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Krishnananda Pralhad Ingle
PhD Research Scholar, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Amit Gulabrao Deshmukh
Assistant professor, Nagarjuna Medicinal and Aromatic Plant Division, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dipika Ashokrao Padole
Junior Research Assistant, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Mahendra Shankarrao Dudhare
Assistant professor, Vasantrao Naik College of Agril. Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Mangesh Pradip Moharil
Assistant professor, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Vaibhav Chandrakant Khelurkar
Junior Research Fellow, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dhiraj Raghunathrao Gangtire
MSc Student, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Correspondence
Krishnananda Pralhad Ingle
PhD Research Scholar, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Bioassay guided fractionation of antifungal activity of *Jatropha curcas*

Krishnananda Pralhad Ingle, Amit Gulabrao Deshmukh, Dipika Ashokrao Padole, Mahendra Shankarrao Dudhare, Mangesh Pradip Moharil, Vaibhav Chandrakant Khelurkar and Dhiraj Raghunathrao Gangtire

Abstract
Bioactive constituents from plants are an integral component of research and development in the pharmaceutical and agrochemical industry. Plants face many stresses in their life cycle and in the process produce secondary metabolites. These secondary metabolites are not important for the metabolic functions of the plant but help to face many stressful conditions like diseases, pests, etc. Some of these secondary metabolites have capacity to fight microorganisms and thus can be used as antimicrobial agents. The present study has exploited the probability of having any antimicrobial molecules present in the *Jatropha curcas.* In the present study root, leaf, bark, seed, and seed coat of *Jatropha curcas* were extracted with methanol using reflux extraction method. The crude methanolic root extract was found to have better antifungal activity (upto 23.1% growth inhibition) against *Rhizoctonia*. The root extract when fractionated by column chromatography showed upto 72.72 % growth inhibition against *Fusarium udum* and 41.17% against *Rhizoctonia bataticola* in toluene fraction 1. It was further screened for HPTLC and FTIR to characterize fraction in functional groups.

Keywords: Bioassay guided fractionation, antifungal activity, *Jatropha curcas*

Introduction
Plants have always been among the common sources of medicines, either processed as traditional preparations or used to extract pure active principles. Because of the large chemical diversity among natural products, many research groups screen plant extracts in their search for new promising therapeutic candidates for infectious diseases (Abreu *et al*. 2004) [1]. Plants are the richest source of organic chemicals on earth and they are claimed to produce a wide variety of secondary metabolites which are used as defensive weapons. The importance of research on several products other than protection chemicals have been realized in recent years due to the hazards of toxic chemicals to human beings and animals. Botanical Possess the great potentialities, being used as bactericide and fungicide without any adverse effect on the environment for the management of plant disease. (Pandya *et al*. 2009) [15]. Because of available antimicrobials failure to treat infectious diseases, many researchers have focused on the investigation of natural products as source of new bioactive molecules (Recio *et al*.1989, Silver *et al*. 1993) [16, 17]. *Jatropha curcas* is commonly called physic nut, purging nut or pig nut (Orwa *et al*. 2009). In the present study *Jatropha curcas* selected for the assessment of antifungal activity. *Jatropha curcas* is a source of secondary metabolites of medicinal importance. The leaf, fruits, latex and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that exhibit wide ranging medicinal properties. The plant product exhibit antimicrobial activity. The seed of the plant are not only the source of biodiesel but also contains several metabolites of pharmaceutical importance. (Debnath and Bisen, 2008). The antifungal property of crude extracts of root, leaf, bark, seed, and seed coat has been studied as part of the exploration for new and novel bio-active compounds.

Material and methods
Collection of samples and culture
The experiment was conducted at Biotechnology center, Department of Agricultural Botany, Dr. PDKV., Akola. The plant materials i.e root, leaf, bark, seed, and seed coat of *Jatropha curcas* were collected from the college of Forestry and two fungal cultures viz., *Rhizoctonia bataticola* and *Fusarium udum* were collected from the Department of Plant Pathology, Post Graduate Institute, Dr. PDKV, Akola.

Methods

Bioassay guided fractionation approach was used to isolate antifungal activities of various tissues of *J. curcas*. The tissues were first extracted with methanol by soxhlet extraction and then crude extracts were assayed for antifungal activity using cup diffusion and poison food technique. The active extract was further purified by solvent extraction method and again assayed for antifungal activity.The antifungal component was further subjected to open column chromatography and the fractions so obtained were subjected to antifungal assays. Appropriate extracts and their fractions were analyzed by phytochemical screening using HPTLC and FTIR.

