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Chemical Composition, Antimicrobial Properties and Toxicity of *Jatropha curcas* Provenances from Diverse Origins

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ABSTRACT

This paper discusses probable therapeutic bases of 14 *Jatropha curcas* provenances based on their cytotoxicity, secondary metabolite profile and antimicrobial activity. Ethanolic extracts of *Jatropha* leaves and bark were subjected to antimicrobial and brine shrimp toxicity assays. The India 2 provenance showed a broad range of antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*. The rest of the provenances also revealed varying degrees of antibacterial activity against the three bacteria except for provenances India 1, Phil 6 and *Tubang bakod,* which did not show antimicrobial action. Brine shrimp lethality assay revealed high toxicity levels for the different *Jatropha* extracts. The presence of alkaloids, flavonoids, leucoanthocyanins, saponins, tannins and phenolics was detected through phytochemical analysis. The results of various assays indicate that *Jatropha* provenances have the potential for the development of novel compounds for the treatment of various ailments and infections.

Key Words: *Jatropha curcas*; Phytochemical screening; Brine shrimp assay; Antibacterial assay

INTRODUCTION

Jatropha curcas or physic nut is a non-edible multipurpose shrub belonging to the family *Euphorbiacea*. It is an uncultivated non-food wild-species which grows in tropical and sub-tropical regions of the world (Meng *et al.,* 2009; Saetae & Suntornsuk, 2010). The Greek root *(jatros)* from which the genus name *Jatropha* was derived means 'doctor' implying ancient medical uses of the plant in its centers of origin in Latin America (Das *et al.,* 2010). Different parts of the plant had been used as ethnomedicine in different countries for centuries (Igbinosa *et al.,* 2009). Many studies have been done to demonstrate the efficacy of *J. curcas* against a wide array of bacteria (Oyi *et al.,* 2007; Igbinosa *et al.,* 2009) and fungi (Ayanbimpe & Fagbemi, 2005; Saetae & Suntornsuk, 2010). Results of several studies also revealed that *J. curcas* has anticancer and antitumor properties (Lin *et al.,* 2003; Aiyelaagbe *et al.,* 2007; Oskouelan *et al.,* 2011). Moreover, phytochemical studies done on *Jatropha* revealed the presence of secondary metabolites, which can be explored in the bio-prospecting for new and novel bioactive compounds. One problem, however, that hinders the exploitation of *J. curcas* to its fullest potential is the presence of toxic components in all parts of the plant. In this research, 14 *J. curcas* provenances from the Philippines, Indonesia, China, India and Mexico (including the Mexican non-toxic provenance) were tested for antimicrobial activity and toxicity using brine shrimp assay.

MATERIALS AND METHODS

Plant materials: The study made use of 14 Philippine and foreign *J. curcas* provenances maintained in the *Jatropha* field genebank of the Philippine Coconut Authority in Davao City. Shown below are the provenances used in the various assays:

1. Phil 1
2. Phil 2
3. India 1
4. Phil 3
5. Phil 4
6. India 2
7. Mexico 1
8. China
9. Phil 5
10. Foreign Unknown
11. Mexico 2
12. Indonesia
13. Phil 6
14. Tubang Bakod (Philippines).

Preparation of ethanolic extract: Fresh leaves and bark of 14 *J. curcas* provenances were air-dried, pulverized to fine powder and subjected to ethanolic extraction. After extraction, the ethanol filtrate was filtered and concentrated to dryness through a water bath kept at 60-65% (near boiling point for ethanol) to remove the solvent. The dried filtrate was then re-dissolved in distilled water in preparation for the different bioassays.

Phytochemical screening: The extracts were subjected to

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phytochemical screening to detect the presence of plant secondary metabolites, alkaloids, flavonoids, leucoanthocyanins, saponins, tannins, phenolics and unsaturated sterols following standard procedures (Trease & Evans, 1989; Harborne, 1998).

Antimicrobial screening: Gram negative *Escherichia coli* and gram positive *Bacillus cereus* and *Staphylococcus aureus* obtained from the biology laboratory of Mindanao State University were cultured on test tube NA slants at 36°C and incubated for 24 h. About 1ml of distilled water was added per test tube to make bacterial inoculums (10^{8-9} CFU/mL). Commercially prepared antibiotics, benzylpenicillin and streptomycin, at concentrations of 5 mg/mL, were used as reference standards. The antimicrobial assay was done using the agar disc diffusion method (Bauer *et al*., 1996). 1 mL suspension of each inoculum was poured in sterile petri plates. About 12-15 mL of pre-cooled agar was introduced and the petri plates were swirled to allow even dispersal of the inoculum. Test discs were made using sterile filter paper discs (6 mm) that were soaked in the *Jatropha* extracts and standard antibiotics until totally saturated. The plates were then incubated upside down for 24 h at 37°C. The zones of inhibition (in mm), which were the areas of clearing emanating from the border of the paper disk were used in the computation of the antimicrobial index (AI). The data on antimicrobial assay were presented in a graph for easy interpretation. Two-factor analysis of variance (ANOVA) was also done to detect significant differences among provenances and bacterial responses.

