



Original Article



Phytochemical Measurements and Anti-microbial Evaluation of Neem Seed Extract

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Email: abdissa694@gmail.com,
desalegn.abdissa@ju.edu.et**Abstract****Background:** The plants can introduce a wide range of phytochemical components which are considered as secondary metabolites. As antioxidants, phytochemicals prevent cell damages which are usually caused by free radicals especially those which are associated with heart disease and cancer. Neem seed has high potential of phytochemicals which is used as traditional medicinal. This study aimed to evaluate bioactive and phytochemical extract of Neem seed.**Methods:** The antimicrobial activities and bioactive compounds of Neem seed extracted with distilled water were investigated in this study. Bioactive components of Neem seed were examined by agar well diffusion method.**Results:** Tannins, quinones and phenols were the highest phytochemical in Neem seed bioactive extract. Anthocyanin, terpenoids, saponins and glycosides were in medium levels. Also, alkaloids and flavonoids were in low level. At the lowest concentration of bioactive (10 mg/mL), *Bacillus subtilis* (G+) was the best controllable compared to others. The optimum concentration of the selected pathogenic species was 55 mg/mL at which the *Salmonella typhimurium* (G-) was highly controllable. *Staphylococcus aureus* (G+), *Bacillus subtilis* (G+), and *Escherichia coli* (G-) were 40 mm, 38 mm and 34 mm, respectively.**Conclusion:** Tannin and phenol were the highest phytochemical concentration in the Neem seed. In general the Neem seed extract seems to be antioxidants and antimicrobials with effective control area.**Keywords:** Antibacterial, Bioactive extract, Pathogenic bacteria, Optimum concentration, Neem seed

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Introduction

In many developing countries, the antibiotic resistance by microorganisms has been documented. A comprehensive investigation of the potential medicinal properties of plants serves as a conscientious and effective scientific endeavor to address drug-resistant microorganisms, thereby becoming a critical focus in medical research.¹

Plants is undoubtedly the main source of food for most herbivores. While plants are known to fulfill the nutritional requirements of living organisms, they also play a significant role on folk remedies for addressing diverse health issues. Moreover, the medicinal properties of numerous plants remain largely unexplored.² Emphasizing the active ingredients is crucial for a comprehensive understanding of the potential inherent in these plants. The medicinal potential of certain plants is attributed to specific chemically active substances found in various plant parts and their extracts.³ These active

ingredients are named the secondary plant substances which can have specific physiological effects on the target organism.⁴

The plants can introduce a wide range of phytochemical components which are considered as secondary metabolites. These secondary metabolites have unique effects which are directly or indirectly used in the pharmaceutical industry. Phytochemicals possess antioxidant properties, mitigating cell damage typically induced by free radicals linked to some conditions like heart disease and cancer.⁵ Phytochemicals exhibiting antioxidant properties include allyl sulfides, flavonoids, polyphenols, and carotenoids.⁶ It is also known that phytochemicals have the ability to interfere with enzyme activity. For example, doles can reduce the risk of breast cancer by stimulating enzymes that reduce the effects of estrogen.⁷ The ability of phytochemicals to interfere with DNA replication has been addressed before.⁸ When harmful cells are presented



in the biological system, phytochemicals prevent them from replicating. Phytochemicals exhibit hormone-like properties. For instance, isoflavones can mimic human estrogen, potentially alleviating symptoms of menopause and contributing to the management of osteoporosis.⁹ Phytochemicals are known for their ability to inhibit the growth of bacteria. They contribute to preventing the adhesion of pathogens to cell walls.¹⁰ Specifically, anthocyanidins with anti-adhesion properties, for instance, contribute to reducing the risk of urinary tract infections and supporting oral health. Due to the presence of diverse phytochemicals in the important oils and different plant extracts, there may be excessive capability for extracts from different flora to have anti-microbial properties. A thorough investigation and effective utilization should lead to the discovery of novel materials and active compounds that are potent against seemingly drug-resistant microorganisms, while simultaneously mitigating many of the side effects commonly associated with synthetic antimicrobials.¹¹ In this study, we assessed the antimicrobial activity of Neem seed essential extracts against the microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The antimicrobial activity of Neem seed crude extract, obtained using distilled water, was evaluated through the agar well diffusion method against the aforementioned microorganisms, and minimum inhibition concentrations were determined.

