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Enhancing antimicrobial activity of antibiotics with *Jatropha gossypifolia* bioactive compounds

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ABSTRACT

Background As resistance to commonly used antibiotics increases, inhibiting bacteria and resolving diseases has become more challenging. This necessitates an in-depth study on the use of secondary metabolites from *J. gossypifolia* in combination therapies with commonly used antibiotics. This study aims to investigate the ethanolic extract of *Jatropha gossypifolia* in conjunction with five antibiotics against two bacterial species (*Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus),* exploring the possibility of either synergistic or antagonistic interactions.

Method: The choice of *J. gossypifolia* was based on an ethnobotanical survey conducted with over 20 participants. Varying concentrations of the plant leaf extract (25mg/mL, 50mg/mL, 100mg/mL, and 150mg/mL) were produced for inoculation. The manufacturer's description was employed to prepare different concentrations of all conventional antibiotics used. Sensitivity testing was performed with agar disc diffusion for the plant extract at different concentrations and their combinations.

Results: Synergistic activity was observed at a Minimum Inhibitory Concentration (MIC50) of 50 mg/mL with all five antibiotics. At 100 mg/mL, the antibacterial effects were significant ($p < 0.05$); however, the combined activity was less than that of the plant extracts alone, highlighting the need for further optimization.

Conclusion: The study results show that the ethanolic leaf extract of *J. gossypifolia* works well with the five antibiotics tested. However, at 100 mg/mL, it may have an opposing effect due to its sensitivity.

Keywords: Antagonism, *Jatropha gossypifolia, Methicillin-resistant Staphylococcus aureus, Staphylococcus aureus*, Synergism,

1. INTRODUCTION

With over 80% of the world's population depending on traditional medicine to treat illnesses, there has been an increased interest in investigating various plants for use in folkloric medicine [1]. Plants, known as rich sources of bioactive compounds, have contributed to the development of over 50% of modern clinical drugs [2, 3]. These plant-derived drugs have contributed immensely to the combat of various diseases, most especially in developing countries [4, 5]. The different plant parts used include the leaves, roots, flowers, twigs, exudates, and modified plant organs, all possessing varied medicinal properties and phytochemicals [6, 7]. The rise of antibiotic resistance has led to increased screening of plant-based natural products for antimicrobial activity as potential alternative treatments used by traditional healers [8]. This has prompted the herbal industry to collect these plants in large quantities for the production of modern drugs. More than 130 drugs have their chemical entities extracted from higher plants or modified synthetically for the economic treatment of diseases. Antibiotics are substances used to inhibit the growth of microorganisms (bacteria, viruses, fungi, protozoa) in high dilution, now an epidemic, as the microbes are resistant to

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them, which has posed a huge problem and remains a threatening concern [9]. The antibacterial agents used against resistant antibiotics are agents used not to kill the pathogenic bacteria but to modify them to produce a phenotype that is susceptible to antibiotics, thus another way of combating resistant antibiotics working in synergism with an antibacterial agent of plant extract, which could either slow down or prevent the emergence of resistant phenotypes [10]. An ethnobotanical survey was conducted in areas such as Ago Iwoye, Ijebu Igbo, Ijebu Ode, and Abeokuta in Ogun State. Information was gathered through questionnaires from traditional healers and herb sellers about the various plants used to treat skin diseases like rashes, pimples, measles, and boils. *Jatropha gossypifolia* (family Euphorbiaceae) has been known traditionally for its usage in the treatment of various illnesses. This study aims to investigate the ethanolic extract of *Jatropha gossypifolia* in conjunction with five antibiotics against two bacterial species, exploring the possibility of either synergistic or antagonistic interactions.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Nutrient Broth, Ethanol, Electric blender, Nutrient Agar, Distilled water, Cotton wool, Petri-dishes, Clavenger, Weighing balance, Crucible, Round bottom flask, Water bath, Stirrer, Rotary Evaporator, Oven.

2.1.2 Biological Materials

Jatropha gossypifolia was collected in an airtight polythene bag a few meters beyond the health center at Olabisi Onabanjo University in Ago Iwoye, Ogun State. The plant was identified at the ELKARF herbarium of Olabisi Onabanjo University, where it was allocated voucher number 7286. The leaves were carefully washed in distilled water to remove dirt and foreign materials. Following the wash, the leaves were spread on a clean surface to air dry for five days. They were then placed in an oven and dried at 15ºC for 30 minutes. Finally, the dried leaves were pulverized into a fine powder using an electric blender.

