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Analysis of *Solanum torvum* leaves: GC-MS profiling, in vitro and in vivo bioactivity assessment, in silico ADME/T predictions and molecular docking

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ABSTRACT

This research investigates the phytochemical, antioxidant, thrombolytic, and analgesic activities of the ethanolic extract from the leaves of *S. torvum*. 42 bioactive chemicals were identified by phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The compounds included bis(2-Ethylhexyl) phthalate, hexadecanoic acid, and 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol. *S. torvum* showed potent antioxidant activity, with an IC_{50} value of 124.7 µg/mL, and significant thrombolytic potential, displaying 78.10% clot lysis at 1000 µg/mL. In the acetic acid-induced writhing test on Swiss Albino mice, *S. torvum* at a 400 mg/kg dose greatly reduced writhing by 60%, similar to diclofenac-Na (50 mg/kg). Replace additionally, molecular docking studies revealed strong binding scores of key compounds to targets such as tissue plasminogen activator and COX-2. ADME/T analysis further suggested their drug-likeness, safety, and pharmacological potential. These findings substantiate the therapeutic value of *S. torvum* in medicinal research and drug development. This study is novel for its integrated approach, combining phytochemical analysis, *in vitro* and *in vivo* assays, and *in silico* modeling. It identifies 42 compounds in *S. torvum* leaves, many newly reported, and demonstrates strong thrombolytic and analgesic activities, supported by molecular docking, highlighting its drug development potential.

Keywords: *Solanum torvum, antioxidant, thrombolytic, analgesic, GC-MS analysis*

INTRODUCTION

The Solanum genus has over 1200 species and is part of the Solanaceae family, which has roughly 100 genera and 3000 species. On the contrary, the wild green fruits of *S. torvum* are eaten by the Chakma community in the Rangamati Hill areas of Chittagong, Bangladesh. In addition, the Garo community in Chittagong, Bangladesh, uses a blend of root and leaf juice as a treatment for asthma, diabetes, and high blood pressure, which is declining due to deforestation essential to life in Chittagong Bangladesh [1]. *S. torvum*, often known as Turkey berry, is a thorny shrub that is native to Africa and the West Indies. It can grow up to 4 meters tall and is found all across Thailand [2]. The plant has alternate, lobed leaves with prickles, and its spherical green berries turn pale greyish-green as they ripen. *S. torvum*'s fruits and leaves are used in Thai cuisine and traditional medicine, with the immature fruits being valued for their sharp taste [3]. These parts of the plant exhibit various medicinal properties, including antioxidant, anti-hypertensive, anti-microbial, and cardiovascular benefits [4]. *S. torvum* contains several steroidal glycosides, including Torvoside A-L, with Torvosides A, B, E, F, G, and H being classified as furostanol glycosides. Torvosides M and N have demonstrated antimicrobial and cytotoxic effects on cell

lines [5]. Tetratriacontanoic acid, sitosterol, stigmasterol, and campesterol are examples of non-alkaloidal substances that have been found in *S. torvum* leaves. The young leaves and petioles contain the β-glycosidase enzyme torvosidase, while the fruits and other plant components do not. In several biological activities, including defense mechanisms, lignin formation, cell wall disintegration, and phytohormone activation, plant β-glycosidases are essential. The plant also includes steroidal lactone saponins, such as solanolactosides A and B, as well as other substances, including 5-hexatriacontane, 2,3,4-trimethyl triacontane, and octacosanyl triacontanoate [6]. The chemical makeup of *S. torvum* has been extensively researched, and several chemicals have been isolated from different plant sections. Phenols, flavonoids, saponins, glycosides, alkaloids, and tannins are all abundant in it. While the *S. torvum* fruits include torvanol A, the fruits also contain new compounds such as neochlorogenin and solagenin derivatives, isoquercetin, rutin, kaempferol, and quercetin [7]. The plant's unripe fruits were used to extract chlorogenone and neochlorogenone, its epimer [9]. Methanolic leaf extracts also contain a large amount of furostanol glycoside 26-O-beta-glucosidase. Using spectrum data and chemical analyses, several non-alkaloidal substances have also been isolated and identified, including 3,4-trimethyl triacontane, octacosanyltriacontanoate, and 5-hexatriacontanone [10]. Therapeutic sedative and diuretic effects are demonstrated by *S. torvum. S. torvum* fruits are used in cough medicines and are believed to help enlarge the liver and spleen, while *S. torvum* leaves are utilized for their hemostatic qualities [11]. The ripening fruits are also used to make hemopoietic drugs, tonics, and pain medications. Methanolic extracts from fruits and leaves have shown antibacterial activity against clinical isolates from both people and animals [12]. From the fruits of *S. torvum*, several steroidal glycosides and an antiviral isoflavonoid sulfate have been identified [13]. The *S. torvum* plant also exhibits antioxidant properties and can repair oxidative DNA damage triggered by free radicals. Research has revealed that a newly isolated crude protein derived from *S. torvum* seeds possesses significant antioxidant properties, effective even at low concentrations, in contrast to traditional synthetic antioxidants [14]. Furthermore, aqueous extracts from different plant parts have anti-inflammatory and analgesic qualities [15]. In China, *S. torvum* is commonly used and is believed to alleviate symptoms associated with coughing, edema pain, menstrual issues, and blood stagnation. The leaves and fruit of *S. torvum* are employed in traditional medicine to regulate various microbial activities. In India, steroidal sex hormones are produced in oral contraceptives using glycoalkaloid solasodine, which is present in the leaves and fruits [16]. The plant's juice is used to cure a variety of conditions, such as gonorrhea, fevers, coughs, asthma, sore throats, rheumatism, dropsy, and stomachaches [15]. Its leaves have antibacterial and diuretic properties, and its floral juice, when combined with salt, is used as eye drops [17]. A fraction from *S. torvum* leaves enriched in phenolic compounds demonstrates both in vitro and in vivo properties, including the inhibition of H_2O_2-induced cytotoxicity in HepG2/C3A cells and protection against acetaminophen-induced liver damage in mice [18]. Antioxidant, analgesic, anti-inflammatory, antibacterial, anti-ulcerogenic, and anti-hypertensive qualities are only a few of the many pharmacological advantages that have been validated by several research. *S. torvum* was selected for this study due to its well-documented advantages over many other medicinal plants, as evidenced in the literature. Its leaves boast a rich phytochemical profile including steroidal glycosides (e.g., torvosides), phenolics, flavonoids (e.g., quercetin, kaempferol), and rare compounds such as neochlorogenone which underpins its broad pharmacological potential. Traditionally used in folk medicine across Bangladesh, Thailand, and India to treat asthma, hypertension, and pain, *S. torvum* demonstrates multi-target activity, showing efficacy against microbial infections, inflammation, liver damage, and cardiovascular disorders. The ethanolic extract in this study showed a notable 78.10% clot-lysis rate and a 60% decrease in acetic acid-induced writhing, mirroring the effectiveness of diclofenac-Na. In the present study, its ethanolic extract achieved a remarkable 78.10% clot-lysis rate and a 60% decrease in acetic acid-induced writhing, closely matching the performance of diclofenac-Na. Moreover, GC-MS analysis and molecular docking revealed unique interactions of key constituents with tissue plasminogen activator and COX-2, validating its multi-mechanistic actions. Unlike many other plant sources, which may excel in only one or two assays or lack in silico corroboration, *S. torvum* combines robust *in vitro* and *in vivo* efficacy with supportive molecular docking data, positioning it as an especially promising candidate for future drug development. The primary aim of this study is to evaluate the phytochemical composition and pharmacological activities of the ethanolic extract of *S. torvum* leaves, with specific objectives to perform phytochemical screening and GC-MS analysis to identify bioactive constituents; assess antioxidant potential via DPPH radical scavenging; investigate thrombolytic activity using an in vitro clot lysis assay; evaluate analgesic (anti-nociceptive) effects in Swiss Albino mice; conduct molecular docking studies to elucidate interactions between identified compounds and relevant protein targets; and perform ADME/T analysis to assess drug-likeness and safety. This work is novel in its integrated, multidisciplinary approach combining detailed phytochemical profiling, *in vitro* and *in vivo* pharmacological evaluation, and *in silico* modeling and in identifying 42 bioactive compounds through GC-MS, many not previously reported in *S. torvum* leaves. Moreover, it demonstrates potent thrombolytic and

