavenge free radicals. The overall reducing power of *S. khasianum* extracts was also determined. The inhibitory activity of *S. khasianum* extracts against erythrocyte hemolysis and lipid peroxidation was also determined *ex vivo*.

Results: The total phenolic and flavonoid contents were significantly higher in the methanolic extract of *S. khasianum* in comparison to the aqueous extract. Consistently, the methanolic extract was found to possess significantly higher free-radical scavenging activities. Both the extracts of *S. khasianum* showed significant inhibitory activities against erythrocyte hemolysis and lipid peroxidation. LC-MS analysis revealed the presence of 35 secondary metabolites from the methanolic extract of *S. khasianum*. Network pharmacology study identified epidermal growth factor receptor (EGFR) as the primary target of bioactive compound specifically Bicuculline, which exhibited notable binding affinity (-9.3 kcal mol^{-1}) with EGFR.

Interpretation: The findings reveal that *S. khasianum* extracts possess antioxidative potential, and highlights the significant potential of Bicuculline as an effective EGFR inhibitor, suggesting its potential candidate for the treatment of oxidative stress-related disorders.

Key words: Antioxidative activity, Bicuculline, EGFR, Molecular docking, Phytochemicals, *Solanum khasianum*



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Introduction

The body's physiological functions, including the regulation of gene expression, immune response, and cellular proliferation are significantly affected by reactive oxygen species (ROS) such as hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and superoxide ($O_2^{\cdot-}$) at low to moderate concentrations (Lalremruati *et al.,* 2019). However, due to their highly reactive nature, excessive ROS formation impairs the antioxidant defense mechanisms of the body, resulting in a condition known as 'oxidative stress' and tissue damage (Nghakliana *et al.,* 2020). Oxidative stress is strongly associated with many lifestyle-related conditions, including arthritis, coronary heart disease, diabetes, neuro-degenerative diseases, lung injury, inflammation, and cancer. In healthy human cells, the excessive oxidative stress damages proteins, DNA, and induces lipid peroxidation (Lalmuansangi *et al.,* 2020). Despite cells' impressive repertoire of enzymatic and non-enzymatic small molecule antioxidants to counterbalance ROS and prevent oxidative stress, overproduction of ROS overwhelms the endogenous antioxidants, causing oxidative cellular damage and likely long-term health problems (Zosangzuali *et al.,* 2021).

Therefore, exogenous antioxidant supplements can assist biological systems in scavenging free radicals and mitigating oxidative stress. Various medicinal plants have been used to isolate several modern drugs to treat certain diseases. The risk of cancer and numerous lifestyle-related disorders has been reported to be reduced by consuming natural antioxidants, such as flavonoids and phenolic compounds derived from plants (Lalremruati *et al.,* 2019). Plants are a rich source of phytochemicals and have significant antioxidant qualities due to their intrinsic capacity to produce non-enzymatic antioxidant molecules such as ascorbic acid, glutathione, and phenolic compounds (Swargiary *et al.,* 2021). Numerous pharmacological studies have been carried out to support the traditional medicinal uses of various plants within the genus *'Solanum'*. A total of 17 species have been reported for their anticancer activities, anti-inflammatory, antioxidant, anti-fungal, anti-diabetic, anti-nociceptive, anti-psoriatic, anti urolithiatic, hepatoprotective, diuretic, and nephrotoxicity (Kaunda *et al.,* 2019). Among *Solanum* species, *S. khasianum*, popularly called "nightshade," possess immense medicinal value, however, report on its antioxidative potential is meager. The plant was discovered in India and is now extensively distributed throughout Asia.

It can thrive in a wide range of climatic and agricultural conditions (Begum *et al.,* 2022). It is also commonly used as traditional medicine in China, Taiwan, and Korea. *S. khasianum* has been traditionally used to treat filaria, smallpox, whooping cough, rheumatism, trachoma, bronchitis, snake bites and skin infections (Chirumamilla *et al.,* 2022). Among the various tribes in India, the fruit and rhizome of *S. khasianum* are used as a traditional medicine to cure toothache, oral health issues, and problems with birth control (Lal *et al.,* 2022). Solasodine, solasonine, solanine, solamargine and khasianine are the potential glycoalkaloids reported in *S. khasianum*. Among the *Solanum* species, *S. khasianum* is mostly preferred for commercial production of solasodine (Chirumamilla *et al.,* 2022). The bioactive components of plants or natural products are in vogue assessed for their therapeutic properties based on their antioxidant activity. *In-vitro* screening and *in-silico* methods have been used recently for the elucidation of chemical compounds, and the study of pharmacological research on medicinal plants (Nghakliana *et al.,* 2025, Lalmuansangi *et al.,* 2025). In view of this, the current investigation was conducted to evaluate the antioxidative potential of aqueous and methanolic extracts of *S. khasianum,* and identify the potential bioactive compounds using *in-silico* methods.

Materials and Methods

Collection of plant material and preparation of extracts: Berries of *S. khasianum* were collected from the Hliappui Village in Mizoram, India. The Department of Horticulture and Aromatic Medicinal Plants at Mizoram University in Aizawl certified its authenticity (Voucher No. 20212807/2022). The berries were shade-dried for 30 days at room temperature, subsequently chopped using a mixer grinder, and then preserved in an airtight plastic container. Extraction was performed using soxhlet apparatus with aqueous and methanolic solvents at their respective boiling points until the solvent became colorless. The liquid extracts were filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator (Buchi, Germany) at 40°C for 5 hr under reduced pressure. Thereafter, the extracts were collected and preserved at -20°C until further use.

Phyto-chemical analysis: The presence of phytochemicals such as alkaloids, cardiac glycosides, saponins, steroids, tannins, terpenoids, and phlobatannins in the aqueous extract and methanolic extract of *S. khasianum* was determined following the standard methods (Doughari, 2012).

Determination of total phenolic and flavonoid contents: The total phenolic and total flavonoid contents were estimated by the method of Sakanaka *et al.* (2005) with slight modifications at different concentrations (1-8 mg ml^{-1}).

