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A COMPARATIVE AND SYNERGISTIC *IN-VITRO* ANTIMICROBIAL EVALUATION OF *NIGELLA SATIVA* AND *AMARANTHUS POLYGONOIDES* SEED EXTRACT

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ABSTRACT

The present study aimed to evaluate the antimicrobial activity of seed extracts of *Amaranthus polygonoides* and *Nigella sativa*, individually and in combination, using the agar disc diffusion method. The extracts were tested at concentrations of 20, 40, and 60 µg/ml, and their efficacy was compared with the standard antibiotic ciprofloxacin. The results demonstrated that both individual and combined extracts exhibited concentration-dependent antimicrobial activity. The *Nigella sativa* extract showed zones of inhibition of 8 mm, 14 mm, and 18 mm, while *Amaranthus polygonoides* exhibited 9 mm, 12 mm, and 16 mm at 20, 40, and 60 µg/ml, respectively. The combined extract displayed improved activity with inhibition zones of 12 mm, 15 mm, and 18 mm at the respective concentrations, indicating a possible synergistic effect. The standard drug ciprofloxacin showed significantly higher antimicrobial activity, with zones of inhibition of 18 mm, 22 mm, and 29 mm at corresponding concentrations. Among the plant extracts, the combined formulation demonstrated comparatively better antimicrobial efficacy than the individual extracts.

1. INTRODUCTION

The rapid emergence of bacterial infections and the increasing prevalence of antibiotic resistance have become major global health concerns. Antibiotics have played a crucial role in modern medicine by enabling the effective treatment of infectious diseases and supporting advanced medical procedures such as organ transplantation, chemotherapy, and complex surgical interventions. However, the widespread and often inappropriate use of antibiotics has contributed to the development of antimicrobial resistance (AMR), making many conventional drugs less effective and posing a significant challenge to public health worldwide [1].

Antimicrobial resistance occurs when microorganisms such as bacteria develop mechanisms that protect them from the effects of antimicrobial agents. As a result, infections become more difficult to treat, leading to prolonged illness, increased healthcare costs, and higher mortality rates. The emergence of multidrug-resistant bacterial strains has intensified the need to discover new and effective antimicrobial agents. In recent years, natural products derived from medicinal plants have gained considerable attention as potential alternatives to synthetic drugs due to their therapeutic efficacy, natural origin, accessibility, and relatively lower side effects.

Medicinal plants are rich sources of biologically active compounds known as phytochemicals, such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and steroids. These compounds exhibit a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. Plant-derived products obtained from different parts such as seeds, leaves, roots, bark, fruits, and flowers have long been used in traditional medicine systems for the treatment of various infectious diseases. Scientific studies have demonstrated that these phytochemicals can inhibit microbial growth by disrupting cell membranes, interfering with enzyme activity, inhibiting nucleic acid synthesis, and altering essential metabolic pathways [2].

Among medicinal plants, *Nigella sativa*, commonly known as black seed or black cumin, has been widely recognized for its diverse pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities. Similarly, *Amaranthus polygonoides* is reported to contain various bioactive constituents that may contribute to

its medicinal potential. Investigating the antimicrobial properties of these plant species may therefore provide valuable insights into the development of natural antimicrobial agents.

Therefore, the present study was undertaken to evaluate the antimicrobial activity of seed extracts of *Nigella sativa* L. and *Amaranthus polygonoides* against environmental bacterial isolates obtained from soil samples. Different solvent extracts, namely hexane, ethyl acetate, and aqueous extracts, were prepared and their antibacterial activity was assessed using the disc diffusion method to determine their potential as natural antimicrobial agents [2].

2. SOURCES OF ANTIMICROBIALS

Antimicrobial agents originate from various natural and synthetic sources. These compounds inhibit or destroy pathogenic microorganisms by targeting essential bacterial structures and metabolic processes such as the cell wall, cell membrane, protein synthesis machinery, nucleic acid synthesis, and metabolic pathways, thereby preventing bacterial growth and replication [6].

2.1 Natural Sources

Natural sources are among the earliest and most important contributors to antimicrobial discovery and include microorganisms, plants, and animals [6].

