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RESEARCH ARTICLE

Phytochemical Screening, HPTLC, GC-MS and *In Vitro* Antioxidant Analyses of Ethanol Extract of *Strychnos potatorum* Seeds

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Abstract

Introduction: Oxidative stress is a key factor in the development of chronic diseases, and plant products with well-established antioxidant properties have been investigated to counteract this phenomenon. *Strychnos potatorum* seeds are used to treat various ailments, including jaundice and diabetes. Moreover, they are used for water purification because of their coagulant properties. **Aim:** To explore the phytochemical constituents of the ethanol seed extract of *S. potatorum* and evaluate its *in vitro* antioxidant activity and *in silico* physicochemical characterisation. **Methods:** This research examined the phytochemical profile, antioxidant properties, HPTLC, and *in silico* characterisation of bioactive compounds identified by GC-MS analysis of the ethanol seed extract of *Strychnos potatorum* (EESP). **Results:** Phytochemical analyses identified the presence of triterpenoids, flavonoids, alkaloids, tannins, glycosides, and saponins. *In vitro* antioxidant assays demonstrated that EESP exhibited concentration-dependent free radical scavenging activity with IC50 values of 70.04 µg/mL (DPPH), 83.05 µg/mL (ABTS), 116.27 µg/mL (FRAP), and 87.47 µg/mL (hydrogen peroxide), compared to ascorbic acid. SwissADME *in silico* analysis showed that 16 of the 17 compounds identified in the GC-MS analysis complied with Lipinski's rule, suggesting drug-like properties and favourable bioavailability. **Conclusion:** These findings suggest that EESP may effectively mitigate diseases associated with oxidative stress and represent a potential candidate for the development of novel therapeutic agents. Further clinical trials are needed to assess the efficacy of EESP-derived compounds in human subjects, which are crucial for translating these findings into therapeutic applications.

Major Findings: *Strychnos potatorum* seed extract shows rich phytochemical content and free radical scavenging activity comparable to ascorbic acid. In silico analysis shows 16 of 17 bioactive compounds follow Lipinski's rule, indicating good bioavailability.

Keywords: Antioxidant Assays, GC-MS, HPTLC, *In Silico* Characterisation, Phytochemistry, *Strychnos potatorum*

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1. Introduction

Oxygen is essential for the survival of living organisms and serves as the foundation for cellular aerobic respiration. However, when oxygen exists in the form of free radicals, it acts as a reactive molecule called reactive oxygen species[1]. The human body maintains an equilibrium between the generation and removal of free radicals. Oxidative stress occurs when this equilibrium is disrupted, adversely affecting cell cycle progression. Oxidative stress is responsible for various age-related diseases, such as malignancy, diabetes, heart disease, neurodegenerative conditions, and inflammatory diseases.

Antioxidants aid in reducing oxidative stress. These antioxidants can be categorised as either endogenous or exogenous. Both endogenous and exogenous antioxidants perform essential functions in preventing the formation of deleterious free radicals by neutralising them or facilitating their decomposition. Utilising products derived from plants is an effective approach to combat oxidative stress. This is largely due to the abundance of phytochemicals in these products, which act as potent sources of antioxidants. Naturally occurring phytochemicals can be found in various plant parts such as vegetables, fruits, bark, leaves, flowers, and roots. These phytochemicals are classified into primary and secondary metabolites, based on their functions in plant metabolism. Alkaloids, polyphenols, terpenoids, and plant sterols are secondary metabolites with diverse chemical structures that serve various functions. Secondary metabolites do not directly contribute to plant growth and development; however, they play a crucial role in defence mechanisms against both biotic and abiotic stressors[2].

India serves as a repository of medicinal plants that are utilised in various traditional medical systems, including *Ayurveda, Unani*, and *Siddha*, for the treatment of diverse ailments. A limited number of medicinal plants have undergone rigorous scientific examination and subsequent validation. Among them, *Strychnos potatorum* is an important plant belonging to the family Loganiaceae. A medium-sized, glabrous tree with a height of 12–13 meters. The fruit is black when mature, globose, 1–2 cm in diameter, whitish, and lustrous, with short yellow sericeous trichomes. The seeds are globose. Previous research had shown that the root, stem bark, and seeds of *S. potatorum* contain various chemical compounds, including alkaloids, flavonoids, phenols, saponins, sterols, glycosides, lignins, and tannins. The examination of *S. potatorum* revealed that tannins were the predominant phytochemicals present in the plant's various parts, including roots, stems, bark, and seeds. Saponins and alkaloids were ranked second and third, respectively, in abundance. Interestingly, the alkaloid compound diaboline was identified in all analysed samples of *S. potatorum*[3].

