Exploration of phytochemistry and their applications of *Sesamum indicum* L. and *Nyctanthes arbor-tristis* extracts

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Abstract. Nepal is one of the richest countries in plant biodiversity since a significantly large variety of medicinal plants are found at different altitudes. This research mainly focuses on searching for phyto-compounds in plant-leaf-derived methanolic extracts and their biological applications. Cultivated *S. indicum* and *N. arbor-tristis* plants were selected to prepare methanol extracts. Phenolics, flavonoids, saponins, alkaloids, etc., as secondary metabolites were identified from phytochemical screening tests, UV-vis, and FT-IR spectroscopic studies. Further, GC-MS analysis identified flavonoid, ester, ether, acid, alcohol, amide, and lactone. The total phenolic and the total flavonoid content were determined by the Folin-Ciocalteu and $AlCl_3$ colourimetric method, respectively, using a 96-well plate. The phenolic content of *S.indicum* and *N. arbor-tristis* extracts was 177.76 ± 3.40 mg/ g GAE and 131.32 ± 5.03 mg/ g GAE, respectively. Similarly, the flavonoid content of *S.indicum* and *N. arbor-tristis* extracts was 77.90 ± 0.78 mg/g QE and 173.75 ± 3.89 mg/ g QE, respectively. Both of these extracts exhibit eco-friendliness according to an oral acute toxicity test. The extracts possess remarkable antioxidant and anti-diabetic properties. However, they did not exhibit anti-fungal and anti-bacterial activity. These results may help in discovering pharmaceuticals and indicate that many plants in Nepalese forests are potent for medicine, and scientific study is essential for their use.

1. Introduction

Medicinal plants have been used in medicine and drug discovery for centuries. Many pioneering medications, such as antimalarials derived from Cinchona bark and opium from *Papaver somniferum*, have been obtained from plants [1]. Almost 80% of people worldwide rely on medicinal herbs for their primary healthcare [2]. Any part of the plant—including roots, stems, leaves, flowers, seeds, and bark—may hold therapeutic value since they are stores of secondary metabolites. This is one of the many benefits of phytomedicine [1]. The biochemical reactions and metabolic pathways within our body continually produce nitrogen and oxygen free radicals. These free radicals are harmful and contribute to ageing, neurological disorders, cancers, and cardiovascular diseases [3]. Treating these diseases has become difficult because synthetic antioxidants are either resistant or have numerous side effects. Plant-based antioxidants are highly effective, with minimal or no side effects, as they neutralise free radicals due to their abundance of phenolics and polyphenols [4]. In Nepal and other parts of the world, type-2 diabetes has become increasingly prevalent at an alarming rate, posing a serious issue. Plant-derived antidiabetics offer promising prospects because they are safer, less prone to resistance, and have fewer or no side effects. *Sesamum indicum* L. belongs to the Pedaliaceae family, which comprises 13 genera and 40 species, of which 36 are listed in the Kewensis index [5]. It is known by names such as tila (Sanskrit) and kalo tila (Nepali). This plant is cultivated in the Terai, Basi, and hill Tars of Nepal, as well as in various regions worldwide, including India, China, America, Africa, Brazil, the southern United States, Northwest and Southwest Ethiopia, North Gondar, Mexico, and Japan [6]. According to Hindu tradition, it appears to have originated in Indonesia. There are three varieties of sesame: black, white, and grey. In Nepal, black sesame is the most commonly cultivated variety. All parts of *S. indicum*—leaves, flowers, and seeds—possess medicinal properties. The decoction of *S. indicum* leaves is utilised to treat chronic dysentery, pain [7], catarrh, eye pain, erupted skin lesions, and bruises [8]. Root, seed, and leaf aqueous extracts showed ineffective inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Xanthomonas compestris*, and *E. coli*. However, methanol extracts from these sections demonstrated anti-*Pseudomonas aeruginosa* activity. Additionally, the methanol extract of seeds showed anti-*Staphylococcus aureus* and anti-*Xanthomonas compestris* activity. Antioxidant activity was present across all sections [9]. The leaf ethanol extract demonstrated excellent antibacterial activity against *Escherichia coli*, with no effect on *Staphylococcus aureus*, and low effectiveness against *Salmonella typhi* and *Klebsiella*

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pneumoniae. Conversely, the aqueous extracts showed little activity against these bacteria [10]. The antimicrobial properties of white *S. indicum* leaf methanol extract from Nigeria were tested against several bacteria, revealing zones of inhibition; however, no zones were observed against *Corynebacterium ulcerans, Escherichia coli*, and *Candida stellatoidea* [11]. Another study investigated the antifungal properties of *S. indicum* extracts [12]. Two flavonoids, quercetin and kaempferol, were isolated from the leaves [13]. A pentacyclic triterpene, 3-epibartogenic acid, along with two flavonoids—epigallocatechin and kaempferol glycoside—was isolated from the methanol extract of *S. indicum* leaves and studied for their antidiabetic activity. The IC_{50} values for 3-epibartogenic acid and epigallocatechin were reported as 146.7 μM and 303.9 μM, respectively [14]. Three iridoids and seven polyphenols have been previously isolated from young *sesame* leaves [15]. Several phenolic compounds—including chlorogenic acid, quercetin, gallic acid, and catechin have been identified in the leaf extract through ultrasound-assisted and solvent extraction techniques [16]. Microwave-assisted extraction and mass spectrometry (LCMS-Q-TOF) of leaves identified fifty polyphenolic compounds [17]. The leaf extract demonstrated inhibitory effects on HT29 and another cell line, as well as in vitro antioxidant activity [18]. A recent molecular docking and simulation study of phytochemicals derived from this plant indicated inhibition of SARS-CoV-2's main protease [19].