Materials and Methods

Plant Bioactive Extract

The Neem seeds have a high potential of phytochemical constituents, and they have been found from the West Hararge, Benishangul-Gumuz, and South West of Ethiopia. The collected Neem seeds were washed with distilled water, and subsequently dried in the sun until complete removal of moisture content. The next step was grounding the dried Neem seed by mortar and pestle up to 0.05 to 1mm of particle size for the extraction. Phytochemical extraction process was distilled water soaking method.¹² The grounded Neem seeds in fine particle were soaked in distilled water for 5 hours at 60 °C. Subsequently, the bioactive extract was separated from the grounded seed cake using filter paper.¹³

Phytochemical Extraction

In this process, the fine powder obtained from grinding of Neem seed was loaded into 1mL volume of distilled water. The fine grounded Neem seed added to the distilled water were 10, 15, 20...95 mg. Extraction process was done at 60 °C, and the different extract concentration were collected after 5 hours.¹⁴

Phytochemical Analysis

The qualitative phytochemical analysis of biochemical extracts from Neem seeds was conducted using the following methods¹⁵:

Carbohydrates Test

Procedure: 1 mL of Molisch's reagent was introduced to 2 mL of the biochemical extract, followed by the addition of a few drops of concentrated sulphuric acid.

Interpretation: The presence of carbohydrates was indicated by the development of a purple coloration.¹⁶

Tannins Test

Procedure: The 1 mL of biochemical extract was mixed with 2 mL of 5% ferric chloride.

Interpretation: The presence of tannins was confirmed by the appearance of a greenish-black coloration.¹⁵

Saponins Test

Procedure: The 2 mL of biochemical extract was mixed with 2 mL of distilled water. It was shaken for 15 minutes or the formation of foam was observed.

Interpretation: The presence of saponins was inferred from the observed foam formation.¹⁷

Flavonoids Test

Procedure: The 1 mL of biochemical extract was mixed with 5 mL of dilute NH_3 solution, followed by the addition of concentrated sulphuric acid.

Interpretation: The detection of a yellow coloration indicated the presence of flavonoids.¹⁸

Anthocyanins and Betacyanin Test

Procedure: The 2 mL of biochemical extract was mixed with 1 mL of 2N NaOH. It was then heated for 5 minutes at 100 °C.

Interpretation: The presence of anthocyanins and betacyanins was affirmed by the appearance of a yellow coloration.¹⁹

Alkaloids Test

Procedure: 2 mL of concentrated HCl was mixed with 2 mL of the biochemical extract, and a few drops of Mayer's reagent were added.

Interpretation: The development of a greenish coloration indicated the presence of alkaloids.¹⁵

Quinones Test

Procedure: The 1 mL of biochemical extract was mixed with 1 mL of concentrated H_2SO_4 .

Interpretation: The confirmation of quinones was evidenced by a red coloration.²⁰

Terpenoids Test

Procedure: The 0.5 mL of biochemical extract was mixed with 2 mL of chloroform and concentrated H_2SO_4 .

Interpretation: The presence of terpenoids was demonstrated by the emergence of a red-brown coloration at the interface.¹⁵

Phenols Test

Procedure: The 1 mL of biochemical extract was mixed

with a few drops of 10% ferric chloride and 2 mL of distilled water.

Interpretation: The detection of a green coloration indicated the presence of phenols.¹⁵

Glycosides Test

Procedure: The 2 mL of biochemical extract was mixed with 3 mL of chloroform and 10% NH₃ solution.

Interpretation: The presence of glycosides was confirmed by the development of a pink coloration.²¹

Antimicrobial Evaluation

The well diffusion agar method was employed to assess the antibacterial activity of biochemical extracts derived from Neem seeds.²² As a technique to estimate the antibacterial efficacy of the oil extract, 0.1 mL of the culture solution of each isolate was incorporated into 18 mL of Mueller-Hinton agar medium within a sterile Petri dish. Additionally, in certain instances, 1000 µL of essential oil was introduced by creating a 4 mm hole in the oil extract.¹⁵ Gentamicin was used as a control on another plate composed of 1000 µL of methanol 70%.²³ The plate was initially kept at ambient temperature for 1 hour to facilitate optimal diffusion of the oil. Subsequently, it was incubated at 37 °C for a duration of 24 hours. This incubation period was chosen to observe the sustained efficacy of the vegetable oil extract, ensuring a significant reduction in its effectiveness to impede the growth of the test isolate. It should be mentioned the experiments were duplicated. Also, suppression zones were measured in millimeters and the averages were recorded.

