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Examine The Antibacterial Properties And Essential Oil Extracts Of Artemisia Afra Leaves Against Gram Negative Bacteria

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Abstract

Artemisia afra, a widely recognized medicinal plant, was investigated for its antibacterial properties and essential oil extracts against various pathogenic bacteria. Essential oils were extracted from the leaves of Artemisia afra using hydrodistillation. To evaluate the antibacterial activity, both essential oil and extracts obtained with ethanol, methanol, petroleum ether, and aqueous solvents were tested against five bacterial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella typhimurium. The disk diffusion method and minimum inhibitory concentration (MIC) assays were employed to determine the efficacy of the extracts. Essential oil exhibited significant antibacterial activity with inhibition zones ranging from 10 to 32 mm, and MIC values between 0.5 and 1.0 µL/mL. Comparatively, solvent extracts showed varied effectiveness, with ethanol and methanol extracts generally demonstrating higher antibacterial activity than petroleum ether and aqueous extracts. The essential oil and ethanol extract were notably effective against Staphylococcus aureus and Salmonella typhimurium. This study underscores the potential of Artemisia afra essential oil and its extracts as viable natural antimicrobial agents, suggesting further exploration for their application in pharmaceutical and therapeutic contexts.

Keywords: Artemisia afra, Essential oil, Antibacterial properties, Disk diffusion assay, Minimum inhibitory concentration (MIC)

1. INTRODUCTION

Medicinal plants have a long and illustrious history in traditional medicine [1]. Pharmacological research on medicinal plants is currently exploding. The primary driving force behind this is the emphasis on medicinal plants as a source of novel antibacterial compounds with therapeutic significance and as a possible drug development tool for vital physiological and physical health care. There are several benefits to using natural medicines instead of synthetic medications. Herbal medicine can help with multi-drug resistance issues [2]. This is partly because, due to their lower potential for side effects, herbal medicines are often seen as safer than synthetic drugs [3].

Antibiotics may weaken immune systems and cause allergic reactions in certain persons [4]. For this reason, using herbal treatments to treat certain illnesses is preferred. The World Health Organization (WHO) reports that the resistance rates of Escherichia coli and Klebsiella pneumoniae to ciprofloxacin were 8.4–92.9% and 4.1–79.4%, respectively. Third-generation cephalosporin resistance was found in 12.11 percent of Staphylococcus aureus and 36.0% of E. coli, according to the World Health Organization. Drug-resistant Candida albicans was the most important fungal infection out of all of them. Notwithstanding the availability of potent antifungal medications, invasive candidiasis still results in 20–50% of patient deaths. Extended hospital stays—two to four weeks on average—can extend up to two months in severe circumstances [3]. Treatment for bacterial and fungal infections is notoriously challenging, which raises the risk of treatment failure, prolongs hospital stays, and dramatically increases treatment costs. Because of the toxicity of current anti-fungal and anti-bacterial treatments as well as the prevalence of drug-resistant illnesses, people are now more aware of the antibacterial activity of natural goods [6].

Artemisia afra, also known as African wormwood, is a plant that is a member of the Asteraceae family, more especially the Compositae [7–13]. In Africa, this plant's essential oil is highly valued for its medicinal properties. It's called "Chigugn" (in Amharic) by the inhabitants [15]. This deciduous subshrub or perennial plant has green or gray leaves and yellow blooms. It is evergreen. The plant has a rich, sweet scent that is released when it is destroyed. At an elevation of 3070–3600 meters, this tree can grow up to 1.5 meters in height.

With many traditional applications, Artemisia afra is a widely used medicinal plant. Numerous conditions have been treated with it, such as: gout, sore throat, asthma, colic, intestinal parasitic diseases, fever, headache, bladder and kidney disorders, diabetes, cough, colds, malaria, rheumatoid swellings, bronchitis, dry dyspepsia, purgative, chills, dandruff, smallpox, stomach pain, dental care, pneumonia, poor appetite, wounds, flu, heart disease, cancer, and more.

Fig. 1: Artemisia afra

The plant's leaves possess many phenolic bioactive compounds that give it their antibacterial properties [17]. This healing plant has gained popularity as a treatment for bacterial ailments like ear infections, sore throats, and bronchial difficulties [18]. A. abortus has been traditionally used to treat viral infections such as influenza and measles, as well as parasitic diseases such as intestinal parasites and malaria. Treating diabetes, heartburn, bronchitis, and asthma are among the other medical conditions for which this plant is used [19]. Recent research indicates that A.afra has an especially potent antibiotic effect against methicillin-resistant Staphylococcus aureus, Mycobacterium smegmatis, and Mycobacterium tuberculosis. Traditionally, patients have relied on medicines to reduce viral and inflammatory illnesses [20]. Therapy for bacterial illnesses is becoming more challenging due to the emergence of more and more antibiotic-resistant bacterial strains. In places with inadequate sanitation, poor personal hygiene, and close quarters, the transmission of illness and other infectious diseases is facilitated.