***In-vitro* antioxidant assay:** The DPPH radical scavenging activity was carried out by the method of Leong and Shui (2002) with a slight modification. The ABTS test was carried out for determining the free radical scavenging activity of *S. khasianum* following the method of Re *et al.* (1999). Nitro Blue Tetrazolium (NBT) reduction method (Hyland *et al.,* 1983) with minor modifications was used to measure the superoxide scavenging activity. The reducing power of aqueous and methanolic extracts of *S. khasianum* were determined by the method of Oyaizu (1986) with a slight modification. Both the extracts of *S. khasianum* were used at the ranges of 1-800 µg ml^{-1} for the determination of their free radical scavenging activities.

***Ex-vivo* antioxidant assay:** Anti-hemolytic activities of aqueous and methanolic extracts of *S. khasianum* were determined by

inhibiting erythrocyte hemolysis (Li *et al.,* 2004) with a slight modification. Lipid peroxidation inhibition assay was performed using $FeCl_2$-H_2O_2 induced lipid peroxidation in the liver of mice (Kaur *et al.,* 2010).

Animals: A colony of inbred Swiss albino mice was maintained at the Animal House, Department of Zoology, Mizoram University under standard environmental conditions (22±5°C; 12/12 hr light: dark) with free access to food and water. The use of animals for this research was approved by the Institutional Animal Ethical Committee, Mizoram University, India (No. MZUIAEC/2020/07) and CPCSEA, New Delhi, India (Registration No. 1999/GO/ ReBi/S/18/CPCSEA).

Liquid Chromatography-Mass Spectrometry (LC-HRMS) analysis: The bioactive secondary metabolites found in the methanolic extract of *S. khasianum* were identified using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). The analysis was performed with an ACCUCORE HPLC (C18,150 X 2.1, 1.7 um) system linked to a triple quadrupole tandem mass spectrometer (ACQ-TQD-QBB1152, Waters acuity PDA detector, Waters Corporation, Milford, MA, USA) using an orthogonal ESI source. The mass acquisition spectra for the methanolic extract were obtained between 150 and 2000 m/z. For ES+ and ES-, the source temperature was set at 120°C and the dissolution temperature at 350°C, respectively. The mobile phases A and B (water and 0.1% formic acid, mixed aceto-nitrile, and 0.1% formic acid, respectively) were used as eluents in a linear gradient flow. Throughout the gradient, the injection volume was 5 µl with a constant flow rate of 0.3 ml min^{-1}. Mass Lynx™ and Open Lynx™ (Waters Corporation, Milford, MA) were used to analyze the data.

ADME prediction and drug likeness analysis: The ADME components of a drug's pharmacokinetic profile are essential for understanding its absorption, distribution, metabolism, and excretion in human body. For further study, the canonical smiles of the phytocompounds identified in the methanolic extract of *S. khasianum* were obtained from PubChem. Pharmacokinetics and physiochemical properties are essential for the identification of drug candidates with therapeutic significance that could function as a safe and effective medication. The druglikeness and bioavailability of the compounds were subsequently screened using Lipinski, Ghose, Veber, Egan, and Muegge's protocols in Swiss ADME software (https://www.SwissADME.ch). The phytocompounds that showed no violation of druglikeness were then evaluated for their pharmacokinetic properties using pkCSM software (https://biosig.lab.uq.edu.au/pkcsm/prediction).

Network pharmacology screening for potential gene target

Screening of potential targets: Swiss prediction database (https:// www.swisstargetprediction.ch/) was utilized for determining the target proteins of the selected phytocompounds. Antioxidant-related proteins were obtained from the GeneCard database (https://www.genecards.org/). Finally, the shared targets of the selected phytocompound and antioxidant were obtained using Jvenn, a plug-in of the jQuery JavaScript library (https://jvenn.toulouse.inrae.fr/app/index.html).

Protein-Protein Interaction (PPI) network construction and core target screening: The STRING database (https://string-db.org/) was then used to generate the protein-protein interaction network for shared targets. The results were downloaded in tsv.format and then incorporated into Cytoscape 3.10.2 software for further study. To find the potential protein targets, the related nodes were ranked based on their degree value using the Cytohubba plug-in.

Molecular docking: The 3D structures of the proteins selected through network pharmacology were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/), while the chemical structures of the chosen bioactive compounds were obtained from the NCBI PubChem database (http://pubchem.nlm.nih.gov/). Molecular docking was done using UCSF CHIMERA plugin by VINA, and the docked result was visualized using Discovery Studio Visualizer.

Statistical analysis: Data are presented as mean±standard error of the mean. Graph Pad prism software ver. 8.0 was used to determine the IC_{50} values by plotting the results against the log doses. To assess for significant differences in the phytochemical contents and antioxidant tests of different extracts, One-way ANOVA was applied, followed by a Tukey multiple comparison of means. For statistical and graphical assessments, SPSS ver.16.0 software (SPSS Inc, Chicago, Illinois, USA) and Graph Pad prism program ver.8.0 were used. The statistically significant estimate with p-value less than 0.05 was evaluated.

Results and Discussion

Qualitative analysis revealed that the aqueous and methanolic extracts of *S. khasianum* contained various bioactive phytochemicals like alkaloids, cardiac glycosides, saponins, steroids, tannins, phlobatannins, and terpenoids (Table 1). However, the aqueous and methanolic extracts of *S. khasianum* contained different phytochemicals of polyphenolic with pharmaceutical potentials. The total phenolic content of the aqueous and methanolic extracts of *S. khasianum* increased in a concentration-manner (Fig. 1a, b). At 8.0 mg ml^{-1}, the methanolic extract had significantly higher ($p < 0.05$) total phenolic content (3993.38 ± 111.97 mg gallic equivalent/g of dry extract) than the aqueous extract (3023.10 ± 72.96 mg gallic equivalent/g of dry extract). At 8.0 mg ml^{-1}, the methanolic extract had significantly higher ($p < 0.001$) total flavonoid content (1889.58 ± 56.29 mg rutin equivalent/g of dry extract) as compared to the aqueous extract (971.05 ± 47.87 mg rutin equivalent/g of dry extract) (Fig. 1c,d). Compounds such as flavonoids, polyphenols, and carotenoids in several medicinal plants are essential phytochemicals with potent antioxidant activities that neutralize free radicals (Shen *et al.,* 2022). These natural compounds derived from medicinal plants, particularly those with antioxidant

