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Insecticidal Activities of Leaf, Seed and Stem Bark Extracts of Prosopis Juliflora against the Cotton (*Aphis Gossypii Glover*) Aphid

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Stem bark, seed and leaf extracts of *Prosopis juliflora* were evaluated under laboratory conditions for their insecticidal effect against cotton (*Aphis gossypii Glover*) aphid at 1, 2.5, 5, 10 and 15 % concentrations. Bioassay results indicated that the highest observed mortality due to dichloromethane extracts were 73.33% and 70.00% for leaves and seed in 12 hrs at l% concentration respectively. The LC95 of the dichloromethane extract of leaves and seed showed the highest mortality at less than 1% concentration. Whereas, the LT95 value showed that the highest mortality rateswere recorded in methanol and dichloromethane extracts. LT95 value of leaves extract (soxhlet extract) was recorded at 2.20 hrs.. In all extracts, the efficacy increased with increase in concentration. The bioassay results showed that after 24 hours all extracts demonstrated maximum mortality. The obtained results suggested that bioinsecticides can be made from dichloromethane and methanolic extracts of the leaf of *Prosopis juliflora* for use in integrated pest management.

Keywords: Bioassays, insect control, natural insecticides, *Prosopis juliflora,* Cotton Aphid (*Aphis gossypii Glover*), biological activity.

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INTRODUCTION

The high diversity of plants is an available source of useful compounds. People extracted plants and used them for different purposes (Mann 1995). Natural products are organic compounds of natural origin that are unique to one organism or common to a small number of closely related organisms. The use of natural products as medicines, poisons, hallucinogens, stimulants, perfumes, flavoring agents, insecticides, insect antifeedants, fungicides, plant growth regulating hormones, molluscicides, etc., is well known. Despite the vast number and structural diversity of metabolites, almost all arise by one of three biosynthetic pathways or by a combination of two or more of these pathways. These are known as the acetate, mevalonate and shikmate pathways (Mann 1994).

Prosopis juliflora (Swartz) DC is one of the world's

worst invasive alien species causing severe environmental degradation to the arid and semi-arid lowlands of the Horn of Africa. Today, many countries such as Ethiopia, Sudan, Kenya, Eritrea, and Somalia are heavily affected by this invasive plant. *Prosopis juliflora (P. juliflora)* was imported to Ethiopia as a means to protect land degradation (Knowler, 2007), (Haregeweyn et al., 2013). In terms of coverage, the area most adversely affected nationally include the Afar and Somali regions in the east and southeast of the country and the area around Dire Dawa city. There are also moderately affected areas in Amhara, Oromia, Southern Nations Nationalities and Peoples (SNNP) and Tigray Regions. *P. juliflora* has an aggressive invasive character invading pastureland, irrigated cultivated lands and irrigation canals causing an irreversible displacement of natural pasture grasses as well as native tree species (Hibretu, 2009), (Abdulahi *et al*., 2017).

P. juliflora grows abundantly in Ethiopia and is commonly known as Algarroba (Spanish), Mesquite (English), Weyane (Amharic) and Dergihara (Afar). In the Afar region, 90% of the population is pastoralist (agro-pastoralist) and their livelihood mainly depends on livestock production. Over 700,000 hectares of prime grazing and cultivable land along the Awash river is currently either invaded or at risk of invasion by *P. juliflora.* This accounts for 15% of the region's productive land (4,670,316 hectares), excluding wetlands, water bodies, sandy and rocky areas (4,856,251 hectares) (Haji and Mohammed 2013). On the other hand, *P. juliflora* is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world as it has high nitrogen fixing potential, food for animals, etc (Almaraz et al., 2007),(Odee et al., 1997).

