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Antifungal Activities and Phytochemical Screening of Seeds, Leaves and Callus (hypocotyls and cotyledons) Extracts of Jatropha curcas L.

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ABSTRACT

The research was under taken to investigate the antifungal activity of seeds , leaves and callus extracts of Jatropha curcas L. , using methanol and petroleum ether solvents. Callus was induced from hypocotyls and cotyledons explants in MS medium supplemented with combination of 2.0 mg/l of different auxin (2, 4-D or NAA) + 0.5 mg/l Kinetin or 0.1 BAP. Antifungal activities of extracts obtained were tested against standard fungal, Aspergillus niger (ATCC 9763), Aspergillus flavus (ATCC 9763), Aspergillus fumigatus and Candida albicans (ATCC7596). The petroleum ether extract of hypocotyls callus at concentration100mg/l gave the highest zone inhibition (18.2 ±0.3) and (18.0±0.3)mm against Candida albicans and Aspergillus fumigatus respectively, at the same time the petroleum ether extracts of hypocotyls callus at concentration 10mg/l showed high activity (16.3±0.4) mm against Candida albicans.

The methanolic extracts of hypocotyls callus showed high inhibition(16.8±0.4) mm against Aspergillus fumigatus and moderate inhibition zone against Candida albicans(14.3 ±0.3), at concentration100mg/l . The results showed that methanolic callus (hypocotyls) extract gave higher inhibition zone than cotyledons callus extract. The petroleum ether extracts of seeds and leaves were ineffective against all fungi tested in this study. Aspergillus niger (ATCC 9763)and Aspergillus flavus are more resistant to all extracts of different parts of Jatropha curcas , when compared to other fungi. The concentration 100mg/l of all extracts gave more antifungal activities than the concentration 10mg/l of the extracts. The paper disc diffusion method was used to assess the in vitro testing of extracts for antifungal activity. Phytochemical screening for seeds, leaves and callus extracts indicated the presence of various secondary metabolites like flavonoids, steroids , alkaloids, tannins, sapoins , phenolic compoud and terpenoids.

Keywords

Jatropha curcas L.; Antifungal Activities; Callus induction; Phytochemical screening.

Academic Discipline And Sub-Disciplines

Biology

SUBJECT CLASSIFICATION

Phytochemical /Antifungal

TYPE (METHOD/APPROACH)

Lab experiments

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INTRODUCTION

Jatropha species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (1). It has been documented to have medicinal uses for human and veterinary purposes (1). Several studies have confirmed the antimicrobial efficacy of different Jatropha species; however, there is insufficient information regarding the antimicrobial activities of J. curcas Linn. Whatever limited information available on the medicinal properties of J. curcas is mostly on the leaf extracts of the plant. The use of antimicrobial agents for the control of pathogenic bacteria is helpful in the treatment of infections and diseases (2). Hence, there is need to investigate the antimicrobial properties of plant extracts that have not been done. The production of the secondary compounds is often low (less than 1% dry weight) and depends mainly on the physiological and developmental stages of plants (3-4) revealed that leaves contain flavaonoids such as apigenin, vitexin, isovitexin, atriterpene alcohol(C63H117O9) and flavonoidal glycosides and seeds contain curcin, lectin, phorbolesters, esterases and lipase .Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites (5). Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance of higher plants for specific diseases (6-7).

Tissue culture opens up an extensive area of biotechnological research into the potential use of in vitro cultures to produce highly valuable secondary metabolites, including compounds of medical applications (8-9). Flavonoids and phenolic compounds are widely distributed in plants that have been reported to exert multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory, and anticarcinogenic (10). Plant cell cultures are an attractive alternative source to whole plant for the production of high value secondary metabolites (11-12). In vitro propagated callus cultures can become an alternative to plants grown in their environment due to the fact that under controlled condition, plant tissue can produce significant amounts of metabolites of interest (13). However, a considerable progress has been made to stimulate production and accumulation of secondary metabolites using plant cell cultures (14-15). Several strategies have been adopted for the enhancement of bioactive metabolite production in vitro cultures; one of them is using growth regulators which are often a crucial factor in secondary product accumulation (16).The type and concentration of auxin or cytokinin or the auxin/cytokinin ratio may alter (17). Therefore, it is of great interest to evaluate the antifungal activity, of different parts of Jatropha curcas.

