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A Comparative Study of the Effects of *Jatropha multifida* and *Euphorbia hirta* and Their Mixture on Pathogenic Growth Rate †

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Abstract: Medicinal plants are used for the treatment of many diseases across the globe. However, some are expensive or not readily available. The increasing prevalence of multidrug-resistant strains of pathogenic microorganisms constitutes an important and growing threat to public health due to the uncontrolled use of synthetic microbial antibiotics. Due to the side effects and the resistance that pathogenic microorganisms develop against common antibiotics, the extraction of biologically active compounds from plants has recently attracted a great deal of attention. This study was carried out to compare the effectiveness of two medicinal plants, *Jatropha multifida*, and *Euphorbia hirta*, with respect to the growth inhibition of pathogenic *Escherichia coli*. The results showed that ethanolic extracts of the mixture of the two plants presented the highest inhibition of the growth of *E. coli*. When used independently, *Euphorbia hirta* presented higher inhibition than *Jatropha multifida*.

Keywords: antibiotics; drugs; herbal; pathogens; resistance

1. Introduction

Since antiquity, plant products have been used as medicine to cure and prevent diseases. They provide a vast array of natural products that have been exploited as medicaments for a variety of diseases due to their many bioactive compounds. The extracts from plants are used for the treatment of many infections.

Since the discovery of antibiotics in the 1950s from microorganismal sources, the use of plants as antimicrobials has diminished. Annually, an average of two to three antibiotics derived from microorganisms are developed [1]. In recent decades, the effective life span of any antibiotic has become limited due to misuse and abuse of antibiotics. The increased resistance of many microorganisms toward established drugs has necessitated the investigation of the chemical compounds within traditional plants. Medicinal plants possess both potential crude antimicrobial drugs and a source of natural compounds that may act as new anti-infective agents in the future [2]

The *Jatropha multifida* tree belongs to the Euphorbiaceae family. Its common names include the *Coral plant* and *Physic nut*, and it is widely distributed in tropical regions throughout the world. It is a multi-purpose medicinal agent [3]. Its leaves, latex, and fruits are used for the treatment of infected wounds and skin infections and such as cicatrizing wounds and ulcers in addition to oral thrush, constipation, and fever. Crude extracts from the plant's roots and stems are used as anticancer, cytotoxic, antitumor, antimalarial, antimicrobial, insecticidal, and molluscicidal agents. Its bark and leaves are used as medicine for neurodermatitis, itchy skin, and skin eczema, and the stems are employed as chewing sticks for dental care. The alcoholic extracts of the roots have antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

E. hirta belongs to the plant family Euphorbiaceae, which is locally known as an asthma plant. It is widespread at low altitudes throughout the tropics and subtropics. The plant

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prefers sunny to lightly shaded dry conditions and is an early colonizer of bare ground. *E. hirta* is a weed of cultivated fields, perennial crops, grasslands, roadsides, gardens, etc.; thus, it is a common plant that can easily be provided in emergency situations [4]. It is used in the treatment of gastrointestinal disorders; bronchial and respiratory diseases like asthma, bronchitis, hay fever, etc.; and conjunctivitis [5]. The aqueous extract exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities. The stem sap is used in the treatment of eyelids, and a leaf poultice is used to treat swelling and boils. The ethanolic extract from the leaves has antifungal and antibacterial activities.

E. coli is a Gram-negative, facultative anaerobic, non-sporulating bacterium with rod-shaped cells. Its cell wall is composed of a thin peptidoglycan layer and an outer membrane. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that the pathogen (*E. coli*) is not damaged by penicillin. Shiga-toxin *Escherichia coli* (STEC) is a type of *E. coli* that produces toxins, known as Shiga-toxins, which cause dysentery [6].

The increasing prevalence of multidrug-resistant strains of pathogenic microorganisms constitutes an important and growing threat to public health due to the uncontrolled use of antibiotics [7].

Over the span of a decade, the rising specter of multidrug-resistant TB (MDR-TB) began to threaten global TB control efforts. This is a disease caused by a strain of *Mycobacterium tuberculosis* resistant to at least Isoniazid and Rifampicin, the two most powerful anti-TB drugs [8].

