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Phytochemical and Biological Evaluation of Defatted Seeds of *Jatropha curcas*

(Penilaian Fitokimia dan Biologi Biji *Jatropha curcas* Nyahlemak)

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ABSTRACT

In this study, the antimicrobial, antioxidant, phytotoxic and phytochemical properties of defatted seeds of Jatropha curcas *were evaluated. A crude methanolic extract of defatted seeds was tested against three fungal strains* - Aspergillus niger, Aspergillus flavus *and* Aspergillus fumigatus - *and five bacteria:* Escherichia coli *and* Klebsiella pneumoniae *(Gram-negative) and* Micrococcus luteus, Bacillus subtilis *and* Staphylococcus aureus *(Gram positive). The methanolic extract was diluted in dimethylsulfoxide to final concentrations of 1, 2, 3, 4 and 5 mg/10 mL. The largest zones of inhibition against* K. pneumoniae, M. luteus *and* B. subtilis *were achieved using the concentration of 5 mg/10 mL. The concentration of 1 mg/10 mL was most effective against* S. aureus *and* E. coli. *In a 1, 1-diphenyl-2-picrylahydrazyl (DPPH) radical scavenging assay, the 5 mg/10 mL concentration of the* Jatropha *seed extract showed the strongest activity. Higher concentrations of the* Jatropha *seed extract (10 mg/50 mL and 5 mg/50 mL) significantly inhibited the germination of radish seeds and had negative effects on radish seedling relative water content, shoot length, root length, seedling fresh weight and seedling dry weight (*$p<0.05$*). Phytochemical analyses of the defatted seeds detected alkaloids (7.3%), flavonoids (0.39%) and soluble phenolics (mg gallic acid equivalents/g extract). Based on these results, it was inferred that* J. curcas *seeds contain active ingredients that are effective against pathogenic microbes and therefore could be used to formulate drugs to treat various diseases.*

Keywords: Antimicrobial; antioxidant; Jatropha curcas*; phytochemical; phytotoxicity*

ABSTRAK

Dalam kajian ini, sifat antimikrob, antioksidan, fitotoksik dan fitokimia biji Jatropha curcas *nyahlemak telah dinilai. Ekstrak mentah metanol biji nyahlemak diuji terhadap tiga strain kulat* - Aspergillus niger, Aspergillus flavus *dan* Aspergillus fumigatus *dan lima bakteria:* Escherichia coli *dan* Klebsiella pneumoniae *(Gram-negatif) serta* Micrococcus luteus, Bacillus subtilis dan Staphylococcus aureus *(Gram positif). Ekstrak metanol dicairkan dalam dimetilsulfoksida hingga ke kepekatan akhir 1, 2, 3, 4 dan 5 mg/10 mL. Zon terbesar perencatan terhadap* K. pneumoniae, M. luteus *dan* B. subtilis *telah dicapai menggunakan kepekatan 5 mg/10 mL. Kepekatan 1 mg/10 mL adalah paling berkesan terhadap* S. aureus *dan* E. coli. *Dalam 1, 1-diphenyl-2-picrylahydrazyl (DPPH) asai skaveng radikal, kepekatan 5 mg/10 mL ekstrak biji* Jatropha *menunjukkan aktiviti yang kuat. Kepekatan tinggi ekstrak biji* Jatropha *(10 mg/50 mL dan 5 mg/50 mL) dengan ketara menghalang percambahan biji lobak dan mempunyai kesan negatif ke atas kandungan air relatif biji lobak, panjang pucuk, panjang akar, berat segar biji dan berat kering biji (*$p< 0.05$*). Analisis fitokimia biji nyahlemak dikesan mengandungi alkaloid (7.3%), flavonoid (0.39%) dan fenol larut (mg asid galik setara/ ekstrak gram). Berdasarkan keputusan ini, dapat disimpulkan bahawa biji* J. curcas *mengandungi bahan aktif yang berkesan terhadap mikrob patogen dan boleh digunakan untuk menghasilkan ubat-ubatan untuk merawat pelbagai penyakit.*