Preparation of extract by Soxhlet extraction

For extraction purpose, root, leaf, bark, seed, and seed coat were used. The plant parts were air dried and powdered using mixer grinder. Ultimately the dried powdered was used for further extraction by solvent. Soxhlet extraction carried out using methanol as solvent using Universal Extraction System (Buchi) (Harborne, 1973).

Soxhlet extraction procedure

Soxhlet extraction was carried out with Universal Extraction System (Buchi). Ten grams dried powder (root, leaf, bark, seed, and seed coat) was taken in glass thimble and extracted with polar and non-polar solvents such as methanol, aqueous methanol, acetone, ethyl acetate and hexane. The procedure was carried out for 10 cycles for each extract and the temperature was adjusted just below the boiling point of the respective solvents. Further the solvent from each extract was evaporated and dried at room temperature (Harborne, 1973). The dried extract weighted and dissolved in universal solvent DMSO (dimethylsulfoxide) and methanol and further used for screening of antifungal activity.

Screening for antifungal activity by cup diffusion assay

The crude methanolic extracts were screened for antifungal activity against selected fungi by cup diffusion method (Kavwnagh, 1963) against fungal cultures viz., *Rhizoctonia bataticola* and *Fusarium udum*. 25 mL of potato dextrose broth was prepared and sterilized in autoclave at 121^0C (15 lbs/ sq. inches) for 20 minutes. The broth was inoculated aseptically with a loop full culture of above mentioned test organisms and incubated for 48 hrs in orbital shaker incubator at 37^0C with continuous shaking at 150 rpm. Potato dextrose agar was dissolved and distributed in 25 ml quantities in 100 ml conical flasks and sterilized in autoclave. This medium was then inoculated with extracts (root, leaf, bark, seed, and seed coat) at various concentration viz.,1%, 2.5%, 5%, 7.5%, 10%, and 15% level using 48 hrs old cultures of the test organism produced as above aseptically and poured into sterile petri dishes of 4 inches size and allowed to set at room temperature for 30 minutes. Five cups of 8 mm diameter at equal distance from each other were made with the help of cork borer in each plate, one cup was used for control i.e. DMSO, other for standard tricyclazol (0.06%), and remaining cups were used for different concentrations of each test samples. The plates so prepared were kept for 90 min at 4^0C in refrigerator for diffusion of the test compounds and incubated for 72 hrs at 29^0C in incubator. After 72 hr, zone of inhibitions were examined and recorded. The experiments were performed in duplicate and average diameter of the zones of inhibition was calculated. The crude methanolic fraction was further purified using bioassay guided fractionation procedure.

Purification of extract by bioassay guided fractionation procedure

Few amount (2 gm) of methanolic root extract was taken and triturate with MeOH:H_20 (4:1) ratio and filtrated, the residue was separated and considered as fraction 1. The filtrate then acidified with 2M H_2SO_4 and was extracted again with $CHCl_3$ for three times. Thus chloroform and aqueous acid layer get separated. Chloroform was dried and evaporated and considered as fraction 2a and aqueous acid layer then basified with the NH_4OH_2 and was extracted again with chloroform and methanol for two times. Finally chloroform-methanol and aqueous basic layer get separated which were considered as fraction 2b and fraction 3 respectively (Harborne, 1998). Finally fraction 2a and 2b mixed together and considered as fraction 2.

Screening for antifungal activity after solvent extraction by poison food techniques

Solvent fractions were screened for antifungal activity against selected fungi by poison food technique (Nene and Thapliyal, 1971) against fungus *Rhizoctonia bataticola.* Calculated amount of plant extracts was incorporated in sterilized molten 100 ml of PDA medium was poured in each sterilized petriplates. A 5 mm disc of *Rhizoctonia bataticola* was inoculated at the centre of the petriplates. The pathogen inoculated medium containing DMSO as a control. Plates were inoculated at 28 ± 1^0C. Experiments were carried out in duplicates. The average growth of the colonies was measured after 72 hr of inoculation. The percent inhibition of the growth was calculated as follows.

$$I = \frac{C - T}{C} X100$$

Where,

I = Percent Inhibition C = Control T = Treatment

Phytochemical screening of solvent extracted fraction

Aqueous basic fraction after solvent extraction were then subjected to phytochemical screening using standard method described by (Harborne, 1998) for the presence or absence of various secondary metabolites.

Partial purification of solvent fractionated bioactive extract by column chromatography

Preparation of column

Thirty five grams of silica gel (mesh 60-120) for column chromatography was activated by keeping it in hot air oven for 2 hrs at 110^0C. The silica gel was cooled and 20% slurry was prepared with methanol. It was kept in ultrasonic bath for 30 min for degassing. The slurry was slowly poured in a column (20:1.5 cm, height: diameter). The silica gel was then allowed to settle with intermittent tapping to remove any air bubble. The column was then equilibrated with toluene.