In addition, high costs and adverse side effects, such as hypersensitivity, allergic reactions, and immunosuppression, are commonly associated with popular synthetic antibiotics, constituting major global issues with respect to treating infectious diseases [9]. Medicinal plants have become the focus of intense study in terms of constituting alternatives capable of matching the threats posed by antimicrobial resistance. This study was conducted to determine the effectiveness of different plant herbs and their mixtures on the growth inhibition of pathogenic bacteria. Their potency in treating pathogenic bacterial infections, if used appropriately, could solve the global multi-drug resistance crisis precipitated by the overuse of antibiotics, and benefit low-income communities due to the high costs of antibiotics and the availability of the studied plants.

2. Materials and Methods

2.1. Study Area

This research was conducted at Gulu University in the Multifunctional Laboratory. Gulu University is located in Pece-Laroo Division, Gulu City, in the Northern part of Uganda. The coordinates of the University's main campus are 2°47′19.0″ N and 32°19′01.0″ E. The dominant ethnic group in the University's surrounding area is the Acholi people, a subset of the Luo people. A study in Gulu Regional Referral Hospital revealed a relatively high frequency of resistance to most antibiotics among the people in the area [10], which is consistent with other studies conducted in the country.

2.2. Study Design

This study employed an experimental design that established the effect of plant extracts on pathogenic growth. The inhibitory extent of the different plant extracts was measured.

2.3. Collection of Plant and E. coli Samples

Fresh *Jatropha multifida* and *Euphorbia hirta* plants were collected (uprooted) from around the main campus of Gulu University. The isolates of *E. coli* bacteria used in this study were obtained from patients diagnosed with gastro-intestinal infections, and they were kept in Lacor Hospital and Gulu University's Multifunctional Laboratory.

2.4. Preparation of Crude Extracts of Euphorbia hirta and Jatropha multifida

After collection, the plants' parts were washed and shadow-dried for three and seven days (for *Euphorbia hirta* and *Jatropha multifida*, respectively). The difference in the number

of days of shadow drying corresponds to *Euphorbia hirta*'s small size, which caused it to dry faster. After drying, plant parts (leaves and stems) were ground into powder using a motor and pestle, and extracts were prepared by separately soaking 50 g of each plant powder in 100 mL of ethanol (non-polar solvent) for 24 h. These mixtures were later filtered, evaporated to dryness, and concentrated using a vacuum evaporator, and stored in a moisture-free container.

To create a concoction consisting of a mixture of *Euphorbia hirta* and *Jatropha multifida*, 50 g of powder of each plant was soaked in 100 mL of ethanol for 24 h. Filtration was conducted, and evaporation and concentration were performed in a vacuum evaporator. Then, the resulting concoction was stored in a moisture-free container.

2.5. Preparation of Nutrient Agar and Physiological Saline

A total of 7 g of nutrient agar powder was suspended in 250 mL of distilled water. The resulting mixture was heated until dissolved and then autoclaved and cooled. Glass plates were incubated to ensure sterility before use. Nutrient agar was poured into each plate, left to solidify, and transferred for storage in an incubator.

Physiological saline was prepared by dissolving 8.5 g of sodium chloride (NaCl) in water; the solution was then autoclaved and cooled to room temperature. Colonies of *E. coli* were transferred from the storage media into physiological saline and mixed to attain turbidity standards.

2.6. Bacterial Culture

Streak plate culturing was conducted [11]. A sterile wire loop that was heated and cooled was used to inoculate the nutrient agar with *E. coli*. *E. coli* was scraped and spread evenly over the surface of the agar and incubated at 37 °C for 24 h. In order to achieve an even distribution of the bacteria, a zigzag pattern was used to spread the *E. coli.* The wire loop was always heated with fire so that it would be sterilized before another inoculation was conducted.

