Effects of Pure Compounds of *Prosopis juliflora* against Groundnut Aphids

Mulate Zerihun[1] and Estifanos Ele[2]
[1]Melkassa Agriculture Research Center, EIAR, P.O. Box 2003, Addis Ababa, Ethiopia,
[2]Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.
Corresponding author e-mail: mulatezerihun@yahoo.com.

Abstract

In the current study the different solvent extracts of Prosopis juliflora (stem bark, seed and leaf) were evaluated for their insecticidal effects on groundnut aphids (Aphis craccivora) at 1, 2.5, 5, 10 and 15 % concentrations. The study was also focused on isolation and characterization of active ingredients of the plant. The analysis of bioactive compounds in the plant extracts involved the applications of common phytochemical screening assays through chromatographic techniques. Three compounds, 4-(2-aminoethyl) phenol (1), 3-O-methyl-chiro-inositol (2) and 4-(2-aminoethyl) phenoxy)-6-methoxycyclohexane-1, 2, 3, 4-tetraol (3) were isolated from the leaves of the methanolic extract. The highest mortality caused by methanol and dichloromethane extracts in 12 h was 83.33% against groundnut aphid. Structural elucidations of the compounds were performed based on their ^{1}H NMR, ^{13}C NMR, UV and IR spectra.

Introduction

The high diversity of plants in different parts of the world is a potential source of useful compounds, which could be extracted and used for various purposes. Primitive man has found these extracts efficient as medicines for the relief of pain or alleviation of the symptoms of disease, as poisons for use in warfare and hunting and as effective agents for euthanasia and capital punishment. They have also been used as narcotics, hallucinogens, or stimulants to relieve tedium, or alleviate fatigue and hunger. Many of these natural products are still used today usually for the same general purpose (Mann, 1995).

Besides, the use of natural products as perfumes, flavoring agents, insecticides, insect antifeedants, fungicides, plant growth regulating hormones, molluscicides, etc. has been well known long ago. Characterization of new plant compounds is usually followed by study of their biological activity and biosynthesis. One of the most exciting things that emerged in the last few years is realization of natural products that have been considered useless do have functions in the organisms from which they originate. It is recognized that many of them have vital roles as mediators of ecological interactions, thereby ensuring the continued survival of a particular organism. Despite the vast number and structural diversity of metabolites, almost all arise by one of the three biosynthetic pathways or by a combination of two or more of

these pathways, known as the acetate, mevalonate and shikmate pathways (Mann, 1994).

Prosopis belongs to the family *Leguminosae* (Fabaceae), sub-family *Mimosoideae*. It contains 44 species (Burkart, 1976) which grow in a wide array of environments and are commonly not restricted by soil type, pH or salinity. They grow in semi-arid and arid tracts of tropical and sub-tropical regions of the world and are spreading fast because the leaves are unpalatable and animals do not digest their seeds (Felker., *et al*. 1981). Most of the species are shrubs or small trees, mainly characterized by the presence of thorns and prickles and highly recognized for their properties as windbreaker, soil binder, and sand stabilizer, as well as their ability to grow in the poorest soils and to survive in areas where other trees cannot do well (Van Klinken and Campbell, 2001). One of the species, *P. juliflora* was introduced in to Ethiopia as a biological soil and water conservation agent during the late 1970s. Now it is becoming a major threat because of its invasive nature. There are reports indicating that *P. juliflora* is widely distributed in Ethiopia (Abdulahi., *et al*. 2017, Birhane., *et al*. 2017). However, extracts of *P. juliflora* are said to be effective against some weed species, insects, nematodes, pathogenic bacteria, fungi and viruses. Its leaf extracts have been found to inhibit germination in a number of species (Sen and Chawan, 1970), including the invasive weed *Parthenium hysterophorus* (Al-Rawahy., *et al*. 2003).Therefore, the objective of this study was to investigate the phytochemical composition of *P. juliflora* and insecticidal activities of its extracts against ground nut aphid.

Materials and Methods

Chemicals and Instruments

Some of the chemicals used in this study were HPLC grade (Methanol, Dichloromethane, n-hexane, DMSO), while the remaining were analytical grades. Fatty acid standards were purchased from Sigma-Aldrich, Germany. All other chemicals were purchased from Fisher Scientific, UK. HPLC experiment was conducted on Agilent-1260 infinity system with UV Daiod Aray detector. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400.13 and 100.6 MHz, respectively. IR Spectra were recorded on a Perkin-Elmer Spectrum 65 instrument in the range of 4000-200 cm^{-1}. UV spectra were recorded on a UV-T60 spectrophotometer.