Preparation of sample and loading

Two grams of the extract was weighed accurately and dissolved in 2 ml methanol and 2 ml DMSO. The extract was concentrated to about 2 ml using vacuum evaporator this was loading sample. It was loaded on the top of the column slowly, without disturbing chromatographic bed.

Solvent systems and column development

Various solvent systems were used to fractionate the root extract and extraction yield was evaluated. These solvent

systems are given in Table 1 in sequence. These fractions were collected for further assay. The fractions were concentrated by vacuum concentrator and yields were recorded for each fraction.

Table 1: Solvent systems for column chromatography of solvent extracted fraction of (Root extract)

SN	Solvent System	Ratio
1	Toluene	100
2	Ethyl acetate	100
3	Methanol	110
4	Methanol :Water	50:50
5	Water	60

High performance thin layer chromatography (HPTLC) studies of extracts

Sample preparation and application on HPTLC plates

Hundred milligrams of each crude methanolic extract (root, leaf, bark, seed, and seed coat), solvent extracted fractions and column chromatography fractions were weighed accurately and added in 5 ml dimethylsulfoxide and diluted to 10 ml by methanol so that the final concentration of the extract was 10 mg/ml. The extracts were applied on 20 x 10 precoated Silica Gel G 60 F_{254} HPTLC plates (Merck) using Linomat V (Camag) sample applicator in the form of 8 mm bands with a distance of 6 mm between the bands (Camag, 1996) [4].

Solvent system and plate development

Solvent system was prepared using Chloroform: Methanol: Ammonia in the ratio of 6.5:2.5:0.5. Ten milliliters of the solvent system was added to the solvent chamber. A Whattman filter paper was kept in the chamber and the chamber was closed with the glass lid for saturation. After half an hour the sample is then applied with the sample applicator machine. In between, HPTLC plate (silica gel coated with aluminium sheet) was kept in the solvent chamber for presaturation with the solvent system. The solvent was allowed to run till 80% of the vertical distance of the plate. The plate was removed and dried using a hair drier so that all the solvent from the plate was evaporated.

Visualization and analysis of secondary metabolites from *J. curcas*

The developed plate was visualized at UV@254 nm and UV@366nm using a photo documentation system (Camag). The densitogram of the developed plate was taken using Scanner 3 (Camag) and the number of bands was measured. Anisaldehyde -sulphuric acid was used as a visualizing agent for derivatization of plates.

Fourier transform infrared spectroscopic analysis of bioactive fraction (FTIR)

Fourier transform infra red spectroscopy is a valuable tool for the identification of functional groups present in the molecule. It helps for identification and structure determination of the molecule. Since good antifungal activity was observed in case of column fraction 1 (Toluene fraction 1) it was subjected to Fourier transform infra red (FTIR) spectroscopic analysis which helps in characterizing the fraction in terms of functional groups (Anonymous, 2009) [2].

Result and discussion

Screening of antifungal activity of crude methanolic extract using cup diffusion method:

The antifungal assay was done using different concentrations **(**1%, 2.5%, 5%, 7.5%, 10%, and 15%) of root, leaf, bark, seed, and seed coat extract. 5% concentration of each extract found better amongst all concentrations used and hence screen for antifungal assay.

Amongst all, methanolic extract of root was found to have better antifungal activity with 23.1% and 17.7% growth inhibition as against standard tricyclazol 32.6% against *Rhizoctonia bataticola* and *Fusariu mudum* respectively. The lowest growth inhibition of *Rhizoctonia bataticola* was observed with bark extract (3.6 %), while in case of *Fusarium udum* it was with seed extract (1.7 %). The percent growth inhibition of different extracts of *J. curcas* mentioned in Table 2 and figure 1.

Table 2: Screening of antifungal activity @ 5 % concentration.

