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Phytochemical screening and evaluation of antioxidant and antimicrobial activity of *Solanum incanum*: medicinal plant from Al-Baha Region

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Abstract. *Solanum incanum* is a prominent medicinal plant, used to treat various ailments. The current study sought to assess the phytochemical profile, as well as the antioxidant and antimicrobial activities, of the crude methanolic extract and the corresponding chloroform, ethyl acetate, n-hexane, and aqueous fractions of the leaves, stem bark, and fruits. Leaf and fruit extracts had the largest levels of polyphenols, flavonoids, tannins, and alkaloids referring to stem-bark. Hexadecanoic acid methyl ester is a major phytochemical component revealed in different plant parts, especially in stem-bark with 31.82% level. The 9,12,15-Octadecatrienoic acid, methyl ester is also revealed in all plant parts, especially in leaf with 20.07%. Fruit and leaf ethyl acetate and water fractions showed the highest antioxidant capacity compared to stem-bark fractions. Using the agar disc diffusion method, antimicrobial activity was evaluated in vitro against four different bacterial species and one fungal species (*Candida albicana*). The ethyl acetate, n-hexane, and chloroform fractions of fruits and leaves showed strong antimicrobial activity against all microorganisms. Aqueous fraction of different plant parts was inactive or partially active against tested microorganisms. The high total content of phenols, flavonoids, tannins and alkaloids, especially in leaves and fruits, correlates positively with the highest antimicrobial and antioxidant activities detected in these parts of the plant.

Key words: GC-MS Analysis, free radical scavenging, solanaceae, phenolic compounds.

INTRODUCTION

Solanum incanum L. is part of the complex and widely distributed *S. incanum* sensu lato group, native to a broad region across Africa and the Middle East (Samuels, 2012). Its distribution spans from North Africa through the savannah regions of sub-Saharan

Africa, the Arabian Peninsula, and into parts of the Middle East and India (Ranil et al., 2017; El-Shaboury et al., 2021). In Saudi Arabia, it is a common component in the flora of the southwestern highlands. This plant is a perennial shrub, well adapted to arid and semi-arid conditions of the desert. This bushy herb is widely distributed throughout Al-Baha area. This plant species is used in traditional medicine to treat throat and chest problems (Zivanayi et al., 2023), as antiseptic for dermatomycosis, and as antihyperlipidemic (Andargie et al., 2022; Ohalete, 2023). Fruit extracts presented too, an antidiabetic activity (Satyanarayana et al., 2022).

While it has usually been harvested from the wild for traditional uses, its biological robustness and the growing interest in its bioactive compounds argue for considerable potential for cultivation. Successful cultivation would ensure a sustainable and standardized supply that is necessary for any future pharmaceutical use, as was explored with other wild species of the genus Solanum (Ranil et al., 2017). Recent phytochemical studies of Solanum species have reported the presence of flavonoids, amides, steroids, steroidal saponins, steroidal alkaloids, and lignans, (Ateshim & Tekle, 2022).

Plant phytochemicals play a vital role in drug discovery as they can be used directly as drugs, as synthetic precursors, or as lead compounds (Kim et al., 2021; Chaachouay et al., 2024). In this context, medicinal plants are a particularly valuable resource, due to their rich diversity of bioactive chemicals (Augšpole et al., 2018; Espro et al., 2021; Yazarlu et al., 2024). In *S. incanum*, the most important classes of bioactive compounds are tannins, flavonoids, alkaloids and phenolic compounds (Lezoul et al., 2020; Roy et al., 2022). Interestingly, the phytocomplex of *S. incanum,* characterized by the presence of fatty acid esters, alkaloids, and sterols, shows a very good correspondence with the bioactive profile reported for other medicinal plants of recognized pharmaceutical relevance. Among others, hexadecanoic acid methyl ester was identified by GC-MS in the sclerotia of Pleurotus tuber-regium (Oseghale et al., 2025), a mushroom with important medicinal properties, and in the leaves of *Heinsia crinita*, traditionally used in medicine (Mgbeje et al., 2016). The fatty acid esters and sterols identified in *S. incanum* are also relevant in the anticancer profile of *Melastomastrum capitatum* (Ukwubile et al., 2019) and in the wild and cultivated specimens of *Leonotis nepetifolia*, a plant with anti-inflammatory and analgesic properties (de Oliveira et al., 2015). This comparative phytochemical profile underlines the potential of *S. incanum* as a source of lead compounds for pharmaceutical development.

In humans, Reactive Oxygen Species (ROS) are generated endogenously through metabolism or exogenously by environmental stresses that can cause oxidative damage to cellular macromolecules, including lipids, proteins, and DNA (Di Meo et al., 2022). It is considered a fundamental contributor to the pathogenesis of many chronic health risks, such as cardiovascular, neurodegenerative, and inflammatory diseases. Lack of antioxidants accelerates the development of degenerative diseases, including cardiovascular diseases, cancers (Rychter et al., 2022), neurodegenerative diseases, Alzheimer's disease, (Pritam et al., 2022) and inflammatory diseases (García-Sánchez et al., 2020). Plant phytochemicals in medicinal plants could serve as a preventive medicine. *S. incanum* along with several other medicinal plants, has been reported with potential antioxidant activity (Akanmu et al., 2021; Chivodze et al., 2021).

Additinally, the antioxidant capacity conferred by polyphenolic and flavonoid content in various plants, especially medicinal plants, represents a significant potential for the salt stress mitigation (Maaroufi-Dguimi et al., 2025).