2.2 Methods

2.2.1 Ethnobotanical survey

An ethnobotanical survey was conducted using a semi-structured questionnaire in Ogun State. Herb sellers and traditional medical practitioners in Ago Iwoye, Ijebu Igbo, and Oru market in Ogun state were interviewed orally about the medicinal plants that are used for the treatment of skin diseases, their mode of administration, plant parts, and how effective these herbs are. The local Yoruba names of these plants and their excipients were given. The scientific names of these plants were sorted out through plant databases such as IPNI and APD, and a plant list was used scientifically to validate these medicinal plant names [11]. For most skin diseases, antibacterial and antifungal agents are used for the treatment of skin diseases

2.2.2 Preparation of Plant Extract

Four hundred grams (400 g) of *Jatropha gossypifolia* leaf powder was macerated in an airtight container with 2,500 mL of ethanol for 72 hours, with intermittent shaking. The mixture was subsequently filtered using Whatman No. 1 filter paper, and the filtrate was evaporated with a rotary evaporator set at 40ºC. The concentrated extract was dried in a crucible on a water bath at 40ºC to achieve a solvent-free crude extract. The yield was then measured to calculate the percentage yield. Finally, the crude extract was stored in a clean bottle at room temperature.

2.2.3 Susceptibility test

The test organisms, Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus*, were sourced from the Nigerian Institute of Medical Research (NIMR) in Lagos, Nigeria. A nutrient broth was prepared and inoculated with the test organisms to create a bacterial suspension, which was incubated at 37ºC for 24 hours. Various concentrations of plant leaf extract (25 mg/mL, 50 mg/mL, 100 mg/mL, and 150 mg/mL) were prepared for inoculation. Following the methodology established by [12], the ethanolic leaf extract was measured at different weights (0.25 g, 0.5 g, 1 g, and 1.5 g) and dissolved in 10 mL of ethanol to achieve homogeneous concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL, and 150 mg/mL. One milliliter of the overnight cultured bacterial suspension was placed into a sterile petri dish, followed by the addition of 10 mL of Mueller-Hinton agar. The mixture was swirled gently to ensure uniform distribution and allowed to solidify before incubation at 37ºC for 24 hours under sterile conditions.

2.2.4 Antibiotics Sensitivity Test

The interaction between the plant extract and antibiotics was assessed by determining the minimum inhibitory concentration (MIC) for *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA). Five antibiotics were utilized in this study, which were prepared according to the specifications provided by the manufacturer. The antibiotics included Ampiclox (30 µg), Erythromycin (10 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), and Streptomycin (30 µg). For this experiment, nutrient agar was freshly prepared and then poured into sterile Petri dishes that were properly labeled. After mixing, the agar was allowed to solidify at room temperature. Utilizing a sterile core borer, three wells of 6 mm each were created in the agar. In the first well, 100 µl of each standard antibiotic was placed. The second well contained 100 µl of the standard antibiotic combined with 100 µl of a 50 mg/mL solution of the plant extract, while the third well included 100 µl of the standard antibiotic mixed with 100 µl of a 100 mg/mL solution of the plant extract. The petri dishes were incubated at 37°C for 24 hours. Following incubation, the presence of clear zones around the wells was observed, indicating the inhibition of bacterial growth. The diameters of these zones were measured and recorded in millimeters. The experiment was conducted in triplicate to ensure the reliability and accuracy of the results.

2.3 Statistical Analysis

Results from the experiments, conducted in triplicate, are presented as means with their corresponding standard error of the mean (SEM). Student's t-test was employed to identify statistically significant differences, with a p-value of less than 0.05 indicating significance.

3. RESULTS

The percentage yield of the crude leaf extract of *Jatropha gossypifolia* was found to be 16%.

