OPEN ACCESS

International Journal of Multidisciplinary Research & Innovation
Volume 2. Issue 1, 2024. University of Kabianga, Kenya. ijmri@kabianga.ac.ke.

Phytochemical Investigation of *Prosopis Juliflora* Leaves Extracts and Their Insecticidal Activity

Too Samuel[1], Kiplimo Joyce[2], Mule Shadrack[3]

[1]University of Kabianga : sammytoo62@gmail.com
[2] University of Kabianga : joycebett01@gmail.com
[3] University of Kabianga : muleshadrack@gmail.com

ABSTRACT

*One known compound trans-ferrulate (compound **1**) together with fatty acids derivatives were isolated from the leaves of Prosopis juliflora. P juliflora is an invasive plant found in dry regions of Kenya mainly Baringo, Turkana and Tana River. The plant has been reported to possess many phytochemicals, some of which are known to possess insecticidal activities. The leaves were collected from Baringo County, then put in cotton bags and transported to University of Kabianga, Biological Sciences Department for taxonomical identification. The leaves were then washed using running water then air-dried. The dried leaves were ground into powder using a mill and then sequentially extracted using methanol 1:1 dichloromethane and methanol for 24 hours in each case. The extracts were then concentrated using rotary evaporator at 35 ^0C – 64.7 ^0C to obtain their respective crude extracts. Repeated column chromatography of fraction 1, yielded trans-ferrulate (compound **1**) a cinamic acid derivative having both methoxy and hydroxy substituents on the phenyl ring. Fractions F1, F10 and F11 exhibited significant activity against adult aphids, with corresponding percentage mortalities of 90 ± 2.03, 95 ± 2.03 and 95 ± 2.25 % respectively. The insecticidal activities of these extracts could be attributed to the conjugation in the benzene rings as well as the activation caused by the ring activators (-OH and -OCH_3 groups) on the benzene ring. The findings of this research credence to the use of the leaves of this plant as natural pesticides that are ecofriendly.*

Keywords: *P. juliflora. trans-ferrulate, insecticidal activity*

1. INTRODUCTION

P. juliflora is a tree native to Mexico and other parts of Northern and Central South America. It has an ability to spread faster in arid and semi-arid areas. *P. juliflora* due to its invasive nature has been declared as noxious weed in many African countries including Kenya (Nadio *et al.*, 2020). It has been considered a threat by the residents due to its undesirable effects to their livestock, however recent studies have reported that *P. juliflora* (Fig 1) can be used to improve livelihoods of the communities from these areas (Nadio *et al.*, 2020). Its uses include timber, charcoal burning, making of animal fodder, generation of power, and many uses in medicine.

Figure 1: Pictures of *P. juliflora* (Nadio *et al.*, 2020)

Its flowers are made into powder then mixed with sugar and *used* to prevent miscarriage during pregnancy. The powder from pods is used in improving weight in infants while the leaves have been used in treatment of oral infections. The concoctions from powdered leaves are used in treatment of irritations and swelling of the eyes (Nadio *et al.*, 2020). In general the plant is a source of natural phytochemicals which can be utilized in agro pharmaceutical approaches.

Vegetables such as kales and cabbages are some of the most staple food crops used by Kenyans in the rural and urban areas. They are the major source of vitamins to the people. However, the attack by aphids and other pests have hampered their production. Farmers incur huge losses due to the attacks. It is reported that in the year 2016 alone, Kenyans lost up to 40 % of their kales due to aphids attack (Van Emden and Harrington, 2017; Olwande *et al.*, 2015; FAO, 2017). A number of synthetic pesticides are used to control these pests but their use has led to a number of side effects in human, environmental pollution affecting human health and even causing death. They are also very expensive to the local farmers who depend on these crops (Mahmood *et al.*, 2016; Okello and Swinton, 2010; Macharia *et al.*, 2009).

It is for this reason that this study aimed at searching for a cheaper environmentally friendly and alternative way of controlling aphids using insecticidal botanicals.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Prosopis juliflora leaves were collected using cotton bags from Mogotio area (0.0208° S, 35.9561° E) in Baringo county

of Kenya. The leaves were identified by a taxonomist at the University of Kabianga biological science lab.

