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Jatropha curcas L: Phytochemical, antimicrobial and larvicidal properties

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ABSTRACT

Objective: To evaluate antimicrobial activities as well as the phytochemical and larvicidal properties of different parts of *Jatropha curcas* L. (*J. curcas*) growing in Mauritius.
Methods: Determination of the presence of phytochemicals in the crude plants extracts by test tube reactions. Disc diffusion method and microdilution method were used to detect the antimicrobial sensitivity and activity (minimal inhibitory concentration). The crude solvent extracts were also tested on the larvae of two insects, *Bactrocera zonata* and *Bactrocera cucurbitae* (Diptera, Tephritidae).
Results: The antimicrobial activities were significantly dependent for the different crude plant extracts on the thirteen microorganisms tested. For the Gram-positive bacteria, the crude ethyl acetate extract was more efficient compared to the Gram-negative bacteria with both solvents being effective. The crude ethyl acetate extract of *J. curcas* bark and mature seed oil showed the highest efficacy. The highest mortality percentage was observed after 24 h for both Diptera flies with (66.67 ± 2.89)% of *Bactrocera cucurbitae* larvae killed by ethyl acetate extract of *J. curcas* bark.
Conclusions: This paper compared the different *J. curcas* plant sections with respect to the effectiveness of the plant as a potential candidate for new pharmaceuticals. The larvicidal effect was also studied in order to demonstrate the dual purpose of the plant.

1. Introduction

Plants derivatives have made a large contribution to human health as they have been used as source of preliminary compound of drugs. Widespread usages of drugs have led to the development of pathogen resistance, hence, urging research of new drugs for the treatment of diseases. Active compound present in the medicinal plants provide the bountiful resource of active compounds for the pharmaceutical, cosmetics and food industries, and more recently in agriculture for pest control [1].

Antimicrobial agents are substances that kill microorganisms or inhibit growth of the microorganisms. They are widely employed to cure bacterial diseases. Antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes [2–4].

Jatropha curcas L. (*J. curcas*) plant originated from Mexico and was spread to Asia and Africa by Portuguese traders as a hedge plant and it belongs to the family Euphorbiaceae. In many subtropical and semi-arid regions, traditionally, *J. curcas* is used for its medicinal properties and its seeds contain semi-dry oil which has been found to be useful for medicinal purposes. It has played a major role in the treatment of various diseases including bacterial and fungal infections. The seeds and leaves extracts of *J. curcas*, have shown molluscidal and insecticidal properties [5,6]. The extracts of many *Jatropha* species including *J. curcas* have shown to display potent cytotoxic, anti-tumour and antimicrobial activities in different assays. The latex of *J. curcas* have shown to possess antibacterial activity against *Staphylococcus aureus*

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(*S. aureus*) [6], however, the antimicrobial activity of the other parts have not been fully investigated [7].

The objectives of this study were to investigate the effectiveness of whole plant of *J. curcas* plant against some selected human pathogens which are known to cause diseases and to compare the extent of antimicrobial properties of the different plant sections of *J. curcas*, hence determining the most active part of the plant. Furthermore, to investigate the dual purpose of the plant extracts, the larvicidal effect tests were carried on two chosen *Bactrocera* species namely *Bactrocera zonata* (*B. zonata*) and *Bactrocera cucurbitae* (*B. cucurbitae*).

2. Materials and methods

2.1. Plant material collection and miscellaneous experimental material

Barks, roots and immature, mature and fully mature leaves, pericarps and seeds of *J. curcas* plant (Accession number MAU 26484) were collected in Mauritius for the study. The analytical grade extraction solvents, media, standard antibiotics (Oxoid®), microplates (Merck®), Whatman filter papers and sterile Petri-dishes from Sigma® chemicals were used for the different assays.

2.2. Plant and oil extraction methods

The plant crude extracts were obtained using the decoction method after the parts were thoroughly washed under running tap water, dried in shade. A total of 20 g of different plant parts were macerated in 40 mL of ethyl acetate (EA) and methanol (ME) moderately polar solvent systems for 48 h, respectively. The concentration process of the crude extracts were done after filtration on 15–18 mm pore size Whatman filter paper in a ventilated fume cupboard at room temperature and then stored at 4 °C in dark bottles. The Soxhlet distillation steps were carried out for the seed oil extraction after the seeds were reduced to powder from the two different solvent systems and again stored at 4 °C until further use.

2.3. Antibacterial and antifungal susceptibility test (disc diffusion method and microdilution method)

For the disc diffusion antimicrobial tests, the test pathogens (100 µL/plate) were spread on Muller Hinton agar plates. Sterile paper discs of (6 mm diameter, 0.09 mm thickness, 5 discs/plate) were aseptically transferred on agar plates and were then soaked with equal volume 5 µL of 0.2 g/mL (w/v) fixed concentration of crude plant extracts and seed oil. The zone of inhibition was measured (in mm diameter) after the plates were incubated at 37 °C for 24–48 h. The pathogens were also tested with both a standard antibiotic tetracycline (30 µg as positive control) and negative controls (solvents – EA and ME). The sterile discs were also solely tested on a sterile plate of Muller Hinton agar.