Nyctanthes arbor-trisits, known as Parijata in Sanskrit. It has other names, such as Har-singer in Hindi, Night Jasmine in English, and Raatko Rani in Nepali. It belongs to the Oleaceae (Nyctaginaceae) family [20]. It is found in tropical and subtropical regions in Nepal, Australia, New Zealand, Japan, India, the USA, China, Mexico, and other countries. The leaves of *N. arbor-tristis* have many pharmacological significances and are used in burns, swelling, treatment of epilepsy, sciatica, rheumatism, chronic fever, internal worms, laxatives, diuretics, and diaphoretics [21]. The leaf extract is used as an antidote for reptile venoms, and combined with honey to cure fever, cough, and stomach problems [22]. The plant has a significant role as an analgesic, anti-inflammatory activity of stem bark [23], antimicrobial and antioxidant activity of essential oil [24], hepatoprotective activity of leaves [25], antibacterial activity of leaves, fruits, and seeds [26], antifungal activity of leaves, stem, bark, seeds, and flowers [27], antiviral [28], antioxidant and anti-inflammatory activity of leaves and flowers [29], the antispasmodic and anthelminthic activity of leaves, barks, flowers and seeds [30], tranquillising, antihistaminic, and purgative [31], anti-nociceptive and antipyretic [32]. The antimalarial activity of leaf extract against *Culex*, *Anopheles*, and *Aedes* mosquitoes has yielded encouraging results [34], and it has anticancer activity against breast cancer cell lines [33]. In addition to flavanol glycosides like astragaline (Kaempferol-3-glucoside) and nicotiflorinkaempferol-3-rhamnoglucoside, leaves have also yielded amorphous resin, mannitol, arborside-C, β-amyrin, β-sitosterol, hentriacontane, and benzoic acid [35].

Recognising the medicinal potential, the present authors have investigated the methanol extracts of *S. indicum* and *N. arbor-tristis* from Nepal to study their phytochemistry and to inform future directions for the pharmaceutical industry. The research is designed to prepare *S. indicum* and *N. arbor-tristis* extracts using a highly polar solvent, methanol, by a cold percolation method to study phytochemistry in qualitative and quantitative ways. Further, the biological applications of these extracts are studied with a target for medicinal applications. This research may be the door to the novel medicinal arena.

2. Materials and methods

2.1 Collection of plants

The *Sesamum indicum* and *Nyctanthes arbor-tristis* leaves were collected from Pigouna-2, Mahottari (*26.65, 85.82*), and Sifal Kathmandu (*27.71, 85.32*), respectively. The voucher codes for these plants, issued by the National Herbarium and Plant Laboratory Godavari, are RDP-03 and KATH-RDP0001, respectively.

Fig. 1. (a) *S. indicum* plant

Fig. 1. (b) *N. arbor-tristis* plant

2.2 Preparation of extract

The leaves were gathered, cleaned with purified water, allowed to dry in the shade, and then powdered using an

electrical grinder. The powdered leaves were soaked in 99.8% methanol (solute: solvent = 1:5 w/v), left at ambient temperature for three days, and shaken occasionally. The filtration was performed first with cotton and then with Whatman paper 41. This process was repeated three times to ensure complete extraction. The solvent was concentrated at 40℃ by rotatory evaporator to obtain the methanol extract, and preservation was done at 4℃ until further use.

2.3 Qualitative phytochemical analysis

The chemical tests for secondary metabolites were performed on these plants' methanol extracts using the protocol described [36]for secondary metabolites. The UV-visible spectra of the plant extracts were recorded in the 200 to 800 nm range using a UV-visible (SPECCORD® 200, PLUS, Germany) double-beam spectrophotometer. The functional groups were inferred from spectral data. The crude methanol extracts were scanned for FTIR analysis in the 4000 to 500 cm^{-1} range using PerkinElmer Spectrum 2 IR Version 10.6.2. The functional groups were inferred from spectral data.

