Original Research Article

EFFECTS OF COMBINING CRUDE ETHANOLIC EXTRACT OF *JATROPHA CURCUS* L. LEAF AND SOME ANTIBIOTICS AGAINST SOME SELECTED MICROORGANISMS

Akanwariwiak W G[1*], Addo-Fordjour P[1], Musah A A[1]

[1]*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*

**Corresponding author: Email: garibawa@yahoo.co.uk, Tel: 233-24-571570, Fax: 233-51-60306*

Received: 02/04/2012; Revised: 22/04/2012; Accepted: 25/04/2012

ABSTRACT

Evidences are mounting concerning the resistance of microorganisms to antibiotics throughout the world. This development has awakened scientists to explore alternative approaches that target and block resistance. One way of accomplishing this has been the combination of plant extracts with antibiotics to increase their activity. The study was therefore, aimed at determining the effects of combining the leaf extract of *Jatropha curcas* L. with some antibiotics on certain selected microorganisms. The antimicrobial activity of the ethanolic extract of *J. curcas* leaf and its combination with selected antibiotics was assessed against certain microorganisms using the agar well diffusion method. The diameter of inhibition zone and minimum inhibitory concentration (MIC) were used as indicators of antimicrobial activity. The plant extract alone showed antimicrobial activity against all the test organisms, with diameter of inhibition zone ranging from 2–13.7 mm. The diameter of inhibition zone of the antibiotics alone ranged from 3.7–23 mm. The activity of the antibiotics varied upon combination with the plant extract, but the diameter of inhibition zone was between 6 and 25 mm. The antimicrobial activity of ciprofloxacin was increased significantly (MICs reduced significantly) when combined with the plant extract whereas that of tetracycline was reduced. In all, ciprofloxacin and ciprofloxacin-plant extract were the most effective treatments recording the lowest MICs. The most significant reduction of MICs was observed in the ciprofloxacin-plant extract combination.

Keywords: antimicrobial activity, crude ethanolic extract, *Jatropha curcus* leaf, diameter of inhibition zone, minimum inhibition concentration (MIC)

INTRODUCTION

Infectious diseases caused by microorganisms are increasing in numbers thereby drawing the attention of researchers. Since the twentieth century, antibiotics have been employed in the treatment of many of these diseases. However, some microorganisms have already become resistant to many antibiotics while more continue to develop resistance to the action of some antibiotics (Lewis et al. 2002). For instance, *Candida albicans* is now reported to be resistant to a standard drug, clotrimazole, which once used to be very effective in tackling candidiasis (Goff et al. 1995; Nolte et al. 1997; Kieren et al. 1998). The problem of microbial resistance to antibiotics is growing and the outlook for the use of antimicrobial drugs in future is still uncertain, as newly developed antimicrobial agents are also being resisted (Coates et al. 2002).

In the midst of increasing resistance of antibiotics to microorganisms, it is imperative to explore alternative approaches that target and block resistance. The use of agents that do not kill pathogenic bacteria but modify them to produce a phenotype that is susceptible to the antibiotic has been suggested as an alternative approach to the treatment of infectious diseases (Taylor et al. 2002). Such agents could render the pathogen susceptible to a previously ineffective antibiotic, and because the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes. One way of accomplishing this has been the combination of plant extracts with antibiotics with the view to reducing the minimum inhibitory concentration (MICs) of the antibiotics significantly, against resistant strains (Darwish et al. 2002; Al-hebshi et al. 2006; Betoni et al. 2006). It is speculated that inhibition of drug efflux and alternative mechanisms of action could be responsible for the interactions between plant extracts and antibiotics (Zhao et al. 2001; Lewis and Ausubel 2006).