2. LITERATURE REVIEW

Nortjie et al. (2024) [21] investigated the antibacterial qualities of coatings produced from plant extracts of Artemisia afra and Eucalyptus globulus. The two plants were subjected to a pulsed ultrasound-assisted extraction technique (PUAE) to extract hexanoic and methanolic chemicals. Artemisia afra yielded less, whereas Eucalyptus globulus yielded the highest quantity, at 22.76% (±0.61%), from the methanol extraction process. Phytochemical screening identified several secondary metabolites in the extracts, including phenols, quinones, and steroids. Comparing the hexanoic extract of Eucalyptus globulus to both Escherichia coli and Staphylococcus aureus, the extract exhibited the highest level of action against the latter in antimicrobial testing, with an average percentage growth decrease of 18.74% (±0.26%). Despite the absence of complete inhibition at dosages below 500 µg/mL, the minimum inhibitory concentration (MIC) values of the extracts were discovered. Some of the extracts even promoted bacterial growth. Significant antibacterial action against Staphylococcus aureus was shown when Eucalyptus globulus methanolic extracts were applied to textiles. In samples coated with polyester, the inhibition zone was 65.97 mm², while samples coated with cotton had the highest inhibition zone, measuring 258.4 mm².

Dogra et al. (2024) [22] investigated the phytoconstituents, antioxidant, anti-inflammatory, cytotoxic, and wound-healing properties of A. vestita leaf extract (ALE). Using Soxhlet extraction and gas chromatography-mass spectrometry (GC-MS), they were able to identify 36 phytochemicals in ALE, of which 22 were the main ones. The antibacterial activity of ALE was evaluated against several bacterial and fungal strains using the agar well diffusion method. With zone diameters of 14.2 ± 0.28 mm against Staphylococcus aureus, 17.6 ± 0.52 mm against Escherichia coli, and 17.6 ± 0.11 mm against Candida albicans, the results showed substantial inhibition. Its ability to significantly scavenge free radicals was demonstrated in tests using DPPH, ABTS, and FRAP, suggesting that it may have antioxidant potential. The anti-inflammatory action was assessed using an enzyme inhibition assay, and the outcomes demonstrated that COX-II was successfully inhibited. ALE was shown to be toxic to HaCaT cells using the MTT assay, which measures cytotoxicity. In addition, in an in vitro scratch experiment evaluating its ability to heal wounds, ALE performed better than the positive control, Cipladine, with a wound closure rate of 94.6% after 24 hours.

Molokoane et al. (2023) [23] investigated the anti-fungal, anti-bacterial, and cytotoxic qualities of compounds derived from Artemisia afra. Using spectroscopic methods such as 3β-taraxerol acetate (B), Liquid Chromatography-Mass Spectrometry (LC-MS), Fourier Transform Infrared Spectroscopy (FTIR), and Nuclear Magnetic Resonance (NMR), the structures of eight isolated compounds were clarified. The remaining chemicals were: Isofraxidin-7-O-β-D-glucopyranoside (H), sitosterol-3-O-β-D-glucopyranoside (F), 3,5-di-O-feruloylquinic acid (G), ferulic acid (D), scopoletin (E), and dodecyl-p-coumarate (C). Together with compounds B–C, F–G, and H, novel compounds A–H were also identified from A. afra roots. The analysis of the separated compounds and extracts revealed strong anti-fungal and anti-bacterial activity in crude extracts of dichloromethane and ethyl acetate, with a minimum inhibitory concentration (MIC) of 0.078 mg/mL. Compound E, in particular, had significant efficacy against Escherichia coli, demonstrating a minimum inhibitory concentration (MIC) of 62.5 µg/mL. Enterococcus faecalis was significantly inhibited by Compounds C and F, with MIC values of 62.5 and 31.25 µg/mL, respectively.

Nortjie et al. (2023) [24] assessed the effectiveness of coatings on textiles for biomedical purposes using plant extracts from South African biomass, specifically Eucalyptus globulus and Artemisia afra. The extracts, which were generated using a pulsed ultrasound-assisted solvent extraction method, were applied to cotton and polyester fabrics by a simple immersion procedure. Numerous metrics were examined in this study, such as phytochemical content, extraction yields, MIC, ZOI, and washing durability in addition to antibacterial activity. Methanolic extractions yielded noticeably more than hexanoic extractions. Phytochemical screening revealed the presence of bioactive components such as quinones, sterols, and phenolic compounds in extracts from A. afra and E. globulus. E. globulus hexanoic extracts showed significant inhibitory zones against Staphylococcus aureus ATCC 33591, making them the most potent antibacterial agent. The second most

efficient extracts were found to be methanolic extracts of E. globulus. Methanol extracts of A. afra were shown to be more efficient as antibacterial agents than hexanoic extracts when tested against S. aureus ATCC 33591. When E. globulus methanolic extract was applied, cotton fabric displayed the largest inhibition zone (258.4 mm2), whereas polyester fabric displayed the smallest inhibition zone (65.97 mm^2). The most notable discovery was the anti-S. aureus bioactivity. The minimum inhibitory concentration (MIC) values of the extracts ranged from 5 to 500 µg/mL. The A. afra methanol extract had the lowest MIC value (5 µg/mL) against S. aureus, demonstrating its high sensitivity.