Chromatography

Analytical TLC was run on Merck Kieselgel 60 F254. Plates were visualized under UV light and by spraying with Vanillin 5% H_2SO_4 in MeOH followed by heating for a few seconds. Silica gel 60 (Merck) with particle size 0.063-0.200 (70-230 mesh ASTM) was used for column chromatography.

Preparation of Plant materials

The stem bark, seeds and leaves of *P. juliflora* were collected from Amibara Woreda (Afar region) in October 2016. The samples were collected in sterile polyethylene bags. The fresh samples were transported in ice box and were preserved in a deep freezer until processing. The stem bark of *P. juliflora* was chopped into small pieces and dried at room temperature for two weeks. The dried seeds and stem bark were milled using a "knife" mill. The fresh leaves were frozen in liquid nitrogen and crushed with a mortar and pestle.

Extraction

Hydrodistillation, Soxhlet and solvent extraction method extracted the powdered plant materials. The crude extracts were evaluated under laboratory condition through different concentrations. Based on preliminary evaluation, phytochemical studies on methanol leaf extract of *P. juliflora* was conducted as follow:

The fresh leaves of *P. juliflora* were cut into small pieces, frozen in liquid nitrogen and ground. The ground leaves (5 kg) were soaked in MeOH(8 L) for 72 h at room temperature with occasional stirring and shaking. The extracts was then filtered first through a fresh cotton plug and then with a Whatman No. 1 filter paper. The solvent was then removed by rotary evaporation at 35 °C to afford a dark green crude extract (309 g). Solvent-solvent partitioning (Figure 1) was done by using the protocol developed by Kupchan and Tsou (Kupchan and Tsou. 1973) and modified by Wagenen., *et al*. (1993). Thus, the crude extract was dissolved in MeOH (400 mL) and defatted by soaking in n-hexane (0.8L) for 48 hwith occasional stirring and shaking. The hexane extract was separated and the solvent was removed to afford 10.7 g of extract. The residue obtained after removal of MeOH was washed with DCM (0.8L) and removal of the solvent afforded 8.9 g of the DCM-soluble material. The residue was dried to afford 289 g of hexane and DCM-insoluble material. Figure 1 summarizes the extraction procedure.

Isolation of compounds

The residue (FR 3, 30g) was applied on a column of silica gel (900g) and eluted with DCM-MeOH mixtures of increasing polarity. Fourt nine fractions (each 100 ml) were collected as shown in Table 1. Analytical TLC with DCM-MeOH solvent system monitored the progress of separation and fractions of similar TLC profiles were combined to give twelve fractions (Table 2).

Fraction 6 was further purified by chromatography over a short silica gel (10 g) column using .EtOAc: MeOH mixtures of increasing polarity. Thirteen fractions (each 10 ml) were collected. From fraction 1 to 5 were collected using EtOAc-methanol (9:1) solvent system. Fraction 6 eluted with EtOAc: MeOH (8:2) resulted in white crystals (31mg), which was identified to be 4-(2-aminoethyl) phenol (1).

Removal of the solvent from Fraction 9' give a residue (989 mg) which was applied on a column of silica gel (30 g) and eluted with $CHCl_3$:MeOH mixtures of increasing polarity. Twenty-eight fractions (each 25 mL) were collected. Fraction 13, which was

eluted with $CHCl_3$: MeOH (7.5:2.5) afforded a white crystalline solid (79 mg) which was identified to be 3-*O*-methyl-chiro-inositol (2).

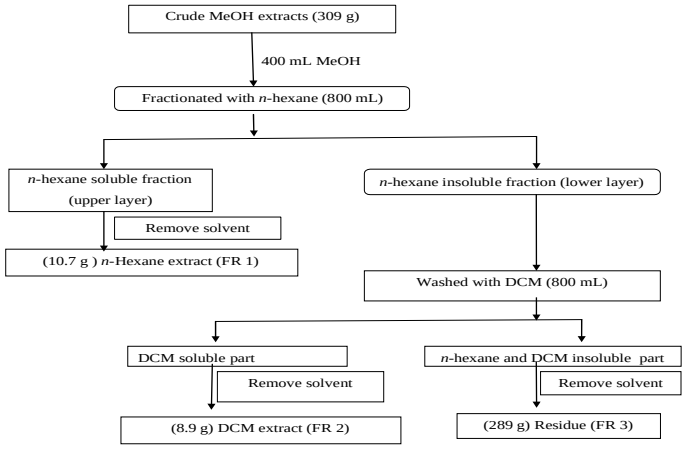


Figure 1. Schematic representation of partitioning of the crude MeOH extract of leaves of *P. juliflora*.