Microorganisms such as bacteria and fungi produce many clinically important antibiotics. For instance, Streptomyces species produce antibiotics such as streptomycin and tetracycline, while fungi like Penicillium produce penicillin. These antibiotics primarily attack bacterial cell wall synthesis or inhibit protein synthesis, which prevents bacterial multiplication and ultimately leads to cell death [6].

Plants are rich sources of antimicrobial compounds due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phenolics, terpenoids, and essential oils. These phytochemicals exert antimicrobial activity by damaging microbial cell membranes, disrupting enzyme activity, interfering with DNA replication, and inhibiting metabolic pathways, thereby restricting bacterial growth [6].

Animal-derived antimicrobial substances also play a role in innate immunity. Compounds such as lysozyme, defensins, and lactoferrin help protect against microbial infections. Lysozyme breaks down the peptidoglycan layer of bacterial cell walls, defensins disrupt microbial membranes, and lactoferrin limits bacterial growth by binding iron required for bacterial metabolism.

2.2 Semi-Synthetic Sources

Semi-synthetic antimicrobials are chemically modified forms of natural antibiotics designed to improve antimicrobial effectiveness and overcome resistance. Examples include ampicillin and amoxicillin, which are derived from penicillin. These drugs mainly inhibit bacterial cell wall synthesis, weakening the bacterial structure and leading to cell lysis, thereby reducing bacterial growth [3-46].

2.3 Synthetic Sources

Synthetic antimicrobial agents are produced entirely through chemical synthesis. Examples include sulfonamides, fluoroquinolones, and nitroimidazoles. These agents target essential bacterial metabolic processes such as folic acid synthesis, DNA replication, and protein synthesis, preventing bacterial cell division and growth [6].

2.4 Chemical and Mineral Sources

Certain inorganic chemicals and mineral compounds also possess antimicrobial activity. Substances such as iodine, hydrogen peroxide, silver salts, and boric acid act as antiseptics and disinfectants. They destroy microorganisms by causing protein denaturation, oxidative damage, and disruption of bacterial cell membranes, thereby preventing bacterial survival and multiplication [6].

2.5 Biotechnological Sources

Biotechnological approaches involve the use of genetic engineering, recombinant DNA technology, and fermentation processes to produce antimicrobial agents. Genetically engineered microorganisms can produce improved antibiotics and antimicrobial peptides that target bacterial membranes, enzymes, and genetic material, helping inhibit microbial growth and overcome drug resistance [6].

3. CLASSIFICATION

Antimicrobial agents can be classified based on their source, target microorganism, mechanism of action, and spectrum of activity. Such classification helps in understanding how these agents interfere with bacterial cell structures and metabolic pathways to control or eliminate microbial infections.

4. LITERATURE REVIEW

Singh R. et. al., (2025) [1]: The review emphasized the significant role of *Nigella sativa* in combating antimicrobial resistance and highlighted its synergistic interactions with conventional antibiotics, suggesting its potential in combination therapies.

Kumar V. et. al., (2025) [2]: The study highlighted the importance of medicinal plants, including *Amaranthus* species, as promising sources for the development of novel antimicrobial agents.

Sharma N. et. al., (2025) [3]: The research reported that phytochemicals present in *Nigella sativa* and *Amaranthus* contribute to strong antimicrobial activity, supporting their therapeutic relevance.

Patel K. et. al., (2025) [4]: The review emphasized the therapeutic potential of herbal medicines, particularly *Nigella sativa*, in the treatment of microbial infections.

Rao S. et. al., (2025) [5]: The study confirmed the antimicrobial efficacy of *Amaranthus* extracts against a wide range of pathogenic microorganisms.

Gupta P. et. al., (2025) [6]: The research highlighted *Nigella sativa* as a potential candidate for antimicrobial drug discovery due to its bioactive constituents.

Das R. et. al., (2025) [7]: The study emphasized the application of *Amaranthus* species in antimicrobial therapy owing to their rich bioactive profile.

Singh T. et. al., (2025) [8]: The findings reported strong microbial inhibition by *Nigella sativa* extracts, supporting its traditional medicinal use.

Verma P. et. al., (2025) [9]: The study highlighted the importance of plant-based antimicrobials, particularly *Amaranthus* species, in modern therapeutic approaches.

