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

Ekangu Gerald and Opio Alfonse *

Faculty of Science, Department of Biology, Gulu University, Gulu P.O. Box 166, Uganda; email1@email.com
* Correspondence: a.opio@gu.ac.ug

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Abstract: Medicinal plants are used for the treatment of many diseases all around the world. Besides, some are expensive or not readily available. The increasing prevalence of multidrug-resistant strains of pathogen microorganisms constitutes an important and growing threat to public health due to the uncontrolled use of synthetic microbial antibiotics. Because of the side effects and the resistance that pathogenic microorganisms build against common antibiotics, much recent attention has been made to extracting the biologically active compounds which are isolated from plants. This study has been carried out to compare the effectiveness of two medicinal plants, *Jatropha multifida* and *Euphorbia hirta* on the growth inhibition of pathogenic *Escherichia coli*. The results showed ethanolic extracts of the mixture of the two plants with the highest inhibition on the growth of *E. coli*. When used independently, *Euphorbia hirta* had higher inhibition than *Jatropha multifida*.

Keywords: antibiotics; drugs; herbal; pathogens; resistance

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1. Introduction

Plants products have been used since ancient times for medicinal reasons to cure and prevent diseases. They provide a vast array of natural products that have been exploited as medicaments for a variety of disease conditions because of the many bioactive compounds. The extracts from plants are used in the treatment of many infections.

Since the launch of antibiotics in the 1950s from microorganism sources, the use of plants as antimicrobials have been diminished. Annually, two or three antibiotics on average derived from microorganisms are launched (Nikaido, 2009). In recent decades, the effective life span of any antibiotic has become limited due to misuse and abuse of antibiotics. Increased resistance of many microorganisms towards established drugs has made the investigation of chemical compounds within traditional plants a necessity. Medicinal plants possess both potential antimicrobial crude drugs as well as a source of natural compounds that act as new anti-infective agents in the future. (Heinrich et al., 2004).

The *Jatropha multifida* tree belongs to the Euphorbiaceae family, its common names *Coral plant* and *Physic nut* widely distributed in tropical regions throughout the world. It is a multi-purpose medicinal agent. (Aiyelaagbe 2001). The leaves, latex, and fruits are used in the treatment of infected wounds, and skin infections and as a cicatrizing, ulcers, oral thrush, constipation, and fever. Crude extracts from roots and stems are used as anticancer, cytotoxic, antitumor, antimalarial, antimicrobial, insecticidal, and molluscicidal. The bark and leaves are used as medicine for neurodermatitis, itchy skin, and skin eczema, and the stems are employed as chewing sticks used 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 locally known as an asthma plant. It is widespread at low altitudes throughout the tropics and subtropics. The plant 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, a common plant that can easily be available for emergency situations. (Kumar et al., 2010) It's used in the treatment of gastrointestinal disorders, bronchial and respiratory diseases like asthma, bronchitis, hay fever, etc., and conjunctivitis (Kumari, 2017). 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 on swelling and boils. Ethanolic extract from leaves has antifungal and antibacterial activities.

E. coli is a Gram-negative, facultative anaerobic, and 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-toxins *Escherichia coli* (STEC) is the type of *E. coli* that produces toxins, known as Shiga-toxins which is responsible for dysenteries (Analia and Nora, 2013).

The increasing prevalence of multidrug resistant strains of pathogen microorganisms constitutes an important and growing threat to public health due to uncontrolled use (Anani et al., 2016).

Over a decade, the rising spectra of multidrug-resistant TB (MDR-TB) began to threaten global TB control efforts. This is a disease caused by *Mycobacterium tuberculosis* resistant to at least Isoniazid and Rifampicin, the two most powerful anti-TB drugs. (Marcus et al., 2009).

In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppression, which are major burning global issues in treating infectious diseases (Michael et al., 2014). Medicinal plants have become the focus of intense study in terms of constituting alternatives matching antimicrobial resistance challenges. This study is to determine the effectiveness of the different plant herbs and their mixture on the growth inhibition of pathogenic bacteria. The potency in treating pathogenic bacterial infections, if used appropriately would solve the global multi-drug resistance created by the overuse of antibiotics and this benefits low income communities due to the high costs of antibiotics, and the availability of the plants.