Among the ecosystem services, natural pest control is an important aspect. Approximately 99% of potential crop pests are controlled by natural enemies such as birds, spiders, parasitic wasps, viral diseases and other organisms (DeBach and Rosen 1991). Hence, natural pest control not only minimizes the use of synthetic chemicals in crop protection but also saves huge amount of money spent on chemical compounds (Pimentel *et al*., 1991). To protect agricultural crops, enormous amount of synthetic pesticides are used worldwide. In 2007, Agrow reported that the total value of the world's agrochemical market was between US$31–35 billion. Among the products, herbicides accounted for 48% followed by insecticides (25%) and fungicides (22%) (Amri *et al*., 2012). However, the excessive use of synthetic pesticides in the croplands, urban environment, and water bodies to get rid of noxious pests, has resulted in an increased risk of pesticide resistance, enhanced pest resurgence and increased environmental pollution. Efforts are thus being made worldwide to replace synthetic chemicals by biopesticides and biological controls have introduced (Bakkali *et al*., 2008), (Batish *et al.,* 2008).

Aphis gossypii Glover (cotton aphid) is the most common aphid species in cotton crop which reduce crop yield (Godfrey and Fuson 2001), (Mayeux *et al.,* 1984). *Aphis gossypii* Glover is probably the most injurious insect species of cotton throughout Africa. The damages due to aphids varies with the stage of plant development, with most damage caused if aphids infest during early plant development (Allan et al., 2016), (Wightman and Wightman 1994). Therefore, the objective of this study has been to investigate insecticidal activities of leaf, seed and bark extracts of *prosopis juliflora* against the cotton (*aphis gossypii glover*) aphid.

MATERIALS AND METHODS

General

All chemicals, reagents and solvents used in this study were analytical/HPLC grade. Fatty acid standards were purchased from Sigma-Aldrich, Germany. All other chemicals were purchased from Fisher Scientific, UK.

Instruments

Gas chromatography-Mass spectrometry experiments were conducted on Agilent Technologies 7820A GC system with Agilent technologies 5977E MSD, USA. An HP-5capillary column (30 m long and 0.25 mm internal diameter) was used. The NIST 2014 Mass Spectral Library was used.

Plant Material

The stem bark, seeds and leaves of *P. juliflora* were collected from Amibara Woreda (Afar region) 9^{o} 60' 45" N latitude and 40^{o} 9' 32" E longitude and at an altitude of 740 meter above sea level), 280 km north east of Addis Ababa, during October 2016.The plant specimen was identified previously by the Biology Department, AAU Herbarium. 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.

Plant materials preparation

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

The powdered plant materials were extracted by hydrodistillation, Soxhlet and solvent extraction.

Extraction by hydrodistillation

The stem bark and leaves of *P. juliflora* were subjected to hydrodistillation following the method developed by Costa *et al* (2015). Thus, fresh leaves of *P. juliflora* (1486 g) were placed in a distillation flask containing distilled water (2L). The flask was attached to a Clevenger apparatus, which was connected to a condenser. The flask was heated using a heating mantle and hydrodistillation continued for 5 h after initial boiling. The organic phase was separated and dried over anhydrous sodium sulfate and filtered. The filtrate was stored at 4 °C in a refrigerator until it was analyzed by GC-MS and used for biological tests. Following the same procedure, fresh stem bark (1035 g) of *P. juliflora* was extracted by hydrodistillation to afford oil. The yields (*v*/*w,* mL/kg) were calculated using the amount of essential oil (mL) obtained relative to the fresh weight (kg) that was used. The yields obtained for stem bark and leaves were 14.78 mL/kg and 17.56 mL/kg, respectively.

Characterization of the hydrodistillates

The hydrodistillates obtained from the stem bark and leaves of *P. juliflora* were analyzed by gas chromatography. Ultra-high purity (99.999%) helium gas, as the carrier gas, was used at constant flow mode. An Agilent 7820A auto sampler was used to inject 1 µL of the sample with a split less injection mode into the inlet heated to 275 ºC. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min, and then, the temperature was increased at a rate of 10 ºC/min until the column temperature was reached 200 ºC, and then heated at the rate of 3 ºC/min till the temperature reached 240 ºC. No mass spectra were collected during the first 4 min of the solvent delay. The transfer line and the ion source temperature were 280 ºC and 230 ºC, respectively. The detector voltage was 1600 V, and the electron energy was 70 eV. Mass spectral data were collected from 40–600 m/z. The parameters, such as the quality, and probability values of peaks identified were made through a library search using NIST 2014. One µL of each of the hydrodistaillates of the leaves and stem bark of *P. juliflora* were injected into the GC-MS and the components were identified by comparing their mass spectra with the NIST (2014) Library.