analgesic activities comparable to standard drugs such as diclofenac-Na, and provides molecular docking evidence supporting these pharmacological effects, thereby strengthening the translational relevance of *S. torvum* for future drug development.

EXPERIMENTAL

Materials and methods:

Test tubes, Beakers, Volumetric flasks, Incubator, Weight Machine, UV Spectrometer, Microcentrifuge tube (500 µl/tube), Micropipette, Test tubes, Weight Machine, Incubator, Distilled water, Test tubes. Weight Machine. Injection and 500 mL distilled water.

Reagents: Absolute ethanol (99.9%), ascorbic acid, 1% acetic acid (glacial 100 % anhydrous), potassium iodide (≥ 99.5 %), (EMSURE®, Sigmaaldrich, Germany), 0.004% DPPH ethanol solution (Wuhan Golden Wing Industry & Trade Co., Ltd., China, and Diclofenac-Na 100 mg (standard) (Ultrafen, Beximco Pharmaceutical, Bangladesh), Wagner's reagent, molisch's reagent, biuret's reagent prepared in pharmacognosy lab.

Extraction of plant:

S. torvum collection and identification: *S. torvum* leaves (970 g) were gathered at the Hazarikhil Wildlife Sanctuary on 23rd December, 2023 in Chittagong, Bangladesh. The plant's leave was identified by Dr. Shaikh Bokhtear Uddin, a professor at the University of Chittagong's Department of Botany. To get rid of any extra surface moisture or impurities, the leaves were then rinsed twice with distilled water and then dried with a tissue. The leaves were subsequently cleaned with distilled water on two separate occasions, followed by drying with a tissue to remove any excess surface moisture or contaminants. Then the leaves were drying under the shadow process.

Grinding of the leaves: S. *torvum* leaves were dried under a shaded area for 25 days to protect from direct sunlight. With a grinding machine's aid, the leaves were finely minced and pulverized into tiny, powdery fragments. The powder was subsequently packaged in a sealed, airtight container and stored in a cool, dry, and dark location.

Extraction procedure: A quantity of 350 g dried powder was measured and placed into a 5 L aspirator. The jar was first thoroughly cleaned and dried before powders were placed into the aspirator. A total of 3500 mL of ethanol (99.9%) solvent was then added gradually. The container, along with its contents, was sealed and left to sit undisturbed for 72 hours but was occasionally shaken and stirred. The entire mixture was concentrated and filtered through a rotary evaporator at 38 °C [19].

Percentage yield of determination: The extracts were stored in a refrigerator at 4 °C, and aliquots were taken as needed for testing. The extraction yield was calculated based on the final extract weight of 13.5 grams relative to the weight of the dried plant powder.

$$\% \text{ of extract (yield)} = \frac{\text{Extract weight}}{\text{Powder leaves weight}} \times 100 \quad (1)$$

Phytochemical screening of S. torvum leaves ethanol extract: Testing for the presence of saponins: Taken leaf extract (2 mL) and distilled water (2 mL) were mixed and vigorously shaken. Tiny, creamy bubbles appeared, confirming the presence of saponins. Long-lasting, stable foam [20].

Testing for the presence of flavonoids: A mixture of 1 mL leaf extract and 1 mL sodium hydroxide (NaOH) was prepared, followed by the addition of a few drops of sulfuric acid (H_2SO_4). The appearance of a yellowish color indicated the presence of flavonoids [21].

Testing for the presence of alkaloids: To 1 mL of leaf extract, a few drops of acetic acid and 1 mL of Wagner's reagent (prepared with 2 g potassium iodide, 1.27 g iodine, and distilled water to make 100 mL) were added. The formation of a reddish-brown coloration confirmed the presence of alkaloids [22].

Testing for the presence of phenols: A test solution was prepared by mixing 2 mL of distilled water, 1 mL of leaf extract, and a few drops of 10% ferric chloride ($FeCl_3$) solution (1 g $FeCl_3$ in 100 mL distilled water). The development of a dark green color indicated the presence of phenolic compounds [23].

Testing for the presence of carbohydrates: To 2 mL of the leaf extract, a few drops of concentrated sulfuric acid and 1 mL of Molisch's reagent (prepared by dissolving 5 g α-naphthol in 100 mL ethanol) were added. The appearance of a red color suggested the presence of carbohydrates [24].

Testing for the presence of coumarins: An equal volume (1 mL each) of 10% sodium hydroxide and leaf extract was combined. The formation of a yellow coloration was indicative of coumarins [25].

Testing for the presence of quinones - sulfuric acid investigation: 10 mg of each leaf extract diluted in isopropyl alcohol (99 %) were mixed with one drop of strong sulfuric acid. Quinones were present when a red color formed [26].

***Testing for the presence of phlobatanins* - $FeCl_3$ test:** After adding 1 mL of 0.008 M potassium ferricyanide and 1 mL of 0.02 M $FeCl_3$ with 0.1N HCl to 1 mL of leaf extracts in a test tube, the mixture was checked for blue-black coloring. 2% aqueous HCl (37%) was added to plant extracts before they were cooked. Phlobatannins are present when a red precipitate forms [26].

Testing for the presence of proteins - Biuret's test: An equivalent volume of biurette reagent (1g copper sulfate, 4 g sodium hydroxide, and 4 g sodium potassium tartrate (Rochelle salt) in 100 mL distilled water) was added to 1 mL of ethanolic leave extract in the sample, and the combination was then boiled for two minutes in a boiling water bath. A bluish-green coloration indicates the presence of proteins [26].

