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Phytochemical analysis and *in vitro* antifungal evaluation of *Jatropha curcas* against Late Leaf Spot disease on groundnut

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1 ABSTRACT

This study was done to evaluate the antifungal efficacy of *Jatropha curcas* leaf extracts against groundnut late leaf spot disease caused by *Phaeosariopsis personata* (*P. personata*) and identify their bioactive compounds responsible for antifungal effects. *Jatropha curcas* leaves extracted sequentially through chloroform, ethyl acetate and methanol solvents were evaluated against the mycelial growth of *P. personata* by food poisoning method. About 0.1, 0.25 or 0.5 mg/ml (plant extract/water) of each extract were mixed in molten PDA poured into Petri dishes. Thereafter solidified amended PDA with extracts was kept at room temperature for 24 hours. A seven-day-old fungal plug (4mm diameter) of *P. personata* was plated at the middle of the Petri dishes in triplicates. Inoculation on PDA plates amended with fungicide Chlorothalonil (720g/L) or water was included as positive and negative control respectively. The results proved that *J. curcas* leaf extracts possessed fungicidal properties since they inhibited the growth of *P. personata*. Moreover the antifungal effect of *J. curcas* leaf extracts increased as concentration increased. Moreover, *J. curcas* leaf extracts highly inhibited mycelial growth by (85.78%) similar to standard fungicide (chlorothalonil) (88.37%) in this experiment. The presence of important compounds found in *J. curcas* leaf extracts by GC-MS supported their ability against *P. personata* pathogen. Among the major compounds identified with antifungal activity were hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecane, n-hexadecanoic acid, octadecanoic acid ethyl ester, phytol and 9, 12-octadecadienoic acid (Z,Z)-methyl ester. The potentiality of *J. curcas* extracts in managing groundnut late leaf spot disease was confirmed by their ability to inhibit the growth of *P. personata* and possession of important phytochemical compounds.

2 INTRODUCTION

Groundnut late leaf spot disease (LLS) caused by *Phaeosariopsis personata* (*P. personata*) is a major limiting factor to groundnut productivity in Tropics and Subtropics (Khedikar *et al.*, 2010). LLS disease causes a considerable damage in the groundnut production leading to severe leaf defoliation hence reduces both pods yields and

haulm by 23-47% (McDonald *et al.*, 1985; Waliyar *et al.*, 2000). Much efforts of managing this plant fungal pathogen have been developed. Fungicides application has remained as a primary strategy in managing plant diseases. Fungicides seem to be effective financially and manage fungal diseases immediately despite their

shortcomings causing pathogen disease resistant and detrimental effects to human and environment (Karaman *et al.*, 2003; Monyo *et al.*, 2009). Application of the natural bioactive compounds originated from plant resources has gained much interest aiming to replace the synthetic compounds (Karaman *et al.*, 2003; Monyo *et al.*, 2009). This interest is based on possession of phytochemicals, which act differently against pathogens (Sharstry *et al.*, 2010; Gurjar *et al.*, 2012). *Jatropha curcas* (*J. curcas*) is cultivated in subtropical and semiarid regions, mainly as potential source traditionally used for medicinal purposes (Fairless, 2007). Moreover *J. curcas* extracts from various parts i.e.

leaves, stem, barks, roots, seed and seed oil have shown antifungal properties (Saetae and Suntornsuk, 2010). According to Siva (2008), *J. curcas* among 20 plant species was proved to have fungicidal property. Also according to Rahman *et al.* (2011), *J. curcas* fruit was reported its antifungal property. Furthermore, *J. curcas* leaf extract reported to inhibit the growth of *C. musae* causing anthracnose disease in banana. These few evidences suggest the fungicidal property of *J. curcas*. The study assessed the effectiveness of *J. curcas* leaf extracts against LLS that causes severe groundnut yield losses also identify the phytochemical compounds responsible for the management of named pathogen.