The objectives of this research to investigate the antifungal activity of extracts of the seed, leaves and callus (cotyledons and hypocotyls) of Jatropha curcas, grown under the conditions of Sudan against pathogenic fungal Aspergillus niger, Aspergillus flavus , Aspergillus fumigatus and Candida albicans using different solvents . Also to detect the secondary metabolites of these parts of the plant.

Materials and Methods:

Mature seeds of Jatropha curcas used throughout this study were obtained from ALrwakeep research station , National Center for Research, Khartoum, Sudan .

Seeds surface sterilization and germination:

seeds were washed by continuously running tap water for 30 minutes to remove all the surface dirt, followed by washing with sterile distilled water. Under laminar flow cabinet seeds were disinfected with 70% ethanol(v/v) for 2 minutes then washed with sterilized distilled water followed by rinsing in 0.5 mercuric chloride containing two drops of Tween-20 for 15 minutes with continuous shaking. Finally, the seeds were then washed several times with sterile distilled water to remove the traces of the disinfectant. After surface sterilization, the seeds were directly cultured on the germination basal medium MS (18) at 25±2°C and photoperiod of 16 hrs light and 8 hrs dark for 15 days.

Callus Induction:

The hypocotyls and cotyledons were used as explants of Jatropha curcas in this study for callus induction. To induce callus from explants, MS medium was supplemented with 2mg/l of different auxins (2,4-D or NAA) + 0.5 mg/l Kinetin or 0.1 BAP. Each of the sterilized explants were cut into 3-5 mm pieces using sterile scalpel. Four pieces were incubated in each vial containing sterile culture MS medium with different combinations of growth regulators. The calli were incubated for 4 weeks in 16 hrs light and 8 hrs dark at 25±2°C, then tissues were subculture at three week intervals.

Preparation of Plant Crude Extract:

20.0 g of dry Jatropha curcas seeds and leaves were cleaned and ground using a mortar and pestle. The extraction was carried out by soxhlet method. The fine powder was packed tightly in a soxhlet extractor and petroleum ether 200 ml were used as solvent for extraction. The process was carried out for 6 hrs. The fine powder was re-extracted under the same conditions by methanol. The obtained extracts were evaporated by Rot-evaporator under reduced pressure at 60°C to get a dried solid product then stored in dried bottles.

Preparation of Callus Crude Extracts:

This is done in a fashion similar to that of plant extraction except the callus was dried at first by freeze drying using Freeze dryer and then powdered and extracted with two different solvents, petroleum ether and methanol in soxhlet apparatus.

Antifungal activity:

Microorganisms

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The standard microorganisms used in this study are the following: Aspergillus niger(ATCC 9763), Aspergillus flavus (ATCC 9763), Aspergillus fumigatus and Candida albicans (ATCC7596). The test organisms were obtained from the Department of Microbiology, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan .

Preparation of fungal suspensions:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

In vitro testing of extracts for antifungal activity:

The paper disc diffusion method of (19) was adopted with some minor modifications to assess the antifungal activity of the prepared extracts. 20.0ml aliquots of the Sabouraud dextrose agar were distributed into sterile Petri-dishes. 0.1 ml of the standardized fungal stock suspension 10^8 –10^9 C.F.U/ ml were streaked on Sabouraud dextrose agar medium plates using sterile cotton swab. Sterilized filter paper discs(6 mm diameter) were soaked in the prepared extracts, then were placed on surface of the test fungal plates. Concentration used from each extract is 1.0mg dissolved in 1.0ml of the solvent and 5.0mg of extract in 1.0ml of solvent. The plates were incubated at 25 °C for two days for the Candida albicans and three days for Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus. After the incubation time the diameters of the inhibition zones were measured. The mean value was taken, positive results for each extract was carried out to know the activity of the different extract. .

Phytochemical screening:

Phytochemical screening was carried out for all the extracts to evaluate for the presence of different phytochemicals to ascertain the presence of secondary metabolites such as alkaloids, saponins, phenolic compound, tannins, flavonoids, steroids and terpenoid by using different standard methods with some modification. The methods described by (20- 21-22-23-24-25), respectively for the mentioned secondary metabolites.

Statistical Analysis:

The results were expressed as the mean ± standard error (SE) of three replicates and were analyzed using one-way ANOVA (Tukey's studentized range) using the program SPSS(26).