Ayurveda claims that *S. potatorum* seeds are acrid, alexipharmic, and lithotriptic, and can be used to treat several disorders, such as strangulation, urinary incontinence, and head and neck diseases[3]. The *Unani* system of medicine advocates seeds for the treatment of liver and kidney disorders as well as gonorrhea and colic. They are bitter, astringent, aphrodisiac, tonic, and diuretic. Acute and chronic toxicity investigations of *S. potatorum* seed powder and seed aqueous extract were performed in Wistar albino mice and rats. No evidence of toxicity or mortality was observed even at the dose of 2000 mg/kg body weight. In a chronic toxicity study of up to 90 days, neither the aqueous extract nor the seed powder of *S. potatorum* at doses of 100–200 mg/kg caused appreciable changes[4]. Furthermore, few studies have evaluated the anti-inflammatory, antimicrobial, and antidiabetic effects of *S. potatorum* based on its antioxidant properties[5–10].

We attempt to explore the essential phytochemical constituents of the ethanol seed extract of *S. potatorum* (EESP) and evaluate its *in vitro* antioxidant activity and *in silico* physicochemical characterisation. This study will strengthen the traditional application of *S. potatorum* and provide valuable insights for future research.

2. Materials and Methods

2.1 Seed Collection and Authentication

Dried *S. potatorum* seeds were procured from a local market, and the samples were identified and authenticated by the *Siddha* Central Research Institute, Arumbakkam, Chennai (Rf. No.: 339.18082201, dated 18/08/2022).

2.2 Extract Preparation

Four kg of dried *S. potatorum* seeds were coarsely ground in a mill, and approximately 2 kg of the coarse powder was soaked in ethanol until it was completely

immersed and kept for 48 hours at room temperature without any disturbance. The extracted material was concentrated and filtered. The filtrate was poured into a Soxhlet extraction chamber. Four cycles per hour were used to extract the material for 12 hours. Following extraction, the extract powder was obtained after solvent removal using a rotary evaporator[11].

2.3 Preliminary Phytochemical Screening

Standard methods were employed to examine EESP for the presence of various phytochemicals. The screening process included tests for alkaloids, flavonoids, tannins, phenolic compounds, phytosterols, glycosides, triterpenoids, carbohydrates, anthraquinone, amino acids, proteins, oils, fats, gums, saponins, and steroids. The methodology employed for these analyses is provided in the Supplementary Material section.

2.4 HPTLC Analysis

Two grams of EESP were dissolved in 25 mL of methanol. 10 µL of the test sample was applied using a micro-syringe as a band (5 mm) on a precoated silica gel 60 F_{254} plate (0.2 mm thick). The plate was then developed in a chromatography chamber using a mobile phase consisting of ethyl acetate, acetic acid, formic acid, and water (10:1:1:1) until it reached a height of 75 mm. After air drying, the plate was photographed at 200-360 nm using a UV cabinet. The layers were dried (100-105 °C, 15 minutes), and the components were observed. Densitometric analysis of the layers was conducted at 350 nm using a Camag TLC scanner 3 integrated with WinCATS software with slit dimensions of 6.00 x 0.45 mm and a scanning speed of 20 mm/s. Deuterium and tungsten lamps were used as radiation sources.

2.5 GC-MS Analysis

The EESP sample was dissolved in methanol (5 ml) and centrifuged at 2000 rpm. A 1 µl sample was obtained for GC injection. An Agilent GC 7890 instrument with a triple-axis 5975 MS detector was used. The analysis was performed using an HP5MS column (50 mm×0.25 mm ID ×0.25 µm) and helium gas as a carrier. The temperatures of the injector and ion source were set at 220°C and 350°C, respectively. A constant flow rate of 1 ml/min was maintained, with both the ion chamber and GC interface set to operate at 250°C. The oven temperature was initially set at 50°C and increased at the rate of 10°C/minute up to 250°C. Substance identification was accomplished by comparing the mass spectra of the samples with those found in the NIST and WILEY GC–MS databases, along with an analysis of their retention times.

2.6 *In Silico* Absorption, Distribution, Metabolism, and Excretion (ADME) Profile

The SwissADME web tool was employed to assess the physicochemical, drug-likeness, and pharmacokinetic characteristics of phytochemical compounds detected through GC-MS analysis of EESP (http://www.swissadme.ch).

2.7 *In Vitro* Antioxidant Assay

For the antioxidant experiment, stock solutions of EESP (1 mg/mL) were serially diluted with distilled water (25, 50, 100, and 200 µg/mL). In all experiments, ascorbic acid was used as a positive control due to its established free radical scavenging activity, and the solvent used for dissolving extracts, devoid of the test substance, served as the negative control. All assays were performed in triplicate. The results are expressed as Mean ± Standard error of mean (SEM).

2.7.1 DPPH Assay

Various concentrations of EESP were combined with Tris-HCl buffer (0.8 ml), and the pH was adjusted to 7.4. To this mixture, DPPH (500 mM) in 1.0 mL ethanol was added, agitated vigorously, and maintained at room temperature for 30 minutes. Using a UV-visible spectrophotometer (LaboMed) set at 517 nm, the absorbance was measured.

2.7.2 ABTS Assay

Different concentrations of EESP were added to ABTS (7 mmol/L), potassium persulfate (2.45 mmol/L) and ethanol mixture, and absorbance was recorded at 734 nm after six minutes.

