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Study of in-vitro antioxidant potential and antimicrobial activity of *Jatropha curcas*- an important medicinal plant of the Indian subcontinent

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Abstract

The study was designed for screening of the biological activities of methanol extract of the root of *Jatropha curcas* (Family: Euphorbiacae) like antimicrobial and free radical scavenging activities. The antimicrobial activity was determined by disk diffusion method against some gram positive and gram negative bacteria as well as fungi. Chloroform soluble fraction exhibited potent antimicrobial activity in terms of zone of inhibition (9.33 - 13.33 mm) and spectrum of activity compared to the reference standard kanamycin (21 - 26.33 mm). Methanolic crude extract and methanol soluble fraction also showed moderate antimicrobial activities against the tested organisms. In the evaluation of DPPH free radical scavenging activity, methanolic crude extract and chloroform soluble fraction showed strong antioxidant activity with IC_{50} value of 35.62 µg/ml and 43.81 µg/ml respectively where the standard antioxidant butylated hydroxytoluene (BHT) showed the IC_{50} value of 18.31 µg/ml. At the same time, highest amount of phenolic contents were found in methanolic crude extract and chloroform soluble fraction having TPC value of 36.37 and 27.01 mg of GAE/ gm of extractive respectively. These results suggested that bioactivity guided isolation can be carried out to separate the bioactive principles.

Key words: Jatropha curcas, antimicrobial activity, disc diffusion method, antioxidant activity, DPPH free radical scavenging activity

Introduction

In recent decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products [1]. Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have been resistant. Moreover, antibacterial pharmaceuticals are not accessible to the majority of the communities in the developing countries [2]. Plants may provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally.

Antioxidants are intimately involved in the prevention of cellular damage- the common pathway for cancer, aging, and a variety of diseases. The scientific community has begun to unveil some of the mysteries surrounding this topic, and the media has begun whetting our thirst for knowledge. Athletes have a keen interest because of health concerns and the prospect of enhanced performance and recovery from exercise.

The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG), and tert-butyl hydroxyl toluene (TBHQ) [3]. However, they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals [4,5]. Therefore, the development and utilization of more effective antioxidants of natural origin are desired [6].

Jatropha curcas (Family: Euphorbiacae; Bengali name: bag verenda) is a small tree or large shrub which is widespread in tropical and subtropical areas, planted as 'living fences' around fields and settlements.Most parts of the plant and their extracts are used in traditional medicine. Oil from the seeds can be used as biodiesel and for producing soap [7], and press cake from the seeds supplies organic manure [8]. Traditionally it is used to cure diseases like cancer, piles, snakes bites, paralysis, and dropsy [9]. However, limited information is available on the pharmacological properties of *Jatropha* species which showed that many species possess antimicrobial activity [10-12].

The present research was designed to study the in-vitro antioxidant potential and antimicrobial activities of various fractions of *J. curcas* (methanolic crude extract, *n*-hexane soluble fraction, chloroform soluble fraction and methanol soluble fraction). This paper describes antioxidant and antimicrobial activities of this important medicinal plant of the Indian subcontinent.

Materials and methods

Collection, identification and preparation of plant material

Sample roots of *Jatropha curcas* was collected from Jessore district of Bangladesh in January 2009 and authentication of the plant sample has been done by the taxonomist of the Department of Botany, University of Dhaka. After proper identification, root samples were cut into small pieces and sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature (40 °C) for better grinding and the dried samples were grinded into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka and stored in an airtight container for further study.

Extraction and fractionation procedure

600 g of the powdered plant material was taken in a clean, round bottomed flask (5 L) and soaked into methanol (4 L). The flask with the container was sealed by cotton plug and aluminium foil and kept for a period of 7 days with occasional shaking and stirring. The mixture was then filtered through cotton followed by Whatman No. 1 filter paper and the filtrate thus obtained was concentrated at 40 °C with a Heidolph rotary evaporation. Finally the concentrated extract was air dried to solid residue

(14.4 g) and later on treated as methanolic crude extract. 5 g of the methanolic crude extract was triturated with 10% water in methanol (100 ml) to dissolve completely. The content was then fractionated using modified Kupchan partitioning method [13] into *n*-hexane (3.65 g), chloroform (2.3 g) and methanol (13.25 g) fractions.

Investigation of antimicrobial activity

Antimicrobial activities of the test samples were evaluated in accordance with the disc diffusion method [14,15] using 13 bacterial strains (including both gram positive and gram negative) and 3 fungal strains (Table 1).