Khan R. et. al., (2025) [10]: The review emphasized the growing importance of medicinal plants like *Nigella sativa* and *Amaranthus* in addressing antimicrobial resistance.

Rahman M.M. et. al., (2024) [11]: The study explained that *Nigella sativa* exerts antimicrobial effects through mechanisms such as membrane disruption and biofilm inhibition.

Kumar S. et. al., (2024) [12]: The research demonstrated that *Amaranthus* extracts possess antibiofilm activity, preventing microbial colonization and resistance.

Sharma P. et. al., (2024) [13]: The study reported enhanced antimicrobial activity of nanoparticles synthesized using *Amaranthus* extracts, indicating their application in nanomedicine.

Ahmed Z. et. al., (2024) [14]: The research confirmed strong antibacterial and antifungal activity of *Nigella sativa* oil, supporting its use in medicinal formulations.

Singh R. et. al., (2024) [15]: The review highlighted *Nigella sativa* as a promising plant-based antimicrobial agent against resistant pathogens.

Patel R. et. al., (2024) [16]: The study revealed that antioxidant compounds in *Amaranthus* contribute significantly to its antimicrobial activity.

Gupta S. et. al., (2024) [17]: The comparative study identified *Nigella sativa* as highly effective against bacterial pathogens among various plant extracts.

Mehta D. et. al., (2024) [18]: The study emphasized the antimicrobial potential of *Amaranthus* species due to their phytochemical composition.

Farooq U. et. al., (2023) [19]: The research demonstrated strong antimicrobial activity of *Nigella sativa* extracts against pathogenic bacteria.

Sahu R. et. al., (2023) [20]: The study reported effective antifungal activity of *Amaranthus* extracts, suggesting their application in healthcare and agriculture.

Patel D. et. al., (2023) [21]: The findings indicated that phenolic compounds in *Amaranthus* contribute to antimicrobial activity by disrupting microbial cell membranes.

Khan S. et. al., (2023) [22]: The study confirmed significant antibacterial activity of *Nigella sativa* against Gram-positive bacteria.

Verma A. et. al., (2023) [23]: The research emphasized the effectiveness of *Nigella sativa* as a natural antimicrobial agent for pharmaceutical and food industries.

Rao M. et. al., (2023) [24]: The study highlighted the antimicrobial potential of both *Nigella sativa* and *Amaranthus* species in traditional medicine and drug development.

Khan M.A. et. al., (2022) [25]: The study identified thymoquinone and phenolics as major antimicrobial compounds in *Nigella sativa*.

Das S. et. al., (2022) [26]: The research demonstrated significant antibacterial activity of *Amaranthus viridis* extracts.

Gupta R. et. al., (2022) [27]: The study reported strong antibacterial activity of *Nigella sativa* compared to other plant extracts.

Singh P. et. al., (2022) [28]: The findings showed effective antifungal activity of *Amaranthus* extracts against plant pathogens.

Hadi S. et. al., (2021) [29]: The study confirmed dose-dependent antimicrobial activity of *Nigella sativa* extracts.

Ali B.H. et. al., (2021) [30]: The review highlighted broad-spectrum antimicrobial activity of *Nigella sativa*.

Peter K.V. et. al., (2021) [31]: The study reported antimicrobial and antioxidant properties of *Amaranthus* species.

Rahman S. et. al., (2021) [32]: The research demonstrated strong antibacterial activity of *Nigella sativa* oil against *Staphylococcus aureus*.

Ahmad A. et. al., (2020) [33]: The study confirmed significant antibacterial activity of *Nigella sativa* due to thymoquinone.

Yimer E.M. et. al., (2020) [34]: The review emphasized antimicrobial mechanisms of *Nigella sativa*, including membrane disruption.

Sarker U. et. al., (2020) [35]: The study reported antimicrobial activity of *Amaranthus* due to phenolics and flavonoids.