Table 1: Phytochemical screening of aqueous and methanolic extracts of *S. khasianum*

Phytochemicals	Reagents	Colour	Methanolic extract	Aqueous extract
Alkaloids	Dragendroff's Reagent	Reddish brown precipitate	-	+
Cardiac Glycosides	Glacial Acetic Acid, Ferric Chloride, Sulphuric Acid	Brown ring	+	-
Steroids	Olive Oil	Whitish emulsion	+	+
Saponins	Sulphuric Acid	Red colour	-	+
Tannins	Ferric Chloride	Brownish green or blue-black	+	+
Terpenoids	Sulphuric Acid	Reddish brown	+	+
Phlobatannins	Hydrochloric Acid	Red precipitate	-	-

('+' indicates the presence of phytochemicals and '-' indicates the absence of phytochemicals).

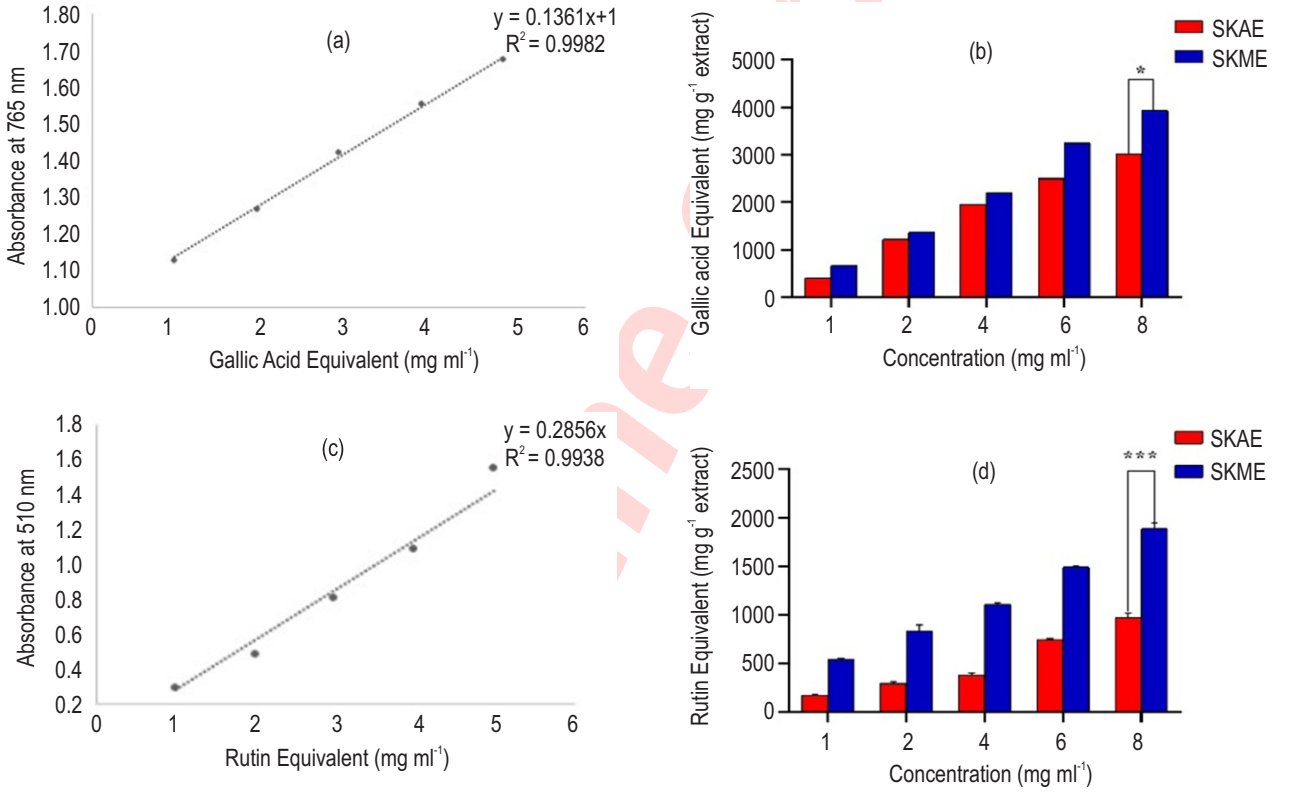

Fig. 1: (a) Standard graph of Gallic acid, (b) Phenolic content of SKAE and SKME determined as Gallic acid equivalent, (c) Standard graph of Rutin, (d) Flavonoid content of SKAE and SKME determined as Rutin equivalent. SKAE: *S. khasianum* aqueous extract; SKME: *S. khasianum* methanolic extract. Values are expressed as Mean ± SEM (n=3). * indicate significant variation at $p < 0.05$, *** indicate significant variation at $p < 0.001$.

properties, can play a significant role in the treatment of diseases associated with oxidative stress by acting as co-factors in enzymatic processes, enzyme inhibitors or stimulators, and scavengers of reactive or toxic substances (Ullah *et al.,* 2020). The present study revealed the presence of various phytochemicals in both the extracts of *S. khasianum*. These phytochemicals belong to polyphenolic compounds and have been reported to possess numerous pharmacological properties including anti-inflammatory, antioxidant, anti-microbial activity, anti-hypertensive and anti-malarial properties (Adu-Amankwaah *et al.,* 2023). Additionally, the quantitative phytochemical analysis also showed that both the extracts of *S. khasianum* possessed significant amounts of phenolic and flavonoid compounds. Phenolic compounds exhibit antioxidant capabilities due to their benzene ring structure, quantity, and position of the OH groups (Zeb *et al.,* 2020). Compounds containing phenols have been