Table 1: Medicinal plants and the frequency in which they were mentioned during the ethnobotanical survey

Scientific names	Family name	Plant part used	Local names	Frequency of outcome
Acacia ataxacantha DC.	Leguminosae	Leaves	Epin pin	1
Aframomum meleguata K.. Schum	Zingiberaceae	Whole plant	Ata re	1
Ageratum conyzoides Linn.	Asteraceae	Whole plant	Imi-esu	3
Alafia bateri Oliv.	Apocynaceae	Leaves	Zoro	4
Alchornea laxiflora (Benth.). Pax & K Hoffin	Euphorbiaceae	Leaves	Opoto	1
Aloe vera (L). Burn. f	Liliaceae	Leaves	Eti Erin	2
Alstonia boonei Dewild	Apocynaceae	Stem barks	Ahun	1
Anogeissus leiocarpus (DC.) Guill.& Perr.	Combretaceae	Seed	Awin	2
Axonopus compressus (Sw.) Beav	Poaceae	Whole plant		3
Bombax buonopozense Beav	Malvaceae	Stem bark	Ponpola	7
Bridelia atroviridis Mull.Arg	Euphorbiaceae	Leaves	Ako-araasa	1
Cajanus cajan(L.) Millsp.	Legunminosae	Leaves	Otili	4
Calotropis procera (Aiton) W.T. Dryand	Apocynaceae	Leaves	Bomubomu	3

Dabergia saxatillis Hook.f.	Legunminosae	Leaves	Ogundu	1
Detarium microcarpum Guill.&Perr	Legunminosae	Leaves	Ogbogbo	2
Entradrophragma utile (Dawne & Sprague) Spague	Meliaceae	Stem bark	Igi jebo	2
Erythrophleum suaveolens (Guill. & Perr.) Brenan	Moraceae	Stem bark	Obo/ Epin	9
Euphorbia unispina NE Br.	Euphobiaceae	Leaves	Enu opiri	19
Ficus platyphlla Del.	Euphorbiaceae	Leaves	Botuje	9
Jatropha gossypifolia L.	Euphorbiaceae	Leaves	Ewe lapalapa pupa, Botuje pupa	2
Khaya grandifolia C.DC	Meliaceae	Stem bark	Oganwo	10
Kigelia africana (Lam.) Benth	Euphorbiaceae	Fruit	Pandoro	1
Lagenaria breviflora (Benth.) Roberty	Curcurbitaceae	Fruit	Egusi bara/ Tagiri	5
Lecanoidiscus cupaniodes Planch	Sapindaceae	leaves	Akika	2
Lippia multiflora Moldenke	Verbanaceae	Whole plant	Efinrin odan	6
Mangifera indica Linn.	Anacardiaceae	Leaves and bark	Mongoro	4
Moringa oleifera	Moringaceae	Leaves	Ewe igbale	4
Momordica charantia Linn	Curcurbiataceae	Seed	Ejinrin	1
Occimum gratissimum Linn	Lamiaceae	Leaves	Efinrin nla	6
Olax subscorpiodea Oliv	Olacaceae	Leaves	Egbon ifon	4
Opuntia dillenii (Ker.Gawl.) Haw.	Cactaceae	Fruit	Oro agogo	15
Oxytenanthera abysinnica (A.rich)Munro;S	Poaceae	Fruit	Parun pupa Parun funfun	2
Plumbago zeylania Linn.	Plumbaginaceae	Fruit	Inabiri	3
Pseudocedrela kotschyi (Schweinf.)Harms	Meliaceae	Leaves and fruit	Emi gbegiri	4
Rauwolfia vomitora Afzel.	Apocynaceae	Leaves	Asofeyeje	3
Securidate longepedunculata Fresen.	Polygalaceae	Leaves	Ipeta	2
Smilax karussiana Meisn.	Smiliacaceae	Leaves	Kaasan	2
Sorghum bicolor (L) Moench	Poaceae	Leaves	Poroporo oka baba	2
Tetrapleura tetrapleura (Schumach.)Taub	Legunminosae	Pod	Aidan	2
Uvaria chamae Beav	Annonaceae	Fruit	Eruju or oko oja	4
Xylopia aethiopica (Dunal) A.rich	Annonaceae	Fruit and seed	Eeru Alamo	3

Table 2: Minimum Inhibitory concentration (MIC) of *Jatropha gossypifolia* ethanol extract

MIC(µg/ml)	Concentration (mg/ml)
S.aureus	50mg/ml
MRSA	100mg/ml

Table 3: Antimicrobial activity of *Jatropha gossypifolia* leaves extract with antibiotics