3 MATERIAL AND METHODS

3.1 Plant leaves: Plant leaves samples (*J. curcas*) were obtained from different parts in Arusha, Tanzania. Thereafter were washed, air-dried and ground to powder for extraction. “Pendo” groundnut variety, which is popular and highly susceptible to LLS disease, was

obtained from Naliendele Agricultural Research Institute, Mtwara, Tanzania. Pendo variety is early maturity (90-100 days), has high yield performances, easy to harvest and pluck (Bucheyeki *et al.*, 2010).



Plate 1: Symptoms of late leaf spot caused by *Phaeoisariopsis personata* on groundnut leaves

3.2 Preparation of leaf extracts: One kilogram of *J. curcas* powdered leaf was separately and sequentially extracted through different solvents in order of polarity chloroform (non-polar), ethyl acetate (mid-polar) and lastly on methanol (polar) at room temperature. Thereafter the leaf extracts were filtered by using Whatman no. 1 thereafter concentrated using

rotatory evaporator to give a sticky semisolid extract, which was kept in the refrigerator at 4°C.

3.3 Isolation of pathogen and culture preparation : Groundnut leaves showing black and nearly circular spots appear on the lower side of the leaflet were obtained from the farmer’s fields from Singida and Dodoma regions, Tanzania. The isolation of the intended pathogen was done in the laboratory by adopting

the scheduled technique (Riker and Riker, 1936). Where the diseased leaf portions were cut into small pieces (1-2mm) sterilized with 0.1% mercuric chloride solution by soaking for 5 minutes then rinsed thrice with sterile distilled water (SDW) and dried on blotter paper. Thereafter those small pieces of leaves were plated of Potato Dextrose Agar (PDA) in a laminar hood then incubated at a room temperature $28 \pm 2^\circ\text{C}$ for 7 days to allow fungi to grow. The emerged fungal colonies were sub cultured to a fresh PDA plates thereafter incubated at a room temperature for 7 days in order to obtain *P. personata* culture. Fungal pathogen *P. personata* was identified by a single spore method. Fungal mycelium from the fresh culture examined under Sterio-microscope (Magnification 40X) by observing their morphological and distinctive images/features (Agrios 2005).

3.4 In vitro test of *J. curcas* leaf extracts on *P. personata*: The antifungal activity of chloroform, ethyl acetate and methanol leaf extracts of *J. curcas* against *P. personata* was measured by using a food poisoning technique by adopting the technique described by Kritzinger *et al.* (2005) with some modification. The appropriate amounts of each stock of extract was added to 100 ml of PDA before pouring into Petri dishes to yield final concentrations of 0.1, 0.25 and 0.5 mg/ml. Plugs (5 ml diameter) of *P. personata* from 7-day-old fungal culture was placed at the centre of the Petri dishes containing PDA amended with either chloroform, ethyl acetate and methanol leaf extracts of *J. curcas* or *P. hysterothorus* leaf extracts. The plates without phytoextract served as negative control and plate along with synthetic fungicide Chlorothalonil (720g/L) served as positive control. Treatments were arranged in a complete randomized design (CRD) with three replications and were conducted twice. The inoculated petri plates were incubated at room temperature and the radial growth was recorded

when the fungus reached the edge of the petri plates. The Percent inhibition of mycelial growth was calculated by comparing with mycelial growth of treatments/extracts and control following a standard proposed formular by Sivakumar *et al.* (2000);

$$I = [C-T/C] \times 100$$

Where;

I = Percent inhibition, **C** = Colony diameter in control, **T** = Colony diameter in treatment

3.5 Phytochemical analysis: The phytochemical analysis of *J. curcas* extracts was done by using Gas chromatography and mass spectroscopy (GC MS) at Tropical Pesticides Research Institute (TPRI), Arusha-Tanzania. The analysis was done using 7890A GC connected to Agilent 5975 MSD (Agilent technology, USA). Helium was used as carrier gas at 1.2ml/min flow rate. The GC was equipped with capillary column (HP 5) length of 30 meters, film 0.25 μm and internal diameter 0.250mm and temperature limit 50°C to 340°C (360°C) was used. The initial oven temperature was 50°C for 2min and then increased by $10^\circ\text{C}/\text{min}$ rise in temperature (i.e. $50\text{-}280^\circ\text{C}$). The injection volume was $1\mu\text{l}$ at a concentration of 1mg/ml of each sample. The mass spectra ionization voltage was 70eV and the total time taken for the analysis was 35min. The inlet temperature was 250°C . Each peak in the chromatography was identified basing on the retention index and compared the fragmentation pattern of the compounds with the mass spectra in the National Institute Standard and Technology (NIST) library.