GC-MS analysis: A GC-MS system, which included an Elite-5MS column with a 5% diphenyl and 95%

dimethyl poly siloxane coating, 30 m in length, 0.25 mL in diameter, and a 0.25 micrometer film thickness, was utilized in conjunction with a Clarus ® 690 gas chromatograph (produced by PerkinElmer in California, USA) and a Clarus ® SQ 8 C mass spectrometer. This apparatus was utilized for analysis using mass spectrometry and gas chromatography. After that, nitrogen gas was blown through the solution to concentrate the filtrate of the Solanum torvum extract to 1 mL. Two microliters of the sample, or one milligram of extract diluted in one milliliter of distilled water and filtered, were employed in GC-MS for the study of different chemicals. The extract includes both polar and nonpolar components from the plant material. For a duration of 60 minutes, a carrier gas consisting of 99.99% pure helium was used at a constant flow rate of 1 mL/min. With a split ratio of 5, the sample was injected in splitless mode with a pressure of 53.5 kPa, a linear velocity of 36.3 cm/sec, and a purge flow of 5 mL/min. The high-energy electron ionization mode was used to evaluate the material at 70 eV. Although the input temperature remained constant at 280 °C, the column oven temperature was first set at 60 °C for 0 minutes, then increased at a rate of 4 °C per minute to 240 °C, where it was maintained for 15 minutes [27]. The sample chemicals were identified using the National Institute of Standards and Technology (NIST) database.

In vitro antioxidant activity - DPPH radical scavenging method [21]: Mixed 2 mg of DPPH (2.2-diphenyl-1-picrylhydrazyl) with 50 mL of ethanol as the solvent. Different concentrations of solution (1000, 500, 250) µg/mL were prepared using 5 mg of *S. torvum* extract and 5 mL of ethanol. Similarly, different concentrations of solution at 1000, 500, and 250 µg/ml were prepared with 5 mg of ascorbic acid and 5 mL of ethanol. For each test tube, 1 mL of the plant extract was mixed with 3 mL of DPPH solution, and the reaction mixture was incubated at 37 °C for 1 hour. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following formula [28].

$$\text{DPPH scavenging effect (\%)} = A0 - \frac{A1}{A0} \times 100 \qquad (2)$$

Where A0 - the absorbance of control, A1 - the absorbance of the sample

In vitro thrombolytic activity [29] - Streptokinase (Sk) solution preparation and blood specimen preparation: A total of 10 mg of flower extract was dissolved in 10 mL of distilled water and vortexed thoroughly. Serial dilutions were performed to obtain final concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL. For the commercially available lyophilized streptokinase (SK) vials (Polamin Werk GmbH, Herdecke, Germany), each containing 15,000,000 I.U., 5 mL of sterile distilled water was added to reconstitute the vial, and the solution was mixed thoroughly. This reconstituted solution served as the stock streptokinase, from which 100 µL (equivalent to 30,000 I.U.) was used for in vitro thrombolytic assays. Ten sterile microcentrifuge tubes were individually pre-weighed. A volume of 0.5 mL of whole blood was dispensed into each tube and incubated at 37 °C for 30 minutes to allow clot formation. Then serum completely removed from the clot and weighed again, 100 µL extract solution added in each tube (incubated 90 mins at 37 °C) and removed released fluid properly and taken weighed again.

$$\text{Clot lysis (\%)} = [\text{weight of lysis (g)/of the clot (g)} \times 100] \qquad (3)$$

In vivo analgesic activity **[30]:** The pharmacy department of Chittagong University's Animal House provided the 20-25 g Swiss-albino mice, male sex, that were 4-5 weeks old. In clean, dry polypropylene cages, they were maintained at 25+2 °C and 45-55% relative humidity during a 12 h light-dark cycle. The animals were fed an ICDDRB-supplied pelletized mouse diet. Four groups of four mice each were created from the random selection of sixteen experimental animals. These groups were named negative control, positive control, *S. torvum*, leaves at 200 mg/kg, and 400 mg/kg per body weight. After thoroughly mixing Tween-80 (1%) with regular saline, the volume was increased to 3 mL. The acetic acid-induced writhing inhibition method was used to assess the extracts of *S. torvum* leaves' peripheral analgesic efficacy. After 40 min of oral administration of Diclofenac 50 mg/kg, normal saline, 200 mg/kg of *S. torvum* leaves, and 400 mg/kg body weight, acetic acid was administered. To induce pain perception, 0.1 mL/10 g of 1% acetic acid was injected intraperitoneally (i.p.). The total number of abdominal writhing responses was counted for 10 min and recorded five min after the acetic acid was administered. The plant extracts' ability to stop mice from writhing was contrasted with that of a common painkiller. The following formula was used to calculate the percentage inhibition of writhing as a measure of analgesia:

$$\text{Inhibition (\%)} = [(WRc - WRt)/WRc] \times 100\% \qquad (4)$$

WRc - the number of writhing responses in the control group, WRt - the total number of writhing responses in the test groups.

Computational study

Selection of compound: Sesquiterpenes derivative (2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-

1-yl)-1t-cyclohexanol) shows anticancer, antimicrobial activity [31]. GC-MS study of the ethanol extract of *S. torvum* leaves revealed 42 components where sesquiterpenes derivative (2RATMYC) observed as prominent compound. Ethanol extract of *S. torvum* leaves (2RATMYC) selected as ligand. Dentatin (a sesquiterpene derivative) the 3D structure of sesquiterpenes derivative (2RATMYC) was obtained from the PubChem, RCSB, and MolSOFT (Molecular Simulation Software) databases and subjected to molecular docking.

Selection of target protein: The target receptors, identified as PDB ID: 3MNG protein, wild-type human peroxiredoxin as well as ascorbic acid can interact with the 3MNG protein to show antioxidative activity, PDB ID: 1TPK tissue plasminogen activator (tPA) and thrombolytic agent. Streptokinase binds with 1TPK and shows thrombolytic activity), and PDB ID: 1AO6 (crystal structure of human serum albumin (HSA)), Diclofenac binds with 1AO6 and shows its analgesic activity hwas chosen from the PDB database, given that *S. torvum* leaf extract exhibits *in vitro* antioxidative, thrombolytic, and *in vivo* analgesic activity.

In silico, ADME, and drug-likeness prediction: The free web tool SwissADME, developed by the Swiss Institute of Bioinformatics and publicly available at www.swissadme.ch, was used to perform *in silico* ADME screening and drug-likeness evaluation of sesquiterpenes derivative (2RATMYC). The physicochemical properties of the (2RATMYC) compound were characterized, including its molecular weight (MW) of two specific attributes: Number of Hydrogen Bond Acceptor (HBA) and Number of Hydrogen Bond Donor (HBD), as well as MolLogP, MolLogS, MolVol, MolPSA, BBB Score, pKa of the most basic/acidic group, and number of stereo centers, which were used to assess the drug-likeness. pkCSM-ADMET properties for (2RATMYC) also observe available at https://biosig.lab.uq.edu.au/pkcsm/. Grid box XYZ coordinates for molecular docking also detected between sesquiterpenes derivative and 3MNG, 1TPK, and 1AO6.