Maximum and Minimum Inhibitory Concentration Analysis

The minimum inhibitory concentration (MIC) was determined using the presented approach after confirming the sensitivity of the bioactive extract to the growth of the

isolate. Additionally, the inhibition zone of the essential extract was determined. A Mueller-Hinton agar solution was prepared by dissolving 38 g of agar in 500 mL of water. The agar solution was then conditioned, dispensed into McCartney bottles, and subjected to sterilization in an autoclave at 121 °C for 15 minutes. Following sterilization, the agar solution was cooled to 45 °C. After preparation, each sequential solution was poured into a Petri dish and left to solidify for a duration of 1 hour. Extracts were created by the serial dilution in concentrations of 95, 90, 85, 80 mg/mL. The plate was then divided into sections and labeled accordingly. Using the sterile tweezers, a 5 mm paper disc was aseptically placed in each marked section of the plate. In each case, an automatic micropipette was used to inject 0.1 mL of isolate into the labeled paper disc on the agar plate. The plates were incubated at 37 °C for 24 hours, and subsequent observation was conducted to assess the growth or viability of the test organisms. The minimum and maximum inhibitory zone and optimum concentrations were determined.

Results and Discussion

Collecting, drying, milling, soaking and separation are respectively the process of antibacterial extract from Neem seed as shown in Figure 1.

As shown in Figure 1, a fresh Neem seed, b dried Neem seed, c milled Neem seed, d soaking in distilled water, and a extracted bioactive were used. Extraction was performed using water soaking methods. The crushed Neem seeds were enclosed in muslin cloth, tied, and placed in the extraction chamber.

Phytochemical Screening

The preliminary tests showed the presence of phytochemical components in Neem seed extract as endemic medicinal plants. Screening was performed for flavonoids, quinones, alkaloids, tannin, phenol, carbohydrate, terpenoid, flavonoid, alkaloid, phenols,

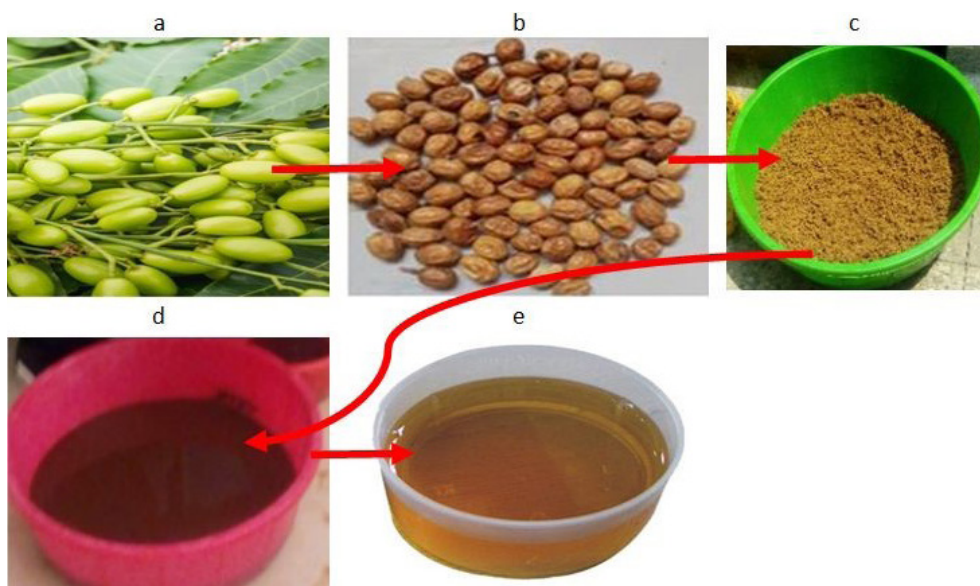


Figure 1. Neem seed and bioactive extract

glycosides and saponin bioactive compounds. Using the titration tests the phytochemical components were qualified with strongly presence by color changes during titration as shown in Table 1.

Figure 2 shows that the presence capacitates of phytochemicals Neem seed extract. There were three layers of quantitative determination of the phytochemical constituents in high, medium and low levels.