2.7.3 Ferric Reducing Antioxidant Power (FRAP)

Varying concentrations of EESP and ascorbic acid were added to freshly prepared FRAP reagent and left for 30 minutes (37°C). The resulting blue-colored complex's absorbance was measured at 593 nm.

2.7.4 Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of various concentrations of EESP and ascorbic acid was performed using the modified Dehpour method. The scavenging activity, expressed as a percentage, was determined using the following formula:

$$\%\,of\ \ Hydrogen\ peroxide\ scavenged = \frac{Absorbance\ of\ \ control - Absorbance\ of\ \ extract}{Absorbance\ of\ \ control} \times 100$$

3. Results

3.1 Phytochemical Screening

Five hundred grams of extract were obtained from 2 kg of coarse powder, yielding 25% w/w. Table 1 displays the outcomes of the initial phytochemical analysis. These findings suggest the presence of alkaloids, flavonoids, triterpenoids, tannins, phenols, phytosterols, saponins, and glycosides in EESP. Based on these findings, EESP was chosen for the HPTLC and GC-MS analyses.

Table 1. Preliminary phytochemical screening of EESP

Phytochemical constituent	Method	Outcome
Alkaloid	Dragendroff's test	+
Alkaloid	Hager's test	+
Alkaloid	Wagner's test	+
Flavonoids	Alkaline reagent test	+
Saponins	Foam test	+
Tannins	Gelatin test	+
Tannins	Lead Acetate test	+
Phenols	Gelatin test	+
Phenols	Ferric chloride test	+
Triterpenoids	Triterpenoids test	+
Phytosterols	Acetic anhydride test	+
Glycosides	Concentrated H_2SO_4 test	+
Steroids	Libermann Buchard reaction	-
Carbohydrates	Molisch's test	-
Anthraquinones	Borntrager's test	-
Amino acid	Ninhydrin test	-
Protein	Biuret test	-
Fixed oil and fat	Soap test/stain test	-
Fixed oil and fat	Spot test	-
Gum	Gum test	-

'+' indicates presence, and '-' indicates absence

3.2 HPTLC Analysis

The EESP chromatogram (Figure 1) revealed the presence of various constituents with Rf values ranging from 0.05 - 1.2, with 10 spots on the TLC plate. The chromatogram displayed a distinct orange zone under the visible light mode, whereas under the 366 nm and 254 nm UV modes, it exhibited green and purple fluorescent zones, respectively. Table 2 presents the Rf values and peak areas for the different components found in the extract. These findings indicated that terpenoids are the most abundant phytochemicals in EESP, followed by tannins, flavonoids, phenols, and alkaloids. This observation was consistent with the preliminary phytochemical screening of EESP and confirmed the presence of these phytochemicals. The numerous pharmacological properties of *S. potatorum*

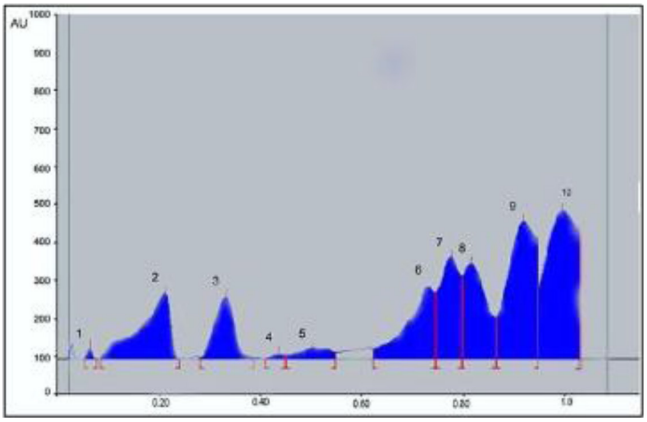


Figure 1. Densitometric scan of the EESP extract.

Table 2. HPTLC analysis of EESP extract

Peak	Constituents	Rf value	Area%
1	Glycosides	0.05	1.25
2	Alkaloids	0.23	5.56
3	Sterols	0.39	1.15
4	Saponins	0.43	4.75
5	Lignins	0.51	2.12
6	Alkaloids	0.72	7.85
7	Phenol	0.78	8.85
8	Flavonoids	0.83	7.25
9	Tannins	0.92	12.20
10	Terpenoids	1.2	15.35

can be ascribed to the presence of these secondary metabolites.

3.3 GC-MS Analysis and Bioinformatics Findings

By comparing the retention times, peak areas (%), heights (%), and mass spectral fragmentation patterns of the bioactive compounds with those of known compounds, the GC–MS chromatograms of EESP revealed a total of 17 peaks (Figure 2). The overall composition and retention duration of the 17 compounds found in EESP are shown in Table 3.

