

A COMPARATIVE EVALUATION OF *IN VITRO* GROWTH INHIBITORY ACTIVITIES OF CRUDE AQUEOUS EXTRACTS OF DIFFERENT PARTS OF *JATROPHA CURCAS* USED SINGLY AND IN COMBINATION WITH OTHER PARTS AGAINST *SALMONELLA TYPHI* AND *ESCHERICHIA COLI*

L. A. OSENI^{1,*} AND B. YUSSIF¹

¹Department of Applied chemistry & Biochemistry, University For Development Studies, Navrongo Campus, P.O. Box 24, Navrongo, U/E, Ghana.
Email: lateefoseni@yahoo.com

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ABSTRACT

The persistent resistance of most disease causing pathogens including *Salmonella typhi* and *Escherichia coli* to synthetic drugs poses a major concern to the general public. This has drawn the attention of the scientific community to traditional medicines prepared from plant products. *Jatropha curcas* has been used in the treatment of ailments related to bacterial infections in traditional medical practices. Reports have shown that the aqueous extracts of *J. curcas* possess little antibacterial activity. The present work was to evaluate the antibacterial activities of aqueous extracts of the following combined parts of *J. curcas*; stem bark-root, stem bark-leaf, root-leaf and stem bark-root-leaf against *Salmonella typhi* and *Escherichia coli*. The results of the combinations were compared with those of the plant parts used singly. Preliminary phytochemical screening revealed the presence of some bioactive principles in extracts of the various combinations as well as in the single plant parts. Antibacterial susceptibility assay showed that the various extracts possess antibacterial activity at a concentration of 50mg/ml. The antibacterial activities of the various combinations however, were not significantly different from those of the single plant parts. We conclude that extracts obtained from combining various parts of the *J. curcas* does not seem to demonstrate synergistic effects against the test organisms.

Keywords: *Jatropha curcas*, Combination therapy, Antibacterial assay, Phytochemistry

INTRODUCTION

People on all continents have used hundreds of thousands of indigenous plants for treatment of ailments since prehistoric times. The use of herbs to treat diseases is almost universal among non-industrialized societies. A number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century: In addition to the use in the developing world, herbal medicine is used in industrialized nations by alternative medicine practitioners such as naturopaths¹. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are in constant search for phytochemicals and lead compounds that could be developed for treatment of various diseases. Thus, plants around us can be investigated for the purpose of identifying those that may be potent against infectious organisms and hence useful in treating ailments caused by pathogens².

The therapeutic activity of medicinal plants could be explained on the basis of the phytochemicals they produce as part of their normal metabolic activities. These phytochemicals are secondary metabolites such as tannins, flavonoids, alkaloids and saponins among others. The functions of secondary metabolites are varied. In addition, the different parts of a plant may contain varied phytochemical constituents and thus may be of different medicinal values.

In many traditions, different medicinal plant parts are either used singly or in combination with other parts of the same plant or of different plants in the treatment of various diseases. Several reports have shown the advantages associated with combining different parts of the same plant or of different plants in the treatment of ailments. The concept of combination of plants in herbal therapy may be beneficial when the individual plants or plant parts in the concoction possess different efficacies that provide additive or synergistic effects. It may also reduce the required doses of the individual components compared with single-component herbal therapy and limit side effects.

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceae. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. Common names include Barbados Nut, Purging Nut, and Physic Nut. *J. curcas* is semi-evergreen shrub or small tree, which grows up to a

height of 6m (20 ft). It also grows in deserts due to its resistance to a high degree of aridity.

J. curcas has several uses in folkloric medical settings including treatment of gonorrhoea, diarrhoea, convulsions, dyspepsia, antihelmintics and dressing for wounds and sores³. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, guinea worm sores and joint rheumatism^{4,5}. Fagbenro-Beyoku et al.⁶ investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity⁷. Studies have also shown that crude ethanol extracts of the stem, root and leaves of *J. curcas* possess antibacterial activity². Several reports have also shown that aqueous extracts of the plant generally showed little antibacterial activities^{8,9,10,11}.