Kata kunci: Antimikrob; antioksidan; fitoketoksikan; fitokimia; Jatropha curcas

INTRODUCTION

Jatropha curcas is a member of the Euphorbiaceae family and is commonly known as Barbados nut, physic nut or purging nut. It is a small tree or bush-like plant that grows up to 5 m high. Extracts from *J. curcas* have been reported to have anti-inflammatory, anti-coagulant, anti-tumor, anti-parasitic and insecticidal properties. This plant has been used for abortion and wound healing (Ejelonu et al. 2010). Jatrophin extracted from the latex has been used to treat various skin diseases, rheumatism and coughs and to promote wound healing (Uche & Aprioku 2008).

Throughout human history, plants have been used to treat different ailments caused by pathogenic microbes. Plant extracts contain a number of biologically active compounds (Khan et al. 2011a). Natural products obtained from different plant sources can have remarkable medicinal value and can be used to cure various diseases. Researchers have attempted to extract the most effective antimicrobial

agents from different kinds of resources, such as plants used in traditional folk medicines (Khan et al. 2011a). For example, the freeze-dried fruit powder of mulberry has been shown to have hypolipidemic and antioxidant effects (Yang et al. 2010). Similarly, artemisene extracted from *Artemisia japonica* was shown to have anthelmintic properties in animals and another product, artemitrene, showed anti-malarial activity, especially against *Plasmodium falciparum* (Hayat et al. 2009). Seeds of guava plants were shown to contain many biologically active compounds including phenolic compounds (Packer et al. 2010). Phenolic compounds have also been detected in seeds of other fruits including apricot (Yiğit et al. 2009), mango (Maisuthisakul 2008) and Citrus (Bocco et al. 1998).

The seed oil of *Jatropha* can be used to produce biodiesel. In fact, *Jatropha* is known as the biodiesel plant in many parts of the world (Belewu 2008; Okujagu et al. 2006). After the extraction of oil from *J. curcas* seeds, a seed meal was obtained as a byproduct. This material could be a rich source of various phytochemicals with biological activity. Several studies have shown that *Jatropha* seed meal contains bioactive components that have activity against sexually communicated diseases, mouth odor and jaundice and have antiseptic properties (Igbinosa et al. 2009; Namuli et al. 2011). *Japtropha* seed extracts have also been shown to have molluscicidal and larvicidal activities (Rug & Ruppel 2000). Recently, James et al. (2011) and Oskoueian et al. (2011) reported that methanolic extracts of the latex, roots, stem bark and leaves of *Jatropha* showed antimicrobial, cytotoxic, wound healing, antioxidant and anti-inflammatory activities. Different parts of the plant have been reported to contain glycosides, alkaloids, tannins, saponins, flavonoids and phenolics (Oskoueian et al. 2011; Thomas et al. 2008). These bioactive components are thought to have potential as anticancer, anti-inflammatory and antimicrobial compounds and as antioxidants (Rathee et al. 2009).

The aims of this study were to determine the phytochemical composition of *J. curcas* defatted seeds and to evaluate the antimicrobial, antioxidant, and phytotoxic properties of extracts prepared from this material.

Materials and Methods

Preparation of defatted seed meal

Fully matured *J. curcas* seeds were collected from the *Jatropha* garden at the University of Science and Technology, Bannu. The seeds were dried in the shade and then powdered using a Wiley mill (60-mesh size). To prepare the defatted seed meal, powdered seed material (100 g) was extracted with *n*-hexane (1 L) in a Soxhlet extractor for 6 h at 60°C (AOAC 1960). Then, the defatted seed meal was dried for further extraction of phytochemicals and biological evaluation.

Extract preparation

In order to prepare the methanolic extract, 20 g seed powder was mixed with 200 mL methanol (Merck Co. Darmstadt, Germany) and macerated at intervals for 3 days at room temperature 25±2°C. The extract was filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The thick, gummy extract obtained was stored in a sealed bottle at 4°C until further use in the experiments. The yield of dry extract (%) was determined in terms of air-dried weight of seed material. The total yield of the methanolic extract was 2.5%.