The ADME parameters of the significant compounds identified by GC-MS were analysed using the SwissADME web tool (Table 4). This web tool provides a predictive model for evaluating the ADME properties

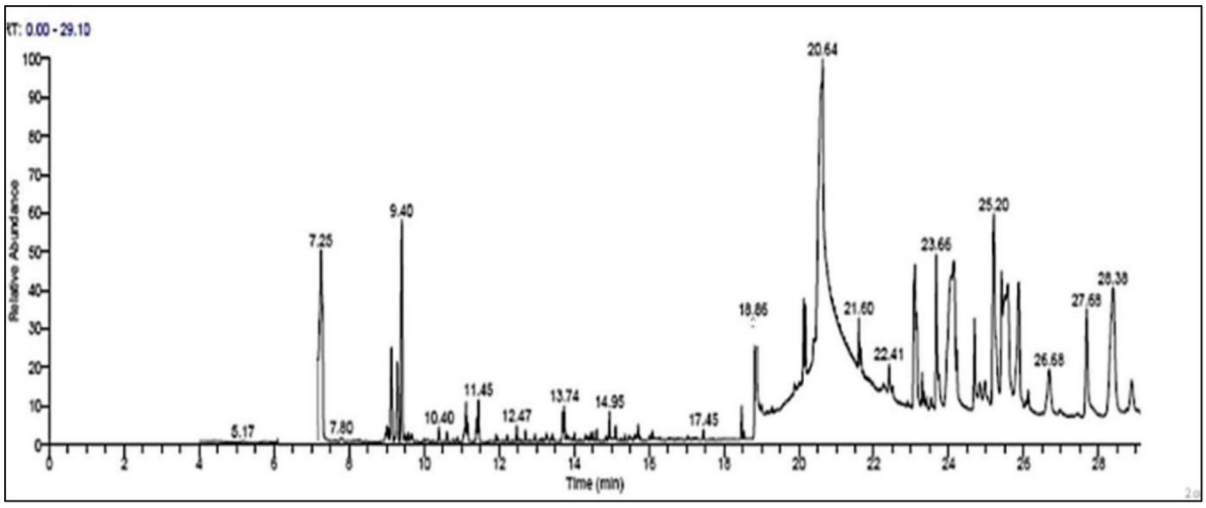


Figure 2. GC-MS analysis spectra of EESP.

Table 3. Bioactive compounds identified in the EESP using the GC-MS technique

S. No.	Compounds	RT	PUBCHEM ID	Molecular formula
1	2H-Indeno(1,2-b) furan-2-one,3,3a,4,5,6,7,8,8b octahydro-8-8-dimethyl	7.25	605626	$C_{13}H_{18}O_2$
2	1-Butanone 1-(2-hydroxylphenyl)	9.4	76157	$C_{10}H_{12}O_2$
3	Imidazole 2-fluoro-1-triacetylribofuranosyl	10.4	536772	$C_{14}H_{17}FN_2O_7$
4	Flavone	11.45	10680	$C_{15}H_{10}O_2$
5	n-Hexadecanoic acid	12.47	985	$C_{16}H_{32}O_2$
6	9,12-octadecdienoic acid	13.74	5322095	$C_{57}H_{98}O_6$
7	But-2-endiamide N, Nbis(4-methoxyphenyl)	14.85	5602-32-4*	$C_{18}H_{18}N_2O_4$
8	Imidazole,2-fluoro-1-triacetylribofuranosyl	17.45	536772	$C_{14}H_{17}FN_2O_7$
9	Dispiro bicyclo heptane	18.86	574859	$C_{14}H_{22}O_4$
10	Thiophene,2,5-dihydro	20.64	137168	C_4H_6S
11	2,3,5,6-Diacetone-4-O-methylmannitol	21.6	538744	$C_{13}H_{24}O_6$
12	1,4, Hexadiene,4-methyl	22.41	5367525	C_7H_{12}
13	Phthalic acid	23.69	7489	$C_8H_6O_4$
14	2-Pyridicarbonitrile, 3-nitro	25.2	45382147	$C_7H_2F_3N_3O_2$
15	Tetracosahexaene	26.2	71339411	$C_{24}H_{38}$
16	1,2-Benzenecarboxylic acid	26.68	181977	$C_8H_4O_4^{-2}$
17	Phosphine	27.68	590903	$C_{22}H_{23}OP$

RT – Retention time, * - CAS Number as the compound is not indexed in the PubChem database

of new compounds before extensive experimental work is carried out, and increases the chances of identifying potential drug candidates. Among the 17 compounds identified, 16 did not violate Lipinski's rule and could be orally absorbed well, as they exhibited good bioavailability scores (0.55 – 0.85) and Topological Polar Surface Area (TPSA) values less than 140 $Å^2$. Except for compound 9, all other compounds were non-P-glycoprotein substrates, suggesting good intestinal absorption and bioavailability. The bioavailability radar images (Figure 3) revealed that at least eight compounds out of the 17 compounds observed for their ADME characteristics fell within the pink area of the polygon, indicating their good oral bioavailability. All 12 compounds, except compounds 12 and 15, had high GI absorption and could cross the blood-brain barrier (except compounds 3, 8, 11, 13, 14, 15, and 16). Compounds 2, 4, 5, 14, and 15 were CYP1A2 inhibitors (cytochrome P450 isoforms). Compounds 4 and 5 were CYP2C19 and CYP2C9 inhibitors, respectively. Compound 17 was found to inhibit CYP2C9, CYP2D6, and CYP3A4. All compounds exhibited negative skin permeability values (log Kp), indicating that they were unsuitable for cutaneous drug delivery.