Radulović et al. (2023) evaluated the antibacterial activity and phenolic compounds present in leaf extracts from five different species of Artemisia: A. alba, A. annua, A. campestris, A. pontica, and A. vulgaris [25]. Although A. annua is a valuable medicinal plant, the study aimed to close a gap in the literature by investigating its biological activity against phytopathogenic strains of the plant. We looked at the phenolic content of the dichloromethane-methanol (1:1) extracts using liquid chromatography-mass spectrometry (LC-MS). Quinic acid and thirteen phenolic compounds were discovered and measured. Chlorogenic acid was the most prevalent compound in all of the extracts; the other major chemicals varied depending on the species and included rutin in A. alba, vitexin in A. annua, and esculin in A. vulgaris. Using assays for mycelial growth and microdilution, the antifungal activity of twelve micromycetes was examined. Penicillium citreonigrum, Botrytis cinerea, and Monilinia laxa all shown high sensitivity, but Fusarium graminearum B1 demonstrated resistance. When examined utilizing well diffusion and microdilution methods, Xanthomonas campestris pv. campestris was the only phytopathogen against which the antibacterial activity was somewhat efficacious; other phytopathogens had less of an effect.

Nikitin et al. (2023) [26] assessed the phytochemical composition and bioactivities of five Artemisia: Artemisia annua cv species. Novichok, Artemisia dracunculus cv. Smaragd, Artemisia santonica cv. Citral, Artemisia abrotanum cv. Euxin, and Artemisia scoparia cv. Tavrida. Gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-MS/MS) were the methods used by the researchers to ascertain the phytochemical profiles of the extracts. Sesquiterpenes, phenolic acids, coumarins, flavonoids, and glycosides were among the main substances found. The study assessing the extracts' antibacterial and nematocidal properties found that activity against phytopathogenic bacteria and nematodes began at 150 µg/mL. A. scoparia cv. Tavrida demonstrated selective effects against Gram-negative bacteria A. tumefaciens and X. arboricola, as well as against fungi A. solani, R. solani, and F. graminearum. It is noteworthy that A. dracunculus cv. Smaragd demonstrated selective antibacterial activity against Gram-positive bacteria R. iranicus and B. subtilis. Additionally, at dosages ranging from 31.3 to 1000 µg/mL, the examined strains A. annua cv. Novichok, A. dracunculus cv. Smaragd, and A. santonica cv. Citral demonstrated nematocidal activity. Their potential as a nematode-targeting drug was proven by interactions with the UNC-63 protein, an N-subtype nicotine receptor.

Umam et al. (2023) [27] addressed the crucial need for novel antibacterial compounds as antibiotic resistance emerged. They discussed the most recent research on the useful and aromatic Artemisia plant in the domains of phytochemistry, pharmacology, and toxicity. By searching the complete body of literature from 2003 to 2022 in databases including PubMed, Google Scholar, Web of Science, and KNApSAcK, they found twenty-five distinct species of Artemisia and seventy-five different chemicals with demonstrated antibacterial action. They specifically drew attention to advancements in our understanding of the chemistry of these phytochemicals, including their structures, sources, and modes of action. Many substances, such as coumarins, terpenoids, and flavonoids, have been demonstrated by thermodynamic studies to be able to pass through the membranes and cell walls of bacteria and interfere with DNA, proteins, and enzymes in order to stop the activity of the bacteria. The data synthesis provided here lends credence to the notion that Artemisia species may be a useful area to start searching for novel antibacterial agents and establish a foundation for future research into their potential medical applications.

Gou et al.'s (2023) [28] examined the antibacterial activity of plants in the Asteraceae family. Many genera were covered in the review, including Baccharis, Calendula, Echinacea, Artemisia, and Centaurea. According to their review, which compiled significant findings from published research, these herbs are frequently used ethnomedically to treat infections, inflammation, and parasitic problems. Essential oils and crude extracts from these plants were revealed to be the primary sources of antimicrobial properties. Particularly, essential oils were discovered to be very significant. The effectiveness of crude extract-coated nanoparticles against MRSA and other drug-resistant bacteria was also discovered by the researchers. Staphylococcus aureus and Escherichia coli have garnered the most interest among multidrug-resistant bacteria, although the Asteraceae family as a whole has been extensively investigated. Antibacterial action mechanism investigations and minimum bactericidal concentration (MBC) studies were underreported, but minimum inhibitory concentration (MIC) values were most frequently employed in assessments of antimicrobial activity. This paper outlines the potential for Asteraceae plants, particularly Artemisia, to combat resistant bacteria and suggests avenues for further research into the mechanisms behind these plants' antibacterial activity.