3.6 Statistical analysis : Data were subjected to 3-way ANOVA (analysis of variance) in factorial arrangement, using STATISTICA program. The treatment means were compared by applying Fischer's least significant difference (LSD) at 5% level of significance.

4 RESULTS

4.1 *In vitro* evaluation of *J. curcas*: The antifungal efficacy of *J. curcas* leaf extracts at three level concentrations (0.1, 0.25 and 0.5mg/ml) was determined by observing the mycelial growth of *P. personata*. The mycelial growth inhibition of *P. personata* differed significantly at ($P \leq 0.001$) under different treatments, solvents and concentrations. The treatments amended with chlorothalonil (standard fungicide) and *J. curcas* leaf extract inhibited *P. personata* mycelial growth highly (88.37%, 85.78%) respectively as compared with the negative control (untreated) (0.00%). Moreover methanolic leaf extracts *J. curcas* inhibited highly the mycelial growth (74.04%) followed by chloroform and ethyl acetate and (57.89%, 56.22%) respectively. Furthermore, the highest concentration of *J. curcas* leaf extracts

(0.5mg/ml) inhibited the *P. personata* mycelial growth highly (78.07%) as compared with the lowest concentration (0.1mg/ml) (54.33%) (Table 1).

4.2 Interactive Effects between Treatments, Solvents and Concentrations:

The mycelial growth of *P. personata* differed highly significantly under interaction of factors; i.e. Treatments and Solvents; and Treatments and Concentrations; (Table 1). Generally, *J. curcas* leaves extracted by methanol inhibited the mycelial growth *P. personata* compared to other solvents. Moreover, the mycelial growth of *P. personata* under different leaf extracts concentration differed significantly ($P \leq 0.001$) where high inhibition was experienced at the highest concentration compared with the lowest concentration.

Table 1: The influence of *J. curcas* extracts on mycelial growth of *P. personata*

Factors	Percent inhibition
Treatments	
<i>Jatropha curcas</i>	85.78±1.64 ^b
Positive control (chlorothalonil)	88.37±0.93 ^a
Negative control	0.00±0.00 ^c
Solvents	
Chloroform	56.22±7.90 ^b
Ethyl acetate	57.89±8.09 ^b
Methanol	74.04±8.37 ^a
Concentrations	
0.1 mg/ml	54.33±7.61 ^c
0.25 mg/ml	58.74±8.20 ^b
0.5 mg/ml	78.07±8.49 ^a
3-way ANOVA (F-value)	
Treatments	6761.46***
Solvents	9.78**
Concentrations	31.33***
Treatments *Solvents	13.21***
Treatments *Concentrations	12.80***
Solvents*Concentrations	0.21ns
Treatments *Solvents*Concentrations	0.77ns

Means with different letters indicate significant differences among treatments according to Fischer's least significant difference (LSD) test. *, **, ***: significant at ($P \leq 0.05$), $P \leq 0.01$, ($P \leq 0.001$) respectively, ns= not significant

