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**Research Paper**

# Chemical Profiling and Bioactivities of *Solanum nigrum* L. and *Solanum melongena* L.: A Comparative Assessment of Two Solanaceae Species

**Atiya Batool[1], Ajwa[1], Saadyia Zia[2], Iqra Saleem[1], Fatma Hussain[1]***

[1]*Department of Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Biochemistry, Postgraduate Agricultural Research Station (PARS), University of Agriculture, Faisalabad, Pakistan*
*Corresponding author's email: fatmah@uaf.edu.pk

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## Abstract

Plants play a vital role in health and beyond traditional medications; these are part of modern pharmaceutical landscape. The present study was designed to compare and contrast phytochemistry and bioactivities of *Solanum nigrum* (SN) and *Solanum melongena* (SM); two species of Solanaceae family. Aqueous leaf extracts were used to assess total phenolic contents (TPC) and total flavonoid contents (TFC), free radical scavenging activity, α-amylase inhibition (antidiabetic activity), antimicrobial and cytotoxic activities along with Chemical profiling by Fourier transform infrared spectroscopy (FTIR). TPC in SN and SM extracts were 32.73 ± 2.08 mg GAE/100g and 43.98 ± 0.12 mg GAE/100g ($p<0.05$) respectively. TFC of SN extract was 69.69 ± 0.538mg CE/100g and TFC of SM was 89.54 ± 0.19mg CE/100g ($p<0.05$). SN and SM extracts showed 70.52 ± 0.73 % and 74.13 ± 0.60 % ($p<0.05$) free radical scavenging capacity respectively. In antimicrobial assay, SN showed antimicrobial activity against *E. coli* with 9mm area of growth inhibition and 12 mm inhibition zone was observed against *S. aureus*. Meanwhile, SM showed 9 mm of inhibition zone against *E. coli* as well as 9.3mm of inhibition zone against *S. aureus*, which indicates the presence of antimicrobial potential. SN and SM exhibited 28.64 ± 3.22% and 28.2 ± 2.21% inhibition of amylase enzyme ($p>0.05$). Aqueous extract of SN showed 10.76% hemolytic activity ($p<0.05$). Aqueous extract of SM being less toxic showed 5.27% hemolytic activity and positive control had 94.87% hemolytic activity. FTIR spectra confirmed the presence of diverse secondary metabolites like phenols, alcohols, alkenes, carboxylic compounds. Both species of Solanaceae family exhibited analogous anti-diabetic and anti-microbial activities. These preliminary findings contribute to existing data, but further validation through animal trials is necessary to establish their medicinal applications.

## Introduction

Bioactive compounds from natural products have been used for centuries in alternative medicine and hold significant potential for developing new treatments for various diseases. Phytochemicals have gained considerable attention in recent years and approximately 75%-80% of people worldwide still rely on herbal medicines (Karahan, 2023).

The World Health Organization has reported that 74% of pharmacologically active medicines are derived from natural sources (Shaheen et al., 2023). Pakistan is 8th in the world in the production of medicinal plants. Over seventy-five percent of Pakistanis depend on medicinal plants for treatment (Arshad et al., 2024). Tomatoes, potatoes and eggplants are members of the *Solanaceae* family, which is one of the largest Angiosperm plant groups. Numerous species have attracted researchers' attention due to their medicinal properties and potential toxicity. Hyoscyamine and scopolamine are the primary pharmacological compounds found in such plants (Zhang et al., 2023). Natural flavonoids with antioxidant, anti-inflammatory, and hypoglycemic effects are potential therapeutic agents for diabetes.