Jatropha curcas (Figures 1 a. & b.) has played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha* spp. including *J. curcas* have displayed potent cytotoxic, antitumor and antimicrobial activities in different assays. For example, the leaves are utilized extensively in West African ethnomedical practice in different forms to cure various ailments like fever, mouth infections, guinea worm sores and joint rheumatism (Irvine 1961; Oliver-Bever 1986). The latex of *J. curcas* is reported to have antibacterial activity against *Staphylococcus aureus* (Thomas 1989), while the methanolic extract of the roots has been shown to exhibit anti-diarrhoeal activity in mice through the inhibition of prostaglandin biosynthesis and reduction of osmotic pressure (Mujumdar et al. 2001). Although the antimicrobial activity of *J. curcas* on some microorganisms has been extensively studied, no work has been conducted on the possible interaction effects produced on microorganisms when extracts of the plant are combined with certain antibiotics. The study was therefore, carried out to determine the effects of combining the leaf extract of *J. curcas* with some antibiotics on certain selected microorganisms.

METHODOLOGY

Plant extraction

Fresh leaves of *Jatropha curcas* were obtained at Maxima, a suburb of Kumasi. The sample was air-dried at room temperature and ground using a hammer mill. Five-hundred and fifty grams of the ground plant material was soaked in ethanol for 48 h after which extraction was done using the Soxhlet extractor. The solvent was removed from the extract with the Buchi rotary evaporator (R152) and the residue dried to a constant weight in an electric oven at 50°C.

The dry plant extract was re-dissolved in methanol to the final graded concentrations of 10, 20, 30 and 40%. Tetracycline, Amoxicillin and Ketoconazole were used as positive control at concentrations of 0.1, 0.05, 0.025 and 0.0125. Ciprofloxacin was also used as a positive control at concentrations of 0.001%, 0.0001%, 0.00001% and 0.000001%.

Figure: 1 a. Fruits of *Jatropha curcus*

b. *Jatropha curcus* in its habitat

Preparation of nutrient agar

An amount of 24.8 g of nutrient agar was weighed into a conical flask. One thousand milliliters of distilled water was added and the mixture was melted over a Bunsen flame. The mixture was then poured into test tubes, 20 ml each and plugged with cotton wool. The cotton wool was covered with cellophane and the test tubes were autoclaved at 1.1 kg/cm^3 steam pressure for 15 min. The nutrient agar was then stabilized in an electric water bath at 45°C for 15 min before use.

Test microorganisms

Six species of bacteria namely, *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Bacillus subtilis* where used for the antimicrobial assay. *C. albicans* was the only fungal species included in the antimicrobial assay. Pure cultures of these organisms were obtained from the Microbiology laboratory of the Department of Pharmaceutics of the Faculty of Pharmaceutical Sciences, KNUST. The following chemotherapeutic agents were used as positive control: Tetracycline, Amoxicillin and Ciprofloxacin for the bacteria and Ketoconazole for the fungus.

Determination of antimicrobial activity

The agar well diffusion method was employed in the assay. Twenty milliliters of stabilized nutrient agar was seeded with microorganisms, palmed and poured into a Petri dish to solidify. A cork borer of 9 mm in diameter was used to make wells in the agar. With the aid of a syringe, the wells were filled with different concentrations of the plant extracts. The extract was allowed to diffuse for 30 minutes and the plates were incubated at 37°C for 24 h. The zone of inhibition of the extract, the clear area around the well was measured in millimeters (mm) using a ruler after 24 h of incubation.

Determination of minimum inhibitory concentration (MIC)

A graph of the diameter of inhibition zones of the plant extract and the antibiotics was plotted against the log of concentration. The MIC of the particular treatment was then calculated as the antilog of the X-intercept from the equation of the line obtained.

Determination of the combined effects of the plant extract-antibiotics combination on the test organisms

The original concentrations of the antibiotics were maintained in combination with a concentration below the lowest MIC of the plant extract against the test organisms. The sub minimum inhibitory concentration of the plant extract, 2% was used as a solvent to dissolve the antibiotics.