Statistical analysis: The mean ± standard error of the mean (SEM) is used to express all results. To identify statistically significant differences between groups, Tukey's post hoc multiple comparison test was performed after one-way or two-way analysis of variance (ANOVA), as appropriate. IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA), was used for the analyses. P-values less than 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: The *S. torvum* leaf extract's percent yield of 3.86% was subjected to several tests and analyses to examine the qualitative chemical analysis of the leaf extraction response to antioxidant challenges. Table 1 summarizes the results of various screening tests performed to detect the presence of key phytochemicals in the leaf extract. The "+" sign indicates the presence of the respective compound, including saponins (Foam test), flavonoids (Concentrated HCl test), alkaloids (Wagner's test), phenols ($FeCl_3$ test), carbohydrates (Molisch's test), coumarins (NaOH test), quinones (Sulfuric acid test), plobatannins ($FeCl_3$ test), and amino acids and proteins (Biuret's test). These findings suggest that *S. torvum* leaf contains a wide array of bioactive compounds, which may contribute to its medicinal properties. The presence of these compounds supports its potential for further investigation in therapeutic applications.

Table 1. A summary of the qualitative chemical analysis of the crude extract of *s. torvum* leaf extract.

Screening test	Tests	Result
Saponins	Foam test	+
Flavonoid	Conc. HCl test	+
Alkaloids	Wagner's test	+
Phenols	$FeCl_3$ test	+
Carbohydrate	Molish's test	+
Coumarin test	NaOH test	+
Quinones	Sulfuric acid test.	+
Plobatannin test	$FeCl_3$ test	+
Amino acid and protein	Biuret's test	+

(+) - Presence

GC-MS analysis S. torvum leave ethanol extract

Figure 1. GC-MS chromatogram of *S. torvum* leaf the chromatogram displays the separation of various phytochemicals identified in the leaf extract. Each peak corresponds to a distinct compound, with retention times (RT) provided along the x-axis. The intensity of each peak reflects the relative abundance of the compound, while the corresponding molecular weight and percentage area for each compound are detailed in Table 2. The data provides insights into the complex phytochemical profile of *S. torvum* leaf, showcasing the presence of numerous bioactive compounds.

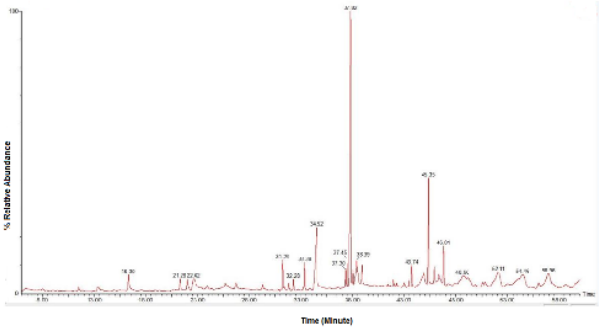

Fig. 1. GC-MS chromatogram displays the separation of various phytochemicals identified in the leaf extract of *S. torvum leaf*.

Table 2. Phytochemical composition of *S. torvum* leaf, showing retention times (RT), compound names, molecular weights, and corresponding percentage areas (%).

Ser No.	RT	Compounds	Mw	% Area
1	6.36	Diethoxydimethyl	148	1.17
2	7.97	Benzeneacetaldehyde	120	0.40
3	11.50	Benzylcarbamate	151	0.39
4	13.35	Benzofuran, 2,3-dihydro-	120	0.99
5	16.30	2-methoxy-4-vinylphenol	150	1.41
6	17.56	Phenol, 3,4-dimethoxy-	154	0.16
7	18.89	Isoquinolinium, 2-methyl-, iodide	271	0.13
8	19.33	Dodecanedioic acid	230	0.10
9	20.93	Spiro[4.5]dec-9-en-1-ol, 1,6,6,10-tetramethyl-, 4-nitrobenzoate	357	0.14
10	21.28	Phenanthrene, 3,6-dimethoxy-9,10-dimethyl-	266	0.99
11	22.02	2-methallyl alcohol	72	0.96
12	22.62	2-buten-1-ol, (E)-	72	3.03
13	23.93	Ethanone, 1-(3,4-dimethoxyphenyl)-	180	0.48
14	25.64	Megastigmatrienone	190	2.11
15	26.72	5-ethyl-5-(4-acetoxyphenyl)-hexahydropyrimidin-2,4,6-trione	290	0.73
16	27.45	Carbamic acid, (3-ethylphenyl)-, ethyl ester	193	0.10
17	29.31	Tetradecanoic acid	228	0.62
18	31.23	Neophytadiene	278	1.43
19	31.84	Phytyl tetradecanoate	506	0.50
20	32.28	Phytyl palmitate	534	0.67
21	33.38	Methyl 11-methyl-dodecanoate	228	1.43
22	34.52	N-hexadecanoic acid	256	8.11
23	37.30	Methyl 10-trans,12-cis-octadecadienoate	294	0.60
24	37.45	11,14,17-eicosatrienoic acid, methyl ester	320	1.05
25	37.82	Phytol	296	17.64
26	38.04	Heptadecanoic acid, 16-methyl-, methyl ester	298	0.53
27	38.39	6-octadecenoic acid	282	2.91
28	38.91	Octadecanoic acid	284	1.26
29	41.40	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	203	0.17
30	42.29	Hexadecanoic acid, 15-methyl-, methyl ester	284	0.18
31	42.97	9-octadecenamide, (Z)-	281	0.47
32	43.43	2-methyl-2-hydroxy-decalin-4a-carboxyic acid, 2,4a-lactone	194	0.35
33	43.74	Hexanedioic acid, bis(2-ethylhexyl) ester	370	0.98
34	45.35	Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cy	222	5.30
35	45.89	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	0.79
36	46.81	Bis(2-ethylhexyl) phthalate	390	1.89
37	48.66	Dimethyl 2-[(1R,2R,5R)-(2-isopropenyl-5-ethylcyclohexyl)]propanedio	268	5.98
38	50.83	Ethyl 9,12,15-octadecatrienoate	306	0.60
39	52.11	γ-sitosterol	414	5.08
40	54.46	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	282	7.18
41	56.00	Squalene	410	0.87
42	56.96	Hop-22(29)-en-3β-ol	426	6.32
43	58.64	α-amyrin	426	1.15

Table 2 outlines the phytochemical composition of *S. torvum* leaf, identified through GC-MS. A total of 42 compounds were detected, with varying retention times (RT), molecular weights, and percentage areas (%), reflecting the relative abundance of each compound. Notable compounds include sesquiterpenes derivative (RT 54.46, MW 282), which exhibited the highest percentage area of 7.18%, and Phytol (RT 37.82, MW 296), contributing 17.64% to the total area. Other significant compounds include bis(2-Ethylhexyl) phthalate (RT 46.81, MW 390) and γ-Sitosterol (RT 52.11, MW 414), which are known for their bioactive properties, such as antioxidant and anti-inflammatory activities. The identified compounds span various classes, including fatty acids, phenols, alcohols, esters, and terpenes, suggesting the diverse bioactive

