WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 6, 42-50. Research Article ISSN 2277– 7105

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCRENNING OF EUPHORBIA LATHYRIS SEEDS

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Article Received on
27 March 2016,
Revised on 18 April 2016,
Accepted on 09 May 2016
DOI: 10.20959/wjpr20166-6229

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ABSTRACT

In this study we have carried out antimicrobial investigation and phytochemical analysis of *Euphorbia lathyris* seeds (Euphorbiaceae), *Euphorbia lathyris* seeds was extracted with 70% ethanol and then was fractionated with different solvents according to polarity start with petroleum ether, chloroform and ethanol, antimicrobial investigation was done for crude extract and all three fractions using cup plate diffusion method by selected six pathogens, four bacterias (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi (*Candida albicans and Aspergilles niger*), crude extract and all fractions show antibacterial and antifungal activity aganist all bacterial and fungal, minimum inhibitory concentration (MIC) was done for crude and fractions compared with two antibiotics gentamycine and ciprofloxacin and also antifungal ketoconazole as standards, activity clear in first and second dilution for crude and fractions,more inhibition zone clear in bacillus .Phytochemical screening is an important step for the isolation of bioactive chemical constituents, phytochemicalScreeningwas done for crude extracts, which was extracted with 70% Ethanol was showed the presence of alkaloids, phytosterols, carbohydrates, flavanoides, coumarine and diterpenes.

KEYWORDS: Antimicrobial, medicinal plants, *Euphorbia lathyris*, MIC, phytochemical screening.

INTRODUCTION

Increased antibiotic resistance has become a global concern, coupled with the problem of microbial resistance, thus highlighting the need to develop novel antimicrobial drugs that are

not only active against drug resistant microbes, but more importantly, kill resistance micro-organisms and shorten the length of treatment.[1] Medicinal plants may constitute a reservoir of new antimicrobial substances to be discovered.[2] Many commercial drugs used in modern medicine were derived from plants following ethno-botanical and ethno-medical knowledge[1] Biological activity of plants is attributed to the class and concentration of phytochemical constituents which makes some plant extracts exhibit a variety of activities[1] *Euphorbia lathyris* is a species of spurge native to southern Europe (France, Italy, Greece, andpossibly southern England), northwest Africa, and eastward through southwest Asia to western China.Other names occasionally used include Gopher Spurge,Gopher Plant or Mole Plant. Its belong to family (Euphorbiaceae).[3] the folk medicinal uses of euphorbia species include the treatment of infections, gonorrhoea, migraine, intestinal parasites, rheumatism, snake-bites, asthma, constipation, cough, sores and skin diseases.[4] the aim of study is to evaluate antimicrobial activity and phytochemical composition of *Euphorbia lathyris* seeds.

MATERIAL AND METHODS

PLANT MATERIAL

Euphorbia lathyris seeds was collected from herbalist,then identified and authenticated by dr. Mubark Ahmed, the medicinal and aromatic plant research institute,Khartoum,sudan.

Preparation of extract: Extraction was done to *Euphorbia lathyris* seeds according to[5] with some modification.

Phytochemical screening: General phytochemical screening was done according to.[6] as follow.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

A-Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

B-Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

C-Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

D-Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

A-Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

A-Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of saponins: A-Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

B-Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols: A-Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

B-Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of flavonoids: A-Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

B-Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of diterpenes

A-Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Antimicrobial activity

Bacterial microorganisms: Two gram positive *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa*.

Fungal microorganisms: Two type of fungal *Candida albicans* and *Aspergillus niger*.

Preparation of the test organisms

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (**7**). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension: 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 suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

In vitro testing of extracts for antimicrobial activity

Testing for antibacterial Activity

The cup-plate agar diffusion method.[8] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes.

The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each of the oils dilutions in methanol using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours.

Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Testing for antifungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for the *Candida albicans* and three days for *Aspergillus niger*.[7]

RESULT AND DISCUSSION

RESULTS

Phytochemical screening

Table(1) phytochemical screening tests of *Euphorbia lathyris* seeds

Phytochemical group	test	reaction
Alkaloides	Mayer's	+
	Wagner's	+
	Dragendroff's	+
	Hager's	+
Carbohydrates	Fehling's	+
Glycosides	Modified Borntrager's	_
Saponins	Froth Test	_
	Foam	_
Phytosterols	Salkowski's	+
	Libermann Burchard's	+
flavonoids	Alkaline Reagent Test	+
	Lead acetate	+
diterpenes	Copper acetate	+

Key: +positive reaction,_negative reaction.

Antimicrobial activity: Antimicrobial avtivity was done for crude extract and three fractions of petroleium ether, chloroform and ethanol, selected four bacteria(*Staphylococcus aureus, Bacillus subtilis, Escherichia coliand Pseudomonas aeruginosa)*and two fugi(*Candida albicans and Aspergilles niger).*

Extracts and all fraction was give the activity aganist all bacteria and low activity aganist fungi in compareson with ciprofloxacin and gentamycin as antibacterial standard and ketoconazol and itraconazol as antifungal standard.

Table(2): invitro antibacterial activity aganist standard organism

(MIDZ)mm				
Sample	***S.aures***	***B.subtillis***	***P.aeruginosa***	***E.coli***
crude	14	19	14	14
p.ether	19	17	17	17
chloroform	13	18	14	14
ethanol	12	17	14	14
ciprofloxacin	21	14	27	20
gentamycin	30	18	21	25

S.aures=Staphylococcus aures, B.subtillis=Bacillus subtillus, P.aeruginosa=Pseudomonas aeruginosa, E.coli=Escherichia coli

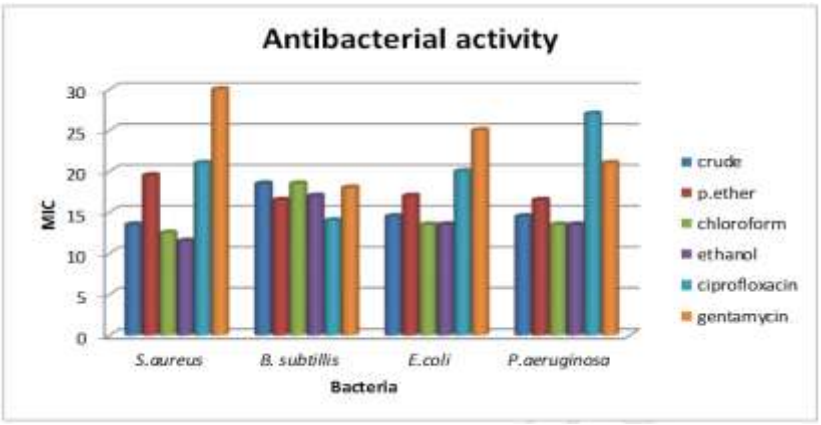

Figure (1)

Table (3): antifungal activity

(MIDZ)mm		
Sample	***C.albicans***	***A.niger***
crude	12	13
p.ether	12	12
chloroform	12	12
ethanol	12	12
ketoconazol	21	16
itracnazol	34	20

C.albicans=Candida albicans, A.niger= Aspergillus niger.

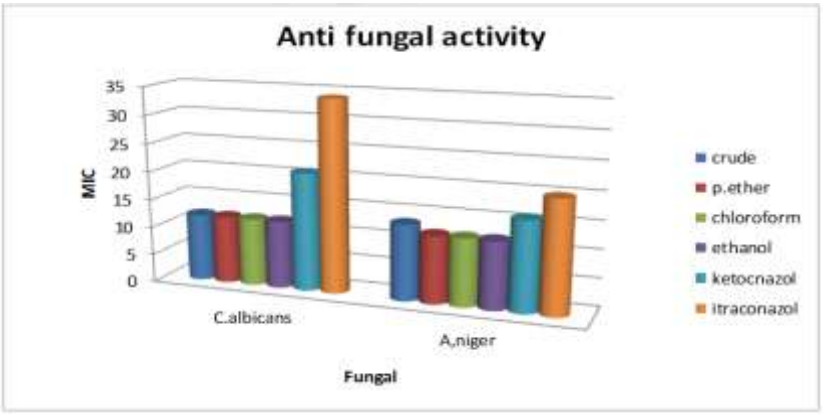

Figure (2)