2.7. Efficacy of Plant Extracts toward Bacteria

The antimicrobial activity of the plant extracts was tested using the disc diffusion method [12]. Petri dishes with bacterial cultures were loaded with plant extracts, which was placed in the center of the petri dish, and the setup was incubated at 37 °C for 24 h. Antibacterial activity was recorded by measuring the diameter of the inhibition zone around each disc (in mm).

3. Results

The mixture of plant extracts showed higher inhibition with a maximum zone of 29.5 mm and a minimum zone of 26.2 mm. When the plant extracts were used individually, *Euphorbia hirta* showed a higher zone of inhibition, with a maximum zone of 21.6 mm and a minimum zone of 18 mm, while *Jatropha multifida* presented a maximum of 13 mm and a minimum of 9.5 mm. The mean and standard deviation of the herbal effects are provided in Table 1.

Table 1. Mean ± Standard deviations of zones of inhibition of aqueous extracts of *Euphorbia hirta, Jatropha multifida,* and their mixture against the growth of *E coli* measured after two days.

	Treatment		
	***Euphorbia hirta* (mm)**	***Jatropha multifida* (mm)**	**Mixture (*Euphorbia hirta* + *Jatropha multifida*) (mm)**
Mean	19.7 ± 1.8	10.8 ± 1.9	27.8 ± 1.7

4. Discussions

Our study assessed the inhibitory effect of the herbal extracts on the growth of *E. coli*, which is an indicator of the presence of pathogenic bacteria. *Escherichia coli* is Gram-negative in nature, possessing an outer membrane made up of peptidoglycan containing lipopolysaccharides and lipoproteins [13]. The presence of an outer membrane acts as a permeability barrier to large molecules and hydrophobic molecules [14]. Ethanolic plant extracts are effective against Gram-positive bacteria because their outer cell membranes can be permeated by hydrophobic molecules. The outer membrane of the cell wall of Gram-negative bacteria appears to act as a barrier to many substances, including synthetic and natural antibiotics.

For many years, people around the world have healed the sick with herbal-derived remedies [9,14]. Our results indicate that herbal extracts are strong inhibitors of *E coli.* The extracts from *Euphorbia hirta* showed higher inhibition of the growth rate of *E. coli* bacteria than the extracts of *Jatropha multifida.* Phytochemical analysis showed that the extracts of *Euphorbia hirta* contain tannins, flavonoids, alkaloids, and cardiac glycosides [15]. These bioactive components have antibacterial activities. Plant tannins are complexed with enzymes or bacterial substrates, via action on the bacterial cell membrane, or via complexation with metal ions [7]. The antibacterial activity of flavonoids results in the lysis of the cell membrane, followed by bacterial cell death. Extracts of *J. multifida* contain alkaloids, phenols, steroids, and tannins and lack coumarins, flavonoids, and saponins [16]. The absence of flavonoids and saponins endows *Jatropha multifida* with only a low level of growth inhibition toward pathogenic bacteria.

In this study, the mixture of the plant extracts showed the highest zone of inhibition of the growth of *E. coli.* This is because both plant extracts contain bioactive components like alkaloids, phenols, tannins, saponins, flavonoids, and carbohydrates, which are antimicrobial. Therefore, mixing increases the concentration of bioactive components, which increases the activity of pathogens. Increased concentrations of bioactive components increase the amount of interaction, leading to increased complexion with enzymes of bacterial substrates, increased lysis of the bacterial cell membrane, and death.

In this study, the greatest inhibition of pathogenic growth was revealed when the two extracts were mixed, implying that *Jatropha multifida* and *Euphorbia hirta* are better when combined than when used independently. We suggest further exploration of the microbial activity of the mixture of *Jatropha multifida* and *Euphorbia hirta* using different solvents in order to determine the adequate concentration of the mixture of the two plant extracts for use in pathogenic growth inhibition.

Author Contributions: The first author, E.G., conceptualized and designed the study under the supervision of the second author, O.A. The draft paper was developed by the first author (E.G.) and later improved by the second author, O.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The proposal was presented and approved by the review committee at the Department of Biology, Gulu University. The first author (E.G.) was then given an introductory letter, which he presented to the authority of the Multifunctional Laboratory at Gulu University. He was then allowed to conduct the research experiment in the laboratory.