To determine the minimum inhibitory concentration (MIC) antibacterial activity of fractionated plant crude extracts and seed oil, the serial microdilution method was used. A total of 100 µL of all tested pathogenic bacterial strains was diluted two-fold with 100 µL sterile distilled water in a sterile 96-well microplate. EA and ME solvents were used as negative control and two-fold dilution of tetracycline (30 µg/mL) was also used as positive control against each bacterium. After 24 h the MIC of the extracts inhibiting total bacterial growth was noted for the different microorganisms on the plates which were incubated at 37 °C. Bacterial growth was assayed with the addition of 40 µL of 0.2 mg/mL iodonitrotetrazolium violet to each well, and then incubated again at 37 °C for 30 min. The experiment included negative controls the solvents ME and EA, and a positive control (tetracycline antibiotic). The plating for each microorganism was done in triplicate.

For the assays, sub-cultures of six Gram-positive [*Bacillus algicola* Acc. 13/5 (*B. algicola*), *Bacillus cereus* ATCC 11778 (*B. cereus*), *Listeria innocua* ATCC 33090 (*L. innocua*), *S. aureus* ATCC 29213, *Staphylococcus epidermis* ATCC 12228 (*S. epidermis*), *Viridibacillus arenosi* LMG 22166 (*V. arenosi*)], six Gram-negative [*Escherichia coli* ATCC 25922 (*E. coli* ATCC 25922), *Escherichia coli* 0145:H28 Acc. No. CP006027.1 (*E. coli* 0145:H28 Acc. No. CP006027.1), *Klebsiella oxytoca* ATCC 43086 (*K. oxytoca*), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Salmonella typhimurium* ATCC 14028 (*S. typhimurium*)] and one fungus [*Candida albicans* ATCC 1023 (*C. albicans*)] were done on sterile nutrient agar plates. After incubation of the bacterium strain in 10 mL of Mueller Hinton broth overnight at 37 °C for 24–48 h, the cultures were standardised to an absorbance of 0.4–0.6 at 600 nm.

2.4. Larvae mortality assays

The experiment was set in a completely randomised manner with 3 replicates of 20 larvae per treatment (food with extracts) and controls (food without extracts, and the two solvents) in sterile Petri-dishes (9-cm diameter) and number of dead larvae per Petri-dishes were recorded after 24, 48 and 72 h incubation period. Three-day-old larvae of *B. cucurbitae* and *B. zonata* were provided by the Entomology Division of the Ministry of Agro-Industry and Food Security. The crude extracts from different plant parts of ME and EA were tested for their effect on the larvae by spraying the natural food formulation with three different concentrations [200, 400 and 800 mg/L (w/v) of plant crude and oil extracts].

2.5. Qualitative screening of the phytochemicals

The screening of secondary metabolites such as flavonoids, alkaloids, saponins, steroids, tannins, coumarins and phenols based on a series of test tube tests.

2.6. Experimentation statistical analysis

Minitab® 17.2.1, NCSS® 10.0 and Microsoft Excel 2010 were used for the statistical, tables and graphs analyses. The antimicrobial assays data were expressed as mean ± SD with One/Two-way ANOVA at 5% and least significant difference test compared the differences between the means. For the mortality, log-probit was used to calculate LC_{50}-lethal concentrations that kill 50% of the treated larvae and χ^2 test to compare the number of death recorded between the different extracts at the three tested concentrations.

3. Results

3.1. Antifungal activity of crude solvent extracts of different parts of local J. curcas plant

The different parts of *J. curcas* plant crude solvent extracts inhibited significantly ($P < 0.05$) the growth of *C. albicans* with varying degrees of effectiveness as compared to the control with zone of inhibition values ranging from (8.40 ± 0.55) to (12.60 ± 1.52) mm. The results also showed that the crude EA extracts of immature and mature leaves prevented the growth of the fungus and its effect was only moderate. The following zone of inhibition results were observed for the both the crude EA mature seed oil [(10.60 ± 1.14) mm] and ME immature [(11.20 ± 0.84) mm] and mature [(10.60 ± 0.89) mm] leaves, root ME [(10.20 ± 1.10) mm] and immature pericarps ME [(11.20 ± 1.64) mm] extracts. The remaining extracts also reduced the fungus growth with zone of inhibition less than 10 mm (Figure 1).

3.2. Antibacterial effect of crude solvent extracts of the different parts of the local J. curcas plant

Twelve bacterial stains were used to screen the possible antimicrobial activities of the different parts of *J. curcas* plant crude solvent extracts. The intensity of antimicrobial activity depends on the plant extract, and tested microorganisms. It was observed that tested plants exhibited better antibacterial activities than antifungal ones as higher zones of inhibition were observed for the different extracts of *J. curcas*. The antibacterial activity of *J. curcas* extracts was statistically highly significant ($P < 0.01$) and the solvent extracts inhibited the growth of both Gram-positive and the Gram-negative bacteria (Figure 2).