Test microorganisms: The test microorganisms used in this experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Here 100 µl suspension of each of the organisms were used containing 10^8 CFU/ml of bacterial strains and 10^4 spores/ml of fungal strains. The bacterial strains were first subcultured in nutrient agar (NA) medium & incubated at 37 °C for 18 h while the fungal strains were subcultured for 72 h at 25 °C.

Preparation of the medium: To prepare required volume of this medium, calculated amount of each of the constituents were taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25 °C) was adjusted at 7.2 - 7.6 using NaOH or HCl. 10 ml and 5 ml of the medium were then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15 lbs. pressure/sq. inch at 121 °C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were used for sensitivity study.

Sterilization procedure: In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glass wares were sterilized by autoclaving at a temperature of 121 °C and a pressure of 15 lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Preparation of subculture: In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37 °C for their optimum growth and which were used later on for the sensitivity test.

Preparation of the test plates: The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petri dishes. The petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

Preparation of discs: Three types of discs were used for antimicrobial screening.

Preparation of sample discs: 8 mg of each of the test samples, methanolic crude extract (MECE), *n*-hexane soluble fraction (NHSF), chloroform soluble fraction (CFSF) and methanol soluble fraction (MESF), were dissolved in 200 µl of methanol to obtain the sample solutions concentration 40 mg/ml in aseptic condition. Sterilized metrical (BBL,

Cocksville, USA) filter paper discs (6 mm in diameter) were taken in a blank petri dish and soaked with 10 µl (400 µg/disc) of the test samples under the laminar hood.

Standard discs: Kanamycin (30 µg/disc) discs were prepared using the same procedure employed for test samples and used as the reference standard. Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test samples.

Blank discs: Negative controls were prepared using the same solvents employed to dissolve the test samples. The negative controls were used to ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.

Diffusion and incubation: The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4 °C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37 °C for 24 hours.

Determination of antimicrobial activity by the zone of inhibition: The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which give clear zone of inhibitions. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. The experiment was carried out in triplicate and the average zones of inhibition in millimeter were calculated.

Evaluation of antioxidant activity

Determination of total phenolics

The amount of total phenolics in the test samples were determined by using Folin-Ciocalteu (Folin C.) reagent as an oxidizing agent and gallic acid as the reference standard [16]. 0.5 ml (2 mg/ml) of each sample solutions in water were added in 2.5 ml (diluted 10 times with water) of Folin-Ciocalteu reagent and 2 ml (7.5% w/v) of sodium carbonate solution. After 20 minutes incubation at room temperature the absorbances were measured at 760 nm using UV-visible spectrophotometer. Total phenolics were calculated by using calibration curve obtained from measuring the known concentrations of gallic acid (0-100 µg/ml) and were expressed as mg of gallic acid equivalent (GAE)/gm of the dried sample.

DPPH free radical scavenging activity

The antioxidant activity of the test samples at different concentrations were evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay [17] with minor modification. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the sample solutions as compared to that of butylated hydroxytoluene (BHT) by UV-visible spectrophotometer. Here butylated hydroxytoluene (BHT) was used as the reference standard. 2 mg of each of the test samples were dissolved in methanol and serial dilution technique was used to obtain the solutions of varying concentrations, like-500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.813 µg/ml, 3.906 µg/ml, 1.953 µg/ml and 0.977 µg/ml. 3 ml of DPPH solution in methanol (20 µg/ml) was mixed to 2 ml of each of the test sample solutions and were allowed to stand for 30 minutes at room temperature in absence of light for completing the reaction. Then absorbances were measured at 517 nm against

methanol as blank by UV-visible spectrophotometer. The corresponding percentages of inhibitions of DPPH free radical were calculated by using the following equation:

$$\% \text{ inhibition} = [1- (ABS_{sample} / ABS_{control})] \times 100$$

Where, ABS_{sample} is the absorbance of the test samples and $ABS_{control}$ is the absorbance of the control reaction (containing all reagents except any test material). Finally, IC_{50} values (sample concentrations providing 50% inhibition) were calculated from the graph obtained by plotting percentage of inhibition against the respective concentrations.

Statistical analysis

All the values are presented as mean ± SD. A difference in the mean values of $p < 0.05$ was considered to be statistically significant.

Results and discussion

Investigation of antimicrobial activity

The antimicrobial activities of methanolic crude extract and different fractions of *Jatropha curcas* on pathogenic bacteria and fungi are presented in Table 1. Previous studies reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents [18-20].

see Table 1.

