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Antioxidant Analysis, Antifungal Activity And Anticancer Activity For Active Compound Valorization From *Jatropha Excisa* Seed Oil

Satish Kumar Kenar[1], Dowluru SVGK Kaladhar[2*]

[1,2*]Microbiology and Bioinformatics, UTD, Atal Bihari Vajpayee Vishwavidyalaya, Bilaspur (CG), India

***Corresponding Author:**
dkaladhar@gmail.com

Abstract
Jatropha belongs to the family of Euphorbiaceae is a commercial and alternate crop for biodiesel production. Fresh seeds of *Jatropha excisa* were collected during summer season and were dried in the sunlight for 24 hours. The oil is extracted by cold press method. The seed oil was shown good antioxidant activity. The seed oil has also shown good antifungal activity with *Candida albicans* and *Mucor indicus.*

Keywords: *Jatropha excisa,* seed oil, Antioxidant analysis, antifungal activity and anticancer activity

INTRODUCTION

Drugs in leaves, barks, fruit bodies, roots, seeds, flowers and other parts of the medicinal plants shows written documents that are preserved monuments from ancient times in healing several diseases (Srivastava, 2018; Iqbal et al., 2017). Medicinal plants are commonly used in Sidda, Homeopathy, UNANI and Ayurveda systems of medicine.
The non-edible like Jatropha oils, residual like waste frying oils, coconut oil, corn oil, palm oil, olive oil, and other vegetable oils like acid oil from soybean soap stock are non-edible are used as fuels and are promoting a circular economy (Re et al., 1999; Bajpai and Tyagi, 2006; Karmakar et al., 2010). Different waste frying oil, raw materials like commercial sunflower and soybean oils, and acid oil from soybean soap stock, and pork fat are using to produce first- and second-generation biodiesel. Flower oil from *Jatropha* species like *Jatropha excisa*, may promote as first- and second-generation biodiesel along with healthcare (Acheampong et al., 2017; Ullah et al., 2015). The present work on *Jatropha excisa* flower oil is applied for analysis of biochemical activities along with *in silico* anticancer and antifungal activities.
Jatropha curcas L. (*J. curcas*) seed kernels shows phenolics, flavonoids and saponins has shown good antimicrobial, antioxidant and anticancer (anticancer therapeutic agents toward breast cancer cells) activities (Oskoueian et al., 2011; Mbakwem–Aniebo et al., 2012). Hence the studies on *J.excisa* has been selected to analyze whether the same properties similar to *J curcus* may be present for antifungal and antioxidant activities.

MATERIAL AND METHODS

Collection of Seeds

Fresh seeds of *J. excisa* was collected during summer season.

Biofuel production

The seeds were air-dried and was used for oil extraction. Oil extraction machine was used for the production process of Biofuel/seed oil.

Secondary metabolite analysis

Secondary metabolite analysis in seed oil has been conducted based on LC-MS method (Table 1 to 4).

Table 1: Instrument Conditions for Phytochemicals

Time	Solvent A (Acetonitrile)	Solvent B (Ammonium)
0	5	95
25	20	80
40	20	80
55	35	65
65	80	20

Table 2: Solvent system

Type of samples	Phytochemical Extracts
Name of column	C18
Wavelength	280
Flow rate	0.2 ml/minute
Solvent System (A)	Acetonitrile
Solvent System (B)	$HCOONH_4$ buffer

EXPERIMENTAL CONDITIONS:
Instrument Details
LC Instrument: XEVO-TQD#QCA1232
Column: SUNFIRE C18, 250 X 2.1, 2.6um

Table 3: HPLC Conditions

A%	0.0 H_2O
B%	5.0 ACN
C%	0.0 MeOH
D%	95.0 0.1% Formic Acid in water
Flow (ml/min)	1.500
Stop Time (mins)	5.0
Column Temperature (°C)	30.0
Min Pressure (Bar)	0.0
Max Pressure (Bar)	300.0

Table 4: The Gradient Table

Time	A%	B%	C%	D%	Flow
0.00	0.0	5.0	0.0	95.0	1.500
1.00	0.0	5.0	0.0	95.0	1.500
6.00	0.0	30.0	0.0	70.0	1.500
12.00	0.0	60.0	0.0	40.0	1.500
16.00	0.0	60.0	0.0	40.0	1.500
20.00	0.0	80.0	0.0	20.0	1.500
26.00	0.0	5.0	0.0	95.0	1.500
30.00	0.0	5.0	0.0	95.0	1.500

Acquisition Mode
Spectra were recorded in negative and positive ionization mode between m/z 150 and 2000.