Table 1. Fractionation of the MeOH extract.

Solvent system	Ratio	Fractions	Volume (mL)
DCM-MeOH	10.0: 0	1&2	200
"	9.5:0.5	3&4	200
"	9.0:1	5-10	600
"	8.5:1.5	11-15	500
"	8.0:2.0	16-21	600
"	7.5:2.5	22-26	500
"	7.0:3.0	27-34	800
"	6.5:3.5	35-39	500
"	6.0:4.0	40-47	800
"	5.0:5.0	48	100
"	0:10.0	49	100

Table 2. Weights of combined fractions

Fractions combined	Code	Weight (mg)
1&2	1'	29
3	2'	107
4-6	3'	307
7-10	4'	141
11-13	5'	826
14-16	6'	234
17&18	7'	241
19-21	8'	203
22-26	9'	989
27-34	10'	2070
35-41	11'	1034
42-49	12'	794

Identification of compounds

4-(2-Aminoethyl) phenol (1).

White crystalline solid.mp 160-163 °C; Lit mp = 164 °C. UV ($\lambda_{max,}$ MeOH) 276 nm.IR υ_{max}(KBr): 3411, 3202, 1613, 1500 ,1229 cm^{-1}.^{1}H NMR: and ^{13}C NMR: The detailed information such as names of the compounds, literature value etc., are given in the result and discussion part.

3-O-methyl-chiro-inositol (2)

White crystalline solid mp158-161°C; UV ($\lambda_{max,}$ MeOH). No absorption band above 200 nm.IR υ_{max} (KBr): 3306, 2913, 1500, 1447, 1369, 1325 and 1246 cm^{-1}. ^{1}H and 13C NMR The detailed information such as names of the compounds, literature value etc., are given in the result and discussion part.

Results and Discussion

Bioassay of extracts of *P. juliflora* against groundnut aphid

The insecticidal activities of extracts of *P. juliflora* were tested against groundnut aphid (*Aphis craccivora*) and insect mortalities in response to application of different concentrations were evaluated after 12, 24, 48, 72 and 96 hrs. In triplicates. Results of the experiment indicated that rate of mortality of groundnut aphids was highly significantly ($P < 0.0001$) affected by concentration of all the extracts (Table 3).

Table 3. Bioassay data of *P. juliflora* extracts against groundnut aphid (*Aphis craccivora*).

	Extract	Plant part and concentration	Mean mortality				
			12 h	24 h	48 h	72 h	96 h
1	Hydro-distillate	Leaves (1%)	3.667^{ij}	8.333^{bcdef}	10.000^{a}	10.000^{a}	10.000^{a}
2		Leaves(2.5%)	5.333^{hi}	8.000^{cdef}	10.000^{a}	10.000^{a}	10.000^{a}
3		Leaves(5%)	6.667^{defgh}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
4		Leaves(10%)	7.000^{cdefgh}	9.667^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
5		Leaves(15%)	8.333^{abcdef}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
6		Stem bark(1%)	3.667^{ij}	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
7		Stem bark(2.5%)	5.333^{hi}	9.333^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
8		Stem bark(5%)	7.000^{cdefgh}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
9		Stem bark(10%)	8.667^{abcde}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
10		Stem bark(15%)	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
11	Soxhlet extract	Leaves(1%)	3.667^{ij}	8.333^{bcdef}	10.000^{a}	10.000^{a}	10.000^{a}
12		Leaves(2.5%)	5.333^{hi}	8.667^{abcde}	10.000^{a}	10.000^{a}	10.000^{a}
13		Leaves(5%)	6.000^{fghi}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
14		Leaves(10%)	6.667^{defgh}	9.667^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
15		Leaves(15%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
16		Stem bark(1%)	2.333^{jkl}	7.000^{f}	10.000^{a}	10.000^{a}	10.000^{a}
17		Stem bark(2.5%)	6.333^{efgh}	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
18		Stem bark(5%)	6.667^{defgh}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
19		Stem bark(10%)	7.000^{cdefgh}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
20		Stem bark(15%)	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
21		Seeds (1%)	5.667^{ghi}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}