SN	Extract	Microorganisms	Growth inhibition %
1	Seed	*Rhizoctonia bataticola*	4.2
		Fusarium udum	1.7
2	Bark	*Rhizoctonia bataticola*	3.6
		Fusarium udum	2.9
3	Seed coat	*Rhizoctonia bataticola*	7.5
		Fusarium udum	2.8
4	Leaves	*Rhizoctonia bataticola*	7.9
		Fusarium udum	8.2
5	Root	*Rhizoctonia bataticola*	23.1
		Fusarium udum	17.7
6	Standard (Tricyclazol 0.06%)	*Rhizoctonia bataticola and Fusarium udum*	32.6

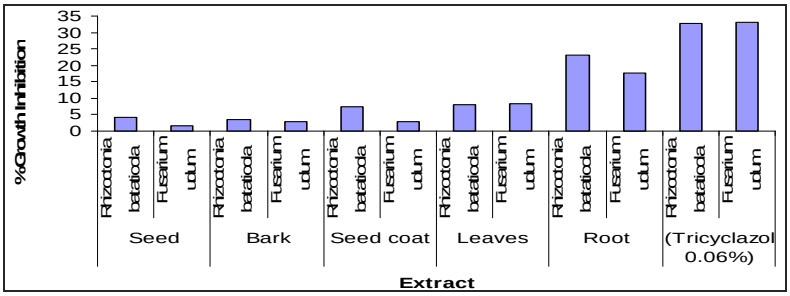
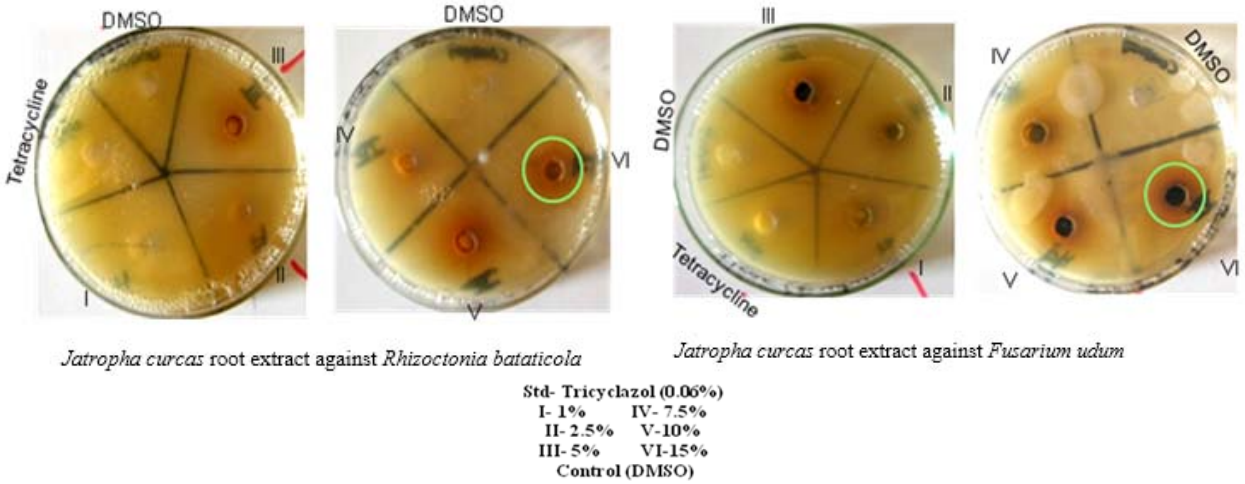


Fig 1: Graphical representation of percent growth inhibition @ 5% concentration of methanolic extract.

Since root extract showed promising antifungal activity against both fungi, it was decided to take a range of concentration to see minimum inhibitory concentration. For this cup diffusion assay was used to calculate zone of inhibition (mm). The root extracts were used at various concentrations such as 1%, 2.5%, 5%, 7.5%, 10% and 15%. Zones of inhibitions were observed at all the concentrations with minimum inhibition at 1% (6.2 mm and 6.5 mm) and maximum inhibition with increasing the concentration (Table 3 and Figure 2) so that a concentration dependent activity was observed.

Table 3: Screening of antifungal activity of methanolic root extract.

SN	Organism	Treatment	Zone of Inhibition (mm)
1	*Rhizoctonia bataticola*	Control	0
		Standard	16.5
		1%	6.2
		2.5%	9.6
		5%	12.4
		7.5%	16.0
		10%	16.1
		15%	16.4
2	*Fusarium udum*	Control	0
		Standard	16.2
		1%	6.5
		2.5%	10.7
		5%	12.0
		7.5%	14.1
		10%	14.5
		15%	15.0



Note: Circle indicates the maximum zone of inhibition

Fig 2: Screening of antifungal activity of crude methanolic root extract using cup diffusion method.

Aiyelaagbe *et al*, 2000 reported antifungal activity of hexane root extract of *J. podagrica* against *C. albicans* and found to be comparable with that of standard miconazole. Igbinosa *et al*, 2009 also reported recently the antifungal activity of *J. curcas* stem alcoholic extract against human pathogenic fungi. However, no literature was observed on the internet regarding the antifungal activity of root extract of *J. curcas* against plant pathogens such as *Rhizoctonia bataticola* and *Fusarium udum.* Since antifungal was observed with methanolic root extract it was decided to fractionate it by solvent fractionation.

