Int. j. adv. multidisc. res. stud. 2026; 6(1):172-176

Received: 17-11-2025
Accepted: 27-12-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

In Vitro Synergistic Antibacterial Activity of Amoxicillin and Ethanol Leaves Extract of *Euphorbia hirta*

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DOI: https://doi.org/10.62225/2583049X.2026.6.1.5540

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Abstract

Euphorbia hirta is a plant belonging to the family (Euphobiaceae). It is used in ethno medicine for the treatment of diarrhea, dysentery and other bacterial infections. This work was aimed at screening the phytochemical constituents and evaluating the *in vitro* combined effects of amoxicillin and ethanol leaves extract of *Euphorbia hirta* against clinical isolates of *Staphylococcus aureus, Pseudomonasa eruginosa, selmonella typhi, Clostridium botulinum* and *klebsiella pneumonia* using broth micro-dilution and Checkerboard techniques. Phytochemical screening of the extract revealed the presence of carbohydrates, flavonoids, tannins, saponins, glycosides, steroids and triterpenes. *Euphorbia hirta* exhibited minimum inhibitory concentration (MIC) range 6.25 mg/mL to 25 mg/mL while Amoxicillin recorded MIC range10 mg/mL to 20 mg/mL against all test organisms. The susceptible antibiotics were used in the checkerboard technique, which was found to be useful for determining antibiotic synergism. The Fractional inhibitory concentration index (FIC) were 0.13, 0.25 and 0.50, this values indicates synergism while FIC value 1.00 for additive effect against the test organisms at 37˚C over night incubation. The result of this study encourages the uses of *Euphorbia hirta*/amoxicillin combination for the treatment of bacterial infection.

Keywords: Synergy, FIC Index, Phytochemical Screening, *Euporbia Hirta*

Introduction

Medicinal plants have been recognized and utilized throughout human history. In order to carry out vital biological tasks and protect themselves from predators like insects, fungus, and herbivorous mammals, plants may synthesize a vast array of chemical compounds [1]. Herbal medicines do not significantly differ from conventional medications in terms of how they function since chemical compounds in plants mediate their effects on the human body through processes that are analogous to those that are already well understood for the chemical compounds in conventional drugs. Because of this, herbal remedies can be just as effective as conventional ones, but they also have the same potential for negative side effects. The health of both individuals and communities is greatly impacted by medicinal plants. These plants have therapeutic properties because they contain certain secondary metabolites known as phytochemicals, which have a specific physiological effect on the human body. These could be non-nutritive substances with protective or disease-prevention qualities [2]. There are several plants in Africa, especially in Nigeria, that are utilized in herbal therapy to treat illnesses and injuries. Numerous biological and pharmacological effects, including anthelminthic, oxytocic, and laxative qualities, are displayed by several of these plants [3].

Euphorbia hirta is often referred to as hairy spurge and asthma weed (English). *Euphorbia hirta* is a common herb found in grasslands, roadsides, walkways, and open waste areas [4]. *E. hirta* is widespread in the hotter regions of Australia, Central America, Africa, and India, and it is frequently found in waste areas by roadways [5].

Euphorbia hirta is locally known as Nonon Kurchiya in Hausa, Harvom in Kaka, Tepel in Fulfulde, Hammocks, and mat (Florida). *Euphorbia hirta* is a widely popular herb among practitioners of traditional medicine [4]. The herb, which is widely used in traditional medicine to treat a number of illnesses, including asthma, coughs, diarrhea, and dysentery, is also known as asthma weed in Asia and Australia [6]. In addition to being used as an antispasmodic, antipruritic, carminative, depurative, diuretic, febrifuge, galactogogue, purgative, and vermifuge, a decoction of the herb is used in east, central, and west Africa to treat asthma, oral thrush, boils, ulcers, skin, and wound infections [6, 7, 8]. The plant's decoction is used in Mauritius to treat

pulmonary conditions, bronchitis, fever, vomiting, and respiratory tract infections [9]. Additionally, ringworm, rashes, toothaches, itching, eczema, and sexual illnesses have all been extensively treated with *M. hirtus* L. [10]. However, a decoction of the plant is used to treat asthma, bronchitis, eczema, athlete's foot, scorpion bite pains, constipation and other stomach issues, and enteric diseases such as diarrhoea and dysentery [8, 11]. There are two types of clinical usage of combined antibiotic therapy for bacterial infections. Infections with strains that are susceptible to one or more specific antibiotics are treated with such medication in the first group. Enhancing activity or achieving a synergistic effect is the main justification for combining two drugs. The term "synergy" refers to an effect that is substantially larger when two agents work together than when each agent works alone [12]. The phytochemical component of *E. hirta* and its synergistic activity with amoxicillin against certain bacterial isolates were the main objectives of this study.