Statistical analysis

Analysis of variance (ANOVA) was used to determine differences between the diameter of inhibition zones on one hand and the MICs on the other hand, between the plant extract, antibiotics and antibiotics-plant extract treatments. The 11th Edition of the GenStat software (VSN International Ltd., Hemel Hempstead, UK) was used for the analysis at a significant level of 5 %.

Table 1: Antimicrobial effect of different concentrations (%) of *J. curcas* leaf extract on the test organisms

Extract	DIZ (mm) at the various concentrations				MIC (%)
	10	**20**	**30**	**40**	
S. typhi	3.7	5.7	7.7	8.7	3.8
C. albicans	6.7	9	11.3	13.7	2.7
P. mirabilis	2	3	3.7	5	4.4
P. aeruginosa	4	5.3	6.7	7.7	2.3
S. aureus	4.3	6.7	9.3	10.7	4.2
K. pneumonia	2.7	3.7	5.3	7.3	4.8
B. subtilis	6.3	7.3	10.3	11.3	2.1

MIC: Minimum inhibition concentration; DIZ: Diameter of inhibition zone

RESULTS

Effects of *J. curcas* leaf extract on the test organisms

The crude extract of *J. curcas* exhibited diverse antimicrobial activity against all the microorganisms used (Table 1). The plant extract inhibited growth of *C. albicans* and *B. subtilis* (ranged from 6.3–13.7 mm) more than the other microorganisms (ranged from 2–10.7 mm). The activity of the plant extracts on all the microorganisms, increased with increasing concentration. There was no significant difference between the activity of the plant extract and that of amoxicillin and ketoconazole ($P > 0.05$). The MIC of the plant extract against *B. subtilis* (2.1%) was smaller compared to that of the other organisms. This was followed by the MIC against *P. aeruginosa* (2.3 %). The highest MIC of the plant (4.8 %) extract against the microorganisms was recorded for *K. pneumoniae*.

Effect of the antibiotics and antibiotics-plant extract combinations on the test organisms

Ketoconazole and ketoconazole-plant extract

The ketoconazole-plant extract combination produced significantly greater diameter of inhibition zones compared to those produced by ketoconazole alone ($p < 0.001$) (Table 2). The MIC of ketoconazole-plant extract combination was lower than that of ketoconazole only.

Ciprofloxacin and ciprofloxacin-plant extract

Ciprofloxacin showed activity against all the bacteria used (Table 3). The diameter of

inhibition zone ranged from 7–22.7 mm for ciprofloxacin. The highest inhibition of growth occurred in *B. subtilis* (ranged from8–22.7 mm). The activity of ciprofloxacin-plant extract was lower than that of the antibiotics alone although the difference was not significant (p = 0.563). The MICs of the ciprofloxacin-plant extract combination were significantly higher than those of ciprofloxacin alone (p = 0.01). The best MIC of the ciprofloxacin-plant extract (1.0×10^{-9}) was recorded against *S. typhi*.

Table 2: Antifungal effects of different concentrations of ketoconazole and ketoconazole-*J. curcas* leaf extract on C. albicans

Concentration (%)	DIZ (mm) of ketoconazole	DIZ (mm) of ketoconazole-plant extract
0.1	11	15
0.05	6.7	13
0.025	5	10
0.0125	3.7	8
MIC	5.148×10^{-3}	1.296×10^{-3}

DIZ: Diameter of inhibition zone

Amoxicillin and amoxicillin-plant extract

Amoxicillin alone and amoxicillin-plant extract combination did not show any activity against *S. typhi, P.mirabilis and P. aeruginosa* (Table 4). The growth of the rest of the microorganisms were however, inhibited by both treatments. The diameter of inhibition zones recorded for amoxicillin against *S. aureaus* and *K. pneumonia*e (10–20 mm) were lower than the diameter of inhibition zones of amoxicillin-plant extract combination against these bacteria (15–23 mm). The difference between the treatments with regard to the diameter of inhibition zones were however, not significant ($p = 0.192$). The MICs of the amoxicillin-plant extract combination were all lower than those of amoxicillin treatment. However, the differences between the MICs of the two treatments were not significant ($p = 0.071$). The lowest MIC for amoxicillin-plant extract combination (3.148×10^{-5}) was recorded against *K. pneumonia*.