Screening of antifungal activity of solvent extracted fractions using poison food technique

The three fractions residues (fraction 1), chloroform-methanol (fraction 2) and aqueous basic layer (fraction 3) were further screened for antifungal activity by poison food technique. The data revealed that at 1% concentration no activity was observed in fraction 1 and fraction 2. The effective fraction 3 was then screened for antifungal activity at different concentrations (1%, 2.5%, 5%, 7.5% and 10%), at 2.5% concentration some inhibition (4.1% and 6.4%) was noted against *R. bataticola* and *F. udum* respectively. The per cent inhibition was drastically increased after 5% concentration (21.7% and 17.3 %) to 7.5% concentration (68.8 % and 56.2%) against *R. bataticola* and *F. udum* respectively. The maximum inhibition was observed at 10% concentration in fraction 3 (88.7% and 71.5%) in case of against *R. bataticola* and *F. udum* respectively. The antifungal activity was found to be concentration depended with 2.5% as minimum inhibitory concentration for Fraction 3 to have any growth inhibition in the tested organism. Fraction 3 is further purified using column chromatography and its antifungal activity was evaluated. The percent zone of inhibition of fraction 3 was found maximum at 10% concentration and minimum at 2.5% concentration. Similar results were reported by Kiran *et al*., in 2011 [11] using aqueous and solvent extract of seeds of P. *corylifolia* against five seed borne fungi of maize viz.,

Curvularia lunata, *Dreschslera halodes*, *Alternaria alternata*, *Cladosporium cladosporioides* and *Rhizopus* sp. They found maximum inhibition in aqueous extract against *Alternaria alternate* (95.4%) and *Curvularia lunata* (86.0%). The zone of inhibition mentioned in Table 4. The antifungal activity against *R. bataticola* depicted in figure 3.

Table 4: Screening of solvent extracted fraction 3 (aqueous basic layer) of *J. curcas* root.

SN	Microorganism	Zone of inhibition (%)*					
		1 %	2.5%	5 %	7.5 %	10 %	Control
1	*Rhizoctonia bataticola*	0	4.1	21.7	68.8	88.7	0
2	*Fusarium udum*	0	6.4	17.3	56.2	71.5	0

* Mean value of two replicates

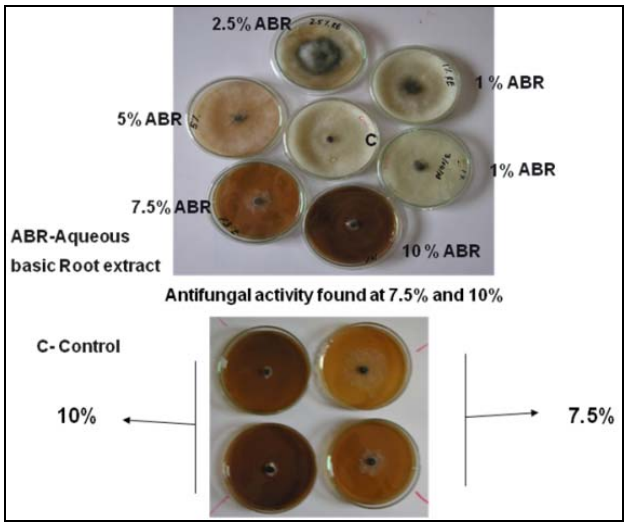


Fig 3: Antifungal activity of solvent fractionated root extract (fraction 3) against *Rhizoctonia bataticola*

Qualitative phytochemical screening of Fraction 3 (aqueous basic root extract/fraction) of root, leaf and seedcoat extract

The observations for phytochemical screening of aqueous basic fractions (Fraction 3) of root extract were shown in Table 5 and figure 4. Phytochemical studies revealed that phenolics were absent in root extract, proteins, gums and mucilages and carbohydrates and glycosides, fats and oils were absent in root extract. Danish *et al*., in 2015 [5] demonstrated the occurrence of alkaloids, tannins, saponins, flavonoids, phenolics, amino acids and terpenes, in the aqueous leaf extract of *J. curcas*.

Table 5: Qualitative phytochemical screening of Fraction 3 of root, leaf and seed coat extract.