Haile et al. (2022) [29] assessed the antibacterial activity of ethanolic, methanolic, and n-hexane extracts from Artemisia afra leaves against four clinical pathogens that have grown resistant to several antibiotics: Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, and Escherichia coli. After the crude extracts were made with these solvents, their antibacterial efficacy was assessed. The study found that the methanolic extract generated the biggest zone of inhibition (25.33 ± 0.58 mm) against E. coli, indicating strong activity. Conversely, the n-hexane extract had the least inhibitory activity (5.67 ± 1.56 mm) against S. aureus. The range of MIC values for the different extracts was 0 to 6.25 mg/mL, while the greatest value was observed for the methanolic extract against all clinical pathogens. For each of the four pathogens, a minimum bactericidal concentration (MBC) of 12.5 mg/mL was found in both the ethanolic and methanolic extracts. It's important to note that the methanolic extract defeated E. coli with a lower MBC of 6.25 mg/mL. The results of this study provide insight into the varying efficaciousness of several A. afra solvent extracts and indicate that its methanolic extract may have potential as an antibacterial agent, particularly against isolates that exhibit resistance to multiple antibiotics.

Janz et al. (2022) [30] addressed the issue of antibiotic resistance, also known as multi-drug-resistant (MDR) microorganisms. Several Artemisia species were reviewed for their antibacterial characteristics, including Artemisia absinthium, which is a necessary element in Absinthe. Methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Escherichia coli are two examples of the multidrug-resistant bacteria that can be effectively combated by Artemisia plant-based medicines, according to a review of current studies. According to the research, standard antibiotics may once again be effective against bacteria that have developed resistance to them because of the compounds obtained from Artemisia. Researchers think that the reason for this improvement is that components of Artemisia interact with bacterial efflux pumps, which are involved in processes of resistance. In the fight against antibiotic resistance, the findings lend support to the hypothesis that biologically active substances made from Artemisia plants could be helpful as adjuvant therapies to conventional antibiotics.

Mbokane et al. (2020) [31] examined the effects of essential oils from Artemisia afra and Moringa oleifera on resistance and haemato-immunological parameters in African Sharptooth catfish (Clarias gariepinus) challenged with Aeromonas hydrophila. White blood cell counts were considerably elevated by supplementing with both essential oils at doses ranging from 3 to 12 percent during the 45-day experiment, both before and after the bacterial challenge. Prior to the challenge, there were no significant differences in red blood cells (RBC), hemoglobin (HGB), or hematocrit (HCT) between the control and M. oleifera groups. However, supplementing with A. afra led to a substantial increase in RBC and HCT. In terms of RBC, HGB, and HCT post-challenge, there was a statistically significant difference between the lowered supplementation and control groups. There were no significant differences in lysozyme activity between the control and treatment groups, except for A. afra, where both NBT and lysozyme activities increased from 6% to 12% supplementation. Both essential oils, however, significantly increased nitro-blue tetrazolium (NBT) levels

from 3% to 12% supplementation. Fish with higher amounts of both oil supplementsation fared better in the wild. While no such alterations were found prior to the test, the kidneys of the control group and the groups that received 3% and 6% supplementation displayed significant histological abnormalities following the challenge.

3. RESEARCH GAP

Based on the literature review, a significant research gap exists in understanding the comparative efficacy and mechanisms of action of plant extracts against a broader range of multi-drug-resistant (MDR) bacterial pathogens, particularly those not yet extensively studied. While current research has identified promising antimicrobial activities and phytochemical profiles in various Artemisia species and other plants, there is limited exploration of their effectiveness against less common but clinically relevant resistant strains. Additionally, while several studies have focused on the antibacterial activities and mechanisms of specific extracts, comprehensive comparative studies that systematically analyze and contrast the performance of different extracts and their components across multiple resistant strains are lacking. Addressing this gap could provide deeper insights into the potential of these plant-based solutions and guide more targeted therapeutic applications in combating MDR bacterial infections.

4. RESEARCH METHODOLOGY

4.1 Collection and Preparation of Plant Material

Artemisia afra leaves will be collected from a verified source to ensure consistency and quality of the plant material. The leaves will be air-dried at room temperature and ground into a fine powder using a mechanical grinder. The powdered leaves will be stored in airtight containers to prevent moisture absorption and degradation.

4.2 Extraction of Essential Oils

Essential oils will be extracted from the powdered Artemisia afra leaves using a hydro distillation method. In this process, the powdered leaves will be subjected to steam distillation using a Clevenger apparatus for a duration of 3 hours. The essential oil obtained will be collected, dried over anhydrous sodium sulfate, and stored in dark glass vials at 4°C until further analysis.

4.3 Preparation of Bacterial Cultures

Selected pathogenic bacterial strains will be procured from a reputable microbiological culture collection. These may include Gram-positive bacteria (e.g., Staphylococcus aureus) and Gram-negative bacteria (e.g., Escherichia coli). The bacteria will be cultured in nutrient broth and incubated at 37°C for 24 hours to achieve a log phase growth.

4.4 Antibacterial Activity Assay

The antibacterial activity of the Artemisia afra essential oil extracts will be evaluated using the disk diffusion method. Sterile filter paper disks will be impregnated with different concentrations of the essential oil and placed on agar plates inoculated with the bacterial cultures. The plates will be incubated at 37°C for 24 hours. Antibacterial activity will be assessed by measuring the diameter of the inhibition zones around the disks.

