Biological Efficiency of *Calotropis procera* Extracts Against the Growth of Selected Fungal Plant Pathogens for Sustainable Agroecosystem Development

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Abstract

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Plant secondary metabolites and plant-based fungicides appear to be one of the better alternatives as they are known to have negligible environmental impact and health hazard to consumers, compared with synthetic pesticides. This study aimed at assessing the antifungal activities of crude extracts obtained from leaf, stem bark, and roots of *Calotropis procera* against the fungal pathogens *Aspergillus niger, Fusarium oxysporum*, and *Fusarium solani*. The stems, roots, and leaves of the selected plant species were shade-dried and ground to powder, and the bioactive components were extracted using ethanol (99.5%), hexane (99.8%), and distilled water. The antifungal activity of the extracts against the three selected pathogens were evaluated using the agar-well diffusion method and inhibitory zones were measured in millimeters at four different concentration levels (30, 40, 50, and 60 mg/ml). Carbendazim and sterile distilled water were used as positive and negative controls, respectively. Among the selected plant materials, stem bark extracts showed the highest yield (10.45%) in ethanol crude extracts, whereas, the lowest yield (1.45%) was for water crude extract from the roots. The bioassay studies revealed that the crude extracts of ethanol, hexane and water had antifungal activity on all three fungal species at all concentrations and with all solvents. Ethanol extracts had the highest growth inhibitory effects as compared to those of the hexane and water crude extracts. In this study, the selected solvent crude extracts had fewer antifungal activities than carbendazim. *A. niger*, *F. oxysporum* and *F. solani* growth was highly affected by ethanol leaf extract as compared with hexane and water extracts. Conversely, stem bark hexane extract showed higher antifungal activity only against *F. oxysporum*. Thus, among the selected plant parts of *C. procera*, leaves showed the highest antifungal activity compared with stem bark and root extract.

Keywords: Antifungal, *Calotropis procera*, organic solvents, plant pathogens.

Introduction

Fungi are the most widespread and destructive plant pathogens. The fungus *Aspergillus niger* is considered worldwide to be one of the most important pathogens of stored onions (black mold). Indications of its presence are the black spores of the organism that are formed on and between the outer papery scales. *Fusarium oxysporum* is an important pathogen which causes tomato vascular wilt disease. Fusarium species also infect potato and cause significant yield loss. The soil- and seed-borne pathogens had a negative impact on yield by preventing the development of potato sprouts (Pepeljnjak & Segvic, 2003). Chemical fungicides cause serious environmental pollution problems. The toxic effect of synthetic chemicals can be overcome by searching for new fungicides, which are eco-friendly and effective. Identification of natural fungicides from plant sources would be a safer alternative to hazardous chemical fungicides. Extracts obtained from traditional medicinal plants are potential sources of new antimicrobial agents (Shahidi *et al*., 2004). Plant secondary metabolites and plant-based pesticides appear to be one of the better alternatives as they are known to have negligible environmental effects and hazards to consumers in contrast to synthetic pesticides (Neerman, 2003).

Numerous plant pathogenic microorganisms and insect pests have established resistance against chemical pesticides. This seriously hinders the management of crop diseases and agricultural products (Satish *et al*., 2007). Because of these adverse features of synthetic fungicides, there is a need to develop substitute treatments that are less hazardous to humans, animals, and are less harmful to the environment. Within this context, plant-produced compounds are of interest as a potential source of safety or more effective substitutes for synthetically produced antimicrobial agents. Although the selected medicinal plant (*Calotropis procera*) has been used traditionally as an herbal remedy, to date, there have been relatively few reports examining the antifungal activity of this plant's extract against several important crops fungal pathogens.

Materials and Methods

Experimental design

The research was designed based on the complete randomized design (CRD) in the laboratory. The treatments included one plant divided into three parts each, and extraction was made by three solvents and tested against three pathogens in three replications. Sterilized distilled water was used as a negative control and carbendazim as a positive control.

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Sample collection and preparation of plant materials

The leaves, roots, and stem barks of *Calotropis procera* (Sodom apple or Tobiaw) were randomly collected from Haramaya University and Dire Dawa. Ethiopia. The plant materials were identified and authenticated at Haramaya University herbarium with the help of a plant taxonomist. The collected plant parts of *C. procera* were separately washed thoroughly with tap water followed by sterile distilled water to remove debris and dust particles and cut into smaller sizes using a sterile knife. Then the plant's leaves, roots, and stem barks were dried under shade on a paper towel for two weeks at room temperature. The resulting dry parts were ground into a fine powder (particle size 20 mesh) with the help of a suitable sterile grinder, and then stored in sterile airtight containers according to Singh *et al*. (2007).