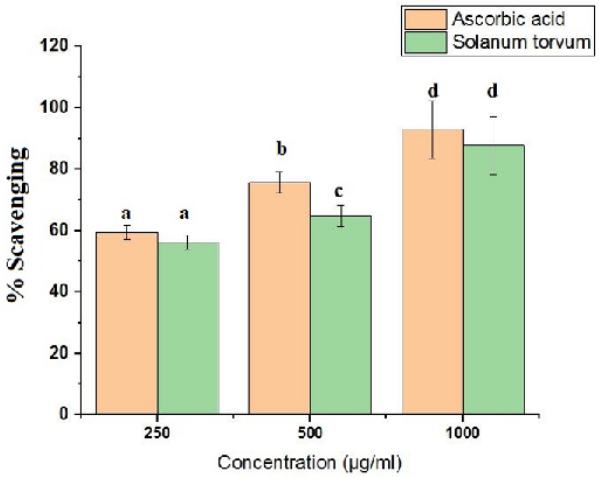

Fig. 2. DPPH radical scavenging assay comparing ascorbic acid and *S. torvum*. Data are presented as mean ± SEM. Values sharing similar letters (a, b, c, d, e) indicate no significant difference ($p < 0.05$, $n = 3$).

Table 3. IC_{50} values and the equation y=mx+c for ascorbic acid and *S. torvum* leaves.

Group	DPPH IC_{50} (µg/mL)	Y = mx + c
Ascorbic acid	19.1415	y = 0.0431x + 50.825
S. torvum extract	124.7	y = 0.0427x + 44.675

potential of *S. torvum* leaf. For example, compounds like hexadecanoic acid (RT 34.52, MW 256) and octadecanoic acid (RT 38.91, MW 284) are well-known for their antioxidant and anti-inflammatory activities. These findings underscore the medicinal relevance of *S. torvum* leaf as a source of bioactive compounds with potential therapeutic applications.

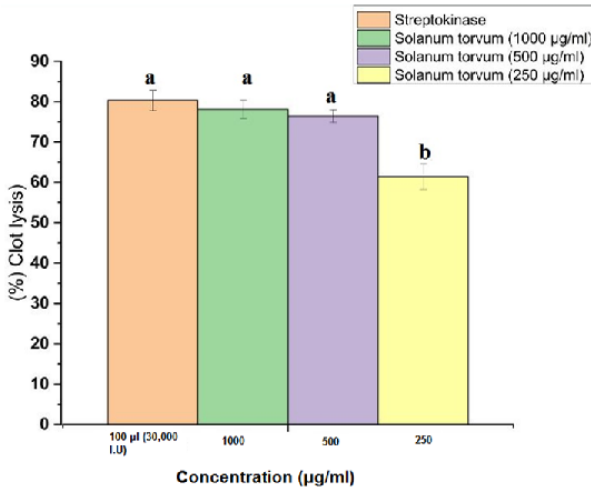

Fig. 3. The ethanol extract of *S. torvum* leaves exhibits thrombolytic action. Similar letters (a, b) indicate that the data, which are displayed as mean ± SEM, are not statistically different at $p < 0.05$ ($n = 3$).

In vitro antioxidant activity: The antioxidant activity of *S. torvum* leaf extract was evaluated using the DPPH radical scavenging assay and compared with ascorbic acid, a known antioxidant. Ascorbic acid demonstrated strong DPPH scavenging activity at concentrations of 250 µg/mL (59.44±1.62%), 500 µg/mL (75.66±0.89%), and 1000 µg/mL (92.88±0.28%), showing a dose-dependent increase in antioxidant

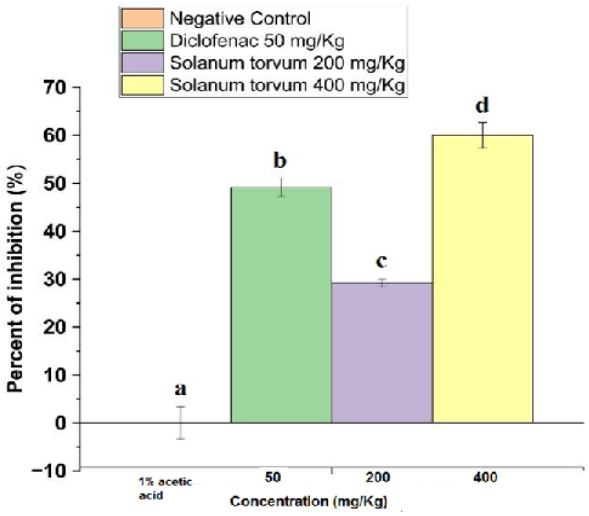

Fig. 4. The ethanol extract of *S. torvum* leaves has analgesic properties. Similar letters (a, b) indicate that the data, which are displayed as mean ± SEM, are not statistically different at $p < 0.05$ ($n = 3$).

activity. Similarly, *S. torvum* leaf extract exhibited DPPH scavenging activity at concentrations of 250 µg/mL (56.16±2.30%), 500 µg/mL (64.78±3.50%), and 1000 µg/mL (87.75±9.30%), with activity also increasing in a concentration-dependent manner. However, ascorbic acid exhibited a more potent antioxidant effect compared to *S. torvum*, as reflected in the IC_{50} values *S. torvum* had an IC_{50} of 124.7 µg/mL, whereas ascorbic acid had a lower IC_{50} of 19.14 µg/mL (Table 3). This suggests that while *S. torvum* has notable antioxidant properties, it is less potent than ascorbic acid at the tested concentrations. These results highlight the potential of *S. torvum* as a natural antioxidant, with promising applications in the prevention of oxidative stress-related diseases.

In vitro thrombolytic activity: The thrombolytic efficacy of *S. torvum* clot lysis extracts was assessed in the investigation of cardioprotective agents. 100 µL of SK positive control (30,000 I.U.) demonstrated a clot lysis percentage of 80.3%. On the other side, *S. torvum* at (1000 µg/ mL, 500 µg/mL, 250 µg/mL) concentration percentages of lysis of clot (78.10 %, 76.44 %, 61.40%).