S.N.	Phytochemical Group	Root Extract
1	Phenolics	-
2	Saponins	+
3	Protein and amino acids	-
4	Gums and mucilages	-
5	Phyto-sterol	+
6	Alkaloids	+
7	Carbohydrates and Glycoside	-
8	Terpe-noids	+
9	Fats and Oils	-
10	Phlobatannins	+
11	Tannins	+

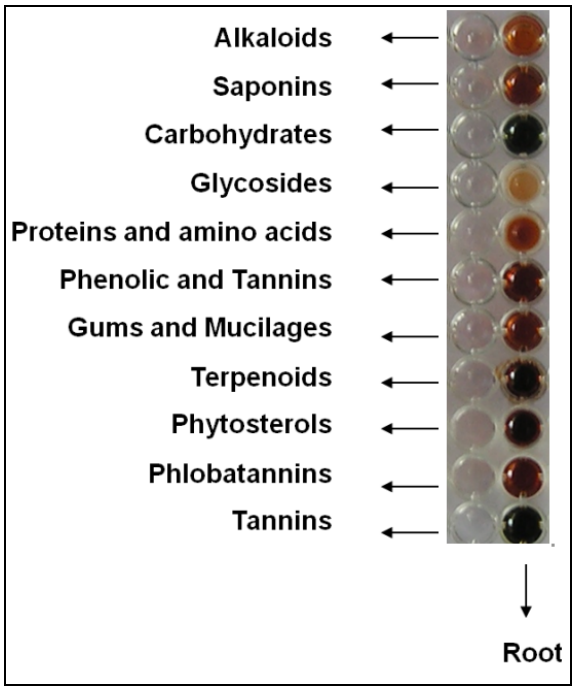


Fig 4: Phytochemical screening of aqueous basic fractions (fraction 3) of root extract

Partial purification of Fraction 3 of root extract (Aqueous basic layer) using column chromatography

The bioactive fraction (Fraction 3) was subjected for further purification using column chromatography. The method was performed using Silica Gel 60-120 mesh and solvents system such as toluene (100 %), ethyl acetate (100 %), methanol (100%), methanol and water (1:1) and water. The solvent system were used in increasing order of polarity so that the first solvent system was non polar while the last system was polar. The maximum yield was obtained in methanol fraction 2 (MF 2, 496 mg) whereas minimum yield was observed in ethyl acetate fraction 2 (EAF 2, 15 mg). The Table 6 shows the details of fraction volume and the dry weight of extract obtained after removal of solvent.

Table 6: Column chromatography of Fraction 3 of root extract.

S. N.	Fractions	Fraction Volume (ml)	Dry wt (mg)
1	Toluene 1 (TF 1)	30	213.7
2	Toluene 2 (TF2)	40	102.9
3	Toluene 3 (TF 3)	30	207.6
4	Ethyl acetate 1 (EAF1)	30	17.3
5	Ethyl acetate 2 (EAF2)	40	15
6	Ethyl acetate 3 (EAF 3)	30	10.6
7	Methanol 1 (MF 1)	30	199.2
8	Methanol 2 (MF 2)	40	496
9	Methanol 3 (MF 3)	40	73.7
10	Methanol: Water 1 (MWF1)	40	193.2
11	Methanol: Water 2 (MWF2)	30	188.9
12	Water (WF1)	60	46.2

Screening of antifungal activity of column chromatography fractions by cup diffusions method

All the column fractions were assessed for antifungal activity against *R. bataticola* by agar well diffusion method and zone of inhibition (mm) was analyzed. DMSO was used as control. Out of 12 fractions, the antifungal activity was observed in

only 3 fractions i.e toluene fraction 1(TF1: 16 mm), methanol fraction1 (MF1: 14 mm) and Methanol: water fraction 1:1 (MWF1:10 mm). The Table 7 and figure 5 showed the zone of inhibition of column fractions.

Table 7: Antifungal activity of column fractions of Fraction 3 of aqueous basic root extract.

SN	Microorganism	Zone of Inhibition (mm)											
		Fr1	2	3	4	5	6	7	8	9	10	11	12
1	*Rhizoctonia bataticola*	16	-	-	-	-	-	14	11	-	10	-	-

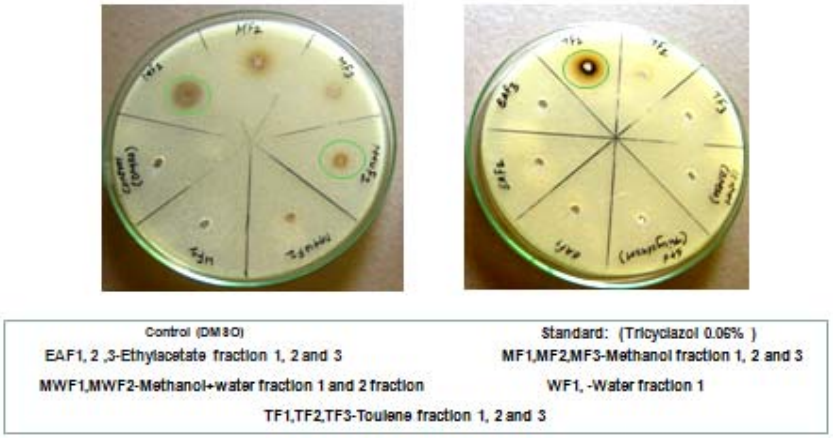


Fig 5: Screening of partially purified column fractions activity against *R. bataticola.*