Antibacterial activity

The antibacterial assay was performed by the agar well diffusion method (Leven et al. 1979). Wells were produced in seeded agar and methanolic extract was introduced directly into the wells. After 24 h of incubation, the zone of inhibition around each well was measured and compared against those of standard antibiotics.

Preparation of stock solution

In order to prepare the stock solution, 5 mg of methanolic defatted seed extract was dissolved in 10 mL dimethyl sulfoxide (DMSO). This solution was further diluted in 4, 3, 2 and 1 mg/10 mL concentrations, respectively, before use in experiments.

Bacterial strains

The antibacterial activity of the extract was tested against two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) and three Gram-positive (*Micrococcus luteus, Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains. All bacterial strains were refreshed in a liquid broth for 24 h and then mixed with physiological saline to equalize the turbidity among samples. The turbidity of all samples was matched to the McFarland 0.5% $BaSO_4$ turbidity standard.

Preparation of agar plates

Autoclaved nutrient agar media (20 g medium in 1 L distilled water) was poured into Petri dishes (14 cm) and allowed to cool and solidify before seeding plates with bacteria. Six wells per Petri plate were made using a sterile cork borer (8 mm). Then, 50 µL of the test sample (five concentration of the extracts in DMSO: 1, 2, 3, 4 and 5 mg/10 mL) a positive control (clarithromycin) and a negative control (pure DMSO) were added to the respective wells.

The Petri plates were incubated at 37°C in a paraffin oven. After 24 h of incubation, the diameter of the clear zone showed no bacterial growth was measured in mm (Collins et al. 1989). Three replicates were tested for each bacterium.

ANTI-FUNGAL ASSAY

Antifungal activity was evaluated by the agar tube dilution method as described by Choudhary et al. (1995). In order to prepare the fungal inocula, fungi were grown on SDA media (Sabouraud dextrose agar). The *Jatropha* extract stock solution was diluted to 1, 3 and 5 mg/10 mL in DMSO. The antifungal agent Terbinafine at concentrations of 1, 3 and 5 mg/10 mL was used as the positive control and pure DMSO served as the negative control. The tubes (marked at 10 cm) were filled with 6 mL media and 100 µL extract and then allowed to cool in a slant position at room temperature. A 4 mm inoculum taken from a 1 week-old culture of each of three strains (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*). Tubes with the diluted extracts, DMSO (negative control) and Terbinafine (positive control) were incubated for 1 week at 28°C. The % inhibition was calculated as follows:

$$\text{Percentage inhibition of fungal growth} = [(100 - \text{linear growth in test sample in mm}) / (\text{linear growth in control in mm})] \times 100. \quad (1)$$

ANTIOXIDANT ACTIVITY

The antioxidant activity was evaluated using the 1, 1-diphenyl-2-picrylahydrazyl (DPPH) method as described by Brand-Williams et al. (1995). A stock solution of DPPH (2 mg in 50 mL methanol) was kept at 20°C until needed. The DPPH stock was further diluted with methanol to obtain an absorbance value of 0.980 ± 0.02 at 517 nm on a U-5100 spectrophotometer (Hitachi, Tokyo, Japan). A 2.8 mL aliquot of the DPPH solution was mixed with 200 µL *Jatropha* seed extract (diluted in DMSO to concentrations of 1, 2, 3, 4 and 5 mg/10 mL). The mixture was incubated in the dark at room temperature and then the absorbance was measured at 517 nm with a U-5100 spectrophotometer (Hitachi). The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following:

$$\text{Scavenging \%} = [(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance}) \times 100]. \quad (2)$$

Ascorbic acid served as the positive control.