Methodology

Sample collection and Treatment

The leave part of *Euphorbia hirta* was collected from Sokoto State University premises, Sokoto State Nigeria. The plant was properly identified and authenticated at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Science, Usmanu Danfodio University, Sokoto Nigeria; the sample was assigned a voucher specimen number PCG/UDUS/EUPH/0003. The plant material was collected and air dried under shade for 14 days and was ground to coarse powder using motor and pestle.

Test microorganisms used

Gram-positive and gram-negative micro-organisms such as *Staphylococcus aureus, Klebsiella pneumonia, Clostridium butolidium, Salmonella typhi, Pseudomonas aeruginosa*, were used for the antimicrobial studies. The microorganisms were obtained from the Department of Microbiology, Sokoto State University, Sokoto.

Extraction of Plant Materials

Approximately, 200 g of the powdered plant material was macerated in 500 mL of ethanol for three days with occasional agitation. The mixture was then filtered using a clean muslin cloth and then Whatman filter paper. The filtrate was then evaporated to dryness using water bath at 40ºC. The concentrated extract was stored at room temperature in air tight bottle for further studies.

Phytochemical Screening

The extract, was subjected to phytochemical screening using standard procedures. Phytochemical screening was carried out for carbohydrate tannin, saponin, cardiac glycosides, steroid, reducing sugar flavonoids and terpenoids [13].

Antibacterial Screening

Preparation of Culture Media

The culture media used was Mueller-Hinton broth (MHB). The media was prepared according to manufacturer specification. The media was prepared by weighing 11.2 g of the powder and dissolved in 500 mL of distilled water in a1000 mL conical flask. The mixture was heated until the powder was completely dissolved. The solution was sterilized in an autoclaving machine at 121 oC for 15 minutes. The solution was allowed to cool at room temperature before usage [14].

Preparation of Extract/Drug Stock Solution

The stock solution of *Euphorbia hirta* leaf extract was prepared on each occasion by careful weighing of 0.8 g and dissolving in 2 mL of Dimethyl sulfoxide (DMSO) to get a concentration of 400 mg/mL. 0.04 mg of pure powder of amoxicillin was dissolved in 1mL of distilled water to get 40 µg/mL of stock solution [15].

Preparation of Standard Inoculums of Test Organisms

The inoculum of the test organisms was prepared by first streaking the organisms on the prepared Mueller Hinton broth in a slant to obtain colonies of the bacteria. A colony was picked and subculture unto sterile nutrient broth and incubated at 37 ºC for 24 hours. From the broth culture, a loopful of each bacterial suspension was transferred into bottles containing sterile distilled water and the turbidity was adjusted to McFarland standard to obtain a bacterial density of 1.5×10^5 CFU/mL [14].

Determination of Minimum Inhibitory Concentration (MIC) of the Drug and Extract

The minimum inhibitory concentration (MIC) of the extracts was determined using the micro dilution methods. About 50 mL of the media (Mueller Hinton broth) was dispensed into the 96 well microtitre plates. 50mL of 200 mg/mL of the extract was dispensed into the wells, followed by a Twofold serial dilutions made by using micropipettes from wells A11–G11 to A1–G1 and 50 µL portions were discarded from the last column. Bacterial suspensions (50µL) were added to all wells to bring the total volume to 100 µL and initial inoculum size of 1to5×105 CFU/mL. Extract-free wells in column A12–G12 were used as positive control. Plates were allowed to stand for 30minutes followed by incubation at 37oC for 24 hrs and examined for the presence or absence of growth using turbidity as criterion. The lowest concentrations in the serial dilution without visible signs of growth after 24 hrs were considered to be the minimum inhibitory concentration (MIC) [16].

Determination of Minimum Bactericidal Concentration (MBC)

BC) was calculated using the results of the minimum inhibitory concentration (MIC). After being dipped into the well that did not exhibit turbidity in the MIC test, a sterile wire loop was stroked over fresh Mueller Hinton broth on a different plate. For a whole day, the plates were incubated at 37 oC. The plates were checked to see if there had been any growth after the incubation period. This was carried out to ascertain whether the samples' antibacterial activity is bacteriostatic or bactericidal [14].