Preparation of plant crude extracts using organic solvents

Twenty grams of each of the coarsely powdered plant materials (leaves, roots, and stem barks) of *C. procera* was suspended in 100 ml of different solvents, i.e., ethanol (99.5%) and hexane (99.8%), separately in 250 ml conical flasks (Cheesbrough, 2002). The suspended plant materials were placed on a rotary shaker at 190-220 rpm for 72 hrs. at room temperature (Newton *et al*., 2002). Muslin cloth was then used to filter the plant residue, and the filtrate obtained was further purified by filtration through Whatman No.1 sterile filter paper, and the resulting filtrates were collected as sources of crude extracts. The filtrates were concentrated under reduced pressure in a rotary vaporizer under vacuum at 40ºC and the gummy residue was further dried in a water bath at 40–50°C for 24 hrs until the solvents were completely removed (Amit *et al*., 2015). After the evaporation of solvents, the remaining crude extracts were weighed using a balance, and the resulting weights were recorded. These crude extracts were further diluted with 10 ml of sterile distilled water and kept in sample vials with stoppers at 4°C until they were used against the test pathogens (Dewanjee *et al*., 2007).

Preparation of water extracts

Crude extracts of the leaf, stem bark, and root of the *Calotropis procera* plant were prepared by adding 100 ml of sterile distilled water to 20 g of coarsely powdered plant material in a 250 ml conical flask. The resulting suspension was then shaked at 121 rpm for 24 hrs. Muslin cloth was then used to filter the plant residue. The filtrate obtained was further purified by filtration through Whatman No.1 sterile filter paper. Then the solution was subjected to hot air water bath evaporation at 35°C. For one week, the remaining crude extracts were weighed and diluted with 10 ml of sterile distilled water, and then preserved in airtight bottles until further use in a refrigerator (De Flora & Izzotti, 2007).

Determination of antifungal activity

Extracts of *Calotropis procera* were tested for antifungal activity against the fungal pathogens *Aspergillus niger, Fusarium oxysporum*, and *F. solani*. The pure culture of selected fungal isolates was obtained from the plant pathology laboratory, Haramaya University, Ethiopia. The antifungal activity of *Calotropis procera* (leaf, stem bark, and root extracts) was determined, using the agar-well diffusion method (Perez *et al*., 1990). The extracts were dissolved in sterile distilled water to obtain a final concentration of 100 mg/ml. 30, 40, 50, and 50 mg/ml concentrations of different extracts were introduced into the well and plates were incubated at 28±2°C for 48 hours. Sterile distilled water was used as a negative control, and carbendazim was used as a standard fungicide at 50 ppm

Results

Yield of crude extracts of *Calotropis procera* leaves, stem bark and roots

Ethanol, hexane and water crude extracts of *C. procera* leaves, stem barks, and roots were obtained, and the results were summarized in Table 1. The yield (amount) of the crude extracts obtained ranged from 1.45 to 13.75% for *C. procera*. The results clearly showed that the percentage yield of the crude extracts of the different plant parts varied from solvent to solvent. In this study, ethanol showed the highest percentage yield of crude extract as compared with hexane and water extract. However, the lowest percentage of crude extract yield was noticed in water extracts. Moreover, among the selected plant parts, stem bark extracts showed the highest percentage yield (10.45% in ethanol extract) and the lowest percentage yield (1.45%) of crude extract was obtained from root extracts, especially in water extract.

Antifungal activities of crude extracts of the stem bark of *Calotropis procera* against the test organisms

In this study, the antifungal activity of the stem bark extract of *Calotropis procera* was observed against the selected fungal pathogens (*Aspergillus niger*, *Fusarium oxysporum* and *F. solani*) and the results obtained are summarized in Table 2. The antifungal activities depend on the level of concentration as well as the solvents used. The diameter of the inhibition zone was directly related to the level of crude extract concentration. The highest zone of inhibition (22.50 mm) was observed at the highest concentration level of crude extract (60 mg/ml). *Aspergillus niger* growth was highly affected by ethanol extract as compared with hexane and water extracts. The largest zone of inhibition (20.66 mm diameter) was noticed for the highest concentration level (60 mg/ml) of the ethanol extract, and the smallest zone of inhibition (7.50 mm diameter) was obtained for the hexane extract (30 mg/ml). Moreover, *Fusarium oxysporum* growth was highly affected by hexane extract as compared with ethanol and water extracts. The largest zone of inhibition (21.50 mm) was observed for the 60 mg/ml concentration in hexane extract. In addition, *Fusarium solani* growth was highly inhibited (22.50 mm zone of inhibition) by ethanol stem bark extract at the highest level of concentration (60 mg/ml).