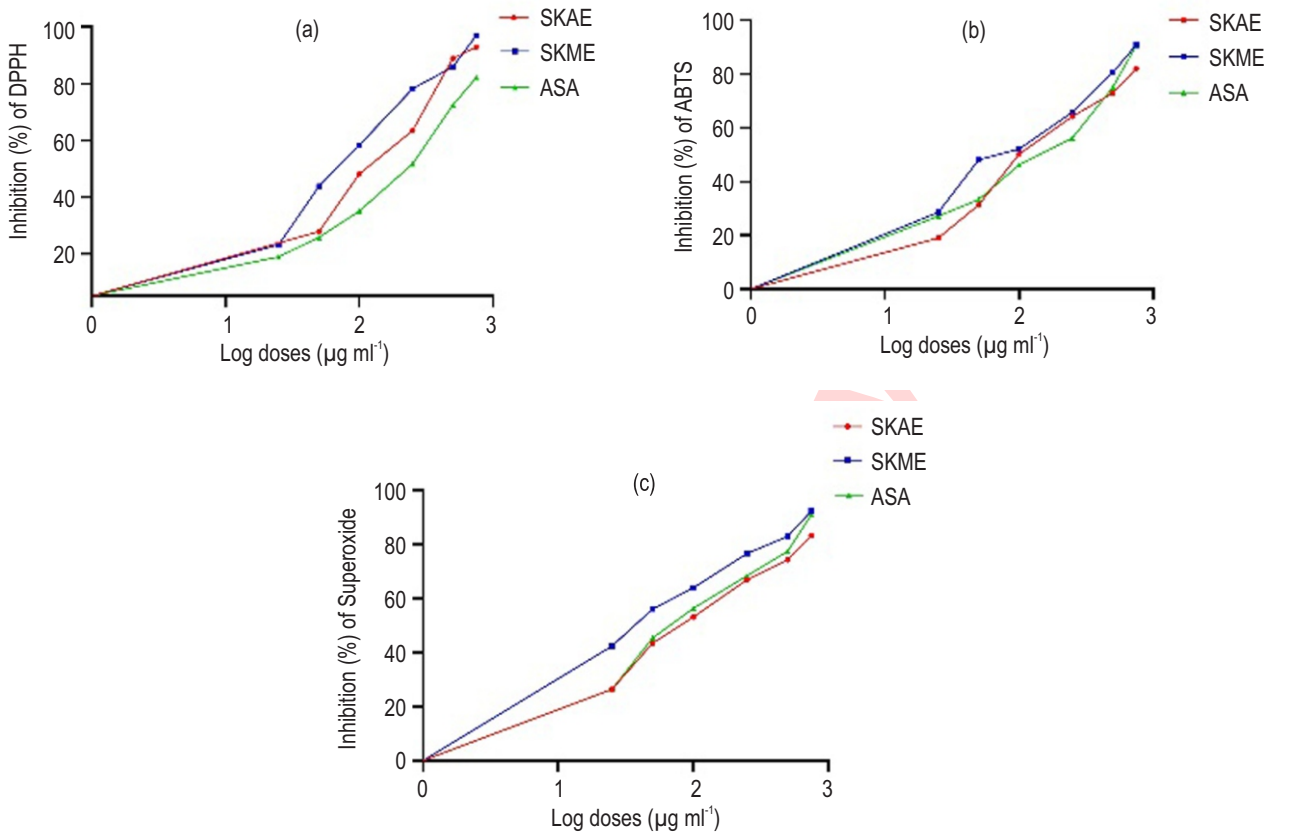

Fig. 2: Plots of log-doses of SKAE, SKME, and standard ascorbic acid (ASA) against %inhibition of (a) DPPH, (b) ABTS, (c) $O_2^{\bullet-}$ for the calculation of IC_{50}. SKAE: *S. khasianum* aqueous extract; SKME: *S. khasianum* methanolic extract.

reported to exhibit anti-inflammatory, antioxidant, antimicrobial, anti-carcinogenic, anti-asthmatic and cardioprotective effects (Sun *et al.,* 2023). Comparatively the flavonoids have been reported to possess antioxidant activity because of their ability to donate hydrogen and prevent the cells from damage caused by free radicals by reducing α-tocopheryl radicals, inhibiting oxidases like lipoxygenase, phosphoinositide 3-kinase [PI3K], xanthine oxidase [XO], and cyclo-oxygenase [COX], and scavenging ROS (Shen *et al.,* 2022). Furthermore, the flavonoids are associated with various health-promoting characteristics and pharmacological effects such as antiviral, anti-inflammatory, antibacterial, and anti-allergic properties (Mutha *et al.,* 2021).

The antioxidative potential of the aqueous and methanolic extracts of *S. khasianum* was assessed by *in-vitro* antioxidant assays using DPPH, $ABTS^{\bullet+}$, $O_2^{\bullet-}$ and reducing power. The free radical scavenging activities of both the extracts increased in a concentration-dependent manner. To determine the IC_{50}, the log-doses of both the extracts and the standard ascorbic acid were plotted against the inhibition (%) of DPPH, $ABTS^{\bullet+}$, and $O_2^{\bullet-}$ radicals (Fig. 2a-c). The scavenging activity of methanolic extract against DPPH (IC_{50}: 70.56 ± 1.16 µg ml^{-1}), $ABTS^{\bullet+}$ (IC_{50}: 75.76 ± 2.05 µg ml^{-1}) and $O_2^{\bullet-}$ (IC_{50}: 38.86 ± 1.89 µg ml^{-1}) were found to be significantly higher than the aqueous extract (IC_{50}: 115.90 ± 1.83 µg ml^{-1} for DPPH; 122.56 ± 1.50 µg ml^{-1} for $ABTS^{\bullet+}$; 87.68 ± 1.36 µg ml^{-1} for $O_2^{\bullet-}$). Despite the non-significant variations, the methanolic extract surpassed standard ASA in DPPH and $O_2^{\bullet-}$ scavenging. Similarly, no significant variation was observed between the methanolic extract and the standard ascorbic acid in $ABTS^{\bullet+}$ scavenging activities (Fig. 3a-c). The reducing activity of both aqueous and methanolic extracts was found to be dose dependent. At 750 µg ml^{-1}, the higher reducing activity was recorded for methanolic extract (0.50 ± 0.007) as compared to the aqueous extract (0.247 ± 0.001). The reducing activity of the methanolic extract was even higher than the standard ASA (0.345 ± 0.002) (Fig. 3d). This study revealed that the elevated ferric-reducing power of the methanolic extract serves as a significant indicator of its potential antioxidant activity. The conversion of methanolic DPPH solution to its non-radical form, DPPH-H, is commonly employed to evaluate the antioxidant activity of various chemical compounds and plant extracts. Both the extracts of *S. khasianum* efficiently reduced the stable DPPH radical to yellow-colored diphenyl-picrylhydrazine, likely because of active compounds capable of donating hydrogen atoms to neutralize free radicals. It has been demonstrated that certain compounds, such as glutathione,

