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Solvent-Dependent In Vitro Antimicrobial Potential of Aqueous and Ethanolic Extracts of *Nicotiana tabacum* and *Sesamum alatum*

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ABSTRACT

This study evaluates the antimicrobial properties, phytochemical composition, and thin-layer chromatographic (TLC) profiles of aqueous and ethanol leaf extracts of *Nicotiana tabacum* and *Sesamum alatum*. The preliminary phytochemical screening showed that alkaloids, tannins, saponins, flavonoids, glycosides, carbohydrates, proteins, terpenoids, and anthraquinones were present in both plants. Phytosterols were detected only in *N. tabacum*. TLC analysis of ethanol extracts revealed five phytocompounds for *N. tabacum* with Rf values of 0.06, 0.08, 0.12, 0.76, and 0.90, and six for *S. alatum* at 0.05, 0.10, 0.13, 0.21, 0.72, and 0.97. Antimicrobial analysis against some selected microorganisms showed that both extracts demonstrated concentration-dependent inhibition activities. In *N. tabacum*, the ethanolic extract showed the highest zone of inhibition (18 mm) against *Staphylococcus aureus*, while the aqueous extract showed more potency against *Proteus vulgaris* (15 mm). For *S. alatum*, the ethanol extract exhibited microbial inhibition against *Klebsiella pneumoniae* (23 mm), while the aqueous extract showed moderate inhibition with a highest value of 14 mm against *Salmonella typhi*. No inhibition was demonstrated at concentrations of 0.1 and 0.3 g/mL each for either plant. The results supports the prospect of antimicrobial potential of these medicinal plants, with ethanol extracts being more active.

Keywords: Antimicrobial Activity; Antibiotics; Inhibition; *Nicotiana tabacum*, *Sesamum alatum*

1. INTRODUCTION

There has been a growing interest in medicinal plants due to their enhanced use in the pharmaceutical industry and the search for potential bioactive molecules. Over the past decades, these plants have been used to cure different diseases. Their historical application in the treatment of diseases like hypertension, asthma, diabetes, and infection is presently confirmed by scientific evidence of the active phytochemicals (Shatnawi et al., 2021). The extensive uses of antibiotics in

human medicine, animal production, and agriculture have mainly been the causative factor for the establishment of antibiotic-resistant microbial populations, which is a serious challenge in managing infectious diseases. In order to find new compounds that can overcome these restrictions, efforts have been made to find alternative antimicrobial agents, particularly from plant sources (Bereksi et al., 2018). For a long time, plants have played an essential role in the treatment of infections and remain a rich source of promising antimicrobial agents. (Cowan, 1999).

Nicotiana tabacum, commonly known as tobacco (Fig. 1), a member of the Solanaceae family (Sharm et al., 2016). Historically, it has been utilized by herbal practitioners primarily as a relaxant, although its ability to induce addiction has limited the use of the plant in modern herbal practices for both internal and external administration. Traditionally, the leaf, when ingested is used as a cholagogue to support liver function (Kidah et al., 2018). Various research findings have shown that *N. tabacum* leaves have several pharmacological activities, including antibacterial, antifungal, antimicrobial, antinociceptive, anthelmintic, and anti-Alzheimer's effects (Sokunvary et al., 2017; Emordi et al., 2015). Tobacco is derived from the cured leaves of *Nicotiana* species and has applications ranging from consumption to use as a pesticide and, in certain forms such as nicotine tartrate, in pharmaceuticals (Rawat et al., 2013). Besides *N. tabacum*, several other species are widely cultivated, including *N. affinis*, *N. rustica*, *N. sanderae*, *N. alata grandiflora*, *N. acuminata*, *N. bigelovii* (Indian Tobacco), *N. longiflora*, *N. noctiflora*, *N. suaveolens*, *N. sylvestris*, and *N. wigandioides* (Binorkar et al., 2012).

Sesamum alatum L. (Fig. 2), which belongs to the Pedaliaceae family, has long held a place in traditional Ayurvedic medicine due to its therapeutic value. Although the black seeds are the most frequently used for medicinal applications, other parts of the plant are also being investigated for their potential health benefits, and the young leaves are widely consumed as leafy vegetables across various African communities (Ellandala et al., 2011; Sundarakumar & Karmegam, 2018).

