



Phytochemical screening and evaluation of traditional herbal medicines for antimicrobial activity

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Abstract

The goal of this research was to find therapeutic plants that might fight against MDR bacteria by testing their phytochemical composition and antimicrobial activity. The study used aqueous and 80% methanolic extraction processes to get the phytochemical components of *Rumex abyssinicus*, *Cucumis pustulatus*, *Discopodium penninervium*, *Lippia adoensis*, *Euphorbia depauperata*, *Cirsium englerianum*, and *Polysphaeria aethiopica*. The hydroalcoholic extract demonstrated notable antibacterial efficacy against microorganisms that are resistant to many drugs, with MIC values between 1.0 and 128.0 µg/ml and IZ widths between 11 and 29 mm. When extracts from *E. depauperata* and *C. englerianum* were tested against MRSA and *Streptococcus pyogenes*, a wide IZ range of 26-29mm was observed. The IZ diameters of *E. coli* and multidrug-resistant *Klebsiella pneumoniae* were 12–25mm and 23–28mm, respectively. While all of the extracts tested were efficient against *T. mentagraphytes*, *C. englerianum* shown to be more beneficial against *S. pyogenes* and *K. pneumoniae*. *Cirsium englerianum*, *L. adoensis*, *D. penninervium*, and *R. abyssinicus* all shown antifungal activities against *Candida albicans* and *Candida mentagraphytes*. Among them, *R. abyssinicus* had the strongest antifungal action, with minimum inhibitory concentration (MIC) values ranging from 32 to 64 µg/ml. There is no difference between the antibacterial activity of the plant extracts and that of modern medications.

Keywords: Phytochemical screening, Antimicrobial, therapeutic, compounds

Introduction

As the world's leading producer of medicinal plants, India has earned the nickname "the botanical garden of the world." In addition to their potential utility in medication research and development, medicinal plants provide a rich natural resource for the discovery of novel therapeutic chemicals. According to the World Health Organization, traditional medicines, which mostly include of natural plant products, are used by 80% of the population in developing countries for their fundamental healthcare needs. One consequence of people becoming more conscious of the importance of natural commodities is the surge in demand for medicinal herbs throughout the world. As opposed to synthetic medications, which may be adulterated and have side effects, they are inexpensive, pharmacologically active, and have low toxicity, making them a simple solution for many human problems. Scientists are on the lookout for substances with possible antibacterial action due to the concerning rise in the illness incidence caused by germs that are resistant to antibiotics. Plants are a treasure trove of medicinal and pharmaceutical goods due to their capacity to

produce antimicrobial-potential chemicals via secondary metabolism. Research on the antimicrobial effects of plant extracts has been conducted on a global scale.

Among the most significant medicinal plants of India, Bael (*Aegle marmelos*) has been recognized since Charak's time (1500 B.C.). Crude extracts of this plant have also been shown to have anti-inflammatory, antibacterial, antispermatogetic, anti-cancer, anti-ulcer, antidiabetic, and antihyperlipidaemic properties in a variety of animal models. Fruits, stem, bark, and leaves of the *Aegle marmelos* plant are all used medicinally to treat a variety of skin and eye problems. When it comes to bioactive chemicals produced as secondary metabolites, the leaf is among the plant components with the greatest accumulation rates. The current investigation set out to assess the phytochemical content and antimicrobial efficacy of aqueous and methanolic leaf extracts from *Aegle marmelos*. Antimicrobial resistance is on the rise, therefore scientists across the globe have been looking for herbal remedies to cure a variety of bacterial diseases. Herbs may be a rich source of potential new treatments. About 80% of the global

population uses herbal medicines as part of their basic healthcare, and the global market for herbal goods reaches over US\$ 60 billion per year, according to the World Health Organization (WHO). Hypersensitivity, immunosuppression, and allergic responses are some of the adverse host effects that antibiotics may cause, and they are becoming more common as antibiotic resistance increases (Graul *et al.*, 2009) [10]. Some natural antimicrobial medicines that effectively combat harmful microorganisms with little side effects were discovered as a result of these unpleasant responses. A potential approach might include the systematic screening of bioactive compounds found in traditional medications in the hopes of finding a new moiety with effective antimicrobial properties. Because of its all-natural composition, lack of psychoactive properties, and relative accessibility, affordability, and lack of adverse effects, plant-based medicines are seeing rising demand in both underdeveloped and industrialized nations.