As the good antifungal activity was found in the Toluene fraction 1 (TF1), methanol fraction 1 (MF1) and Methanol+ Water fraction 1 (MFW1), these fractions were selected for further confirming antifungal activity by poison food technique against *Rhizoctonia bataticola* and *Fusarium udum*. It was observed that toluene fraction 1 (TF1) has potential antifungal activity as compared to other. The details are shown in Table 8. It was interesting to note that the antifungal activity is present in toluene fraction 1 against both the organism while methanol fraction and methanol:water fraction exhibited antifungal activity against only *F. udum* and not against *R. bataticola* (figure 6). Gopalakrishnan *et al*., in 2010 [7] fractionated the organic fraction of *Jatropha* biowash with eluent 5%, 10%, 20%, 40%, 60%, 80% and 100% MeOH fractions and found only 80% methanol (MeOH) fraction to inhibit *S. rolfsii*.

Table 8: Antifungal activity of active column fractions of aqueous basic root extract @ 1 mg/ml concentration.

SN	Microorganism	% Inhibition		
		Fraction 1 (Toluene)	Fraction 7 (Methanol)	Fraction 10 (Methanol: Water)
1	*Rhizoctonia bataticola*	41.17	-	-
2	*Fusarium udum*	72.72	45.45	15.15

Note: (Values are mean of 2 replications)

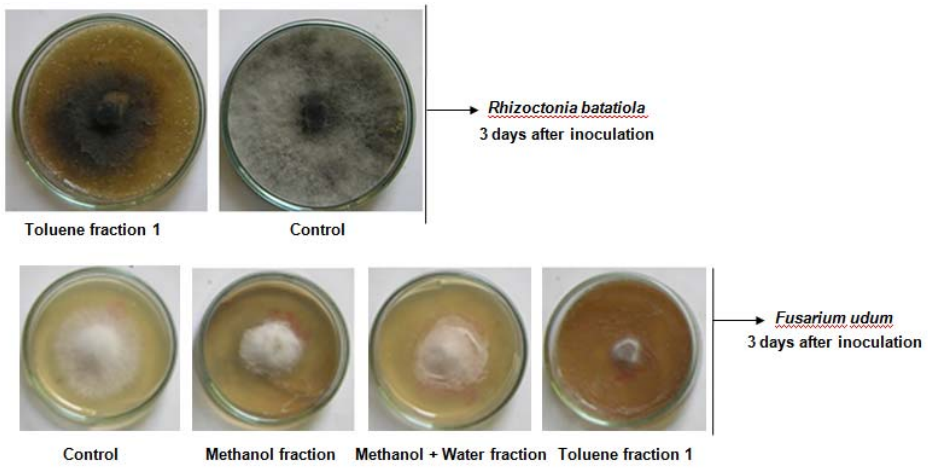


Figure 6: Screening of antifungal activity of selected column fractions of aqueous basic root extract against *R. bataticola* and *F. udum*

HPTLC analysis of column chromatography fraction of aqueous basic root extract

The clarified column fractions after solvent extraction were subjected for HPTLC analysis. Toluene fraction showed the good as 2, 8 and 3 bands @ 254 nm, 366 nm and after derivatization of plate respectively. Thus, it can be conclude that one of the band amongst all could be associated with inhibition activity against *R. bataticola* and *F. udum*. Similar

results were reported by Gopalakrishnan *et al*., in 2010 [7] in chickpea and sorghum and further chromatographed the active methanol fractions which showed three clear bands @254 nm and concluded that one of these three bands could be the active ingredients that inhibited *S. rolfsii* and can be further exploited as a bio-fungicide. Since good characterization was observed in toluene fraction 1, it was further selected for the FTIR analysis. The data was given in Table 9 and figure 7.

Table 9: No of bands/ spots detected in column fractions by HPTLC.