Solanum incanum has significant antibacterial activity (Chivodze et al., 2021; Karanja et al., 2021; Musyimi et al., 2021). The antimicrobial activity of *S. incanum* has been reported in plant extracts (Vaou et al., 2021), essential oils (Ramsey et al., 2020) or isolated compounds such as alkaloids (Ti et al., 2021), flavonoids (Sok Yen et al., 2021), sesquiterpene lactones (Liu et al., 2021), diterpenes (Mohammed et al., 2022), triterpenes (Renda et al., 2022) or naphthoquinones (Ahmadi et al., 2020).

In the Al-Baha region (Fig. 1), *S. incanum* is used in traditional medicine, yet there is no scientific evidence supporting its bioactivity or biological safety. The lack of scientific information in the literature regarding the phytochemical analysis and bioactivity of different parts of this plant prompted the current study. The aim is to establish the phytochemical profile and evaluate the antioxidant and antimicrobial activities of methanolic extracts from the leaves, stem-bark and fruits.

Figure 1. Map of Kingdom of Saudi Arabia showing Al-Baha Province.

MATERIALS AND METHODS

Plant material

Solanum incanum was collected in October 2023 of five localities from Al-Baha region in south western Saudi Arabia located in the Sarawat Mountains (Fig. 1). In each locality and within the area of collection, around 10 individual mature plants were sampled for the creation of a composite sample for each part: leaves, stem-bark, and fruits. The plants that were sampled were mature, characterized by fully expanded leaves, and immature to mature fruits. Although there is variation in individual plant architecture, as noted in studies of Saudi *S. incanum* populations (AL-Rowaily et al., 2021), the general morphology of the mature plants sampled was 3–5 main stems with numerous leaves (estimated > 30–50 per plant) and fruit clusters (Fig. 2). Plant materials collected from different locations were combined and thoroughly homogenized into one composite sample for each respective plant part to ensure representativeness for chemical and biological analyses, accounting for natural intra-species variability. The pooling strategy herein adopted is a common approach in phytochemical screening in order to have a representative profile of the species from the region.

The specific collection sites were at an altitude of approximately 2,000 meters above sea level. In period collection, the region experiences a climate with temperatures ranging from 24 °C to 35 °C and an average rainfall of about 18 mm. The plants were growing in an open area with full sunlight exposure on soils with textures ranging from clay loam to loamy to sandy loam.

The plants were identified according to the key diagnostic morphological features described for the *S. incanum* complex by Samuels, 2012; Kannan et al., 2023. These features include: the presence of sharp, broad-based prickles on stems and midribs; ovate to elliptic leaves with sinuate or lobed margins covered in stellate hairs. Fruits were spherical berries, approximately 2–3 cm in diameter, exhibiting characteristic white streaks on a green background. Such a morphological profile agrees with the description of *S. incanum* from the southwestern highlands of Saudi Arabia given by El-Shaboury et al., 2021 and ALshaqhaa et al. 2023. Such a plant was taxonomically recognized and verified by Dr. Haidar Abd Al Gadir of the Biology Department, Al-Baha University, where voucher specimens (voucher number: ABUSI-1023) were deposited in the university herbarium for future reference.

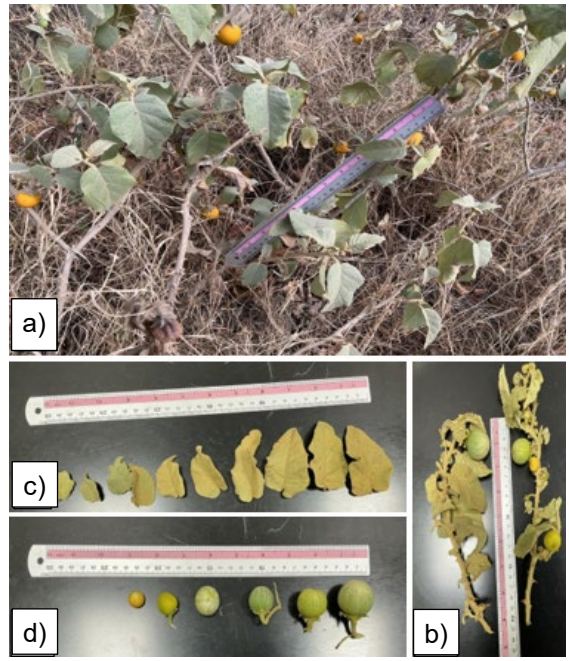

Figure 2. *S. Incanum* (a) whole plant in field (b) branch of plant (c) leaves (d) Fruits.

Determination of phytochemical components

Plant samples were shade dried and separately ground into fine powder using a mechanical grinder and then stored in airtight containers. To ensure a representative sample for chemical and biological analyses, the plant materials (leaves, stem-bark, and fruits) collected from the various locations were combined and thoroughly homogenized into a single composite sample for each respective plant part. A 15 g of powder from each plant part (leaves, stem-bark and fruits) was defatted with n-hexane then extracted three times by exhaustive maceration in 150 mL of 80% methanol for 48 hours on an orbital shaker (150 rpm). Following filtration, the filtrates were concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator (Buchi, USA). The resulting crude extract was stored in amber glass vials at 4 °C to protect it from light and degradation for pending analysis (Fig. 3).

Figure 3. Different plant parts (leaves, fruits and stem-barks) ground and their extracts.