5. MATERIALS & METHODS

5.1. Sample collection

The seeds of *Nigella sativa* and *Amaranthus polygonoides* were collected from market in Chittoor (AP), India, during the month of December. The plant materials were authenticated by a qualified taxonomist Dr. K. Madhava Chetty, from the Department of Botany, Sri Venkateswara University Tirupati – 517502, A.p., India. The collected samples were cleaned to remove dust and foreign particles, shade dried, and powdered for further extraction. A voucher specimen was preserved in the departmental herbarium for future reference.

FIGURE 1. Extraction process

The seeds of *Nigella sativa* and *Amaranthus polygonoides* were thoroughly washed with distilled water to remove dust and impurities and shade dried at room temperature. The dried seeds were powdered using a mechanical grinder and stored in airtight containers for further use.

5.2. Hexane Extract

A 10 g quantity of powdered seed material was extracted using 100 mL of hexane by the cold maceration method. The mixture was kept at room temperature for 48–72 hours with occasional shaking to ensure proper extraction. After maceration, the mixture was filtered through Whatman No.1 filter paper and the filtrate was concentrated to obtain the hexane extract.

5.3. Ethyl Acetate Extract

Similarly, 10 g of powdered seed material was macerated with 100 mL of ethyl acetate for 48–72 hours with intermittent shaking. The mixture was filtered using Whatman No.1 filter paper, and the filtrate was evaporated to obtain the ethyl acetate extract.

5.4. Aqueous Extract

For the aqueous extract, 10 g of powdered seed material was macerated with 100 mL of distilled water using the cold maceration method for 48–72 hours. The mixture was filtered and the filtrate was concentrated to obtain the aqueous extract.

All the obtained crude extracts were stored in airtight containers at 4°C until further use. For antimicrobial evaluation, the dried extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to prepare the required concentrations for experimental analysis.

5.5. Isolation and Preparation of Microbial Inoculum

Environmental bacterial isolates obtained from soil samples were isolated using the serial dilution technique under sterile conditions. Approximately 1 g of soil sample was suspended in 9 mL of sterile distilled water and mixed thoroughly to obtain a 10^{-1} dilution. From this suspension, 1 mL was transferred into another test tube containing 9 mL of sterile distilled water to obtain a 10^{-2} dilution. The serial dilution process was continued sequentially up to the 10^{-6} dilution.

An aliquot from the 10^{-6} dilution was aseptically spread onto sterile nutrient agar plates and incubated at 37 °C for 24 hours to allow bacterial growth. Distinct colonies appearing on the agar plates were observed based on colony morphology such as size, shape, color, and texture, and representative colonies were carefully selected and subcultured to obtain pure bacterial cultures.

The purified bacterial cultures were transferred into sterile nutrient broth and incubated at 37 °C for 24 hours to prepare the bacterial inoculum. The turbidity of the broth culture indicated active microbial growth. These actively growing cultures were subsequently used as test microorganisms for *in-vitro* antimicrobial activity assays of the plant extracts.

5.6. Disc Diffusion Method

The antimicrobial activity of the plant seed extracts was evaluated using the Disc Diffusion Method. Sterile nutrient agar medium was prepared and poured into sterile Petri plates and allowed to solidify. The prepared bacterial inoculum obtained from environmental bacterial isolates was adjusted to obtain an approximate microbial density of ~10^6 CFU/mL (colony-forming units per milliliter) to ensure uniform bacterial growth.

The standardized bacterial suspension was evenly spread over the surface of the nutrient agar plates using a sterile cotton swab to form a uniform bacterial lawn. Sterile filter paper discs (approximately 6 mm in diameter) were impregnated with the plant extract solutions prepared from *Nigella sativa* and *Amaranthus polygonoides* seeds dissolved in 10% dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL. Approximately 20 µL of the extract solution was applied to each sterile disc.

The impregnated discs were placed on the inoculated agar surface using sterile forceps and gently pressed to ensure proper contact with the agar surface. A disc containing 10% DMSO was used as the negative control. The plates were then incubated at 37 °C for 24 hours.

After incubation, antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (mm) surrounding each disc. The presence of a clear zone around the disc indicated the antibacterial activity of the plant extracts against the tested environmental bacterial isolates.