The rising incidence of infectious diseases and the growing interest by scientists in herbal medicine have drawn attention to the significance of compounds derived from plants used for alternative or supplemental treatments (Kebede et al., 2021). The current study examines the antibacterial properties of these medicinal plants against selected microbial pathogens. This study aims to assess the phytochemical composition and antimicrobial properties of *N. tabacum* and *S. alatum* leaf extracts. This study aims to find promising plant-based compounds for therapeutic development as well as to offer scientific support for their traditional uses.

Fig. 1 *N. tabacum* plant

Fig. 2 *S. alatum* plant

2. METHODOLOGY

2.1. Chemicals and Reagents

All chemicals and reagents used, unless otherwise noted, were of analytical grade and acquired from the Scientific Laboratory in Jimeta-Yola, Adamawa State, Nigeria.

2.2. Plant Sample Collection and Identification

N. tabacum and *S. alatum* leaves were collected from the wild in Sukur Kingdom, Madagali LGA, Adamawa State, Nigeria. The plant specimens were identified by Professor Dimas Kubmarawa, a specialist in Natural Products Chemistry at Modibbo Adama University, Yola.

2.3. Plant Sample Pre-analysis Treatment

The plant leaves were washed and air-dried under shade at room temperature in Chemistry Laboratory 2, Faculty of Science Complex, Adamawa State University, Mubi. The samples were air dried in the laboratory before pounding to a fine powder using a pestle and mortar to a mesh size of about 60 and then stored in a dry container (Kubmarawa et al., 2009).

2.4. Plant Preparation and Extraction

One hundred grams of powdered leaves from each plant were weighed and extracted with ethanol in air-tight containers for 24 hours. The resultant mixture was filtered with filter paper (Whatman No. 1) under gravity. The filtrate was dried at 60 ^{0}C in a water bath to yield the aqueous extract residue (Adamu et al., 2018).

2.5. Thin Layer Chromatography (TLC) Analyses

Thin-layer chromatography (TLC) was performed on the ethanolic extracts using silica gel pre-coated glass plates. Two grams of the concentrated dry extracts of N. tabacum and S. alatum were each dissolved in 70% ethanol before spotting. Each sample was diluted to make up a volume of 1 mL and then spotted onto preparative TLC plates (Giri et al., 2020). A microsyringe was used to uniformly apply 10 µL of each extract onto the TLC plate. The spots were then allowed to dry at room temperature. A mobile phase consisting of ethanol, methanol, and ethyl acetate (5:3:2) was used to develop the plates in a chromatographic tank. After development, the plates were air-dried at room temperature. The retardation factor (R_f) for each component was calculated (Kumar et al., 2013).

2.6. Phytochemical Screening

Standard methods were used to carry out the preliminary phytochemical screening. By using different specific reagents, the presences of leading groups of natural products were detected in ethanolic extracts of *N. tabacum* and *S. alatum* according to the following: Alkaloids (Banu and Cathrine, 2015); Phenolic Compounds and Tannins (Rashed et al., 2019); Saponins and Terpenoids (Oscar et al., 2020); Flavonoids (Namadina et al., 2020); Glycosides (Rajitha et al., 2022); Phytosterols, Proteins and Amino Acids (Khanal et al., 2015); Reducing Sugars (Ayoola et al., 2008); and Anthraquinones (Shegute et al., 2020).

2.7. Antimicrobial Test

2.7.1. Collection of Microorganisms

The microorganisms used in the study were collected from the stock cultures of the General Hospital, Infinity Medical Laboratory, Ecogate Clinic & Maternity, and New Life Hospital, all located in Mubi Local Government Area of Adamawa State-Nigeria. The microorganisms used include *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris, Streptococcus pneumoniae, Candida albicans,* and *Pseudomonas aeruginosa*.

2.7.2. Solvent Extraction

A total of 200 g of each plant powder was extracted with 200 mL of 9 5% ethanol (w/v) in airtight containers for 24 hours. The mixture was then filtered, and the resulting extracts were concentrated to dryness at 60 °C using a water bath. The same procedure was used for aqueous extractions. The extracts were then refrigerated at 4 °C before use. Amounts of 1, 3, 5, 7, and 9 g of the extract were reconstituted in 10 mL of sterile distilled water to obtain solutions of 0.1, 0.3, 0.5, 0.7, and 0.9 g/mL concentrations, respectively while the reference drug, ciprofloxacin USP 500 mg prepared at 0.1, 0.3, 0.5, 0.7 and 0.9 mg/mL were used for the antimicrobial screening (Ogunsola and Fasola, 2014).