Literature Review

Lalitha, G. & T.H, Nazeema & Sharmila, L. (2015) [9]. Drugs derived from medicinal plants have many benefits, including being easy to use, having a wide range of effects, and a stronger focus on prevention. Their active ingredients are effective in curing a wide range of human ailments. The purpose of this research was to evaluate *Andrographis paniculata*'s potential antimicrobial, antioxidant, anticoagulant, and fibrinolytic effects. Phytochemical analysis of *A. Paniculata* (leaf) showed that it included steroids, phenols, glycosides, saponins, and flavanoids. The antioxidant and antibacterial effects of the plant component, the leaf, were solvent-dependent. In comparison to other organic solvents and water-based extracts, the research also showed that ethanolic extract was highly antibacterial and that methanolic extract was antioxidant. In comparison to the ethanol and methanol extracts, the anticoagulant activity was much higher in the water-based extract. The fibrinolytic activity was much higher in the ethanol and methanol extracts compared to the water-based one.

Y, Padma & Raju, Venkata. (2013) [4]. Acanthaceae member *A. nallamalayana* (root) extracts are the focus of this article's screening and assessment efforts for biomolecules with antibacterial potential. The eastern Ghats in India are home to the uncommon and endemic *A. nallamalayana*, which is only found in the woods of the Nallamalais. Oral ulcers, leucorrhoea, and infertility/abortion have all been treated with it in traditional medicine. The test species have not been published for their phytochemical screening or antibacterial activity as of yet. Flavonoids, alkaloids, phenols, steroids, and triterpenoids were all identified in the phytochemical analysis of these extracts. Results from testing the antibacterial activity of three different *A. nallamalayana* extracts (petroleum ether, ethyl acetate, and methanol) showed that the root methanolic extract inhibited all test pathogens to the greatest extent, followed by ethyl acetate. *Bacillus subtilis* was shown to be resistant to the test extracts, whilst *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Staphylococcus aureus* were determined to be the most susceptible.

Essien, Emmanuel & Antia, Bassey & Udoh, B.I. (2015) [3]. The purpose of this study was to examine the antimicrobial and phytochemical characteristics of seed extracts obtained

from fully grown short-hybrid bottle gourds (*Lagenaria siceraria*) in Nigeria. Strong antibacterial and antifungal activity against a range of pathogens was established by varied concentrations of *L. siceraria* seed extracts using the agar well diffusion technique. *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas* sp., *Escherichia coli*, *Candida* sp., and *Aspergillus niger* were among these pathogens. Between 0.002 and 0.100 g/ml were the minimal inhibitory concentrations (MIC). Phytochemical analysis of different seed extracts revealed varying levels of phenols, cardiac glycosides, flavonoids, terpenoids, deoxy-sugar, carbohydrates, and reducing sugars. It has been shown that *Lagenaria siceraria* seeds may contain pharmacologically significant antibacterial drugs.

Rathee, Deepti *et al.* (2012) [2]. Bioactive components associated with antibacterial characteristics were identified during the phytochemical screening of *Picrorhiza kurroa* Benth rhizomes. Glycosides, sterols, and phenolic substances were found in several *P. kurroa* rhizome extracts, according to chemical tests and thin-layer chromatography investigations. Iridoid glycosides and cucurbitacins, two types of triterpenoids, were the primary chemical components identified in the methanolic extract. Infections caused by harmful bacterial and fungal strains may be treated using the plant component. While the methanolic extract had only moderate antibacterial action against *E. coli*, *B. subtilis*, and *M. luteus*, it exhibited substantial activity against *P. aeruginosa* and *S. aureus*. This crude extract has a long history of use in traditional medicine for the treatment of skin conditions, urinary tract infections, diarrhea, and gastrointestinal disorders. While the water-based extract showed reduced efficacy against some bacterial strains, it had no effect on fungus. Compared to the standard antibiotic ciprofloxacin, the efficacy of the methanolic extract was lower. The content of iridoids was evaluated using HPTLC tests; picroside I had a content of 3.66 ± 0.11 and kutkoside had a content of 4.44 ± 0.02 , respectively.