The crude EA extracts proved to be a better solvent for the Gram-positive bacteria while for Gram-negative bacteria both solvents were effective. The most significant extracts were the crude EA of *J. curcas* bark and mature seed oil against *S. aureus*, *B. algicola* and *E. coli* 0145:H28 Acc. No. CP006027.1 with zone of inhibition of (23.40 ± 2.19), (20.40 ± 0.55) and (17.60 ± 0.55) mm, respectively. Gram-positive bacteria *B. algicola*, *B. cereus*, *L. innocua* and *S. aureus* were also susceptible to the following crude EA extracts of the immature and mature leaves, immature and mature seed oil and root with moderate inhibition zones lying between 13.00 and 14.60 mm. Methanol crude extracts of fully mature leaves and seed oil, immature leaves, seeds oil and pericarps, mature leaves pericarps, bark and root had a moderate antibacterial effect on Gram-negative bacteria *E. coli* ATCC 25922, *E. coli* 0145:H28 Acc. No. CP006027.1, *K. oxytoca*, *P. mirabilis*, *P. aeruginosa*, and *S. typhimurium* with zone of inhibition values ranging from 12.00 mm to 14.60 mm (Figure 2).

3.3. MIC assays

The antibacterial activity of the different parts of *J. curcas* was determined against both Gram-negative and Gram-positive bacteria and a fungus. The summarized results showed that the tested extracts displayed significant ($P < 0.05$) selective antibacterial activities (Tables 1 and 2). Both ME and EA extracts

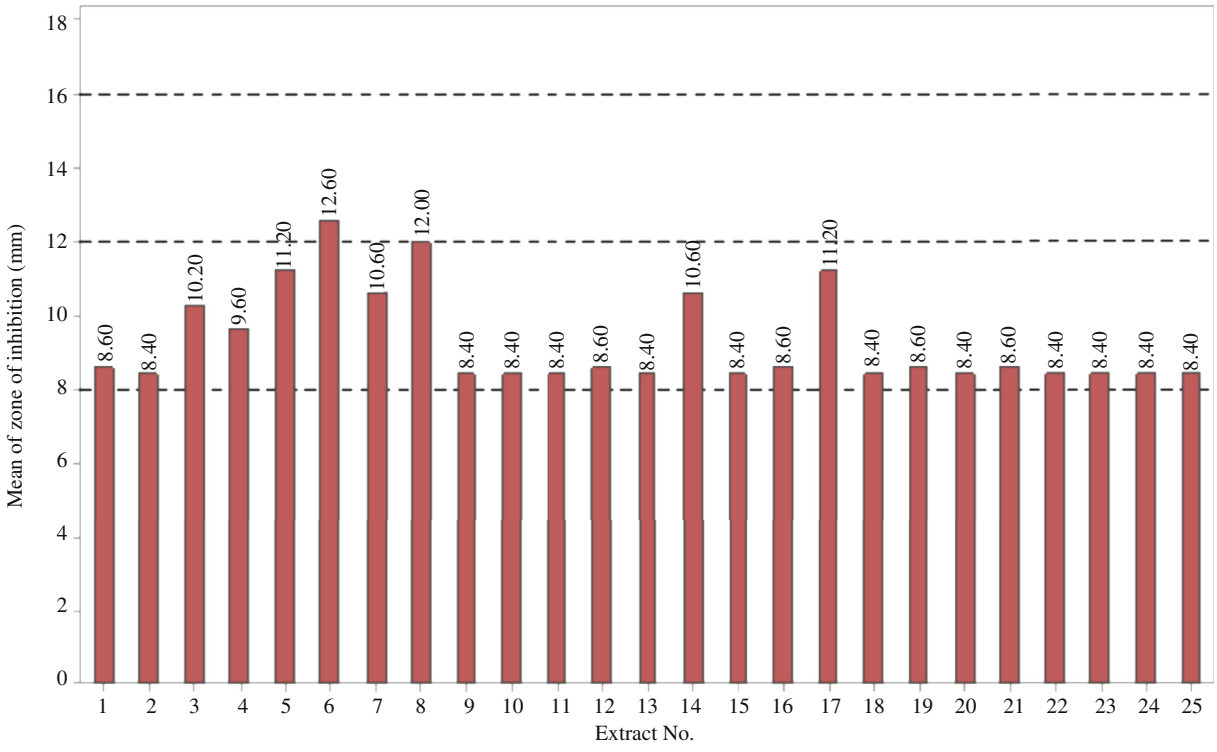

Figure 1. Antifungal effect of *J. curcas* extracts on *C. albicans*.
1: Bark ME; 2: Bark EA; 3: Root ME; 4: Root EA; 5: Immature leaves ME; 6: Immature leaves EA; 7: Mature leaves ME; 8: Mature leaves EA; 9: Fully mature leaves ME; 10: Fully mature leaves EA; 11: Immature seed oil ME; 12: Immature seed oil; EA 13: Mature seed oil ME; 14: Mature seed oil EA; 15: Fully mature seed oil ME; 16: Fully mature seed oil EA; 17: Immature pericarp ME; 18: Immature pericarp EA; 19: Mature pericarp ME; 20: Mature pericarp EA; 21: Fully mature pericarp ME; 22: Fully mature pericarp EA; 23: Negative control-ME; 24: Negative control-EA; 25: Positive control-tetracycline; T: Tetracycline; NC: Negative control; PC: Positive control.