Antimicrobial Evaluation

The major cause of human health problems in life is often attributed to bacteria. So, in this study, the extract was experimentally applied on the bacteria. The presence of these bioactive compounds recovered from the traditional medicinal plants were found to inhibit the growth of both reference strains and clinical isolate microbes. Antimicrobial activity of the aqueous and organic extracts of plant samples were evaluated by the paper disc diffusion method as shown in Figure 3. For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto 15 cm diameter nutrient agar (Oxoid) plates.

The cultures were inoculated onto Sabouraud Dextrose Agar plates. Sterile filter paper discs (with a diameter of 6 mm for bacteria) containing reconstituted extract in a minimal amount of solvent at concentrations of 10 mg/mL were then positioned on the culture plates previously inoculated with bacterial cultures at 0.5 McFarland and 106 cfu/mL. Bacterial cultures (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*) were then

incubated at 37 °C for 24 hours. Paper discs impregnated with a 10 mg/mL solution of cotrimoxazole and chloramphenicol (as standard antimicrobials for bacteria) were used for comparison. Antimicrobial activity was determined by measurement of inhibition zone around each paper disc. For each extract three replicate trials were conducted against each organism.

Different concentrations of isolated dissolved in extracts were added to tested bacteria. Antimicrobial activity was determined by measurement of inhibition zone.

Determination of Minimum and Maximum Control Diameter

The MIC of the extracts was determined for each organisms in triplicates. Two milliliters of nutrient broth was added to 0.5 mL of varying concentrations of the extracts (10, 15, 20 25, 30, 35, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90 and 95 mg/mL). These dilution proportions were applied on each selected microbial species including *E. coli*, *S. aureus*, *B. subtilis*, and *S. typhi*. The system was applied frequently to the experimental organisms using ordinary antibiotics (cotrimoxazole and chloramphenicol for bacteria). The bacterial culture was incubated for 24 hours at 37 °C. After 24 hours incubation, the microbial expansion areas were determined by looking turbidity.

As seen in Table 2, the maximum and minimum inhibition zones were determined for each bacterial species. The maximum inhibition zones were indicated at the concentration of 50 to 60 mg/mL. Similar phytochemical (bioactive extracts) with the same concentration were

Table 1. Antimicrobial Activity of Extracts of Oil From Neem Seed

Determination	Carbohydrates	Tannins	Saponins	Flavonoids	Anthocyanins	Alkaloids	Quinones	Terpenoids	Phenols	Glycosides
Strongly presence		a					a		a	
Moderately presence	b		b		b			b		b
Low presence				c		c				

^a The phytochemical in high concentration.
^b The phytochemical in medium concentration.
^c The phytochemical in low concentration.

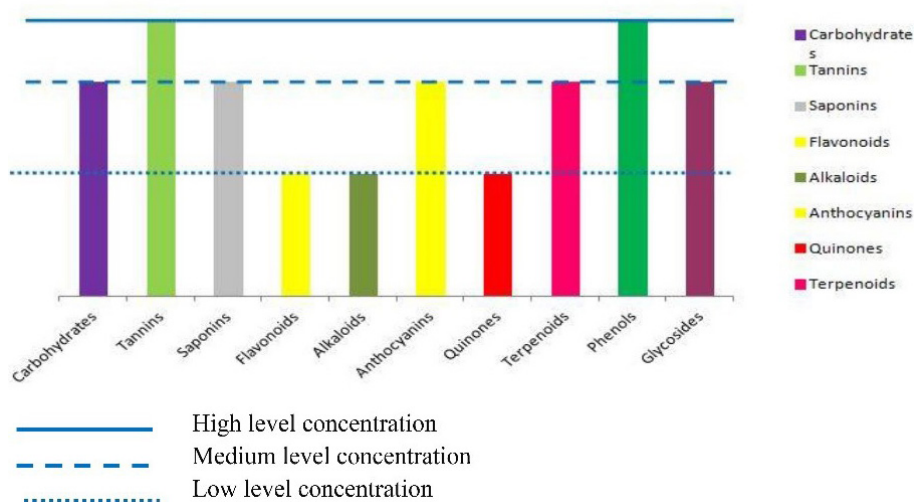


Figure 2. Qualitatively Phytochemical Identification and Color Indicators of Neem Seed Extracted Oil

applied on the difference bacterial species, and the different results were found from the bacterial resistivity against the Neem extracts.