6. RESULTS AND DISCUSSION

6.1. 1 Percentage yield using 10gm sample

The percentage yield of extracts obtained from *Amaranthus polygonoides* and *Nigella sativa* using different solvents is shown in Table 1. In *Amaranthus polygonoides*, the highest yield was observed in ethyl acetate extract (13.1%), followed by aqueous (12.9%) and hexane (12.8%) extracts. In *Nigella sativa*, the hexane extract showed the highest yield (20.5%), followed by ethyl acetate (15%) and aqueous extract (12.4%). The variation in yield may be due to differences in solvent polarity and phytochemical composition of the plants.

6.2. Physical parameter of Extract

The colour and consistency of the extracts are presented in Table 2. The ethyl acetate extracts were brown to dark brown and semi-solid.

TABLE 1. Data showing percentage yield of extract of powered Amaranthus polygonoids & *Nigella sativa*

Extract	% yield w/w	
	Amaranthus polygonoids	***Nigella sativa l***
Ethyl acetate	13.1%	15%
Hexane	12.8%	20.5%
Aquous	12.9%	12.4%

TABLE 2. Data showing the colour, consistency of different extract of Amaranthus polygonoids & *Nigella sativa*

Extract	***Amaranthus polygonoids***		***Nigella sativa***	
	Colour	**Consistenc**	**Colour**	**Consistenc**
Ethyl acetate	Brown or dark greenish-brown	Semi-solid	Yellowish-brown to dark brown	Semi-solid or sticky
Hexane	Light yellow or greenish	Oily or slightly viscous	Pale yellow to light brown	Oily or semi-viscous
Aquous	Dark brown or greenish-brown	Thick paste or gummy	Brown to dark brown	Thick, gummy, or viscous

TABLE 3. Data showing the Proximate Analysis of *Amaranthus polygonoids & Nigella sativa*

Parameter	Concentration %	
	Amaranthus polgonoids	***Nigella sativa***
Moisture Content	20	16
Ash content	25	15
Acid insoluble	4	2
Water soluble ash	17	10
Crude lipid	8	30
Crude protein	15	25
Carbohydrate	45	14
Calorific value(kcal/100gm)	372	426

TABLE 4. Data showing the preliminary Phytochemical screening of Amranthus polygonoids & *Nigella sativa* lextract.

Phytocompounds	Ethyl acetate	Hexane	Aq
Alkaloids	+	-	+
Glycosides	+	-	+
Flavonoids	+	-	+
Phenols	+	-	+
Tannins	-	-	+
Saponins	-	-	+
Terpenoids	+	+	-
Steroids	+	+	-
Quinones	+	-	+
Coumarins	+	-	+
Carbohydrates	-	-	+
Proteins	-	-	+
Aminoacids	-	-	+
Fixed oils & fats	-	+	-

("+") indicates presence while ("-") indicates absence

TABLE 5. Antimicrobial Activity of *Nigella sativa* seed extract by Disc Diffusion Method

Concentration (µg/ml)	Zone of Inhibition of *Nigella sativa* seed extract (mm) (Mean ± SD)	Zone of Inhibition of Ciprofloxacin (Std)
20	8	18
40	14	22
60	18	29

The hexane extracts appeared pale yellow and oily due to the presence of non-polar compounds such as oils and fats. The aqueous extracts were dark brown and viscous, which may be due to the presence of polar compounds such as tannins, carbohydrates, and proteins.

6.3. Proximate analysis of *Nigella sativa* and Amaranthus polygonoids seed extract

The proximate composition and ash values of *Amaranthus polygonoides* and *Nigella sativa* were determined and are presented in Table 3.The moisture content was found to be higher in *Amaranthus polygonoides* (20%) compared to *Nigella sativa* (16%), indicating relatively higher water content in the former.The total ash content, which represents the total inorganic matter, was observed to be 25% in *Amaranthus polygonoides* and 15% in *Nigella sativa*, suggesting a greater mineral composition in *Amaranthus polygonoides*.The acid-insoluble ash, indicating the presence of siliceous matter such as sand and soil, was found to be 4% in *Amaranthus polygonoides* and 2% in *Nigella sativa*. This shows comparatively higher earthy impurities in *Amaranthus polygonoides*. The water-soluble ash values were 17% for *Amaranthus polygonoides* and 10% for *Nigella sativa*, indicating a higher proportion of water-soluble inorganic salts in *Amaranthus polygonoides*.In terms of nutritional composition, *Nigella sativa* exhibited a significantly higher crude lipid content (30%) compared to *Amaranthus polygonoides* (8%), confirming its oil-rich nature. Similarly, the crude protein content was higher in *Nigella sativa* (25%) than in *Amaranthus polygonoides* (15%). On the other hand, total carbohydrate content was considerably higher in *Amaranthus polygonoides* (45%) compared to *Nigella sativa* (14%), indicating its potential as a carbohydrate-rich plant source. The calorific value was calculated to be higher in *Nigella sativa* (426 kcal/100 g) than in *Amaranthus polygonoides* (372 kcal/100 g), which may be attributed to its higher lipid and protein content.