22		Seeds(2.5%)	6.000^{cdefgh}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
23		Seeds(5%)	6.333^{bcdefgh}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
24		Seeds(10%)	6.667^{defgh}	9.667^{ef}	10.000^{a}	10.000^{a}	10.000^{a}
25		Seeds(15%)	6.667^{fghi}	9.667^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
26	DCM extract	Leaves(1%)	6.667^{defgh}	7.667^{def}	10.000^{a}	10.000^{a}	10.000^{a}
27		Leaves(2.5%)	7.667^{abcdefgh}	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
28		Leaves(5%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
29		Leaves(10%)	9.333^{bcdefgh}	10.000^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
30		Leaves(15%)	9.667^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
31		Stem bark(1%)	3.667^{ij}	8.333^{bcdef}	10.000^{a}	10.000^{a}	10.000^{a}
32		Stem bark(2.5%)	7.000^{cdefgh}	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
33		Stem bark(5%)	7.667^{abcdefgh}	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}
34		Stem bark(10%)	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
35		Stem bark(10%)	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
36		Seed(1%)	6.667^{defgh}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
37		Seeds (2.5%)	7.000^{cdefgh}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
38		Seeds (5%)	7.667^{ghi}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
39		Seeds (10%)	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
40		Seeds (15%)	9.000^{abcd}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
41	MeOH extract	Leaves(1%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
42		Leaves(2.5%)	9.333^{abcdefgh}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
43		Leaves(5%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
44		Leaves(10%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
45		Leaves(15%)	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
46		Stem bark(1%)	8.667^{abcde}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
47		Stem bark(2.5%)	9.333^{abc}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
48		Stem bark(5%)	9.000^{abcdefg}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
49		Stem bark(10%)	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
50		Stem bark(15%)	9.667^{abcdef}	10.000^{a}	10.000^{a}	10.000^{a}	10.000^{a}
51		Seeds(1%)	6.000^{fghi}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
52		Seeds(2.5%)	8.333^{abcdef}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
53		Seeds(5%)	9.333^{abc}	9.667^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
54		Seeds(10%)	9.333^{abcd}	9.667^{abc}	10.000^{a}	10.000^{a}	10.000^{a}
55		Seeds(15%)	9.667^{ab}	9.667^{ab}	10.000^{a}	10.000^{a}	10.000^{a}
56	Control	1%	1.333^{jkl}	1.333^{ghi}	1.667^{cde}	2.000^{cd}	3.000^{bc}
57		2.5%	1.667^{jkl}	1.667^{ghi}	2.000^{bcd}	2.333^{bc}	3.000^{bc}
58		5%	1.667^{jkl}	1.667^{ghi}	2.000^{bcd}	2.333^{bc}	3.000^{bc}
59		10%	2.000^{jkl}	2.333^{gh}	2.667^{b}	2.667^{b}	3.000^{bc}
60		15%	2.667^{jk}	2.667^{g}	2.667^{b}	2.667^{b}	3.333^{b}
61		DMSO	2.000^{jkl}	2.333^{gh}	2.333^{bc}	2.333^{bc}	2.333^{de}
62		blank	0.667^{kl}	0.667^{i}	1.000^{ef}	1.6667^{de}	2.333^{de}
63		distilled water	0.667^{kl}	0.667^{i}	1.000^{ef}	1.6667^{de}	1.667^{fg}
Mean			5.742	7.227	5.742	7.227	7.733

CV(%)	7.345	5.017	4..345	3.017	2.211
LSD(0.05)	2.348***	1.518***	2.348***	1.518***	0.899***

*Figures followed by the same letter (s) within a column are not significantly different at P ≤ 0.05. ***= very high significant difference, DMSO= dimethyl sulfoxide,. CV = coefficient of variance; LSD = least significant difference*

The results presented in Table 3 summarize the bioassay of the extracts of *P. juliflora* that promoted significant mortality (Ghosh., *et a*l. 2012) for at least a given concentration when compared to the control. Insecticidal activities higher than 50% at any tested concentration were considered significant. The highest mortality of groundnut aphid was recorded for 1% concentration in 24 hrs. At this concentration, the groundnut aphids encountered total death. There were significant correlations between mortality of groundnut aphid, type of plant extract and its concentration. Hence, highly significant differences were observed between different extracts and concentrations. This means that aphid mortality is greatly affected by plant extract concentration and type of extract used.