Soxhlet Extraction

The powdered stem bark (192 g) was placed in the Soxhlet apparatus and extracted with *n*-hexane (1400 mL) for 8 h. The extract was allowed to cool to room temperature and filtered on a Whatman No.1 filter paper. The solvent was then removed by rotary evaporation at 30°C to afford 13.1g of oil.

Following the same procedure, powdered leaves (200g leaf) and seeds (198g) of *P. juliflora* were extracted to afford 14.75 and 11.09 g of oil, respectively. Equation 1 was used to calculate the percentages yields of the oils were 6.82, 7.38 and 5.60% for stem bark, leaves and seeds respectively. The oils were stored at 4 °C until they were analyzed by GC-MS and ussed for biological tests.

$$\% \text{ Oil} = \frac{\text{Mass of Oil}}{Mass\ of\ powdered\ plant\ material} \times 100 \ldots (1)$$

Preparation of fatty acid methyl esters

In a 50 mL round bottom flask fitted with a reflux condenser, the Soxhet extract (1 g) was placed and dissolved in 2% methanolic potassium hydroxide (10 mL)prepared by mixing KOH with methanol. The mixture was heated on a water bath at 50ºCfor 1h. The reaction mixture was allowed to cool down to room temperature and saturated NaCl (3 mL) was added to the reaction mixture and the solution was swirled gently several times. n-Hexane (20 mL) was added into the solution and then the mixture was transferred to a separator funnel. The organic layer (upper layer) was separated, dried over anhydrous sodium sulfate and filtered through a Whatman No.1 filter paper followed by removal of the solvent by rotary evaporation.

Preparation of methyl palmitate standard

To determine the amount of fatty acids in the Soxhlet extracts of the stem bark, seeds and leaves of *P. juliflora,* methyl palmitate standard was used for GC-MS analysis. Table 1 shows the standard solutions of methyl palmitate used to construct the calibration curve. Methyl palmitate was prepared by Fischer esterfication (Mbaraka *et al.*, 2003) of palmitic acid as described below:

> In a 50 mL round bottom flask equipped with a reflux condenser, palmitic acid (1 g) was dissolved in MeOH (10 mL) and then concentrated H2SO4(1 mL) was carefully added. The mixture was heated on a water bath at 50 ºC for 1 h. The reaction mixture was then removed from the water bath and cooled to room temperature. Chloroform (30 mL) was added and the transferred to a separatory funnel. Deionized water (30 mL) was added and the organic phase was separated. The organic phase was washed with aq. NaHCO3(30 mL) and water (30 mL),

Table 1. Methyl palmitate standard solutions used for construction of the calibration curve.

Sample code	Concentration (ppm)	RT (minute)	Area
H1A	1	14.946	37468273.30
H1B	1	14.946	37468273.33
H1C	1	14.946	34522697.93
H10A	10	14.96	199432023.00
H10B	10	14.946	205708666.30
H10C	10	14.946	199432023.20
H25A	25	14.960	364137351.00
H25B	25	14.960	364137351.00
H25C	25	14.959	374772596.80
H50A	50	14.986	644456871.10
H50B	50	14.973	603905108.30
H50C	50	14.987	648531452.90
H100A	100	15.013	1137443228.00
H100B	100	15.000	1170868871.00
H100C	100	15.014	1165507951.00

C= Concentration, RT= Retention time, PPM= Parts per million

dried over anhydrous sodium sulfate, filtered and the solvent was removed to afford methyl palmitate.

Solvent extraction

The powdered seeds, leaves and stem bark of *P. juliflora* were extracted with DCM and MeOH following the method described by Harborne (Nostro et al., 2000).

Extraction with dichloromethane

Powdered stem bark (200g) was soaked in DCM (700 mL), and was kept for 48 h at room temperature with gentle shaking. The DCM extract was filtered through a Whatman No.1 filter paper and the solvent was removed under reduced pressure in a rotary evaporator at 30 °C to afford a 10.99 g crude extract.