Gupta, Divya & Dubey, Jyotirmay & Kumar, Mukesh. (2016) [1]. This study set out to examine the synergistic effects of five different medicinal plant extracts with suitable antibiotic discs, in addition to their antibacterial activity and phytochemical content. To evaluate the compounds, researchers used two different diffusion methods: agar well and disc. *Terminalia arjuna* (in chloroform and methanol), *Camellia sinensis* (in ethanol), and *Polyalthia longifolia* (in petroleum ether) exhibited the highest levels of in vitro inhibition of the microorganisms tested, including *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*. By inhibiting *Escherichia coli* with an inhibition zone of 35 mm, levofloxacin achieved the greatest antibacterial activity. Additionally, when combined with lincomycin, the powerful plant extracts demonstrated synergistic beneficial effects against *Staphylococcus aureus*. Phytochemical research uncovered glycosides, terpenoids, flavonoids, tannins, proteins, carbohydrates, and saponins. According to the research, *Polyalthia longifolia*, *Terminalia arjuna*, and *Camellia sinensis* are all powerful natural antibacterial agents that potentially replace synthetic medications on the market with fewer negative side effects.

Research Methodology

Experimental testing was conducted in vitro to assess the phytochemical screening and antimicrobial status of *R. abyssinicus*, *C. pustulatus*, *E. depauperata*, *C. englerianum*, *L. adoensis*, *C. aethiopica*, *D. penninervium*, and *E. depauperata* against clinical isolates of multidrug-resistant bacteria and their reference strains.

Standards for the selection of medicinal plants

Researchers in this research followed the advice of traditional healers and farmers in the Ethiopian Berbere area to collect therapeutic herbs. Just 17 peasant groups call these 526 miles from Addis Abeba area home, and basic services including roads and clinics are severely lacking. Skin conditions, diabetes, UTIs, hepatitis, STDs, cancer, hypertension, erectile dysfunction, and contraception are some of the medical conditions treated by locals using medicinal plants. According to the research, seven peasant groups were chosen for their extensive traditional knowledge. Traditional healers often use a variety of herbs to alleviate bacterial illnesses.

Plant collection

Seven plants-*P. aethiopica*, *E. depauperata*, *C. englerianum*, *L. adoensis*, *C. pustulatus*, *D. penninervium*, and *R. abyssinicus*-were collected and evaluated for their phytochemical bioactive components and antibacterial capabilities on common human illnesses. Standard storage methods were followed while handling the gathered samples, which included wrapping them in plastic sheets. Every sample was authenticated and then deposited.

Preparation of plant extracts

The plant materials were prepared for extraction by washing, rinsing, air-drying, and reducing them. After three days of soaking in distilled water, the aerial powder was macerated with 80% methanol. The extraction was carried out three times after being separated and filtered. The rotary evaporator and hot air oven were used to concentrate the filtrates at a temperature of 40 °C. By allowing it to

evaporate, the organic solvent was extracted. After drying the extracts, the watery residue was passed through a lyophilizer to eliminate non-polar solvents. The freeze-drying process significantly concentrated the extract. We collected the dry extract, sealed it in bottles, and placed each bottle in a glass vial. The desiccator was placed on silica gel to retain the dry material, which was then chilled to -4 °C until it was needed. To get the yield, we divided the dry extract's weight by the entire dry plant materials' weight.