To the best of our knowledge, the antimicrobial activity of extracts obtained from a combination of different parts of *J. curcas* has not been reported. These combinations may yield an improved inhibitory activity against known microbes. Thus the aim of the present study was to evaluate the antibacterial activities of crude aqueous extracts of the following combinations; stem-leaf, stem-root, leaf-root and stem-leaf-root against *Salmonella typhi* and *Escherichia coli*. The antibacterial activities of these combinations were compared with those of the plant parts used singly.

MATERIAL AND METHODS

Reagents

All reagents used were of analytical grade unless otherwise stated and were purchased from Sigma-Aldrich.

Plant material

Samples of the fresh stem bark, root and leaves of *Jatropha curcas* were collected from Navrongo area of Upper East region of Ghana. The samples were identified and authenticated at Department of Applied Biology, University for Development Studies, Navrongo campus.

Preparation of extracts

About 200g each of the powdered stem bark, leaves and roots were separately cold extracted in distilled water for five days. About 100g

each of the stem bark, root and leaves were used in the preparation of the following combinations; 200g stem bark-root, 200g stem bark-leaf, 200g root-leaf and 300g stem bark-root-leaf. These combinations were separately cold extracted in distilled water for five days. The extracts were then separately filtered through Whatman's No. 1 filter paper and the filtrates were concentrated to dryness *in vacuo* using a rotary evaporator to remove the solvents.

Test for phytochemicals

Test for the phytochemical were carried out using standard procedures^{12,13} with little modification.

Test for flavonoids

About 1ml of the extract was measured into a test tube and 3 drops of NaOH was added. An intense yellow color was observed in the test tube which gradually became colorless upon adding few drops of dilute HCl indicating the presence of flavonoids.

Test for alkaloids

About 1ml of the extract was measured into a test tube and few drops of 1% HCl were added on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with a drop of Mayer's reagent. The extract became turbid indicating the presence of alkaloid.

Test for tannin

About 0.5ml of the extract was heated in a steam bath for about 5minutes and 2 drops of 5% FeCl₃ was added. A greenish precipitate indicates the presence of tannins.

Test for terpenoid

About 5ml of the extract was measured into a separating funnel and 3 drops of diethyl ether added and shaken gently. It was allowed to stand for about 10minutes and then evaporated to dryness. The residue was dissolved in 0.5ml acetic anhydride and then 0.5ml chloroform was added. The solution was transferred into a dry test tube and 2ml of concentrated sulphuric acid added and shaken gently.

Test for cynogenic glycosides

About 2ml of the extract was measured with a pipette and transferred into a test tube. 1ml of chloroform was added and a piece of picric acid paper was inserted into the test tube just above the extract. The test tube was stopped and warmed at about 35°C in a water bath for about 30 minutes. The picric paper changed from yellow to different shades of red which indicated the presence of cynogenic glycosides

Test for saponin

About 2ml of the extract was measured into a test tube and shaken vigorously. A honeycomb like foam was formed and persisted for about 30 minutes indicating the presence of saponin in the extract.

Test for reducing sugars

About 0.5ml each of Fehling's solution A and B measured into a test tube and 0.5ml of the extract was added. The solution was heated in a water bath. Development of brick red precipitate was an indication of the presence of reducing sugars.

Test for Phytosterol

About 5ml of the extract was measured into a separating funnel and 3 drops of diethyl ether was added and shaken gently. It was allowed to stand for about 10 minutes and then evaporated to dryness. The residue was dissolved in 0.5ml acetic anhydride and then 0.5ml chloroform was added. The solution was transferred into a dry test tube and 2ml of concentrated sulphuric acid was added and shaken gently.

Test for Steroids

About 2 ml of acetic anhydride was added to 0.5 g of the extract followed by the addition of 2 ml of H₂SO₄. The change in color from

violet to blue or green in the extract indicates the presence of steroids.