Brine shrimp toxicity assay: The bioactivity of the extracts was monitored by the brine shrimp lethality test. Brine shrimp eggs were hatched in a tank prepared with artificial sea water under constant aeration and illumination for 48 h. The newly emerged nauplii were then collected from the hatching vessel for bioassay. Ten nauplii were drawn through a pipette and placed in individual test tubes containing 4.5 mL brine solution. For the toxicity assay, 0.5 mL of *Jatropha* extract was added to each tube and the set up was maintained at room temperature under light. The set up was examined closely at 6 h intervals for the number of surviving nauplii. The brine shrimp toxicity data were presented in graphs, where the concentration of the extracts was plotted against % mortality of the *Artemia salina* nauplii. The LC_{50} values, which represent the concentration resulting in 50% mortality of the nauplii were then calculated by graphical interpolation (Vanhaecke *et al.,* 1981).

RESULTS

Phytochemical and antimicrobial screening: Two-factor ANOVA revealed significant differences in the bacterial responses but failed to detect significant differences among provenances at α=0.05. Fig. 1 shows the differential responses of the 14 *J. curcas* provenances against the *B. cereus, S. aureus* and *E. coli*. The India 2 provenance showed a broad range of bactericidal activity regardless of gram reaction. It can also be seen that Phil 2 and India 2 compared favorably with the commercial antibiotic Streptomycin against *E. coli*. The other provenances were ineffective against this gram negative bacterium. As for the antimicrobial activity of provenances Mexico 1, China and Phil 5 against *B. cereus,* this has been highlighted in Figure 1 through the extremely wide zones of inhibition of these three provenances, which contrasted dramatically with that of the antibiotic Benzylpenicillin. Furthermore, the antimicrobial action of Phil 1, Phil 2, Phil 3, Phil 4, Foreign, India 2, Mexico 2 and Indonesia provenances against *B. cereus* ranged from weak to moderate. For the gram positive bacterium *S. aureus*, only provenances Phil 3, India 2 and Phil 4 exhibited appreciable antibacterial action. Provenances India 1, Phil 6 and *Tubang bakod*, meanwhile did not show any antibacterial action against *E. coli*, *S. aureus* and *B. cereus*. For this assay, *B. cereus* was found out to be the most susceptible to *Jatropha* leaf/bark ethanolic extracts, while the gram negative *E. coli* was discovered to be least susceptible. Qualitative chemical analysis (Table I) done on the 14 provenances revealed the presence of alkaloids, flavonoids, leucoanthocyanins, saponins, tannins and phenolics. Tests were negative for unsaturated sterols.

Brine shrimp assay: The results of the brine shrimp assay lend further support to the reported toxicity of *Jatropha* in literature. At 10000 ppm for all provenances, all the brine shrimp larvae used in the triplicate tests died after only 6 h of exposure implying that this concentration is highly toxic to brine shrimp nauplii (Fig. 2). Of the 14 provenances, Mexico 2, India 1 and Phil 4 were found to be least toxic with LC_{50} values of 100 ppm. Provenances India 2 and Mexico 1 proved to be most toxic with 78% mortality at 100 ppm after 6 h of observation. The other provenances also demonstrated varying levels of lethality against *Artemia* nauplii. Mexico 2, the non-toxic provenance from Mexico also showed toxicity against *Artemia* nauplii albeit not as high as the other provenances.

DISCUSSION

The extensive use of *J. curcas* as folkloric medicine in many places around the globe provided the impetus for this research. A very close association between antibacterial action and cytotoxity was discovered in the study. Provenance India 2, which exhibited very high toxicity on brine shrimp nauplii was also very effective against *B. cereus, E. coli* and *S. aureus*. Similarly, Mexico 1, which also registered very high ratings in the toxicity scale also showed very strong antimicrobial action against *B. cereus*. Inversely, India 1, which showed least toxicity in the brine shrimp assay also proved to be ineffective against the three bacteria. The non-toxic provenance from Mexico (Mexico 2) was ineffective against *S. aureus* and *E. coli* and showed slight antibacterial action against *B. cereus*. Phil 4, which also scored low in the toxicity test was moderately

Table I: Phytochemical Screening of Ethanolic Extracts of 14 *Jatropha* Provenances

Sample Number	Alkaloids	Unsaturated Sterols	Flavonoids and Leucoanthocyanins	Saponins	Tannins and Phenolics
Phil 1	Positive	Negative	Positive	Positive	Positive
Phil 2	Positive	Negative	Positive	Positive	Positive
India 1	Positive	Negative	Positive	Positive	Positive
Phil 3	Positive	Negative	Positive	Positive	Positive
Phil 4	Positive	Negative	Positive	Positive	Positive
India 2	Positive	Negative	Positive	Positive	Positive
Mexico 1	Positive	Negative	Positive	Positive	Positive
China	Positive	Negative	Positive	Positive	Positive
Phil 5	Positive	Negative	Positive	Positive	Positive
Foreign Unknown	Positive	Negative	Positive	Positive	Positive
Mexico 2	Positive	Negative	Positive	Positive	Positive
Indonesia	Positive	Negative	Positive	Positive	Positive
Phil 6	Positive	Negative	Positive	Positive	Positive
Tubang Bakod	Positive	Negative	Positive	Positive	Positive

effective against *B. cereus* and *S. aureus* and ineffective against *E. coli*.