2.7.3. Preparation of Media

Two grams of nutrient agar were dissolved in one liter of distilled water using a water bath at 100 °C. The prepared nutrient agar solution was autoclaved at 121 °C for 15 minutes, then allowed to cool to 45 °C before being poured into sterile Petri dishes for subsequent bacterial plating (Ogunsola & Fasola, 2014).

2.7.4. Evaluation of Antimicrobial Test

The susceptibility of each test organism was assessed using the agar well diffusion method. The nutrient agar was prepared in line with the manufacturer's guideline, autoclaved and, dispensed into sterile Petri dishes and allowed to set before use. The test isolates were used to inoculate the plates, after which a sterile cork borer (5 mm in diameter) was used to punch wells into the nutrient agar. Each appropriately labeled well was then filled with 0.2 mL of the extract. For 30 minutes, the inoculated plates were kept at room temperature to allow the extract to diffuse into the agar and subsequently incubated at 37 °C for 24 hours. Antimicrobial activity was determined by zones of inhibition, and quantified by measuring the diameter of the zone of inhibition in (mm) using a meter rule (Peter et al., 2019).

3. RESULTS

3.1. Thin Layer Chromatography (TLC) Profiles of *N. tabacum* and *S. alatum*

As shown in Fig. 3, TLC analysis of the ethanol extract of *N. tabacum* revealed five distinct bands with retention factor (R_f) values of 0.06, 0.08, 0.12, 0.76, and 0.96 while six compounds were separated from *S. alatum* on the TLC plate, with R_f values of 0.05, 0.10, 0.13, 0.21, 0.72, and 0.97. The distance moved by the solvent, DS = 12cm. The differences in the phytochemical composition of these plant extracts are reflected in the variations in their Rf values.

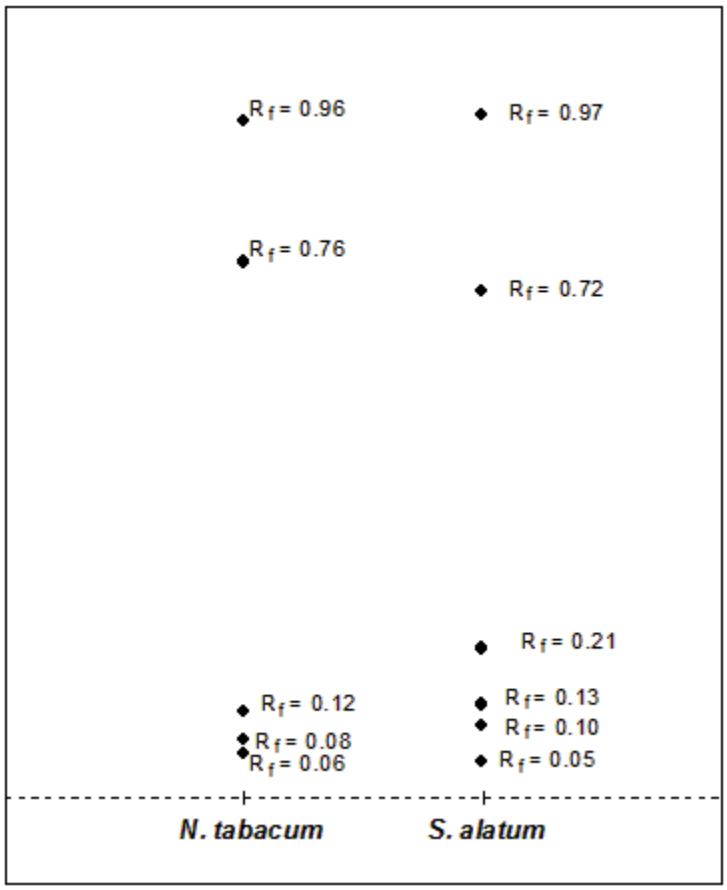

Fig 3. TLC sketch of the *N. tabacum* and *S. alatum* ethanolic extracts (created with ChemDraw)

3.2. Phytochemical Composition of *N. tabacum* and *S. alatum*

As shown in Table 1, the phytochemical screening of the ethanol extract of *N. tabacum* revealed a rich presence of various secondary metabolites, including alkaloids, tannins, saponins, flavonoids, glycosides, phytosterols, carbohydrates, proteins, terpenoids, and

anthraquinones. In contrast, the ethanol extract of *S. alatum* contained all the above-mentioned phytochemicals except phytosterols, which were absent.