Tetracycline and tetracycline-plant extract

The diameter of inhibition zones produced by tetracycline-plant extract combination against *P. mirabilis, P. aeruginosa* and *S. aureus* were higher than those produced by tetracycline alone (Table 5), although the differences in diameter of inhibition zones of the two treatments were not significant ($p = 0.725$). All the MICs produced by tetracycline-plant extract combination were significantly lower than those produced by tetracycline only ($p = 0.003$).

Table 3: Antimicrobial effects of different concentrations of ciprofloxacin and ciprofloxacin-*J. curcas* leaf extract on the test organisms

Microorganism	DIZ (mm) of ciprofloxacin				MIC	DIZ (mm) of ciprofloxacin-plant extract				MIC
	10^{-3}	10^{-4}	10^{-5}	10^{-6}		10^{-3}	10^{-4}	10^{-5}	10^{-6}	
S. typhi	15.7	12.3	9.3	6.3	1.015×10^{-8}	12	10	8	6	1.0×10^{-9}
P. mirabilis	18	14.3	11	7	1.086×10^{-8}	15	11	8.3	7.7	1.71×10^{-9}
P. aeruginosa	20	15.3	11.3	7.3	1.992×10^{-8}	10.3	7.3	5.7	4.7	4.96×10^{-9}
S. aureus	19	12.7	9	6.3	4.887×10^{-8}	24.7	19	13.3	11	7.37×10^{-9}
K. pneumonia	21.7	17.3	12.3	9	1.005×10^{-8}	20	15.7	11.3	9	5.71×10^{-9}
B. subtilis	22.7	18.3	13.3	8	2.128×10^{-8}	21.7	18	14	11	1.05×10^{-9}

MIC: Minimum inhibition concentration; DIZ: Diameter of inhibition zone

Table 4: Antimicrobial effects of different concentrations of amoxicillin and amoxicillin-*J. curcas* leaf extract on the test organisms

Microorganism	DIZ (mm) of amoxicillin				MIC	DIZ (mm) of amoxicillin-plant extract				MIC
	0.1	0.05	0.025	0.0125		0.1	0.05	0.025	0.0125	
S. typhi	0	0	0	0	0	0	0	0	0	0
P. mirabilis	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0	0	0	0	0
S. aureus	18.3	15	12.3	11	6.674×10^{-4}	23	20.7	17.7	17	5.623×10^{-5}
K. pneumonia	17	14	12	10	6.643×10^{-4}	20.3	18	16.7	15	3.148×10^{-5}
B. subtilis	20	18	15.7	12.7	3.110×10^{-4}	18	15.7	14.7	13	4.701×10^{-5}

MIC: Minimum inhibition concentration; DIZ: Diameter of inhibition zone

Table 5: Antimicrobial effects of different concentrations of tetracycline and tetracycline-*J. curcas* leaf extract on the test organisms

Microorganism	DIZ (mm) of tetracycline				MIC	DIZ (mm) of tetracycline-plant extract				MIC
	0.1	0.05	0.025	0.0125		0.1	0.05	0.025	0.0125	
S. typhi	21	18	15	12.7	5.72×10^{-4}	17	14	12	10	6.64×10^{-4}
P. mirabilis	13	12	11	10	1.26×10^{-5}	18	14.7	13	12	2.26×10^{-4}
P. aeruginosa	15	12	10	9	6.69×10^{-4}	18.7	15.3	12.3	10.3	1.10×10^{-3}
S. aureus	15	13.7	12.7	11.3	1.92×10^{-5}	18.7	16.3	14.3	12.3	2.35×10^{-4}
K. pneumonia	22	20	17.7	16.3	4.10×10^{-5}	20	19	14	13	4.43×10^{-4}
B. subtilis	23	21.3	19.7	17	2.82×10^{-5}	25	21.3	17.3	15.3	5.78×10^{-4}

