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Evaluation of Antimicrobial Activity of Select Medicinal Plant Extracts Against Various Pathogenic Strains.

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KEYWORDS
water extracts, ethanolic extracts, combined extracts, antibacterial efficacy, minimum inhibitory concentrations.

ABSTRACT:

The aim of this study is to evaluate the antibacterial effectiveness of water extracts, ethanolic extracts, as well as combined water and ethanolic extracts from the leaves of Jatropha gossypifolia and Holoptelea grandis against three pathogenic bacteria: Staphylococcus aureus, Bacillus cereus, and Bacillus subtilis. The minimum inhibitory concentrations (MIC) of these plant extracts against both gram-positive and gram-negative bacteria were determined using the agar-cup plate method.

The findings suggest that the combined ethanolic extract of Jatropha gossypifolia and Holoptelea grandis leaves exhibited greater efficacy in inhibiting the growth of Staphylococcus aureus, and Bacillus subtilis, compared to their individual water extracts, ethanolic extracts, and combined water extracts. Conversely, the combined water extract of Jatropha gossypifolia and Holoptelea grandis leaves showed higher efficacy in inhibiting the growth of Bacillus cereus compared to their individual water extracts, ethanolic extracts, and combined ethanolic extracts.

In terms of antibacterial activity, the zone of inhibition of the extracts was compared to that of amoxicillin.

Introduction:

High mortality rates are often attributed to infectious diseases, which have become more prevalent due to factors such as increased pollution, population growth, and changing environmental conditions.[1] These conditions have led to decreased immunity in people, making them more susceptible to bacterial and fungal illnesses. Furthermore, prolonged use of antimicrobial drugs has led to the emergence of drug-resistant microorganisms, posing a significant threat to both human and animal health. [2]

Motivated by these challenges, we aimed to develop a novel herbal remedy with potent antibacterial and antifungal properties to address various microbial infections effectively. [3] Previous literature has indicated the antimicrobial activity of Jatropha gossypifolia leaves and Holoptelea grandis leaves. However, scientific information on this topic is currently limited.

Our objective was to investigate the antimicrobial profiles of Jatropha gossypifolia leaves and Holoptelea grandis leaves against various microbial species. These medicinal plants contain phytochemicals such as

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flavonoids, alkaloids, tannins, and terpenoids, which are known for their antibacterial and antioxidant properties. [4]Through our research, we aimed to explore the potential of these plant extracts as effective antimicrobial agents in combating microbial infections. [5,6]

Additionally, our study sought to fill the existing gap in scientific knowledge by providing comprehensive data on the antimicrobial activity of these plant extracts. By conducting a series of experiments, including antimicrobial susceptibility testing against different microbial strains, we aimed to elucidate the effectiveness of Jatropha gossypifolia and Holoptelea grandis extracts in inhibiting bacterial and fungal growth. [7,8]

Furthermore, we aimed to identify the specific phytochemicals present in these plant extracts that contribute to their antimicrobial properties. Flavonoids, alkaloids, tannins, and terpenoids are known to exhibit antimicrobial activity by disrupting microbial cell membranes, interfering with cellular processes, and exerting antioxidant effects. Understanding the composition of these plant extracts can provide insights into their mechanism of action and potential therapeutic applications.

Overall, our research aimed to contribute to the development of natural, plant-based alternatives to conventional antimicrobial drugs. By harnessing the antimicrobial potential of medicinal plants, we aimed to provide safer and more sustainable treatment options for microbial infections, ultimately improving public health outcomes.

Plant Profile:

A. Jatropha gossypifolia L [9-11]

Synonym: Jatropha curcas Linn.

Biological Source: Jatropha gossypifolia is a flowering plant belonging to the family Euphorbiaceae. It is commonly known as bellyache bush or cotton-leaf physic nut.