Antibiotics	Bacteria	Zones of inhibition (mm) 100µl of standard drug	100µl of std drug and 50mg/ml of extract	100µl of std drug and 100mg/ml of extract
Ampiclox	MRSA	20.0±4.0	19.0±5.0 *1.0	21.5±3.5 **1.5
	S.aureus	29.5±3.5	30.0±1.0 **0.5	27.5±2.5 *2.0
Ciprofloxacin	MRSA	29.5±0.5	33.5±2.5 **4.0	29.0±5.0 *0.5
	S.aureus	38.0±0.9	31.5±3.5 *6.5	33.0±4.0 *5.0
Erythromycin	MRSA	20.5±4.5	25.5±0.5 **5.0	27.0±3.0 **6.3
	S.aureus	25.0±1.0	25.5±0.5 **0.5	22.0±2.0 *3.0
Gentamycin	MRSA	24.0±0.0	26.0±1.0 **2.0	26.5±1.5 **2.5
	S.aureus	32.5±4.5	34.5±0.5 **2.0	32.5±3.5 *0
Streptomycin	MRSA	21.0±0.0	23.5±0.5 **2.5	19±5.5 *2.0
	S.aureus	34.0±0.0	31.0±2.0 *3.0	28±3.0 *6.0

All the values are mean ± standard deviation of three determinations; Keys: * = antagonism; ** = synergism