3.4 *In Vitro* Antioxidant Activity

Table 5 presents the scavenging activities of various EESP concentrations against ABTS, DPPH, FRAP, and hydrogen peroxide, compared to ascorbic acid, the reference standard. A greater percentage of inhibition was associated with greater antioxidant activity. IC_{50} of EESP was 83.05 µg, 70.04 µg, 116.27 µg and 87.47 µg against ABTS radical, DPPH radical, FRAP radical and hydrogen peroxide, respectively. Although EESP showed lower antioxidant properties than ascorbic acid, both compounds demonstrated the ability to scavenge free radicals in a dose-dependent manner.

4. Discussion

Although various parts of *S. potatorum,* such as the leaves, stem bark, and roots, are used in alternative and complementary medicine, the seeds are the most commonly used parts for water purification. Ethanol, a polar solvent, was selected for this study to extract hydrophilic compounds, as it demonstrated favourable solubility for phenolic compounds[12].

Approximately nine studies were conducted on seed EESP from different locations using different concentrations of ethanol (Table 6). Although information regarding the yield of the ethanol extract was not provided in every study, it varied from 3.4% to 21.50%. However, in the present study, absolute ethanol was used as the solvent, and the yield was 25%, which was substantially higher than that reported in other studies. The extraction yield is determined by various factors such as solvent polarity, pH level, temperature, duration of extraction, and sample composition. Various aspects, such as the plant part used, maturity level, seasonal variation, mechanical processing, and prolonged storage, can influence the composition of the sample[13]. The variation in the yields between different studies could be attributed to variations in the extraction methodology, particularly the choice of solvent and extraction conditions. Furthermore, the higher yield obtained in this study might be due to the unique properties of absolute ethanol, which could have enhanced its ability to extract specific compounds from the seeds. It is also possible that the seed samples used in this study had higher concentrations of extractable compounds than those used in previous studies.

Furthermore, in the preliminary phytochemical screening, the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, sterols, and glycosides was observed in the present study, which correlated well with the majority of previous studies (Table 6). However, few studies have reported the presence of fixed oils, proteins/amino acids, carbocyclic acids, coumarins, or lignins. Secondary metabolites are chemicals prepared by plants to protect themselves. The presence of different secondary metabolites from the same species in different geographical locations is possible depending on various factors such as climatic conditions, time of collection, and method of extract preparation. These variations can lead to the production of novel phytochemical compounds and a lack of standardisation, which are considered the strengths and potential weaknesses of phytochemical research, respectively. A study by Mallikharjuna *et al.* conducted a quantitative analysis of phytochemicals in the seeds of *S. potatorum*. Their findings revealed that tannins were the most prevalent compounds, followed by saponins, alkaloids, flavonoids, and phenols in descending order of abundance[14]. In this study, HPTLC analysis revealed

Table 4. *In silico* analysis of physicochemical and pharmacokinetic properties of selected phytochemicals identified using GC-MS analysis of EESP

Mol. No.	Molecular Weight (g/mol)	No. of heavy atoms	No. Aromatic heavy atoms	Fraction Csp3	No. of rotational bonds	No. of H-bond acceptors	No. of H-bond donors	Molar refractivity	TPSA (Å²)	Consensus Log $P_{o/w}$	Lipinski's rule	Bioavailability Score	GI absorption	BBB permeation	P-glycoprotein substrate	Inhibition					
																CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	log Kp (cm/s)
1	206.28	15	0	0.77	0	2	0	58.81	26.3	2.68	Yes	0.55	High	Yes	No	No	No	No	No	No	-6.02
2	164.20	12	6	0.30	3	2	1	48.27	37.30	2.32	Yes	0.55	High	Yes	No	Yes	No	No	No	No	-5.24
3	344.29	24	5	0.57	8	9	0	74.34	105.95	0.79	Yes	0.55	High	No	No	No	No	No	No	No	-7.8
4	222.24	17	16	0	1	2	0	67.92	30.21	3.18	Yes	0.55	High	Yes	No	Yes	Yes	No	No	No	-5.13
5	256.42	18	0	0.94	14	2	1	80.8	37.3	5.2	Yes#	0.85	High	Yes	No	Yes	No	Yes	No	No	-2.77
6	879.38	63	0	0.74	50	6	0	277.12	78.90	-	Violation	-	-	-	-	-	-	-	-	-	-
7	326.35	24	12	0.11	8	4	2	91.91	76.66	2.24	Yes	0.55	High	Yes	No	No	Yes	Yes	No	Yes	-6.79
8	344.29	24	5	0.57	8	9	0	74.34	105.95	0.79	Yes	0.55	High	No	No	No	No	No	No	No	-7.80
9	254.32	18	0	1	0	4	0	64.85	36.92	2.15	Yes	0.55	High	Yes	Yes	No	No	No	No	No	-6.78
10	86.16	5	0	0.5	0	0	0	26.35	25.3	1.45	Yes	0.55	High	Yes	No	No	No	No	No	No	-6.04
11	276.33	19	0	1	4	6	1	67.04	66.38	0.96	Yes	0.55	High	No	No	No	No	No	No	No	-7.95
12	96.17	7	0	0.43	2	0	0	34.81	0	2.5	Yes	0.55	Low	Yes	No	No	No	No	No	No	-4.76
13	166.13	12	6	0	2	4	2	40.36	74.6	1.13	Yes	0.85	High	No	No	No	No	No	No	No	-5.89
14	217.1	15	6	0.14	2	7	0	42.78	82.5	1.2	Yes	0.55	High	No	No	Yes	No	No	No	No	-6.47
15	326.56	24	0	0.50	15	0	0	114.64	0	7.52	Yes#	0.55	Low	No	No	Yes	No	Yes	No	Yes	-2.73
16	164.11	12	6	0	2	4	0	36.48	80.26	0.3	Yes	0.56	High	No	No	No	No	No	No	No	-6.78
17	334.39	24	12	0.32	4	1	0	103.44	30.66	4.81	Yes#	0.55	High	Yes	No	No	No	Yes	Yes	Yes	-4.63