2.2 Extraction of secondary metabolites

The shade-dried and ground leaves of *P. juliflora* were extracted with 100 % methanol and methanol 1:1 DCM mixtures. About 1000 g of the ground powder were soaked in methanol 1:1 dichloromethane, and then extracted to exhaustion, where 240.22 g of the crude extract was obtained. Another 1000 g of the ground powder soaked in distilled methanol yielded 180.43 g of the crude extract. The resulting organic layers were then concentrated under reduced pressure using rotary evaporator to yield their respective crude extracts. The crude extracts were then taken for further purification on column chromatography.

2.3 Isolation and purification of secondary metabolites from plant materials

The crude extracts were separated on silica gel column chromatography. Dry samples were reconstituted using distilled solvents used on their extractions to form slurry like paste, then loaded on evenly packed column chromatography. The columns were then eluted using a step gradient of hexane, dichloromethane, ethyl acetate gradient, starting with 100 % hexane stepped to 10 %, 20 %, 30 %, 50 %, 80 % and 100 % dichloromethane, followed by 20 % and 30 % ethyl acetate in dichloromethane. Fractions of about 50 mL were collected and then thin layer chromatography (TLC) was used to check on the purity of the fractions. Those that showed similar profiles on TLC plates were pooled together of which in total twelve (12) fractions were obtained; F1 (20.32 mg), F2 (13.23 mg), F3 (25.13 mg), F4 (40.44 mg), F5 (27.03 mg), F6 (14.15 mg), F7 (16.14 mg), F8 (4.43 mg), F9 (100.89 mg), F10 (42.15 mg), F11 (67.14 mg) and F12 (78.44 mg). Each fraction was purified using column chromatography, F4 was re-chromatographed using 20:80 of dichloromethane :ethylacetate, fraction one (F1) yielded compound 1.

2.4 General experimental procedure

The ^{1}H, ^{13}C, DEPT, HSQC, COSY and HMBC NMR spectra were recorded on the Bruker Avance 500 MHz NMR spectrometer. The readings were done in deuterated chloroform and their chemical shifts assigned by comparison with the residue proton and carbon resonance of the solvent. Tetramethyl-silane (TMS) was used as an internal standard and chemical shift given as δ (ppm). The off-diagonal elements were used to identify the spin-spin coupling interactions in the ^{1}H-^{1}H COSY (Correlation spectroscopy). The proton-carbon connectivity, up to three bonds away, was determined using ^{1}H - ^{13}C HMBC (Heteronuclear Multiple Bonds Coherence), whereas HSQC spectrum (Heteronuclear Single Quantum Coherence) was used to determine the connectivity of hydrogen to their respective carbon atoms.

For gas chromatography-mass spectrometry (GC-MS) analyses, the samples were analysed on an Agilent GC–MSD apparatus equipped with DB-5SIL MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. Helium (at 2 ml/min) was used as a carrier gas. The mass spectrometry (MS) was operated in the EI mode at 70 eV

2.5 Leaf disc bioassay

Leaf disc bioassay was used in assessing insecticidal activities of *P. juliflora* extractives against adult aphids (*Brevicoryne brassicae*).

2.6 Media preparation

A concentration of 28 g/l of nutrient agar was prepared. The media was sterilized at 15 lbs of pressure at 121 °C for 15 minutes in an autoclave, removed and allowed to cool to 45 °C, then plated on sterile Petri dishes. The Petri dishes containing the media were then allowed to cool and solidify in a laminar flow, before bioassay procedures.

2.7 Insecticidal test

Geometric concentration of 100 % (10 mg/ml), 50 % (5 mg/ml) and 25 % (2.5 mg/ml) of crude, fractions and pure compounds were prepared by diluting serially. Using metal tube, leaf-discs were generated from clean, untreated host plant leaves, with a diameter of 2 mm less than that of the petri dish. Leaf discs were then dipped individually for 10 minutes in the different concentrations, after which they were surface dried on a paper towel. Dry leaf discs containing the samples were transferred to the petri dishes containing the agar, having the abaxial surface facing up. About 25 apterous adult aphids were transferred onto each of the leaf discs using fine paint brush and hand lens. Each case unit was sealed with a ventilated lid then stored upright in an incubator set at 25 °C. An assessment of mortality was done after 72 hours using hand lens. Malathion (10mg/ml) was used as positive control while distilled water was used as negative control. All the tests for each geometric dilution were performed in triplicate.

