COMPARATIVE ASSESSMENT OF BIOLOGICAL ACTIVITIES OF *SESAMUM INDICUM* AND *SESAMUM RADIATUM* EXTRACTS AND CHEMICAL CHARACTERIZATION.

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ABSTRACT: Current research focuses on investigating the medicinal properties of the aqueous extract obtained from the seeds of *Sesamum indicum* and *Sesamum radiatum*. Various tests were conducted to evaluate the antioxidant, antidiabetic, antimicrobial and hemolytic activities of both plants. The extraction yields for *S. indicum* and *S. radiatum* seed extracts were found to be 21.87% and 44.63%, respectively. Both *S. indicum* and *S. radiatum* was the most active antioxidant and showed maximum total phenolic content (55.97±2 and 65.28±1.1 mg GAE/100 g), total flavonoid content (64.0±0.4 and 68.8±1.9 mg CE/100 g) and free radical scavenging activity (46.26% and 36.62 %) respectively. Both plants showed no antibacterial activity, with a zone of inhibition of 0 mm against both *E. coli* and *S. aureus*. For alpha-amylase inhibition, the aqueous extract of *S. indicum* showed a mean inhibition of 37.13%, while *S. radiatum* showed 33.02%. The % hemolysis of *S. indicum* and *S. radiatum* was 4.231±1.35 and 5.790±1.35 respectively. The extracts were chemically characterized using FTIR. Every activity's results were reported as mean ± S.D and the T-test was used to determine whether the results were statistically significant. However, further research is required to comprehensively assess their therapeutic properties and explore their potential applications.

Keywords: Aqueous extracts, antidiabetic, antimicrobial, antioxidant, FTIR.

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INTRODUCTION

Using plant materials for therapeutic reasons, such as seeds, berries, roots, leaves, bark, or flowers, is known as herbal medicine, sometimes known as botanical medicine or phytomedicine. The goal of antimicrobial research is to develop new antibacterial and antifungal substances since natural remedies are more benign and have fewer side effects (Koshta and Sharma, 2023). Schistosomiasis, a neglected parasitic worm disease affecting 200 million people globally, is primarily found in deprived communities lacking safe drinking water and sanitation. The WHO suggests developing alternative drugs like praziquantel and incorporating herbal remedies in mainstream clinical practice (Asanti-Kwatia *et al.*, 2023). Medicinal plants contain biologically active ingredients like terpenoids, alkaloids, glycosides and volatile oil used for treating various infections with low side effects and low cost (Alqahtani and Sahat, 2022).

Sesamum indicum L. is an important oilseed plant that has been grown for 5000 years in subtropical Asia and Africa. It is utilized in food, tahin and animal feed. Sesame seeds are stable and have a long shelf life due to their high oil content, antioxidants, and protein content. They can promote the growth of mushroom mycelium and are rich in minerals and amino acids (Talebi *et al.*, 2023). Sesame is beneficial for maintaining vascular endothelial function, adipocytokine balance, blood pressure and platelet aggregation. Ancient Chinese used it to boost vitality, soothe the mind, and delay ageing. It has estrogenic, anti-inflammatory and heart-protective properties and contains lignans, phenolic compounds, protein, and fat (Hadipour *et al.*, 2023). Sesame offers medicinal benefits, such as hepatoprotection, hypocholesterolemia and anti-cancer capabilities. To cure injuries and avoid malnutrition, it provides vital micronutrients like calcium, potassium and phosphorus. To relieve pain, use sesame oil (Nagar *et al.*, 2022). The phytochemical sesame, which is abundant in substances like sesamin, tocopherol and fatty acids, has a substantial nutritional benefit as a source of protein and an edible oil. Due to its nutritional content and health benefits, its commercial value has increased (Yaseen *et al.*, 2021).

Sesamum radiatum (black sesame) is a leafy vegetable grown in Asia and Africa that is high in polyunsaturated fats, vitamins, minerals and antioxidants. It also has a high oil content (Gbonjubola and Francis, 2023). Seeds and seed oil contain phytoconstituents like carvacrol, sesamol, eicosanoic acid, palmitic acid, oleic acid, ricinoleate and flavonoids (Astalakshmi *et al.*, 2022). Its leaves are rich in phenolic compounds, high in macro- and micronutrients, protein and antioxidants. n-hexadecanoic acid, which is beneficial for bruising,

fungal and bacterial infections, male infertility, constipation and cardiovascular and oestrogenic action, is present in the essential oil extracted from dried leaves. The bactericidal potential of the leaves has been investigated (Narzary *et al.,* 2023). This research indicated that aqueous extract of *Sesamum indicum* and *Sesamum radiatum* has extraordinary pharmacological activities and in-depth studies are required to further evaluate the remarkable properties and therapeutical properties of these medicinal plants.