PHYTOTOXICITY ASSAY

The phytotoxicity test was conducted as reported by Arzu and Camper (2002) and Atta-ur-Rehman (1991). Radish (*Raphanus sativus* L.) seed germination and various growth parameters of radish seedlings were assayed with five different concentrations of the methanolic *J. curcas* defatted seed extract (diluted in DMSO to 0.625, 1.25, 2.5, 5 and 10 mg/50 mL). Radish seeds were sterilized by rinsing with 0.1% mercuric chloride solution for 2 min and then washing three times with autoclaved distilled water. A 5 mL aliquot of each concentration of the extract was added to an autoclaved Petri plate containing sterilized Whatman No. 1 filter paper. The methanol was vacuum evaporated and then 5 mL of sterile distilled water was added to each plate. For the negative control, a filter paper in a Petri plate was moistened with methanol (5 mL), which was removed by evaporation and then 5 mL autoclaved distilled water was added. For the positive control, only 5 mL autoclaved distilled water was added to each plate. Ten seeds were placed in each plate and then the plates were placed in the dark at 25°C to germinate. The germinated seeds were counted each day until 90% seeds in the control had germinated. Seeds were considered to be germinated when the radical reached 5 mm in length. After 10 days, the seedlings were harvested and various growth attributes were analyzed.

DETERMINATION OF SEED GERMINATION

Seed germination (%) was determined as follows:

$$\text{germinated seeds / total seeds} \times 100. \quad (3)$$

DETERMINATION OF SEED GERMINATION INDEX

The seed germination index was calculated as follows:

$$\text{number of seeds germinated at first count + number of seeds germinated at final count / days of first count + days of final count/} \quad (4)$$

GERMINATION RATE INDEX

The germination rate index (GRI) was calculated as follows:

$$\text{germination index/germination percentage.} \quad (5)$$

DETERMINATION OF SEEDLING RELATIVE WATER CONTENT

Seedling relative water content was determined as follows:

$$\text{SFW - SDW / STW - SDW} \times 100,$$

where SFW is seedling fresh weight; SDW is seedling dry weight; and STW is seedling turgid weight.

PHYTOCHEMICAL ANALYSIS

Identification of the bioactive components in defatted *J. curcas* seeds was carried out using standard procedures (Krishnaiah et al. 2007; Mattilla et al. 2007).

QUALITATIVE ANALYSIS

TEST FOR FLAVONOIDS

The methanolic extract (0.5 g) was treated with petroleum ether to remove traces of fatty materials. The residue

was dissolved in 80% ethanol (20 mL) and filtered. An aliquot of the filtrate (3 mL) was mixed with aluminum chloride (1%, w/v) prepared in methanol and the color was observed. The formation of a yellow color indicated that flavonoids were present.

TEST FOR ALKALOIDS

An aliquot of the methanolic extract (0.6 g) was mixed with 1% (v/v) HCl (8 mL) and then the mixture was slightly warmed and filtered. A 2 mL aliquot of the filtrate was treated separately with Maeyer's and Dragendorff's reagents. Turbidity or precipitate formation indicated the presence of alkaloids.

TEST FOR PHENOLS

A 2 g aliquot of the extract was dissolved in 1 mL diethyl ether and then left to stand at room temperature for 2 h. The sample was then heated and boiled with 50 mL ether for 15 min. A 5 mL aliquot of the mixture was added to 10 mL distilled water and 2 mL ammonium hydroxide solution. The mixture was incubated for 30 min at room temperature for the development of color.

QUANTITATIVE ANALYSES

FLAVONOID ANALYSIS

A 10 g portion of the defatted seed sample was extracted with 100 mL 80% (v/v) aqueous methanol. The solution was filtered and then the filtrate was evaporated in a water bath to dryness and weighed (Mattila & Hellström 2007; Williamson & Manach 2005).

ALKALOID ANALYSIS

A portion of the defatted seed sample (5 g) was mixed with 200 mL 10% CH_3COOH in ethanol and incubated for 4 h at room temperature. The mixture was then filtered and ammonium hydroxide (concentrated) was added to the filtrate until a precipitate formed. The precipitate obtained was washed, diluted with ammonium hydroxide and filtered. The residue, which consisted of alkaloids, was dried and weighed.