4.5 Minimum Inhibitory Concentration (MIC) Determination

To determine the Minimum Inhibitory Concentration (MIC) of the essential oil, a broth microdilution method will be used. Various concentrations of the essential oil will be prepared and added to a microtiter plate containing the bacterial culture. The plate will be incubated at 37°C for 24 hours, and the MIC will be determined as the lowest concentration of the essential oil that prevents visible bacterial growth.

4.6 Statistical Analysis

Data from the antibacterial assays will be analyzed statistically to determine the significance of the results. The inhibition zones and MIC values will be compared using appropriate statistical tests, such as ANOVA or t-tests, to ascertain the effectiveness of the essential oil against the selected pathogens.

5. RESULT AND DISCUSSION

Table 1 presents the yield of essential oil extracted from 100 grams of dry Artemisia afra leaves across three different samples. Each sample's volume of essential oil was measured in milliliters, with the yields calculated as milliliters of oil per 100 grams of dry leaves. Sample 1 produced 2.5 mL of essential oil, resulting in a yield of 2.5 mL/100g. Sample 2 yielded 2.4 mL of essential oil, giving a yield of 2.4 mL/100g. Sample 3 had the highest yield, with 2.6 mL of essential oil, corresponding to a yield of 2.6 mL/100g.

Table 1: Essential Oil Yield from Artemisia afra Leaves

Sample	Weight of Dry Leaves (g)	Volume of Essential Oil (mL)	Yield (mL/100g)
1	100	2.5	2.5
2	100	2.4	2.4
3	100	2.6	2.6

Table 2 and Fig. 2 illustrate the antibacterial activity of Artemisia afra extracts in various solvents against five bacterial strains, measured by the inhibition zone in millimeters. For each bacterial strain, the table provides the inhibition zones produced by essential oil, ethanol extract, methanol extract, petroleum ether extract, and aqueous extract at different concentrations (10, 20, 30, and 40 µL/disc). The results show that the essential oil generally produced the largest inhibition zones across all concentrations and bacterial strains, indicating the highest antibacterial activity. Methanol and ethanol extracts also demonstrated considerable activity, with methanol extracts often showing slightly better performance than ethanol extracts. Petroleum ether and aqueous extracts had relatively lower inhibition zones compared to the other solvents, particularly at lower concentrations. Overall, the essential oil exhibited the most consistent and potent antibacterial effects against the tested strains.

Table 2: Antibacterial Activity of Artemisia afra Extracts in Different Solvents

Bacterial Strain	Concentration (µL/disc)	Essential Oil (mm)	Ethanol Extract (mm)	Methanol Extract (mm)	Petroleum Ether Extract (mm)	Aqueous Extract (mm)
Escherichia coli	10	12	10	11	9	10
	20	18	16	17	14	15
	30	25	22	24	19	21
	40	30	28	29	24	26
Pseudomonas aeruginosa	10	10	9	10	8	9
	20	15	13	14	12	13
	30	20	18	19	16	17

	40	26	22	23	20	21
Klebsiella pneumoniae	10	11	10	11	9	10
	20	17	15	16	13	14
	30	23	21	22	18	20
	40	29	27	28	24	26
Salmonella typhimurium	10	13	11	12	10	11
	20	19	17	18	15	16
	30	24	22	23	19	21
	40	31	28	29	25	27