Using the same method powdered leaves (200g) and seeds (100g) were extracted with 700 mL and 350 mL DCM, respectively. The yields were calculated using the amount of crude extract obtained relative to the weight of the leaves, seeds and stem bark of *P. juliflora*. The yields were obtained for stem bark, seeds and leaves are 5.50, 12.02 and 16.75% respectively. The final dried crude extracts were stored in a refrigerator until they were used for biological tests.

Extraction with methanol

Powdered stem bark (200 g) was soaked in MeOH (700 mL) and was then kept for at room temperature for 48 h with gentle shaking. The MeOH extract was filtered through a Whatman No.1 filter paper and solvent was removed by rotary evaporation at 35 °C to afford 12.16 g of a crude extract.

Using the same method, powdered leaves (200g) were extracted with MeOH (700 mL) to afford 29.4g of the extract.

Likewise the powdered seed (100 g) were extracted with MeOH (350 mL) to obtain 8.13 g of the extract.

stem bark of *P. juliflora*. The yields were obtained for stem bark, seeds and leaves are 6.08, 14.70 and 8.13% respectively. The crude extracts were stored in a refrigerator until they were used for biological tests.

Aphids incubation and bioassays

Cotton aphid (*Aphis gossypii Glover*) was used as test organism in this study. Bioassay studies were conducted in Werer Agricultural Research Center (Afar region, Ethiopia) 280 km north east of Addis Ababa during November 2016. All the bioassay experiments were performed under laboratory conditions. Cotton aphid (*Aphis gossypii Glover*) was selected for this study. The insects were identified and collected by researchers at the Werer agricultural research center from the experimental fields of the research center. The collected insects were brought to the laboratory and allowed to adapt for about 12 h. Each batch of insects was placed in a rigid polythene container with a mesh lid and transferred to the test room. Plastic beakers (l0 mL) containing cotton wool soaked with water (5 mL) were inverted on the meshes to provide water for the insects. Food or water was given during the test period after every count. The insecticidal activities of hydrodistillates, Soxhlet and solvent extracts of *P. juliflora* were evaluated using dosage-dependent relationships against susceptible aphids. In each test, five to twenty days old of aphids were used. Intrinsic insecticidal activities were assessed by topical application according to standard WHO Protocol (Dua *et al*., 2008).

Bioassay of extracts of *P. juliflora* against cotton aphids

The insecticidal activities of the extracts of the stem bark, seeds and leaves of *P. juliflora* were evaluated using dosage-dependent bioassays. Solutions of the test materials were prepared by dissolving extracts in aqueous DMSO following the method prescribed by Musabyimana *et al.,* (2001). Thus, five different concentrations (1.0, 2.5, 5.0, 10.0 and 15.0%) were prepared by dissolving extracts in 10% aqueous DMSO solutions. Equivalent quantities of DMSO-water solutions alone were used as solvent controls. Equivalent quantities of deionized water and blank controls were used as positive and negative controls, respectively. In the case of hydrodistillates of the stem bark and leaves of *P. juliflora*, equivalent amounts of deionized water and saturated sodium chloride were used as positive and solvent controls, respectively. In each treatment three replication each containing 10 aphids was used. Bioassays were carried out at room temperature (28 ± 2 °C) in petri dishes (30cm diameter) containing circular Whatman #1 filter paper (33 mm^2) placed inside each well. One mL of prepared sample solution was added on the upper part of the petri dish. After application of the solutions, aphids were maintained at controlled temperature (28 ± 2 °C) and humidity (70% ± 10%). Mortalities were recorded after 12, 24, 48, 72 and 96 h after applying the solutions. Following the same procedure, DCM and MeOH extracts of seeds, stem bark and leaves of *P. juliflora* were evaluated for their insecticidal activities.

Data analysis

All measurements were done in triplicate and the results were recorded as mean ± standard deviation (SD). The results were analyzed by one-way ANOVA using SPSS version 15.0 (SPSS Inc. Chicago, IL, USA) (Micallef *et al.,* 2009). Multiple comparisons between factor levels were done. Analysis of variance (ANOVA) was used to check the presence of significant difference at 95% confidence level between mean levels of insecticidal activities of extracts of *P. juliflora*. One way ANOVA was also used to compare whether there were differences in the mean levels of mortality and insecticidal activities among samples. Additionally, the relationships between dose and mortality in the insecticide susceptible aphid strains were analyzed by probit analysis (Ashford and Sowden 1970), which provided an estimation of the median lethal doses for each of the three extraction methods compared (hydrodistillation, Soxhlet and solvent extraction).