TOTAL PHENOLICS CONTENT

The Folin-Ciocalteu method (Adam & Liu 2002) was used to determine the total phenolics content of all the extracts used in the phytotoxicity assay. For each assay, 125 µL methanolic extract was mixed with 500 µL distilled water and then 125 µL Folin-Ciocalteu reagents was added. The mixture was left to stand for 6 min and then the volume was completed to 3 mL by adding 1.25 mL 7% aqueous sodium carbonate solution and 1 mL distilled water. The mixture was incubated for 90 min in the dark and the absorbance was measured at 760 nm on a U-5100 spectrophotometer (Hitachi). A standard curve was prepared using different concentrations of gallic acid. The concentration of total phenolics was determined as mg gallic acid equivalents/g sample.

STATISTICAL ANALYSIS

The phytotoxicity data were analyzed by one-way analysis of variance (ANOVA) according to Steel and Torrie (1980). Mean values were compared among treatments using Duncan's multiple range test (DMRT) and coefficients of correlation among growth attributes were calculated using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA).

RESULTS AND DISCUSSION

The methanolic extract of *Jatropha* seeds was tested for its antibacterial activity against five bacterial strains (*E. coli*, *K. pneumoniae*, *M. luteus*, *B. subtilis* and *S. aureus*) using the agar well diffusion method. As shown in Figure 1, the methanolic extract inhibited the growth of all the

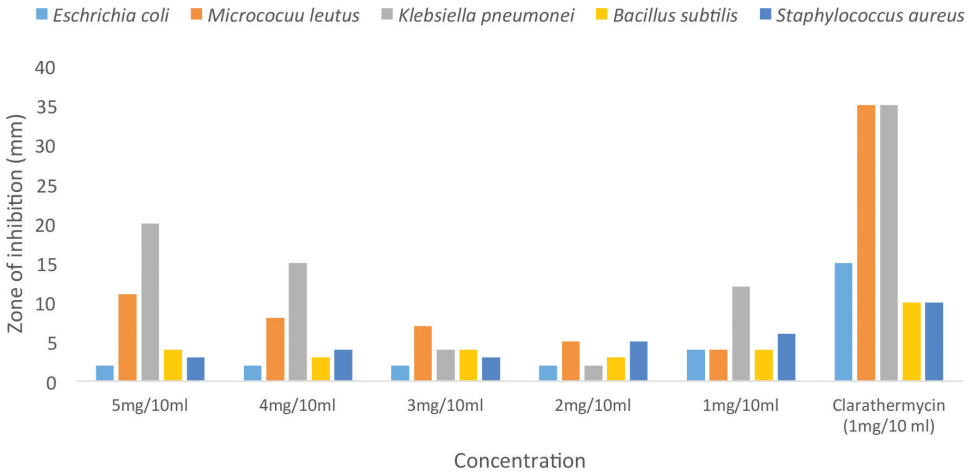


FIGURE 1. Antibacterial activity of the *J. curcas* defatted seed. The data represent mean of three replicates

tested bacterial strains at all concentrations. The largest zones of inhibition for *K. pneumonei* (20 mm), *M. luteus* (11 mm) and *B. subtillis* (4 mm) were achieved using the concentration of 5 mg/10 mL. The largest zones of inhibition for *S. aureus* and *E. coli* were achieved using the dose of 1 mg/10 mL. All of the tested bacterial stains were highly susceptible to clarithromycin (positive control), as exhibited by the maximum zones of inhibition. The antibacterial activity of the *Jatropha* seed extract may be attributed to the presence of several proteins and other bioactive compounds in the extract (Idris et al. 2013; Khan et al. 2011b).