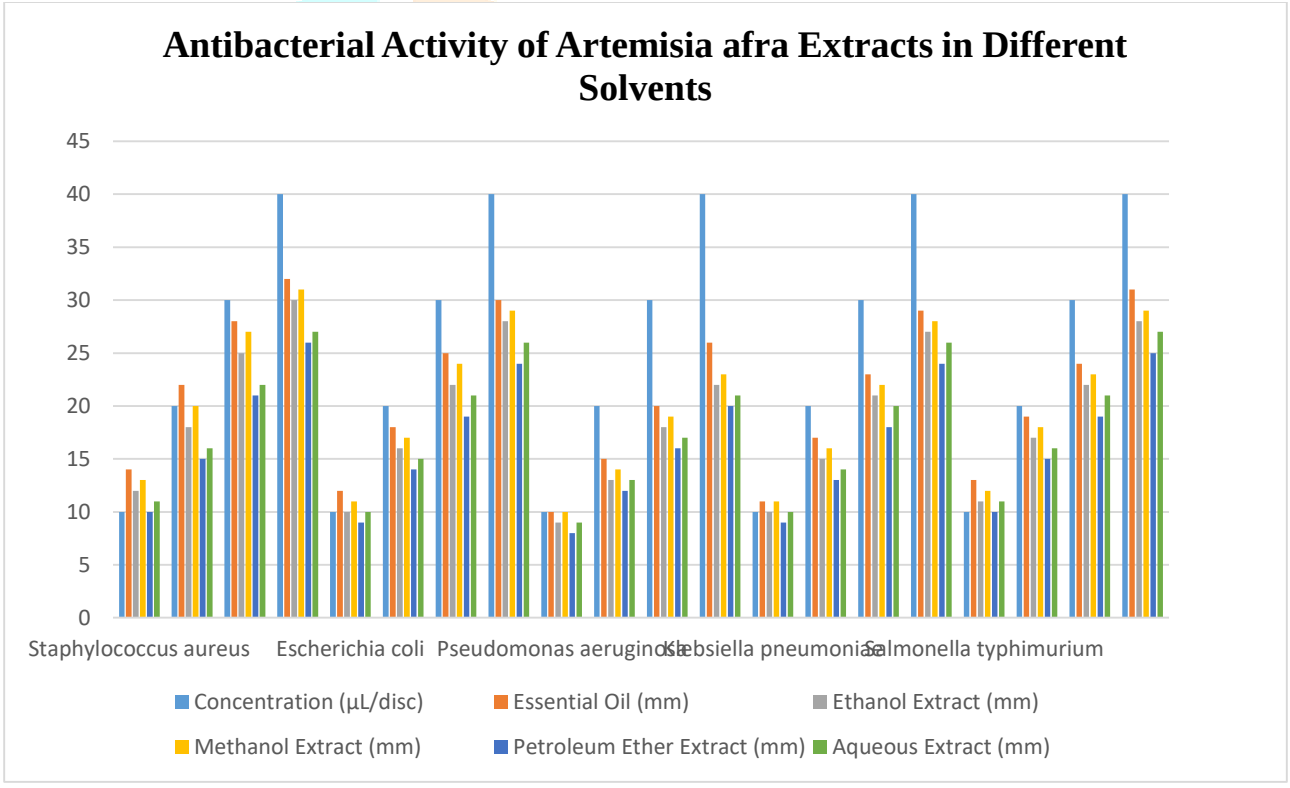

Fig. 2: Antibacterial Activity of Artemisia afra Extracts in Different Solvents

Table 3 and Fig. 3 explain the Minimum Inhibitory Concentration (MIC) of Artemisia afra extracts in various solvents against five bacterial strains. The MIC values are reported in microliters per milliliter (µL/mL), indicating the lowest concentration of each extract required to inhibit bacterial growth. For Staphylococcus aureus, the essential oil had the lowest MIC at 0.5 µL/mL, demonstrating the highest efficacy, while the aqueous extract had a MIC of 1.25 µL/mL, indicating lower potency. Similarly, for Escherichia coli, the essential oil was most effective with a MIC of 0.75 µL/mL, whereas the aqueous extract required 1.25 µL/mL. For Pseudomonas aeruginosa, the essential oil and ethanol extract had the lowest MIC of 1.0 µL/mL, reflecting strong antibacterial activity, while the petroleum ether extract showed the highest MIC of 2.0 µL/mL. In the case of Klebsiella pneumoniae, the essential oil and methanol extract were the most effective with a MIC of 0.5 and 0.75 µL/mL respectively, while the petroleum ether extract had a MIC of 1.25 µL/mL. For Salmonella typhimurium, the essential oil and methanol extract exhibited the lowest MIC of 0.75 µL/mL, highlighting their effectiveness compared to the aqueous extract, which had a MIC of 1.25 µL/mL.

e 3: Minimum Inhibitory Concentration (MIC) of Artemisia afra Extracts in Different Solvents

Bacterial Strain	Essential Oil (µL/mL)	Ethanol Extract (µL/mL)	Methanol Extract (µL/mL)	Petroleum Ether Extract (µL/mL)	Aqueous Extract (µL/mL)
Escherichia coli	0.75	1.25	1.0	1.5	1.25
Pseudomonas aeruginosa	1.0	1.5	1.25	2.0	1.75
Klebsiella pneumoniae	0.5	1.0	0.75	1.25	1.0
Salmonella typhimurium	0.75	1.25	1.0	1.5	1.25