EXPERIMENTAL:

Collection and Identification of Plant Materials Collection of the seeds were done from the local market. Certification and the checking of both plants were done from the Botany Department, UAF.

Preparation of Samples: The seeds of *S. indicum* and *S. radiatum* were collected, dried and ground into fine powders. Distilled water was used as a solvent for extraction. Samples were placed in beakers and the aqueous solvent was preserved for three days. After filteration, semi-solid samples were stored in falcon tubes for further experiments (Abubakar and Haque, 2020).

Total Phenolic Content (TPC): FC reagent was used to assess TPC by oxidizing phenols in a solution of phosphomolybdic and phosphotungstic acids. The maximum absorption at 765 nm was measured, relating to the total amount of phenolics (mg GAE/100g) present (Hussain *et al.,* 2022).

Total Flavonoid content (TFC): In a 96-well plate, test samples, NaNO2 and distilled water were incubated for 10 minutes. Further 5 minutes incubation was done after addition of NaNO2 and 10% AlCl3. A UV/Visible spectrophotometer measured absorbance at 510 nm to express TFC as mg CE/100g (Hussain *et al.,* 2022).

DPPH Radical Scavenging Assay: Sample (2.5 mL) and DPPH solution mixture was incubated (35 minutes) and absorbance (A) was taken at 517nm (Hussain *et al.,* 2022). The radical scavenging capabilities were determined using the formula: [A (control) – A (sample)/ A (control)] x 100

Antimicrobial Assay: The antibacterial properties of *Sesamum indicum* and *Sesamum radiatum* seeds were assessed using the agar diffusion assay. Agar solution was prepared by adding 2.66 g agar in 70mL aqueous. The agar solution and petri plates were autoclaved and 100µL bacterial strains were added. Petri plates were packed with inoculum and three wells were created in gel. 80µL of both samples and ciprofloxacin were added as a positive control. After incubation at 35-37^{o}C for 16-18 hours, the diameter of the inhibition zone was measured (Ahmed *et al.,* 2022).

Alpha-Amylase Inhibition Assay: Sample (25 µL) and standard acarbose (25 µL) were incubated for 10 minutes with alpha amylase solution. Substrate starch solution was added and incubated again (30 minutes). Then 1 M HCl and iodine solution were added. Absorbance was measured at 580 nm against a blank (Ali *et al.,* 2022). Percentage inhibition = [1-A (cntrl) / A (smpl)] × 100

Hemolytic Assay: Human blood was centrifuged (five minutes). RBC and plant sample (S) were mixed and the supernatant was diluted with cold PBS (phosphate buffer saline). PBS was negative control (NC) and positive control (PC) was Triton X-100. Absorbance (A) was observed at 576 nm (Kauser *et al.,* 2018). Formula for the calculation of percentage inhibition of hemolysis is given below: % inhibition = [A(S) – A (NC)/ A (PC)] × 100

Structural Analysis

Fourier Transformed Infrared Spectroscopy: FTIR analysis was performed on plant extracts, ground with potassium bromide and examined in the 400-4000 cm-^{1} range (Kamran *et al.,* 2019).

Statistical Analysis: Data is expressed as mean ±S.D. Analysis by T-test with level of significance as $p<0.05$ was done using the Minitab Statistical software version 17 (Montgomery, 2019).

RESULTS AND DISCUSSION:

Antioxidant Activity: Results of TPC, TFC and DPPH were expressed in table 1. Higher TPC content observed in aqueous extract of *S. radiatum* 65.28±1.66mgGAE/g than *S. indicum* 65.28±1.66mg GAE/g. Maximum total flavonoid content showed by *S. radiatum* 131.8±198mgCE/100g than *S. indicum* 110±0.45mgCE/100g. Higher DPPH activity was shown by *S. indicum* 46.2±0.24% than *S. radiatum* 36.62±0.24%.

According to Agbemade *et al.* (2022) the aqueous extract exhibited the highest TPC (17.12 ± 0.041 mg GAE/ g of dried extract) and TFC (64.27 ± 4.711 mg CE/ g of dried extract). The antioxidant activities of various *S. indicum* extracts were measured using the DPPH radical scavenging activity. The aqueous extract of *S. indicum* exhibited the strong DPPH activity (52.8%). while in this study the aqueous extract of *S. indicum* has TPC value 55.97 ± 2 and TFC has 110.0 ± 0.45 and then DPPH has 46.265 ± 0.24.