SN	Extracts	No. of spots detected		
		@ 254 nm	@ 366 nm	After derivatization
1	Toluene fraction 1	2	7	3

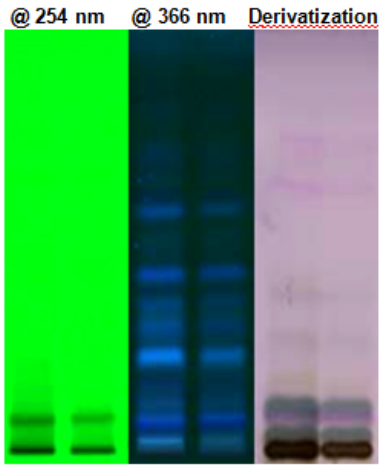


Figure 7: HPTLC analysis of column fractions of aqueous basic root extract

Fourier transform infrared spectroscopic analysis of bioactive fraction (FTIR)

Fourier transform infra red spectroscopy is a valuable tool for the identification of functional groups present in the molecule. It helps for identification and structure determination of the molecule. Since good antifungal activity was observed in case of column fraction 1 i.e toluene fraction 1, it was subjected to Fourier transform infra red spectroscopic analysis which helps in characterizing the fraction in terms of functional groups. The details of the analysis are given in Table 10 and Table 11. The peak pattern depicted in figure 8.

Table 10: Peak of Toluene fraction 1

Peak List	Center	Area	Height	Left Edge	Right Edge
2	1083.996	-513.458	11.473	1126.245	1071.283
5	1633.805	-4822.77	43.219	1754.937	1581.372
8	3347.461	-1.24	0.051	3367.164	3344.986

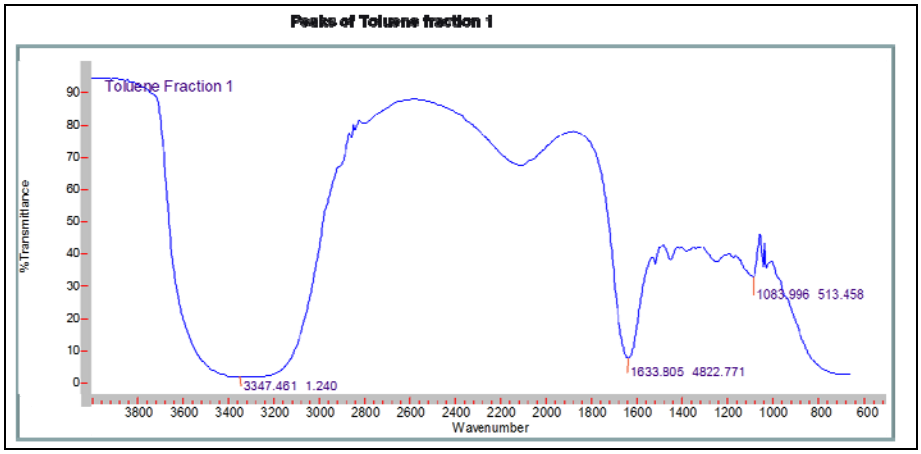


Fig 8: FTIR analysis of toluene fraction 1 (Column chromatography) of aqueous basic root extract

Table 11: FTIR analysis of Column fractions of aqueous Basic Root Extracts

SN	Sample	Peak List	Wave Number	Peak assignment	Functional Group
1	Toluene fraction 1	2	1083.996	C-O	Alcohols, esters, ethers, carboxylic acid, anhydrides
		5	1633.805	C=O	Amide
		8	3347.461	O-H	Alcohol, Phenols H –Bonded

FTIR spectrum is useful as a qualitative finger print check for identification of the functional groups present in the organic compounds (Boeriu *et al*., 2004) [3]. The data showed the presence of acidic, alcoholic and phenolic groups. Phenolic compounds are known to possess antimicrobial activity. Further purification of this fraction is necessary for more clarification regarding the active molecule. Naengchomnong *et al*., in 1994 [12] isolated *jatrophol*, *marmesin*, *propacin*, and *jatrophin* from polar fraction of crude extract of the root of *J. curcas* and structure was elucidated by FTIR. The data so obtained will help in further identification of potential bioactive biomolecule. Based on results it can be concluded that *J. curcas* root is a potent medicinal plants which showed a strong antifungal activity against both fungi *R. bataticola* and *F. udum* both in aqueous and solvent extract. A further work is necessary to isolate a bioactive compound and to test its potentiality against all the phytopathogenic fungi.

Conclusion

This study has given a direction to search new molecules with antimicrobial activity from *Jatropha curcas*. Further purification of extracts, characterization of active biomolecules and confirmation of bioactivity against a wide range of insects and plant pathogens will be helpful to identify new source of insecticidal and antimicrobial activity which can be exploited for product development.

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