For the quantitative assays, the total phenolic, flavonoid, tannin, and alkaloid content were determined spectrophotometrically. Each extract was analyzed in triplicate (n = 3 analytical replicates), and the results are expressed as the mean ± standard deviation (SD).

Determination of total polyphenols. The quantification of total phenolic content was performed by means of the Folin-Ciocalteu calorimetric (Cicco et al., 2019). A 200 µl of extract (0.5 mg mL^{-1}) in methanol was added with 1.5 mL diluted Folin-Ciocalteu reagent (1:10) and mixed and allowed to equilibrate for 1–2 minutes. A 2.5 mL sodium carbonate (2.0 10^{-1}) was added to the test tube and incubated at room temperature for 30 minutes. The absorbance of the generated solution was measured at 760 nm using water as a blank. The results were calculated based upon a gallic acid standard curve ($y = 0.7962x – 0.0372$, $R^2 = 0.9794$).

Determination of total flavonoids. The total flavonoid content was determined using the aluminum chloride colorimetric method (Pothitirat et al., 2009). In short, 1 mL of each extract was combined with 1 mL of the aluminum chloride solution (2.0 10^{-2}). The mixture was incubated at room temperature for 10 min, and the absorbance was read at 415 nm. Quantification was based on a standard curve for quercetin ($y = 1.4712x – 0.2389$, $R^2 = 0.9887$).

Determination of tannins. The tannin content was assessed through the vanillin-HCl method (Rebaya et al., 2014), by mixing 12.5 µl of the extract with a solution of 1% vanillin (w/v) at a volume of 750 µl, and adding 375 µl of 8% HCl (v/v). After 15 minutes of incubation at room temperature, the absorbance was read at 500 nm. Catechin standard curve = ($y = 2.2648x + -0.2069$; $R2 = 0.9934$).

Determination of total alkaloids. According to the procedure given by Shamsa et al. in 2008, total alkaloid content was determined for each suspension. To prepare the samples, 1 mg of each respective plant extract was dissolved in 2N HCl and filtered. From the filtrate, 1 mL was taken with 5 mL of phosphate buffer (pH 4.7), to which 5 mL of bromocresol green (BCG) solution was added at the same time (final concentration was 10-4 M). For the extracts, dilution was done with chloroform (*10), and the absorbance was measured at 470 nm. Atropine was used as the standard ($y = 0.9011x – 0.1097$ $R^2 = 0.9923$).

Biological activities and Gas Chromatography-Mass Spectrometry analysis (GC-MS)

The plant powder of each part was soaked in ethanol (70%, two weeks, 3 times) at room temperature. The resulting residues were filtered, pooled and evaporated to dryness under reducing pressure at 45 °C to afford a green viscous syrup. The crude extract so obtained, was transferred on Petri plate, allowed to dry and finally weighed. The crude ethanol extract was suspended in distilled water and defatted with n-hexane. The defatted ethanol extract was further fractionated by using Chloroform, ethyl acetate and n-butanol, respectively to afford n-hexane fraction, Chloroform fraction, ethyl-acetate fraction, and water fraction. It is important to point out that the use mixture of solvents (Ethanol 70%) throughout all extraction process to assure the extraction of all polar and non-polar compounds.

Gas chromatography-mass spectrometry analysis. An analysis using GC-MS was performed on a Shimadzu GCMS-QP2010-Ultra, featuring an Rtx-5ms capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas, with a flow rate of 1.61 mL min^{-1}. A 1 µl sample was injected in split mode (injector temperature: 300 °C). The temperature program was as follows in the GC oven: 60 °C (held for 0 min), ramped up to 300 °C at 10 °C min^{-1}, and held for 3 min (total run time: 26 min). The MS interface was maintained at 250 °C and the ion source was kept at a temperature of 200 °C. Mass spectra were obtained throughout the entire scan range of m/z 40–500. Compounds were identified from mass spectral and retention index values from the NIST (National Institute of Standards and Technology) mass spectral library. GC-MS analysis was performed on a single injection per composite sample for the qualitative identification of constituents. The relative abundances reported are based on the peak area percentage of the total ion count from a single run and should be interpreted as indicative.

Antimicrobial assay. The antimicrobial potential of the plant extracts was examined through the agar disc diffusion method, as previously described by the Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards (NCCLS), 2006). To obtain bacterial suspensions, the density of bacterial cell suspensions was adjusted to 10^8 CFU mL^{-1} (0.5 McFarland standard) using a sterile saline. A 100 µl sample of each suspension was evenly spread on the surface of Mueller-Hinton Agar (MHA) plates and allowed to dry in a sterile cabinet for 5 min. Following this, discs of sterile filter paper (6 mm diameter) were placed onto the inoculated agar and impregnated with 20 µl of the extract's solution. The plates were inverted and incubated at 37 °C for 24 hours. The same methodology was used for the control plates inoculated with pathogen strains resistant to the extracts. Following incubation the diameters of inhibition zones were measured. Antimicrobial activity was interpreted according to the following criteria: inactive (< 9 mm), partially active (9–12 mm), active (13–18 mm), and very active (> 18 mm; Mukhtar & Ghori, 2012).

Antioxidant activity. To assess antioxidant potentials, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging test is used. DPPH radical scavenging was carried out using the method established by Shimada et al., 1992. Using a 96 well plate, test samples were allowed to react with stable free radical (DPPH) at 37 °C for 30 minutes. DPPH concentration was held constant at 300 µM. Test samples were prepared in dimethyl sulfoxide (DMSO), while DPPH was dissolved in ethanol. After the incubation period, the decrease in absorbance was measured at 517 nm using a multiplate reader spectrophotometer. The % radical scavenging activity of samples was calculated as compared to the control group which was treated with DMSO. All tests and analyses were performed in triplicate.