Family: Euphorbiaceae

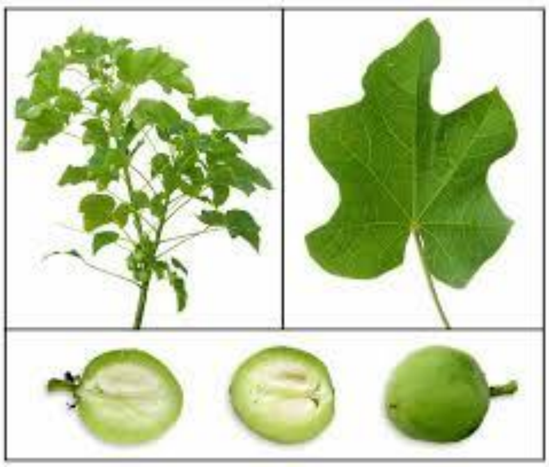

Figure 1.1 Jatropha gossypifolia L

Chemical Constituents:

Jatropha gossypifolia contains various chemical constituents, including:

Diterpenoids: Jatropholone A, jatropholone B, and jatropholone C.

Flavonoids: Quercetin, kaempferol, and rutin.

Alkaloids: Jatrophine, jatrophamidine, and jatrophone.

Triterpenoids: Betulinic acid and lupeol.

Phenolic compounds: Gallic acid, ellagic acid, and chlorogenic acid.

Uses:

Medicinal Uses: Jatropha gossypifolia has a long history of medicinal use in traditional systems of medicine. It is used for various ailments, including:

Treatment of diarrhea and dysentery: The plant possesses anti-diarrheal and anti-dysenteric properties.

Antipyretic properties: It is used to reduce fever.

Anti-inflammatory properties: Jatropha gossypifolia extracts have shown anti-inflammatory activity.

Treatment of skin diseases: The plant is used topically for skin conditions such as eczema and dermatitis.

Analgesic properties: It is used to alleviate pain.

Treatment of respiratory disorders: The plant is used for coughs and respiratory infections.

Insecticidal Properties: Extracts from Jatropha gossypifolia have shown insecticidal properties and are used in traditional agriculture for pest control.

Wound Healing: The plant has been traditionally used for wound healing due to its antimicrobial and anti-inflammatory properties.

Anti-cancer Potential: Some studies have suggested that compounds present in Jatropha gossypifolia possess anti-cancer properties, although further research is needed to validate these claims.

Biofuel Production: Like its close relative Jatropha curcas, Jatropha gossypifolia has potential as a biodiesel feedstock due to its high oil content in its seeds. However, caution should be exercised as the plant is toxic and invasive in some regions.

Ornamental Use: In some regions, Jatropha gossypifolia is cultivated as an ornamental plant due to its attractive foliage and flowers.

It's important to note that while Jatropha gossypifolia has several medicinal and other potential uses, caution should be exercised due to its toxicity. Proper dosage and administration should be followed under the guidance of trained healthcare professionals. Additionally, its invasive nature in certain ecosystems should be carefully monitored to prevent ecological disturbances.

B. Holoptelea grandis [12-14]

Synonym: Holoptelea integrifolia

Biological Source: Holoptelea grandis is a deciduous tree belonging to the family Ulmaceae. It is commonly known as Indian elm or chilbil.

Family: Ulmaceae

Figure 1.2 Holoptelea grandis

Chemical Constituents:

Holoptelea grandis contains various chemical constituents, including:

Triterpenoids: Grandinin, hopenone, and holoptelol.

Flavonoids: Quercetin, kaempferol, and rutin.

Alkaloids: Holopteline and holoptelin.

Steroids: β-sitosterol and stigmasterol.

Phenolic Compounds: Gallic acid and ellagic acid.

Uses:

Medicinal Uses: Holoptelea grandis has a rich history of medicinal use in traditional systems of medicine. It is utilized for various therapeutic purposes, including:

Anti-inflammatory Properties: Extracts from the bark and leaves have demonstrated anti-inflammatory activity, making it useful in the treatment of inflammatory conditions.

Antioxidant Properties: The presence of flavonoids and phenolic compounds contributes to its antioxidant activity, which may help protect cells from oxidative stress.

Anti-microbial Activity: Holoptelea grandis extracts have shown antimicrobial activity against various pathogens, suggesting potential use in the treatment of infectious diseases.

Anti-diabetic Potential: Some studies have indicated that extracts from Holoptelea grandis may possess hypoglycemic properties, which could be beneficial in managing diabetes.

Wound Healing: The plant is traditionally used for wound healing due to its antimicrobial and anti-inflammatory properties.