According to Nigam *et al.* (2015) the amount of total phenols in the black sesame methanolic seed extract was 19.48 ± 0.98 mg GAE /g of the dry weight of the sample. Astalakshmi *et al*. (2022) observed that the total flavonoid content of *Sesamum radiatum* seeds was calculated by using the standard curve and was expressed in terms of mg CE/g. The result of total flavonoid content

in *Sesamum radiatum* seeds is 4.585mg CE/g in aqueous extract which is contradict to current research.

Pathak *et al.* (2020) observed *S. radiatum* exhibits high inhibition of free radical formation (83.52%) and highest scavenging capacity 25.1 ± 0.4 for black sesame seeds in aqueous and ethanol extracts.

According Milli *et al.* (2021) ethanolic sesame seed extracts have more antioxidant activity than aqueous extracts. The larger number of phenolic compounds and flavonoids is a factor in this research.

Table 1. Comparative Analysis of TPC, TFC and DPPH.

	Sesamum indicum			***Sesamum radiatum***		
Solvent	**TPC mgGAE/100g**	**TFC mgCE/100g**	**DPPH %**	**TPC mgGAE/100g**	**TFC mgCE/100g**	**DPPH %**
Aqueous	55.97±2	110.0±0.45	46.265±0.24	65.28±1.66	131.8±1.98	36.626±0.24

Antimicrobial Activity: Results of antimicrobial activity expressed in table 2, according to table positive control (Ciprofloxacin) of *E. coli* has a zone of inhibition of 27mm while *Streptococcus aureus* showed 28 mm zone of inhibition. *S. indicum* showed same antimicrobial activity against *E. coli* and *S. aureus* with 8mm of inhibition zone. Meanwhile, *S. radiatum* showed 8mm of inhibition zone in *E. coli* as well as 8mm of inhibition zone in *S. aureus*.

Nigam *et al.* (2015) observed the significant antibacterial activity against *S. aureus* and *E. coli* in the methanolic seed extract of black sesame at various doses. At 500 mg/mL, it was most strongly inhibited. A zone of inhibition of 13 ± 0.871 mm was found to exhibit the highest inhibition against *S. aureus* at a dosage of 500 mg/mL. At 500 mg/mL concentration, a zone of inhibition of 10.17 ± 0.946 mm was observed against *E. coli*. Gentamicin, an antibiotic, demonstrated zones of inhibition against *E. coli* and *S. aureus* of 35 and 36 mm, respectively. This zone of inhibition is similar to current study.

Manogaran *et al.* (2022) showed *Sesamum indicum* has strong antimicrobial potential, and the increasing incidence of microbial resistance to conventional antibiotics calls for exploring new antimicrobial agents. This study evaluated the in-vitro inhibitory potential of *Sesamum indicum* seed extract against *S. aureus* and *E. coli* using the well diffusion method. Results showed SSE (Sesame Seed Extract) had a good inhibitory effect on bacterial growth, while SDS (synthetic derivative of sesamol), synthesized through eugenol amination, and displayed high inhibitory potential.

Akanmu *et al*. (2019) found that *S. radiatum* aqueous extract did not respond favorably to any isolates. However, the methanolic extract was effective against *S. typhi* and *P. aeruginosa* at dosages of 200, 400 and 600 mg/mL with 8.00±0.00mm zone of inhibition which is contradict to current research.

Table 2. Antimicribial activity.

Bacterial Strain	**Positive control (Ciprofloxacin)**	***Sesamum indicum***	***Sesamum radiatum***
E. coli	27mm	8mm	8mm
S. aureus	28mm	8mm	8mm

Antidiabetic and Cytotoxic activity: Table 3 showed comparative analysis of alpha amylase percentage inhibition and Hemolytic assay of seeds of both *S. indicum* and *S. radiatum*. Aqueous extract of *S. indicum* and *S. radiatum* showed 37.13% and 33.02% inhibition of alpha amylase respectively. Positive control (Glucobay) showed 78.05% inhibition while the hemolytic percentage of both *S. indicum* and *S. radiatum* showed 4.23% and 5.79% and positive control (Titron-X-100) showed 84.87% hemolytic activity.

Reshma *et al*. (2013) indicated that methanolic extract of *Sesamum indicum* showed inhibitory effect of 16.95%. while current study showed 37.1% alpha amylase inhibition of *Sesamum indicum*. Another experiment performed showed the alpha amylase inhibitory effect of n-butanol fraction of black sesame seeds extract was 24.95% (Amutha and Godavari, 2016).