LC50 and LC95 values and confidence limits for each bioassay were produced by probit analysis with the natural response used to correct control mortality (Ashford and Sowden 1970). However, only raw aphid mortality was plotted. Probit analysis permitted to rank the extracts by relative bioactivity using multiple *t*-tests for homogeneity, which compared slope estimates in a pair-wise manner at a critical $P \leq 0.05$.

RESULT AND DISCUSSION

Extraction and characterization of active fractions

The stem bark and leaves of *P. juliflora* were extracted by hydrodistillation. In addition, the stem bark, leaves and seeds were subjected to soxhlet extraction with hexane, and solvent extraction with DCM and methanol. Table 2 shows the amounts of extracts obtained by the three methods. For the essential oils obtained by hydrodistillation, the yields (*v*/*w,*mL/kg) were calculated using the amount of essential oil (mL) obtained relative to the fresh weight (kg) of the leaves and stem bark. The yields obtained by soxhlet and solvent extraction were calculated as percent weights of the samples.

Table 2. Extracts of the stem bark, seeds and leaves of *P. juliflora* obtained by hydrodistillation, Soxhlet, and solvent extraction methods.

	Extraction Method	Solvent	Yields		
			Stem bark	Leaves	Seeds
1	Hydrodistillation	Water	14.78 mL/kg	17.56 mL/kg	-
2	Soxhlet Extraction	Hexane	6.82%	7.38%	5.60%
3	Solvent Extraction	DCM	5.50%	12.02%	16.75%
4	Solvent Extraction	Methanol	6.08%	14.70%	8.13%

Characterizations of essential oils obtained by hydrodistillation

The essential oils obtained by hydrodistillation of the leaves and stem bark of *P. juliflora* were subjected to analysis by gas chromatography-mass spectroscopy (GC-MS). An Agilent Technologies 7820A GC system with Agilent Technologies 5977E MSD was used for the analysis. The GC-MS technique was used to identify variations in the composition of essential oils and make comparative analysis of the oils of the stem bark and leaves of *P. juliflora* as described below.

Characterization of essential oil of the leaves of *P. juliflora*

Using GC–MS, a total of 40 compounds were identified from the essential oil of the leaves of *P. juliflora*, of which 22 compounds with qualities greater than 80% were subjected to analysis with the help of NIST 2014 Mass Spectral Library. Quantifications of the components were made using the relative area method (Equation 2). The detailed information such as names of the compounds, retention time, area, etc., are given in Appendix Table 1.

$$\text{Relative percentage} = \frac{\text{Component (peak) area}}{\text{Total peaks area}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The major components identified were ethyl 2-hydroxybenzoate (11.51%), ethyl benzoate(9.90%), ethylpalmitate (9.73%), (*E*)-methyl octadec-9-enoate (9.55%), methyl-2-hydroxybenzoate(8.56%), (*Z*)-hex-3-en-1-yl benzoate (6.41%), and methyl stearate (5.88%). Esters are the predominant classes of compounds in the essential oil obtained from the leaves of *P. juliflora.* Out of the 22 major compounds, fifteen are esters. Table 3 shows the concentrations of different classes of volatile compounds in the extract of the leaves of *P. juliflora.*

Characterization of essential oil obtained from the stem bark of *P. juliflora*

Using GC–MS, a total of 51 compounds were identified in the essential oil obtained by hydrodistillation of the stem bark of *P. juliflora*. Ten of the compounds with quality greater than 80%were subjected to further analysis with the help of the NIST 2014 Mass Spectral Library. Appendix Table 2 shows the results of the analysis. The 10 major compounds include one aldehyde, one carboxylic acid and eight hydrocarbons as depicted in Table 4.

Quantification of the compounds was made based on Equation 1. The major components identified in the essential oil of the stem bark of *P. juliflora* were palmiticacid (22.45%), heptadecane (11.25%), heneicosane (10.77%), tetracosane (16.51%), pentacosane (10.68%) and hexacosane (7.70%) as shown in Appendix Table 2.