Numerous secondary metabolites are found in different plant parts (Bernela et al., 2023). *Solanum nigrum* L. (SN) known as black nightshade (Makoh khushk) is commonly grown wild or cultivated in temperate, subtropical and tropical temperature zones. With a long history of usage as a Chinese folk medicine for antipyretic and diuretic reasons, reports have mentioned the biological role of crude extracts and chemicals derived from SN against cervical cancer, hepatic large intestine and breast cancers. Phytochemical research on SN revealed the presence of saponins, amide alkaloids and phenolics that might operate as antiproliferative, hepatoprotective, anti-inflammatory, antiulcerogenic, antioxidant and neuroprotective agents (Deng et al., 2023). *Solanum melongena* (SM), often known as brinjal (Baingan) is an edible fruit that is widely utilized in numerous cuisines. It is commonly grown in tropical, Asian and European regions. SM is well-known for its nutritional value as well as its therapeutic benefits. It contains anthocyanins, phenols, steroids, tannins, glycosides, triterpenes, sugars and glycoalkaloids in high concentrations (Mansurat et al., 2023). It is an excellent source of nutrition for persons suffering from diabetes and liver disease as it has antidiabetic, antioxidant and antimicrobial effects (Khalawi, 2022).

Fruits of SN and SM are regularly consumed in Pakistani cuisines (Arshad et al., 2024) while other parts of these plants especially leaves are either used in homeopathy or wasted during

domestic use. Anatomy of leaves in both species is quite similar (Okeke et al., 2020). Although a few studies have explored the medicinal potential of these plants (Ramesh et al., 2015; Okeke et al., 2020; Mansurat et al., 2023), data on antioxidant, antimicrobial, antidiabetic and hemolytic efficacy is limited. A comprehensive assessment of their role in preventing and treating microbial infections, oxidative stress, and diabetes is necessary to bridge this knowledge gap. It is a hypothesis that one of the specie will exhibit better bioactivities than the other. Therefore, research was planned to compare the bioactivities of SN and SM leave extracts using different bio-analytical techniques. Comparative investigation will give a better understanding of phytoconstituents, their antioxidant, anti-microbial, antidiabetic and cyto-protective effects.

## Materials and Methods

Leaves of *Solanum nigrum* and *Solanum melongena* were taken from local areas of Farooq Abad (Latitude: 31.7333° N, Longitude: 73.9833° E), Sheikhupura city. Sample authentication (physical: manually check for accuracy, physical appearance) was done at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The leaves were thoroughly washed, shade-dried and ground into a fine powder. The sample was mixed with distilled water (1:10 ratio) and placed on shaking water bath (50 rpm at 58$^{o}$C) to facilitate the extraction of bioactive compounds. Afterward, the mixture was filtered with Whatman paper and the filtrate was left at room temperature until it formed a semisolid consistency. The *S. nigrum* aqueous (SNA) and *S. melongena* aqueous (SMA) extracts were used for further experiments (Noreen et al., 2020).

## Phyto-chemistry

Total phenolic content (TPC) as mg gallic acid equivalent (GAE)/100g was determined by using Folin-Ciocalteu (FC) reagent. Sodium carbonate (100 µL, 7%), 125 µL test samples (S) and 25 µL FC reagent were incubated for two hours at ambient temperature. The absorbance was measured at765 nm. For total flavonoid content (TFC), plant extract (38 µL), 9.5 µL $NaNO_2$ and 156 µL distilled water were mixed and incubated (10 minutes) at ambient temperature. Then 19 µL (10%) $AlCl_3$ was added and incubated for 5 minutes. Absorbance was noted at 510 nm and TFC were expressed as mg catechin (CE)/100g. TPC, TFC results were compared against standard curves. For antioxidant activity, 250 µL DPPH (2,2-diphenyl-1-picrylhydrazyl) solution and 2.5 µL sample were incubated for 30 minutes with ascorbic acid as control (C). Absorbance at 570 nm

was measured to calculate percentage antioxidant activity as: [A(C) – A(S)/A(C)] x 100 (Hussain et al., 2021).