Antioxidant activity by DPPH method

About 5µl of different stock of the test compound (0µg/ml-2500 µg/ml) was added to 0.1 ml of 0.1mM DPPH solution in a 96 well plate. The reaction was set in triplicate form and duplicates of blank was prepared containing 0.2 ml DMSO/Methanol and 5µl compound of different concentrations (0µg/ml-2500 µg/ml).The plate was incubated for 30 min in dark. At the end of the incubation, the decolorization was read 495 nm using a micro plate reader (iMark, BioRad). Reaction mixture containing 20µl of deionized water was served as Control. The scavenging activity was presented as '% inhibition' with respect to control.

Antimicrobial activity

Microbes from MTCC (Microbial Type Culture Collection) have been used in the present study. Fungi used in the work are *Candida albicans MTCC* 227 and *Mucor indicus* MTCC 4349.
Fungi was grown in Sabour and Dextrose Media (Himedia Pvt. Ltd., Mumbai., India) at 25°C for 72 hours, and were maintained on nutrient agar slants at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth for overnight. The overnight broth cultures were sub cultured in fresh nutrient broth and grown for 3 hours to obtain log phase culture. The agar plates were prepared by pour plate method using Sabourand Dextrose agar (SDA) Media for fungi. The sterile SDA medium cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism ($1x10^8$ cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 8mm size were made with sterile borer and test extracts were added. The SDA plates were incubated at 25°C for 72 hrs for fungi. The diameter of zones of inhibition was measured in mm using HiMedia zone reader. Fluconazole is selected as standard antibiotic.

Docking

The compounds that are analyzed based on LC-MS from seeds of *J excisa* was shown in table 5.

Table 5: Compounds analyzed from LC MS

Compounds from LCMS Seeds
3-Indolylacetonitrile
L-Histidine
Methyl Jasmonate
Thiabendazole
6-(gamma,gamma-Dimethylallylamino)purine
O-Acety-L-carnitine hydrochloride
Fusaric acid
4-Hydroxy-3-methoxycinnamaldehyde
Caffeine, Anhydrous
N-Stearoyl-D-erythro-Sphingosine
Adenosine-3',5'-cyclicmonophosphate
Quercetin-3,4'-O-di-beta-glucopyranoside
isorhamnetin-3-rutinoside
(-)-Riboflavin
S-Lactoylglutathione
Malvin chloride
Solasodine
L-saccharopine
L-Carnosine
2'-Deoxyinosine
gamma-Linolenic acid
D-Glucosamine-6-phosphate sodium salt
alpha-D-Galactose-1-phosphate Dipotassium Salt

Acacetin
Xanthosine
Luteolin
6-Phosphogluconic acid Barium salt hydrate

The compound/ ligand structures are retrieved from https://hmdb.ca/metabolites/HMDB0006524. Candida protein was retrieved from PDB with id 5JPE (Figure 1). iGEMDOCK v2.1 are the free software that has good protein-ligand activity. The minimum energy obtained during docking process will be the good ligand.