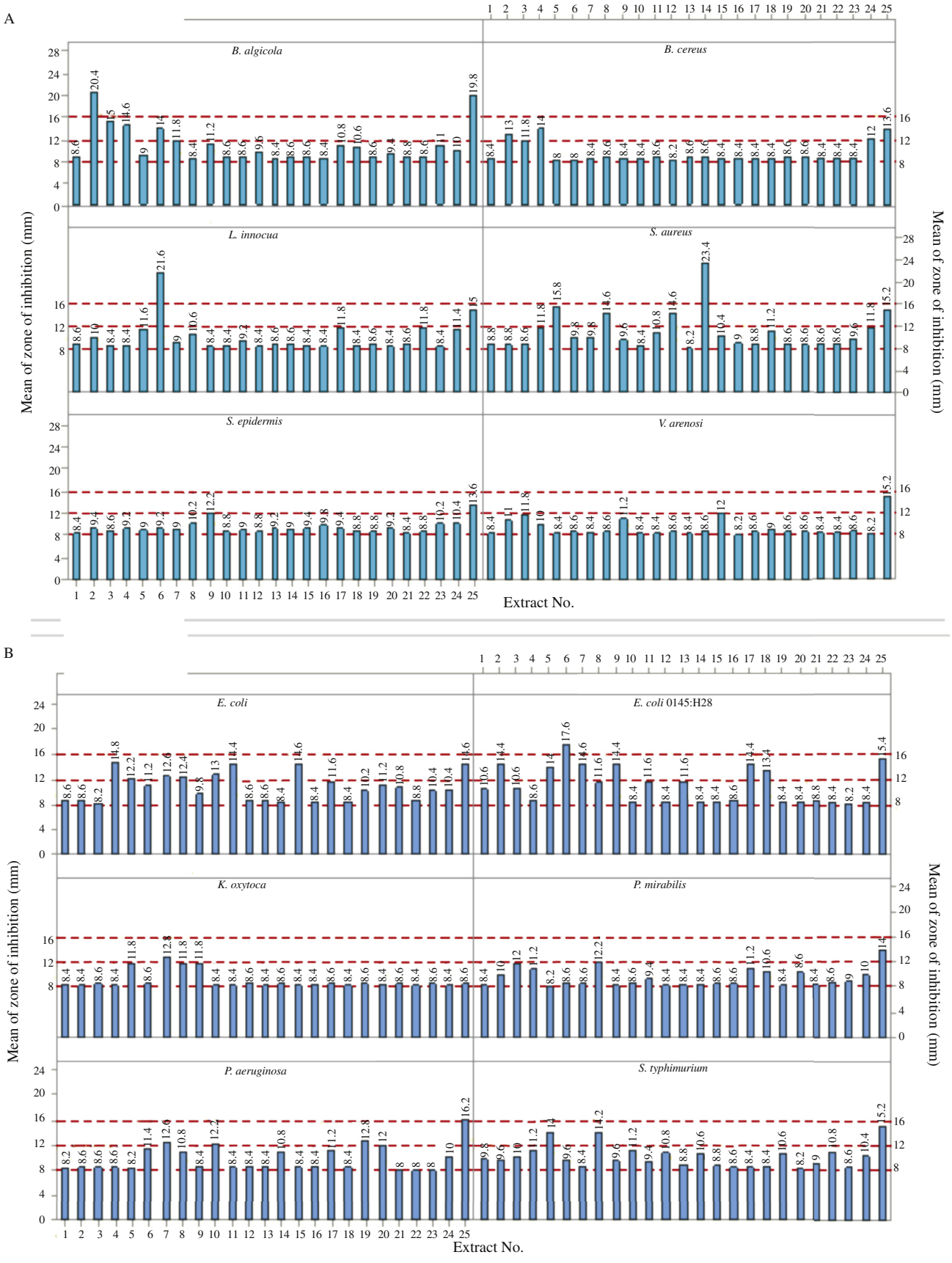

Figure 2. Antibacterial effect of *J. curcas* crude solvent extracts against both Gram-positive (A) and Gram-negative (B) bacteria.
1: Bark ME; 2: Bark EA; 3: Root ME; 4: Root EA; 5: Immature leaves ME; 6: Immature leaves EA; 7: Mature leaves ME; 8: Mature leaves EA; 9: Fully mature leaves ME; 10: Fully mature leaves EA; 11: Immature seed oil ME; 12: Immature seed oil EA; 13: Mature seed oil ME; 14: Mature seed oil EA; 15: Fully mature seed oil ME; 16: Fully mature seed oil EA; 17: Immature pericarp ME; 18: Immature pericarp EA; 19: Mature pericarp ME; 20: Mature pericarp EA; 21: Fully mature pericarp ME; 22: Fully mature pericarp EA; 23: Negative control-ME; 24: Negative control-EA; 25: Positive control-tetracycline; T: Tetracycline; NC: Negative control; PC: Positive control.

Table 1

Mean MIC of the aerial and roots parts of *J. curcas* L. plants crude and oil solvent extracts for the Gram-positive strains. µg/mL.