YP *et al.* (2018) indicated PHZ(phenyl hydrazine) injections in rats led to hemolytic anemia, causing a decrease in red blood cells, Hb concentration, and hematocrit percentage. Treatment with *S. indicum* reversed these effects, suggesting its effectiveness in managing anemia. According to study conducted by kouacou *et al.* (2022) *Sesamum radiatum* aqueous extract is not toxic to rats, causing no death, suffering, or behavioral changes. The extract did not significantly alter organ morphology or weight compared to controls. Which is contradict to current research.

Table 3. Comparative analysis of Alpha amylase inhibition assay and hemolytic assay of aqueous extract of *S. indicum* and *S. radiatum.*

	Sesamum indicum		*Sesamum radiatum*		Positive control	
Solvent	Inhibition %	Hemolysis %	Inhibition %	Hemolysis %	Glucobay	TitronX100
`Aqueous	37.131±0.15	4.231±1.35	33.022±0.48	5.790±1.35	78.059±0.00	84.877±0.00

Fourier Transformed infrared spectroscopy: Table 4 showed the FTIR analysis of *S. indicum* and *S. radiatum* revealed various functional groups. *S. indicum* consist of various compounds including lignins, phenolic compounds and flavonoids. The absorption values ranged from 3270-9009cm-^{1}, with notable peaks indicating carboxylic acid, hydrogen bonds, alkenes, ketones, isocyanates, allene, aromatic compounds, δ-lactone, esters, ketones, conjugated alkene, amine, cyclic alkene, nitro compounds, fluoro-compounds, C-F bonds and sulfoxides. Additionally, a weak peak at 9009.8cm-^{1} indicated the presence of aromatics. The FTIR absorption values of *S. radiatum* also revealed various functional groups, with a strong peak at 3274.5cm^{-1} indicating alcohol and an alkene compound at 3009.8cm^{-1} indicating alkane. The presence of nitro compounds, carboxylic acid, alkanes, phenol and amines was also observed.

Alarfaj *et al.* (2021) obtained IR spectra of *S. indicum* oil from 599-4000cm^{-1} and indicated the presence of different functional group in these bands. He identifies -OH functional group at the wavenumber peak of 3416cm^{-1} which indicate the presence of sesamin, sesaminol and sesamol while wavenumber of 2354cm^{-1} indicated the presence of -C=O group which showed the presence of unsaturated fatty acids.

Selvaraj *et al.* (2019) describe different vibrational peaks around 3430cm^{-1}- 1040cm^{-1}. A peak at 1626cm^{-1} represent the carbonyl stretching vibration of C=O groups. And two vibrational peaks recorded at 1394cm^{-1} and 1040cm^{-1} indicated the presence of functional groups.