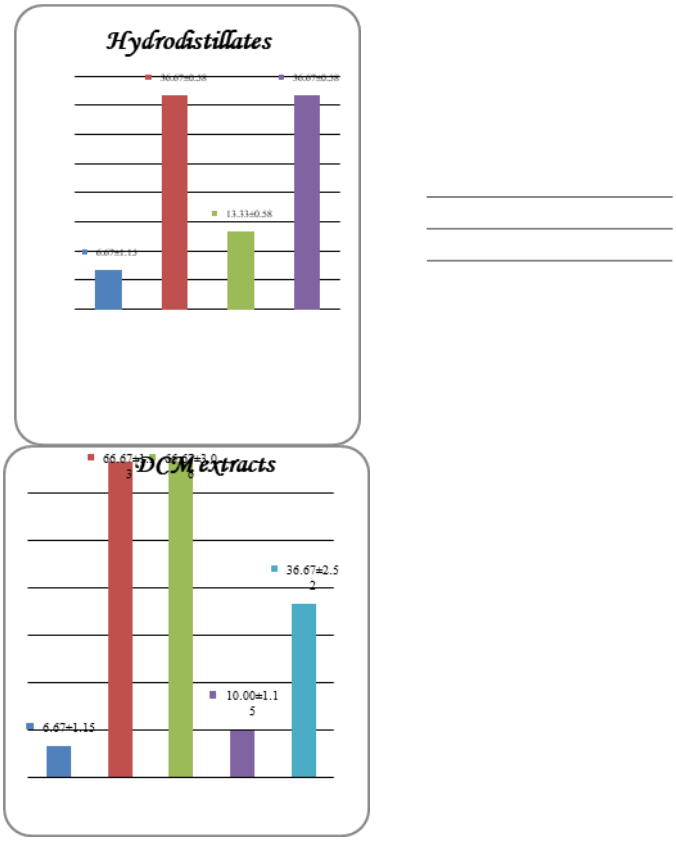


Figure 2. Mean mortality rates of groundnut aphids exposed to different extracts at the lowest concentration (1%) after 12 h. 952 8agm381.66 8ag7.3 952 agm381.66 ag7.3 952 368.1

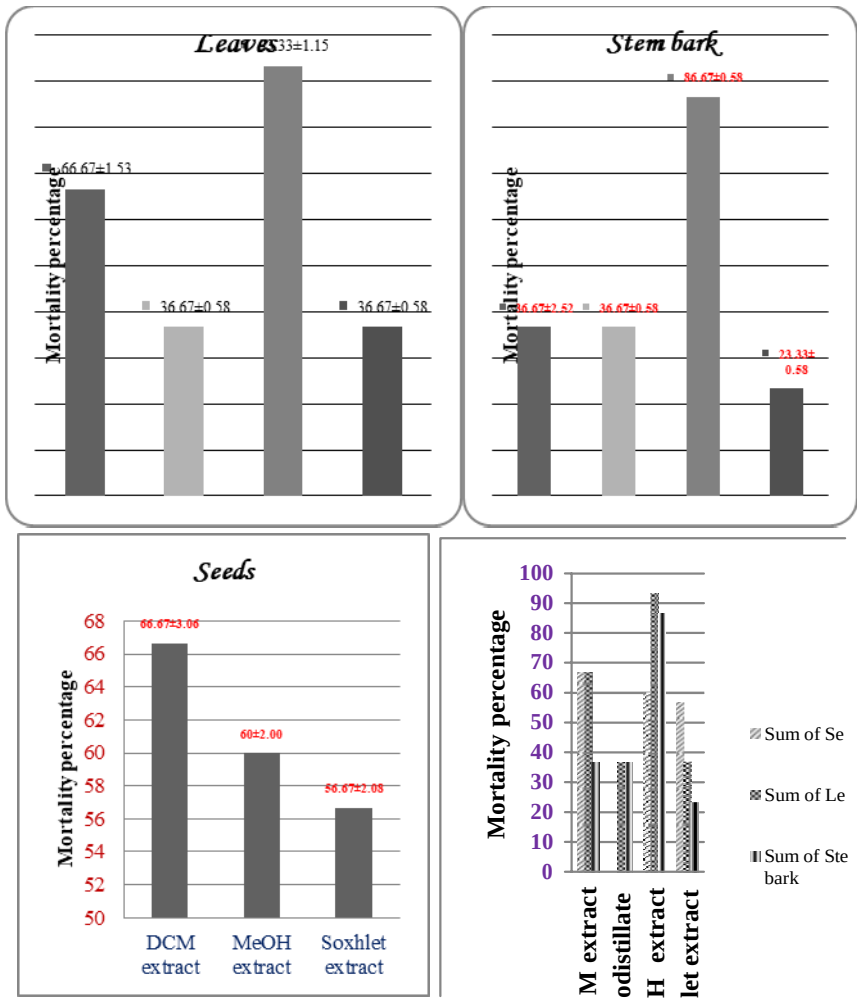


Figure 3. Mean mortality rates of groundnut aphids due to extracts from different parts of *P. juliflora* at the lowest concentration (1%) after 12 h.