Statistical analysis

All quantitative experiments were performed with at least three independent replicates ($n \geq 3$). Data are presented as the mean ± standard deviation (SD). At the probability level < 0.05, significant differences between the means of the various treatments were determined using ANOVA analysis and Tukey's *HSD* tests. SPSS software version 20.1 (IBM version 20.0.2004) was used.

RESULTS AND DISCUSSION

Total polyphenols content

Alkaloids, phenols, tannins, and flavonoids have been found in various plant parts of *S. incanum*, according to a number of recent studies (Akanmu et al., 2021; Karanja et al., 2021; Musyimi et al., 2021). The content of phytochemical components in different plant parts was illustrated in Table 1. Results showed a higher content of polyphenols in all plant parts referring to flavonoid, tannin and alkaloid levels. As summarized in Table 1, the leaves consistently contained the highest levels of all quantified phytochemicals, polyphenols, flavonoids, tannins, and alkaloids, followed by the fruits. The stem-bark exhibited significantly lower concentrations of these bioactive compounds compared to the other plant parts.

Table 1. Total polyphenol, flavonoid, tannin and alkaloid contents in different parts of *Solanum incanum.* Columns with different letters (a, b, c) for each variable differ significantly according to Tukey's test ($p < 0.05$)

Plant part	Poyphenols (mg GAE/g DW)	Flavonoids (mg QE/g DW)	Tannins (mg CE/g DW)	Alkaloids (mg AE/g DW)
Leaves	9.0 ± 0.01^{a}	7.6 ± 0.12^{a}	3.6 ± 0.08^{a}	4.6 ± 0.04^{a}
Stem-bark	3.3 ± 0.02^{c}	1.8 ± 0.00^{c}	1.6 ± 0.02^{b}	2.2 ± 0.03^{c}
Fruits	7.6 ± 0.05^{b}	5.8 ± 0.05^{b}	3.0 ± 0.06^{a}	3.1 ± 0.01^{b}

As reported in Table 1 and according to Chivodze et al., qualitative phytochemical analysis showed that various plant parts of *S. incanum* contained phenols, flavonoids, coumarins, and condensed tannins. These diverse phytochemical types have important medicinal uses and could help researchers create new medications against various disorders (Saboon et al., 2019). Furthermore, the environment, climate, and plant organ all had a significant impact on the biochemical components, particularly polyphenolics and flavonoids (Ivanova et al., 2022).

Gas chromatography-mass spectrometry analysis (GC-MS) in hexane fraction in different plant parts

Table 2 listed the 19 compounds that were present in leaves. The two main compounds found in the leaf hexane fraction were 9, 12, 15-Octadecatrienoic acid, methyl ester (20.07%), and hexadecanoic acid, methyl ester (26.84%). In leaf hexane fraction, the 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester and squalene levels were about 12.09%. Some trace compounds were also reported. Eleven compounds were identified via volatile metabolites in the fruit hexane fraction (Table 3). The predominant component was 9-Octadecenoic acid (Z)-, methyl ester (48.78%), followed by Hexadecanoic acid, methyl ester (18.92%). Table 4 displayed the identification of 20 compounds within the stem-bark n-hexane fraction. The predominant compound was Hexadecanoic acid methyl ester, which constituted 31.82% of the total fraction. Additionally, the 9, 12-Octadecadienoic acid (Z, Z)-methyl ester was present at a significant level of 29.08%. Other compounds were found in smaller quantities.

GC-MS analysis of different plant parts was illustrated in Table 2, 3 and 4. Results showed two major compounds in all plant parts of hexane fraction, with different level.

Table 2. GC-MS Analysis showing the chemical constituents in leaf extract of n-hexane fraction

Peak No.	Compound	Retention time (RT)	Relative abundance (%)	Formula	Major mass spectral data m/z
5	Hexadecanoic acid, methyl ester	16.387	26.84	$C_{17}H_{34}O_2$	41, 57, 74, 87, 143
11	9,12,15-Octadecatrienoic acid, methyl ester	18.216	20.07	$C_{19}H_{32}O_2$	41, 55, 67, 79, 95, 108
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.139	12.09	$C_{19}H_{34}O_2$	41, 55, 67, 81, 95, 109
19	Squalene	24.329	12	$C_{30}H_{50}$	41, 69, 81, 95
13	Methyl stearate	18.404	6.46	$C_{19}H_{38}O_2$	41, 43, 57, 74, 87, 298
12	Phytol	18.324	5.51	$C_{20}H_{40}O$	41, 57, 71, 95, 123
10	9-Octadecenoic acid (Z)-, methyl ester	18.182	4.69	$C_{19}H_{36}O_2$	41, 55, 69,74, 97, 98
18	Gamma-Sitosterol	23.982	4.53	$C_{29}H_{50}O$	41, 43, 57, 81, 95, 107, 145, 161, 213, 303, 414
4	4-Hexadecen-6-yne (Z)-	16.159	1.4	$C_{16}H_{28}$	41, 43, 67, 79, 93, 107
17	Stigmasterol	22.84	1.39	$C_{29}H_{48}O$	41, 55, 69, 83, 91, 105, 119, 133, 145, 159, 412
1	Methyl tetradecanoate	14.175	1.01	$C_{15}H_{30}O_2$	41, 57, 74, 87
8	Heptadecanoic acid, methyl ester	17.418	0.85	$C_{18}H_{36}O_2$	41, 57, 74, 87
14	Eicosanoic acid, methyl ester	20.26	0.85	$C_{21}H_{42}O_2$	41, 43, 57, 74, 87, 143
16	Docosanoic acid, methyl ester	21.961	0.67	$C_{23}H_{46}O_2$	41, 43, 57, 74, 87, 143
15	Phenol, 2-2'methylenebis[6-(1,1-dimethylethyl)-4-methyl-Cresol	21.285	0.45	$C_{23}H_{32}O_2$	57, 149, 164, 177, 340
6	Hexadecanoic acid, 14-methyl-, methyl ester	17.134	0.44	$C_{18}H_{36}O_2$	27, 41, 57, 74, 87
3	2-Pentadecanone,6,10,14-trimethyl-Hexahydrofarnesyl acetone	15.542	0.37	$C_{18}H_{36}O$	41, 43, 58, 71, 85
2	Pentadecanoic acid, methyl ester	15.307	0.18	$C_{16}H_{32}O_2$	41, 57, 74, 87
7	Cis-10-Heptadecenoic acid, methyl ester	17.206	0.18	$C_{18}H_{34}O_2$	27, 41, 55, 69, 97, 98