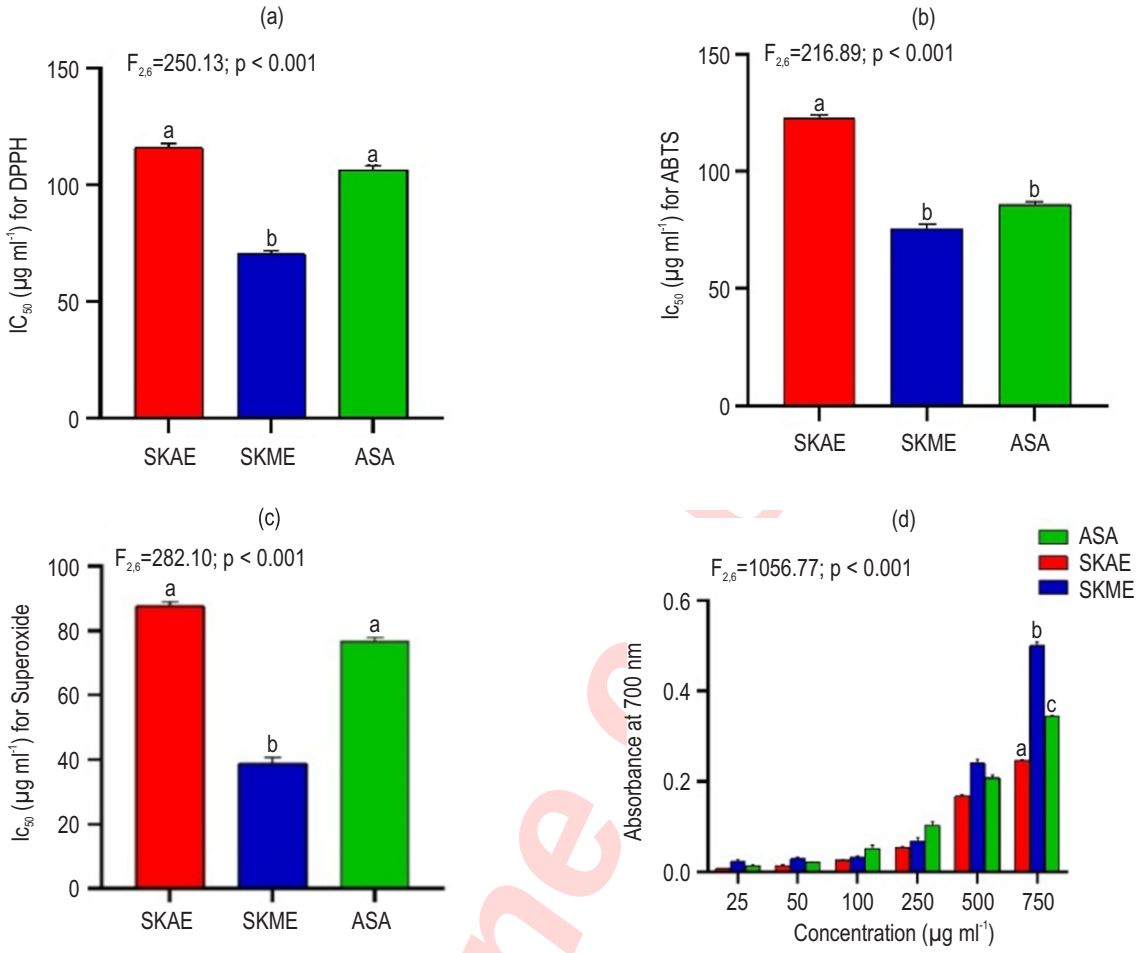

Fig. 3: IC_{50} (µg ml^{-1}) for (a) DPPH, (b) ABTS, (c) $O_2^{\bullet-}$, and (d) Reducing power of aqueous and methanolic extracts of *S. khasianum* and standard ascorbic acid. SKAE: *S. khasianum* aqueous extract; SKME: *S. khasianum* methanolic extract; ASA: Ascorbic acid. Values are expressed as Mean ± SEM (n=3). Different letters indicate significant variation.

polyhydroxy aromatic compounds, cysteine, tocopherol, and ascorbic acid, can reduce DPPH by hydrogenation (Gulcin and Saleh, 2023). ABTS assay is also an effective and simple method for evaluating the antioxidant activity of hydrogen-donating and chain-breaking antioxidants. Comparable to the principle of the DPPH method, ABTS can also form a stable free radical, and decolorization occurs as antioxidants reduce the pre-formed $ABTS^{\bullet+}$ (Ruan *et al.,* 2022). In the present study, the scavenging activity of *S. khasianum* extracts against $ABTS^{\bullet+}$ occurred in a concentration dependent manner. According to Shahid *et al.* (2022), the presence of phenolic hydroxyl groups contributes to the anti-radical activity of phenolic compounds.