Bacteriological Analysis

Preparation of media

About 38g of Muller Hinton broth was measured into a beaker and 1000ml of distilled water added. The mixture was then agitated and heated to boil on a hot plate. While, heating the mixture were constantly stirred with a magnetic stirrer until the powder had dissolved completely. The media was autoclaved at 121°C for 15minutes. It was then allowed to cool to about 50°C and stored in a refrigerator for later use.

Bacteria culture

The organisms, *E. coli* and *S. typhi* were cultured in a nutrient agar and incubated at 37°C for 24 hours at the microbiology laboratory of the University For Development Studies.

Antimicrobial susceptibility test

The agar well diffusion method was used according to reported standard procedures (Cruickshank et al¹⁴, Garrod et al¹⁵). The Muller-Hinton agar media was poured in the sterilized petri dishes and allowed to solidify. About 0.1ml each of the *Salmonella typhi* and *Escherichia coli* was spread uniformly over the surface of the Muller-Hinton media with a sterile glass rod spreader. A hole was bored by 5mm cork borer in the middle of each inoculated agar plate. About 0.1ml of each extract (50mg/ml in DMSO) was pipetted into the respective holes. Ciprofloxacin was used as a positive control. Triplicates of plates were allowed to stand for about 1 hour to allow the extracts to diffuse into the media. The plates were then incubated upside down at 37°C for 24 hours. The diameters (mm) of the zones of inhibition were measured from underneath the plates using a pair of dividers and a ruler and their means were also recorded.

RESULTS AND DISCUSSIONS

Phytochemical screening

The extracts obtained from the various plant parts when used alone or in combination with other parts showed varying phytochemical constituents (Table 1).

Alkaloids, tannins and reducing sugars were found to be present in all extracts in significant quantities. This result is consistent with those from previous investigations^{2,16}. Alkaloids and tannins have been implicated as antibacterial compounds in several reports and their presence justifies the use of the plant for treatment of bacterial infections. Alkaloids have been reported extensively for their anticancer activities¹⁷. Tannins are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery¹⁸. Therefore its presence may have a significant effect in the inhibition of the test organisms. Phytosterols and cyanogenic glycosides were absent in all extracts. Saponins were present in all but in ASE. Saponins are a class of compounds that are known to be effective for the treatment of syphilis and other venereal diseases¹⁹. Flavonoids were present only in ALE and ASLE. Terpenoids were present in moderate quantities in ARE but in low amounts in ASRE, ARLE and ASRLE. This result suggests antagonist effect when the root is combined with other parts of the plant. Similarly, Steroids which were found in moderate amounts in ALE were found to be present in low quantities in ASLE, ARLE as well as in ASRLE. This observation also suggests that the stem bark and root may contain compounds capable of masking the steroids in the leaf extract.

Bacteriological analysis

Antibacterial activity assay revealed that the various extracts possess some antibacterial properties (Table 2). The result may be attributed to the presence of the phytochemicals which are bioactive. *Salmonella typhi* was the most susceptible to the extracts with ASLE demonstrating the highest antibacterial activity with zone of inhibition of 12.90mm against *S. typhi*. Of all the extracts, ALE demonstrated the least antibacterial activity (zone of inhibition =

5.80mm) against *E. coli*. This extract contains most of the phytochemicals tested. The ALE also showed a high level of antibacterial activity against *S. typhi*. The result suggests that the compounds found in ALE are very active against *S.typhi* than *E.coli*. None of the combinations demonstrated synergistic antibacterial effects against any of the test organisms.

ARE demonstrated a zone of inhibition of 8.95 mm against *E. coli*. However, the root-leaf combination showed a zone of inhibition of 7.60 mm against the same organism. This result compared to the plant parts used alone (ALE zone of inhibition = 5.60 mm, ARE zone of inhibition = 8.95 mm) reveals that the leaf extract may be

antagonistic to the activity of the root extract against the test organism. Similarly, the root extract (ARE) was antagonistic to the leaf extract (ALE) against *S. typhi*. Combining the stem bark, root and leaf of *J. curcas* in one extraction did not show superior antibacterial activity compared with the individual parts used alone.