The 9, 12, 15-Octadecatrienoic acid, methyl ester was revealed in all plant parts, but the highest level was identified in fruit extract (48.78%). The hexadecanoic acid, methyl ester presented a major compound in all plant parts, but the highest level was revealed in stem-bark fraction (31.82%). Usually, these bioactive substances were associated with possible antibacterial and antioxidant properties. Recently, Shaaban et al 2021 reported that hexadecanoic acid methyl ester had effective antibacterial activity against multidrug-resistant bacteria. Another study reported that seed oil from *Lepidium sativum* exhibited broad-spectrum antimicrobial activity and showed antioxidant and anti-inflammatory activities (Alqahtani et al., 2019). *Lepidium sativum* seed oil was

found to contain both hexadecanoic acid methyl ester and 9,12-Octadecadienoic acid methyl ester (Alqahtani et al., 2019).

Table 3. GC-MS analysis showing the chemical constituents in fruit extract of n-hexane fraction

Peak No.	Compound	Retention time (RT)	Relative abundance (%)	Formula	Major mass spectral data m/z
6	9-12-Octadecadienoic acid (Z,Z)-, methyl ester	18.141	48.78	$C_{19}H_{34}O_2$	41, 55, 67, 81, 95, 294
2	Hexadecanoic acid, methyl ester	16.387	18.92	$C_{17}H_{34}O_2$	41, 57, 74, 87, 143
9	Methyl stearate	18.402	9.26	$C_{19}H_{38}O_2$	41, 43, **57**, **74**, **87**, **298**
7	9-Octadecenoic acid (Z)-, methyl ester	18.181	7.78	$C_{19}H_{36}O_2$	41, 55, 69, 74, 97, 98
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- methyl ester	18.214	6.29	$C_{18}H_{30}O_2$	55, 67, 79, 95, 108
10	11,14-Eicosadienoic acid, methyl ester	18.628	4.71	$C_{21}H_{38}O_2$	41, **55**, 67, 81, 95, 109
3	n-Hexadecanoic acid	16.851	2.24	$C_{16}H_{32}O_2$	**43**, 60, 73, 85, 129
11	Eicosanoic acid, methyl ester	20.251	1.05	$C_{21}H_{42}O_2$	41, 43, 57, 74, 87, 326
5	Heptadecanoic acid, methyl ester	17.416	0.61	$C_{18}H_{36}O_2$	41, **57**, 74, 87, 143
1	9-Hexadecenoic acid, methyl ester, (Z)	16.19	0.23	$C_{17}H_{32}O_2$	41, 55, 69, 74, 96
4	Hexadecanoic acid, 14-methyl-, methyl ester	17.039	0.13	$C_{18}H_{36}O_2$	41, 57, 74, **87**, **284**

In the leaves of pink flowered *c. roseus*, GC-MS analysis reported the presence of 9,12,15-Octadecatrienoic acid, methyl ester. Additionally, pink-flowered *C. roseus* exhibited higher levels of total flavonoid and phenolic content, which were linked to significant antioxidant activity (Rani & Kapoor, 2019). Recently, Ilozue et al., 2024 reported on the GC-MS profiling of wo herbal mixtures marketed for the treatment of Staphylococcus infection and typhoid and malaria. The study revealed the presence of 9-Octadecenoic acid (Z)-, methyl ester, among other active compounds. The presence of this compound, along other active compounds, helps to explain the various pharmacological activities of these herbal drugs.

GC-MS analysis of leaf extract of *Catharanthus roseus* and *Moringa oleifera* revealed the presence of 9-Octadecenoic acid (Z)-, methyl ester. The presence of this compound, along with other active compounds, has been associated with important antioxidant and antimicrobial activity (Syeda & Riazunnisa, 2020).