The phytochemical screening results are shown in Table 3. The extracts were found to contain various phytoconstituents such as alkaloids, flavonoids, phenols, glycosides, terpenoids, steroids, and coumarins. The aqueous extract showed the presence of tannins, saponins, carbohydrates, proteins, and amino acids, while hexane extract mainly contained terpenoids, steroids, and fixed oils. These compounds may be responsible for the biological activities of the plants.

6.4. Antimicrobial activity

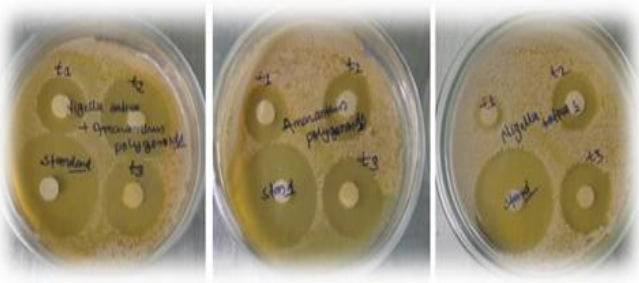
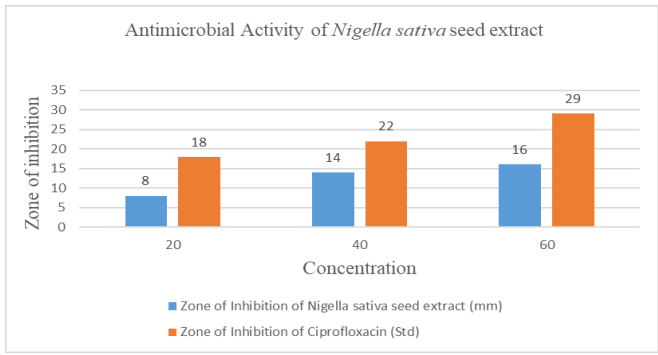


FIGURE 2. Zone of Inhibition of Amaranthus polygonoids and *Nigella sativa* seed extract

6.5. Antimicrobial Activity of *Nigella sativa*

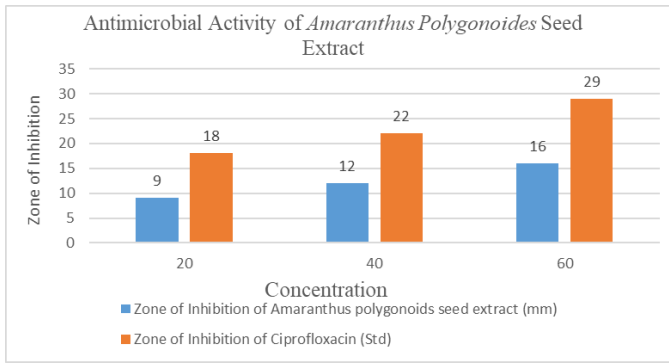
The antimicrobial activity of the combined extract of *Nigella sativa* was evaluated using the disc diffusion method and the results are shown in Table No.5. The extract exhibited concentration-dependent antimicrobial activity. The zone of inhibition increased from 8 mm at 20 µg/ml to 14 mm at 40 µg/ml and reached a maximum of 18 mm at 60 µg/ml. The standard antibiotic ciprofloxacin showed zones of inhibition of 18 mm, 22 mm, and 29 mm at the respective concentrations, indicating significantly higher antibacterial activity than the plant extract.