Extracts	*B. algicola*	*B. cereus*	*L. innocua*	*S. aureus*	*S. epidermis*	*V. arenosi*
Barks EA	4.9 ± 2.0^{f}	10.51 ± 6.4^{c}	83.3 ± 28.9	100.0 ± 0.0^{a}	21.5 ± 6.1	100.0 ± 0.0
Barks ME	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	75.0 ± 43.3	83.3 ± 23.9^{ab}	41.7 ± 14.4	58.3 ± 38.2
Fully mature leaves EA	100.0 ± 0.0^{a}	75.0 ± 73.3^{a}	52.4 ± 46.4	12.1 ± 4.2^{ef}	100.0 ± 0.0	100.0 ± 0.0
Fully mature leaves ME	50.0 ± 0.0^{cd}	58.3 ± 38.2^{abc}	23.8 ± 22.9	41.7 ± 14.4^{cd}	14.5 ± 0.0	10.4 ± 12.6
Fully mature pericarps EA	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	50.0 ± 0.0	33.3 ± 14.4^{de}	100.0 ± 0.0	100.0 ± 0.0
Fully mature pericarps ME	100.0 ± 0.0^{a}	83.3 ± 28.9^{a}	50.0 ± 43.3	66.7 ± 28.9^{bc}	100.0 ± 0.0	83.3 ± 28.9
Fully mature seeds oil EA	100.0 ± 0.0^{a}	75.0 ± 43.3^{a}	33.3 ± 14.4	100.0 ± 0.0^{a}	83.3 ± 28.9	41.8 ± 51.9
Fully mature seeds oil ME	83.3 ± 23.9^{ab}	83.3 ± 28.9^{a}	66.7 ± 28.9	3.8 ± 0.0^{f}	66.7 ± 28.9	71.4 ± 49.5
Immature leaves EA	9.6 ± 4.2^{f}	83.3 ± 28.9^{a}	17.8 ± 6.2	41.7 ± 14.4^{cd}	7.2 ± 0.0	50.0 ± 43.3
Immature leaves ME	83.3 ± 28.9^{ab}	50.0 ± 0.0^{abc}	26.1 ± 20.7	33.3 ± 14.4^{de}	9.6 ± 4.2	21.4 ± 6.2
Immature pericarps EA	41.7 ± 14.4^{de}	66.7 ± 28.9^{ab}	66.7 ± 28.9	41.7 ± 14.4^{cd}	21.5 ± 24.7	83.3 ± 28.9
Immature pericarps ME	66.7 ± 28.9^{bc}	75.0 ± 43.3^{a}	50.0 ± 43.3	33.3 ± 14.4^{de}	4.9 ± 2.0	33.5 ± 28.6
Immature seeds soil EA	100.0 ± 0.0^{a}	53.3 ± 45.1^{abc}	75.0 ± 43.3	83.3 ± 23.9^{ab}	83.3 ± 28.9	75.0 ± 43.3
Immature seeds soil ME	100.0 ± 0.0^{a}	66.7 ± 58.9^{ab}	66.7 ± 28.9	7.2 ± 0.0^{ef}	100.0 ± 0.0	20.0 ± 8.7
Mature leaves EA	100.0 ± 0.0^{a}	75.0 ± 43.3^{a}	66.7 ± 28.9	3.8 ± 0.0^{f}	33.3 ± 14.4	9.5 ± 4.1
Mature leaves ME	21.4 ± 6.2^{ef}	75.0 ± 43.3^{a}	41.7 ± 14.4	6.1 ± 2.0^{ef}	3.8 ± 0.0	17.8 ± 6.2
Mature pericarps EA	100.0 ± 0.0^{a}	75.0 ± 43.3^{a}	66.7 ± 28.9	21.5 ± 6.1^{def}	83.3 ± 28.9	58.3 ± 38.2
Mature pericarps ME	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	75.0 ± 43.3	41.7 ± 14.4^{cd}	100.0 ± 0.0	33.3 ± 14.4
Mature seeds soil EA	66.7 ± 28.9^{bc}	83.3 ± 28.9^{a}	66.7 ± 28.9	83.3 ± 23.9^{ab}	100.0 ± 0.0	70.0 ± 52.0
Mature seeds soil ME	66.7 ± 28.9^{bc}	66.7 ± 28.9^{ab}	66.7 ± 28.9	83.3 ± 23.9^{ab}	25.0 ± 0.0	46.4 ± 14.2
Negative control-EA	33.3 ± 14.5	58.3 ± 38.2	75.0 ± 43.3	33.3 ± 14.4^{de}	100.0 ± 0.0	83.3 ± 28.9
Negative control-ME	21.5 ± 6.1	66.7 ± 28.9	83.3 ± 28.9	21.5 ± 6.1	100.0 ± 0.0	66.7 ± 28.9
Positive control-Tetracycline	6.1 ± 2.0	50.7 ± 64.8	83.3 ± 28.9	7.2 ± 0.0	9.6 ± 4.2	100.0 ± 0.0
Roots EA	21.5 ± 6.1^{ef}	10.5 ± 6.4^{c}	41.7 ± 14.4	6.1 ± 2.0^{ef}	100.0 ± 0.0	46.4 ± 46.7
Roots ME	3.8 ± 0.0^{f}	17.8 ± 6.2^{bc}	66.7 ± 28.9	66.7 ± 28.9^{bc}	29.8 ± 18.2	75.0 ± 43.3

One-way ANOVA with least significant difference at 5%, $n = 3$; Values were expressed as mean ± SD; Means with the same letter are significantly.

inhibited the growth of the microorganisms. The MIC values for the Gram-positive and Gram-negative bacteria were between 3.75 µg/mL and 100.00 µg/mL and *C. albicans* between 17.80 µg/mL and 83.30 µg/mL. The very high values for several extracts indicated limited antibacterial efficacy.