**FTIR-based Fingerprinting Analysis**

In Fourier transform infrared spectroscopy (FTIR), the samples were finely powdered with potassium bromide, subjected to extreme pressure (5-15 tons/cm$^2$) using compression in the die zone till the pellet was formed. The identification of bioactive compounds was done by Bruker Tensor 27 FTIR spectrometer in the range of 400-4000 cm$^{-1}$ (Raza et al., 2024).

**Antimicrobial activity**

In agar well diffusion method, plant extract was reconstituted in dimethyl sulfoxide (DMSO) solution (5mg extract in 1mL DMSO). In petri plates with nutrient agar (4mm thick), wells were created and 100 µL (105 CFU/mL) inoculum (bacterial strains: *Staphylococcus aureus*, *Escherichia coli*) was added. Then 100µL test samples (2.5mg/mL) were poured. Ciprofloxacin was positive control. After 18 hours incubation period, diameter (mm) of growth inhibition zone was measured (Raza et al., 2024).

α -**Amylase Inhibition Assay**

To determine antidiabetic potential, 30 µL test sample (S) and acarbose (positive control sample) were incubated at room temperature for 10 minutes with 10 µL α- amylase (source: *Bacillus subtilis*) solution. Then substrate 40 µL (1% starch) was mixed for incubation (30 minutes). Subsequently, 20 µL of 1 M HCl and 75 µL iodine solution were added. Phosphate buffer saline (PBS) was negative control (N-C). The absorbance at 580 nm was used to estimate percentage enzyme inhibition by the formula: [A (N-C) – A (S)]/A (N-C)] × 100 (Ali et al., 2022).

**Hemolytic Assay**

Cytotoxic effects were determined by hemolytic assay (Kauser et al., 2018). Blood was centrifuged for five minutes and washing was done with 5mL PBS thrice to isolate red blood cells (RBC). Then, RBC (180 µL) and plant extract (20 µL) were combined and the supernatant (100 µL) was diluted with 900 µL cold PBS after 5 minutes of centrifugation. Triton X-100 was positive control (P-C) and PBS as the negative control (N-C). At 576 nm, the absorbance (A) was noted to assess percentage inhibition of RBC hemolysis: [A(S) – A (N-C)/A (P-C)] × 100

## Statistical analysis

Data (n=3) was expressed as mean ± S.D. To estimate significance, unpaired, one-tailed T-test was used by using Minitab statistical software version 17 with 95% level of significance.

## Results

Percentage yields of SN and SM extracts in aqueous solvent were 14.14% and 22.6% respectively. Results are presented in table 1. Significant TFC, TPC and antioxidant results were observed in SMA as compared to SNA sample. Antimicrobial activities of SNA and SMA are shown in fig 1(A, B) and table 1.

Table 1 Phytochemistry, antidiabetic, antimicrobial and cytotoxic activities of *Solanum nigrum and Solanum melongena* leave extracts

| **Sample** | **Phytochemistry** | | | **Antidiabetic activity** | **Cyto-toxicity** | **Anti-microbial activity** | |
|---|---|---|---|---|---|---|---|
| | **TFC** | **TPC** | **DPPH** | | | ***E. coli*** | ***S. aureus*** |
| **SNA** | 69.69 ± 0.538 | 32.73 ± 2.08 | 70.52 ± 0.736 | 28.64 ± 3.22 | 10.7 6±0.68* | 9 | 12 |
| **SMA** | 89.54 ± 0.19* | 43.98 ± 0.12* | 74.13 ± 0.60* | 28.2 ± 2.21 | 5.27 ± 0.68 | 9 | 9.3 |
| **Control** | - | - | 90.16 ± 0.00 | 79.05 ± 0.00 | 94.87 ± 0.00 | 27 | 28 |