2.4 Quantitative phytochemical analysis

2.4.1 Total phenolic content analysis

The extract's total phenolic content was ascertained using the Folin-Ciocalteu reagent (FCR) [37]. 20 µL (1 mg/mL) of the extract and 100 µL (1:10 dilution in distilled water) of FCR reagent were placed in 96-well plates. For five minutes, the mixture was kept at room temperature in the dark. After that, 80 µL of sodium carbonate (7.5% in distilled water) was added to the mixture. The absorbance was measured at 765 nm using a microplate reader (BioTek, USA; Synergy/LX, multimedia reader) after the plates were incubated for 15 minutes in the dark. The results were reported as mg/g gallic acid equivalent, with gallic acid (1 mg/mL in ethanol) serving as a standard. Every experiment was carried out three times. The TPC is calculated using the formula,

TPC = C x V/m… (1), where V is the extract's volume in millilitres, m is its mass in grams, and C is the gallic acid concentration (mg/mL).

2.4.2 Total flavonoid content analysis

The $AlCl_3$ method was used to determine the extracts' total flavonoid content [38]. In short, a 96-well plate was filled with a 130 µL solution of aluminium chloride (20 µL extract + 110 µL distilled water) in triplicate. Initial absorbance was measured at 415 nm after standard quercetin 130 µL (0.1 mg/mL in methanol) was also taken in triplicate. Each plate was filled with 5 µL of $AlCl_3$, 5 µL of potassium acetate, and 60 µL of ethanol. The mixture was then allowed to stand at room temperature in the dark for half an hour. Using a microplate reader (Synergy/LX, multimedia reader BioTek, USA), the final absorbance was determined at 415 nm. The standard calibration curve was plotted for different quercetin concentrations in methanol. The TFC results were given in mg/g QE. Every experiment was carried out three times. TFC is calculated using the formula, TFC = C x V/m… (2),
Where C is the quercetin concentration in milligrams per millilitre, V is the extract volume in millilitres, and m is the extract mass in grams.

2.4.3 Identification of the chemical compositions

A Shimadzu GC-MS-QP2010 plus gas chromatograph with an AOC-20i autosampler was used to perform the GC-MS analysis. Helium was used as the carrier gas, and the separation was carried out on an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with primary pressures ranging from 500 to 900. The following temperatures were used: 60°C for the column oven, 200°C for the ion source, 280°C for the interface, 280°C for the injection, and splitless injection mode. The pressure was 80 kPa, the purge flow was 3.0 mL/min, the column flow was 2.89 mL/min, the total flow was 5.9 mL/min, and the linear velocity was 92.3 cm/sec. The mass (m/z) scan range was 40-500, the run time was 14.67 minutes, and the MS acquisition mode was set to normal scanning (scan speed 2500).

2.5 Applications of plant-based extracts

2.5.1 Acute oral toxicity

Albino mice were used in the acute oral toxicity test of plant leaf extract/powder following the OECD TG 425 Up and Down Procedure, 2001 [39]. Three female Albino mice of the same sex were given a single dose of leaf extract or powder based on their body weight using this technique. After that, they were watched for 14 days. During this observation period, special attention was given to the first four hours and then for the next twenty-four hours. The plant extract or powder is toxic if any of the three mice die within the allotted fourteen days; otherwise, it is non-toxic. To confirm the toxicity, additional observations with the lower dose are required if the initial result is toxic. The LD_{50} value serves as the quantitative basis for toxicity analysis. If there is no mortality at a single dose level of more than 2000 mg/kg body weight, the plant leaf extract or powder is considered non-toxic. If mortality is noted at a dose level greater than 2000 mg/kg body weight, the plant leaf extract or powder is toxic. This is ensured by repeated observations with a decreased dose twice and thrice

2.5.2 Antioxidant assay

Leaf extracts were subjected to an antioxidant assay using the 1, 1-diphenyl 1, 2-picrylhydrazyl (DPPH) assay [40]. A 96-well plate was filled with 100 µL of 0.1 mM DPPH solution (Sigma Aldrich, Germany) and 100 µL of plant extracts at different concentrations. After the

plate was left in the dark for 30 minutes, its absorbance at 517 nm was measured using a microplate reader. Ascorbic acid was used as a reference to calculate the antioxidant capacity, and the result was expressed in µg/mL. The percentage scavenging was calculated using the following formula:

% scavenging = Ao-At /Ao ×100 %… (3), where Ao is the absorbance of the control solution (DPPH without sample) and At is the absorbance of the mixture solution or test sample and DPPH. The following formula is used to determine percentage inhibition: % inhibition $=A_{control}-A_{sample} / (A_{control}) \times 100$ %... (4).

2.5.3 Antidiabetic assay

The leaf extracts' in-vitro α-amylase inhibition activity was examined following the protocol [41]. The working sample was made by preparing 0.5 mg of α-amylase in 1 millilitre of phosphate buffer with a pH of 6 to 9. The concentrations of 100 µg/mL, 75 µg/mL, 50 µg/mL, and 25 µg/mL were obtained by further dissolving the plant extract (1 mg/mL) in methanol in phosphate buffer. 200 µL of each plant extract solution was combined with 200 µL of α-amylase, and the mixture was incubated for 10 minutes at 30°C. Each tube received 200 µL of a 1% w/v starch solution in water, which was then incubated for three minutes. Two millilitres of DNSA reagent (3, 5-dinitrosalicylic acid) were added to stop the reaction. The solution was added to the reaction mixture to create the positive control, which contained 1 mg/mL of metformin. The plant extract solution was not added when creating the 100% estimated enzyme activity tube. After that, each tube was incubated for ten minutes at 85°C. After 10 minutes, 5 mL of distilled water was added to each tube, and absorbance was taken at 540 nm. The following formula calculates the inhibition rate,

$$\frac{\text{Absorbance of 100\% enzyme activity} - \text{Absorbance of sample reaction}}{\text{Absorbance of 100\% enzyme activity}} \ldots\ldots (5)$$