3.3. Antimicrobial Activity of *N. tabacum* and *S. alatum*

The study evaluates the antimicrobial activity of aqueous and ethanol leaf extracts of *S. alatum* (Table 2) and *N. tabacum* (Table 3) against selected pathogenic microorganisms at concentrations of 0.9, 0.7, and 0.5 g/mL. No microbial inhibition occurred at 0.3 and 0.1 g/mL for any of the extracts tested, although these concentrations were included in the assay.

For *N. tabacum*, the ethanol extract exhibited a broader and more potent spectrum of antimicrobial activity compared to its aqueous counterpart. At 0.9 g/mL, the highest zone of inhibition (18 mm) was recorded against *S. aureus*, while the lowest (13 mm) was observed against *K. pneumoniae*. At 0.7 g/mL, *K. pneumoniae* and *S. pneumoniae* showed no inhibition, while inhibition was limited to *P. vulgaris* and *E. coli* at 0.5 g/mL. The aqueous extract of *N. tabacum* showed its highest activity against *P. vulgaris* (15 mm) at 0.9 g/mL. At 0.7 g/mL, only *P. vulgaris* was inhibited (10 mm). At the same time, *K. pneumoniae, S. aureus, S. typhi, E. Coli, S. pneumoniae,* and *P. aeruginosa* showed no response to the aqueous extract at this concentration.

In contrast, *S. alatum* displayed a different antimicrobial profile. The ethanol extract exhibited potent activity against *K. pneumoniae*, producing a maximum inhibition zone of 23 mm at a concentration of 0.9 g/mL. At the same concentration (0.9 g/mL), the lowest inhibition zone of 9 mm was recorded against *E. coli*. At 0.7 g/mL, *K. pneumoniae*, *S. pneumoniae*, and *S. typhi* each exhibited a 14 mm zone of inhibition, while *Candida albicans* showed the highest activity with a zone of 17 mm. At 0.5 g/mL, inhibition was observed only against *K. pneumoniae* (9 mm) and *S. aureus* (10 mm). The aqueous extract of *S. alatum* showed moderate inhibition against *S. typhi*, with maximum zones of 14 mm and 10 mm at concentrations of 0.9 and 0.7 g/mL, respectively, while *P. vulgaris* and *E. coli* were not inhibited by the aqueous extract at all concentrations tested assays.

In summary, both plant extracts demonstrated concentration-dependent antimicrobial activity, with the ethanol extracts generally showing higher potential and broader activity in all the tested pathogenic-microorganisms compared to the aqueous extracts.

4. DISCUSSION

4.1. Phytochemical Constituents of *N. tabacum* and *S. alatum*

N. *tabacum* thrives in environments with stable temperatures and relative humidity levels ranging between 80–85%. Its seeds are small, oval, and brown, while most other parts of the plant have been discovered to contain the alkaloid nicotine, the seeds does not (Chinweuba, 2013). According to the reports of Wilsan et al. (2025), qualitative phytochemical screening of *N. tabacum* leaf extract revealed fthe presenceof our phytochemicals: flavonoids, saponins, steroids, and triterpenoids. In a related research work, the ethanol extract of *N. tabacum* contained flavonoids, tannins, saponins, steroids, alkaloids, and glycosides, while terpenoids and carbohydrates were absent (Chaudhary et al., 2023). The leaves of *N. tabacum* are rich in pyridine alkaloids, glucosides, organic acids, terpenes, and potentially carcinogenic substances (Rawat et al., 2013). Phytochemical screening of both the aqueous and methanolic extracts of *S. radiatum* revealed the presence of flavonoids, terpenoids, cardiac glycosides, and cardenolides. The aqueous extract does not contain saponin, whereas carbohydrates were present in the methanolic extract (Akanmu et al., 2019). The aqueous extract of *S. radiatum* has been found to contain phenols, flavonoids, lignans, and sterols in high proportion (Osibote et al., 2010). Rosni et al. (2024) reported that specific phytochemical components such as flavonoids, alkaloids, tannins, cyanogenic glycosides, and anthraquinones may possess antibacterial properties that serve as natural defenses for plants against disease causing microorganisms and insect predators.