Data are represented as mean ± SD or percentage (n=3). * Significant at $p < 0.05$. SNA: *S. nigrum* aqueous, SMA: *S. melongena* aqueous, TPC: total phenolic contents (mg gallic acid equivalents [GAE]/100 g dry weight); TFC: total flavonoid contents (mg catechin equivalents [CE]/100 g dry weight); percentage DPPH: 2, 2-diphenyl l-picrylhydrazyl. Antimicrobial activity (mm inhibition of microbial growth, Hemolytic activity expressed as percent hemolysis of red blood cells, Antidiabetic activity expressed as percent α-amylase inhibition. Positive control: Ascorbic acid (DPPH activity), Ciprofloxacin (antimicrobial activity), Triton-X 100 (hemolytic activity), Acarbose (antidiabetic activity)

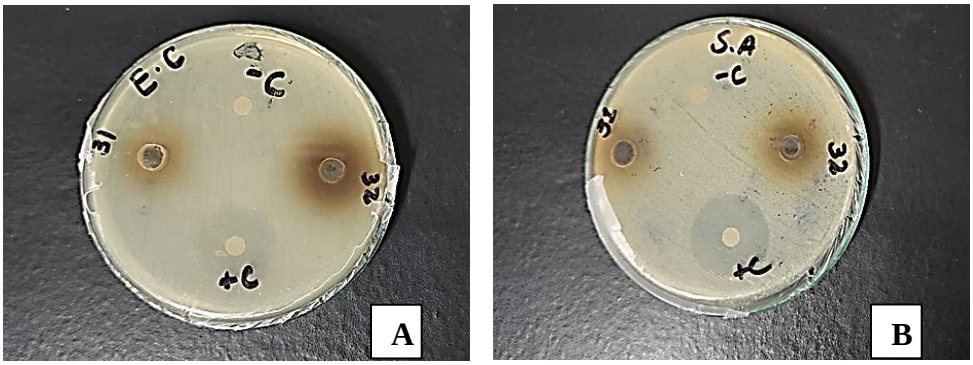


Figure 1 Antimicrobial activity of *S.nigrum* (A) and *S.melongena* (B) against *E. coli* (E.C) and *S. aureus* (S.A)

+C: positive control Ciprofolxacin, -C: negative control DMSO (dimethyl sulfoxide**)**

**Fourier transformed infrared spectroscopy**: Both cultivars SN and SM were structurally characterized using FTIR, to identify the bioactive chemical groups.