Antifungal activities of crude leaf extracts of *Calotropis procera* against the test organisms

The ethanol leaf extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Aspergillus niger* (21.43 mm) and a minimum inhibition zone at 30 mg/ml

(14.80mm). Likewise, the hexane leaf extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Fusarium oxysporum* (25.50 mm) and a minimum inhibition zone at 30mg/ml (20.66 mm). The water leaf extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Fusarium oxysporum* (10.66 mm) and a minimum inhibition zone at 30 mg/ml (7.16 mm). In addition, *Fusarium solani* growth was highly inhibited (22.00mm zone of inhibition) by ethanol leaf extract at the highest level of concentration (60 mg/ml) (Table 2).

Antifungal activities of crude extracts of the *Calotropis procera* roots against the test organisms

The different solvent root extracts of *C. procera* were tested for their antimicrobial properties against the test pathogens (Table 2). The diameters of the zone of inhibition of the ethanol, hexane, and water root extracts were in the range of 6.13–14.33, 6.33–10.83 and 4.50–6.66 mm, respectively. All root extracts at concentrations of 30, 40, 50 and 60 mg/ml showed significant antibacterial activity against all three tested pathogens. The results obtained indicated that the ethanol root extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Fusarium solani* (14.33 mm) and a minimum zone of inhibition at 30 mg/ml against *Aspergillus niger* (6.13±0.31 mm). Similarly, a hexane root extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Aspergillus niger* (10.83 mm), and a minimum inhibition zone at 30 mg/ml against *Fusarium oxysporum* (5.46 mm). Moreover, a water root extract of *C. procera* showed a maximum inhibition zone at 60 mg/ml against *Fusarium solani* (6.66±0.41 mm), and a minimum inhibition zone at 30 mg/ml against *Aspergillus niger* (4.50±0.43 mm).

Discussion

This study has evaluated the antifungal activity of the *Calotropis procera* leaf, stem bark, and root plant extracts against three plant pathogens (*Aspergillus niger, Fusarium oxysporum* and *F. solani*) associated with important crops such as onion and potato. The biological activity of plant extract is mostly dependent on the plant material, the choice of solvent used, and the extraction procedure (Diego *et al*., 2021). Plant materials that may be fresh or dried can be used as a source for the extraction of secondary metabolites.

In this study, the extraction method used involved successive extraction with solvents of increasing polarity from a non-polar to polar solvents (hexane, ethanol, and water) to extract a wider polarity range of active compounds from different parts. Water extract showed the lowest percentage of crude extract yield compared with ethanol and hexane extracts. Among the different plant parts, stem bark showed the highest percentage of crude extract yield when extracted by ethanol, which is in agreement with previous findings (Cely-Veloza *et al*., 2023; Salim *et al*., 2023).

The present study revealed that the plant extracts obtained using organic solvents such as ethanol and hexane showed better results than water extracts. Cowan (1999) reported that most of the antibiotic compounds already identified in plants were aromatic or saturated organic molecules, which can easily be solubilized in organic solvents. Similar results also showed that alcoholic extracts had better antimicrobial activity than water extracts (Preethi, 2010). Previous studies also confirmed that various parts of *C. procera* (root, leaf, flower, and stem bark) showed antimicrobial activities (Kawo *et al*., 2011).

The changes in antifungal properties of plant extracts are attributable to the stage of the plant used, freshness, cleanliness of plant materials, in addition to physical factors such as temperature, light, soil type, and water, and dosage level (Brij *et al*., 2015).

This study showed that the different parts of *Calotropis procera* possess antifungal potential against *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium solani*, this may be due to the interruption of the cell wall formation which subsequently causes the outflow of cytoplasmic constituent as reported by several workers (Chima *et al*., 2016; Yahaya *et al.*, 2017). However, the higher efficacy of ethanol extract compared to the hexane and water extracts may be because different solvents have different polarities, hence different degrees of solubility of the various phyto-constituents. Based on the limited spectrum of activity of the other extracts (acetone and water) compared with the ethanol extracts, it was suggested that the active components are more soluble in ethanol than in the other solvents (Ekaiko *et al*., 2015). Highest inhibition of *Aspergillus niger*, *Fusarium oxysporum, and Fusarium solani* was obtained by the ethanol extract of *Calotropis procera* leaf. However, the least inhibition was exhibited by water extracts of roots against all the tested organisms. This may be due to the poor ability of water to extract the antifungal compounds present in the plant parts (Yahaya *et al*., 2017).