Evaluation of Combined Effects of Amoxicillin and *Euphorbia hirta* extract

One 96-well plate was used to test for two antibiotics at a time, each one alone and in double Combinations against each of the microorganism briefly; rows A to E were used for each of the microorganism for the double combination. All wells were filled with 50 µL of cation adjusted Muller Hinton broth (MHB). 25 µLof each of the extract and the drug at four times (4x) of the highest tested minimum inhibitory concentrations in Mueller Hinton broth was

delivered to wells A11–E11. Twofold serial dilutions were made by using micropipettes from wells A11–E11toA1–E1 and 50 µL portions were discarded from the last column. Bacterial suspensions (50µL) were added to all wells to bring the total volume to 100µL and were used as positive control. Plates were incubated at 37 oC for 24 hours after being let to stand for 30 minutes. The most effective antibiotic was used to calculate the minimum bacteriostatic and bactericidal concentrations in combinations. Each isolate underwent repeated trials, and the results were only taken into account if the MIC agreed in at least one of the three wells [17].

The chequerboard approach was used to confirm the double combination results achieved by the method given. The combination response was evaluated by calculation of the fraction inhibitory indices (FIC) as follows, [15].

$$\text{FIC index} = \text{FIC of extract} + \text{FIC of Amoxicillin} \quad (1)$$

$$\text{FIC of Extract} = \frac{\text{MIC of Extract incombination with amoxicillin}}{MIC\ of\ extract\ alone} \quad (2)$$

$$\text{FIC of Amoxicillin} = \frac{\text{MIC of Extract incombination with amoxicillin}}{MIC\ of\ amoxicillin\ alone} \quad (3)$$

Result and Discussions

Result of the Phytochemical Screening

Table 3.1: Phytochemical screening of ethanol extract of Euphorbia hirta

Phytoconstituents	Test	Inference
Carbohydrate	Molisch test Fehling test	+ +
Cardiac glycoside	Keller–killiani test	+
Saponins	Frothing test	+
Steroids & Triterpenes	Liberman Burchard test Salkwoski test	+ +
Flavonoids	Sodium hydroxide test Shinoda test	+ +
Tannins	Ferric chloride test	+
Alkaloids	Dragendoffs test Mayers test Wagners test	+ ND ND

KEY+ = Present ND = Not detected

Minimum Inhibitory, Bacteriostatic and Bactericidal Concentration

Table 3.2: Results of MIC and MBC of euphorbia hirta and the amoxicillin (mg/mL)

Organisms	Extract		Drug	
	MIC (mg/mL)	MBC (mg/mL)	MIC (µg/mL)	MBC (µg/mL)
S. aureus	12.50	12.50*	20	20*
P. aeuriginosa	6.25	6.25*	20	20*
C. botulinum	25.00	25.00*	10	10*
S. typhi	12.50	12.50*	20	20*
K. pneumoniae	25.00	25.00µ	20	20*

Key: MIC = Minimum Inhibitory Concentration MBC = Minimum Bactericidal Concentration
*=Bactericidal µ=Bacteriostatic

Combined activity of Amoxicillin and Ethanol Leaf Extract of *Euphorbia hirta*

Table 3.3: Result of the combined effect of amoxicillin and ethanol extract of E. hirta

Organisms	E. hitra MIC mg/mL	Amoxicillin MIC Mg/mL	E. hirta MIC Mg/mL	Amoxicillin MIC Mg/mL	FIC Index	Inference
S. aureus	0.78	1.25	0.06	0.06	0.13	Synergy
P. eurigenosa	0.78	2.50	0.13	0.13	0.26	Synergy
C. botulinum	6.25	2.50	0.25	0.25	0.50	Synergy
S. typhi	3.13	5.00	0.25	0.25	0.50	Synergy
K. pneumonia	12.5	10.0	0.50	0.50	1.00	Additive

Key: MIC = Minimum inhibitory concentration FIC = Fractional inhibitory concentration

The results of the systematic and scientific evaluation of the *in vitro* effects of *Euphorbia hirta* leaf extract and amoxicillin have been presented in this paper (Table 3.3). Synergy was defined as FIC index values less than 1, and the degree of synergy grows as the value approaches zero. Additive effects are indicated by FIC index values of 1, indifference is shown by values greater than 1 but less than 2, and antagonism is indicated by values larger than 2 [15].

Antimicrobial studies and phytochemical screening are important methods for proving a chosen plant species' ethnomedical claims. Some bioactive components, including tannins, carbohydrates, reducing sugar, glycosides, cardiac glycosides, saponins, flavonoids, steroids/triterpenes, and alkaloids, were found in the ethanol leaf extracts of *E. hirta* during phytochemical screening (Table 1). The study's findings are consistent with those published in the literature [18]. When the same plant's methanol extract was employed for phytochemical screening, similar phytoconstituents were discovered [19]. These substances may be used to combat human pathogens, such as those that cause intestinal infections [4].

Numerous authors have linked the antibacterial properties of crude plant extracts to the presence of these bioactive substances. The plant's usage in ethnomedicine is supported by the presence of glycosides, saponins, cardiac glycosides, and flavonoids, which are known to prevent tumour growth and protect against gastrointestinal infections [6]. The plant's ethnomedical use for wound healing, diarrhoea, dysentery, and anticoagulant was further supported by the presence of tannins [6, 20, 21].