Log $P_{o/w}$ partition coefficient between n-octanol and water; GI, Gastrointestinal; BBB, Blood-brain barrier; CYP, Cytochrome P450; #1 violation: MLOGP>4.15

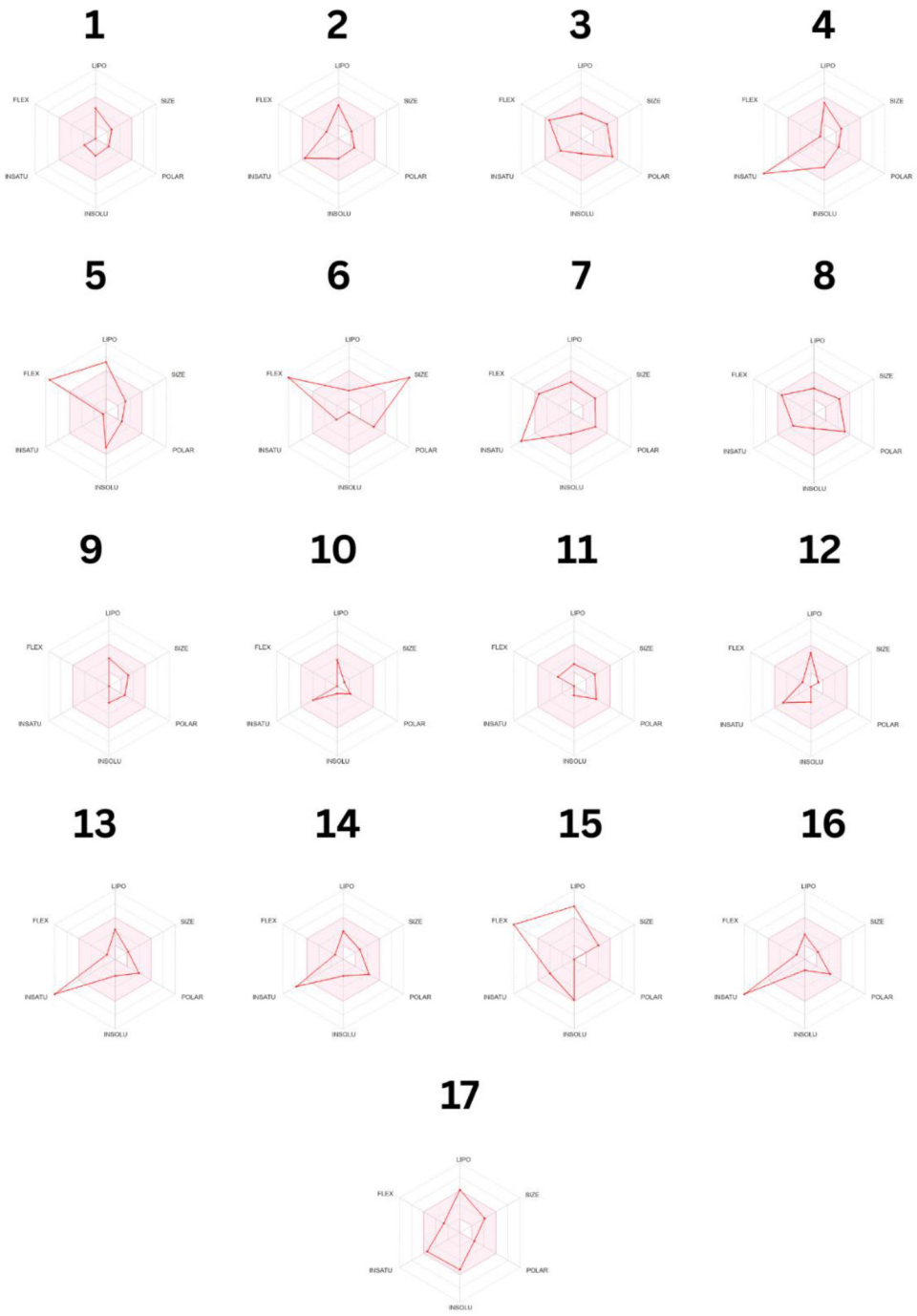


Figure 3. Bioavailability radar images of selected compounds identified in the EESP using the GC-MS technique. The pink zone is ideal for oral bioavailability. LIPO (lipophilicity: –0.7 < XLOGP3 < p 5); SIZE (molecular weight: 150 g/mol < mol wt < 500 g/mol); POLAR (polarity: 20 Å2 < TPSA < 140 Å2); INSOLU [insolubility: 0 < Log S (ESOL) < 6]; NSATU (insaturation: 0.25 < fraction C sp3 < 1); and FLEX (flexibility: 0 < number of rotatable bonds < 9).

that terpenoids were the most abundant phytochemicals, followed by tannins, flavonoids, phenols, and alkaloids. Among alkaloids, diaboline is the most abundant and is considered the key molecule responsible for its established pharmacological properties[14].

Arunthathi *et al.*, in the year 2023, have done a GC-MS analysis of EESP and found similar results. Compounds 3, 9, 10, 11, 12, 13, 14, 16, and 17 (Table 4) were common to both studies. However, the presence of bicyclo [2.2.1]heptan-2-ol,

Table 5. *In vitro* antioxidant activity of EESP

Conc (µg/ml)	Percentage of inhibition							
	ABTS Radical Scavenging Activity		DPPH radical scavenging activity		Hydrogen peroxide scavenging activity		FRAP radical scavenging activity	
	EESP (%)	Ascorbic Acid (%)	EESP (%)	Ascorbic Acid (%)	EESP (%)	Ascorbic Acid (%)	EESP (%)	Ascorbic Acid (%)
25	18.0 ± 1.5	29.9±1.8	12.39 ± 2.1	22.5 ± 1.4	15.45 ± 1.8	18.59 ± 2.1	11.9 ± 1.0	19.5 ± 1.2
50	30.1 ± 2.1	39.3±2.8	35.50 ± 1.6	41.0 ± 1.7	35.44 ± 1.7	39.50 ± 1.6	27.4 ± 1.3	38.7 ± 2.2
100	64.3 ± 3.2	65.5±2.2	59.34 ± 1.8	62.3 ± 2.4	62.11 ± 2.3	63.69 ± 1.8	43.0 ± 3.8	64.2 ± 3.5
200	76.8 ± 2.9	83.8±4.1	70.18 ± 2.5	84.2 ±3.1	71.56 ± 4.9	83.32 ± 3.1	77.5 ± 7.5	89.3 ± 5.7
Negative control 0.0 ± 0.0								

The results are expressed as mean ± SEM (n=3).

Table 6. Yield and phytochemicals present in EESP seeds of different studies

References	Year	Location	Type of extract	Yield	Phytochemicals												
					Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Sterols	Terpenoids	Tannins	Fixed oil	Proteins/ amino acids	Carbocyclic acid	Coumarins	Lignins
Sharmila C *et al.*[5]	2020	Tamil Nadu	95% Ethanol	18.50%	+	+	+	+	+	+	+	+					
Thavaranjit AC *et al.*[8]	2016	Sri Lanka	Hot extract Cold extract		+	+	+		+	+	+	+					
Gangwar M *et al.*[12]	2017	Madhya Pradesh	Ethanol	3.4%		+	+		+		+			+			
Arunthathi K *et al.*[15]	2023	Tamil Nadu	Ethanol		+								+	+	+	+	
Sharma SK *et al.*[20]	2018	Rajasthan	Ethanol	10.60%	+	+		+	+	+							
Varghese R *et al.*[21]	2011	Tamil Nadu	Ethanol	12.70%	+	+	+	+	+	+		+					+
Mishra S *et al.*[22]	2014	Uttar Pradesh	50% Ethanol	21.50%	+	+	+	+	+	+			+				
Sharma L *et al.*[23]	2019	Tamil Nadu	Ethanol		+	+		+			+	+					
Chattopadhyay R *et al.*[24]	2022	West Bengal	30, 50, 60, 70 and 80% Ethanol		+	+	+	+			+						
Present study	2024	Tamil Nadu	Ethanol	25%	+	+	+	+	+	+	+	+					

'+' indicates presence

1,3,3-trimethyl, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, and bicyclo [2.2.1]hept-2-ene, 2-methyl was reported by Arunthathi *et al.*, in addition to the above-mentioned compounds[15]. Similarly, in the present study, we reported the presence of 2H-Indeno(1,2-b) furan-2-one,3,3a,4,5,6,7,8,8b octahydro-8-8-dimethyl, flavone, 9,12-octadecadienoic acid, But-2-endiamideN, Nbis(4-methoxyphenyl), and tetracosahexaene, which have not been reported previously. Prolonged seed storage results in the interconversion of certain compounds into their derivatives, which could explain the differences observed in these two studies[14]. Among these, the significant compounds include imidazole, 9,12-octadecadienoic acid, 1-butanone 1-(2-hydroxyphenyl), imidazole 2-fluoro-1-triacetylribofuranosyl, and flavone, which

together exhibit antioxidants, anti-inflammatory[16], and antitumor properties[17]. N-Hexadecanoic acid is known for its antioxidant and antibacterial activities[18]. Furthermore, indenoic compounds have been clinically acknowledged for their effectiveness against tuberculosis and their ability to reduce inflammation[19].