The effectiveness of antioxidants having phenolic hydroxyl groups in their structure to scavenge free radicals greatly depends on the bond dissociation energy between the phenolic hydrogen and oxygen. The superoxide anion radical, a highly reactive species, contributes to cellular damage by triggering lipid peroxidation. Superoxide ($O_2^{\bullet-}$) radicals can break down to form stronger reactive oxygen species (ROS), including hydroxyl radicals and singlet oxygen (Untea *et al.,* 2023). Therefore, scavenging of $O_2^{\bullet-}$ could inhibit the production of additional ROS and protect cells from damage caused by oxidative stress. It has also been reported that the antioxidant properties of some polyphenolic compounds, particularly flavonoids, are effective mainly through the scavenging of superoxide anion radicals (Cañas *et al.,* 2023). Extracts of *S. khasianum* were found to inhibit the production of superoxide radicals in a concentration-dependent manner, probably due to the presence of substantial amount of flavonoids, and the $O_2^{\bullet-}$ scavenging effect of the methanolic extract was even better than that of the standard ascorbic acid. The reducing power was determined using the ferricyanide reduction method. Fe^{3+} to Fe^{2+} reduction is a key indicator of electron-donating activity, which is a significant component of antioxidant action (Baliyan *et al.,* 2022). The reducing ability of a chemical compound can serve as a key

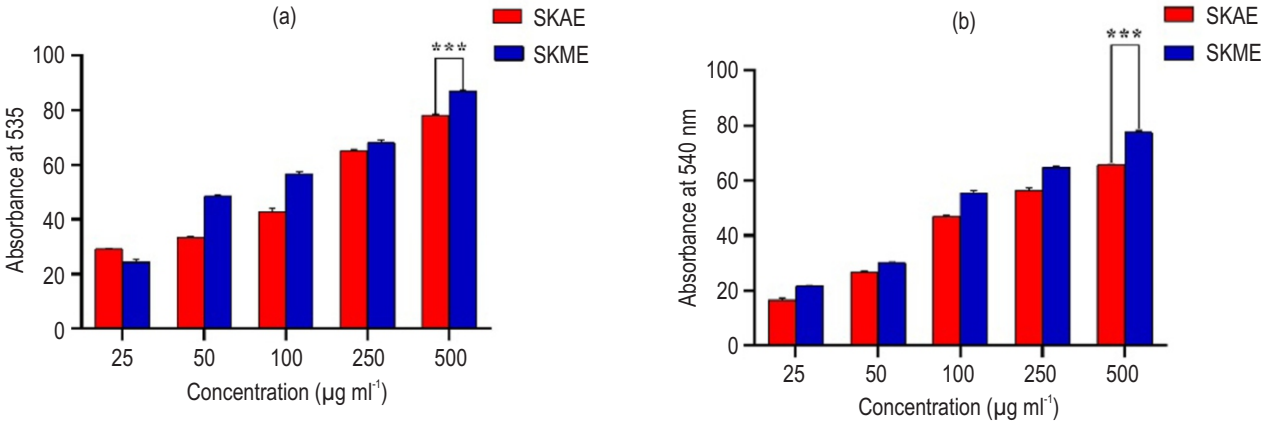

Fig. 4: (a) Anti-hemolytic and (b) lipid peroxidation inhibition of aqueous and methanolic extracts of *S. khasianum*. SKAE: *S. khasianum* aqueous extract; SKME: *S. khasianum* methanolic extract; ASA: Ascorbic acid. Values are expressed as Mean ± SEM (n=3). *** indicate significant variation at $p < 0.001$.

indicator of its antioxidant potential. The dose-dependent increase in the reducing power of both *S. khasianum* extracts also suggested its potent antioxidant activity. The concentration of aqueous and methanolic extracts of *S. khasianum* determines the potential of their anti-hemolytic activity and ability to inhibit lipid peroxidation (Fig. 4a-b). At 500 µg ml^{-1}, methanolic extract possessed significantly higher ($p < 0.001$) inhibitory activity against erythrocyte hemolysis with an inhibition rate of 86.75% when compared to the aqueous extract with an inhibition rate of 78.08%. Our result showed a significant variation among both the extracts in their inhibitory activity against lipid peroxidation.

Higher inhibitory activity was recorded for methanolic extract with an inhibition rate of 77.61% compared to the aqueous extract with an inhibition rate of 65.75%. The oxidative damage to the membrane system caused by ROS induces the hemolysis of red blood cells. This could lead to increased cell membrane fluidity by rendering it more permeable and resulting in material leakage (Lalmuansangi *et al.,* 2020). Lipid peroxidation generates specific breakdown products, such as malondialdehyde (MDA), which are regarded as the primary cause of cell membrane disintegration and damage to cells. In this study, lipid peroxidation in mice liver homogenate was induced by $FeCl_2$-H_2O_2. Thus, the formation of MDA is used as an indicator of lipid peroxidation, and subsequently, oxidative stress (Nghakliana *et al.,* 2020). The extracts of *S. khasianum* also exhibited notable anti-hemolytic effects and the ability to inhibit lipid peroxidation, likely due to their rich content of phenolic and flavonoid compounds. It has been shown that certain flavonoids and phenolic compounds interact with cell membranes, impeding the passage of free radicals and therefore reducing the chain reaction of free radicals. Flavonoids, by adhering to the membrane, have the ability to enhance the integrity of erythrocytes against lysis and reduce lipid peroxidation inside the cells (Lalremruati *et al.,* 2019).

The LC-HRMS analysis of methanolic extract revealed the presence of major compounds that mostly belong to the phenolics, steroids, terpenoids, carotenoids, alkaloids, and numerous other phytocompounds. The precise mass, retention time, m/z ratio, and molecular formula of the deprotonated molecules of the identified compounds in methanolic extract of *S. khasianum* are presented in (Table 2). LC-HRMS has been an essential tool for bio-prospecting plant bioactive compounds. Compounds identified from the methanolic extract have been reported to possess various pharmacological properties. Among the compounds identified from the methanolic extract, (S)-Canadine has been reported to act as a potential inhibitor against atherosclerosis through inhibition of PCSK9, Latifoline has been reported to possess antioxidant properties (Hasibuan *et al.,* 2024). Mananimbine shows strong anticancer activity against a wide range of cancer cell lines such as A549, Hs172 and MCF-7 cells (Chan *et al.,* 2024). Solanidine, a well-known phyto-constituent has also been reported to possess multiple biological properties like antimicrobial, cardio-protective effect, anti-inflammatory and anti-proliferative effects against HL-60 human leukemia cell and human lung fibroblast cell line (MRC-5) (Patel *et al.,* 2017).