Statistical analysis

An analysis of the triple data reading values of inhibitory zones in diameter and concentration values (MIC and MBC) was conducted using the Statistical Packages for the Social Sciences (SPSS) application, Window version 22, in accordance with CLSI. All experimental values were represented by their respective means and standard deviations. To check for a statistically significant difference, we used a Student t-test. Next, we compared the p-values. Results were considered significant for this study if the p-value was less than 0.05.

Analysis

Antibacterial and antifungal activity against human infections were evaluated using 80% methanol extracted from powdered and dried leaves of *P. aethiopica*, *C. englerianum*, *E. depauperata*, *L. adoensis*, *D. penninervium*, and roots of *C. pustulatus* and *R. abyssinicus*. Results against *E. coli*, *Klebsiella*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Candida albicans*, and *Candida mentagrophytes* were encouraging.

Antibacterial effect of extracts on multidrug-resistant bacteria

When tested against two or more strains of human pathogenic bacteria, including those that are resistant to several drugs, almost all of the extracts shown antibacterial activity. Extraction from *E. depauperata*, *R. abyssinicus*, and *L. adoensis* reduced *E. coli* growth and exhibited the widest range of activity.

Table 1: Agar disc diffusion technique was used in 2018 to measure the inhibitory zone in diameter on human pathogens by methanolic extracts.

Scientific name	µg/ml	Inhibition zone in diameter (mm)									
		Gram-positive bacteria				Gram-negative bacteria				Fungi	
		MRSA1	MSSAR	<i>S. pyogen</i> 1	<i>S. pyogen</i> R	<i>E. coli</i> 1	<i>E. coli</i> R	<i>K. pne</i> 1	<i>K. pne</i> R	<i>C. aR</i>	<i>T. rR</i>
<i>C. englerianum</i>	30	28(0.8)	28(3.0)	29(1.3)	29(1.5)	22(0.7)	23(0.2)	28(0.8)	28(0.9)	23(0.7)	11(0.3)
	10	17(0.4)	18(0.1)	20(0.9)	19(1.0)	11(0.3)	15(1.4)	18(2.0)	19(0.1)	13(1.1)	-
<i>E. depauperata</i>	30	26(1.0)	27(2.4)	28(0.9)	28(0.5)	15(0.1)	14(0.4)	27(1.2)	27(0.7)	-	25(0.7)
	10	17(0.7)	17(0.6)	18(2.0)	19(0.3)	-	-	19(0.1)	19(0.7)	-	13(0.9)
<i>L. adoensis</i>	30	27(1.8)	27(2.4)	26(0.5)	26(1.5)	25(2.1)	24(0.3)	29(0.1)	29(0.7)	15(0.9)	23(0.1)
	10	19(0.1)	18(1.2)	19(1.1)	18(0.1)	10(0.7)	14(0.2)	20(0.6)	20(0.3)	-	11(0.3)
<i>D. penninervium</i>	30	25(2.0)	25(0.5)	24(0.1)	22(1.0)	27(0.8)	27(0.1)	28(0.7)	29(0.4)	20(0.9)	21(0.4)
	10	16(0.1)	16(0.3)	17(0.4)	17(0.7)	20(0.7)	19(1.0)	18(0.3)	18(0.2)	13(0.3)	9(1.6)
<i>R. abyssinicus</i>	30	23(0.4)	23(0.6)	27(0.4)	27(1.1)	12(0.8)	12(0.1)	24(0.1)	24(0.6)	22(0.5)	26(0.1)
	10	12(0.8)	13(0.9)	15(1.1)	15(1.0)	-	-	14(1.0)	15(0.7)	10(0.2)	14(0.4)
<i>C. pustulatus</i>	30	22(0.8)	22(0.8)	24(0.5)	23(0.8)	23(0.4)	18(0.9)	25(1.0)	24(0.4)	18(0.1)	20(0.8)
	10	13(0.2)	12(1.0)	12(0.8)	15(1.2)	15(0.2)	10(0.8)	15(0.9)	14(0.8)	12(0.8)	11(0.1)
<i>P. aethiopica</i>	30	19(0.1)	19(0.2)	20(0.1)	21(0.1)	-	-	19(0.3)	19(0.4)	-	-
	10	10(0.5)	9(0.2)	12(0.6)	12(0.6)	-	-	12(0.4)	11(0.9)	-	-
Modern drug	-	-	28(0.1)C	NT	NT	-	26(2.1)C	-	32(1.2)c	28(0.3)F	26(1.3)F