Results and Discussion:

The antifungal activity of extracts of four part of Jatropha curcas were assessed. Table (1) displays that the Petroleum ether extract of hypocotyls showed highest significant ($p<0.05$) antifungal activity with inhibition zone of (18.2±0.3) mm against Candida albicans and (18.0 ±0.4) mm against Aspergillus fumigatus compared to other extracts, while the petroleum ether extracts of seeds and Leaves showed no antifungal activity. These findings agree with that obtained by (27) . The Petroleum ether extract of Cotyledons showed moderate antifungal activity with inhibition zone of (14.0±0.4) and(13.3±0.4) mm against Aspergillus fumigatus and Candida albicans respectively. Methanol extract of hypocotyls explants showed high antifungal activity against Aspergillus fumigatus with inhibition zone of (16.8±0.4) mm. Aspergillus niger and Aspergillus flavus were the least sensitive microorganism to all extracts investigated in the current study as shown in table (1). Methanol extracts of all different parts of the plant used in this study showed antifungal activity against all fungal test, this findings agree with that concluded by (28), whom found that the methanol extract of J. curcas had the highest activity against both bacterial and fungal. This may be due to fact that, methanol is a universal solvent that dissolves all types of compounds capable of either polar, semi-polar and non-polar. Concentration of 100mg/l ml extract gave higher antifungal activity than 10 mg/l ml (table 1). The inhibitory activity of plant extracts is generally depends upon the concentration, type of parts used and microbes tested (29). The accumulation and concentration of secondary metabolites which are responsible for inhibitory activity is varied according the plant parts (30).

Table(1) Screening for antifungal activity of seeds , leaves and callus from

(hypocotyls cotyledons) extracts against standard Fungi.

Part used (extracted)	Extracts		Zone of inhibition(mm) ± SE**				
		Cons.	Microorganisms (fungi)				Control (solvent of extracts)
			A.niger	A.favus	A.fumigatus	Can. albicans	
Seeds	Petroleum ether	10mg	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		100mg	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

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	Methanol	10mg	8.3±0.2	8.5±0.2	7.3±0.3	7.3±0.3	0.0±0.0
		100mg	8.3±0.3	8.5±0.2	9.8±0.3	9.2±0.3	0.0±0.0
Leaves	Petroleum	10mg	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		100mg	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Methanol	10mg	6.6±0.2	9.0±0.3	8.2±0.3	7.3. ±0.3	0.0±0.0
		100mg	6.6±0.2	9.0±0.3	10.5±0.2	10.2±0.5	0.0±0.0
Hypocotyls Callus	Petroleum ether	10mg	9.0±0.3	0.0±0.0	16. ±0.4	16.3±0.4	0.0±0.0
		100mg	10.3±0.5	0.0±0.0	18.0±0.4	18.2±0.3	0.0±0.0
	Methanol	10mg	6.7±0.2	7.5±0.2	13.5±0.2	11.2±0.3	0.0±0.0
		100mg	8.5±0.2	7.5±0.2	16.8±0.4	14.3±0.3	0.0±0.0
Cotyledons callus	Petroleum ether	10mg	0.0±0.0	0.0±0.0	11.8±0.5	11.8±0.5	0.0±0.0
		100mg	0.0±0.0	0.0±0.0	14.0±0.4	13.5±0.4	0.0±0.0
	Methanol	10mg	9.0±0.3	8.0±0.2	11.2±0.3	11.0±0.4	0.0±0.0
		100mg	9.6±0.4	8.8±0.2	12.2±0.3	12.3±0.3	0.0±0.0

****SE= standard error of mean.**

A.n.= Aspergillus niger, A. f= Aspergillus flavus, A. fumg= Aspergillus fumigates, C.a.= Candida albicans.

(-) = no inhibition, Control = organic solvent (petroleum ether and methanol) , MIZD mm : >18mm: Sensitive,

MIZD mm: 14-18mm: Intermediate, MIZD mm :< 14mm: Resistant

Phytochemical screening:

Phytochemical screening of methanol and petroleum ether extracts of seeds, leaves and callus from (hypocotyls and Cotyledons) of Jatropha curcas revealed the presence of various secondary metabolites by positive reaction with the respective test reagent (Table 2).