Because the plant parts were combined before extraction, very soluble compounds from one plant part in the mixture could saturate the solution of the extract thereby preventing further extraction of active principles from other plant parts in the mixture. This could result in no additive or synergistic effects in extracts of the various combinations as observed in the results.

Table 1: Phytochemical screening of aqueous extracts of *Jatropha Curcas*

Phytochemical constituents	Various extracts of <i>Jatropha curcas</i>						
	ASE	ARE	ALE	ASRE	ASLE	ARLE	ASRLE
Flavonoids	-	-	++	-	+	-	-
Alkaloids	+	++	++	++	++	++	++
Tannin	++	+	++	++	++	++	++
Terpenoid	-	++	-	+	-	+	+
Cyanogenic Glycosides	-	-	-	-	-	-	-
Saponins	++	+++	++	+++	++	++	++
Reducing Sugar	++	++	++	+	+	+	++
Phytosterol	-	-	-	-	-	-	-
Steroids	-	-	++	-	+	+	-

+ = phytochemical present ++ = phytochemical moderately present
 +++ = phytochemical highly present. - = phytochemical absent.
 ASRE= Aqueous stem bark-root extract ASLE = Aqueous stem bark-leaf extract
 ARLE = Aqueous root-leaf extract. ASRLE = Aqueous stem bark-root-leaf extract
 ASE = Aqueous stem bark extract ARE = Aqueous root extract
 ALE = Aqueous leaf extract

Table 2: Efficacy of the aqueous extracts of *Jatropha Curcas* on *Salmonella Typhi* and *Escherichia coli*

Extracts at 50mg/ml	Mean Diameter of zones of inhibition (mm)	
	<i>S. typhi</i>	<i>E. coli</i>
ASE	8.45	7.75
ARE	9.50	8.95
ALE	12.60	5.80
ASRE	10.80	8.50
ASLE	12.90	9.45
ARLE	9.00	7.60
ASRLE	9.60	7.90
DMSO	0.00	0.00
Ciprofloxacin (10mg/ml)	15.50	13.00

ASRE= Aqueous stem bark-root extract ASLE = Aqueous stem bark-leaf extract
 ARLE = Aqueous root-leaf extract ASRLE = Aqueous stem bark-root-leaf extract
 ASE = Aqueous stem bark extract ARE = Aqueous root extract
 ALE = Aqueous leaf extract

CONCLUSION

The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial remedies ²⁰. From time immemorial, it has been observed and thoroughly demonstrated that phytochemicals from various plants and plant parts, when treated as mixtures, exhibits augmented/ suppressed biological activities, under *in vitro* conditions ²¹. Scientists are in constant search for novel antibacterial remedies from medicinal plants. Extracts prepared from various parts of *Jatropha curcas* at concentration of 50mg/ml showed varying inhibitory activities against *Salmonella typhi* and *Escherichia coli* as they contained different types and amount of phytochemicals. Aqueous extracts from *J. curcus* have been reported to exhibit low

antibacterial activity. The present study was to evaluate antibacterial activities of stem bark-root aqueous extract, stem bark-leaf aqueous extract, leaf-root aqueous extract and stem bark-root-leaf aqueous extract against *Salmonella typhi* and *Escherichia coli*. Results from the combinations were compared to those of the single parts.

The antibacterial activities of extracts from the various combinations were not significantly different from extracts of the plant parts used alone. Thus the present investigation suggests no synergistic effects in the antibacterial activities of the extracts from the various combinations against the test organisms. We therefore recommend a further investigation to evaluate the antibacterial activities of the various parts of *J. curcas* when their extracts are combined after individual extractions.

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