2.5.4 Antibacterial assay

The Central Department of Microbiology, T.U., provided the gram-positive bacteria *Bacillus subtilis* (ATCC-6051) and *Staphylococcus aureus* (ATCC-25293), as well as the gram-negative bacteria *Escherichia coli* (ATCC-8739) and *Klebsiella pneumonia* (ATCC-13883). For the antibacterial assay, the well-diffusion method was employed [42]. Two different kinds of media were made: nutrient broth (NB) and nutrient agar (NA) plates. Following autoclaving, the media were allowed to cool to approximately 37°C. The 25 mL of media was poured into a 90 mm-diameter petri dish. Before being used, the prepared media plates were kept in the refrigerator. The sterile inoculating loop was used to aseptically cultivate the microorganisms in liquid nutrient media, and they were incubated for 24 hours at 37°C. After plating 100 µL of each strain's culture broth onto a nutrient agar plate, it was kept at 37°C for 15 minutes. The 10 µL sample of methanol extract was pipetted out and put into a 6 mm well-bored nutrient agar plate.

2.5.5 Antifungal assay

To evaluate the antifungal activity, the disk diffusion method was used to measure the zones of inhibition (ZOI) that methanol extracts produced on specific fungi [43]. At the Central Department of Microbiology, T.U. *Candida albicans* (ATCC-2091) fungi were gathered. The Nepal Agricultural Research Centre (NARC) provided *Alternaria* and *Fusarium* species. On nutrient agar, 100 µL of the strain's culture broth was plated, and it was incubated for 15 minutes at 37°C. After 15 minutes of incubation at 37°C, 10 µL of the methanol extract was pipetted out and poured onto a nutrient agar disk. All of this was done overnight at 37°C. The extract's antifungal properties against *Alternaria*, *Fusarium* and *Candida albicans* species were examined the following day. Cycloheximide 50 mg/mL as a positive control, DMSO as a negative control and the plant extract 50 mg/mL were employed in this investigation.

2.6 Statistical analysis

Every experiment was conducted three times. Standard deviation ± mean was used to express the data. The linear regression coefficient (R^2) values were calculated using the Origin Pro 2025 learning edition for Windows.

3. Results and discussion

3.1 Qualitative phytochemical analysis

The secondary metabolite profiling of crude methanolic extracts of *S. indicum* and *N. arbor-tristis* was performed following a standard protocol to explore them qualitatively. The results are listed in the following Table 1.

Table 1. Phytochemical Profiling of crude methanol leaf extracts.

S.N.	Secondary Metabolites	Test	*S.indicum* Extract	*N.arbor-tristis* extract
1.	Alkaloids	Dragendroff's	+	+
2.	Flavonoids	Shinoda's	+	+
3.	Glycosides	Keller-Killani's	+	+
4.	Polyphenols	Ferric chloride	+	+
5.	Fat/ Fixed oil	$CuSO_4$ test	-	-
6.	Tannins	Braemer's test	+	+
7.	Saponins	Foam	+	+
8.	Terpenoids	Salkowski test	+	+
9.	Quinones	NaOH test	+	+
10.	Coumarins	NaOH test	+	+

11.	Proteins	Biuret test	-	+
12.	Reducing sugars	Molisch's test	+	-

(Note: '+' refers to the presence of secondary metabolites, and '-' refers to the absence of secondary metabolites.)

Alkaloids, flavonoids, glycosides, polyphenols, tannins, saponins, terpenes, quinones and coumarins were reported in both extracts. Similarly, fat and volatile oils are absent in both extracts. Also, proteins were reported in *N. arbor-tristis* extract and reducing sugars were observed in *S. indicum* extract. Our results of *S. indicum* extracts' secondary metabolites align with previous reports [44-45]. Also, *N.arbor-tristis* extract results were compared and found to be similar to phytochemical results reported in [46].

The UV-Vis spectra of both extracts predict the presence of secondary metabolites, as observed in phytochemical testing. The metabolites, like alkaloids, flavonoids, phenolic and tannins, may be indicated by peaks in the UV-visible region [47]. The peaks observed in the UV range of 200-400 nm indicate the presence of unsaturated systems and heteroatoms such as oxygen, nitrogen, halogen, silicon and sulphur [48]. The peaks in the visible range of 400-600 nm indicate the presence of multiple bonds or heteroatoms in aromatic systems such as β-carotene or Lycopene systems [49].