The best activity was recorded with crude solvent extracts with the immature parts of *J. curcas* plants with MIC values ranging from 4.17 µg/mL to 21.50 µg/mL against 77% tested bacteria. MIC values below or equal to 10 µg/mL were also recorded for several extracts against *B. algicola* ATCC No. 13/5,

Table 2

Mean MIC of the aerial and roots parts of *J. curcas* L. crude and oil solvent extracts for the Gram-negative strains and one fungus. µg/mL.

Extracts	ECA	EC0	*K. oxytoca*	*P. mirabilis*	*P. aeruginosa*	*S. typhimurium*	*C. albicans*
Barks EA	41.7 ± 14.4	21.4 ± 6.2	41.7 ± 14.4	66.7 ± 28.9	83.3 ± 28.9	66.7 ± 28.9	33.3 ± 14.4
Barks ME	100.0 ± 0.0	75.0 ± 43.3	83.3 ± 28.9	33.3 ± 14.4	83.3 ± 28.9	83.3 ± 28.9	100.0 ± 0.0
Fully mature leaves EA	100.0 ± 0.0	83.3 ± 28.9	17.8 ± 6.2	17.8 ± 6.2	100.0 ± 0.0	26.3 ± 20.5	83.3 ± 28.9
Fully mature leaves ME	75.0 ± 43.3	50.0 ± 0.0	83.3 ± 28.9	50.0 ± 43.3	41.7 ± 14.4	27.4 ± 21.5	75.0 ± 43.3
Fully mature pericarps EA	100.0 ± 0.0	100.0 ± 0.0	66.7 ± 28.9	33.3 ± 14.4	100.0 ± 0.0	100.0 ± 0.0	66.7 ± 28.9
Fully mature pericarps ME	100.0 ± 0.0	100.0 ± 0.0	83.3 ± 28.9	50.0 ± 43.3	100.0 ± 0.0	100.0 ± 0.0	66.7 ± 28.9
Fully mature seeds oil EA	21.5 ± 6.1	75.0 ± 43.3	100.0 ± 0.0	83.3 ± 28.9	100.0 ± 0.0	18.0 ± 6.1	83.3 ± 28.9
Fully mature seeds oil ME	100.0 ± 0.0	58.3 ± 38.2	50.0 ± 0.0	83.3 ± 28.9	33.3 ± 14.4	19.1 ± 10.3	25.0 ± 0.0
Immature leaves EA	66.7 ± 28.9	66.7 ± 28.9	21.4 ± 6.2	29.7 ± 18.4	41.7 ± 14.4	75.0 ± 43.3	71.5 ± 49.4
Immature leaves ME	21.5 ± 6.1	83.3 ± 28.9	21.4 ± 6.2	5.9 ± 2.4	4.2 ± 2.9	83.3 ± 28.9	71.5 ± 49.4
Immature pericarps EA	83.3 ± 28.9	75.0 ± 43.3	50.0 ± 0.0	66.7 ± 28.9	21.5 ± 6.1	50.0 ± 0.0	75.0 ± 43.3
Immature pericarps ME	33.3 ± 14.4	29.7 ± 18.4	50.0 ± 43.3	75.0 ± 43.3	100.0 ± 0.0	83.3 ± 28.9	58.3 ± 38.2
Immature seeds soil EA	83.3 ± 28.9	66.7 ± 28.9	25.0 ± 0.0	29.7 ± 18.4	100.0 ± 0.0	50.0 ± 0.0	41.7 ± 14.4
Immature seeds soil ME	43.0 ± 49.4	83.3 ± 28.9	100.0 ± 0.0	41.7 ± 14.4	6.1 ± 2.0	66.7 ± 28.9	83.3 ± 28.9
Mature leaves EA	50.0 ± 43.3	17.8 ± 6.2	50.0 ± 0.0	5.8 ± 2.3	4.5 ± 2.4	41.7 ± 14.4	66.7 ± 28.9
Mature leaves ME	75.0 ± 43.3	75.0 ± 43.3	11.9 ± 4.0	83.3 ± 28.9	19.1 ± 10.3	100.0 ± 0.0	83.3 ± 28.9
Mature pericarps EA	50.0 ± 43.3	33.3 ± 14.4	21.4 ± 6.2	50.0 ± 0.0	100.0 ± 0.0	33.3 ± 14.4	100.0 ± 0.0
Mature pericarps ME	66.7 ± 28.9	58.3 ± 38.2	10.5 ± 6.4	50.0 ± 43.3	66.7 ± 28.9	100.0 ± 0.0	41.7 ± 14.4
Mature seeds soil EA	43.0 ± 49.4	83.3 ± 29.9	50.0 ± 43.3	25.0 ± 0.0	3.8 ± 0.0	58.3 ± 38.2	58.3 ± 38.2
Mature seeds soil ME	100.0 ± 0.0	50.0 ± 0.0	66.7 ± 28.9	38.1 ± 20.7	83.3 ± 28.9	41.7 ± 14.4	58.3 ± 38.2
Negative control-EA	66.7 ± 28.9	66.7 ± 28.9	25.0 ± 0.0	100.0 ± 0.0	21.5 ± 6.1	50.0 ± 0.0	41.7 ± 14.4
Negative control-ME	100.0 ± 0.0	66.7 ± 28.9	41.7 ± 14.4	25.0 ± 0.0	14.5 ± 0.0	50.0 ± 0.0	83.3 ± 28.9
Positive control-Tetracycline	26.3 ± 20.5	17.8 ± 6.2	6.8 ± 6.4	17.8 ± 6.2	12.2 ± 4.0	66.7 ± 28.9	12.1 ± 4.2
Roots EA	83.3 ± 28.9	21.4 ± 6.2	10.7 ± 12.5	41.7 ± 14.4	19.2 ± 10.1	41.7 ± 14.4	50.0 ± 0.0
Roots ME	100.0 ± 0.0	41.7 ± 14.4	66.7 ± 28.9	33.3 ± 14.4	83.3 ± 28.9	100.0 ± 0.0	21.5 ± 6.1