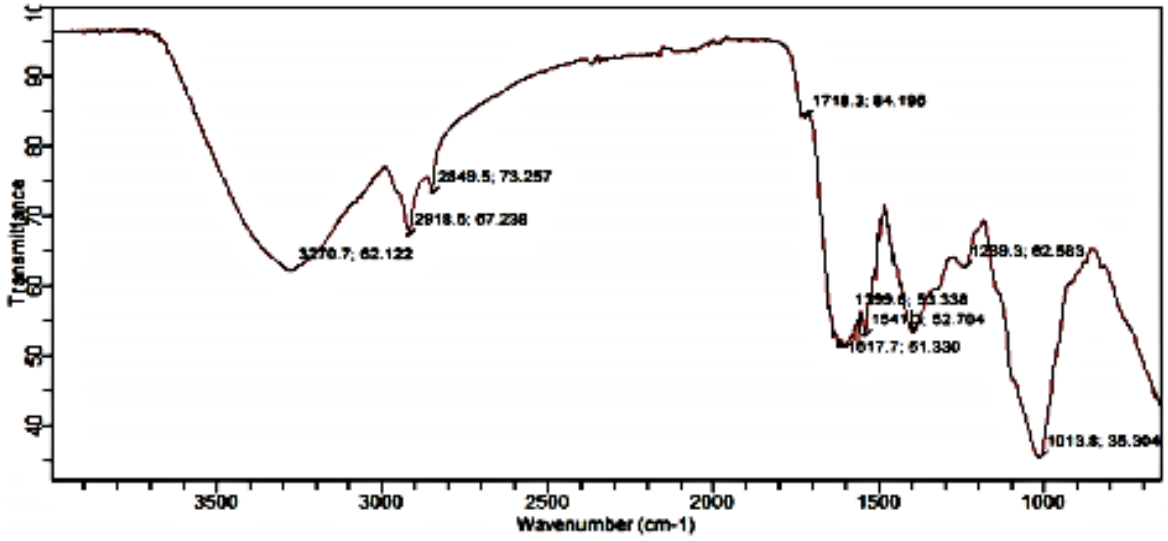


Figure 2 FTIR Spectra of *Solanum nigrum*

FTIR spectra of SN leaf ranges from 3270.7 $cm^{-1}$ to 1013.8 $cm^{-1}$ (Fig 2). Different broad band with prominent absorption represent stretching vibration of different primary and secondary molecules (Table 2).

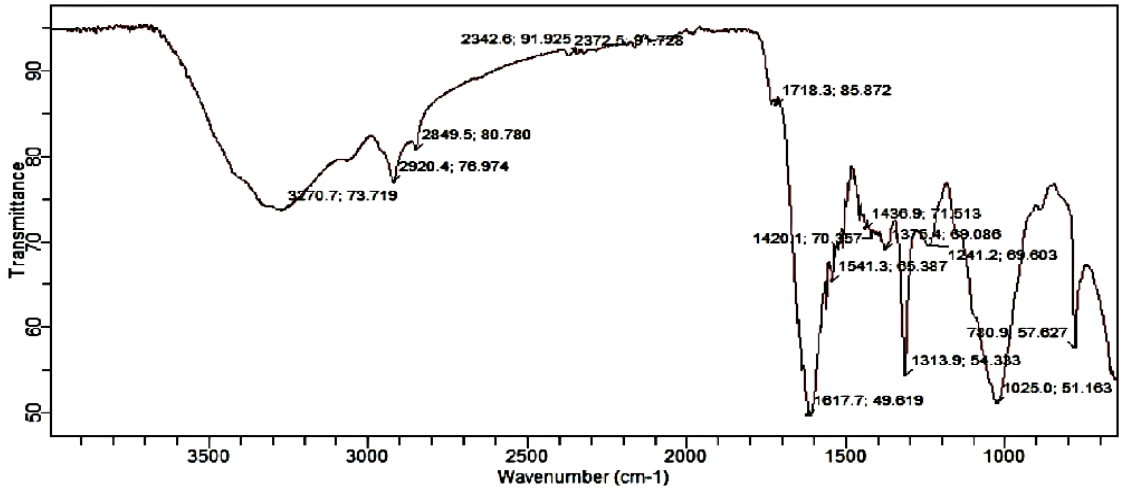


Figure 3 FTIR Spectra of *Solanum melongena*

FTIR spectra of SM ranges from 3270.7cm$^{-1}$ to 1541.3 cm$^{-}$1 (Fig 3). Broad and streching peaks spectra shows presence of different functional groups (Table 2).

Table 2 Functional groups in *Solanum nigrum* and *Solanum melongena* leaves

| ***Solanum nigrum*** | | | | ***Solanum melongena*** | | |
|---|---|---|---|---|---|---|
| **Sr. no** | **Absorption** | **Compound** | **Functional group** | **Absorption** | **Compound** | **Functional group** |
| 1 | 3270.7 | Alcohol, phenols, Carboxylic acid | O-H C-H | 3270.7 | Alcohol, phenols, Carboxylic acid | O-H |
| 2 | 2918.5 | Alkyne Alkanes, carboxylic acid Amine salt | C-H N-H | 2920.4 | Alkyne Alkanes, Carboxylic acid Amine salt | C-H O-H N-H |
| 3 | 2849.5 | Aldehyde, alkane, Carboxylic acid. | C-H | 2849.5 | Aldehyde, Carboxylic acid | C-H O-H |
| 4 | 1718.3 | Ketone, Carboxylic acid α,β-unsaturated ester | C=O | 2342.6 | Alkanes | C-H |
| 5 | 1617.7 | Alkene, α,β-unsaturated ketone | C=C | 2372.5 | Alkanes | C-H |