MIC: Minimum inhibition concentration; DIZ: Diameter of inhibition zone

DISCUSSION

The leaf extract of *J. curcas* showed some levels of activity against all the test organisms by inhibiting their growth. This suggests that the extract contained antimicrobial substances which were responsible for its activity (Srinivasan 2001). The effect of the plant extract varied from one microorganism to another. *Candia albicans* and *B. subtilis* were more susceptible to the extract than the rest of the microorganisms. The activity of the plant extract was concentration dependent, increasing with increasing concentration. Although the antimicrobial activities of ciprofloxacin and tetracycline were significantly higher than the plant extract ($p < 0.001$), the plant extract had inhibiting effects that were similar to those of amoxicillin and ketoconazole ($p > 0.05$).

The leaf extract of *J. curcas* interacted with the antibiotics to produce varying effects on the tested microorganisms. The plant extract and antibiotics contained active ingredients which when combined with each other, resulted in additive, synergistic or antagonistic effects (Delaquis et al. 2002; Fu et al. 2007). While the activity of some of the antibiotics was improved upon combination with the plant extract, the activity of others was reduced. The improved antimicrobial activity strength (indicated by the zone of inhibition size) of the combined treatments varied across the various treatments and tested organisms. The ketoconazole-plant extract combination produced significantly greater inhibition of growth of *C. albicans* compared to that produced by ketoconazole alone ($p < 0.001$). Amoxicillin alone was not able to inhibit the growth of *S. typhi, P. aeruginosa* and *P. mirabilis.* Although the plant extract alone was able to inhibit the growth of these organisms, its combination with amoxicillin did not produce any different effect from that of amoxicillin. However, amoxicillin alone showed some levels of activity against the other microorganisms, and this activity became slightly better when combined with the plant extract. Compared to tetracycline, tetracycline-plant extract inhibited the growth of *P. mirabilis, P. aeruginosa* and *S. aureus* more. Generally however, the differences in diameter

of inhibition zones of these treatments were not significant ($p = 0.725$). These results suggest that there are possibly some phytochemicals in the plant extract which either decreased the resistance of the microorganisms or increased the mechanisms of action of the antibiotics (Al-hebshi et al. 2006). There was no significant combining effect (of ciprofloxacin and ciprofloxacin-plant extract) on the inhibitory effect of ciprofloxacin against the microorganisms ($p = 0.153$).

The MICs of the standard drugs were relatively lower than those of the plant extract due to the crude nature of the extract. When the antibiotics were combined with the ethanolic extract of *J. curcas* leaf at a sub minimum inhibitory concentration, the MICs of ciprofloxacin were decreased substantially ($p = 0.01$) against the test organisms. This reflects the interaction effects between the treatments (Cha et al., 2009). The MIC of ketoconazole was also slightly decreased when combined with the plant extract. On the contrary, the combination between tetracycline and the sub minimum inhibitory concentration of the plant extract caused a significant increase ($p = 0.003$) in the MICs of the drug. This may indicate that, some active ingredients in the extract interfered with the mechanism of action by which the antibiotic works. In all, ciprofloxacin and ciprofloxacin-plant extract were the most effective treatments since they had the lowest MICs against all the microorganisms. By far, the best combining effects was observed in the ciprofloxacin-plant extract.

The susceptibility of microorganisms to both the antibiotics and antibiotics-plant extract combinations varied tremendously. For instance, *S. typhi* was most susceptible to both ciprofloxacin alone and ciprofloxacin-plant extract treatments since it required the least dose to be inhibited. On the other hand, *S. aureaus* was least susceptible to these treatments requiring higher doses for inhibition.