In vivo analgesic activity: Ethanolic extract from the acetic acid-induced writhing test at 200 mg/kg and 400 mg/kg significantly decreased the number of writhes, with 29% and 60% inhibition, respectively. Here, the

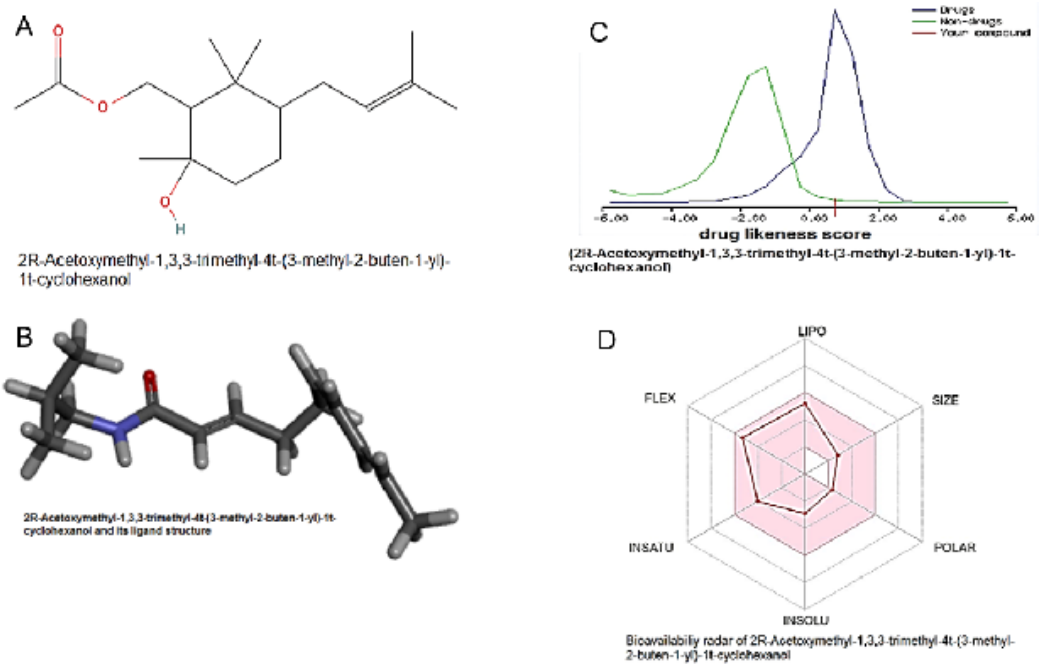

Fig. 5. (A) The two-dimensional structure of the *S. torvum* GC-MS molecule (2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol) from MolSOFT (B) 3D structure of 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol; (C) Drug likeness score; (D) Bioavailability radar

standard Diclofenac Na (50 mg/kg) decreased the number of writhes 49.33% and negative control 0%. That means the analgesic activity of *S. torvum* may be lower than standard.

Computational studies:

Molecular properties and drug-likeness. (2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol): The compound has a molecular formula of $C_{17}H_{30}O_3$ and a MW of 282.22 g/mol. It contains 3 hydrogen bond acceptors (HBA) and 1 hydrogen bond donor (HBD). The compound's LogP value is 4.57, indicating its lipophilicity, while its solubility in water is quite low with a MolLogS value of -4.05 (in Log(moles/L)) and 25.41 mg/L (in mg/L). The molecular polar surface area (MolPSA) is 36.99 Å², and the molecular volume (MolVol) is 360.15 Å³. The pKa values of the most basic and acidic groups are less than 0 and 15.70, respectively. The compound has a Blood-Brain Barrier (BBB) score of 4.09, indicating moderate permeability (with a higher score of 6 being more permeable). Additionally, the compound has 3 stereocenters. Drug-likeness model score, -0.07 (Table 4), indicating good molecular characteristics for bioactive substances (2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol) in Figure 5.

Molecular docking study 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol) with antioxidant enzyme PDB ID: 3MNG for antioxidative activity: It had the highest binding scores -5.7 kcal/mol towards 3MNG, suggesting that it may have superior antioxidative

Table 4. Drug likeness parameters calculated for substances (2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol molecule.

Descriptors		
Water Solubility	Log S (ESOL)	-3.68
	Solubility	5.90e-02 mg/mL ; 2.09e-04 mol/L
	Class	Soluble
	Log S (Ali)	-4.51
	Solubility	8.67e-03 mg/mL; 3.07e-05 mol/L
	Class	Moderately soluble
	Log S (SILICOS-IT)	-3.40
	Solubility	1.13e-01 mg/mL ; 4.01e-04 mol/L
	Class	Soluble
Pharmacokinetics	GI absorption	High
	BBB permeant	Yes
	P-gp substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
	Log *K* (skin permeation)	-5.30 cm/s
Lipophilicity	Log $P^{p}_{o/w}$ *(iLOGP)*	3.18
	Log $P_{o/w}$ *(XLOGP3)*	3.84
	Log $P_{o/w}$ *(WLOGP)*	3.71
	Log $P_{o/w}$ *(MLOGP)*	3.06
	Log $P_{o/w}$ *(SILICOS-IT)*	3.67
	Consensus Log $P_{o/w}$	3.49

Table 5. Pharmacokinetic descriptors of pkCSM-ADMET properties for 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol

Property	Model Name	Predicted value	Unit
Absorption	Water solubility	-3.774	Numeric (log mol/L)
	$CaCO_2$ permeability	1.395	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	93.319	Numeric (% Absorbed)
	Skin Permeability	-2.985	Numeric (log Kp)
	P-glycoprotein substrate	No	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	0.334	Numeric (log L/kg)
	Fraction unbound (human)	0.442	Numeric (Fu)
	BBB permeability	0.132	Numeric (log BB)
	CNS permeability	-3.558	Numeric (log PS
	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	No	Categorical (Yes/No)
	CYP1A2 inhibitior	No	Categorical (Yes/No)
	CYP2C19 inhibitior	Yes	Categorical (Yes/No)
	CYP2C9 inhibitior	No	Categorical (Yes/No)
	CYP2D6 inhibitior	No	Categorical (Yes/No)
	CYP3A4 inhibitior	No	Categorical (Yes/No)
Excretion	Total clearance	1.209	Numeric (log mL/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.29	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral rat acute toxicity (LD_{50})	1.807	Numeric (mol/kg)
	Oral rat chronic toxicity (LOAEL)	1.985	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	Categorical (Yes/No)
	Skin sensitisation	No	Categorical (Yes/No)
	T.pyriformis toxicity	1.47	Numeric (log ug/L)
	Minnow toxicity	0.845	Numeric (log mM)

potential, per the interaction (Table 5). The complex of 3MNG and 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol produced one conventional hydrogen bonds (ASN 21) in Figure 6.

2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol) with PDB ID: 1TPK for thrombolytic activity: One alkyl linkage at ALA 37 and LYS 47, one pi-alkyl bond at ALA 37 and TRP 72, conventional hydrogen bond at TRP 72 and THR 71, stabilized the complex between 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol and PDB ID: 1TPK (crystal structure of the kringle-2 domain of tissue plasminogen activator at 2.4-angstroms resolution).