The physicochemical properties of the compounds found in EESP revealed drug-like molecules based on Lipinski's rule. Furthermore, the compounds are predicted to be well absorbed by the gastrointestinal tract and are predicted to have good oral bioavailability. Arunthathi *et al.*, as a part of estimating the antidiabetic potential of compounds derived from EESP, performed a docking study against 6YYE and 6Y6C proteins and found Thiophene, 2,5- dihydro- had good antidiabetic activity[15]. The permeability of the blood-brain barrier was predicted for seven compounds, indicating their potential effectiveness in treating neurological disorders. Similarly, the bioinformatics data from this report could be utilised for further molecular docking studies to assess the interactions between these phytochemical compounds and disease-specific protein molecules, resulting in the identification of therapeutic targets. This could save time and resources for developing drug candidates from new compounds before conducting extensive preclinical and clinical research.

In the present study, *in vitro* antioxidant assays revealed the concentration-dependent radical-scavenging activity of EESP, which was comparable to that of ascorbic acid. However, the IC50 values of EESP were significantly higher than those of ascorbic acid, indicating lower antioxidant potency compared to the standard. Similarly, using the phosphomolybdenum method, the total antioxidant activity of EESP was found to be 275 g/TAE mL-1[15]. Additionally, a diabetes investigation revealed that EESP played a positive role in regulating antioxidant enzymes in diabetic animals. These enzymes, including lipid peroxide, glutathione peroxidase, superoxide dismutase, catalase, and hydroperoxide, were positively modulated in the plasma, liver, and pancreas[6]. The antioxidant properties of EESP suggest its potential use in managing chronic diseases where oxidative stress is a contributing factor, including conditions like cancer and diabetes. Numerous studies suggest a link between the antioxidant properties of *S. potatorum* and its potential anti-inflammatory[7], hypotensive, anticonvulsant, antiprotozoal, antimicrobial, antimalarial[8,14,15,20], antidiabetic[6,9,15], hepatoprotective[10], and nephroprotective[21] benefits.

This study expands our understanding of the phytochemical compounds present in EESP and highlights potential avenues for future research. Further investigations should focus on quantitative analysis of these phytochemicals, *in vivo* validation of their antioxidant properties, and molecular docking studies to identify therapeutic targets. Additionally, the isolation of bioactive compounds and mechanistic studies will provide valuable insights into their roles in various diseases. Finally, conducting clinical trials to assess the safety, efficacy, and pharmacokinetics of EESP-derived compounds in human subjects is crucial for translating these promising *in vitro* and *in vivo* findings into viable therapeutic applications.

4.1 Limitations

The present study does not involve the quantification of individual phytochemicals, which could have helped prioritise therapeutic applications based on their chemistry. Although bioinformatic analysis proved the generalised potential drug-likeness of EESP, it did not focus on any particular disease model. Furthermore, the antioxidant assays performed were *in vitro* in nature. These results should be validated using *in vivo* models for application in preclinical and subsequent clinical studies.

5. Conclusion

This study provides a comprehensive phytochemical analysis of EESP, revealing a rich profile of secondary metabolites including terpenoids, alkaloids, flavonoids, phenols, tannins, and saponins. The HPTLC analysis confirmed that terpenoids were the most abundant class of compounds. GC-MS analysis identified 17 bioactive compounds, some previously unreported, that exhibit promising pharmacological potential, such as antioxidant, anti-inflammatory, antibacterial, and antitumor properties. Bioinformatics evaluation showed that the majority of these compounds adhered to Lipinski's rule of five, indicating good drug-like properties and oral bioavailability.

EESP exhibited significant *in vitro* antioxidant activity, comparable to that of ascorbic acid, suggesting

its potential therapeutic use in treating oxidative stress-related disorders, including cancer and diabetes. Bioinformatics findings further suggest that these compounds could serve as valuable leads for molecular docking studies aimed at discovering disease-specific protein interactions. This study significantly enhances our understanding of the bioactive compounds in *S. potatorum* seeds and opens new avenues for their application in drug development. We emphasise that these findings are preliminary and necessitate further mechanistic and clinical validation to reach definitive conclusions.

6. Author Contribution

Conceptualization: Abarajitha S, Manickam S; Investigations: Abarajitha S, Senthil Kumar A, Jones Eben Raj T, Nithya V; Formal analysis: Abarajitha S, Jones Eben Raj T, Nithya V; Writing original draft: Abarajitha S, Senthil Kumar A, Ravisankar P; Writing final draft and supervision: Manickam S. All authors have read and agreed to the published version of the manuscript.

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