Table 2. UV-Vis Spectra analysis for secondary metabolites in extracts

Wavelength	Secondary metabolites	
	S. indicum extract	*N. arbor-tristis* extract
415	-	Unsaturated chromophoric group
445	Chlorophyll group	
472	Highly conjugated aromatic systems	
533	Tannins	-
605	Anthocyanins	-
663	Chlorophyll	

Similarly, the idea of functional groups present in crude extracts was obtained by analysing absorption peaks in the FT-IR spectrum, as assigned in Table 3.

Table 3. FT-IR Spectra analysis for assignments of functional groups in extracts

Wavenumber (cm^{-1})		Assigned for Functional Groups
S.indicum extract	*N.arbor-tristis* extract	
3330	-	O-H stretch (Alcohols and Phenols)
-	3006	C-H symmetric Stretch (due to *Cis*-double bond vibration).
2920	2920	C-H symmetric stretch(Alkane)
2845	-	CH_2 andCH_3(vibrations of phospholipids, cholesterol and Creatine)
	2339	C≡N of nitrile
2083	-	C≡C of alkyne
-	1746	C=O ester of lipid and fatty acid
1695	-	C=C Stretch
-	1598	C=C stretch
-	1450	O-CH_2 bending vibration (due to in-plane deformation)
-	1370	O-H bending vibration (may be due to phenolic hydroxyl
1267	-	C-O stretch (may be due to lignin and Xylan)
1164	-	
-	1023	C-O-C bending vibration
806	-	C-H out-of-plane bending due to aromatic ring
-	710	C-H out of the plane bending

Therefore, qualitative chemical and spectroscopic analysis validated the presence of alkaloids, flavonoids, and phenolics as secondary metabolites, which justifies both extracts as suitable for biological applications and further studies.

3.2 Quantitative Phytochemical Analysis

As phenols and flavonoids were reported in both extracts, the total phenolic and total flavonoid content were quantified using the calibration curve of gallic acid and quercetin, respectively, as depicted in Figures. 2(a) and 2(b).

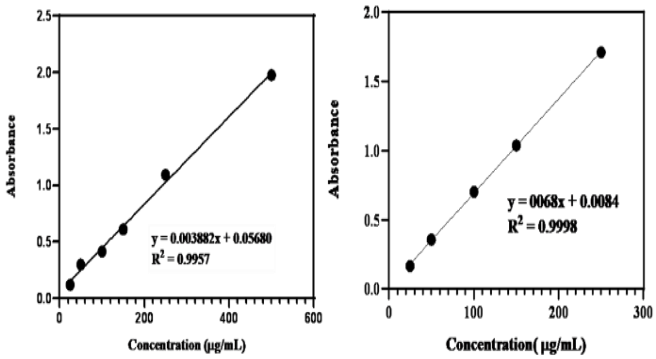

Fig. 2: Standard calibration curve for (a) gallic acid and (b) quercetin**.**

From the equations, y = 0.00388x+0.05680, and y = 0.0068x+0.0084, the value of C was determined from y as the average absorbance recorded from standard calibration curves. The phenolic content of *S. indicum* extract was 177.76 ± 3.40 mg/ g GAE, and *N. arbor-tristis* extract was 131.32 ± 5.03 mg/ g GAE. Therefore, a 26.12% higher TPC was determined in *S. indicum* extract than *in N. arbor-tristis* extract. This indicates that *S. indicum* extract should contain many phenolic compounds as secondary metabolites. Similarly, higher flavonoid content was responsible for the antioxidant properties of methanol extracts [50]. Between two experimental extracts, *N. arbor-tristis* extract was found to be richer in flavonoid content by 55.16% than *S. indicum* extract, since the flavonoid content in *N. arbor-tristis* extract is 173.75 ± 3.89 mg/ g QE and *S. indicum* extract is 77.90 ± 0.78 mg/g QE. In *S. indicum*, the total phenolic content is lower, and the flavonoid content is higher than our value, as reported in the other study [18]. Similarly, in *N.arbor-tristis*, a higher total phenolic content (260.3 ± 12.2 mg/g

GAE) than in our study has been reported elsewhere [51]. This is due to the differences in the extraction method, determination protocol, and climatic variation of the plant.

3.3 Chromatographic analysis

The biological applications of plant-based extracts are due to secondary metabolites. Hence, it is better to identify the compounds if possible. For this reason, GC-MS is an effective tool for determining compounds present in plant extract from retention time, area percentage, and mass [52]. The spectra of *S. indicum* reveal the presence of different secondary metabolites, as suggested by FT-IR and UV-Vis Spectroscopy. They are listed in Table 4 in tabular form.