Table (4): Minimumm inhibitory concentration of crude and fractions

	MIC			
	crude	**p.ether**	**chloroform**	**ethanol**
S.aureus	100	25	100	100
B.subtillus	25	12.5	25	12.5
E.coli	50	50	100	50
P.aeruginosa	50	50	100	50
C.albicans	100	100	100	100
A.niger	100	100	100	100

S.aures=Staphylococcus aures, B.subtillis=Bacillus subtillus, P.aeruginosa=Pseudomonas aeruginosa, E.coli=Escherichia coli, C.albicans=Candida albicans, A.niger= Aspergillus niger.

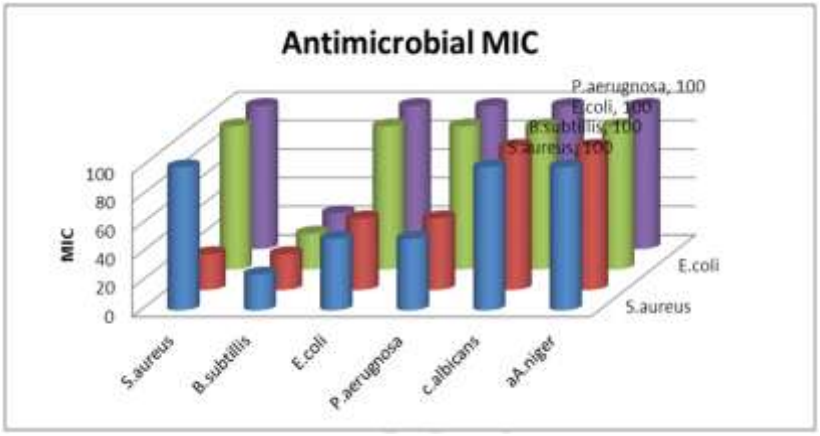

Figure (3)

DISCUSSION

In phytochemical screening of *Euphorbia lathyris* presence the alkaloids, carbohydrates, phytosterol, flavanoids and diterpenes this agree with[9] and disagree in the presence of glycoside.and absence of glycoside may be due to unadiquite temperature to hydrolysis.Also phytochemical screening agree with.[10]

Antimicrobial activity of ethanolic crude extract and their fractions give activity on selected four bacteria and low activity aganist two fugal. Crude and fractions give high activity aganist gram positive bacteria than gram negative. *Bacillus subtillus* (crude 19mm,25mg/ml.petroleum ether17mm, 12.5mg/ml. chloroform18mm, 25mg/ml. ethanol17mm, 12.5mg/ml), *Staphylococcus aureus* (crude14mm, 100mg/ml. petrolium ether19mm, 25mg/ml. chloroform13mm, 100mg/ml. ethanol12mm, 100mg/ml) *Escherichia coli* (crude14mm, 50mg/ml. petrolium ether14mm. 50mg/ml. chloroform14mm, 100mg/ml. ethanol14mm, 50mg/ml) *Pseudomonas aeruginosa* (crude14mm, 50mg/ml. petrolium ether17mm, 50mg/ml. chloroform14mm, 100mg/ml.etanol14mm, 50mg/ml). for fungal tow *Candida albicans* and *Aspergillus niger* give the same results for crude and fraction(12mm.100mg/ml).

Presence of phenolic compounds can obtain antimicrobial activity more than in results ,this indicate compound is alead compound.

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