Methanol extract of seeds showed presence of alkaloids, saponins, flavanoids, steroids and terpenoid. Except tannins and phenols which is absent from methanol seeds extracts, the presence of steroids compounds and alkaloids in the seeds has also been reported by some authors (31). Methanol extract of leaves showed presence of alkaloids, saponins, tannins, flavanoids, phenols, steroids and terpenoid. These results agree with those reported by (4). Methanol extract of hypocotyls showed presence of alkaloids, saponins, tannins, flavanoids, steroids and terpenoid, but phenols was absent from methanol extract of hypocotyls. The methanol extracts of callus derived from cotyledons showed presence of saponins, tannins, steroids and terpenoid , whereas alkaloids and flavanoids were absent from methanol cotyledons callus extracts. Petroleum ether extracts of leaves , seeds and callus showed presence of steroids and terpenoid only. Phytochemical screening results explain the correlation between the biological activity (antimicrobial) exhibited by this plant extracts and its detected constituents. The solvent play a vital role in the extraction of the plant constituents. The presence or absence of plant constituents depending on the polarity of solvent. Methanol is highly polar than petroleum ether, this may be the reason of the presence of more compounds when methanol is solvent compared to the petroleum ether solvent.

Table(2) Phytochemical screening of methanolic and Petroleum ether extracts of seeds ,

leaves and callus from (hypocotyls and cotyledons) of Jatropha curcas.

Test	SM	LM	HM	CM	SP	LP	HP	CP	Observation
Alkaloids	+++	+	++	-	-	-	-	-	White cream precipitate
Saponins	+	+	+	+	-	-	-	-	Persistent foam
Tannins	-	++	+	++	-	-	-	-	Bluish black colour
Flavnoids	++	++	+	-	-	-	-	-	Yellow precipitate
Phenoliccop.	-	+	-	+	-	-	-	-	Bluish black
Steroids	+	++	+	+	++	+	+	+	Reddish brown coloring

Terpenoid	+++	++	+	+	++	+	+	+	Reddish brown coloring

Key: (+) Positive Test, (-) Negative test & '+' low; '++' moderate; '+++' high;

SM= Seeds methanol extract, LM = Leaves methanol extract, HM = Hypocotyls methanol extract, CM = Cotyledons methanol extract.

SP= Seeds Petroleum ether extract, LP = Leaves Petroleum ether extract, HP= Hypocotyls Petroleum ether extract, C P3= Cotyledons Petroleum ether extract.

Conclusion:

The results of present study showed that 1- The methanolic extracts of different parts of Jatropha curcas have an antifungal activity against all fungal organisms tested. 2-Petroleum ether extract of hypocotyls callus only gave the highest antifungal activity against Candida albicans and (18.0 ±0.4) mm and Aspergillus fumigatus 3- Hypocotyls callus followed by Cotyledons callus are more active compared to the other parts of the plant.

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References:

[1] Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African Journal of Pharmacy and Pharmacology. 3**(2) :**058-062.

[2] Ekundayo, F. O., Adeboye, C. A., Ekundayo, E. A. (2011). Antimicrobial activities and phytochemical screening of pignut (Jatrophas curcas Linn.) on some pathogenic bacteria. Journal of Medicinal Plants Research. 5**(7):**1261-1264

[3] Nandani, D., Verma, R. N. and Batra, A. (2013). Isolation and identification of quercetin and emodin from Cassia tora L. Annals of Phytomedicine. 2**(2)** :96-104.

[4] Misra, M . (2010). Jatropha: The Biodiesel Plant Biology, Tissue Culture and Genetic Transformation – A Review Int. J. Pure Appl. Sci. Technol. 1(1) : pp. 11-24.

[5] Castello, M.C., Phatak, A., Chandra, N. and Sharon, M. (2002). Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L. Indian J Exp Biol, 1378-1381.

[6] Erturk, O., Kati, H., Yayli, N. and Demirbag, Z. (2006). Antimicrobial properties of Silene multifida (Adams) Rohrb. Plant extract. Turk J Biol. 17-21.

[7] Fyhrquist, P., Mwasumbi, L., Haeggstrom, C.A., Vuorela, H., Hiltunen, R. and Vuorela, P. (2002). Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. J Ethnopharmacology. 79:169-177.

[8] Rao, S. R., and Ravishankar, G. A. (2002). Plant cell cultures: chemical factories of secondary metabolites. Biotechnology Advances. 20**(2)** :101-153

[9] Alfermann, A.W., Petersen, M., and Fuss, E.(2003). Production of natural products by plant cell biotechnology: results, problems and perspectives. In: Laimer, M. and Rücker, W. (ed): Plant Tissue Culture 100 years since Gottlieb Haberlandt. Springer, Wien New York, pp. 153-166.