The methanolic extract showed antifungal activity against *A. fumigatus*, *A. flavus* and *A. niger*. The concentration of 5 mg/10 mL showed the strongest antifungal activity (Figure 2). Next, the antioxidant activity of methanolic *Jatropha* seed extract was determined using the DPPH radical scavenging assay. As shown in Figure 3, the concentrations of the *Jatropha* seed extract could be ranked, from strongest antioxidant activity to weakest, as follows: 5 mg/10 mL > 4 mg/10 mL > 3 mg/10 mL > 2 mg/10 mL > 1 mg/10 mL. The strong antioxidant activity of the *Jatropha* seed extract might be owing to its high phenolic content.

In order to determine whether the *Jatropha* seed extract had phytotoxic effects, the extract was applied to seeds of radish and germination and early seedling growth were evaluated. Such phytotoxicity assays can determine whether natural or synthetic compounds are beneficial or harmful for the growth and development of plants (Badshah et al. 2015). As shown in Figure 3(a), the high concentration of *Jatropha* seed extract (10 mg/50 mL) significantly inhibited seed germination (%) as compared with that of the control. Other germination indices such as germination index and germination rate index were not significantly affected by the extract at all concentrations (Figure 3(b) & 3(c)). Higher concentrations of the extract (10, 5 and 2.5 mg/50 mL) had significant negative effects on seedling relative water content, as compared with that of the control (Figure 3(d)). Similarly, higher concentrations of the *Jatropha* extract (10, 5 and 2.5 mg/50 mL) significantly reduced shoot length, root length, seedling fresh weight, and seedling dry weight, as compared with those of the control (Figure 4(a)-4(d)).

The results of this study showed that *J. curcas* seeds contain some active ingredients that inhibited the growth and development of radish plants at higher concentrations. Allelopathy is a phenomenon in which an organism

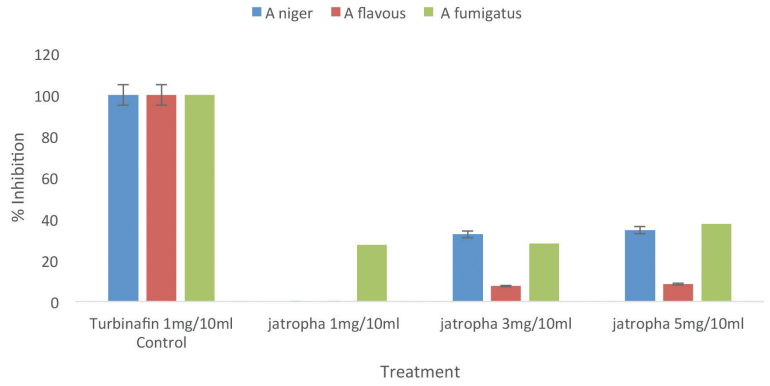


FIGURE 2. Antifungal activity of the *J. curcas* defatted seed.
The data represent mean of three replicates