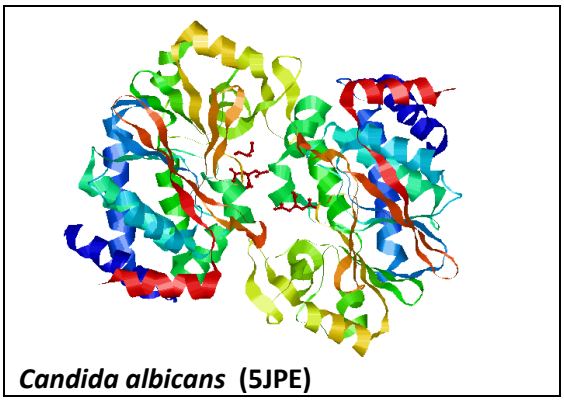

Figure 1: Selected receptor in present study

RESULTS AND DISCUSSION

The LCMS spectra obtained was shown in Figure 2

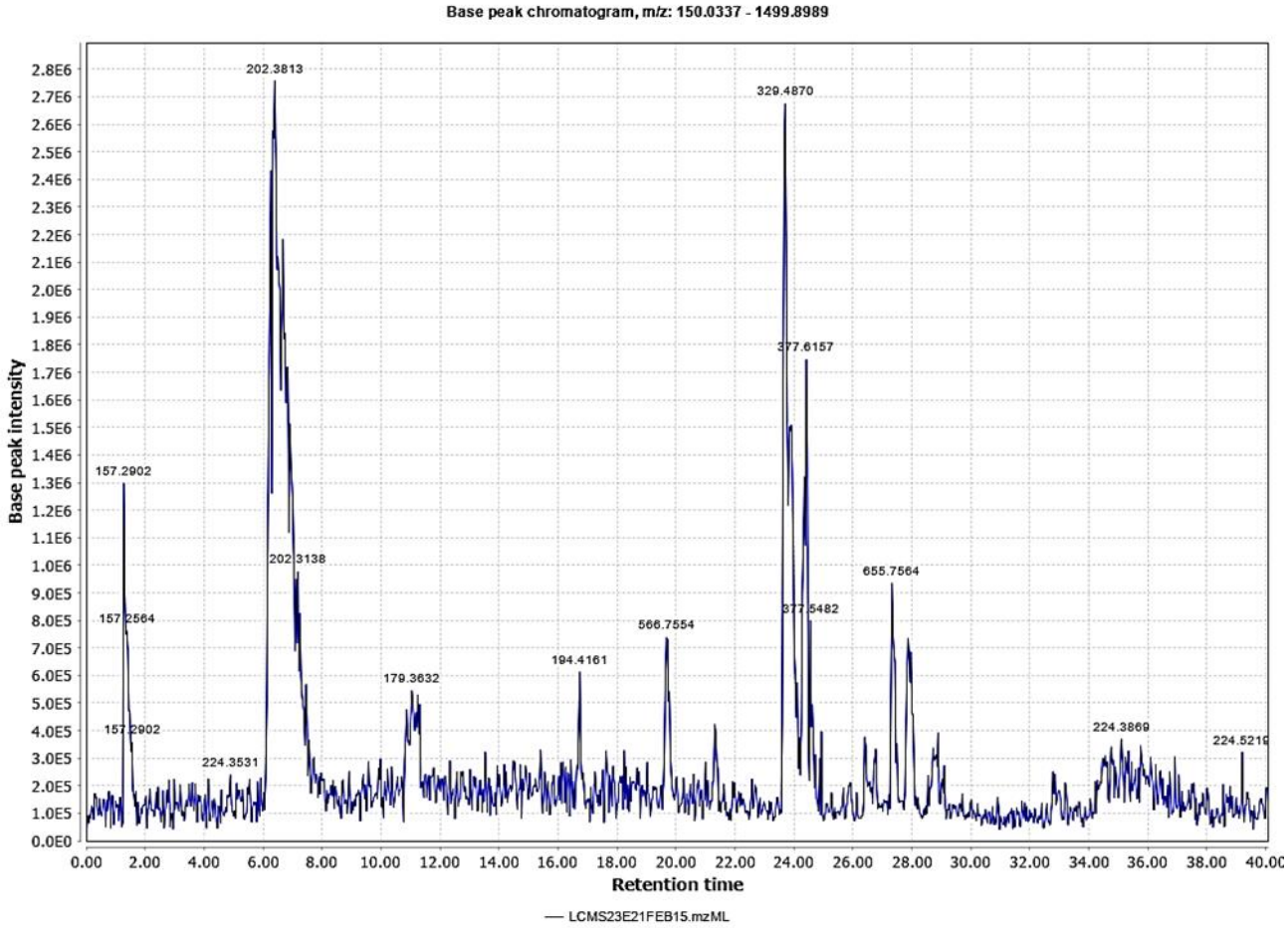

Figure 2: LCMS Spectra

Table 6: OD DPPH results

CON.	CONTROL	ASCORBIC ACID			F			S		
		OD1	OD2	OD3	OD1	OD2	OD3	OD1	OD2	OD3
20	0.765	0.763	0.761	0.76	0.653	0.652	0.651	0.658	0.656	0.655
40	0.765	0.762	0.762	0.761	0.564	0.564	0.564	0.583	0.583	0.582
60	0.765	0.737	0.735	0.734	0.537	0.535	0.534	0.555	0.554	0.552
80	0.765	0.661	0.66	0.658	0.424	0.423	0.422	0.481	0.479	0.478
100	0.765	0.518	0.516	0.509	0.387	0.383	0.382	0.454	0.451	0.451