Additionally, GC-MS analysis has indicated a rich profile of fatty acid methyl esters in *S. incanum,* dominated by Hexadecanoic acid methyl ester (palmitic acid) and different octadecadienoic/trienoic acid methyl esters (linoleic/ linolenic acids) in all analyzed parts of the plant. Comparison of these results with other medicinal plants, and specifically with the Lepidium genus, adds to the potential bioactivity of the studied extracts. Accordingly, the main compounds identified in *S. incanum*, have a close similarity to the various Lepidium species reported for their pharmacological activities. For example, Hexadecanoic acid, methyl ester, which is a major component in the *S. incanum* stem-bark (31.82%), leaves (26.84%), and fruits (18.92%), has been reported as a major compound in *Lepidium sativum* seed oils by Getahun et al. (2020) and Akash

& Singh (2017), and Kumar et al. (2020). This is commonly known for its antimicrobial and anti-inflammatory activities (Shaaban et al., 2021; Alqahtani et al., 2019). Also, linoleic acid was a dominant constituent in *S. incanum* stem-bark (29.08%) and leaves (12.09%). This compound is also a major component of the *L. sativum* seed oil, as proved in different studies (Getahun et al., 2020; Kumar et al., 2020). 9,12,15-Octadecatrienoic acid, methyl ester (linolenic acid), dominant in *S. incanum* leaves at 20.07% and fruits at 6.29%, is also reported in *L. sativum* (Kumar et al., 2020).

Table 4. GC-MS analysis showing the chemical constituents in stem-bark extract of n-hexane fraction

Peak No.	Compound	Retention time (RT)	Relative abundance (%)	Formula	Major mass spectral data m/z
8	Hexadecanoic acid methyl ester	16.39	31.82	C17H34O2	41, 57, 74, 87, 143
11	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.142	29.08	C19H34O2	41, 55, 67, 81, 95
13	9,12,15-Octadecatrienoic acid, methyl ester	18.221	12.23	C19H32O2	79, 67, 95, 93, 108
15	Methyl stearate	18.406	7.85	C19H38O2	41, 43, 57, 74, 87, 143
20	Gamma-Sitosterol	23.981	4.63	C29H50O	41, 43, 57, 81, 95, 107, 145, 213, 303, 414
14	Phytol	18.325	2.57	C20H40O	41, 43, 57, 71, 123
12	9-Octadecenoic acid (Z)-, methyl ester	18.17	1.93	C19H36O2	41, 55, 69, 74, 97, 98
16	Eicosanoic acid, methyl ester	20.26	1.56	C21H42O2	41, 43, 57, 74, 87, 143
17	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl)-4-methyl-cresol	21.285	1.53	C23H32O2	41, 57, 91, 121, 149, 161, 177, 340
19	Stigmasterol	22.841	1.39	C29H48O	55, 69, 83, 97, 255, 412
1	Methyl tetradecanoate	14.18	0.95	C15H30O2	41, 55, 57, 74, 87
10	n-Nonadecanol-1	18.029	0.84	C19H40O	41, 55, 83, 97, 111
9	Heptadecanoic acid methyl ester	17.422	0.81	C18H36O2	41, **57**, **74**, **87**, **143**
3	5-Octadecenoic acid, methyl ester	15.145	0.55	C19H36O2	41, 67, 74, 96, 98
4	Pentadecanoic acid, methyl ester	15.31	0.45	C16H32O2	41, 57, 74, 87
18	Docosanoic acid methyl ester	21.96	0.44	C23H46O2	41, 43, 57, 74, 87, 143
5	2-Pentadecanone, 6,10,14-trimethylpentadecane	15.544	0.39	C18H36O	41, 43, **58**, 71, 85
6	Pentadecane	16.077	0.33	C15H32	41, 43, 57, 71, 85
7	9-Hexadecenoic acid, methyl ester, (Z)-methyl palmitoleate	16.195	0.33	C17H32O2	55, 69, 87, 98
2	Tetradecane	14.986	0.31	C14H30	41, 43, 57, 71, 85

Compounds in *S. incanum*, like Methyl stearate and Pentadecanoic acid, methyl ester, have also been recorded in *Lepidium cartilagineum* (Dávid et al., 2024). This comparative analysis underlines the fact that the high concentration of shared bioactive fatty acid esters between *S. incanum* and well-documented Lepidium species suggests a significant antioxidant and antimicrobial activities. Thus, the presence of such compounds known to contribute to the therapeutic effects of established medicinal plants reinforces the potential of S. *incanum* as a valuable source of natural bioactive agents.

Antimicrobial activity of different plant parts

In this study, the antimicrobial activity of four selected fractions of *Solanum incanum* was assayed *in vitro* by agar disc diffusion method against four bacterial species (*E. coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus*) and one fungus (C*andida. albicana*). The Fig. 4 showed results of antimicrobial activity of different fractions of each plant part (leaves, fruits and stem-bark). The chloroform, ethyl acetate, and n-hexane fractions of the leaves exhibited the broadest spectrum of inhibitory activity, with mean inhibition zones ranging from 14 to 18 mm against all tested bacterial strains and *C. albicans* , indicating strong antimicrobial potential. The three fractions of fruits (chloroform, ethyl acetate and n-hexane) had good activity against all microorganisms with inhibition zone ranged between 15–18 mm. Aqueous fraction of fruit was inactive against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Stem-bark aqueous fraction was partially active against *Staphylococcus aureus* and *Candida albicans.* The ethyl acetate and n-hexane fractions of stem-bark were active against all tested microorganisms. Chloroform fraction exhibited good activity against *Staphylococcus aureus* and C*andida albicana* only. The aqueous fraction of the strain was partially active against *Staphylococcus aureus* and *Candida albicans* and not active against the other microorganisms tested.