Tetrahydropalmatine and Levallorphan have been well recognized for their anti-inflammatory and neuro-protective properties (Aryal *et al.,* 2022). Bicuculline is also well known for its probing of GABA-mediated synaptic inhibition in the central nervous system (Johnston, 2013). Among the 35 phytocompounds identified in the methanolic extract of *S. khasianum*, 20 compounds exhibited no violations of the druglikeliness rules (Table 3). Following the druglikeness and ADMET evaluation, Bicuculline from the methanolic extract was selected for *in-silico* analysis. A total of 100 possible molecular targets of the compounds from the methanolic extract of *S. khasianum* were identified by analyzing their targets in the Swiss Target Prediction database. A total of 5,109 protein targets associated with antioxidants were retrieved and compiled from the GeneCard database. The mutual gene matching between the target genes of *S. khasianum* compounds and those associated with antioxidants resulted in the identification of 70 genes. The 70 protein targets were loaded into the STRING database for PPI

Table 2: Compounds identified in the methanolic extract of *S. khasianum* using LC-HRMS

Family	Compounds	Molecular weight	Retention Time	m/z ratio	Molecular Formula
Acetamide	4-(beta-Acetylaminoethyl) imidazole	153.18	31.134	153.12	$C_7H_{11}N_3O$
	L-Selenomethionine	196.12	16.135	196.95	$C_5H_{11}NO_2Se$
Amino Acid	L-Tyrosine methyl ester 4-sulfate	275.28	9.349	275.03	$C_{10}H_{13}NO_6S$
	O-Phospho-4-hydroxy-L-threonine	215.10	12.131	215.0	$C_4H_{10}NO_7P$
	S-(4-Bromophenyl)-L-cysteine	276.15	17.561	275.0	$C_9H_{10}BrNO_2S$
	(-)-Sympatol	167.2	30.880	167.1	$C_9H_{13}NO_2$
	Solanidine	397.6	28.793	397.36	$C_27H_{43}NO$
	Haplopine	245.23	6.328	245.1	$C_{13}H_{11}NO_4$
	Ajmaline	326.4	30.625	326.23	$C_{20}H_{26}N_2O_2$
	Bicuculline	367.4	8.975	367.14	$C_{20}H_{17}NO_6$
	(S)-Canadine	339.4	23.092	339.15	$C_{20}H_{21}NO_4$
	Latifoline	393.4	18.273	393.18	$C_{20}H_{27}NO_7$
	Lucidine B	467.7	29.675	467.37	$C_{30}H_{49}N_3O$
Alkaloid	Elaeokanine C	211.3	16.034	211.17	$C_{12}H_{21}NO_2$
	Levallorphan	283.4	22.888	283.18	$C_{19}H_{25}NO$
	Heliotrine	313.39	15.491	313.21	$C_{16}H_{27}NO_5$
	Evocarpine	339.5	31.439	339.29	$C_{23}H_{33}NO$
	Mescaline	211.26	26.417	211.13	$C_{11}H_{17}NO_3$
	Tetrahydropalmatine	355.4	9.925	355.2	$C_{21}H_{25}NO_4$
	N-Methylpelletierine	155.24	11.554	155.16	$C_9H_{17}NO$
	Alpinine	415.5	31.847	415.21	$C_{23}H_{29}NO_6$
	Mahanimbine	331.4	22.210	331.2	$C_{23}H_{25}NO$
	Prunasin	295.29	2.664	295.08	$C_{14}H_{17}NO_6$
Cyanogenic glycoside	Taxiphyllin	311.29	34.222	311.1	$C_{14}H_{17}NO_7$
Phosphorodiamide	4-Hydroxycyclophosphamide	277.08	15.525	275.98	$C_7H_{11}Cl_2N_2O_3P$
	3,4-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione	316.4	6.702	316.14	$C_{19}H_{24}O_4$
Phenol	Sinapate	224.21	11.826	224.09	$C_{11}H_{12}O_5$
	Chlorogenate	354.31	15.117	354.08	$C_{16}H_{18}O_9$
Alkylbenzene	Fenpropimorph	167.16	27.961	303.2504578	$C_{20}H_{33}NO$
Amine	Trihexyphenidyl	301.5	21.327	301.244	$C_{20}H_{31}NO$
	Cholest-4-en-3-one	348.6	26.553	385.37	$C_{27}H_{44}O$
	3beta,5alpha,6beta-Cholestanetriol	420.7	29.064	420.39	$C_{27}H_{48}O_3$
Steroid	Kurchessine	372.6	25.976	372.38	$C_{25}H_{44}N_2$
	Ergosterol	396.6	28.861	397.37	$C_{28}H_{44}O$
	Tomatidine	415.7	25.060	415.32	$C_{27}H_{45}NO_2$

Table 3: Druglikeness analysis of drug candidates. 'Yes', indicate zero violation

Rule	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Veber	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Egan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Muegge	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.6	0.55	0.55	0.55	0.55	0.6	0.55	0.55	0.6	0.55	0.55	0.11	0.55	0.55	0.55	0.55

network analysis. The resulting network comprises nodes and 474 edges with an enrichment p-value < 1.0e-16. The network was then imported into Cytoscape software and analyzed by determining centrality and other metrics. The Cytohubba plug-in revealed the top ten genes ranked by degree,and the top core gene target for Bicuculline of the methanolic extract was confirmed to be EGFR. Bicuculline was selected and assessed for its inhibitory potential of EGFR by utilizing the molecular