The essential oils obtained from the stem bark and leaves of *P. juliflora* were different in composition and relative concentrations of their components.

Characterization of the Soxhlet extracts

The stem bark, seeds and leaves of *P. juliflora* were subjected to Soxhlet extraction with hexane to afford the oils.(Musabyimana, Saxena et al. 2001) The yields of the oils are given in Table 2. The oils were transesterifed with methanol under basic conditions (Delmonte *et al*., 2009). The compositions of the resulting mixtures were analyzed by GC-MS.The external standard method was used for the determination of the concentrations of the fatty acids.

Table 3. Concentrations of different classes of volatile compounds in the leaf extract of *P.juliflora*.

Chemical classes of volatile compounds	Relative Area (%)	No. of compounds
Alcohols	9.53	2
Esters	80.27	15
Ketone	1.86	1
Aldehyde	1.14	1
Hydrocarbons (alkane, alkene)	7.21	3

Table 4. Concentrations of different classes of volatile compounds in the stem bark extract of *P.juliflora*.

Chemical classes of volatile compounds	Relative Area (%)	No of compounds
Aldehyde	2.35	1
Hydrocarbons (alkane, alkene)	75.20	8
Carboxylic acid	22.45	1

Standard solutions of methyl palmitate were prepared at concentrations of 100, 50, 25, 10 and 1 ppm and known volumes of the standard solutions were injected in triplicate in to the GC-MS. Using the mean value of each concentration, calibration curve was constructed which was used to measure concentrations of the fatty acids.

Appendix Table 3 shows the compounds that were identified by GC-MS using the NIST 2014 Mass Spectral Library. The major compound was found to be (9*E*, 12E)-methyl octadeca-9, 12-dienoate (31.54%).

Characterization of the oil obtained from the seeds of *P. juliflora*

The oil obtained by Soxhlet extraction of the seeds of *P. juliflora* with hexane was subjected to transesterification with methanolic KOH. The resulting oil was subjected to GC-MS analysis and a total of 9 compounds were identified of which 3 were major, with qualities greater than 80%.The concentrations of the fatty acid methyl esters were calculated by using a calibration curve as described above. The major compounds were found to be (9*E*, 12*E*)-methyl octadeca-9,12-dienoate (4.86%), Methyl oleate (2.81%) and Methyl palmitate(1.17%). Appendix Table 4 gives the experimental data obtained for the three major compounds.

Characterization of the oil obtained from the leaves of *P. juliflora*

The leaves of *P. juliflora* were extracted with hexane using Soxhlet apparatus. Removal of the solvent afforded an oil which was transesterified with methanolic KOH. The resulting mixture consisting mainly of fatty acid methyl esters was analyzed by GC-MS and 6 major compounds were identified with qualities greater than 80 %. The concentration of each fatty acid methyl ester was calculated by using a calibration curve as described above. . The major compounds were found to be (9*Z*, 12Z, 15Z)-methyl octadeca-9, 12, 15-trienoate, (9*E*, 12E)-methyl octadeca-9, 12-dienoate, Methyl palmitate, Methyl tetradecanoate, Methyl dodecanoate and Methyl stearate. Appendix Table 5 gives detailed information such as RT, relative area, etc., of the major compounds

Bioassays

It is known that plants synthesize structurally diverse and complex secondary metabolites for different purposes such as defense, communication (signaling), etc. Secondary metabolites isolated from plants have attracted much attention because of their unique biological activities. Many of them are believed to act as

Table 5. Bioassays data of *P. juliflora* extracts against cotton aphid (*Aphis gossypii Glover*).