Table 1. The percentage yields of the crude extracts of the leaves, roots, and stem barks of *Calotropis procera*.

	Weight and percentage yield of crude extracts by different solvents					
	Ethanol		Hexane		Water	
Plant part	**Weight (g)**	**Yield (%)**	**Weight (g)**	**Yield (%)**	**Weight (g)**	**Yield (%)**
Leaf	1.65	8.25 cB	1.9	5.45 aB	0.53	2.65 aB
Stem bark	2.09	10.45 cC	1.32	6.60 bB	0.59	2.95 aB
Root	1.03	5.15b A	1.12	5.20 bA	0.29	1.45 aA

Values followed by the same small letters in the same row and values followed by the same capital letters in the same column are not significantly different at P=0.05.

Table 2. Antifungal activities of crude extracts of the stem bark of *C. procera* against the test organisms.

Tested organisms	Conc. of crude extracts (mg/ml)	Zone of inhibition (mm)				
		EsE	HsE	WsE	SDW	Carbendazim (0.1 mg/ml)
Stem bark						
Aspergillus niger	30	14.66±0.57 Cd	7.50±0.50 Ab	8.96±0.90 Aa	-	24.90 ±0.1 A
	40	15.21±0.02 Dd	8.42±0.00 Bb	9.81±0.33 Ca	-	
	50	19.33±0.12 Fd	10.30±0.31 Cb	10.32±0.42 Da	-	
	60	20.66±0.57 Gd	14.00±0.28 Db	13.16±0.28 Fa	-	
Fusarium oxysporum	30	10.70±0.26 Ac	14.66±0.57 Ed	8.66±0.57 Aa	-	24.50±0.50 A
	40	12.41±0.32 Bc	16.45±0.14 Fd	9.51±0.67 Ca	-	
	50	17.12±0.23 Ec	18.55±0.00 Gd	10.01±0.66 Da	-	
	60	18.83±0.76 Fc	21.50±0.50 Hd	11.33±0.58 Ea	-	
Fusarium solani	30	10.83±0.76 Ab	14.83±0.76 Ed	9.00±0.90 Ba	-	24.10±10 B
	40	12.55±0.12 Bb	15.00±0.00 Ed	11.50±0.43 Ea	-	
	50	15.45±0.22 Db	16.60±0.65 Fc	13.10±0.55 Fa	-	
	60	22.50±0.50 Hc	19.00±1.00 Gb	14.00±1.00 Ga	-	
Leaf						
Aspergillus niger	30	14.80±0.34 Ad	11.43±0.40 Aa	10.80±0.72 Da	-	24.90 ±0.1 A
	40	18.51±0.06 Bd	15.42±0.01 Db	11.77±0.22 Ea		
	50	19.43±0.13 Cc	18.60±0.33 Fb	13.00±0.32 Fa	-	
	60	21.43±0.45 Fd	19.83±0.76 Gb	14.66±0.57 Ga	-	
Fusarium oxysporum	30	20.66±0.57 Ed	14.20±0.34 Cb	7.16±0.57 Aa	-	24.50±0.50 A
	40	21.41±0.41 Fd	17.00±0.33 Eb	8.55±0.77 Ba	-	
	50	22.98±0.53 Gc	20.00±0.02 Gb	9.11±0.26 Ca	-	
	60	25.50±0.45 Hd	21.83±0.76 Hb	10.66±0.57 Da	-	
Fusarium solani	30	18.53±0.50 Bc	11.50±0.36 Aa	10.33±0.57 Da	-	24.10±10 B
	40	20.15±0.44 Dc	13.01±0.05 Ba	11.40±0.93 Ea	-	
	50	21.87±0.66 Fc	15.50±0.76 Da	14.10±0.65 Fa	-	
	60	22.00±1.00 Gc	17.80±0.72 Ea	15.66±0.57 Ga	-	
Root						
Aspergillus niger	30	6.13±0.31 Ab	6.33±0.41 Bb	4.50±0.43 Aa	-	24.90 ±0.1 A
	40	8.11±0.00 Bb	7.89±0.58 Db	4.87±0.44 Aa	-	
	50	9.33±0.17 Cb	9.70±0.63 Fb	4.99±0.52 Aa	-	
	60	11.06±0.11 Eb	10.83±0.28 Hb	5.43±0.40 Ba	-	
Fusarium oxysporum	30	6.26±0.27 Ac	5.46±0.45 Ab	4.73±0.30 Aa	-	24 .50±0.50 A
	40	8.61±0.74 Bc	6.10±0.11 Bb	4.85±0.97 Aa	-	
	50	11.68±0.93 Fd	8.00±0.05 Db	5.61±0.76 Ba	-	
	60	12.23±0.40 Gc	9.50±0.50 Fb	6.20±0.20 Ca	-	
Fusarium solani	30	10.76±0.68 Dc	5.50±0.45 Ab	4.93±0.35 Aa	-	24. 10±10 B
	40	11.16±0.47 Ec	7.01±0.00 Cb	5.40±0.13 Ba	-	
	50	13.47±0.16 Hc	8.55±0.66 Eb	6.08±0.62 Ca	-	
	60	14.33±0.57 Ic	10.23±0.32 Gb	6.66±0.41 Da	-	