Figure 3 shows the mean mortality rates of groundnut aphids at the lowest concentration. Among the treatments, methanol extract from both leaves and stem bark caused death largely. On the other hand, among the extracts of seeds, DCM extracts killed aphids largely compared to other extracts. In this study, the highest recorded of mortality was due to leaf extracts. As shown in Figure 3, at the lowest concentration (1%) the MeOH extract of leaves of *P. juliflora* showed the highest insecticidal activity after 12 h of treatment application.

The LC_{50}, LC_{95},LT_{50} and LT_{95} values were obtained through probit analysis.(Ashford and Sowden 1970) .The LC_{50} values showed that MeOH extracts of all parts of the plant had higher efficacies at the lowest concentration. The DCM extract of stem bark was the least active while the MeOH extract of the leaves was the most active compared to the others.

Table 4. Efficacy of *P. juliflora* stem bark, seed and leaf extracts against groundnut aphids for lethal concentration LC_{50} and LC_{95} at the shortest time (12 h) and for lethal time LT_{50} and LT_{95} at the smallest concentration (1%) after treatments.

Extraction method	Plant part	Curve equation, M= yC+ b	Lethal concentration (%)		Lethal time (h)	
			LC_{50}	LC_{95}	LT_{50}	LT_{95}
Hydro-distiliation	Seeds	-	-	-	-	-
	Stem bark	M=14.000C+11.334	2.76	5.98	1.46	4.76
	Leaves	M=10.999C+18.004	2.91	7.00	1.63	4.77
Soxhlet extraction	Seeds	M=2.667C+52.000	HS	16.12	HS	4.54
	Stem bark	M=15.335C+2.660	3.09	6.02	2.44	4.89
	Leaves	M=12.666C+11.336	3.05	6.61	1.63	4.77
DCM extraction	Seeds	M=6.666C+52.004	HS	6.45	HS	4.25
	Stem bark	M=15.666C+14.004	2.30	5.17	1.63	4.77
	Leaves	M=7.666C+54.670	HS	5.26	HS	4.70
MeOH extraction	Seeds	M=8.334C+51.996	HS	5.16	HS	4.44
	Stem bark	M=2.334C+83.998	HS	4.71	HS	3.12
	Leaves	M=1.334C+89.328	HS	4.25	HS	1.25

HS= out of the range (highly significant), M = mortality , y = slope , C = concentration, b =y-intercept

Phytochemical investigation of the leaves of *P. juliflora*

In the course of this work, attempts were made to isolate and characterize secondary metabolites from the leaves of *P. juliflora*. Thus, three compounds were isolated by using silica gel column chromatography and characterized by spectroscopic techniques as described below.

Characterization of Compound 1

Compound one had a melting point of 160-163 °C. Its UV spectrum in MeOH displayed an absorption maximum (λ_{max}) at 276 nm, which is characteristic of a phenolic nucleus *(*Sayed, *et al*. 2006). The IR spectrum (KBr) indicated the presence of an aromatic ring (1613, 1500, 1229 cm^{-1}), a hydroxy group (3202 cm^{-1}) and an amine (3411 cm^{-1}).

The ^{1}H NMR spectrum of compound 1 (Table 8) showed doublets at δ 7.10 and 6.79 (J = 8.0 Hz) integrating for two protons each due to aromatic protons on a 1,4-disubstituted benzene ring. Two triplets were observed at δ 3.12 and 2.87 (J = 8.0 Hz) integrating for two protons each indicating the presence of two adjacent methylene groups, presumably attached to the aromatic ring at one end and to an electron withdrawing group on the other.

Table 5. Comparison of ^{1}H NMR data of compound 1 with literature values (in D_2O).

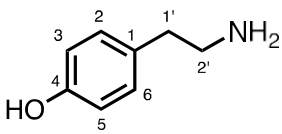
Proton	Comp. 1 (δ_{ppm})	Literature (Sato., et al. 1970), (Samsonova., et al. 2004) (δ_{ppm})
2, 6	7.12 (2H, *d*, *J*= 8.0 Hz, H-2 and 2')	7.24
3, 5	6.79 (2H, *d*, *J*=8.0 Hz, H-3 and 3')	6.90
1'	2.87 (2H, *t*, *J*=8.0 Hz, H-6)	2.94 (2H)
2'	3.12 (2H, *t*, *J*=8.0 Hz, H-5)	3.16 (2H)

The ^{13}C NMR spectrum (Table 6) of compound 1 showed six carbon resonances of which four are in the aromatic region and the remaining two are in the aliphatic region. The DEPT-135 spectrum revealed that the signals at δ 129.4 and 115.3 are due to aromatic methine carbons while the signals at δ 40.9 and 32.4 are due to aliphatic methylene carbons. The quaternary carbon signals appeared at δ156.4, 127.0. The ^{13}C NMR spectrum together with the ^{1}H NMR spectrum allowed for the assignment of the signal at δ 129.4 to the two equivalent aromatic carbons C-2 and C-6. The signal at δ 115.3 can be attributed to C-3 and C-6 *ortho* to an electron-donating group at C-4. It is thus evident that the side chain is attached to C-1 whose carbon resonance appeared at δ 127.0.