| *Solanum nigrum* | | | | *Solanum melongena* | | |
|---|---|---|---|---|---|---|
| **Sr. no** | **Absorption** | **Compound** | **Functional group** | **Absorption** | **Compound** | **Functional group** |
| 6 | 1541.3 | Nitro compound | N-O | 1718.3 | Ketones α,β-unsaturated ester | C=O |
| 7 | 1399.6 | Fluoride | C-X | 1420.1 | Carboxylic acid, Alcohol | C-H O-H |
| 8 | 1239.3 | Alcohol, ethers, Easter, carboxylic acid, anhydride. | C-O, C-N, C-X | 1617.7 | Alkene | C=C |
| 9 | 1013.8 | Fluoride | C-X | 1541.3 | Nitro compound | N-O |

## Discussion

### Antioxidant activity

TPC in SNA extract was 32.73 ± 2.08 mg GAE/100g and 43.98 ± 0.12 mg GAE/100g for SMA. Statistical analysis indicates highly significant difference ($p<0.05$) in antioxidant results of SNA and SMA samples. SM exhibited higher antioxidant activity than SN due to its chemical moieties like phenolic, flavonoid, keto, ester and alcoholic groups as observed during FTIR analysis**.** Similar to current results, TPC value of SN leaves aqueous extract was 35.73 ± 2.52 mg GAE/100g previously (Yimer et al., 2023). Another study however, reported higher TPC (2454.9 ± 135 mg GAE/100g) in aqueous extract of SM (Contreras-Angulo *et al*., 2022). TFC of SNA extract was 69.69± 0.538mg CE/100g and TFC of SMA was 89.545± 0.19mg CE/100g. Contrary to these results, 0.85 ± 0.03 to 11.25 ± 0.01 mg CE/100g TFC was observed by Yimer et al. (2023). While another study reported 39.3 ± 7 mg CE/100g TFC in aqueous extract of SMA (Contreras-Angulo et al., 2022). Regarding antioxidant activity, SNA and SMA extracts showed 70.52 ± 0.73 % and 74.13 ± 0.60 % free radical scavenging capacity respectively. Which were quite less as compared to that showed by positive control (89.63%). In previous studies by Gasti et al., (2020) and Asante et al., (2024), 28.12% and 42.86 - 55.80 mg/mL DPPH radical scavenging activities of SN and SM were reported respectively.

## Antimicrobial Activity

Positive control (Ciprofloxacin) had 27mm and 28 mm zones of inhibitions against *E. coli* and *S. aureus* respectively. SNA showed antimicrobial activity against *E. coli* with 9mm area of growth inhibition and 12 mm inhibition zone was observed against *S. aureus*. Meanwhile, SMA showed 9 mm of inhibition zone against *E. coli* as well as 9.33mm of inhibition zone against *S. aureus.* SNA exhibits slightly better antimicrobial activity due to its alkaloid content and specific phenolic compounds as shown in FTIR data. These are weak inhibitions, suggesting that samples have antimicrobial potential, but not capable to inhibit the microbial growth completely. Additionally, higher sample doses may have strong bactericidal effects. These patterns suggest that samples don't have clinical application against infectious diseases. It can be used in combination therapy along with synthetic antibiotics or for food preservation processes. Different factors like concentration of sample (as higher concentration may give better growth inhibition), variable levels of susceptibility shown by different strains and experimental conditions also affect outcomes. Previously reported data was inhibitory zone by SNA against *Escherichia coli* as 7.33 mm and 16.33 mm for *S. aureus* (Singh et al., 2023). Dunkwu-Okafor et al., (2020) stated that the inhibition zone of SMA against *Escherichia coli* was 5.67 mm and for *S. aureus* 6.67 mm.