EsE = Ethanol stem extract, HsE= Hexane stem extract, WsE= Water stem extract, Carbendazim = positive control, SDW= sterile distilled water (negative control). - = no inhibition zone. Values followed by the same small letters in the same row and values followed by the same capital letters in the same column are not significantly different at P=0.05.

الملخص

بيرغاشيوا، أ.، ك. أميها وم. مانيكاندان. 2025. الكفاءة الحيوية لمستخلصات نبات العشار (*Calotropis procera*) ضدّ نمو بعض مسببات أمراض النبات الفطرية لتطوير نظم زراعية بيئية مستدامة. مجلة وقاية النبات العربية، 43(1):126−131. https://doi.org/10.22268/AJPP-00129

تعدّ المستقلبات الثانوية النباتية ومبيدات الفطور من أصلٍ نباتي واحدةً من أفضل البدائل بوصفها ذات تأثيرات بيئية ضئيلةً ومخاطر صحية قليلة على المستهلكين مقارنةً بالمبيدات الحشرية المصنعة. هدفت هذه الدراسة إلى تقييم أداء المستخلصات الخام المضادة للفطور والتي تمّ الحصول عليها من أوراق ولحاء الساق وجذور نبات العشار (*Calotropis procera*) ضدّ مسببات الأمراض الفطرية *Aspergillus niger*، *Fusarium oxysporum* و *Fusarium solani*. تمّ تجفيف السوق

والجذور والأوراق في الظلّ وطحنها إلى مسحوق، واستخلاص المكونات النشطة حيوياً باستخدام الإيثانول 99.5%، الهكسان 99.8% والماء المقطر. تمّ تقييم النشاط المضاد للفطور للمستخلصات ضدّ مسببات الأمراض الثلاثة المختارة باستخدام طريقة الانتشار عبر الآجار، وتمّ قياس المناطق المثبطة بالملليمتر عند أربعة تراكيز مختلفة (30، 40، 50 و 60 مغ/مل). استُخدم مبيد الكاربندازيم والماء المقطر المعقم كشواهد إيجابية وسلبية، على التوالي. من بين المواد النباتية المختارة، أظهر مستخلص لحاء الساق الايثانولي أعلى إنتاجية 10.45%، بينما كانت أقل إنتاجية 1.45% لمستخلص الجذور المائي. أظهرت دراسات الاختبارات الحيوية أن المستخلصات الخام للإيثانول، الهكسان والماء ذات نشاط مضاد للفطريات إزاء الأنواع الفطرية الثلاثة وبجميع التراكيز ومع جميع المذيبات. كان لمستخلصات الإيثانول أعلى التأثيرات المثبطة للنمو مقارنة بتلك الخاصة بمستخلصات الهكسان والماء. في هذه الدراسة، كان للمستخلصات الخام المذيبة المختارة نشاطاً مضاداً للفطور بنحوٍ أقل من الكاربندازيم. تأثر نمو *Aspergillus niger*، *Fusarium oxysporum* و *Fusarium solani* بشكل كبير بالمستخلص الإيثانولي للأوراق مقارنةً بمستخلصات الهكسان والماء. على العكس من ذلك، أظهر مستخلص الهكسان للحاء الجذع نشاطاً مضاداً للفطور بنحوٍ أعلى فقط ضدّ الفطر *F. oxysporum*. كما أظهرت الدراسة أن مستخلص أوراق نبات *C. procera* ذو نشاط مضاد للفطور أعلى مقارنةً مع مستخلصي لحاء الجذع والجذر.

كلمات مفتاحية: مضاد للفطور، *Calotropis procera*، المذيبات العضوية، مسببات الأمراض النباتية.

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