CONCLUSION

The leaf extract of *J. curcas* showed antibacterial and antifungal activities against all the micro-organisms. The antimicrobial activity of ciprofloxacin was increased significantly (MICs reduced significantly) when combined with the plant extract. On the other hand, the activity of tetracycline was reduced significantly (increased MICs) when combined with the plant extract. In all, ciprofloxacin and ciprofloxacin-plant extract were the most effective treatments with the lowest MICs. The most significant reduction of MICs was observed in the ciprofloxacin-plant extract combination.

ACKNOWLEDGEMENTS

Logistical support for the study was provided by the Department of Pharmaceutics, KNUST, Kumasi, Ghana.

REFERENCES

Al-hebshi N, Al-haroni M, Skaug N (2006). In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. *Arch. Oral Biol.* 51:183–188.

Betoni JEC, Mantovani RP, Barbosa LN, Di-Stasi LC, Fernandes A (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Mem. Inst. Waldo Cruz.* 101 No. 4.

Cha JD, Moon SE, Kim JY, Jung EK, Lee YS (2009). Antibacterial activity of sophoraflavanone G isolated from the roots of *sophora flavescens* against methicillin-resistant *staphylococcus aureus*. *Phytother. Res.* 23(9):1326–1331.

Coates A, Hu Y, Bax R (2002). The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discovery* 1:895–910.

Darwish RM, Aburjai T, Al-Khalil S, Mahafzah A (2002). Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus*. *J. Ethnopharm.* 79:359–364.

Delaquis PJ, Stanich K, Girard B, Mazza G (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food. Microbiol. 74:101–109.

Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, Efferth T (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother. Res.* 21: 989–994.

Goff DA, Koletar SL, Buesching WJ, Barnishan J, Fass RJ (1995). Isolation of fluconazole resistant *Candida albicans* from human immunodeficiency virus-negative patients never treated withazoles. *Clin Infect Dis.* 20(1):77–83.

Irvine ER (1961). Woody plants of Ghana with special reference to their uses. 2nd Ed. *O.U.P. London* p. 233–237.

Kieren AM, Lyons CN, Rustad T, Bowden RA, White TC (1998). Rapid, Transient Fluconazole Resistance in *Candida albicans* Is Associated with Increased mRNA Levels of *CDR Antimicrob. Agents Chemother.* 42: 2584–2589.

Lewis K, Ausubel FM (2006). Prospects for plant-derived antibacterials. *Nat. Biotechnology.* 24(12): 1504–1507.

Lewis R, Gaffin D, Hoefnagels M, Parker B (2002). Life. 4th Ed. McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York p. 275.

Mujumdar AM, Miser AV, Salaskar MV, Upadhye AS (2001). Antidiarrhoeal effect of an isolated fraction of *Jatropha curcas* roots in mice. *J. Nat. Remedies* 1: 98–93.

Nolte FS, Parkinson T, Falconer DJ, Dix S, Williamsm J, Gilmore C, Geller R, Wingard JR (1997). Isolation and characterization of fluconazole- and amphotericin B- resistant Candida albicans from blood of two patients with leukemia Antimicrob. *Agents Chemothe.* 41: 196–199.

Oliver-Bever B (1986). Medicinal plants in tropical West Africa, Cambridge University Press, London.

Srinivasan D, Nathan S, Suresh T, Perumalsamy O (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.* 74:217–220.

Taylor PW, Stapleton PD, Luzio JP (2002). New ways to treat bacterial infections. *Drug. Discov. Today* 7: 1086–1091.

Thomas OO (1989). Re-examination of the antimicrobial activities of *Xylopia aethiopica*, *Carica papaya*, *Ocimium grastissimum* and *Jatropha curas. Fitoterapia* 60:147–161.

Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T (2001). Mechanism of synergy between Epigallochatechin gallate and *B*-Lactams against methicillin resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemotherapy* 45(6): 1737–1742.

Source of Support: Nil **Conflict of Interest: None Declared**