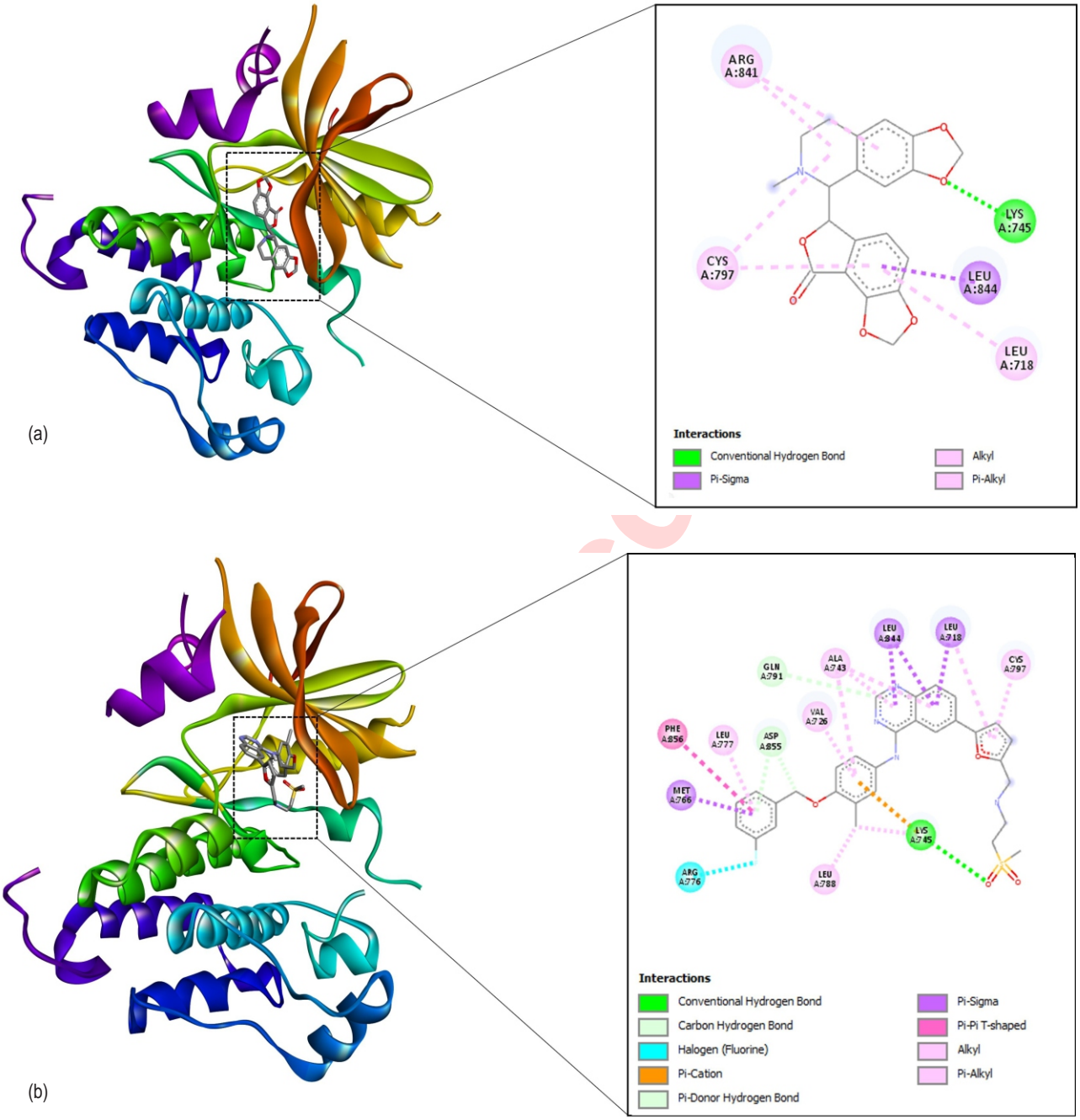

Fig. 5: 3D structure of EGFR showing binding site and the main residues involved with ligands (a) Bicuculline and (b) standard Lapatinib.

docking technique. The AutoDockVina tool was employed to execute molecular docking to evaluate the potential interaction between Bicuculline (CID 10237) and standard Lapatinib (CID 208908), and the primary target EGFR (PDB ID 1XKK). The result shows that Bicuculline can bind strongly with the active site of EGFR with a binding affinity of -9.3 kcal mol^{-1} by interacting with different amino acids in the active pocket of the receptor (Fig. 5a). The binding affinity of Bicuculline with the receptor was comparable with the standard Lapatinib which possessed a binding affinity of -11.2 kcal mol^{-1} (Fig. 5b). Nonetheless, the strong binding of Bicuculline suggests its strong inhibitory potential against EGFR. The EGFR (epidermal growth factor receptor) is a membrane-spanning protein containing an intracellular tyrosine kinase domain. Apart from regulating cellular

functions, including cell proliferation, differentiation, and survival, EGFR activation has been reported to be associated with the induction of oxidative stress (Mushtaq *et al.,* 2021). More recently, the development of other chronic diseases such as cardiomyopathy, insulin resistance, and diabetic nephropathy has also been linked to the EGFR pathway. EGFR inhibitors have been shown to alleviate angiotensin 2-induced renal damage by reducing inflammation and oxidative stress (Fang *et al.,* 2016). The molecular docking analysis revealed the high binding affinity of Bicuculline with EGFR, rendering it a promising option for the development of next-generation EGFR inhibitors.

According to the present study, the berries of *S. khasianum* extracts have potent antioxidant and antiradical properties. The high concentration of polyphenols and phytochemical components contributed to their higher antioxidant activity, as demonstrated by their dose-dependent inhibition of free radicals, and inhibition of lipid peroxidation and hemolysis. The result from the molecular docking study highlights the therapeutic potential of *S. khasianum* extract in targeting EGFR to combat oxidative stress-related diseases. Furthermore, our findings might identify future drug candidates that could assist researchers in developing target-specific therapeutic agents for controlling and treating diseases associated with oxidative stress.

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