4.3 Chemical Composition of Leaf Extracts: This study reveals that the use of organic solvents in extraction of selected plants

has identified different compounds by GC-MS. From chloroform leaf extracts of *J. curcas* the following important phytochemical compounds

were identified (Table 2), the major compounds were *n*-hexadecanoic acid (7.89%), phenol, 2,4-bis (1, 1-dimethylethyl) (4.04%), cyclotetracosane (1.23%), hexadecane (1.20%) and octacosane (1.02%). The following major phytochemical compounds were identified from ethyl acetate leaf extracts of *J. curcas*; phytol (9.31%), hexadecanoic acid ethyl ester (3.97%), phenol 2, 4-bis (1, 1-dimethylethyl) (3.37%) and 5-icosene, (E) (2. 11%), (Table 3). The following phytochemical compounds were

identified from methanolic leaf extracts of *J. curcas*; phytol (26.75%), hexadecanoic acid methyl ester (14.32%), octadecanoic acid, methyl ester (2.79%), 9, 12-octadecadienoic (Z,Z)-methyl ester (2.33%) (Table 4). The detected phytochemical compounds with antifungal property from chloroform, ethyl acetate and methanolic leaf extracts of *J. curcas* with their retention times, peak area (%), molecular formula and formula are presented in Table 2, 3 and 4.

Table 2: Reported antifungal activity of phytochemical compounds obtained from *J. curcas* chloroform leaf extract

Retention time(min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
10.629	dodecane, 2,6,11-trimethyl-	C ₁₅ H ₃₂	212.41	(Zhang <i>et al.</i> , 2015)
11.745	2-tetradecene, (E)-	C ₁₄ H ₂₈	196.37	(Shirani <i>et al.</i> , 2017)
11.905	tetradecane	C ₁₄ H ₃₀	198.39	(Begum <i>et al.</i> , 2016)
12.460	pentadecane	C ₁₈ H ₃₈	254.49	(Zhang <i>et al.</i> , 2015)
12.958	octacosane	C ₂₈ H ₅₈	394.76	(Zhang <i>et al.</i> , 2018)
13.192	sulfurous acid butyl decyl ester	C ₁₆ H ₃₄ O ₃ S	306.50	(Sharma, 2016)
13.267	heneicosane	C ₂₁ H ₄₄	296.57	(Ebrahimabadi <i>et al.</i> , 2016)
13.461	phenol 2,4-bis(1, 1-dimethylethyl)	C ₁₄ H ₂₂ O	206.32	(Manikandan <i>et al.</i> , 2017)
14.011	2-bromo dodecane	C ₁₂ H ₂₅ Br	249.23	(Manikandan <i>et al.</i> , 2017)
14.503	hexadecane	C ₁₆ H ₃₄	226.44	(Zhang <i>et al.</i> , 2015)
15.041	heptadecane, 9-octyl-	C ₂₅ H ₅₂	352.68	(Musa <i>et al.</i> , 2015)
15.401	heptacosane	C ₂₇ H ₅₆	380.73	(Bouzabata <i>et al.</i> , 2018)
16.002	2,4-dimethyldodecane	C ₁₄ H ₃₀	198.38	(Begum <i>et al.</i> , 2016)
16.488	pentadecane	C ₁₅ H ₃₂	212.41	(Yuan <i>et al.</i> , 2012)
17.009	ethanol, 2-(octadecyloxy)-	C ₂₀ H ₄₂ O ₂	314.50	(El-Din Mohy and Mohyeldin, 2018)
18.142	hentriacontane	C ₃₁ H ₆₄	436.84	(Ruban and Gajalakshmi, 2012)
18.457	geranylgeraniol	C ₂₀ H ₃₄ O	290.48	(Ashraf <i>et al.</i> , 2017)
18.542	octadecane	C ₁₈ H ₃₈	254.49	(Zhang <i>et al.</i> , 2018)
18.869	<i>n</i> -hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	(Omoruyi <i>et al.</i> , 2014)
19.584	12-methyl-E-E-2, 13-octadecadien-1-ol	C ₁₉ H ₃₆ O	280.00	(Vijayabaskar and Elango, 2018).
20.013	tetradecanal	C ₁₄ H ₂₈ O	212.37	(Passos <i>et al.</i> , 2003)
29.037	cyclotetracosane	C ₂₄ H ₄₈	336.64	(Buglio <i>et al.</i> , 2017)