## Antidiabetic activity (Alpha-amylase Inhibition Assay)

Almost analogous results were shown by both plants. SNA and SMA exhibited 28.64±3.22% and 28.2±2.21% inhibition of amylase enzyme action. Whereas, positive control showed 79.06% inhibition. Statistical analysis indicates highly non-significant difference ($p>0.05$) was observed in amylase inhibition assays of SNA and SMA. It is suggested that samples have moderate enzyme inhibition potential but were unable to block enzyme action completely. FTIR analysis has revealed different chemical entities with potential enzyme inhibition capacity. However, various rate limiting factors such as reactivity, stability, conformation, steric hindrance of functional groups at the active site of enzyme and phytoconstituents, along with solvent type and extraction method applied can reduce the reaction. These samples can be used to regulate blood glucose level especially in high glycemic index starchy foods. Earlier, 73.15% amylase inhibition by SN and 40.11% inhibition by SM were observed (Oluwagunwa et al., 2021) which are higher than current results.

## Cytotoxic activity (Hemolytic Assay)

Aqueous extract of SN showed 10.76% hemolytic activity, aqueous extract of SM showed 5.27% hemolytic activity and positive control had 94.87% hemolytic activity. Statistical analysis indicates highly significant difference ($p<0.05$) was observed in hemolytic assay of *Solanum nigrum* and *Solanum melongena*. Phenols as identified in chemical profiling (FTIR) can cause hemolysis by binding to cell membranes. It is assumed that higher doses can lead to higher erythropoiesis. Safety and efficacy studies are required to identify safer dose for therapeutic purpose. Alternate formulations like nanoencapsulations are suggested. Kumar *et al*. (2016) performed biocompatibility test in terms of erythrocyte hemolysis, confirmed 1.34% cell lysis potential of SN. Similar inferences were shared in another study that SM did not cause hemolysis and therefore it is not toxic towards red blood cells (Khalawi, 2022).

## Fourier transformed infrared spectroscopy

FTIR analysis show different compounds like phenols, carboxylic, aldehydes, ketones, esters, ether and amino compounds which correlate with different bioactivities. Further research is required for the specification and quantitative measurement of these compounds. The discrepancies in FTIR spectra across studies could be due to differences in solvent selection, extraction conditions (temperature, pH, and duration), sample preparation methods, and plant part used. Akhtar et al., (2024) observed that FTIR spectra of *Solanum nigrum* extracts had peaks at 3323 and 3685 $cm^{-1}$ due to stretching of OH group. Another study showed peak at 1404 assigned carboxylic side in amini redidue having streaching symmetry. The band at 1209 $cm^{-1}$ indicated C-N and band at 1126, 1030 and 605 $cm^{-1}$ bandes of carbonate. While in current study it indicated halide, easter, ether and anhydride (Ramesh et al., 2015). Study observed FTIR spectrum of SM expose peak on 3200-3600 $cm^{-1}$ indicate OH and $NH_3$ stretching vibration. Band at 2921 $cm^{-1}$ showed methyl group CH with symmetrical stretching vibration. Another peak at 1610 $cm^{-1}$ indicated carbonyl group and ketones. Other bands at 1026 and 1086 attributed asymmetric C-O-C stretching band. All these observations indicated various functional groups like hydroxyl, amine, carboxyl etc. (Emeribe & Ogbuehi, 2024).

Limitations of the current research include one solvent (aqueous) and *in vitro* bioanalytical techniques. More comprehensive understandings demand use of whole plants, different solvents, multiple extraction techniques and *in vivo* trial.

## Conclusion

In context of main objective to compare both species, antioxidant contents and activity of *S. melongena* was better than *Solanum nigrum* that has potential against oxidative stress induce diseases. Both the species of Solanaceae family exhibited analogous anti-diabetic and anti-microbial activities along with similar chemical fingerprints that are responsible for their medicinal properties. However, *S. melongena* was slightly less toxic as compared to *Solanum nigrum*. These are preliminary assessments that have contributed to the existing data but in real-world scenario, animal trials can better reflect their application in medicine.

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