This investigation demonstrated promising broad-spectrum antimicrobial activity of methanolic crude extract as well as chloroform and methanol soluble fractions of the crude extract against the tested microorganisms. Whereas *n*-hexane soluble fraction showed no inhibitory effect on the tested pathogenic microbes. The antibacterial activities of the chloroform extract (zone of inhibition 9.33 - 13.33 mm) compared favorably well with that of standard kanamycin (21 - 26.33 mm) and have appeared to be broad spectrum as its activities were independent of the gram action. The antimicrobial activities of the methanolic crude extract and the methanol soluble fraction showed moderate activities in terms of both zone of inhibition (7 - 11.33 mm and 7.67 - 10.33 mm) and spectrum of activity against the microorganisms.

Phytoconstituents present in plants, namely-flavonoids, alkaloids, tannins and triterpenoids have demonstrated exciting potentials for the modern chemotherapies against a wide spectrum of microorganisms [21,22]. The results of this study showed that all the fractions except *n*-hexane soluble fraction exhibited varied antimicrobial activities against the tested organisms. The samples tested were effective against a group of microorganisms that are implicated in either typhoid fever and/or other gastrointestinal infectious diseases such as dysentery. Among all the samples, chloroform soluble fraction showed the strong antimicrobial activity.

Total phenolics and DPPH free radical scavenging activity

In case of screening for antioxidant activity (Table 2), the MECE and CFSF of roots exhibited strong free radical scavenging activity with IC_{50} values of 35.62 µg/ml and 43.81 µg/ml respectively. At the same time, reference standard BHT showed the antioxidant potential having the IC_{50} value 18.31 µg/ml. For the test samples, highest amount of phenolic contents were found in MECE and CFSF having TPC value of 36.37 and 27.01 mg of GAE/ gm of extractive which also demonstrated significant free radical scavenging activity (IC_{50} = 35.62 µg/ml and 43.81 µg/ml respectively). Therefore a positive correlation was seen between the total phenolic content and free radical scavenging activity and it supports the fact that phenolic compounds have potent free radical scavenging activity [23,24].

see Table 2.

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Antioxidants minimize the oxidation of lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides, arising from linoleic acid oxidation, that are known to be carcinogenic. Polar extracts exhibited stronger activity than non-polar extracts, indicating that polyphenols or flavanones and flavonoids may also play important roles in the activity and the plant could be suggested as a natural source of antioxidant and antiaging factor.

Conclusion

From the study, it is evident that the test extracts/fractions demonstrated significant antimicrobial activities as well as strong *in-vitro* antioxidant activities. These investigations are useful for understanding about its active ingredients, and also reveal the potential of *J. curcas* for use as an important traditional medicinal plant. Further bio-assay guided investigation can be done to isolate the bioactive principles and to know their mechanisms of actions.

References

1. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. P Natl Acad Sci USA 1993; 90(17):7915-7922.
2. World Health Organisation. Global strategy for containment of antibiotic resistance. Geneva: WHO 2001; p. 99.
3. Sherwin FR. Antioxidant. In: Branen R, ed. Food Additice, Marcel Dekker, New York, NY, USA, 1990:139-193.
4. Grice HC. Safety evaluation of butylated hydroxyl toluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem Toxicol 1986; 24:1127-1130.
5. Wichi HP. Enhanced tumor development by butylated hydroxyanisole (BHA) form the prospective of effecton forestomach and oesophageal squamous epithelium. Food Chem Toxicol 1988; 26:717-723.
6. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. LWT-Food Sci Technol 2003; 36(2):263-271.
7. Henning R, Von Mitzlaff K. Produktion und Nutzung von Purgieröl als Kraftstoff und Rohstoff für die lokale Seifenherstellung im Sahel. Der Tropenlandwirt 1995; 53:137-151.
8. Sherchan DP, Thapa YB, Khadka JT, Tiwari TP. Effect of green manure on rice production. Pakhribas Agric 1989; 2:12-15.
9. Okujagu TF, Etatuvie SO, Eze I, Jimoh B, Nweke C, Mbaoji C. Medicinal Plants of Nigeria; South-west Nigeria. In collaboration with Lagos state Traditional Medicine Board and Botany Department, University of Lagos. 1st ed. 2006; 1: 20.
10. Aiyelaagbe OO, Adeniyi BA, Fatunsin OF, Arimah BD. In vitro Antimicrobial activity and photochemical analysis of Jatropha curcas roots. Intern J Pharmacol 2007; 3(1):106-110.
11. Aiyelaagbe OO. Antibacterial activity of Jatropha multifida roots. Fitoterapia 2001; 72:544-546.
12. Thomas OO. Re-examination of the antimicrobial activities of Xylopia aethiopica, Carica papaya, Ocimum gratissimum and Jatropha curcas. Fitoterapia 1989; 60:147-155.
13. Van Wagenen BC, Larsen R, Cardellina JH, Ran Dazzo D, Lidert ZC, Swithenbank C. Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri. J Org Chem 1993; 58:335-337.
14. Bauer AW, Kirby WMM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. J Am Clin Pathol 1966; 45:493-496.
15. Rahman MS, Rashid MA. Antimicrobial activity and cytotoxicity of Eclipta prostrata. Orient Pharm Exp Med 2008; 8:47-52.
16. Skerget M, Kotnik P, Hadolin M, Hras AR, Simonic M, Knez Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 2005; 89:191-198.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995; 28:25-30.
18. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62:183-193.
19. Eloff JN. Which extract should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol 1998; 60:1-8.
20. Lin J, Opoku AR, Geheeb-Keller M, et al. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol 1999; 68:267-274.
21. Lutterodt GD, Ismail A, Basheer RH, Baharudin HM. Antimicrobial effects of Psidium guajava extracts as one mechanism of its antidiarrhoeal action. Malaysian J Med Sci 1999; 6:17-20.
22. Marjorie MC. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12:564-582.
23. Pietta A, Sionetti P, Mauri P. Antioxidant activity of selected medicinal plants. J Agric Food Chem 1998; 46:4487-4490.
24. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. Crit Rev Food Sci 1992; 32:67-103.