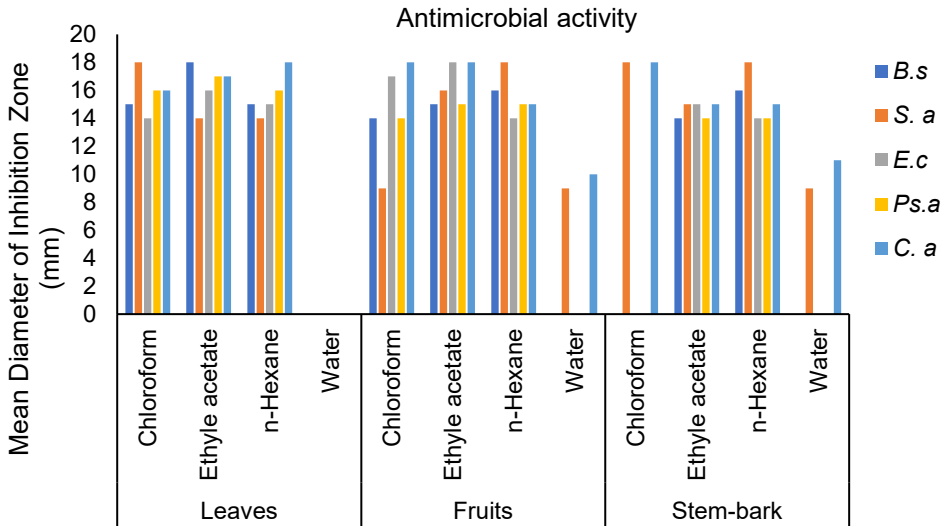

Figure 4. Antimicrobial activity of *Solanum incanum* against standard microorganisms.

(E. c.: Escherichia coli ATCC 25922; P. a.: Pseudomonas aeruginosa ATCC 27853; S. a.: Staphylococcus aureus ATCC 25923; B. s.: Bacillus subtilis ATCC 2836; C.a: Candida albicans). Mean diameter of growth inhibition zone in (mm) (conc. 2 mg/disc).

The current study found that ethyl acetate and n-hexane fractions of stem-bark were active against all microorganisms tested. Only *Staphylococcus aureus* and *Candida albicana* were susceptible to the chloroform fraction. The aqueous fraction of stem-bark was partially active against *Staphylococcus aureus* and *Candida albicans* but inactive against the other microorganisms tested. The significant inhibition zones produced by the ethyl acetate and n-hexane fractions, particularly from fruits and leaves, suggest the

presence of potent antimicrobial compounds. While the disc diffusion method effectively identifies bioactive extracts, future research is essential to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of these promising fractions to fully quantify their antibacterial and antifungal efficacy.

Solanum incanum has been shown in several studies to have antimicrobial activity (Karanja et al., 2021). Microbial activity in *Solanum incanum* varied according to plant part and extract fraction. In 2017, Sahle & Okbatinsae discovered anti-fungal and antibacterial activity in ethyl acetate, ethanol, chloroform, petroleum ether, and water fruit extracts. Ethyl acetate, ethanol, and chloroform fruit extracts outperformed aqueous extracts. In the present study, the aqueous fraction was less effective than the chloroform, ethyl acetate, and n-hexane fractions in leaves, stem-bark, and fruits. While in stem-bark and fruits, the aqueous fraction was partially active against *Staphylococcus aureus* and *Candida albicans*. The efficacy of fruit extracts is consistent with previous reports; for instance, Karanja et al. (2021) demonstrated their activity against plant pathogens, while Indhumathi & Mohandass (2014) reported potent antimicrobial activity in a crude ethanol extract. The crude ethanol extract showed the highest antibacterial activity (26 mm) against *Staphylococcus aureus* and also showed good antibacterial activity (10–25 mm) against all pathogenic bacteria.

The hydroacetone extract was also reported to be very efficient for the prevention and treatment of microbial diseases in poultry. (Séré et al., 2022). Furthermore, Karanja et al reported that *S. incanum* had remarkable antimicrobial activity (Karanja et al., 2021). Additionally, Ateshim et al. found that the chloroform extract from *S. incanum* roots revealed important antibacterial activity against *S. aureus* (Ateshim, & Tekle, 2022). In a study by Jepkoech & Gakunga, methanolic extracts from *S. incanum* fruits displayed greater antibacterial activity compared to aqueous extracts against *S. aureus* (Jepkoech & Gakunga, 2017). In 2021, Musyimi et al. reported that seed ethanoic extract was the most effective for antimicrobial activity against *E. coli* and *S. aureus*. Furthermore, in 2024, Melliti et al., reported that stem and leaf methanolic extract of tunisian *Pancratium maritimum* was rich in alkaloids and showed excellent antibacterial and antifungal potentials within its alkaloid fractions (Melliti et al., 2024).

Antioxidant Activity

DPPH was used to measure the antioxidant activity of different fractions. Strong antioxidant activity was demonstrated by various fractions of ethanolic extracts of leaves, stem bark, and fruits. Among all tested fractions, the ethyl acetate fractions of fruits (89 ± 0.01%) and leaves (86 ± 0.07%) exhibited the highest antioxidant activity (Fig. 5). The aqueous fraction of fruits (83 ± 0.01) and leaves (85 ± 0.01) also showed significant antioxidant activity. Whereas, the stem-bark's n-hexane fraction had the lowest antioxidant activity (09 ± 0.0). The ethyl acetate fraction of fruits and leaves showed the best antioxidant activity, which can be attributed to the higher content of polyphenolic compounds detected in leaves and fruits (Table 1). Škrovánková et al found a strong positive correlation between free polyphenols levels and antioxidant capacity in pseudocereals such as amaranth, buckwheat, and quinoa (Škrovánková et al., 2020). Additionally, Chivodze et al, demonstrated that ethyl acetate fraction of leaves exhibited a high reducing antioxidant capacity, which was associated with the presence

of high level of polyphenols in leaves (Chivodze et al., 2021). Leaf and fruit extracts contained high levels of flavonoids (Table 1).