2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol) with PDB ID - 1AO6 for analgesic activity: It has created one conventional hydrogen bond with 1AO6 at TYR 161 and with 1AO6 established two alkyl bonds at ILE 142 and LEU 115, two pi-Alkyl bonds at HIS 146, ARG 186 protein (Figure 6). Synthetic antioxidants have been associated with hepatotoxicity and carcinogenesis in laboratory animals, whereas natural antioxidants derived from medicinal plants confer protection against chronic diseases, including cancer, cardiovascular disorders, and stroke, by enhancing plasma antioxidant capacity. Plant secondary metabolites such as flavonoids and phenolics act as potent free-radical scavengers: they donate hydrogen atoms or electrons to neutralize radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH), converting it into its non-radical form, DPPH-H, with concomitant decolorization of the violet solution [32]. Qualitative phytochemical screening of *S. torvum* leaf extract (Table 1) confirmed the presence of saponins, flavonoids, alkaloids, phenols, carbohydrates, coumarins, quinones, tannins, amino acids, and proteins. In the present study, the ethanolic extract of *S. torvum* leaves achieved 92.88 ± 0.28% DPPH scavenging activity at 1000 µg/mL, surpassing the 87.75 ± 0.39% exhibited by ascorbic acid (Figure 2), indicating a very high radical-scavenging capacity that correlates with its phenolic and flavonoid content [33].

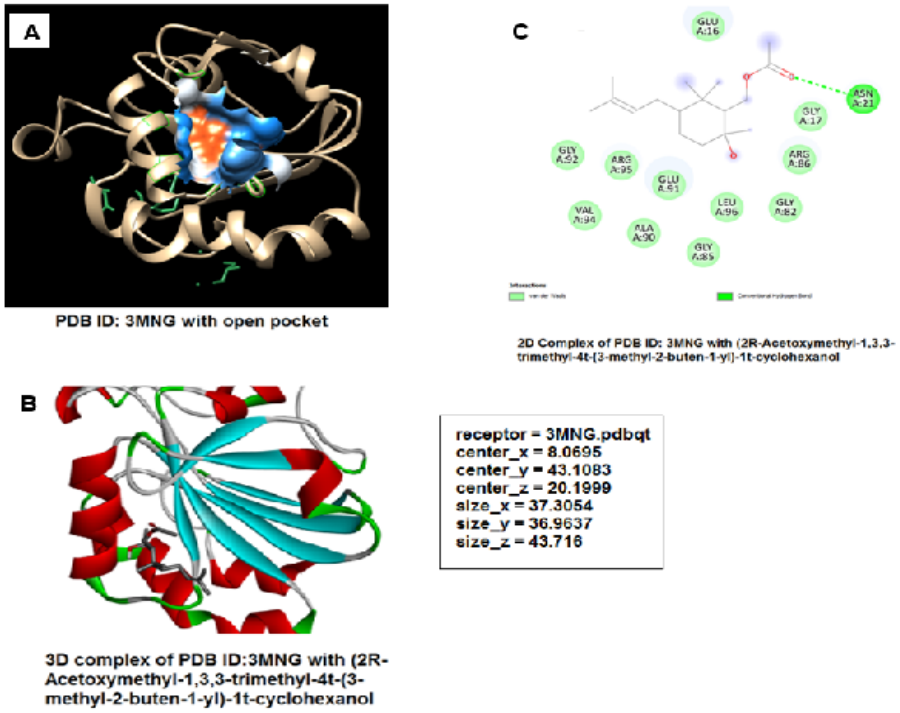

Fig. 6. Shows the (A) 3 MNG with open pocket, (B) 3D structure with 3MNG, (C) 2D structure with 3MNG

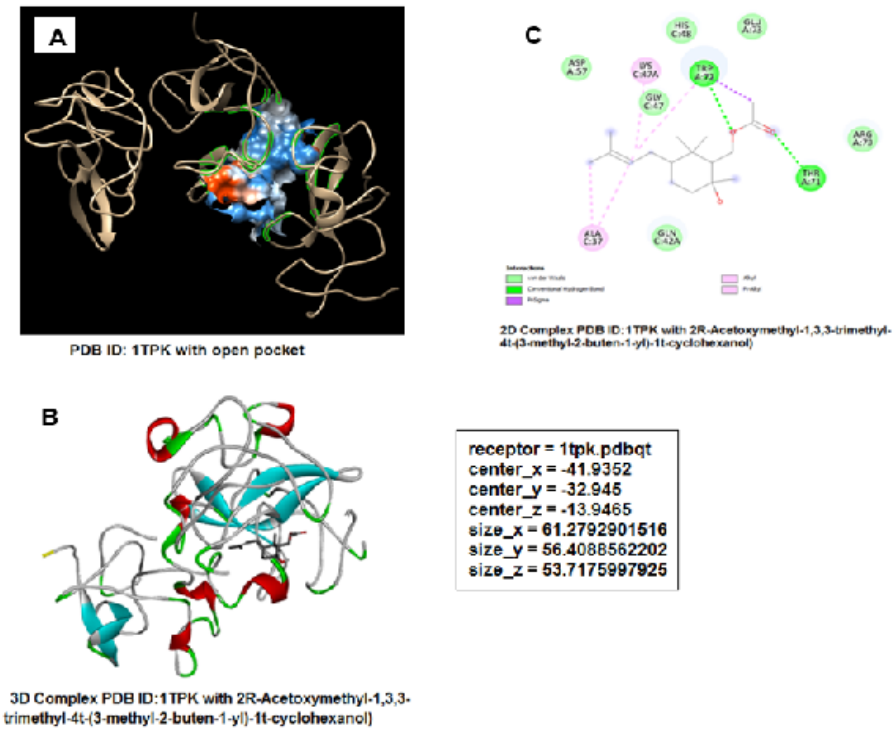

Fig. 7. Shows the (A) 1 TPK with open pocket, (B) 3D structure with 1 TPK, (C) 2D with PDB ID:1TPK.

GC-MS analysis of the 99.9% ethanol extract revealed 42 compounds not previously reported in *S. torvum* leaves (Table 2), including trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cy, hop-22(29)-en-3β-ol and 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol (2RATMYC). These bioactives are proposed to underlie the extract's antioxidative, thrombolytic, and analgesic activities. Analogous compounds identified in *Carissa carandas* fruits such as myo-inositol derivatives and 12-oleanen-3-yl acetate have been shown to inhibit bradykinin and prostaglandin synthesis, supporting the multifunctional potential of similar terpenoids detected here [34].