Table 7: % inhibition based on DPPH results

Con	% INHIBITION ACTIVITY								
	AA			F			S		
	OD1	OD2	OD3	OD1	OD2	OD3	OD1	OD2	OD3
20	0.261438	0.522876	0.653595	14.64052	14.77124	14.90196	13.98693	14.24837	14.37908
40	0.392157	0.392157	0.522876	26.27451	26.27451	26.27451	23.79085	23.79085	23.92157
60	3.660131	3.921569	4.052288	29.80392	30.06536	30.19608	27.45098	27.5817	27.84314
80	13.59477	13.72549	13.98693	44.57516	44.70588	44.8366	37.12418	37.38562	37.51634
100	32.28758	32.54902	33.46405	49.41176	49.93464	50.06536	40.65359	41.04575	41.04575

Table 8: Average % inhibition based on DPPH results

CON.	AVERAGE		
	AA	F	S
0	0	0	0
20	0.479303	14.77124	14.20479
40	0.43573	26.27451	23.83442
60	3.877996	30.02179	27.62527
80	13.76906	44.70588	37.34205
100	32.76688	49.80392	40.91503

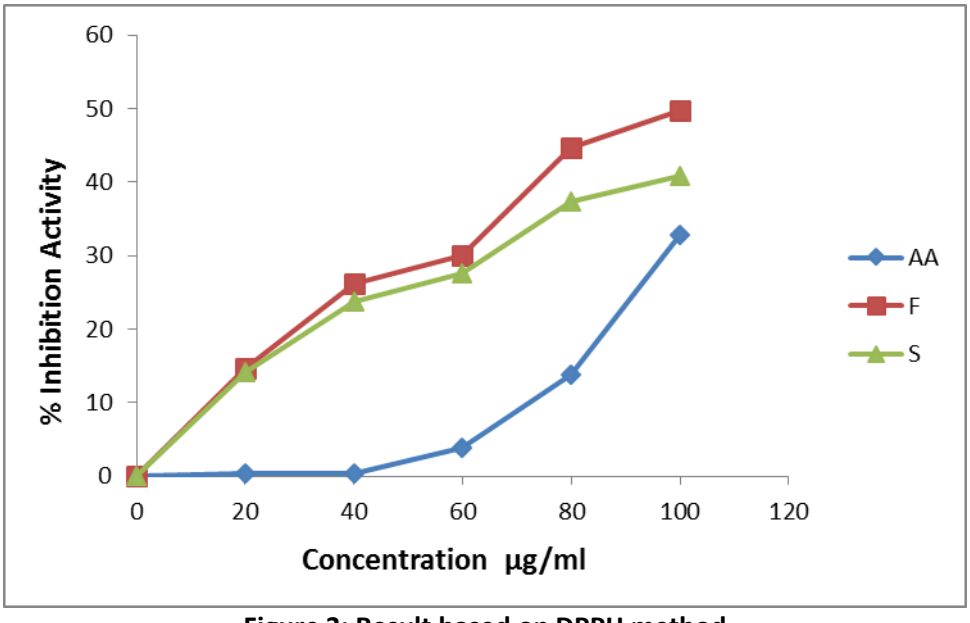

Figure 3: Result based on DPPH method

DPPH method is one of the well-known method for evaluating antioxidant *in vitro* activity. Table 6 to Table 8 and Figure 3 had showed the scavenging activities of Flower and Seed on the DPPH radical compared with ascorbic acid. At the lowest concentration (20 µg/mL), ascorbic acid, Flower and Seed had a scavenging effect of 0.48, 14.77 and 14.20 percent respectively for DPPH. At the highest concentration (100 µg/mL),

ascorbic acid, Flower and Seed had a scavenging effect of 32.77, 49.8 and 40.92percent respectively for DPPH. Data are shown as the mean and SD (*n* = 3)