2.8 Data analysis

Experiment was done in triplicates and its results presented as the average. The mortality rate was then calculated using Abbott's equation. The raw data obtained was subjected to one-way ANOVA (Analysis of Variance) using SPSS version 22 to show if there is statistical relationship on the insecticidal activities of the extracts against both negative and positive controls.

3. RESULTS AND DISCUSSION

3.1 Structure elucidation of the isolated compound

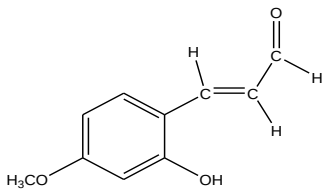


Figure 2: structure of compound 1

Compound **1** was isolated as white crystals from gradient elution using 20:80 of DCM: ethyl acetate (F1). Its Molecular mass was established to be 194.18 amu, with molecular formulae of $C_{10}H_{10}O_4$. This indicates a hydrogen deficiency index (HDI) of five, attributed to the three (3) conjugated double bonds in the benzene ring, a *trans* double bond on the side chain and a carbonyl carbon of the carboxylic acid moiety (C-9). The ^{1}H NMR spectrum indicates the presence of benzylic protons resonating at δ_H 7.26 (H-2), δ_H 6.90 (H-5) and δ_H 6.92 (H-6) corresponding to carbon signals at δ_C 111.6 (C-2), δ_C115.0 (C-5) and δ_C 129.7 (C-6) in the HSQC spectrum. Also present are the methylene protons with varied multiplicities at δ_H 7.45 (H-7) and δ_H 6.33 (H-8) corresponding to carbon signals at δ_C144.9 (C-7) and δ_C 119.2

in HSQC spectrum. Methoxy protons were observed at δ_H 3.79, corresponding to carbon signal at δ_C 51.6 in HSQC spectrum. In addition, a hydroxyl proton resonating at δ_H 5.30 was also observed.

In the ^{13}C NMR and DEPT Spectrum shows that Compound 1 has a total of 10 Carbons, 5 methine carbons resonating at δ_C 111.6 (C-2), δ_C 115.0 (C-5), δ_C 129.7 (C-6), δ_C 144.9 (C-7) and δ_C 119.2 (C-8) (Table 1). Three quaternary carbons at δ_C 129.5 (C-1), δ_C 149.2 (C-3) and δ_C 147.5 (C-4). One carbonyl carbon resonating at δ_C 209.4 (C-9) and a methoxy carbon at δ_C 51.6 (Table 1).

The HMBC spectrum showed that proton resonating at δ_H 7.26 (H-2) correlates with carbon signals; C-1, C-3, and C-6. Consequently, proton resonating at δ_H 6.90 (H-5) correlates with carbon signals; C-3, C-4 and C-6. Also observed in the HMBC spectrum was a strong correlation between methoxy protons resonating at δ_H 3.79 and carbon signal at δ_C 149.2 (C-3). In the COSY spectrum correlations between proton at δ_H 6.90 (H-5) with that at δ_H 6.92 (H-6) was observed, also olefinic protons at δ_H7.45 (H-7) and δ_H 6.33(H-8) correlated. Other HMBC and COSY spectra are shown in Fig 1 and Fig 3. Compound 1, was assigned IUPAC name as (*E*)-3-(4-hydroxy-3-methoxyphenyl) acrylic acid and a common name *trans* ferrulate bearing the methoxy and hydroxyl groups at position 3 and 4 respectively on the phenyl ring.

Table 1: NMR data of Compound 1

	13C	DEPTH	HSQC	HMBC	COSY
1.	127.5	C	-	-	-
2.	111.6	CH	7.26	1,3,6,7	-
3.	149.2	C	-	-	-
4.	147.5	C	-	-	-
5.	115.0	CH	6.90	3,4,6	6
6.	129.7	CH	6.92	1,4,5	5
7.	144.9	CH	7.45	1,2,8,9	8
8.	119.2	CH	6.33	1,7,9	7
9.	209.4	C	-	-	-
O-CH_3	51.6	CH_3	3.79	3	-

3.2 Leaf disc bioassay

The results showed that most of the extracts had insecticidal activities against adult aphids at different concentrations (Table 2). Methanol 1:1 DCM crude extract was more active than methanol crude extract, having its highest percentage mortality at 85.33 ± 2.52 % as compared to 76.00 ± 5.29 % of methanol extract (Table 2). This is so because combination of solvents during extraction, isolate most of the secondary metabolites as opposed to one solvent extraction.