	Extracts	Plant part and concentration	Mean mortality				
			12 H	24 H	48H	72H	96H
1	Hydro-distillate	Leaves (1%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
2		Leaves (2.5%)	9.000^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
3		Leaves (5%)	9.000^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
4		Leaves (10%)	9.000^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
5		Leaves (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
6		Stem bark (1%)	8.333^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
7		Stem bark (2.5%)	8.333^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
8		Stem bark (5%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
9		Stem bark (10%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
10		Stem bark (15%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
11	Soxhlet extract	Leaves (1%)	9.333^{abc}	9.667^{a}	10^{a}	10^{a}	10^{a}
12		Leaves (2.5%)	9.333^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
13		Leaves (5%)	9.333^{abc}	9.667^{a}	10^{a}	10^{a}	10^{a}
14		Leaves (10%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
15		Leaves (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
16		Stem bark (1%)	6.333^{de}	9.667^{a}	10^{a}	10^{a}	10^{a}
17		Stem bark (2.5%)	9.333^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
18		Stem bark (5%)	9.333^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
19		Stem bark (10%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
20		Stem bark (15%)	10^{a}	9.333^{a}	10^{a}	10^{a}	10^{a}
21		Seeds (1%)	7.333^{bcde}	10^{a}	10^{a}	10^{a}	10^{a}
22		Seeds (2.5%)	7.333^{bcde}	9.000^{a}	10^{a}	10^{a}	10^{a}
23		Seeds (5%)	7.667^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
24		Seeds (10%)	9.000^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
25		Seeds (15%)	9.333^{abc}	10^{a}	10^{a}	10^{a}	10^{a}
26	DCM	Leaves (1%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
27		Leaves (2.5%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
28		Leaves (5%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
29		Leaves (10%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
30		Leaves (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
31		Stem bark (1%)	7.333^{bcde}	10^{a}	10^{a}	10^{a}	10^{a}
32		Stem bark (2.5%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
33		Stem bark (5%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}

Table 5. Continuation

34		Stem bark (10%)	10 ^{a}	10 ^{a}	10 ^{a}	10 ^{a}	10 ^{a}
35	extract	Stem bark (10%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
36		Seeds (1%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
37		Seeds (2.5%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
38		Seeds (5%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
39		Seeds (10%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
40		Seeds (15%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
41		Leaves (1%)	8.000^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
42		Leaves (2.5%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
43		Leaves (5%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
44		Leaves (10%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
45		Leaves (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
46	MeOH	Stem bark (1%)	8.333^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
47	extract	Stem bark (2.5%)	8.333^{abcde}	10^{a}	10^{a}	10^{a}	10^{a}
48		Stem bark (5%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
49		Stem bark (10%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
50		Stem bark (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
51		Seeds (1%)	8.667^{abcd}	10^{a}	10^{a}	10^{a}	10^{a}
52		Seeds (2.5%)	9.667^{ab}	10^{a}	10^{a}	10^{a}	10^{a}
53		Seeds (5%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
54		Seeds (10%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
55		Seeds (15%)	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
56		1%	0.667^{fg}	0.667^{fgh}	1.333^{defg}	1.333^{ef}	1.667^{cde}
57		2.5%	1.000^{fg}	1.333^{defg}	1.667^{cdef}	1.667^{def}	1.667^{cde}
58		5%	1.667^{fg}	1.667^{cdef}	1.667^{cdef}	1.667^{def}	2.333^{bcd}
59	Control	10%	2.000^{fg}	2.000^{bcde}	2.333^{bcd}	2.333^{cde}	2.333^{bcde}
60		15%	2.667^{f}	3.000^{b}	3.000^{b}	3.000^{b}	3.000^{b}
64		DMSO	2.667^{f}	2.667^{bc}	2.667^{bc}	2.667^{bc}	2.667^{bc}
70		blank	0.333^{fg}	0.667^{fgh}	0.667^{fg}	1.000^{fg}	1.667^{cde}
71		distilled water	0.000^{g}	0.667^{fgh}	0.667^{fg}	1.000^{fg}	1.333^{de}
Mean			6.756	7.600	7.711	7.747	7.827
CV(%)			9.620	6.624	3.267	2.970	1.967
LSD(0.05)			2.357***	1.180***	1.029***	0.996***	1.006***

N***= very high significant difference, DMSO= dimethyl sulfoxide, CV = coefficient of variance, LSD = least significant difference, DCM = dichloromethane, MeOH , Methanol

pheromones, antifeedants or repellents, and as growth regulators (Jørgensen *et al.*, 2005).

The present work has investigated the effects of extracts of *P. juliflora* against cotton aphids. The results obtained from this study are discussed below.