Table 6. Comparison of ^{13}C NMR data of compound 1 with literatures (Sato., *et al.* 1970), (Samsonova., *et al.* 2004) values (in DMSO-d_6).

Carbon	Comp. 1 (δ_{ppm})	Literature (Sato., *et al.* 1970), (Samsonova., *et al.* 2004) (δ_{ppm})
1	127.0	127.2
2,5	129.4	129.4
3, 6	115.3	115.4
4	156.4	156.2
1'	32.4	30.6
2'	40.9	38.7

The UV, IR and NMR data allowed for the identification of comp. 1 as4-(2-amino ethyl) phenol. Comparison of the ^{1}H and ^{13}C NMR data of comp. 1 with those reported for 4-(2-amino ethyl) phenol in the literature (Sato., *et al.* 1970) (



al.

Characterization of Compound 2

Compound two had a melting point of 158-161°C. The UV spectrum of two in MeOH did not show any absorption band above 200 nm, which indicated that there are no chromophores, and that it might be a saturated hydrocarbon. The IR spectrum (KBr) indicated the presence of hydroxyl groups (3306 cm^{-1}). An aliphatic C-H stretching band appeared at 2913 cm^{-1}, O-C stretching band was observed at 1500 cm-1 while bands between 1246, and 1447 cm^{-1} are due to bending vibration of O-C group.

The ^{1}H NMR spectrum of compound 2 (in D_2O) showed nine proton resonances between δ 3.25 and 3.88 indicating that all of the carbon atoms to which the protons are attached are oxygenated. The proton resonances at δ 3.88, 3.70, 3.67, 3.62, 3.55, and 3.24 integrated for one proton each due to methine protons, while the signal at δ 3.46 integrated for three protons and is due to a methoxy group. The ^{1}H NMR spectrum recorded in DMSO-d_6 (Table 7) showed five additional proton resonances at δ 4.73 (*d*), 4.69 (*d*), 4.54 (*d*), 4.49 (*d*), 4.35 (*d*) which integrated for one proton each. These signals disappeared upon addition of drops of D_2O and therefore revealed the presence of five hydroxyl groups.

The ^{13}C NMR spectrum of two showed seven carbon resonances between δ 60.1 and 84.2. The DEPT-135 spectrum revealed that the signal at δ 60.1 is due to a methoxy group while the remaining carbon resonances are attributable to methine carbons.

It was evident from the spectroscopic data generated for compound 2 that it is a polyhydroxylated hydrocarbon. From the ^{1}H, ^{13}C and DEPT-135 NMR spectra, the molecular formula $C_7H_{14}O_6$ could be deduced for compound 2. The rings plus double bonds calculated for this molecular formula is 1. This, coupled with the spectroscopic data, clearly suggested the presence of only one ring in compound 2. It was also evident that the ring is six-membered.

Based on the above experimental data, compound 2 was identified to be 3-*O*-chiro-inositol (Figure 4). The HH COSY and HSQC spectra established correlations, which agreed with the proposed structure. Comparison of the ^{1}H and 2D NMR data of 2 with those reported in the literature (Della Greca., *et al.* 2007)' (Abraham., *et al.* 2005) for 3-*O*-methyl-chiro-inositol revealed a very close resemblance.

Table 10. ^{1}H NMR (400.13 MHz) data of D_2O.

Carbon	Comp. 2
1	72.4
2	71.3
3	84.2
4	73.0
5	70.5
6	72.9
7	60.1

Table 11.^{13}C NMR (100.6 MHz) data (δ_{ppm}) of compound 2 in compound 2 in DMSO-d_6.

Proton	Comp. 2	Literature (Della Greca., *et al.* 2007), (Abraham., *et al.* 2005)
1	3.70	3.89
2	3.62	3.62
3	3.24	3.25
4	3.46	3.35
5	3.67	3.73
6	3.88	3.91
7	3.55	3.58

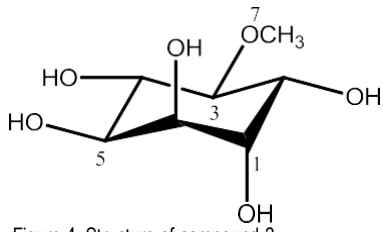


Figure 4. Structure of compound 2.