F10 and 11 showed the highest mortalities with corresponding percentages of 93.67 ± 1.15 % and 93.67 ± 1.15 % at a concentration of 10 mg/ml respectively. This could be attributed to the presence of a higher concentration of insecticidal compound in it. On the other hand, F6 and 7 showed poor insecticidal activities with their corresponding highest % mortalities against adult aphids at 13.67 ± 4.04 and 10.00 ± 2.52 % respectively, which conversely indicates a lower concentration of insecticidal in the samples.

Paired t-Test statistical analysis at $P \leq 0.05$ showed that insecticidal activities of methanol crude, Methanol 1: 1 DCM, F1, F9, F 10, F11 and F12, had insecticidal activities that were not statistically different from that of Malathion (positive control) with their p vales more than 0.05 (Table 2), while that of F7 and F6 were not different from that of negative control (distilled water) Table 2**.** Other mortality results are shown in Table 2 and Figure 3.

The insecticidal activities of compound 1 are attributed to the conjugation in the benzene rings as well as the activation caused by the ring activators (-OH and -OCH_3 groups) on the benzene ring. Consequently, the Trans, double bond on the site chain of the carboxylic acid moiety offers a greater reactive stability to the compound hence can be used individually as an insecticide or a precursor in production of other compounds.

Table 2: Mortality rates of extractives at different geometric dilution against adult aphids

Samples	%M ± SD	Malathion %M ± SD	P value, α= 0.05
Methanol crude 100 %	76.00 ± 5.29	97.67 ± 2.52	0.0060
50%	56.00 ± 6.56	85.00 ± 5.00	0.0200
25 %	36.00 ± 6.56	69.33 ± 4.04	0.0300
Methanol 1:1 DCM 100 %	85.33 ± 2.52	97.67 ± 2.52	0.0100
50%	64.00 ± 0.58	85.00 ± 5.00	0.0100
25 %	40.33 ± 2.52	69.33 ± 4.04	0.0500*
F1 100 %	89.33 ± 1.15	97.67 ± 2.52	0.0100
50%	69.33 ± 1.15	85.00 ± 5.00	0.0500*
25 %	56.67 ± 2.89	69.33 ± 4.04	0.0100
F2 100 %	50.00 ± 5.00	97.67 ± 2.52	0.0030
50%	26.00 ± 3.61	85.00 ± 5.00	0.0060
25 %	10.00 ± 2.52	69.33 ± 4.04	0.0030
F3 100 %	61.67 ± 2.89	97.67 ± 2.52	0.0008
50%	29.33 ± 4.04	85.00 ± 5.00	0.0060
25 %	8.67 ± 1.1.5	69.33 ± 4.04	0.0009
F4 100 %	84.33 ± 4.04	97.67 ± 2.52	0.0200
50%	62.67 ± 2.52	85.00 ± 5.00	0.0100
25 %	42.67 ± 2.52	69.33 ± 4.04	0.0010
F5 100 %	29.33 ± 4.04	97.67 ± 2.52	0.0020
50%	15.33 ± 4.04	85.00 ± 5.00	0.0040
25 %	2.67 ± 2.52	69.33 ± 4.04	0.0002
F6 100 %	13.67 ± 4.04	97.67 ± 2.52	0.0006
50%	3.67 ± 1.15	85.00 ± 5.00	0.0080
25 %	0.00 ± 0.00	69.33 ± 4.04	0.0010
F7 100 %	10.00 ± 2.52	97.67 ± 2.52	0.0009
50%	0.00 ± 0.00	85.00 ± 5.00	0.0010
25 %	0.00 ± 0.00	69.33 ± 4.04	0.0010
F8 100 %	61.00 ± 1.73	97.67 ± 2.52	0.0020
50%	34.33 ± 4.04	85.00 ± 5.00	0.0100
25 %	17.67 ± 2.52	69.33 ± 4.04	0.0010
F9 100 %	86.67 ± 2.89	97.67 ± 2.52	0.0700*
50%	47.67 ± 2.52	85.00 ± 5.00	0.0100
25 %	28.33 ± 2.89	69.33 ± 4.04	0.0080
F10 100 %	93.67 ± 1.15	97.67 ± 2.52	0.1900*
50%	67.00 ± 1.73	85.00 ± 5.00	0.0400