ECA: *E. coli* ATCC 25922; EC0: *E. coli* 0145:H28 Acc. No. CP006027.1; One-way ANOVA with least significant difference at 5%, $n = 3$; Values were expressed as mean ± SD; Means with the same letter are significantly.

S. aureus, *E. coli* ATCC 25922, *E. coli* 0145:H28 Acc. No. CP006027.1, *K. oxytoca* and *S. typhimurium*. The lowest MIC value was (3.75 ± 0.00) µg/mL observed with both crude EA and ME of root, mature seed oil and leaves extracts against *B. algicola*, *S. aureus*, *E. coli* 0145:H28 Acc. No. CP006027.1 and *E. coli* ATCC 25922. The ME extracts exhibited stronger and broader spectrum of action compared to EA extracts and negative and positive controls. The Gram-negative strains were the most sensitive with MIC values up to 100.00 µg/mL (Tables 1 and 2).

3.4. Mortality bioassays

Twenty-two extracts from the different parts of *J. curcas* were evaluated for their larvicidal potencies against *B. cucurbitae* (melon fruit fly larvae) and *B. zonata* (peach fly larvae). The mortality (LD_{50}) bioassays for two Diptera species, were significant ($P < 0.05$) for the crude solvent extracts of *J. curcas* plants. The calculated LD_{50} values for *B. cucurbitae* and *B. zonata* larvae were between (290 ± 5) and (1 770 ± 20) mg/L, and (180 ± 5) and (3 290 ± 100) mg/L, respectively (Table 3). The lowest LD_{50} determined was for crude ME fully mature leaves crude ME extract and the highest was for the bark ME extract.

Table 3

LC_{50} of *J. curcas* crude solvent extracts against Diptera fruit flies. mg/L.

Extracts	*B. cucurbitae*		*B. zonata*	
	EA	ME	EA	ME
Bark	510 ± 20	510 ± 60	2360 ± 100	3290 ± 100
Fully mature leaves	630 ± 10	970 ± 20	350 ± 20	180 ± 5
Fully mature pericarp	1480 ± 10	290 ± 5	1260 ± 10	920 ± 30
Fully mature seed oil	930 ± 20	410 ± 10	610 ± 10	500 ± 10
Mature leaves	1770 ± 20	700 ± 20	470 ± 10	750 ± 25
Mature pericarp	700 ± 10	570 ± 10	350 ± 5	460 ± 10
Mature seed oil	470 ± 10	470 ± 10	740 ± 10	870 ± 20
Immature leaves	1580 ± 10	600 ± 50	1240 ± 40	340 ± 10
Immature pericarp	700 ± 20	1030 ± 50	240 ± 5	2150 ± 200
Immature seed oil	540 ± 10	1050 ± 50	1090 ± 40	1110 ± 45
Roots	880 ± 20	820 ± 20	570 ± 10	1530 ± 55

The tested solvent plant extracts exerted promising significant larvicidal activity ($P < 0.05$) after 24 h, 48 h and 72 h (Figure 3) periods of the different concentration (200, 400 and 800 mg/L) crude extracts exposure. The highest mortality

Figure 3. Anti-insecticidal activity of crude solvent extracts of different *J. curcas* parts on larvae of *B. cucurbitae* and *B. zonata* at the concentration of 200, 400 and 800 mg/L for 24 h, 48 h and 72 h.

1: Bark ME; 2: Bark EA; 3: Root ME; 4: Root EA; 5: Immature leaves ME; 6: Immature leaves EA; 7: Mature leaves ME; 8: Mature leaves EA; 9: Fully mature leaves ME; 10: Fully mature leaves EA; 11: Immature seed oil ME; 12: Immature seed oil; EA 13: Mature seed oil ME; 14: Mature seed oil EA; 15: Fully mature seed oil ME; 16: Fully mature seed oil EA; 17: Immature pericarp ME; 18: Immature pericarp EA; 19: Mature pericarp ME; 20: Mature pericarp EA; 21: Fully mature pericarp ME; 22: Fully mature pericarp EA; 23: NC-ME; 24: NC-EA; 25: PC: Normal feed; NC: Negative control; PC: Positive control; n = 3; BC: *B: cucurbitae*; BZ: *B: zonata*.

percentage was observed for the most of the crude solvents extracts after 24 h for both Diptera flies. After 24 h, the highest percentage of larvae killed (66.67 ± 2.89)% was obtained for *B. cucurbitae* and EA bark extract and similarly (70.00 ± 8.66)% for *B. zonata* and ME fully mature leaves extract, and (75.00 ± 5.00)% for *B. cucurbitae* and ME fully mature seed oil extract at 200, 400 and 800 mg/L concentration.