Characterization of Compound 3

Compound three had a yellowish color and melting point of 175-180 °C; Lit mp = 178 °C. The characteristics of compound 3 were the combination of the two compounds.

Conclusion

The present study was conducted to evaluate the insecticidal activities of extracts of *P. juliflora* against groundnut aphids (*Aphis craccivora*). All extracts of *P. juliflora* showed high percentage mortality at 1% concentration in 24 h against groundnut aphids. Extracts of the plant showed significant insecticidal activity at 0.001% level of confidence. Thus, *P. juliflora* has the potential to be used as a bio-insecticide. As part of this research, phytochemical analysis was conducted on the MeOH extract of the leaves of *P. juliflora* and three compounds, namely, 4-(2-aminoethyl) phenol (1), 3-*O*-methyl-chiro-inositol(2)and4-(2-aminoethyl)phenoxy)-6-methoxycyclohexane-1,2,3,4-tetraol (3) were isolated and characterized. In general, it was observed that *P. juliflora* is a rich source of flavonoids, alkaloids and saponins. A more rigorous

bioactivity guided phytochemical work on *P. juliflora* might lead to the isolation and characterization of novel secondary metabolites with insecticidal properties.

References

Abdulahi M and Regasa T. 2017. "Prosopis juliflora L: DISTRIBUTION, IMPACTS AND AVAILABLE CONTROL METHODS IN ETHIOPIA." Tropical and Subtropical Agroecosystems **20**(1): 75-89.

Abraham RJ, J Byrne, L Griffiths, and R Koniotou.2005. "1H chemical shifts in NMR: Part 22—Prediction of the 1H chemical shifts of alcohols, diols and inositols in solution, a conformational and solvation investigation." Magnetic Resonance in Chemistry **43**(8): 611-624.

Al-Rawahy SH, K Al-Dhafri, and S Al-Bahlany. 2003. "Germination, growth and drought resistance of native and alien plant species of the genus Prosopis in the sultanate of Oman." Asian J. Plant Sci. **2**: 1020-1023.

Al-Shakh-Hamed W and M Al-Jammas.1999. "The antimicrobial activity of alkaloidal fraction of Prosopis juliflora." Iraqi Journal Veterinary Science **12**(2): 281-287.

Ashford J and R Sowden.1970. "Multi-variate probit analysis." Biometrics **26**: 535-546.

Birhane E, AC Treydte, Eshete A, Solomon N, and Hailemariam M.2017. Carbon stocks of communal grazing lands invaded by Prosopis juliflora." Journal of Arid Environments 141: 60-67.

Burkart A. 1976. "A monograph of the genus Prosopis (Leguminosae subfam. Mimosoideae)." Journal of the Arnold Arboretum **57**: 450-525.

DellaGreca M, A Fiorentino, A Izzo, F Napoli, R Purcaro, and A Zarrelli.2007. "Phytotoxicity of secondary metabolites from Aptenia cordifolia." Chemistry & biodiversity **4**(2): 118-128.

Felker P, PR Clark, A Laag, and P Pratt.1981. "Salinity tolerance of the tree legumesgrown in sand culture on nitrogen-free media." Plant and Soil **61**(3): 311-317.

Ghosh A, Nand

Sen D and D Chawan.1970. "Ecology of Desert Plants and Observations on Their Seedlings: III: The Influence of Aqueous Extracts of Prosopis juliflora DC. on Euphorbia caducifolia Haines." Vegetatio **21**: 277-298.

Silva AM, AR Silva, AM Pinheiro, SR Freitas, VD Silva, CD Souza, RD Hughes, El- Bachá, MD Costa, and ED Velozo.2007. "Alkaloids from Prosopis juliflora leaves induce glial activation, cytotoxicity and stimulate NO production." Toxicon **49**(5): 601- 614.

Singh S and V Swapnil. 2011. "Antibacterial properties of alkaloid rich fractions obtained from various parts of Prosopis juliflora." Int. J. Pharm. Sci. Res. **2**(3): 114-120.

Van Klinken R and S Campbell. 2001. "The biology of Australian weeds. Prosopis L. species." Plant Protection Quarterly **16**(1): 2-20.

VanWagenen BC, R Larsen, JH Cardellina, D Randazzo, ZC Lidert,and C Swithenban.1993. "Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri." The Journal of Organic Chemistry **58**(2): 335-337.

Wise R, B Van Wilgen, and D Le Maitre. 2012. "Costs, benefits and management options for an invasive alien tree species: the case of mesquite in the Northern Cape, South Africa." Journal of Arid Environments **84**: 80-90.