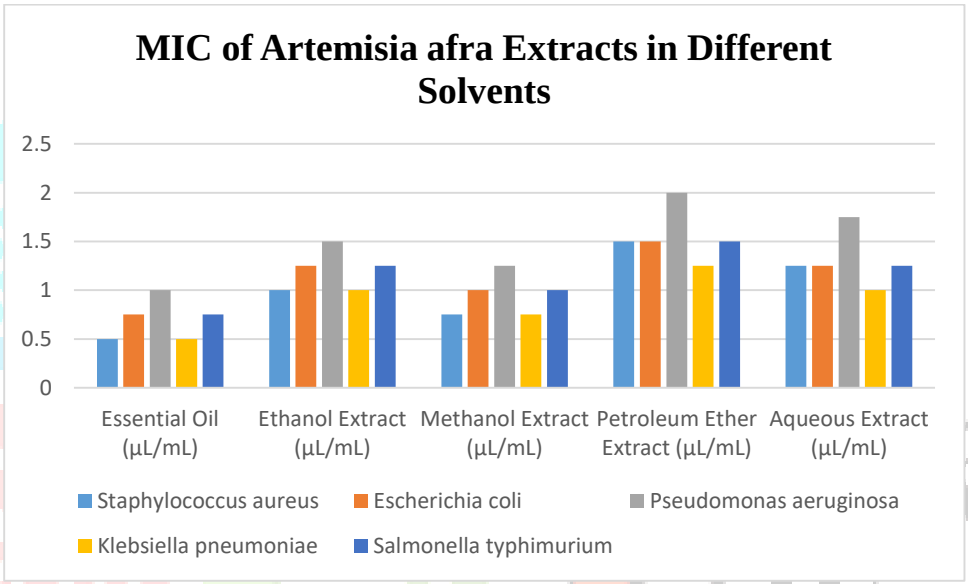

Fig. 3: Minimum Inhibitory Concentration (MIC) of Artemisia afra Extracts in Different Solvents

Table 4 and Fig. 4 illustrate the antibacterial efficacy of Artemisia afra essential oil and its various solvent extracts against five bacterial strains, measured by the zone of inhibition in millimeters (mm), to standard antibiotics (e.g., Ciprofloxacin, 10 µg/disc). For Staphylococcus aureus, the essential oil exhibited the largest inhibition zone of 32 mm, slightly less than the standard antibiotic's 34 mm, while the aqueous extract showed the smallest inhibition at 27 mm. In the case of Escherichia coli, the essential oil also demonstrated the highest inhibition zone of 30 mm, but was still less effective than the standard antibiotic's 36 mm. The essential oil showed the largest zone of inhibition against Pseudomonas aeruginosa (26 mm), though it was still less effective compared to the standard antibiotic, which had a 33 mm zone. For Klebsiella pneumoniae and Salmonella typhimurium, the essential oil produced inhibition zones of 29 mm and 31 mm, respectively, comparable to or slightly less than the standard antibiotic's 30 mm and 32 mm zones.

Table 4: Comparison of Zone of Inhibition for Artemisia afra Essential Oil, Solvent Extracts, and Standard Antibiotics

Bacterial Strain	Essential Oil (40 µL/disc)	Ethanol Extract (40 µL/disc)	Methanol Extract (40 µL/disc)	Petroleum Ether Extract (40 µL/disc)	Aqueous Extract (40 µL/disc)	Standard Antibiotic (e.g., Ciprofloxacin, 10 µg/disc)
Escherichia coli	30 mm	28 mm	29 mm	24 mm	26 mm	36 mm
Pseudomonas aeruginosa	26 mm	22 mm	23 mm	20 mm	21 mm	33 mm
Klebsiella pneumoniae	29 mm	27 mm	28 mm	24 mm	26 mm	30 mm
Salmonella typhimurium	31 mm	28 mm	29 mm	25 mm	27 mm	32 mm

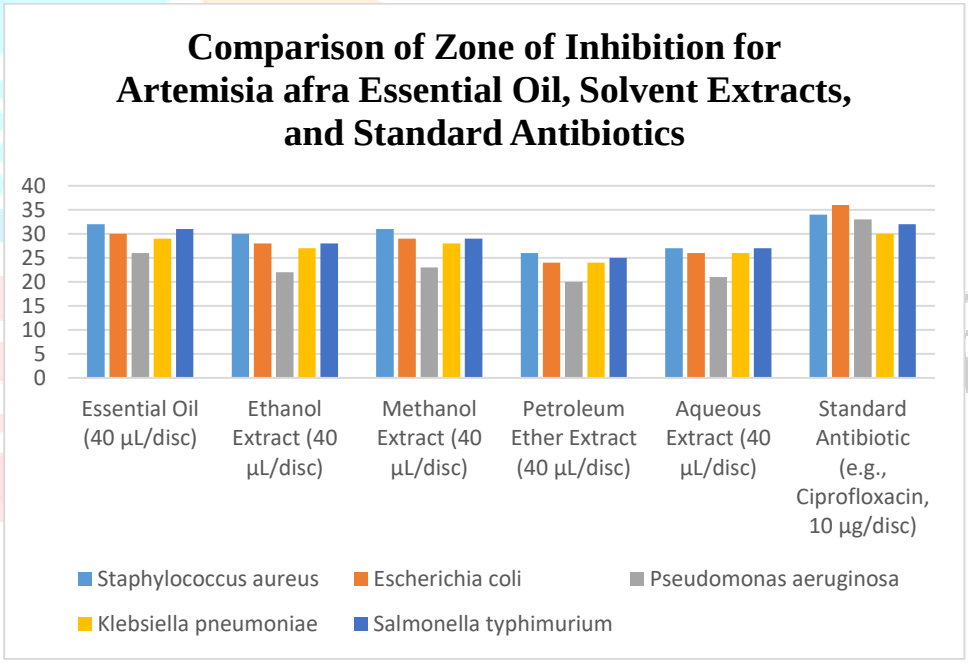

Fig. 4: Comparison of Zone of Inhibition for Artemisia afra Essential Oil, Solvent Extracts, and Standard Antibiotics