Mean (standard deviation) data are shown; NT stands for "not tested," and "-" denotes the "no inhibition zone." The result is 1 for multidrug-resistant bacteria that are less than 16mm IZ, and R for reference strains that are susceptible and more than 21mm IZ. What does C.a stand for? The letters C for ciprofloxacin, F for ketoconazole, K. pne for *Klebsiella pneumoniae*, T.r for *Candida albicans*, and C. The p-value for MRSA treated with cefoxitin is less than 0.001. Table 1 indicates that when comparing K. pneumoniae, *E. coli*, *S. aureus*, and *S. pyogenes*, as well as K. pneumoniae and *E. coli* treated with current medicine, there are significant differences ($p < 0.05$, $##p < 0.01$).

Methanolic extract measurements showed that *C. englerianum* had an inhibitory zone of 28 mm, *L. adoensis* 27 mm, and *E. depauperata* 26 mm on the MRSA. A new antibacterial action against *S. pyogenes* was detected in the methanolic extract of *C. englerianum*. When tested against both the sample and reference strains, it exhibited an inhibition zone of 29 mm. Moreover, *S. aureus* exhibited an impressive inhibitory zone (Fig 1). With inhibition zones of 27 mm and 26 mm, respectively, against MSSA and MRSA, the methanolic extract of *E. depauperata* exhibited strong antibacterial activity, as shown in Table 1.