2. Materials and Methods

2.1. Study Area

The 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 ethnic group in the University's surrounding area is the Luo- Acholi people. A study in Gulu Regional Referral Hospital reveals relatively high frequencies of resistance to most antibiotics (Odongo et al., 2013), consistent with other studies done in the country.

2.2. Study Design

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

2.3. Collection of Plant and E. coli Samples

The fresh plants of *Jatropha multifida* and *Euphorbia hirta* were collected (uprooted) from around the surroundings of the main campus, Gulu University. The isolates of *E. coli* bacteria used in this study were those obtained from patients diagnosed with gastrointestinal infections and kept in Lacor Hospital and Gulu University, Multifunctional Laboratory.

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

After collection, plants parts were washed and shadow dried for three (3) days for *Euphorbia hirta* and seven (7) days for *Jatropha multifida.* The difference in the days of shadow drying was because *Euphorbia hirta* is small hence it dried faster. After drying, plant parts (leaves and stems) were grounded 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. This was later filtered and evaporated to dryness and concentrated using a vacuum evaporator, and stored in moisture free container.

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

2.5. Preparation of Nutrient Agar and Physiological Saline

Seven (7) g of nutrient agar powder was suspended in 250 mL of distilled water, and the mixture was heated to dissolve, 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 the incubator.

Physiological saline was prepared by dissolving 8.5 g sodium chloride (NaCl) in water, 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 done (Humphries 1974). A sterile wire loop that was heated and cooled was used to inoculate the nutrient agar with the *E. coli* bacteria. *E. coli* is scrapped and spread evenly over the area of agar surface and incubated at 37 °C for 24 h. In order to achieve even distribution of bacteria, the zigzag pattern was used to spread the *E. coli.* The wire loop was always flamed to sterilize before another inoculation was done.

2.7. Efficacy of Plant Extracts on Bacteria

The antimicrobial activity of the plant extracts was tested using disc diffusion method (Maddocks, S 2019). Petri dishes with the bacterial cultures loaded with plant extract and concentrated plant extract placed on the center of the petri dish, incubated at 37 °C for 24 h. The 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 was at 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 the minimum zone of 18 mm, and *Jatropha multifida* with a maximum of 13 mm and a minimum of 9.5 mm. The mean and standard deviation of the herbal effects are 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 inhibition effect of the herbal extract on the growth of *E. coli* which is an indicator of the presence of pathogenic bacteria. *Escherichia coli* is gram-negative in nature with an outer membrane made up of peptidoglycan containing lipopolysaccharides and lipoproteins (Schnaitman, 1970). The presence of an outer membrane acts as a permeability barrier to large molecules and hydrophobic molecules (Anne. 2016). The ethanolic plant extracts are effective on gram-positive bacteria due to the outer cell membrane being permeable to the entry of hydrophobic molecules. The gram-negative bacteria cell wall outer membrane 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 (Michael et al., 2014; Mohammad et al., 2018). Our results indicate that herbal extracts were a strong inhibitor of *E coli.* The extracts from *Euphorbia hirta* showed high inhibition on the growth rate of *E. coli* bacteria than the extracts of *Jatropha multifida.* Phytochemical analysis shows that extracts of *Euphorbia hirta* contain Tannins, Flavonoids, Alkaloids, and Cardiac glycosides (Rajan, 2015). These bioactive components have antibacterial activities. Plant tannins are complex with enzymes or bacterial substrates or by action on the bacterial cell membrane or by complexion to metal ions (Anani et al., 2016). The antibacterial activity of flavonoids result in the lysis of the membrane, followed by bacterial cell death. Extracts of *J. multifida* contain alkaloids, phenols, steroids, and tannins and lack coumarins, flavonoids, and saponins (Rampadarath et al., 2014). The absence of flavonoids and saponins leads to low growth inhibition of *Jatropha multifida* on the pathogenic bacteria.

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, phenol and tannins, saponnins, flavonoids, and carbohydrates which are antimicrobial. Therefore, mixing increases the concentration of bioactive components which increases the activity of pathogens. Increased concentration of bioactive components increases the interaction leading to the increased complexion with enzymes of bacterial substrates and increases lysis of the bacterial cell membrane and death.

The study reveals the greatest inhibition of pathogenic growth, 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 to be used in pathogenic growth inhibition.

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