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Test microorganisms	Diameter of zone of inhibition (mm)				
	MESF	NHSF	CFSF	MECE	Kanamycin
Gram positive bacteria					
Bacillus cereus	9 ± 2.65	-	9.33 ± 1.53	9 ± 2	25 ± 1
Bacillus megaterium	8 ± 3.61	-	11.33 ± 2.52	11.33 ± 1.53	25 ± 1
Bacillus subtilis	9 ± 3	-	10.33 ± 1.53	8.67 ± 3.06	26 ± 1
Staphylococcus aureus	8.33 ± 1.53	-	11.67 ± 3.06	9 ± 2.65	26.33 ± 1.15
Sarcina lutea	8.67 ± 1.15	-	12.33 ± 0.58	10 ± 3.61	24.33 ± 0.58
Gram negative bacteria					
Escherichia coli	7.67 ± 3.21	-	10 ± 1	7.33 ± 2.08	23.67 ± 2.08
Pseudomonas aeruginosa	8.67 ± 3.06	-	12.33 ± 2.52	-	25.67 ± 0.58
Salmonella paratyphi	-	-	9.33 ± 1.53	7 ± 1.73	22 ± 1.73
Salmonella typhi	10.33 ± 2.52	-	13.33 ± 1.53	7.33 ± 1.53	22.67 ± 2.08
Shigella boydii	-	-	12.33 ± 2.52	-	22.33 ± 2.52
Shigella dysenteriae	8.67 ± 0.58	-	12 ± 3	-	23 ± 2
Vibrio mimicus	9 ± 2	-	12 ± 2	8 ± 2	23 ± 1
Vibrio parahemolyticus	10.33 ± 2.52	-	11 ± 1.73	8.33 ± 3.51	21 ± 1
Fungus					
Candida albicans	10 ± 2	-	9.33 ± 2.08	8.33 ± 3.06	21.67 ± 1.53
Aspergillus niger	9 ± 2.65	-	9.33 ± 2.52	-	23.67 ± 0.58
Sacharomyces cerevacae	8.67 ± 1.53	-	11 ± 3.61	11.33 ± 3.51	25.67 ± 1.53

Table 1: Antimicrobial activities of Jatropha curcas extracts by disc-diffusion method

Note: The diameters of zone of inhibition (mm) are expressed as mean ± SD (n=3); a diameter less than 7 mm was considered inactive and denoted by '-'; MECE: methanolic crude extract, NHSF: *n*-hexane soluble fraction, CFSF: chloroform soluble fraction, MESF: methanol soluble fraction

Sample	TPC (mg of GAE/ gm of extractives)	IC_{50} (µg/ml)
BHT	-	18.31 ± 1.38
NHSF	14.55 ± 2.04	123.27 ± 3.69
MESF	11.27 ± 2.05	146.34 ± 4.42
CFSF	27.01 ± 2.08	43.81 ± 3.11
MECE	36.37 ± 3.04	35.62 ± 2.98

Table 2: Total phenolic contents and free radical scavenging activity of extracts

BHT: butylated hydroxytoluene