Antifungal activity

The seed oil (19mm) has shown good activity compared with flower oil (12mm) with *Candida albicans.* (Table 9; Figure 4). The seed oil (15mm) has shown good activity compared with flower oil (14mm) with *Mucor indicus*

Table 9: Antifungal activity in *J excisa* seed and flower

Microorganism	Standard	Seed oil	Flower oil	Blank
Candida albicans MTCC 227	20	19	12	0
Mucor indicus MTCC 4349	22	15	14	0

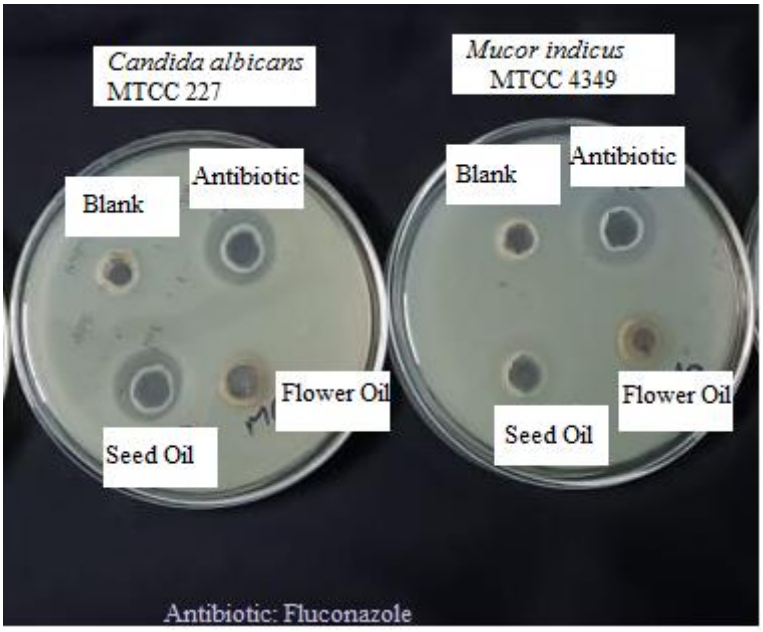

Figure 4: Antifungal activity in *J excisa* seed and flower

The seed and flower oil extracts collected from plants *Jatropha excisa* has been searched further compounds based on LSMS analysis. Screening of compounds for antifungal activities has been conducted (Table 10).

Table 10: *in silico* antifungal activity *Jatropha excisa* seed oil

Compound	Energy	VDW	HBond	Elec
cav5jpe CANDIDA_FLC-HMDB0000033 (Carnosine)-1.pdb	-86.0563	-51.9137	-28.9443	-5.19833
cav5jpe CANDIDA_FLC-HMDB0000045 (Adenosine monophosphate)-0.pdb	-110.788	-62.8998	-45.0555	-2.83223
cav5jpe CANDIDA_FLC-HMDB0000252 (Sphingosine)-0.pdb	-69.9141	-53.4423	-16.4718	0
cav5jpe CANDIDA_FLC-HMDB0000279 (Saccharopine)-1.pdb	-97.2407	-49.8847	-37.2865	-10.0695
cav5jpe CANDIDA_FLC-HMDB0000293 (Xanthosine 5-triphosphate)-1.pdb	**-132.585**	**-44.9305**	**-69.5463**	**-18.1082**
cav5jpe CANDIDA_FLC-HMDB0000645 (Galactose 1-phosphate)-1.pdb	-115.183	-58.3755	-54.3087	-2.49927
cav5jpe CANDIDA_FLC-HMDB0001066 (S-Lactoylglutathione)-1.pdb	-96.6022	-59.6357	-38.2713	1.30478
cav5jpe CANDIDA_FLC-HMDB0001254 (Glucosamine 6-phosphate)-0.pdb	-109.372	-50.5664	-47.7943	-11.0116
cav5jpe CANDIDA_FLC-HMDB0001316 (6-Phosphogluconic acid)-1.pdb	-108.708	-36.4299	-63.5787	-8.69946
cav5jpe CANDIDA_FLC-HMDB0001520 (Flavin mononucleotide) (-)-Riboflavin-0.pdb	-112.431	-59.7966	-51.4838	-1.15023
cav5jpe CANDIDA_FLC-HMDB0001847 (Caffeine)-0.pdb	-68.7131	-41.5771	-27.136	0
cav5jpe CANDIDA_FLC-HMDB0003073 (gamma-Linolenic acid)-0.pdb	-56.5766	-33.3689	-17.3267	-5.88097