The plant's biological and pharmacological significance, including its antipyretic, analgesic, anticancer, and anti-inflammatory properties, was shown by the confirmation of saponins in the plant extract [6]. The analgesic, anti-inflammatory, anti-malarial, anti-microbial, and anticancer properties are attributed to the presence of steroids and triterpenes; certain compounds with phenolic nuclei also possess antiseptic and antioxidant properties [22]. The antibacterial activity of the ethanol leaf extract was evaluated by determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Minimum inhibitory concentration is

the lowest concentration at which the extract inhibits 90% growth of the organism. The minimum inhibitory concentration (MIC) of the extract was tested against three gram negative bacteria (*Pseudomonas aeruginosa, selmonella typhi, klebsiella pneumoniae*) and two gram positive bacteria (*Clostridium botulinum and Staphylococcus aureus*). The MIC for *Pseudomonas aeruginosa* (6.25 mg/mL), *Selmonella typhi* (12.5 mg/mL), *klebsiella pneumonia* (25mg/mL), *Clostridium botulinum* (25 mg/mL) and *Staphylococcus aureus* (12.5 mg/mL) (Table 3.2). This is in conformity with the findings by Saravanan *et al*., [23] which reported that the MIC of the ethanol leaf extract of *E. hirta* against *S. aureous, S. typhi* and *E. coli* ranged between 20 mg/mL to 25 mg/mL. *P. aeruginosa* shows low resistance to the extract and as such the extract inhibits the growth at very low concentration. The MIC showed that ethanol leaf extract of *E. hirta* can be used in the treatment of infection caused by the entire test organism and more effectively in any infection caused by *P. aeruginosa*. The MIC of the amoxicillin for the entire organism is 20 µg/mL with the exception of *C. botulinum* which recorded MIC of 10 µg/mL.

The minimum bactericidal concentration (MBC) is the lowest concentration at which the extract completely kills the organisms. The MBC values were 12.5 mg/mL, 6.25 mg/mL, 12.50 mg/mL, 25 mg/mL and 12.50 mg/mL for *S. aureus, P. aeruginosa, C. botulinum, K. pneumoniae* and *S.typhi* respectively. El Mahmood *et al*., [4] have reported that the MBC values can either be the same or higher than the MIC values. In this study, the MIC values were either the same or slightly lower than the MBC values, similar to the results obtained by Karou *et al*., [24]. The MIC and MBC values are predictive of the efficacy of agents' *in vitro*.

The evaluation of the combined effect of the extract and the drug was determined using the fractional inhibitory concentration indices (FIC_{index}) as shown in equation 1 above. The fractional inhibitory concentration (FIC_{index}) were 0.13, 0.50, 0.25, and 0.50 for *S. aureus, C. botulinum, P. aeruginosa* and *S. typhi* respectivey indicating synergism while 1.00 for *K. pneumoniae* which shows additive effect. The result of the combined effect shows that ethanol leaf extract of *E hirta* can be used in combination with amoxicillin in boosting the activity to effectively treat bacterial infection cause by *S. aureus, C. botulinum, P. aeruginosa* and *S. typhi*. FIC index values < 1 were considered as synergy and the degree of synergy increases as the value tends towards zero. FIC index values of 1 indicate additive effect, values greater than 1 but less than 2 represent indifference while values greater than 2 show antagonism [15]. This affirms the work reported by Micheal *et al*., and Jackson *et al*., [15, 25] that *E hirta* shows synergistic activity at certain combination with erythromycin and nystatin. The combination of antibiotic is often recommended to prevent resistance that may occur during treatment and to achieve higher efficacy in the treatment of infectious diseases [26].

Conclusion

The results of this *in vitro* test indicated that the combination of *Euphorbia hirta* leaf extract and amoxicillin at a given concentration has a possible clinical significance in the treatment of bacterial infections caused by *S. aureus*, *C. botulinum*, *P. aeruginosa* and *S. typhi.* Unguided and indiscriminate combination may lead to an effect which has no clinical significance. Moreover, this herbal extract is widely available, cheap and quite safe.

Conflict of Interest

There is no conflict of interest among the authors.

Funding

This research is funded by the Tertiary Education Trust Fund (TETFUND) Through the Institution-Based Research (IBR) with reference number TETF/DR&D/CE/UNIV/SOKOTO/IBR/2025/VOL.1

Acknowledgement

The authors would like to express their gratitude to Malam Bello of the Department of Microbiology, Sokoto State University, Sokoto, for their helpful contributions that made this research a success.

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