Informed Consent Statement: Professor Echodu Richard (Director) of the Multifunctional laboratory, Gulu University, agreed to the use of the laboratory facilities on the condition that all laboratory rules are adhered to. The laboratory also provided a pure sample of *E. coli* for the experiment.

Data Availability Statement: Data is available with the first author on request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nakaido, H.; Ahmer, B.M. Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. *Mol. Microbiol.* **2009**, *52*, 933–945. [CrossRef]
2. Heinrich, J.; Topp, R.; Brasche, S. Rhinitis and blood pressure in adults. *Am. J. Respir. Crit. Care Med.* **2004**, *168*, 1243–1245. [CrossRef] [PubMed]
3. Aiyelaagbe, O.O. Antibacterial activity of *Jatropha multifida* roots. *Fitoterapia* **2001**, *72*, 544–546. [CrossRef] [PubMed]
4. Kumar, S.; Malhotra, R.; Kumar, D. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacogn. Rev.* **2010**, *4*, 58. [CrossRef] [PubMed]
5. Kumari, I. Effect of crude extracts of *Euphorbia hirta* L. against human pathogenic bacteria *Staphylococcus aureus*. *Vegetos Int. J. Plant Res.* **2017**, *30*, 8. [CrossRef]
6. Etcheverría, A.; Padola, N.L. Shiga toxin-producing *Escherichia coli*, Factors involved in virulence and cattle colonization. *Virulence* **2013**, *4*, 366–372. [CrossRef] [PubMed]
7. Anani, K.; Adjrah, Y.; Ameyapoh, Y.; Karou, S.; Agbonon, A.; de Souza, C.; Gbeassor, M. Antimicrobial, anti-inflammatory and antioxidant activities of *Jatropha multifida* L. (Euphorbiaceae). *Pharmacogn. Res.* **2016**, *8*, 142. [CrossRef] [PubMed]
8. Marcus, V.; de Souza, N. Promising current drug candidates in clinical trials and natural products against multidrug-resistant tuberculosis. In *New Strategies Combating Bacterial Infection*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2009; pp. 71–87. [CrossRef]
9. Michael, C.A.; Dominey-Howes, D.; Labbate, M. The antimicrobial resistance crisis: Causes, consequences, and management. *Front. Public Health* **2014**, *2*, 145. [CrossRef] [PubMed]
10. Odongo, C.O.; Anywar, D.A.; Luryamamoi, K.; Odongo, P. Antibiograms from community-acquired uropathogens in Gulu, northern Uganda - a cross-sectional study. *BMC Infect. Dis.* **2013**, *13*, 2–8. [CrossRef] [PubMed]
11. Humphries, J. *Bacteriology*, 1st ed.; Johns Marry Publishers Limited: London, UK, 1974.
12. Jenkins, R.; Maddocks, S. Fundamental skills for infectious disease research. In *Bacteriology Methods for the Study of Infectious Diseases*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 1–26. [CrossRef]
13. Schnaitman, C.A. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. *J. Bacteriol.* **1970**, *104*, 890–901. [CrossRef]
14. Lawal, U.; Mohammed, R.T.; Gidado, S.M.; Kankara, S.S. Ethnobotanical survey and nutritional composition of medicinal plants used for traditional galactagogue practice in Katsina State, Nigeria. *Adv. Tradit. Med.* **2022**. [CrossRef]
15. Rajan, D.R.J. Antimicrobial screening of *Euphorbia hirta* L. and *Pedalium murex* L. A comparative study. *J. Adv. Nat. Sci.* **2015**, *3*, 193–199. [CrossRef]
16. Rampadarath, S.; Puchooa, D.; Ranghoo-Sanmukhiya, V.M. Antimicrobial, phytochemical and larvicidal properties of *Jatropha multifida* Linn. *Asian Pac. J. Trop. Med.* **2014**, *7*, S380–S383. [CrossRef] [PubMed]