Table 3: Reported antifungal activity of phytochemical compounds obtained from *J. curcas* ethyl acetate leaf extract by GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-ropanetriol, monoacetate	C ₅ H ₁₀ O ₄	134.13	(Teoh and Mashitah, 2012)
8.460	2,5-pyrrolidinedione	C ₈ H ₁₃ NO ₂	331.32	(Takayama <i>et al.</i> , 1982)
8.826	hexadecane	C ₁₆ H ₃₄	226.44	(Adeleye <i>et al.</i> , 2010); (Oliveira <i>et al.</i> , 2014)
9.273	methyl salicylate	C ₈ H ₈ O ₃	152.15	(Pawar and Thaker, 2006)
11.321	triacetin	C ₉ H ₁₄ O ₆	218.21	(Osuntokun and Olajubu, 2014)
11.813	heptadecane	C ₁₇ H ₃₆	240.5	(Zhang <i>et al.</i> , 2015)
11.899	8-hexadecenal, 14-methyl-, (Z)-	C ₁₇ H ₃₂ O	252.4	(Osuntokun and Olajubu, 2014)
12.952	undecane	C ₁₁ H ₂₄	156.31	(Wanxi <i>et al.</i> , 2013)
13.467	phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₇ H ₃₀ OSi	278.50	(Jun <i>et al.</i> , 2018)
13.993	1-naphthalenol	C ₁₀ H ₈ O	144.17	(Kumar <i>et al.</i> , 2012)
14.337	2,6,10,14,18,22-tetracosahexaene	C ₂₄ H ₃₈	326.6	(Devakumar <i>et al.</i> , 2017)
15.658	heptadecane	C ₁₇ H ₃₆	240.48	(Zhang, <i>et al.</i> , 2015)
16.591	1H-indene, 1-ethylideneoctahydro-7- <i>a</i> -methyl-	C ₁₂ H ₂₂	166.30	(Wang <i>et al.</i> , 2013)
16.889	E-14-hexadecenal	C ₁₆ H ₃₀ O	238.41	(Devakumar <i>et al.</i> , 2017)
17.106	1-tetradecene	C ₁₄ H ₂₈	196.37	(Tayung and Jha, 2014)
17.896	tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.50	(El-Din Mohy and Mohyeldin, 2018)
18.868	<i>n</i> -hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	(Tyagi and Agarwal, 2017)
18.983	9,12-octadecadienoic acid (Z,Z)-	C ₁₉ H ₃₄ O ₂	280.40	(El-Din Mohy and Mohyeldin, 2018)
19.109	5-eicosene, (E)-	C ₂₀ H ₄₀	280.50	(Adibe <i>et al.</i> , 2019)
19.172	hexadecanoic acid ethyl ester	C ₁₈ H ₃₆ O ₂	284.47	(El-Din Mohy and Mohyeldin, 2018)
19.338	2-methyl-Z,Z-3,13-octadecadienol	C ₁₉ H ₃₆ O	280.50	(Adibe <i>et al.</i> , 2019)
20.179	9,17-octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.40	(Adibe <i>et al.</i> , 2019)
20.413	phytol	C ₂₀ H ₄₀ O	296.54	(Pejin <i>et al.</i> , 2014)
21.008	9,12,15-octadecatrienoic acid ethyl ester, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306.48	(El-Din Mohy and Mohyeldin, 2018)
21.186	heptadecanoic acid ethyl ester	C ₁₉ H ₃₈ O ₂	298.50	(Bashir <i>et al.</i> , 2019)
23.869	eicosane	C ₂₀ H ₄₂	282.50	(El-Naggar <i>et al.</i> , 2017)