25 %	48.67 ± 2.52	69.33 ± 4.04	0.0020
F11 100 %	93.67 ± 1.15	97.67 ± 2.52	0.0600*
50%	72.00 ± 1.73	85.00 ± 5.00	0.0200
25 %	45.33 ± 2.52	69.33 ± 4.04	0.0070
F12 100 %	94.33 ± 1.15	97.67 ± 2.52	0.0600*
50%	70.33 ± 2.52	85.00 ± 5.00	0.0300
25 %	51.33 ± 5.77	69.33 ± 4.04	0.0100
Malathion 100%	97.67 ± 2.52	-	-
50%	85.00 ± 5.00	-	
25 %	69.33 ± 4.04	-	-
Distilled water	0 ± 0.00	-	-
	0 ± 0.00	-	-
	0 ± 0.00	-	-

The values given represent average percentage mortality rate of different extracts at different geometric concentrations ± Standard Deviation and their lethal activities against adult aphids as compared to positive control (Malathion). The coding, F1-F12 represents fractions eluted from column chromatography.

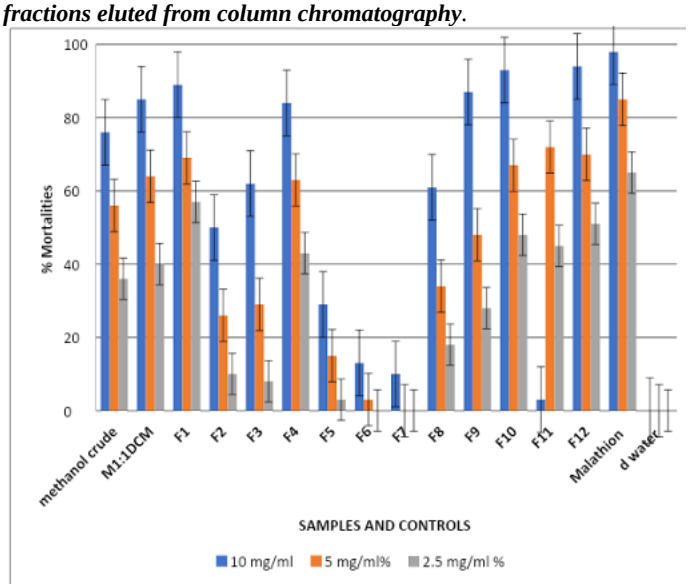


Figure 3: A graphical representation of mortalities against adult aphids of extracts and controls

The bar graph represents average percentage mortality rate of different extracts at different geometric concentrations ± Standard Deviation and their lethal activities against adult aphids as compared to positive control (Malathion). The coding, F1-F12 represents fractions eluted from column chromatography

3.3 LC_{50} determination

Lethal concentration at 50 % was analyzed using probit analysis; this was done by first transforming concentration as logarithm to base of 10. Percentage mortalities were transformed into probit values using conversion table (Appendix 1). Regression analysis of Probit values (Y-axis) and log of concentrations (X-axis) corresponding to each extract was done, at 95 % confidence limit. The corresponding regression results was then recalculated using straight line equation (Eq. 1)

$$Y = MX + C$$

where Y represents the y value, X- the x value, M- gradient and C- the y intercept

LC_{50} values for respective extracts were calculated by replacing Probit value at 50 % mortality, which correspond to 5.00 and placed in equation 1 i.e., 5.00 = MX + C, the values for M and C were obtained from regression summaries then an anti-log of the resulting figures was done to obtain their corresponding LC_{50} values respectively. LC_{50} values were calculated and tabulated in Table 3 and compared in Figure 4 as shown below.