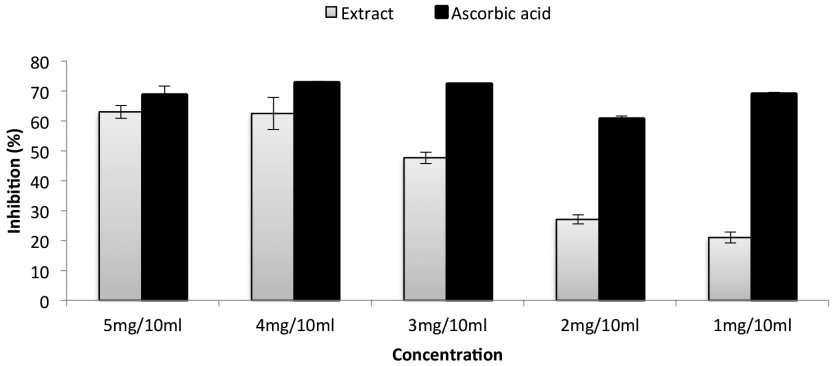
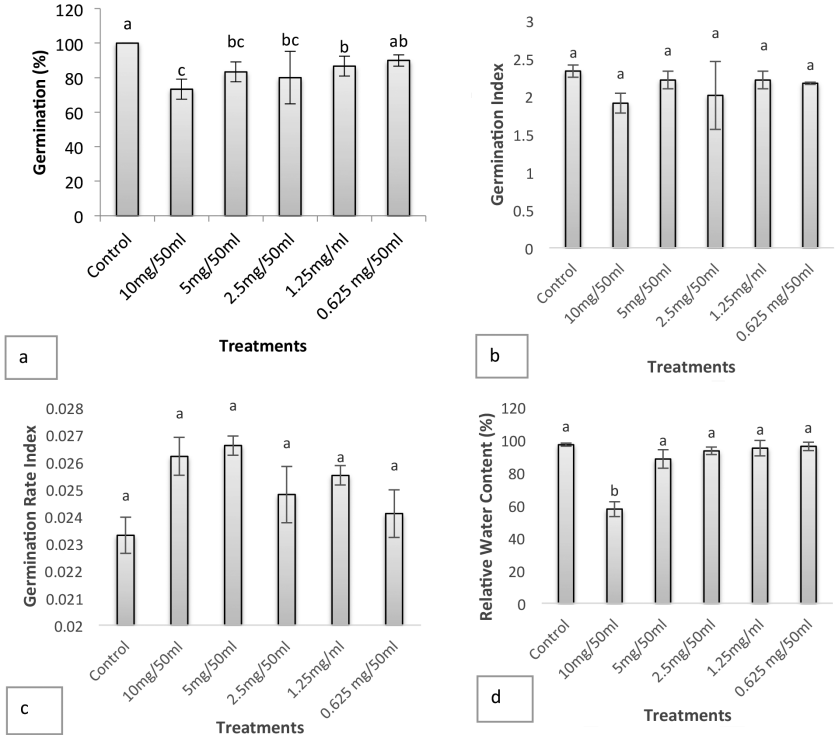
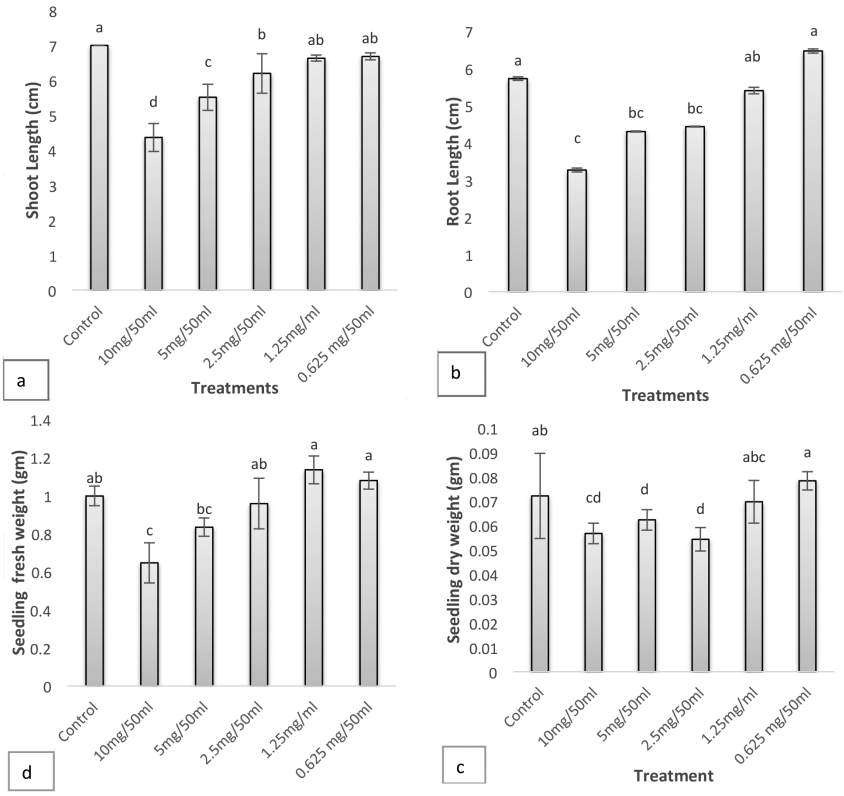