GRAPH 1. Antimicrobial Activity of *Nigella sativa* seed extract

6.6. Antimicrobial Activity of *Amaranthus polygonoides*

The antimicrobial activity of the combined extract of *Amaranthus polygonoides* by disc diffusion method is presented in Table No.6. The extract showed increasing antimicrobial activity with increasing concentration. The zones of inhibition were 9 mm, 12 mm, and 16 mm at 20, 40, and 60 µg/ml, respectively. The standard antibiotic ciprofloxacin showed zones of inhibition of 18 mm, 22 mm, and 29 mm at the respective concentrations, indicating significantly higher antibacterial activity than the plant extract.



GRAPH 2. Antimicrobial Activity of *Amaranthus polygonoides* seed extract

6.7. Antimicrobial Activity of Combined Extract of *Amaranthus polygonoides* & *Nigella sativa*

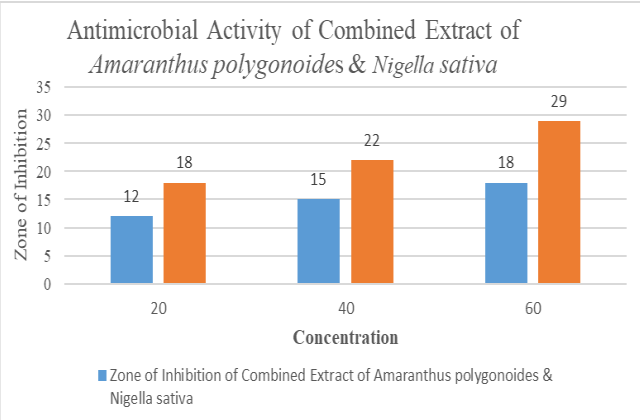
The antimicrobial activity of the combined extracts of *Amaranthus polygonoides* and *Nigella sativa* using the agar disc diffusion method is shown in Table No.7. The extract produced 12 mm, 15 mm, and 18 mm zones of inhibition at 20, 40, and 60 mg/ml, respectively. The standard antibiotic ciprofloxacin showed zones of inhibition of 18 mm, 22 mm, and 29 mm at the respective concentrations, indicating significantly higher antibacterial activity than the plant extract.

TABLE 6. Antimicrobial Activity of *Amaranthus polygonoides* seed extract by Disc Diffusion Method

Concentration (µg/ml)	Zone of Inhibition of Amaranthus polygonoids seed extract (mm) (Mean ± SD)	Zone of Inhibition of Ciprofloxacin (Std)
20	9	18
40	12	22
60	16	29

TABLE 7. Antimicrobial Activity of Combined Extract of *Amaranthus polygonoides* & *Nigella sativa* by Disc Diffusion Method

Concentration (µg/ml)	Zone of Inhibition of Combined Extract of *Amaranthus polygonoides* & *Nigella sativa* (mm) (Mean ± SD)	Zone of Inhibition of Ciprofloxacin (Std)
20	8	18
40	14	22
60	18	29



GRAPH 3. Antimicrobial Activity of Combined Extract of *Amaranthus polygonoides* & *Nigella sativa*

6. CONCLUSION

The present study evaluated the *in-vitro* antimicrobial activity of aqueous, ethyl acetate, and hexane extracts of *Nigella sativa* and *Amaranthus polygonoides* using disc diffusion and agar well diffusion methods. The results demonstrated that both plant extracts possess significant antimicrobial potential against the tested bacterial strains.

Among the different solvent extracts, ethyl acetate and hexane extracts showed comparatively higher antimicrobial activity than the aqueous extract, as evidenced by the larger zones of inhibition. The antimicrobial activity was also found to be concentration-dependent, with higher concentrations producing greater inhibition of bacterial growth.

Furthermore, the combined extracts of *Nigella sativa* and *Amaranthus polygonoides* exhibited enhanced antimicrobial activity compared to the individual extracts, suggesting a possible synergistic effect between the phytoconstituents present in both plants.

Overall, the findings of this study support the potential use of these medicinal plants as natural sources of antimicrobial agents. However, further studies including isolation of active compounds, mechanism of action studies, and in-vivo evaluations are necessary to confirm their therapeutic potential and possible pharmaceutical applications.

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