Table 4: Reported antifungal activity of phytochemical compounds obtained from methanolic leaf extract of *J. curcas* by GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-propanetriol monoacetate	C ₅ H ₁₀ O ₄	134.13	(Teoh and Mashitah, 2012)
9.273	methyl salicylate	C ₈ H ₈ O ₃	152.15	(Essien <i>et al.</i> , 2015)
10.549	2-undecanone	C ₁₁ H ₂₂ O	170.29	(Bisht and Chanotiya, 2011)
10.841	indole	C ₈ H ₇ N	117.15	(Sumiya <i>et al.</i> , 2017)
10.898	decanoic acid methyl ester	C ₁₁ H ₂₂ O ₂	186.29	(Belakhdar <i>et al.</i> , 2015)
11.121	2-methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150.17	(Guo <i>et al.</i> , 2008)
11.287	tert-hexadecanethiol	C ₁₆ H ₃₄ S	258.50	(Yang <i>et al.</i> , 2016)
11.653	phenol, 2,6-dimethoxy-	C ₈ H ₁₀ O ₃	154.16	(Yang <i>et al.</i> , 2016)
11.813	tetradecane	C ₁₄ H ₃₀	198.39	(Begum <i>et al.</i> , 2016)
11.905	cyclotetradecane	C ₁₄ H ₂₈	196.37	(Afrouzan <i>et al.</i> , 2018)
11.991	pentanoic acid ethyl ester	C ₇ H ₁₄ O ₂	130.18	(Sumiya <i>et al.</i> , 2017)
12.248	2-propenoic acid 3-phenyl-, methyl ester	C ₁₀ H ₁₀ O ₂	162.18	(Umaiyambigai <i>et al.</i> , 2017)
12.334	diphenyl ether	C ₁₂ H ₁₀	170.21	(Zhang <i>et al.</i> , 2018)
13.198	pentadecane	C ₁₅ H ₃₂	212.41	(Zhang <i>et al.</i> , 2015)
13.272	tridecane	C ₁₃ H ₂₈	184.36	(Yuan <i>et al.</i> , 2012)
14.503	hexadecane	C ₁₆ H ₃₄	226.44	(Oliveira <i>et al.</i> , 2014)
16.706	heptadecane	C ₁₇ H ₃₆	240.47	(Musa <i>et al.</i> , 2015)
16.797	17-pentatriacontene	C ₃₅ H ₇₀	490.93	(Zhang <i>et al.</i> , 2015)
16.889	1-nonadecene	C ₁₉ H ₃₈	266.50	(Asong <i>et al.</i> , 2019)
17.015	E-15-heptadecenal	C ₁₇ H ₃₂ O	252.43	(Begum <i>et al.</i> , 2016)
17.192	8-hexadecenal 14-methyl-,	C ₁₇ H ₃₂ O	252.40	(Aja <i>et al.</i> , 2014)
17.787	cyclopentadecane	C ₁₅ H ₃₀ O	210.40	(Nakashima <i>et al.</i> , 2014)
18.474	hexadecanoic acid methyl ester	C ₁₇ H ₃₄ O ₂	270.45	(Belakhdar <i>et al.</i> , 2015)
18.777	1-octadecene	C ₁₈ H ₃₆	252.48	(Omoruyi <i>et al.</i> , 2014)
18.868	2-methyl-Z, Z-3, 13-octadecadienol	C ₁₉ H ₃₆ O	280.49	(Phatangare <i>et al.</i> , 2017)
18.983	oleic acid	C ₁₈ H ₃₄ O ₂	282.46	(Walters <i>et al.</i> , 2004)
19.486	9,17-octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.40	(Adibe <i>et al.</i> , 2019)
19.836	2-methyl-Z,Z-3,13-octadecadienol	C ₁₉ H ₃₆ O	280.28	(Adibe <i>et al.</i> , 2019)
20.288	9, 12-octadecadienoic acid (Z,Z)-methyl ester	C ₁₉ H ₃₄ O ₂	294.47	(Chukwunonye <i>et al.</i> , 2015)
20.413	phytol	C ₂₀ H ₄₀ O	296.0	(Hema <i>et al.</i> , 2011)
20.556	octadecanoic acid methyl ester	C ₁₉ H ₃₈ O ₂	298.50	(Banaras <i>et al.</i> , 2017)
21.129	behenic alcohol	C ₂₂ H ₄₆ O	326.60	(Chandrasekaran <i>et al.</i> , 2011)
21.186	octadecanoic acid ethyl ester	C ₂₀ H ₄₀ O ₂	312.53	(El-Din Mohy and Mohyeldin, 2018)
21.380	3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.53	(El-Din Mohy and Mohyeldin, 2018)