Pesticidal Uses: Extracts from Holoptelea grandis have shown insecticidal properties and are used in traditional agriculture for pest control.

Timber: The wood of Holoptelea grandis is used in construction, furniture making, and for various other woodworking purposes due to its strength and durability.

Fuelwood: The tree is also utilized as a source of fuelwood in some regions.

Environmental

Uses: Holoptelea grandis is planted for its ability to provide shade and help in soil conservation due to its extensive root system.

While Holoptelea grandis offers several medicinal and other potential uses, caution should be exercised in its utilization. Proper dosage and administration should be followed under the guidance of trained healthcare professionals. Additionally, sustainable harvesting practices should be employed to ensure the conservation of this valuable plant species.

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MATERIALS AND METHODES: [15,16]

Plant Collection and Authentication

The plant materials, including Jatropha gossypifolia leaves and Holoptelea grandis leaves, were gathered from their natural habitats and subjected to validation procedures following Ayurvedic practices. Additionally, pharmacognostic authentication was conducted to ensure the optimal selection of plant specimens.

Material -

- Culture plates
- Sterile cork borer
- Ampicillin (Merck)
- 70% ethanol (Merck)
- Nutrient agar (HiMedia)
- Nutrient broth
- Wireloop
- Bacterial Test Cultures
- Autoclave
- Incubators

Phytochemical Investigation of Jatropha gossypifolia leaves

Creating the inoculums:[17]

A single colony of the strain was introduced into 20 ml of nutrient broth in a conical flask to create a suspension containing all the organisms. The strain was activated by incubating the mixture at 37°C for 24 hours. The suspension was then adjusted to contain approximately 1 x 10^6 cells per milliliter, as determined using data obtained from cell counts in Neuber's chamber.

Cultural medium:[18]

The medium was prepared by mixing 1000 ml of distilled water with 13 grams of nutrient broth, adjusting the pH to (7.3 ± 0.2), and subsequently sterilizing it in an autoclave at 121°C for 15 minutes.

Microorganisms:

Standard cultures of the following microorganisms were obtained from the Department of Microbiology at Government Medical College, Nagpur, Maharashtra State, India:

Gram-positive Bacteria:

Staphylococcus aureus

Bacillus cereus

Bacillus subtilis

The microorganisms were identified using staining techniques and were maintained by regular subculturing on nutrient agar medium.

Determination of the zone of inhibition by the agar cup plate method: [19,20]

The antibacterial activity of ethanolic extract, water extract, combined water extract, and combined ethanolic extracts of Jatropha gossypifolia leaves and Holoptelea grandis leaves was assessed using the agar cup-plate method. To begin, 0.1 ml of the above-diluted culture was added to each sterile Petri plate (10x10 cm), followed by the addition of 20 ml of sterile nutritional agar medium. The plates were then dried for 30 minutes at 37°C and allowed to set. Using a sterile cork borer, 6 mm diameter holes were created in the inoculated agar. The plant extracts (2.5 mg/ml and 5 mg/ml) were then added to the bores. As standards and controls, amoxicillin (1 mg/ml) and 70% ethanol were utilized. The Petri plates were kept in the refrigerator for 2 hours to allow for uniform diffusion of plant extracts into the agar medium. Finally, all the plates were incubated for 48 hours at 37°C. At the end of the incubation period, the clear zone of inhibition around the bores was measured in millimeters (mm).

Methods:

Extract preparation:[21]

The shade-dried leaves of Jatropha gossypifolia and Holoptelea grandis were coarsely powdered and subjected to extraction. Water extraction was carried out by maceration for 48 hours, while ethanol (95%) extraction was performed using a Soxhlet apparatus for 4-5 hours. Both extracts were concentrated under low pressure using a rotary flash evaporator, followed by air drying.

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Result and Discussion:

The management and treatment of bacterial infections can significantly benefit from the utilization of plants and plant extracts. Not only are phytomedicines cost-effective and readily available, but they also pose a lower risk of hypersensitive reactions compared to allopathic medications. Despite their effectiveness, the popularity of herbal medicines in therapy witnessed a notable decline with the rise of allopathic treatments.