The antimicrobial activity and toxicity of the 14 *Jatropha* provenances can be attributed to the presence of secondary metabolites. Alkaloids are found to inhibit the central processes in the cell, a requisite property against pathogens (Likhitwitayawuid *et al.*, 1993). They exhibit toxicity against foreign cells in culture and are widely studied in cancer research. Saponins on the other hand, disrupt the cell wall giving toxic materials free access to the cell. The damage to the cell wall also cause leakages of vital cell constituents that are otherwise stored inside (Oyi *et al.*, 2007). Tannins, whose potent action involves the coagulation of cell wall proteins and inhibition of protein synthesis, also have a wide array of applications (Parekh & Chanda, 2007). These compounds exhibit significant bactericidal and anticancer properties making them potential sources of active principles for the treatment and prevention of cancer (Oyi *et al.*, 2007). Phenolics, flavonoids and leucoanthocyanins act as cytoplasmic poisons with their inhibitory effect on cellular enzymes (Iwu, 1985). Flavonoids are also known for their antimicrobial, cytostatic and anti-inflammatory properties (Hodek *et al.*, 2002). The antimicrobial action of most of the Jatropha provenances can also be explained by the presence of phenolics (Kowalski & Kedzia, 2007; Igbinosa *et al.*, 2007). These compounds behave as acids with high antimicrobial action (Oyi *et al.*, 2007). Aside from their very potent antibacterial action, phenolics also exhibit anticancer, antitumor and cytotoxic properties (Aiyelaagbe *et al.*, 2007; Igbinosa, 2009).

The antimicrobial properties of most of the *Jatropha* provenances studied make them good candidates for bioprospecting for novel antibiotics. The results of the brine shrimp lethality test also suggest the presence of compounds with potential anticancer properties. Mclaughlin and Anderson (1988) reported that results of brine shrimp toxicity tests generally correlate well with cytotoxic and antitumor properties. Proper caution, however, should be exercised in the utilization of *J. curcas* extracts especially at high concentrations because of provenance-specific toxicities.

Fig. 1: Antimicrobial action of *Jatropha* Provenances

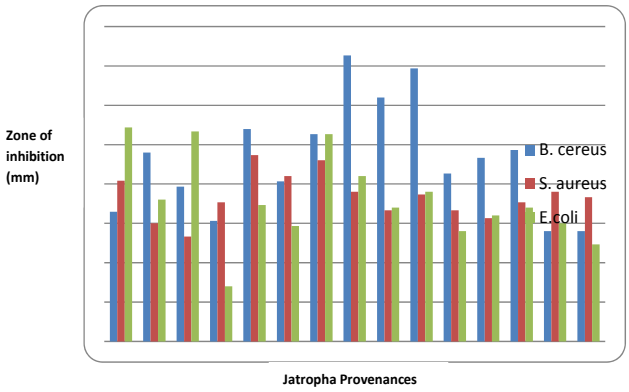
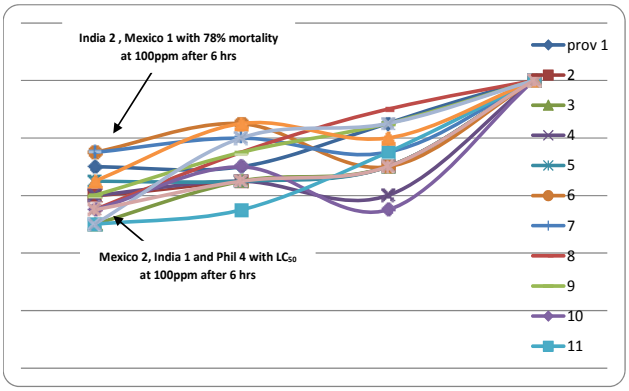


Fig. 2: Toxicity of 14 *Jatropha* Provenances on Brine shrimp larvae after 6 h



CONCLUSION

The results of the study support the folkloric use of *J. curcas* as ethnomedicine and suggest that the plant can be exploited for potent antibiotics and anti-cancer drugs. The detection of different phytochemicals also reveals that the different *Jatropha* provenances constitute a rich but still largely untapped pool of bioactive compounds. More biochemical and pharmaceutical studies are therefore,

warranted to isolate the active components and to test the extracts to a wider range of bacterial, fungal and possibly viral pathogens. The *Jatropha* extracts should also be subjected to quantitative phytochemical analysis so that the results of the antimicrobial and brine shrimp assay can be fully explained. Finally, this study will help in augmenting the knowledge base of the tribal community by integrating scientific findings with indigenous ethno-medical practices.

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