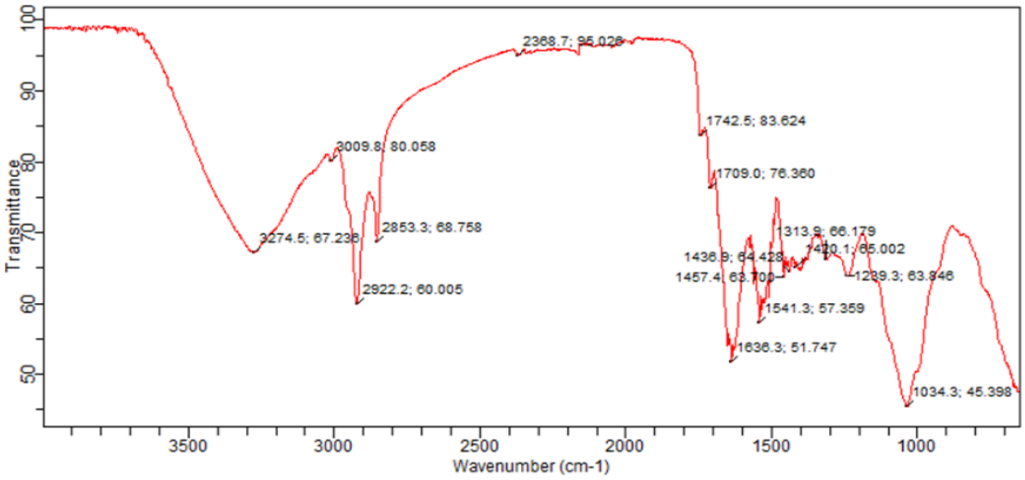

Fig 1: FTIR spectra of *Sesamum indicum*

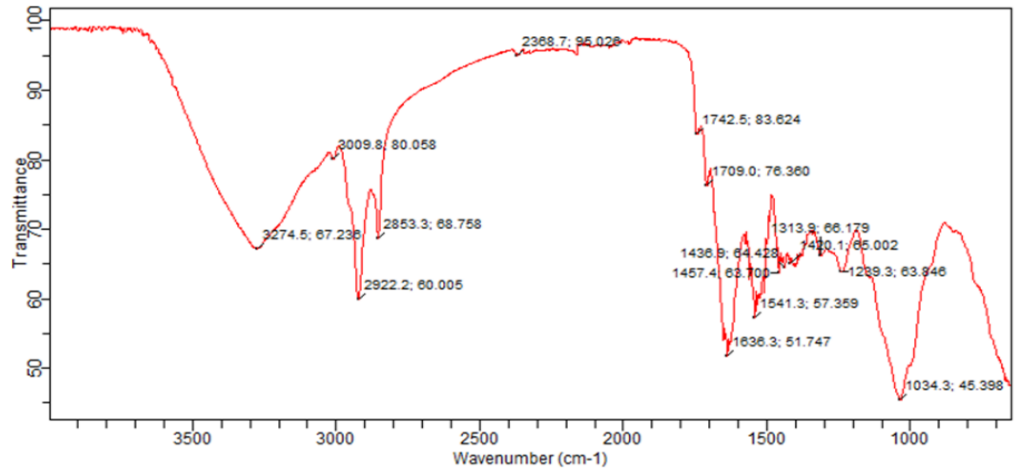

Fig 2: FTIR spectra of *Sesamum radiatum*

Table 4. FTIR Results for identification of functional groups.

Sesamum indicum			*Sesamum radiatum*		
Characteristics absorption	**Compound class**	**Identification functional group**	**Characteristics absorption**	**Compound class**	**Identification functional group**
3270.7	Alcohol, Phenols, Hydrogen bonded, Carboxylic acid	O-H C=O	3274.5	Alcohol, Phenol, carboxylic acid, Primary and Secondary amines and amides.	O-H, COOH, N-H
2924.1	Carboxylic acid	C=O	3009.8	Alkene	C-H
2853.3	Carboxylic acid, Aldehyde, Amine salt, Alkane.	C=O N-H C-H	2922.2	Alkane, carboxylic acid, Alcohols.	C-H O-H
2163.7	Alkenes, Ketones, Isocyanates	X=C=Y	2853.3	Carboxylic acid, Aldehyde, Amine salt, Alkane.	C=O N-H C-H
1913.9	Allene, Aromatic	C=C=C C-H	2368.7	Carbon dioxide	O=C=O
1742.5	δ-lactone, Esters	C=O	1742.5	δ- lactone, Esters	C=O
1709.0	Carboxylic acid, Ketones	C=O	1709.0	Carboxylic acid, ketones.	C=O
1636.3	Conjugated alkene, Amine, Cyclic alkene	C=C N-H C=C	1636.3	Conjugated alkene, Amine, Cyclic alkene.	C=C N-H
1541.3	Nitro compound, Phenyl, Aromatics	N-O C=O C=C	1541.3	Nitro-compound Phenyl, Aromatics	N-O C=O C=C
1457.4	Nitro (R-NO_2)	N=O	1457.4	Nitro (R-NO_2)	N=O
1420.1	Carboxylic acid, Alcohol, Alkanes-CH_3, Nitro	O-H C-H N=O	1436.9	Alkanes, Carboxylic acid, Methyl and Methylene groups	C-H O-H -CH_3
1399.6	Fluoro-compound Fluoride	C-F C-X	1420.1	Carboxylic acid, Alcohol, Alkanes-CH_3 Nitro (R-NO_2)	O-H C-H N=O
1239.3	Alcohol, ethers, esters, carboxylic acid, anhydrides, sulfones, sulfonyl chlorides, sulfates, sulfonamides	C-O, S=O	1313.9	Amines, Sulfones, Sulfonyl chloride, Sulfate, Sulfonamide, Fluoride.	C-N S=O C-X
1153.6	Fluoro- compound	C-F	1239.3	Alcohol, ethers, esters, carboxylic acid, anhydrides, sulfones, sulfonyl chlorides, sulfates, sulfonamides	C-O, S=O
1036.2	Sulfoxide	S=O	1034.3	Sulfoxide, Amine	S=O C-N
9009.8	Alkenes, Aromatics	C=H C-H	-	-	-

Conclusion: The present study investigated some selected biological efficacies *S. radiatum* and *S. indicum* extracts. This research indicated that *S. indicum* and *S. radiatum* have extraordinary pharmacological potential and in-depth studies are required to further evaluate the remarkable properties and therapeutically properties of these medicinal plants.

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