Eighteen compounds were reported when the chromatogram obtained from GC-MS was analysed and compared with the NIST library database. The major compounds are 9, 12-Octadecadienoyl chloride, (*Z, Z*) -, (33.65 %), hexadecanoic acid, methyl ester (16 .16%), 9, 12-Octadecadienoic acid *(Z, Z*) - (12.94 %), 2-tetradecanone (9.77 %), D-erythro-Pentose, 2-deoxy- (6.18 %), Eicosanoic acid, methyl ester (5.03 %), Hexanoic acid, cyclohexyl ester (3.65 %), n-Tridecanoic acid, methyl(tetramethylene)silyl (2.88 %), and 2-hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, acetate (2.16 %). The compounds identified by GC-MS in our study are different from the previously reported compounds from leaves. Previously, twenty-six lignans from aerial organs and seeds [14], 50 phenolic compounds from leaves [15], and nineteen polyphenols from various organs were reported. Eight phenols were found in the seeds and aerial parts. Eight anthraquinones and three naphthoquinones were reported. Three iridoids lambalbid, Sesamoside and Shanzhiside methyl ester were also reported from the leaf [14]. The compounds identified from *N. arbor-tristis* methanolic extract are listed in Table 5.

Table 4. Compounds identified in the methanol extract of *S. indicum* from the GC-MS spectrum

Peak number	Retention Time (RT)	Area %	Base m/z	Name and Molecular formula	Mol wt.
1	3.060	0.64	44.00	Tetrahydro-4H-pyran-4-ol ($C_5H_{10}O_2$)	102.13
2	3.517	0.52	61.05	Diglycerol ($C_6H_{14}O_5$)	166.17
3	4.037	2.88	41.05	n-Tridecanoic acid, methyl(tetramethylene)silyl ($C_{18}H_{30}O_2Si$)	306.52
4	4.223	6.18	57.05	D-erythro-Pentose, 2-deoxy- ($C_5H_{10}O_4$)	134.13
5	4.323	0.90	75.05	4-(2-Fluorobenzoylamino)piperidine-1-carbo	
6					
7	4.410	2.16	43.05	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-, acetate ($C_{22}H_{42}O_2$)	338.58
8	4.484	3.65	43.05	Hexanoic acid, cyclohexyl ester ($C_{12}H_{22}O_2$)	198.31
9	4.571	16.16	74.05	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)	270.46
10	4.806	9.77	58.05	2-Tetradecanone ($C_{14}H_{28}O$)	212.38
11	4.924	33.65	55.05	9,12-Octadecadienoyl chloride, (Z, Z)- ($C_{18}H_{31}ClO$)	298.90
12	5.234	12.94	41.05	9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$)	280.45
13	5.559	5.03	74.05	Eicosanoic acid, methyl ester ($C_{21}H_{42}O_2$)	326.57
14	5.878	0.63	129.10	Hexanedioic acid, dioctyl ester ($C_{22}H_{42}O_4$)	370.57
15	5.950	0.61	87.05	3-O-Methyl-d-glucose ($C_7H_{14}O_6$)	194.18
16	6.422	0.56	74.05	Docosanoic acid, methyl ester ($C_{23}H_{46}O_2$)	354.62
17	8.001	1.23	69.10	Squalene ($C_{30}H_{50}$)	410.73
18	8.490	0.16	43.05	2,4-bis(dimethylamino)-7,9-diethyl-10-imino-8-sulfanylidene-1,7,9-triazaspiro[4.5]deca-1,3-dien-6-one ($C_{15}H_{24}N_6OS$)	336.5

Table 5. Compounds identified in the methanol extract of. *Arbor-tristis* from GC-MS spectrum.

Peak number	Retention Time (RT)	Area %	Base m/z	Name and Molecular formula	Mol wt.
4	3.372	1.71	105.05	Benzoic acid ($C_7H_6O_2$)	122.12
5	3.500	0.18	120.05	Bezofuran, 2,3-dihydro- (C_8H_8O)	120.15
7	3.756	0.42	164.10	*trans*-Isoeugenol ($C_{10}H_{12}O_2$)	164.20
8	3.896	1.55	60.00	d-Gluco-heptulosan($C_7H_{14}O_7$)	210.18
9	4.002	0.86	41.05	13,16-Octadecadienoic acid, methyl ester($C_{19}H_{34}O_2$)	294.48
10	4.094	1.85	194.05	n-Decanoic acid ($C_{10}H_{20}O_2$)	172.27
11	4.152	0.54	68.05	Neophytadiene ($C_{20}H_{38}$)	278.52
12	4.184	1.32	43.05	2-Undecanone, 6,10-dimethyl- ($C_{13}H_{26}O$)	198.35
13	4.267	0.58	41.05	Pentadecanoic acid ($C_{15}H_{30}O_2$)	242.40
14	4.309	4.67	74.00	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)	270.46
15	4.391	2.33	216.10	Valerenic acid, methyl ester ($C_6H_{10}O_2$)	114.14
16	4.441	8.62	43.05	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	256.43
17	4.583	0.90	58.05	Tramadol ($C_{16}H_{25}NO_2$)	263.38
18	4.714	19.60	79.05	9,12,15-Octadecatrienoic acid, methyl ester, (*Z*) ($C_{19}H_{32}O_2$)	292.46
19	4.747	13.11	71.05	Phytol($C_{20}H_{40}O$)	296.54
20	4.894	21.81	79.05	9,12,15-Octadecatrienoic acid, (*Z,Z,Z*)- ($C_{18}H_{30}O_2$)	278.44
21	5.010	3.73	69.05	*trans*-Farnesol ($C_{15}H_{26}O$)	222.37
22	5.103	1.68	157.05	(*3R,6R*)-3-hydroperoxy-3-methyl-6-prop-1-en-2-ylcyclohexene ($C_{10}H_{16}O_2$)	168.24
23	5.166	1.52	79.05	Methyl (*Z)*-5,11,14,17-eicosatetraenoate ($C_{21}H_{34}O_2$)	318.50
24	5.307	1.48	74.00	Eicosanoic acid, methyl ester ($C_{21}H_{42}O_2$)	326.57
32	7.256	0.76	117.05	Doconexent ($C_{22}H_{32}O_2$)	328.50