Table 3: Summary of LC_{50} values

Sample	LC_{50} (mg/ml) ± standard Error
Methanol crude	4.04 ± 0.92
Methanol 1:1 DCM	3.30 ± 0.06
F1	2.17 ± 0.11
F2	10.00 ± 0.10
F3	7.81 ± 0.07
F4	3.20 ± 0.10
F5	12.84 ± 0.92
F6	14.19 ± 0.88
F7	20.30 ± 0.96
F8	7.59 ± 0.08
F9	4.37 ± 0.19
F10	2.87 ± 0.16
F11	2.87 ± 0.08
F12	2.63 ± 0.16
Malathion (positive control)	1.80 ± 0.39

The values represent LC_{50} of different extracts at different geometric concentrations ± Standard Error and their lethal activities against adult aphids as compared to positive control (Malathion). The coding, F1-F12 represents fractions from column chromatograph.

ANOVA single factor analysis for the results in Table 3 above are indicated in Table 4 below. The value of p = 0.001982 is less than p ≤0.05 and therefore the correlations between the LC_{50} of Methanol + crude, Methanol + DCM, fractions (F1 to F12) and Malathion (positive control) are statistically significant and the correlation of LC_{50} for the fractions and LC_{50} for Malathion is real.

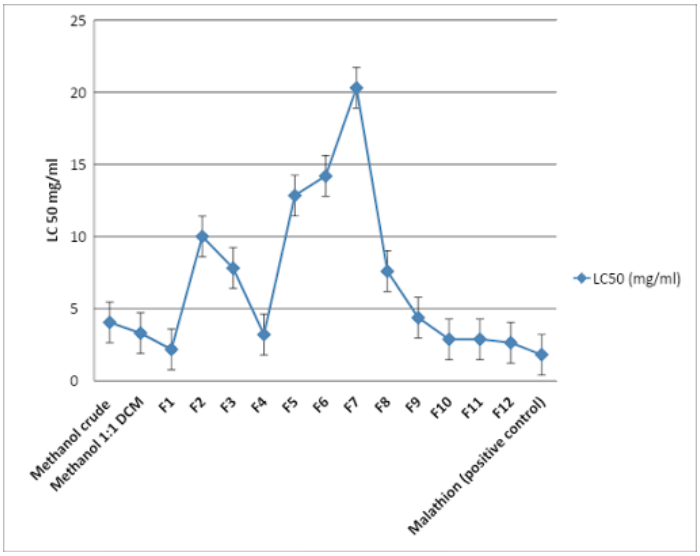
Table 4: Anova: Single Factor analysis for LC_{50}.

Groups	*Count*	*Sum*	*Average*	*Variance*
A	13	94.14	7.241538	31.96033
B	1	23.4	1.8	2.14E-31

ANOVA

Source	*SS*	*Df*	*MS*	*F*	*P-*	*F crit*

e of Variation					*value*	
Between Groups	192.4672	1	192.4672	12.04413	0.001982	4.259677
Within Groups	383.524	24	15.98017			
Total	575.9912	25				



LC_{50} usually shows the toxic level of a substance based on the concentration of the active ingredient to suppress or kill. The lower the LC_{50} values the more toxic it is to the test agents. In the present study **F1** showed the lowest LC_{50} value, which indicates that a little amount of the active ingredient is required to kill the aphids. This state can be explained based on the concentration of phytochemicals that can act against the aphids. As compared to this study, *n*-hexane crude extracts from *P. juliflora* seeds had a significant insecticidal activity against adult with corresponding LC_{50} values of 0.472 % in 12 hours, this is so because the oil extracts contain active insecticidal components that acts against the test organism (Tura and Bezuneh, 2015). The DCM crude extracts from *P. juliflora* leaves showed a desirable insecticidal activity against cotton aphids (*Aphis gossypii, Glover*), with a corresponding mortality of 1 % in 12 hours, it was also evident from this study that insecticidal activities were dosage dependent (Zerihun and Ele, 2020).

It is reported that cinnamic acid derivatives have been considered as more effective than benzoic acid derivatives, due to the resonance stabilization enhanced by the conjugation between π electrons of the ring and the π-bond of the side-chain, (Jing *et al*., 2012). This explains why compound 1 showed potent insecticidal activity, other researchers found stronger activity of ferulic acid than that of *iso*-ferulic acid attributed to the presence of a hydrophobic OCH_3 group in the meta-position for para–OH phenolic acid (Jing *et al.*, 2012), these substituents were present in compound 1.