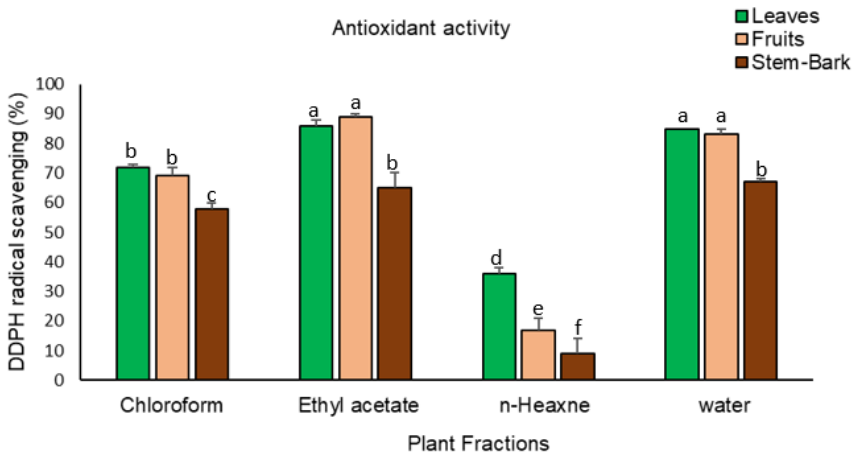

Figure 5. *In vitro* antioxidant activity of different *Solanum incanum* fractions measured by DPPH radical scavenging assay. Data are presented as mean % scavenging ± standard deviation (n = 3). Different lowercase letters above the bars indicate statistically significant differences among the fractions as determined by ANOVA followed by Tukey's HSD test ($p < 0.05$).

To quantitatively establish the relationship between phytochemical composition and antioxidant efficacy, a Pearson correlation analysis was done. The resulting matrix, shown in Table 5, indicates an extremely strong positive correlation between the total phenolic content and the antioxidant activity across most fractions, especially for the chloroform and water fractions, with r = 0.999 and 0.991, respectively.

Table 5. Pearson correlation matrix (r-values) between the antioxidant activity of solvent fractions and the phytochemical content of *Solanum incanum* extracts. Abbreviations: AOX: Antioxidant activity; CHF: Chloroform fraction; EA: Ethyl acetate fraction; HEX: n-Hexane fraction; W: Water fraction; POLY: Total polyphenols; FLAV: Total flavonoids; TANN: Total tannins; ALK: Total alkaloids. Values of |r| > 0.87 indicate a very strong positive correlation ($p < 0.05$)

Variable	AOX-CHF	AOX-EA	AOX-HEX	AOX-W	POLY	FLAV	TANN
AOX-EA	0.949						
AOX-HEX	0.853	0.645					
AOX-W	0.995	0.977	0.794				
POLY	**0.999**	0.938	0.869	0.991			
FLAV	0.995	0.912	0.902	0.979	0.998		
TANN	0.996	0.916	0.897	0.981	0.998	1.000	
ALK	0.895	0.710	**0.996**	0.844	0.909	0.936	0.932

Similarly, flavonoid and tannin content showed very strong associations, establishing that these polyphenolic compounds are the major contributors to the radical scavenging activity of *S. incanum*. The sole notable exception was the n-hexane fraction, whose antioxidant activity was less strongly linked with polyphenols but very strongly associated with alkaloid content (r = 0.996), suggesting a solvent-dependent bioactivity

of non-polar alkaloids as dominant antioxidants in the n-hexane fraction, while polyphenols were dominant in more polar fractions.

Flavonoids are phenolic substances derived from a variety of vascular plants that act as antioxidants and antimicrobials (Hassanpour & Doroudi, 2023). Akanmu et al. 2021 reported that aqueous and methanolic extracts from the fruits of *S. incanum* had significant DPPH radical scavenging activity, especially the aqueous extract. Current study revealed too, that leaf and fruit extracts had the highest tannin content referring to stem-bark (Table 1). The high level of alkaloids in fruits and leaves could be related too to the high antioxidant capacity in these plant parts (Table 1). Antasionasti et al. suggested that antioxidant activity is strongly influenced by the tannin, total flavonoid, and total phenolic contents (Antasionasti et al., 2021).

CONCLUSIONS

Current results indicated that methanolic extracts of various plant parts of *S. incanum* collected from the Al-Baha region are rich in polyphenols, flavonoids tannins and alkaloids, especially leaves and fruits. In addition, GC-MS analysis revealed the presence of two major compounds across all plant parts: the 9, 12, 15-Octadecatrienoic acid, methyl and the hexadecanoic acid, methyl ester. The presence of these compounds, along with other active constituents, explains the diverse pharmacological activities of the studied plant. Interestingly enough, high levels of total phenolic, flavonoid, tannin, and alkaloid content, especially in the leaves and fruits correspond to the significant antimicrobial and antioxidant activities observed in these parts. In general, the strong inhibitory activity observed against several pathogens positions the leaf and fruit extracts, especially in ethyl acetate and n-hexane fractions, as prime candidates for further isolation of active compounds and determination of their MIC)/ MBC values.

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