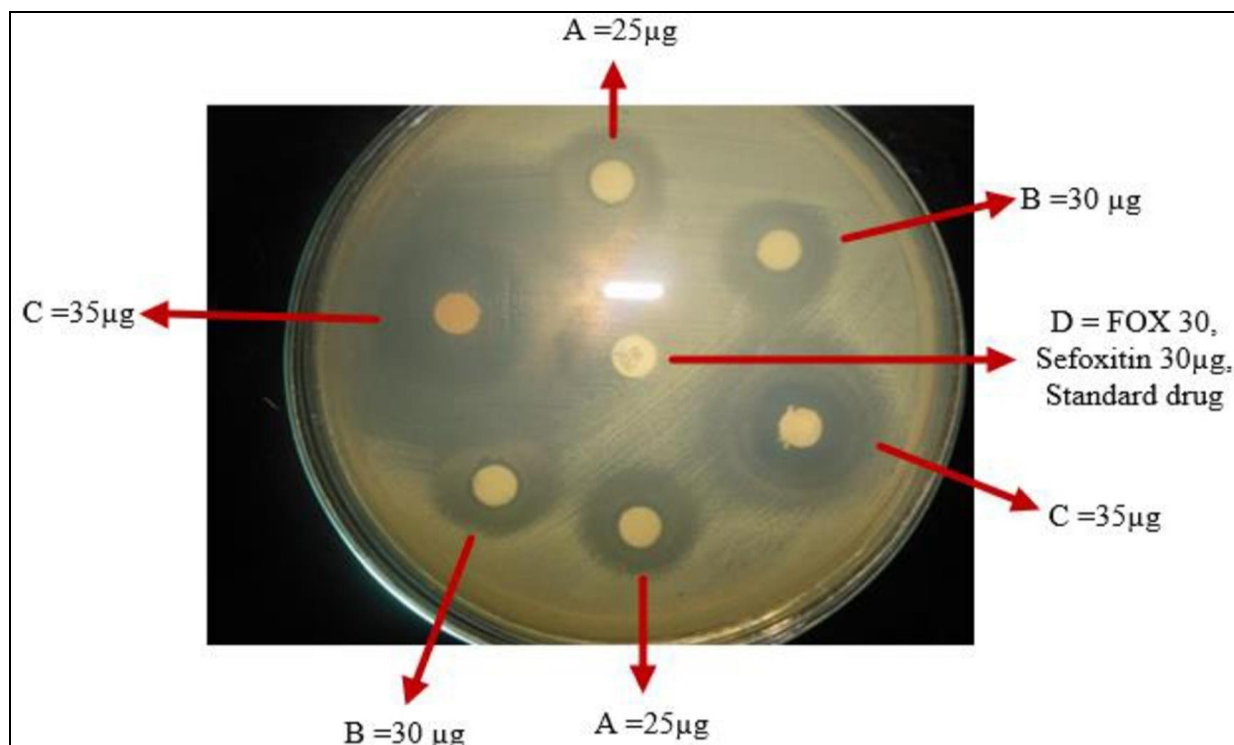


Fig 1: Analysis of the antimicrobial activity of *Cirsium englerianum* against MRSA at several dosages

Phytochemical screening from the medicinal plants extract

Medicinal plant percentage yields varied significantly throughout the trial, with *C. englerianum* exhibiting the greatest yield at 38%. These plant extracts included a variety of phytochemicals, including tannins, alkaloids, terpenoids, anthraquinones, phenolic compounds, flavonoids, cardiac glycosides, and cardiac glycosides. The

investigated human pathogens showed bactericidal or fungicidal effects against these bioactive substances.

Phytochemical screening from the medicinal plants extract:

A wide range of percentage yields were observed when medicinal plants were macerated in 80% methanol. The yield was found to be 38% in *C. englerianum* and 22% in *E. depauperata*, as shown in Table 2.

Table 2: Research on medicinal plants based on ethnobotany and percentage yields against certain human diseases.

Scientific name	Family	Local name	Part Used	% yield, mean (SD)	Locally used to treat
<i>Polysphaeria aethiopica</i> Verdc.	Rubiaceae	Qarraruu	Leaf	36(2.3)	Toothache, scabies, oral inflammation, TB
<i>Euphorbia</i>	Asteraceae	Adaddoo	Leaf	38.1(1.0)	Gonorrhea, Tonsillitis, syphilis, wound amebiasis, malaria, diarrhea
<i>Euphorbia</i>	Euphorbiaceae	Gurii	Bark	22.3	Skin rash, ringworm, bloody
<i>Lippia adoensis</i>	Verbenaceae	Urgoo	Leaf	27.3 (1.7)	Stomach ache, diarrhea, wound, and cough
<i>Discopodium penninervium</i>	Solanaceae	Maraaro	Leaf	33.9 (0.4)	Eczema, wound, scabies, urine retention
<i>Cucumis pustulatus</i>	Cucurbitaceae	Haadhatu	Root	29.1 (2.8)	Cough, TB and chest pain, cold disease, Pneumonia
<i>Rumex abyssinicus</i>	Polygonaceae	Dhangagoo	Root	24.0 (0.3)	Gonorrhea, skin diseases, diarrhea, abscesses, ringworm, pain-relieving, diuretic effect, hypertension, anti-cancer, malaria, wound healing

Conclusion

Infections such as *Staphylococcus aureus*, which are multidrug-resistant (MDR), lead to lengthier hospital admissions and higher rates of patient morbidity and death. An alarming number of *S. aureus* isolates (up to 55%) are multidrug-resistant, and the human race faces a grave danger from MDR pathogenic coliform bacteria such as *Klebsiella pneumoniae* and *Escherichia coli*. Research and development of novel treatment techniques for MDR infections is, thus, urgently needed. A variety of traditional medicinal herbs are available in Bale Zone, thanks to its rich plant richness and status as a natural pharmacy powerhouse. Among a number of medicinal plant extracts tested, the majority showed encouraging antibacterial activity against multidrug-resistant microorganisms. In order to make valid findings, more comprehensive studies are needed to examine plant extracts that have shown antimicrobial responses against multidrug-resistant (MDR) human pathogens. These studies should include toxicity testing, clinical effectiveness trials, safety tests, and cost evaluations. Discovering novel and more effective antibacterial compounds from the extracts relies heavily on these assays.

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