Twenty one compounds were reported when the chromatogram obtained from GC-MS was analysed and compared with the NIST library database. The major compounds are 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (21.81%), 9, 12, 15-Octadecatrienoic acid, Methyl ester, (Z) (19.60%), Phytol (13.11 %), n-hexadecanoic acid (8.62 %), hexadecanoic acid, methyl ester (4.67 %), trans-farnesol (3.73 %), and valerenic acid, methyl ester (2.33 %). The compounds identified by the GC-MS in our study differ from the previously reported compounds from the leaf, flower, seed and stem [53-55]. Our methanol extract GC-MS data were also compared with ethanol extract data in another study [17].

3.4 Applications of plant-based extracts

Both extracts and their powdered form were subjected to acute oral toxicity tests with albino mice with a single dose. No mortality was observed in any of the cases tabulated in Table 6 up to 14 days of observation. According to OECD guidelines, all extracts and original leaf powder are safe for humans. Therefore, these extracts become crucial for the development of novel biological applications. This result is aligned with another study [56]

Table 6. LD_{50} values for the leaf powder and extract

Sample	LD_{50} value
Leaf powder of *S. indicum*	>2000 mg/kg BW
Leaf powder of *N. arbor-tristis*	>2000 mg/kg BW
S. indicum extract	>2000 mg/kg BW
N. arbor-tristis extract	>2000 mg/kg BW

Since both extracts are expected to be strong antioxidants, the DPPH method was used to analyse the antioxidant properties, using ascorbic acid as a reference. Antioxidant efficacy is correlated with IC_{50} values; a lower IC_{50} value indicates more antioxidant activity [57]. Ascorbic acid's standard calibration curve is used to evaluate IC_{50} values and is expressed as a percentage of inhibition versus concentration, as in Figure 3

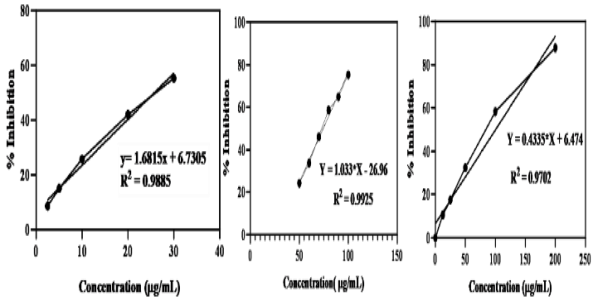

Fig. 3. % inhibition vs concentration curves for a) standard ascorbic acid, (b) *S. indicum* extract, and (c) *N. arbor-tristis* extract for antioxidant activity.

Thus, the IC_{50} values for ascorbic acid, methanolic crude extracts of *S. indicum*, and *N. arbor-tristis* calculated were 25.74 μg/mL (Figure 3a), 74.47 μg/mL (Figure 3b), and 100.40 μg/mL (Figure 3c), respectively. In our case, the IC_{50} value for *S. indicum* extract is nearly three times higher and for *N. arbor-tristis* extract, four times higher than standard ascorbic acid. The % inhibition for methanol extract of *S. indicum* ranges from 24.33% - 75.34%, which is higher than the values 31.60%- 49.42% reported by Sharma *et al*. [9]. The antioxidant efficacy of the plant extract has been divided into four levels, IC_{50} < 50 μg/mL (very strong antioxidant), IC_{50} = 50-100 μg/mL (strong antioxidant), IC_{50} = 100-150 μg/mL (moderate antioxidant) and IC_{50} = 151-250 μg/mL (weak antioxidant) [58]. Hence, *S. indicum* and *N. arbor-tristis* have strong antioxidant potential, as described elsewhere [59].