Table 1. Phytochemical Screening of *N. tabacum* and *S. alatum* ethanolic extracts

Phytochemicals	*Nicotiana tabacum*	*Sesamum alatum*
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	+	+
Phytosterols	+	-

Reducing sugars	+	+
Proteins	+	+
Terpenoids	+	+
Anthraquinones	+	+

4.2. Antimicrobial Activity of *Sesamum alatum* and Related Species

Research on *Sesamum* species, including *S. radiatum*, *S. indicum*, and *S. alatum*, has demonstrated varying degrees of antimicrobial activity. In a study by Bankole et al, ethanolic extracts of *S. radiatum* and *S. indicum* showed no inhibitory activity against *S. aureus*, although they exhibited both antibacterial and antifungal effects against *S. pneumoniae* and *C. albicans (*Bankole et al., 2007). The ethanolic extract of *S. radiatum* exhibited mild activity against *S. pneumoniae* and *C. albicans*, but showed no inhibition against *S. aureus*, *P. aeruginosa*, and *E. coli* (Shittu et al., 2007). Aqueous extracts demonstrated no antimicrobial activity against any of the tested organisms. Sesamol, a compound found in sesame, has shown a minimum inhibitory concentration (MIC) of 2 mg/mL against *B. cereus* and *S. aureus* but only reduced *P. aeruginosa* growth by 80 % at the same concentration (Kumar & Singh, 2014; Andargie et al., 2021). The assessment of the antifungal activity of sesame seed alcoholic extract exhibited 51% growth reduction in *Trichophyton rubrum* at 3% concentration and increased to 86.03% at 10% (Al Subaihawi et al., 2024). The methanolic seed extract of *S. indicum* exhibited significant antibacterial activity, showing the highest zones of inhibition against *Staphylococcus aureus* (13.0 ± 0.87 mm) and *Escherichia coli* (10.17 ± 0.95 mm) at a concentration of 500 mg/mL (Nigam et al., 2015). Akanmu et al. (2015) also found that aqueous extracts of *S. radiatum* had antifungal properties, while methanolic extracts were effective against *Salmonella typhi* and *P. aeruginosa*. Similarly, the methanolic leaf extract of *S. indicum* inhibited *S. aureus*, *S. pneumoniae*, *S. Typhi*, *C. albicans*, *C. krusei*, and *C. tropicalis* at a concentration of 2.5 mg/mL (Ogwuche et al., 2015). Sesame oil demonstrated notable antimicrobial activity, producing inhibition zones against certain test organistas follows: *P. vulgaris* (20 mm), *S. Typhi* (25 mm), *E. coli* (15 mm), and *S. aureus* (19 mm) at a concentration of 50 µL (Saleem, 2011). These findings supports the reports of Bila et al. (2022), who suggested that the antimicrobial effects of sesame species may result from synergistic interactions among multiple bioactive compounds. Similarly, the ethanolic leaf extract of *S. alatum* exhibited antibacterial activity in disc diffusion and resazurin dye reduction assays (Sundarakumar & Karmegam, 2018). Further GC-MS analysis identified sulfathiazole, a well-known antibacterial compound as potential contributor to this activity (Sundarakumar et al., 2018).

Table 2. Antimicrobial activities of *S. alatum* extracts

	Zone of Inhibition (mm)					
	Aqueous extract			**Ethanol extract**		
Concentration (g/mL)	**0.9**	**0.7**	**0.5**	**0.9**	**0.7**	**0.5**
P. vulgaris	-	-	-	-	-	-
K. *pneumoniae*	11	10	-	23	14	9
S. aureus	10	-	-	15	13	10
S. typhi	14	11	-	16	14	-
E. coli	-	-	-	9	-	-
S. pneumoniae	11	10	-	19	14	-
C. Albicans	12	-	-	20	17	-
Ciprofloxacin (mg/mL)	**22**	**15**	**14**	**29**	**22**	**20**