FIGURE 3. DPPH Free radical scavenging activity by the *J. curcas* defatted seed.
The data represent mean of three replicates



Means sharing common English letters are statistically similar

FIGURE 4a. Effect of methanolic extract of *Jatropha curcas* seed on (a) seed germination (%) (LSD: 10.271), (b) Germination index (LSD: 0.6285), (c) Germination rate Index (LSD: 0.03) and (d) Relative water content (LSD: 24.676)



Means sharing common English letters are statistically similar

FIGURE 4b. Effect of methanolic extract of *Jatropha curcas* seed on (a) shoot length (LSD: 0.4849), (b) root length (LSD: 1.1968), (c) seedling fresh weight (LSD: 0.2122) and (d) seedling dry weight (LSD: 0.0142)

TABLE 1. Qualitative Analysis of *J. curcas* defatted seed

Sample code	Alkaloids	Flavonoids	Phenols
J. curcas defatted seed	+ +	+ +	+ + +

+ = present, ++ = moderate, +++ = highly present

TABLE 2. Quantitative analysis of *J. curcas* defatted seed

Sample code	Alkaloids (%)	Flavonoids (%)	Total soluble phenolics (mg gallic acid eq./g extract)
J. curcas defatted seed	7.3	0.39	11

suppresses or stimulates the growth, survival and/or reproduction of other organism through the production of compounds known as allelochemicals (Khattak et al. 2015; Stamp & Nancy 2003; Ullah et al. 2014). Allelochemicals are diverse secondary metabolites that are not required for normal metabolism and growth of the allelopathic organism (Badshah et al. 2015). In the present study, the toxic effect of the extract was concentration-dependent. Only higher concentrations of the extract had toxic effects on radish. These findings are consistent with those of other studies in which the toxicity depended on the concentration of the extract (Ademiluyi 2013; Barkatullah et al. 2015; Khan et al. 2011; Sher at al. 2014). Allelochemicals reduce the moisture content in the shoots and leaves of susceptible test species. In the present study, the radish seedling relative water content was reduced after treatment with the extract at higher concentrations. This decrease in seedling relative water content may be because the allelochemicals produced drought-stress conditions inside the plant. In another study, the poor growth and biomass production of a susceptible plant was attributed to its failure to absorb sufficient water from the medium and the subsequent loss of turgor (Barkosky et al. 2000).

Qualitative analyses of *J. curcas* defatted seeds showed that alkaloids, flavonoids and phenols were present (Table 1). Moderate amounts of alkaloids and flavonoids and large amounts of phenols were detected. Further quantitative analyses confirmed that the defatted seeds contained alkaloids (7.3%), flavonoids (0.39%) and total soluble phenolics (11 mg gallic acid eq/g extract) (Table 2).

The phytochemical composition of defatted *J. curcas* seeds suggested that this material could be suitable for formulating various drugs. Alkaloids have been shown to have defensive activities against various diseases in experimental animals (Edeoga & Eriata 2001; Martin et al. 2015). Alkaloids have been shown to have anti-inflammatory and analgesic activities and to improve resistance against diseases (Gupta 1994). Phenols have been reported to have antiviral, antioxidant and antimicrobial activities (Vasantha et al. 2012). The presence of alkaloids, flavonoids and phenols in defatted seeds of *J. curcas* may explain the antimicrobial, antioxidant, and phytotoxic activities of the methanolic extract of this abundant by-product.

CONCLUSION

An extract of defatted *J. curcas* seeds exhibited antimicrobial, antioxidant and phytotoxic activities. Higher concentrations of the extract inhibited the growth of radish seedlings. Phytochemicals such as alkaloids, flavonoids and phenols were present in the defatted seeds. It was inferred that *J. curcas* seeds contain active ingredients that are effective against pathogenic microbes and therefore, could be used in the formulation of drugs to treat various diseases.

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