Figure 4 shows the extract of bioactive Neem seed applications on the different pathogenic bacteria species. The factors that had effect on the control inhibition zone were the Neem seed extract concentration, resistivity of bacteria species and diffusivity of phytochemical extracts. In this study the bacterial resistivity towards the phytochemical extract were determined. As shown in Figure 4B, the control of Neem seed extract on *B. subtilis*



Figure 3. Inoculation Preparation and Antimicrobial Evaluation of Neem Seed Oil Extract

Table 2. Phytochemical Control Zone at Different Concentration and Bacteria Species

Concentration (mg/mL)	Control Diameter (mm)			
	<i>Escherichia coli</i> (G-)	<i>Staphylococcus aureus</i> (G+)	<i>Bacillus subtilis</i> (G+)	<i>Salmonella typhimurium</i> (G-)
10	12	13	16	5
15	18	15	19	8
20	24	19	24	10
25	25	21	27	15
30	28	23	29	20
35	30	25	31	28
40	31	32	33	33
45	32	37	35	39
50	34	39	38	40
55	34	40	37	41
60	32	40	37	41
65	30	37	36	40
70	25	34	32	36
75	20	30	26	31
80	15	21	16	23
85	13	12	15	13
90	10	6	13	8
95	8	6	9	7

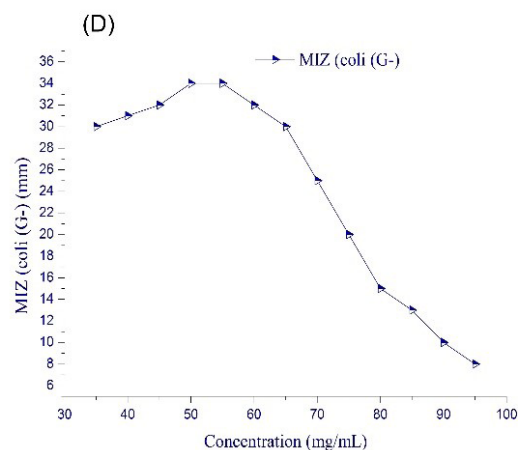
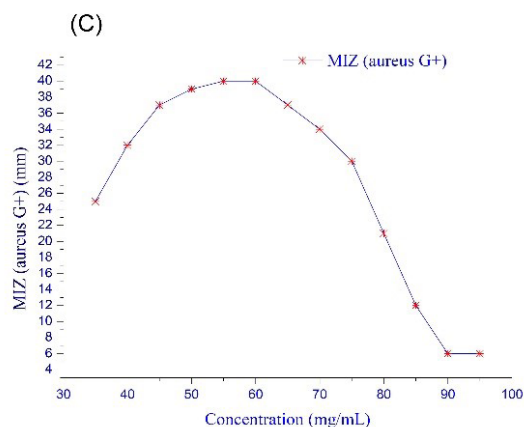
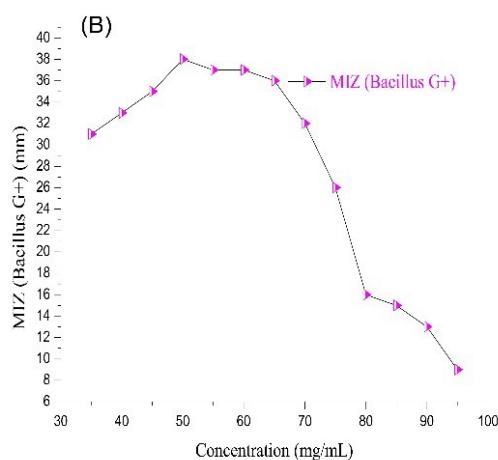
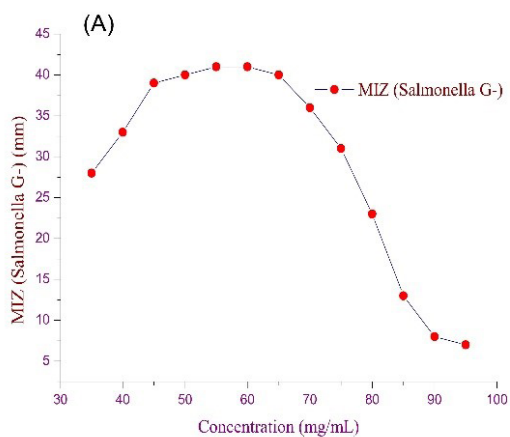