4.3. Antimicrobial Activity of *N. tabacum* and Related species

The antimicrobial abilities of *N. tabacum*, especially its ethanol and methanol extracts, have been evaluated by several researchers. To start with among others, Khan et al. (2023) reported that the *K399* variety showed vigorous antifungal activity, producing a zone of inhibition of 16.5 ± 0.2 mm against *C. albicans*, although it was less effective against *A. niger* (7.0 ± 0.2 mm). Prommaban et al. (2022) evaluated the ethanolic extracts of two varieties of sesame: Virginia exhibited inhibition zones of 14.00 mm and 14.23 mm against *S. aureus* and *P. aeruginosa*, respectively, while Turkish variety exhibited a more antimicrobial activities with inhibition zones of 16.00 mm and 14.53 mm against the same test organismsas Virginia variety. On the other hand, Ameya et al. (2017) evaluates that the MICs of *N.*

tabacum extracts against biofilm-forming strains of *E. coli*, *Klebsiella* sp., and *S. aureus* with values of 62.5 µg/mL, 125 µg/mL, and 500 µg/mL, respectively. Tariq et al. (2020) also reported antimicrobial activity extracts of *N. tabacum* and *N. rustica* against *S. aureus*, while no activity was recorded against *E. coli*. The bioactive potential of *N. tabacum* is attributed to its chemical composition. The methanol extract contains phenols, flavonoids, saponins, alkaloids, steroids, and glycosides, whereas the ethanol extract contains tannins, and phenols are absent. (Chaudhary et al., 2023). Both extracts were devoid of terpenoids and carbohydrates. The leaves are particularly rich in pyridine alkaloids (nicotine), glucosides, organic acids such as chlorogenic and caffeic acids, and terpenes, of which some are potentially carcinogenic (Rawat et al., 2013). Further findings by Zaidi et al. (2012) showed that synthetic zinc(II)-nicotine complexes derived from *N. tabacum* possess broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Sharma et al. (2016) reported that the aqueous, ethanol, acetone, and methanol stem extracts of *N. tabacum* were active against *Bacillus amyloliquefaciens*, *S. aureus*, *E. coli*, and *P. aeruginosa*. In traditional medicine, *N. tabacum* has also been used externally for the treatment of insect and snake bites, ulcers, tetanus, and skin infections such as ringworm, further supporting its historical antimicrobial relevance (Kishore, 2014).

Table 3 Antimicrobial activities of *N. tabacum* extracts

	Zone of Inhibition (mm)					
	Aqueous extract			**Ethanol extract**		
Concentration (g/mL)	**0.9**	**0.7**	**0.5**	**0.9**	**0.7**	**0.5**
P. vulgaris	15	10	-	16	11	8
K. *pneumoniae*	9	-	-	13	-	-
S. aureus	10	-	-	18	13	-
S. typhi	9	-	-	15	12	-
E. coli	11	-	-	14	12	9
S. pneumoniae	-	-	-	-	-	-
P. aeruginosa	14	-	-	17	14	-
Ciprofloxacin (mg/mL)	**24**	**19**	**16**	**33**	**28**	**25**

5. CONCLUSION

The findings of this study demonstrate that both *Nicotiana tabacum* and *Sesamum alatum* possess bioactive compounds with notable antimicrobial properties, particularly in their ethanolic extracts. The observed differences in antimicrobial activity between aqueous and ethanol extracts reveals the influence of solvent polarity on phytochemical extraction and efficacy. These results support the potential use of these plants as sources of natural antimicrobial agents, providing a basis for further isolation and characterization of their active constituents.

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None.

Authors' Contributions

Authors CA and LL designed the study, performed the statistical analysis, wrote the protocol, and AC wrote the first draft of the manuscript. Both authors managed the analyses of the study. Author AC managed the literature searches. All authors read and approved the final version of the manuscript.

Ethical Approval

In this article, as per the plant regulations followed in the Department of Chemistry, Adamawa State University, Mubi, Nigeria; the authors observed the solvent-dependent in vitro antimicrobial potential of aqueous and ethanolic extracts of *Nicotiana tabacum* and *Sesamum alatum*. The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation.

Informed Consent
Not applicable.

Conflicts of interests
The authors declare that there are no conflicts of interests.

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Data and materials availability
All data associated with this study are present in the paper.

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