22.096	9,17-octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.40	(Chukwunonye <i>et al.</i> , 2015)
23.875	eicosane	CH	282.50	(Shirani <i>et al.</i> , 2017)
24.241	docosanoic acid methyl ester	C ₂₃ H ₄₆ O ₂	354.61	(Aida <i>et al.</i> , 2017)

5 DISCUSSION

The effect of *J. curcas* leaf extracts of chloroform, ethyl acetate and methanolic against *P. personata* was more similar to the standard fungicide. This is attributed by their ability to produce toxins, which act on named pathogen by reducing disease development (Kagale *et al.*, 2004; Gupta *et al.*, 2008). This agrees with the findings by Muklesur *et al.* (2011) *J. curcas* leaf extract inhibited the mycelial growth *C. gloesporioides* by 50% on rubber tree. Moreover, the results obtained from *in vitro* trial found that the antifungal activity of *J. curcas* extracts varied with the type of solvent used for extractions. The results showed that polar solvent (methanol) gave greater antifungal effects on mycelial growth of *P. personata* as compared to intermediate and non-polar extract (ethyl acetate and chloroform) respectively. Possibly the polar compounds extracted through methanol had higher antifungal properties than polar compounds. This corresponds with the study done by Sharma *et al.* (2016), the methanolic fraction of *J. curcas* marked antifungal activities against four pathogenic fungus strains. Furthermore, correspond with the findings by (Krishnananda *et al.*, 2017) where *J. curcas* methanolic root extract shown antifungal activity up to 23.1% growth inhibition against *Rhizoctonia*. In addition, the study showed that the mycelial growth of *P. personata* was highly inhibited at highest concentration of *J. curcas* extracts than lowest concentration this shows that they are more fungitoxic at higher

6 CONCLUSION

This study showed that *J. curcas* leaf extracts has antifungal effect against *P. personata* since they possess important bioactive compounds such as hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester,

concentrations. This study corresponds with the investigation by (Amah *et al.*, 2009) where *J. curcas* extract inhibited the growth of *F. oxysporum* by 54% inhibition at highest concentration (80 mg/ml) as compared with 10% inhibition at the lowest concentration 20mg/ml. Likewise according to Bajpai *et al.* (2012); disease severity was lowered as the concentration of plant extracts increased in all tests. Furthermore, the fungal growth was minimized as plant extract concentration increased (Goel and Sharma, 2013). GC-MS analysis was performed on *J. curcas* leaf extracts through chloroform, ethyl acetate and methanol as these exhibited antifungal activities. The major phytochemical compounds identified from this study were hexadecanoic acid ethyl ester, hexadecane, *n*-hexadecanoic acid, hexadecanoic acid methyl ester, octadecanoic acid ethyl ester, phytol and 9, 12-octadecadienoic acid (Z,Z)-methyl ester. Amongst hexadecanoic acid ethyl ester, hexadecanoic acid methyl ester-, octadecanoic acid ethyl ester, hexadecane, *n*-hexadecanoic acid, hexadecane, *n*-hexadecanoic acid, and 9, 12-octadecadienoic acid (Z,Z)-methyl ester are fatty acid with exceptional to phyto being diterpene alcohol. According to Hema *et al.* (2011); Belakhdar *et al.* (2015); (Chukwunonye *et al.* (2015); (Banaras *et al.* (2017) the identified compounds play a great role as antifungal agent. Normally, the fatty compounds absorb the fungus since it has lipophilic nature (Bassey *et al.*, 2013).

hexadecane, *n*-hexadecanoic acid, phytol and 9, 12-octadecadienoic acid (Z,Z)-methyl ester . Hence *J. curcas* is an important agent for managing the groundnut late leaf spot disease aiming to improve groundnut production.

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