Figure 4. Determination of Inhibition Zone for Each Bacterial Species by Concentration and Inhibition Zone (A: *Salmonella typhimurium* G-; B: *Bacillus subtilis* G+; C: *Staphylococcus aureus* G+; D: *Escherichia coli* G-)

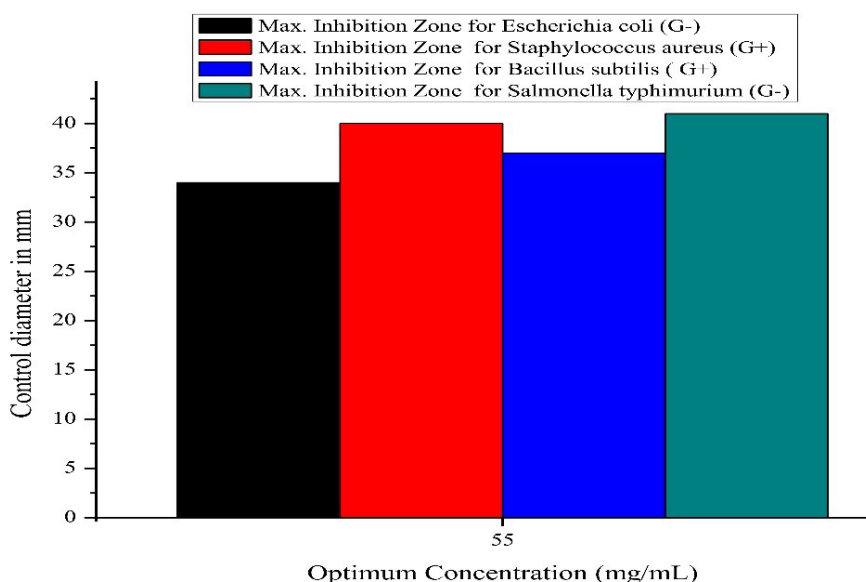


Figure 5. Optimum Concentration of Neem Seed Extract for Maximum Diameter of Bacterial Control

(G+) was the highest at the lowest concentration of extract showing the lowest resistivity. While *S. typhimurium* (G-) had the highest resistivity against Neem seed extracts which was only 5 mm control diameter at the lowest extract concentration. At the highest extract of Neem seed concentration, *S. aureus* (G+) had the highest resistance capacity while *E. coli* (G-) had the lowest resistance towards Neem seed extract (Figure 4C and Figure 4D).

The optimum concentration of Neem seed extract was 55 mg/mL for all pathogenic bacteria species (Figure 5). At the optimal concentration, Neem seed extract demonstrated higher effectiveness against *S. aureus* (gram-positive) compared to *S. typhimurium* (gram-negative), with a recorded diameter of 41 mm in the controls. Furthermore, this concentration exhibited reduced efficacy against *B. subtilis* (gram-positive) in comparison to its activity against *E. coli* (gram-negative).

Conclusion

Tannin and phenol were the highest phytochemical concentration in the oil extract of Neem seed, while the quinones, alkaloid and flavonoids were the minimum. The antimicrobial activity of Neem seed extracts was examined on *Klebsiella* specie, *E. coli*, *S. aureus*, *B. subtilis* (G+), and *S. typhimurium* (G-). At optimum concentration (55 mg/mL), the selected pathogenic species were effectively controlled. In general, the extract of Neem seed had the antioxidant and antimicrobial effect with effective control area.

Authors' Contribution

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Methodology: Desalegn Abdissa Akuma, Ketema Beyecha Hundie.

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Supervision: Desalegn Abdissa Akuma, Ketema Beyecha Hundie, Abraham Bekele Bayu.

Validation: Desalegn Abdissa Akuma, Ketema Beyecha Hundie.

Visualization: Desalegn Abdissa Akuma, Ketema Beyecha Hundie, **Writing-original draft:** Desalegn Abdissa Akuma, Abraham Bekele Bayu.

Writing-review & editing: Desalegn Abdissa Akuma, Abraham Bekele Bayu.

Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

Not Applicable.