cav5jpe CANDIDA_FLC-HMDB0003537 (2'-Deoxyinosine triphosphate)-1.pdb	-128.256	-50.6133	-62.7904	-14.8522
cav5jpe CANDIDA_FLC-HMDB0006524 (3-Indoleacetonitrile)-0.pdb	-66.4876	-53.0699	-13.4177	0
cav5jpe CANDIDA_FLC-HMDB0014868 (Thiabendazole)-0.pdb	-75.1419	-59.6299	-15.512	0
cav5jpe CANDIDA_FLC-HMDB0028887 (Histidylhistidine)-1.pdb	-97.3592	-57.5133	-33.5385	-6.30736
cav5jpe CANDIDA_FLC-HMDB0035282 (Solasodine)-0.pdb	-94.3032	-83.5785	-10.7247	0
cav5jpe CANDIDA_FLC-HMDB0036583 (Methyl jasmonate)-0.pdb	-75.0832	-44.1581	-30.9251	0
cav5jpe CANDIDA_FLC-HMDB0037748 (Isorhamnetin 3-rutinoside 4'-rhamnoside)-1.pdb	-100.643	-62.7694	-37.8734	0
cav5jpe CANDIDA_FLC-HMDB0038009 (Malvin)-0.pdb	-115.538	-80.3132	-35.2253	0
cav5jpe CANDIDA_FLC-HMDB0141782 (Coniferaldehyde) 4-Hydroxy-3-methoxycinnamaldehyde-0.pdb	-70.9619	-49.447	-21.5149	0
cav5jpe CANDIDA_FLC-HMDB0240773 (DL-Acetylcarnitine)-0.pdb	-78.4964	-50.8469	-21.576	-6.07347
cav5jpe CANDIDA_FLC-HMDB0245646 (N-(3-Methylbut-2-EN-1-YL)-9H-purin-6-amine)-1.pdb	-78.2787	-60.003	-18.2757	0
cav5jpe CANDIDA_FLC-HMDB0252556 (Fusaric acid)-1.pdb	-79.3692	-53.9384	-22.5847	-2.84614
cav5jpe CANDIDA_FLC-HMDB0302597 (Luteolin 7-glucuronylglucoside)-1.pdb	-110.748	-60.652	-50.5457	0.449222
cav5jpe CANDIDA_FLC-HMDB0302751 (Acacetin 7-glucoside)-1.pdb	-107.243	-74.8117	-32.4309	0
cav5jpe CANDIDA_FLC-HMDB0304730 (Quercetin 7,4'-O-diglucoside)-0.pdb	**-141.82**	**-87.6704**	**-54.1491**	**0**

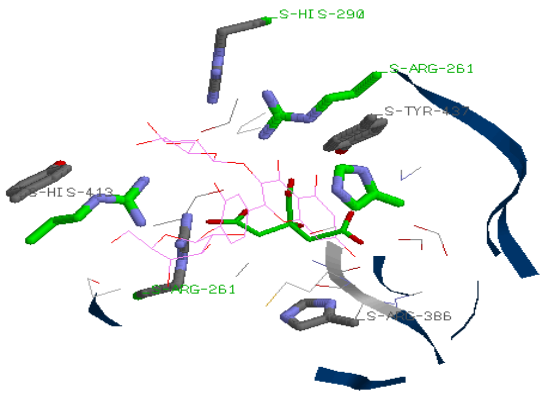

Figure 5: Docking result of CANDIDA with Quercetin 7,4'-O-diglucoside

There are no reports on antioxidant and antifungal activities in *J.excisa* seed oil based on previous research. The *in vitro* and *in silico* studies of compounds from *Jatropha excisa* species has good antioxidant and antifungal activities. The *in silico* docking studies of Quercetin 7,4'-O-diglucoside from *Jatropha excisa* seed oil was shown good antifungal activity (Table 9; Figure 5).

CONCLUSION

The dried seeds of *Jatropha excisa* L were kept in the seeds to oil machine and the oil was extracted by heat press method. The *in silico* docking studies of compounds from *Jatropha excisa* species has good antioxidant and antifungal activities.

Acknowledgement

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Conflict of interest

There is no conflict of interest.

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