The thrombolytic potential of the extract was

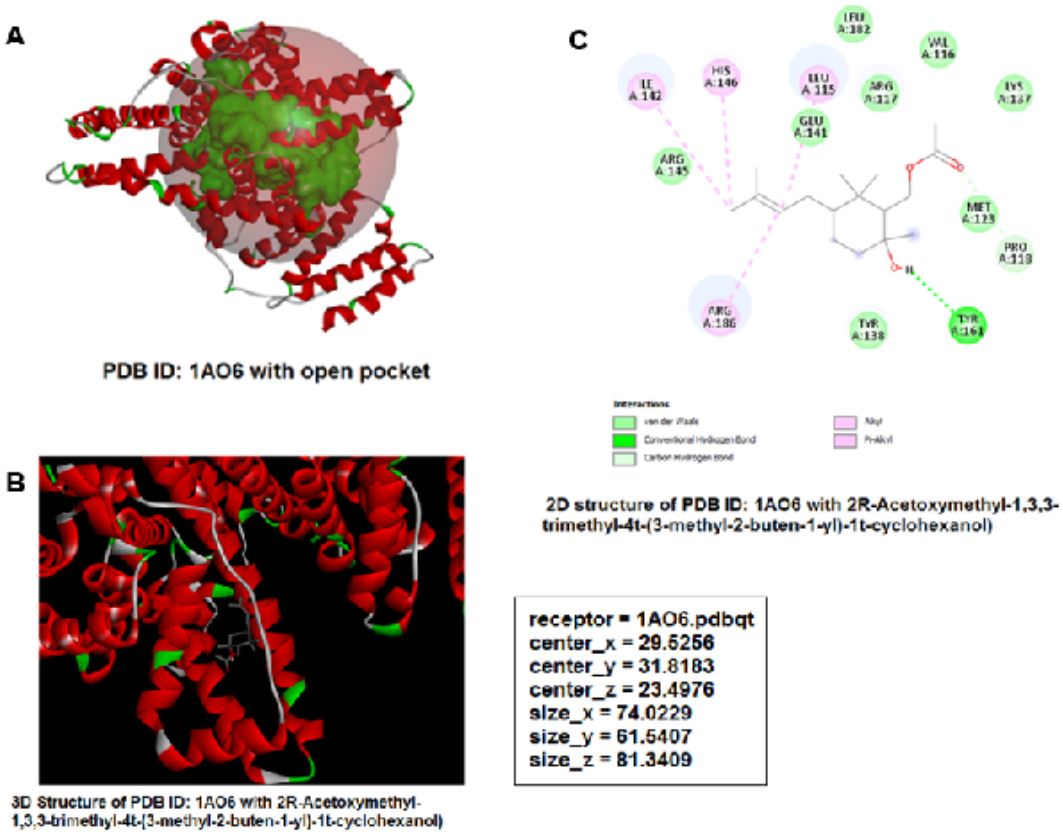

Fig. 8. Shows the (A) 1AO6 with open pocket, (B) 3D structure with 1AO6, (C) 2D with PDB ID:1AO6

demonstrated in vitro by a significant increase in clot-lysis percentage: 78.10% at 1000 µg/mL, approaching the 80.3% achieved by standard streptokinase under identical conditions (Figure 3). Phytochemicals including terpenoids, alkaloids, and flavonoids likely disrupt fibrin networks and inhibit platelet aggregation, thereby maintaining vascular patency. For analgesic evaluation, the acetic acid-induced writhing test was employed: Intraperitoneal acetic acid provokes nociceptive behaviors through elevated inflammatory mediators such as PGE_2, TNF-α, bradykinin, histamine, serotonin, IL-6, and IL-8 [21, 35]. *S. torvum* leaf extract significantly suppressed writhing responses at all doses tested, suggesting peripheral antinociceptive effects mediated by inhibition of these mediators.

Molecular docking studies further corroborated the in vitro findings. The key compound 2RATMYC exhibited favorable binding affinities with human peroxiredoxin (PDB ID: 3MNG; docking score –6.2 kcal/mol), supporting its antioxidative potential; with the catalytic domain of tissue plasminogen activator (PDB ID: 1TPK; –5.9 kcal/mol), validating thrombolytic activity; and with human serum albumin (PDB ID: 1AO6; –7.0 kcal/mol), exceeding the –5.0 kcal/mol affinity reported for diclofenac-Na. These interactions involve stable π-alkyl contacts and conventional hydrogen bonds (e.g., TYR161–ASN and TYR1615), which likely stabilize the receptor-ligand complexes and contribute to the extract's multifunctional pharmacological effects.

CONCLUSIONS

S. torvum leaf extract exhibits significant peripheral analgesic activity in the acetic acid-induced writhing model, suggesting effective inhibition of nociceptive mediators at the site of inflammation. To determine whether the extract also exerts central antinociceptive effects, further behavioral assays such as the hot-plate and tail-flick tests should be conducted alongside motor coordination assessments (e.g., rotarod and grip-strength tests) to rule out sedative or locomotor side effects. Moreover, bioassay-guided fractionation using chromatographic techniques (e.g., column chromatography, preparative HPLC) is warranted to isolate and structurally characterize the individual phytomolecules responsible for the observed thrombolytic, analgesic, and antioxidant activities. Preliminary molecular docking studies demonstrated that key constituents bind to human peroxiredoxin (PDB ID: 3MNG), tissue plasminogen activator (PDB ID: 1TPK), and human serum albumin (PDB ID: 1AO6) with moderate affinities, supporting their multifunctional pharmacological potential. These *in silico* findings, coupled with *in vitro* and *in vivo* data, justify further mechanistic investigations such as enzyme-inhibition

assays, receptor-binding studies, and *in vivo* thrombosis models to fully elucidate the molecular pathways and optimize these compounds for the development of novel analgesic, antioxidative, and thrombolytic therapeutics.

LIMITATIONS OF THE STUDY

Present study has some limitations that should be addressed in future research. Firstly, the pharmacological screening was limited to preliminary evaluations, focusing only on peripheral analgesic effects without exploring potential central mechanisms of action. Secondly, detailed toxicity assessments, including acute and chronic toxicity studies using in vivo models, were not conducted, which limits the understanding of the extract's safety profile. Additionally, while GC-MS analysis identified multiple bioactive compounds, no efforts were made to isolate or structurally characterize individual molecules to directly correlate them with the observed pharmacological activities. The study also employed a limited comparison with standard drugs only diclofenac-Na was used as a reference, without including other commonly used agents like aspirin or streptokinase for a more comprehensive evaluation. Finally, the research utilized only an ethanolic extract of *S. torvum* leaves, and did not consider alternative solvents such as water or methanol, which might extract different sets of bioactive constituents and provide a more complete pharmacological profile.

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This research was not received any fund from any research institution or individual funding agencies.

CONFLICT OF INTEREST

All authors declare that they have no financial or non-financial affiliations, interests, or relationships with any organizations or entities that could be perceived to influence the content or subject matter discussed in this manuscript.

AUTHOR CONTRIBUTION

The experiment design was done by Nazmun Nahar and Abul Kalam Azad. Funding was provided by Abul Kalam Azad. All of the tests, data collecting and data analysis were carried out by Nazmun Nahar, Beethi Barua, Joya Data Ripa, and Ankita Dutta. Data interpretation was carried out by Nazmun Nahar and Abul Kalam Azad. Supervised by Nazmun Nahar. Paper writing and draft preparation were carried out by Abul Kalam Azad and Nazmun Nahar. Review and draft editing for final submission was done by Abul Kalam Azad. Each of the authors proofread, reviewed, and approved the final draft.

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