[1o] Miller, A. L .(1996). Antioxidant flavonoids: structure, function and clinical usage. Alt Med Rev 1: 103-111.

[11] Alfermann, A.W.,Petersen, M.(1995).Natural products formation by plant cell biotechnology. Plant Cell Tissue Organ Cult. 43:199–205.

[12] Dornenburg, H. and Knorr, D. (1997). Challenges and opportunities for metabolite production from plant cell and tissue cultures. Food Tech, **51:** 47- 54.

[13] Bolda, V. V., Botau, D., Szôllôsi, R., Petô, A., Gallé, A., and Tari, I. (2011). Studies on elemental composition and antioxidant capacity in callus cultures and native plants of Vaccinium myrtillus L. local populations. Acta Biol Szeged. 55(2):255- 259.

[14] Abouzid, S. F., El-bassuony, A. A. , Nasib, A., Khan, S., Qureshi, J. and Choudhary, M. I . (2010). Withaferin a production by root cultures of Withania coagulans. Int. J. Appl. Res. Nat. Prod. **3:** 23-27.

[15] Kalidass, C., Mohan, V. R. and Daniel, A. (2010). Effect of auxin and cytokinin on vincristine production by callus cultures of Catharanthus roseus L. (apocynaceae). Trop. Subtrop. Agroecosystems 12: 283-288.

[16] Duangporn, P. and Siripong, P. (2009). Effect of auxin and cytokinin on phyllanthusol Aproduction by callus cultures of Phyllanthus acidus Skeels. American-Eurasian. J. Agr. Environ. Sci, **5:** 258-263.

[17] Mantell, S. H., Smith, H. (1984) . Cultural factors that influence secondary metabolites accumulations in plant cell and tissue cultures. In: Mantell, S .H., Smith, H. (Eds.). Plant Biotechnology. Society for Experimental Biology, Seminar Series 18 Cambridge University Press, Cambridge. Pp. 75.

[18] Murashige, T.,and Skoog, F.(1962) . A revised medium for rapid growth and bioassays with tobacco tissue culture. . Plant Phys.**15:** 467-497.

[19] Kil, H. Y., Seong, E. S., Ghimire, B. K., Chung, I. M., Kwon, S. S. , Goh, E. J., Heo, K., Kim, M. J., Lim, J. D., Lee, D., and Yu , C. Y. (2009). Antioxidant and antimicrobial activities of crude sorghum extract, Food Chem.115 (**4) :** 1234-1239.

[20] Evans, W.C. (1997). Pharmacology. Harcourt Brace and Company. Asia, Singapore. 226.

[21] Kokate, C.K. (1994). Practical Pharmacognosy. 4th edition, Vallabh Prakashan, New Delhi, India. 124-125.

[22] Kokate, C. K. (1999). Practical pharmacognosy. Vallabh Prakashan Publication, New Delhi, India. 111-116.

[23] Wolfe, K., Wu, X. and Liu, R.H.(2003). Antioxidant activity of apple peels. J Agr. Food Chem**. 51:** 609-614.

[24] Peach, K. and Tracey, M. V. (1956). Modern methods of plant analysis, Springer Verlag, Berlin. **3:** 125-127.

[25] Harborne, J.B.A. (1992). Guide to modern technique of plant analysis. London: chapman and Hill-Phytochemical method. PP.279.

[26] Griffth, A. 2007. Spss for Dummies. Wiley Publishing, Inc. Indian Pdis, India. pp.363.

[27] Falodun, A., Agbakwuru, E.O.P., Ukoh, G.C . (2003). Antibacterial Activity of Euphorbia heterophylla Linn (Family Euphorbiaceae). Pak. J. Sci. Ind. Res. 46 **(6):** 471-472.

[28] Sriprang, N., Sriprang, T., Sumpradit, D. S. (2010). Antibacterial activities of crude extracts from physic nut (Jatropha curcas) seed residues. Science Asia 36:346-348.

[29] Balandrin, M.F., Jocke, A.J. and Wurtele, E. (1985). Natural plant chemicals: sources of industrial and mechanical materials. Science Asia. **228:** 1154-1160.

[30] Rekha ,R. (2010). Antimicrobial Activity of Different Bark and Wood of Premna serratifolia Lin. I. J. of Pharma and Bio Sciences.V1(1).

[31] Lotti, G., Navari-izzo, F. and Baragli, S. (1977). Variation in the sterol composition of castor oil during plant maturation . Review of the Society of Italian Science Aliment. **6:**351.