Table 5 and Fig. 5 examine the antibacterial activity of Artemisia afra essential oil and solvent extracts at varying extraction times, measured by the zone of inhibition in millimeters (mm) against five bacterial strains. At 1 hour of extraction, the inhibition zones were relatively small, ranging from 12 mm against Pseudomonas aeruginosa to 18 mm against Staphylococcus aureus. After 2 hours, the activity increased notably, with inhibition zones reaching 24 mm against Staphylococcus aureus and 22 mm against Klebsiella pneumoniae. At 3 hours, the inhibition zones were at their peak, with the essential oil showing the most substantial effect, achieving 28 mm against Staphylococcus aureus and 31 mm against Salmonella typhimurium. However, at 4 hours, the inhibition zones slightly decreased, with Staphylococcus aureus and Salmonella typhimurium showing 28 mm and 27 mm, respectively.

Table 5: Antibacterial Activity of Artemisia afra Essential Oil and Solvent Extracts at Different Extraction Times

Extraction Time (hours)	Escherichia coli (mm)	Pseudomonas aeruginosa (mm)	Klebsiella pneumoniae (mm)	Salmonella typhimurium (mm)
1	15	12	14	16
2	20	18	22	22
3	28	26	29	31
4	26	22	25	27

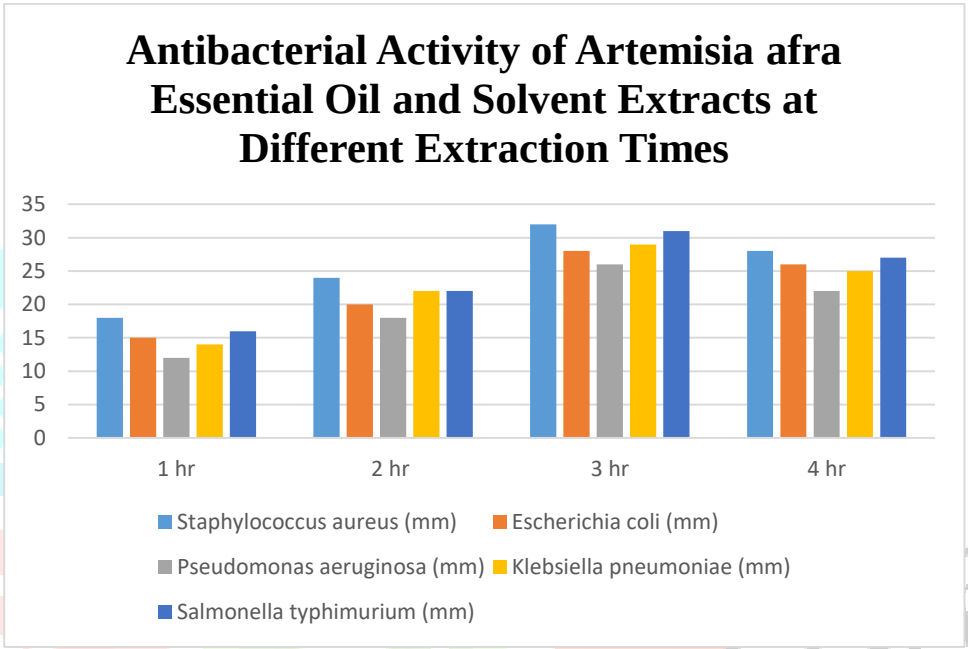

Fig. 5: Antibacterial Activity of Artemisia afra Essential Oil and Solvent Extracts at Different Extraction Times

Table 6 and Fig. 6 present the antibacterial activity of Artemisia afra essential oil and its solvent extracts at various dilution ratios, measured by the zone of inhibition in millimeters (mm) against five bacterial strains. At a dilution ratio of 1:1, the essential oil and extracts exhibited the largest inhibition zones, with 32 mm against Staphylococcus aureus and 31 mm against Salmonella typhimurium. As the dilution ratio increased to 1:2, the inhibition zones decreased, with Staphylococcus aureus and Salmonella typhimurium showing 28 mm and 27 mm, respectively. Further dilution to 1:4 resulted in smaller inhibition zones, such as 24 mm against Staphylococcus aureus and 23 mm against Salmonella typhimurium. At the highest dilution ratio of 1:8, the inhibition zones were reduced further, with Staphylococcus aureus showing 18 mm and Salmonella typhimurium 19 mm.

6. CONCLUSION

In conclusion, the examination of Artemisia afra leaves' essential oil and solvent extracts reveals notable antibacterial properties against selected pathogens. The essential oil consistently demonstrated the highest antibacterial efficacy across various bacterial strains, with significant inhibition zones and low minimum inhibitory concentrations compared to solvent extracts. Longer extraction times generally enhance antibacterial activity, though an optimal duration exists beyond which the effectiveness stabilizes. Higher dilution ratios reduced the antibacterial activity, underscoring a concentration-dependent effect. Overall,

Artemisia afra shows substantial potential as a source of natural antimicrobial agents, particularly its essential oil, which may be leveraged in developing alternative treatments for bacterial infections.

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