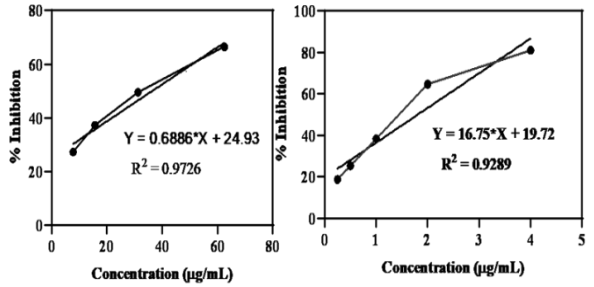

Fig. 4. % inhibition vs concentration curves for (a) *S. indicum* extract, (b) *N. arbor-tristis* extract

Since both extracts possess potent antidiabetic properties due to their high polyphenol content, the antidiabetic properties were analysed using the α-amylase inhibition method. Antidiabetic efficacy can be related to IC_{50} values; that is, lower IC_{50} values indicate greater antidiabetic efficacy. Therefore, the standard calibration curve of metformin is expressed as % inhibition vs concentration to estimate IC_{50} values, as in Figs. 4. Thus, IC_{50} values for methanolic crude extracts of *S. indicum*, *N. arbor-tristis* and metformin are 36.46 μg/mL (Figure 4a), 1.80 μg/mL (Figure 4b) and 19.4 ± 1.4 μM (Figure 4c), respectively. Low IC_{50} values indicate better antidiabetic efficacy of plant-based extracts compared to the standard metformin. Both extracts possess anti-diabetic properties, but the *N.arbor-tristis* extract possesses a lower IC_{50} value than standard metformin. Hence, it possesses better antidiabetic properties than the standard.

Correlation between TPC and TFC, determined as quantitative phytochemistry and applications as antioxidant and antidiabetic properties, was done with statistical approaches using Origin Pro, 2025 (Figure 5). The Karl Pearson correlation analysis was performed between the above parameters. A statistical analysis that shows how two variables relate to a change in one variable is called correlation analysis. There is a positive correlation between two variables if they change in the same direction. A negative correlation occurs when they are moving in the opposite direction. Studies indicate that the TPC and TFC are linearly correlated with antioxidant properties [18]. TPC is nearly orthogonal, i.e., -vely correlated to the antioxidant property with R^2 = -0.933(a high negative value, so there is a strong negative correlation) and p-value 0.020 (very significant).

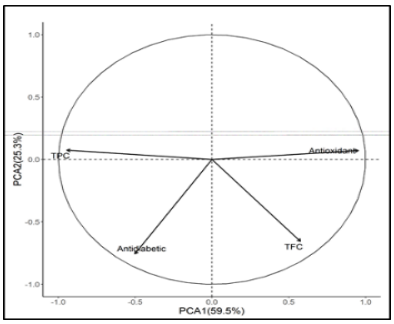

Fig. 5. Loading Plot between variables TPC, TFC, Antioxidant and Antidiabetic property

Similarly, the antibacterial and antifungal activities were assessed, but neither exhibited a zone of inhibition (ZOI). Therefore, they lack antibacterial and antifungal activity against the tested bacteria and fungi. These results are aligned with those of Sharma *et al*. [9]

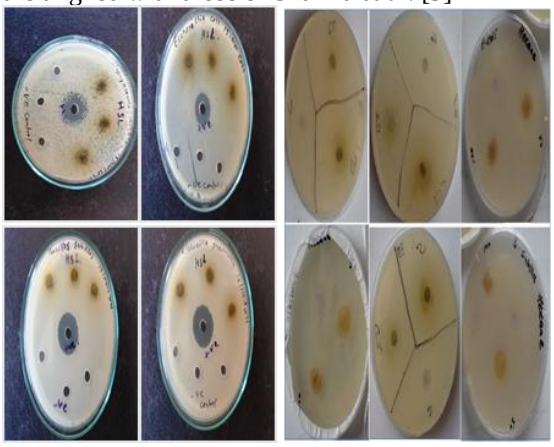

Fig. 6. Antimicrobial Test for (a) S. indicum and (b) N. arbor-tristis

4. Conclusions

Methanolic extracts of two Nepal-based plants, *S. indicum* and *N. arbor-tristis*, were examined for their phytochemistry. Both extracts confirmed the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, tannins, saponins, quinones, and others

through phytochemical screening tests. UV and FT-IR spectroscopy supported these findings. Quantitatively, *N. arbor-tristis* extract was found to be richer in flavonoids by 55.16% compared to *S. indicum*, with flavonoid contents of 173.75 ± 3.89 mg/g QE and 77.90 ± 0.78 mg/g QE, respectively. Conversely, *S. indicum* extract contained a higher amount of phenolic compounds; a 26.12% higher total phenolic content (TPC) was measured in *S. indicum* than in *N. arbor-tristis*. Both extracts were non-toxic to albino mice in acute toxicity tests. Compounds such as 9, 12-Octadecadienoyl chloride, (Z, Z), 9, 12-Octadecadienoic acid (Z, Z), and hexadecanoic acid methyl ester were identified via GC-MS analysis. Both exhibited strong antioxidant activity, with IC_{50} values of 74.47 µg/mL and 100.40 µg/mL, respectively, for *S. indicum* and *N. arbor-tristis*. Anti-diabetic properties were also observed, with IC_{50} values of 36.46 µg/mL and 1.80 µg/mL, respectively. The extracts did not demonstrate anti-fungal or anti-bacterial activity against the tested fungi and bacteria. These findings could represent significant advancements for the pharmaceutical industry.

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