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References

- McEwen SA, Collignon PJ. Antimicrobial resistance: a one health perspective. *Microbiol Spectr*. 2018;6(2). doi: 10.1128/microbiolspec.ARBA-0009-2017.
- Singh S, Singh DB, Singh S, Shukla R, Ramteke PW, Misra K. Exploring medicinal plant legacy for drug discovery in post-genomic era. *Proc Natl Acad Sci India Sect B Biol Sci*. 2019;89(4):1141-51. doi: 10.1007/s40011-018-1013-x.
- Pavela R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—a review. *Plant Prot Sci*. 2016;52(4):229-41.
- Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012;135(5):581-98.
- Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, et al. Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pac J Cancer Prev*. 2014;15(11):4405-9. doi: 10.7314/apjcp.2014.15.11.4405.
- Sarker U, Oba S. Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and

- antioxidant activity in *Amaranthus tricolor*. Appl Biochem Biotechnol. 2018;186(4):999-1016. doi: 10.1007/s12010-018-2784-5.
7. Sood A, Lang DK, Kaur R, Saini B, Arora S. Relevance of aromatase inhibitors in breast cancer treatment. Curr Top Med Chem. 2021;21(15):1319-36. doi: 10.2174/1568026621666210701143445.
 8. Jasek K, Kubatka P, Samec M, Liskova A, Smejkal K, Vybohova D, et al. DNA methylation status in cancer disease: modulations by plant-derived natural compounds and dietary interventions. Biomolecules. 2019;9(7):289. doi: 10.3390/biom9070289.
 9. Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. Molecules. 2019;24(6):1076. doi: 10.3390/molecules24061076.
 10. Klančnik A, Šimunović K, Sterniša M, Ramić D, Smole Možina S, Bucar F. Anti-adhesion activity of phytochemicals to prevent *Campylobacter jejuni* biofilm formation on abiotic surfaces. Phytochem Rev. 2021;20(1):55-84. doi: 10.1007/s11101-020-09669-6.
 11. Russell A. Anthropology of Tobacco: Ethnographic Adventures in Non-Human Worlds. Taylor & Francis; 2019.
 12. Aziz MA. Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits. J Integr Med. 2015;13(3):173-84. doi: 10.1016/s2095-4964(15)60179-0.
 13. Pathan AA, Shah RH, Vaghela HM. The Biogenic Synthesis of Au, Pd and Pt Nanoparticles AND Its Medicinal Applications: A Review. Cambridge Scholars Publishing; 2018.
 14. Chava VR, Manjunath SM, Rajanikanth AV, Sridevi N. The efficacy of Neem extract on four microorganisms responsible for causing dental caries viz *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: an in vitro study. J Contemp Dent Pract. 2012;13(6):769-72. doi: 10.5005/jp-journals-10024-1227.
 15. Babatunde DE, Otusemade GO, Ojewumi ME, Agboola O, Oyeniyi E, Deborah Akinlabu K. Antimicrobial activity and phytochemical screening of Neem leaves and lemon grass essential oil extracts. Int J Mech Eng Technol. 2019;10(3):882-9.
 16. Jain A, Joshi A, Joshi J, Tatawat M, Saeed S, Telang S, et al. Comparative study of phytochemical screening and antibacterial activity of four medicinal plants. J Med Plants. 2019;7(4):81-9.
 17. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014;2(5):115-9.
 18. Moonmun D, Majumder R, Lopamudra A. Quantitative phytochemical estimation and evaluation of antioxidant and antibacterial activity of methanol and ethanol extracts of *Heliconia rostrata*. Indian J Pharm Sci. 2017;79(1):79-90.
 19. Sharma T, Sidhu MC. Cytomorphological and preliminary phytochemical screening of *Eclipta alba* (L.) Hassk. Int J Green Pharm. 2017;11(1):S23-32. doi: 10.22377/ijgp.v11i01.855.
 20. Kumar RS, Balasubramanian P, Govindaraj P, Krishnaveni T. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. roots (coriander). J Pharmacogn Phytochem. 2014;2(6):74-8.
 21. Varadharajan V, Janarthanan UK, Krishnamurthy V. Physicochemical, phytochemical screening and profiling of secondary metabolites of *Annona squamosa* leaf extract. World J Pharm Res. 2012;1(4):1143-64.
 22. Sinaga M, Ganesan K, Nair SK, Gani SB. Preliminary phytochemical analysis and in vitro antibacterial activity of bark and seeds of Ethiopian Neem (*Azadirachta indica* A. Juss). World J Pharm Pharm Sci. 2016;5(4):1714-23.
 23. Pereira V, Dias C, Vasconcelos MC, Rosa E, Saavedra MJ. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). Ind Crops Prod. 2014;52:1-7. doi: 10.1016/j.indcrop.2013.09.032.