4. DISCUSSION

A semi-structured questionnaire was served to twenty respondents (herb sellers, traditional healers, and elders) with over forty plant species belonging to sixteen families, with Euphorbiaceae and Leguminosae being most frequently represented for the treatment of skin diseases and a potential source of antibiotics, as seen in Table 1. This survey corresponds with some of the medicinal plants mentioned in the work of [11]. The ethanolic leaf extract of *Jatropha gossypifolia* gave a yield of 16%, with the minimum inhibitory concentration (MIC) for both Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* detailed in Table 2. The results indicate that *S. aureus* exhibits greater sensitivity to the plant extract at a concentration of 50 mg/mL, which is consistent with the findings of Idowu *et al* [13]. This underscores the potential effectiveness of *J. gossypifolia* in treating *S. aureus* infections, highlighting its therapeutic benefits. The phytochemical screening of *Jatropha gossypifolia* has been exhaustively dealt with, reporting the presence of several phytoconstituents like alkaloids, glycosides, flavonoids, glycosides, phenols, saponins, tannins, and terpenoids. The presence of alkaloids, flavonoids tannins, and saponins has been suggested to be responsible for its antimicrobial activities [14,15]. The ethanol leaf extract of *Jatropha gossypifolia* has demonstrated notable antibacterial activity against *Staphylococcus aureus,* as noted by Parkialashimi and Archana [16]. In a study by Yakubu *et al* [14], where ethanol was utilized as the solvent for extraction, the extract produced the highest zone of inhibition against *Staphylococcus aureus* when compared to the standard antibiotic Ciprofloxacin. This highlights the potential of *J. gossypifolia* as a viable alternative in the fight against antibiotic-resistant strains of bacteria. Table 3 shows that both microorganisms had moderate interactions with the plant extract, as they either synergized or antagonized with the antibiotics. In the context of Gram-positive bacteria such as *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA), the effectiveness of antibiotics like Ampiclox has been highlighted by Okiti and Osuntoken [17]. This effectiveness is attributed to the relatively simple structure of their cell wall, which contributes to the observed synergistic effects when combined with plant extracts at a concentration of 100 mg/mL against MRSA. However, an antagonistic effect was noted at a lower concentration of 50 mg/mL, indicating a concentration-dependent response. The potency of the plant extract against MRSA may stem from ethanol's capability to extract a greater variety of phytochemical constituents, as Ruchi and Renu [15] suggested. This property enhances the antibacterial efficacy of the leaf extract at both concentrations (100 mg/mL and 50 mg/mL) when tested against *S. aureus*. Such findings underscore the potential of combining plant extracts with conventional antibiotics to improve treatment outcomes against resistant bacterial strains. This result is in alignment with the report of Dhale *et al* [18] 'Preliminary screening of antimicrobial and phytochemical studies of *Jatropha gossypifolia* Linn. Gram-positive bacteria, because of their thinner cell membrane, enable easy penetration, which makes them more susceptible to the plant extract than Gram-negative bacteria. Ciprofloxacin, a class of antibiotics known to be effective against gram-positive and gram-negative bacteria, had a modified interaction with the antibacterial potency of *Jatropha gossypifolia* against MRSA at 50mg/ml, and antagonism was observed against *S. aureus.* Antibiotics from the Aminoglycosides class, including Gentamicin and Streptomycin, were utilized in this research. Gentamicin displayed a synergistic effect with the extract at both concentrations for Methicillin-resistant *Staphylococcus aureus* (MRSA) but exhibited antagonism against *Staphylococcus aureus* at 50 mg/mL. Conversely, Streptomycin showed antagonistic effects against *S. aureus* at both concentrations while synergistically enhancing the plant extract's activity at 50 mg/mL for MRSA. These findings contribute valuable insights to the existing literature, corroborating earlier studies by Naziri *et al* and Cunningham-Oakes *et al* [19, 20] which explored the antimicrobial efficacy of Aminoglycosides against *Staphylococcus aureus.* Their research confirms that both Gentamicin and Streptomicin are effective against a range of Gram-positive and Gram-negative bacteria. This highlights the critical need to investigate the synergistic interactions between herbal remedies, like *J. gossypifolia* extracts, and traditional antibiotic therapies, especially in light of the ongoing challenges presented by antibiotic-resistant bacterial strains. The findings in this study further confirm that the ethanolic extract of *J. gossypifolia* leaves has high antibacterial potency against *S. aureus* and should be used solely without the interference of antibiotics, as it antagonizes when used with antibiotics. Erythromycin, belonging to the class of antibiotics Macrolides, was the only antibiotic out of the five antibiotics tested against *S. aureus* that was significantly modulated by synergistically aligning with the plant extract, inhibiting the growth of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* at 50mg/ml as well as 100mg/ml. This finding is contrary to the report of Faparusi and Adewole [21], where no zone of inhibition was seen when ethanolic leaf extract was tested against *S. aureus* and also on interactions with Ciprofloxacin. According to Falodun *et al*. [22], the sensitivity of these organisms is a result of the differences in their morphological constitution, and upon interactions with the plant extract, is a result of their cell wall breakdown. Antimicrobial properties of *Jatropha gossypifolia* leaf can also be attributed to its phytochemical constituents, and this was assessed by Das *et al* [23], who did phytochemical research on three Jatropha species (*Jatropha curcas, Jatropha gossypifolia*, *Jatropha multifida),* and from *Jatropha gossyifolia,* two macrocyclic diterpenes Jatrophenone and Citlalitrione were isolated. Although the investigation led to the isolation of thirty diterpenes, fourteen of which were novel including Jatrophenone from *J. gossypifolia.* Jatrophenone was reported to exhibit significant antibacterial activity against *Staphylococcus aureus* in comparison to Penicillin G. [23,24]reported on the phytochemical properties of *Jatropha gossypifolia* ethanolic leaf extract and found out that leaf extract had a significant amount of alkaloids and cardiac glycosides which can be responsible for the potent antimicrobial effect of the plants. Jeje and Ileola [25] also did a comparative study of phytochemicals in *Jatropha gossypifolia* and *Vernonia amydalina* and they reported the presence of flavonoid and its effective antibacterial and antiviral potential.

5. CONCLUSION

Jatropha gossypifolia has been identified as a potentially effective modifying agent that may enhance the efficacy of certain antibiotics against multidrug-resistant microorganisms, including Methicillin-Resistant *Staphylococcus aureus* (MRSA). This research successfully identified the concentrations at which the antimicrobial potency of *Jatropha gossypifolia* is most effective. This study successfully determined the specific concentrations at which *Jatropha gossypifolia* exhibits optimal antimicrobial potency, laying a foundation for future exploration in the realm of combination therapies.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Contribution of Authors

Olorunsola, O.E conducted the research and wrote the manuscript; Aremu, O.K edited the manuscript and gave it a proper outlook; Adeleye, O.A supervised the project and the writing of the manuscript; Effiong, A.P edited the manuscript; Mada, B, O helped to conduct the survey and analyze data.

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