4. CONCLUTIONS

In the study, Methanol 1:1 DCM, crude extract yielded 12 fractions on column chromatography. F1 appeared as a pure white crystalline after evaporation on rotary evaporator under a reduced pressure. Fraction 1 yielded one known pure compound 1, which showed a high lethality against the test organism at 2.17 % in 12 hours. Leaf disc bioassay showed that, most of the extractives were active against the adult aphids (*Brevicoryne brassicae)* at different concentrations. Fractions F1, F10 and F11 showed greater insecticidal activities that were not statistically different as that of positive control (malathion), Probity analysis was used in determining the LC_{50} values for both crude and fraction, F1 had the least LC_{50} value of 2.17 %, showing that little amount of the extract is lethal to *Brevicoryne brassicae*, hence is considered effective against the aphids.

5. SCONFLICT OF INTEREST

The authors have no conflict of interests.

6. ACKNOWLEDGMENTS

The authors wish to thank Prof. Dulcie Mulholland of the University of Surrey UK, for NMR and MS analysis and Mr. Towett Erick University of Kabianga for his guidance on insecticidal tests.

Appendix 1: Transformation of percentage to Probit's table

		1	2	3	
4	5	6	7	8	9
0		2.67	2.95	3.12	
3.25	3.36	3.45	3.52	3.59	
3.66					
10	3.72	3.77	3.82	3.87	
3.92	3.96	4.01	4.05	4.08	
4.12					
20	4.16	4.19	4.23	4.26	
4.29	4.33	4.36	4.39	4.42	
4.45					
30	4.48	4.50	4.53	4.56	
4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	
4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	
5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	
5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	
5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	
5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	
6.55	6.64	6.75	6.88	7.05	7.33
	0.0	0.1	0.2	0.3	
0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	
7.51	7.58	7.65	7.75	7.88	8.09

REFERENCES

1) FAO, (2017), State of Food Insecurity in the World 2017 - Building resilience for peace and food security, FAO, Rome, retrieved at: http://www.fao.org/3/aI7695e.

2) Jing P, Zhao SJ, Jian WJ, Qian BJ, Dong Y and Pang J (2012). Quantitative studies on structure
3) DPPH scavenging activity relationships of food phenolic acids. Molecules 17,12911–12924.

4) Mahmood I, Imadi SR, Shazadi K, Gul A, Hakeem KR (2016). Effects of pesticides on environment. In: Hakeem K, Akhtar M, Abdullah S, editors. Plant, soil and microbes: volume 1: implications in crop science. Cham: *Springer International Publishing*; 2016. pp. 253–269.

5) Nadio EC, Agevi H, and Obiri J (2020). Impacts of *Prosopis juliflora* on Abundance and Species Diversity of Forage Species in Turkana County, Kenya. *Current Journal of Applied Science and Technology*. 39(33): 112-125.

6) Okello JJ, and Swinton SM, (2010). From circle of poison to circle of virtue: pesticides, export standards and Kenya's green bean farmers. *Journal of agricultural economics*, 61(2), 209-224.

7) Olwande J, Smale M, Mathenge MK, Place F and Mithöfer D. (2015). Agricultural marketing by smallholders in Kenya: A comparison of maize, kale and dairy. *Food Policy*, 52, 22-32.

8) Sindhu M. and Emilia AT. (2006) Bioconversions of ferulic acid, an hydroxycinnamic acid. *Critical Reviews in Microbiology*, 32:115–125.

9) Tura AM and Bezuneh TT, (2015). Insecticidal activity of *Prosopis juliflora* seed oil against
   a. termite (*Odontotermes obesus*) and Cockroach (*Blattella germanica*) *Journal of Natura*
10) *l Sciences Research* 5 (13) 32-36

11) Van Emden HF and Harrington R. (Eds.) (2017). Aphids as crop pests. CABI Publishing, London.

12) Zerihun M and Ele E, (2020). Bioactive compounds and biological assays of different *Prosopis juliflora* extracts against groundnut aphid. Open Journal of Chemistry, 6(1): 021-029. DOI: https://dx.doi.org/10.17352/ojc.000020