After 72 h, crude EA of bark and immature pericarp extracts, the cumulative percentage of killed larvae observed was 96.97% at 200 mg/L, 100% for crude EA of mature pericarp extract at 400 mg/L and 93.33% and 95.00% for EA of mature seed oil and ME of fully mature pericarp extracts at 800 mg/L for *B. cucurbitae* and *B. zonata* respectively. The cumulative lowest mortality was 66.67% for EA immature and seed oil extracts.

3.5. Preliminary phytochemical test for J. curcas

The main phytochemicals constituents detected from the different parts of *J. curcas* plants were alkaloid, steroids, tannins, flavonoids, phenol and coumarins (Table 4). All the six secondary metabolites were present in mature pericarp ME of *J. curcas*. Twelve crude solvent extracts: bark EA, roots EA, immature leaves EA, mature leaves ME, fully mature leaves ME, fully mature leaves EA, mature seed oil ME, mature seed oil EA, fully mature seed oil EA, immature pericarp ME, mature pericarp EA, fully mature pericarp ME had five of the metabolites. Ethyl acetate solvent was better than ME in extracting most of the tested secondary metabolites. Only alkaloids and coumarins were noted in mature leaves EA extract.

Table 4
Phytochemical constituents of *J. curcas* extracts.

Extracts	Alkaloid	Coumarins	Flavonoids	Steroids	Tannins	Phenol
Bark ME	−−	++	++	++	++	−−
Bark EA	++	++	++	−−	++	++
Roots ME	++	−−	++	++	++	−−
Roots EA	++	++	−−	++	++	++
Immature leaves ME	++	−−	++	−−	++	++
Immature leaves EA	++	++	++	++	−−	++
Mature leaves ME	++	−−	++	++	++	++
Mature leaves EA	++	++	−−	−−	−−	−−
Fully mature leaves ME	++	++	−−	++	++	++
Fully mature leaves EA	++	++	++	−−	++	++
Immature seed oil ME	++	−−	++	++	−−	++
Immature seed oil EA	−−	−−	++	++	−−	++
Mature seed oil ME	++	++	++	−−	++	++
Mature seed oil EA	++	++	−−	++	++	++
Fully mature seed oil ME	++	++	++	−−	−−	++
Fully mature seed oil EA	++	++	−−	++	++	++
Immature pericarp ME	−−	++	++	++	++	++
Immature pericarp EA	−−	−−	++	++	++	++
Mature pericarp ME	++	++	++	++	++	++
Mature pericarp EA	++	++	++	−−	++	++
Fully mature pericarp ME	++	++	++	++	++	−−
Fully mature pericarp EA	−−	++	++	++	++	−−

++: Presence; −−: Absence.

4. Discussion

J. curcas is a versatile plant having potential uses in the medicinal field. These medicinal uses of different plant parts had been intensively investigated and studied by several researchers. The plant contains mixtures of different chemical compounds that may act individually, additively or in synergy as a medicine [8]. Numerous biologically active substances have been isolated and characterised from all parts of the *Jatropha* plant. Their action mechanisms have been studied in associate to a great number of applications of *J. curcas* in traditional medicines. However, the different parts of the whole *J. curcas* plant have been for the first time reported as a promising candidate for their effects as potential antimicrobial and larvicidal agents.

Furthermore, solvents used for extraction played an important role in the extraction of the phytochemicals. The plant section that exhibited the best antimicrobial properties was those extracted using EA extracts from the bark and seed oil. This could be due to the higher presence of biologically active metabolites in the EA extracts than the ME [9]. This is in agreement with the findings of Srinivasan *et al.* [8] who reported that different solvents have different extraction capacities and different spectrum of solubility for the phyto-constituents which are known to be biologically active.

The susceptibility of several fungi to *J. curcas* extracts was found significant, but in this study the antibacterial properties of the plant extracts were better than the antifungal activity. These secondary metabolites exert antibacterial and antifungal activity through different mechanisms. The production of secondary metabolites by plants is dependent on environmental conditions and the secondary plant metabolites are affected by gene level and the genetic diversity of plant metabolites. Therefore, this could be a few reasons explaining the difference of previous and present reported results of the antibacterial/antifungal activities.

Another secondary metabolite compound observed in *J. curcas* was alkaloid, widely present in the plant. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms [9]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects, hence explaining the effectiveness of *J. curcas* on both Gram-negative and Gram-positive bacteria. The absence of

alkaloids in *J. curcas* leaf extracts had been reported by Kubmarawa *et al.* [10], whereas this work report the presence of alkaloids in the three different stages of the leaves, irrespective of solvents used for extraction. Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbol ester which prevent animals from feeding on it. Hence, the presence of these compounds in *J. curcas* corroborates with both the antimicrobial activities and larvicidal effect observed.

The inhibitory effect of the extracts of *J. curcas* against pathogenic bacterial strains and larval development can promote the plant as a potential candidate for the treatment of ailments caused by these pathogens. The role of *J. curcas* in medicinal uses should be taken into consideration as it shows promising future in the pharmaceutical field. Commercializing on the medicinal product derived from *J. curcas* may turn out to be more profitable than using *Jatropha* as fuel substitution. The economics of making and marketing for these products should be further explored and encouraged.

Conflict of interest statement

We declare that we have no conflict of interest.

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