Antibacterial Activity of *Jatropha gossypifolia*leaves, & *Holoptelea grandis* leaves

Extract: -

The results of the comparison with the standard antibiotic Amoxicillin and the zone of inhibition of the ethanolic extract, water extracts, combined water extract and combined ethanolic extracts of *Jatropha gossypifolia* leaves, and *Holoptelea grandis* leaves were shown in Tables 1, 2, 3,4,5, and 6, respectively.

Comparison to their water extract, ethanolic extracts, and combined water extract, the combined ethanolic extract of Jatropha gossypifolia leaves and Holoptelea grandis leaves was more effective at inhibiting the growth of Staphylococcus aureus, and Bacillus subtilis.

When compared to each plant's individual water extract, ethanolic extract, and combination ethanolic extracts, the combined water extract of Jatropha gossypifolia leaves and Holoptelea grandis leaves was more successful at suppressing the growth of Bacillus cereus.

Against the examined species, the extract demonstrates potential antibacterial activities comparable to those of regular amoxicillin.

CONCLUSION:

Staphylococcus aureus, Bacillus cereus, and Bacillus subtilis were among the common pathogenic bacterial strains utilized to evaluate the antibacterial activity of the extracts. The testing was conducted using sterile top agar and the agar cup-plate method. The zones of inhibition produced by the extracts at concentrations of 2.5 mg/ml and 5 mg/ml were compared to those of conventional amoxicillin prepared at a concentration of 1 mg/ml in DMSO.

The results indicate that the combined ethanolic extract of Jatropha gossypifolia leaves and Holoptelea grandis leaves exhibited greater effectiveness in inhibiting the growth of Staphylococcus aureus, Bacillus subtilis, and Escherichia coli compared to their individual water extracts, ethanolic extracts, and combined water extracts. On the other hand, the combined water extract of Jatropha gossypifolia leaves and Holoptelea grandis leaves demonstrated superior effectiveness in inhibiting the growth of Bacillus cereus compared to their individual water extracts, ethanolic extracts, and combined ethanolic extracts.

Table 1. Antibacterial Activity of Water Extract of *Jatropha gossypifolia*leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Water Extract of *Jatropha gossypifolia*		Amoxicillin
	2.5mg/ml	5mg/ml	1mg/ml
Staphylococcus aureus	07	11	34
Bacillus cereus	10	15	32
Bacillus subtilis	07	12	32

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Table 2. Antibacterial Activity of Ethanolic Extract of *Jatropha gossypifolia* leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Ethanolic Extract of *Jatropha gossypifolia*		Amoxicillin
	2.5mg/ml	5mg/ml	1mg/ml
Staphylococcus aureus	12	20	36
Bacillus cereus	13	22	34
Bacillus subtilis	13	19	34

Table 3. Antibacterial Activity Of Water Extract of *Holoptelea grandis* leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Water Extract of *Holoptelea grandis*.		Amoxicillin
	2.5mg/ml	5mg/ml	1mg/ml
Staphylococcus aureus	11	14	34
Bacillus cereus	13	17	34
Bacillus subtilis	12	14	34

Table 4. Antibacterial Activity of Ethanolic Extract of *Holoptelea grandis* leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Ethanolic Extract of *Holoptelea grandis* .		Amoxicillin
	2.5mg/ml	5mg/ml	1mg/ml
Staphylococcus aureus	13	20	36
Bacillus cereus	14	21	34
Bacillus subtilis	13	19	34

Table 5. Antibacterial Activity Of Combined Water Extracts of *Jatropha gossypifolia* leaves and *Holoptelea grandis* leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Combined Water Extracts of *Jatropha gossypifolia* and *Holoptelea grandis*		Amoxicillin
	2.5mg/ml each	5mg/ml each	1mg/ml
Staphylococcus aureus	17	22	36
Bacillus cereus	30	32	34

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Bacillus subtilis	16	21	34

Table 6. Antibacterial Activity of Combined Ethanolic Extract of *Jatropha gossypifolia* leaves and *Holoptelea grandis* leaves.

Bacteria	Diameter of Zone of Inhibition in mm		
	Combined Ethanolic Extracts of *Jatropha gossypifolia*and *Holoptelea grandis*		Amoxicillin
	2.5mg/ml each	5mg/ml each	1mg/ml
Staphylococcus aureus	27	32	36
Bacillus cereus	20	24	34
Bacillus subtilis	29	32	34

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