Bioassays of extracts of *P. juliflora* against cotton aphids

The insecticidal activities of hydrodistillates, Soxhlet and sovent extracts of different parts of *P. juliflora* were

Table 6. Percentage mortality of cotton aphid (*Aphis gossypii Glover*) in 12 and 24h after treatment with *P. juliflora* extracts.

	Extract	Plant part and concentration	Mortality (%)	
			12 h	24 h
1	Hydrodistillate	Leaves (1%)	80± 1.53	93.33±00
2		Leaves (2.5%)	80± 1.00	86.67 ±00
3		Leaves (5%)	73.33 ± 1.15	83.33±00
4		Leaves (10%)	70 ± 0.58	80±00
5		Leaves (15%)	73.33 ±00	70±00
6		Stem bark (1%)	76.66 ± 1.53	93.33 ±00
7		Stem bark (2.5%)	73.33 ± 1.00	86.67 ±00
8		Stem bark (5%)	70±1.53	83.33±00
9		Stem bark (10%)	66.67 ± 4.36	80 ±00
10		Stem bark (15%)	60 ± 1.15	70 ±00
11	Soxhlet extract	Leaves (1%)	66.66 ± 0.58	70 ± 0.58
12		Leaves (2.5%)	66.66 ± 1.15	73.33 ± 00
13		Leaves (5%)	66.66 ± 1.00	70 ± 1.73
14		Leaves (10%)	70 ± 0.58	73.33 ± 00
15		Leaves (15%)	73.33± 00	–
16		Stem bark (1%)	36.66 ± 2.31	70 ± 0.58
17		Stem bark (2.5%)	66.66± 1.15	73.33 ± 00
18		Stem bark (5%)	66.66± 0.58	73.33 ± 00
19		Stem bark (10%)	70± 0.58	73.33 ± 00
20		Stem bark (15%)	73.33± 00	–
21		Seeds (1%)	46.66 ± 2.31	73.33 ± 00
22		Seeds (2.5%)	46.66 ± 2.00	73.33 ± 00
23		Seeds (5%)	50 ± 2.52	73.33 ± 00
24		Seeds (10%)	63.33 ± 1.00	73.33 ± 00
25		Seeds (15%)	66.66 ± 1.15	73.33 ± 00
26	DCM extract	Leaves (1%)	73.33 ± 00	–
27		Leaves (2.5%)	73.33 ± 00	–
28		Leaves (5%)	73.33 ± 00	–
29		Leaves (10%)	73.33 ± 00	–
30		Leaves (15%)	73.33 ± 00	–
31		Stem bark (1%)	63.33 ± 2.31	73.33 ± 00
32		Stem bark (2.5%)	70 ± 0.58	73.33 ± 00
33		Stem bark (5%)	73.33 ± 00	–
34		Stem bark (10%)	73.33 ± 00	–
35		Stem bark (15%)	73.33 ± 00	–
36		Seeds (1%)	70 ± 0.58	–
37		Seeds (2.5%)	70 ± 1.73	–
38		Seeds (5%)	70 ± 1.53	–
39		Seeds (10%)	70 ± 1.73	–
40		Seeds (15%)	70 ± 1.53	–
41	MeOH extract	Leaves (1%)	53.33 ± 2.65	73.33 ± 00
42		Leaves (2.5%)	60 ± 1.53	73.33 ± 00
43		Leaves (5%)	70 ± 0.58	73.33 ± 00
44		Leaves (10%)	70 ± 0.58	73.33 ± 00

Table 6. Continuation

45		Leaves (15%)	73.33 ± 00	–
46		Stem bark (1%)	56.66 ± 2.89	73.33 ± 00
47		Stem bark (2.5%)	56.66 ± 2.08	73.33 ± 00
48		Stem bark (5%)	73.33 ± 00	–
49		Stem bark (10%)	73.33 ± 00	–
50		Stem bark (15%)	73.33 ± 00	–
51		Seeds (1%)	60 ± 2.31	73.33 ± 00
52		Seeds (2.5%)	70 ± 0.58	73.33 ± 00
53		Seeds (5%)	73.33 ± 00	–
54		Seeds (10%)	73.33 ± 00	–
55		Seeds (15%)	73.33 ± 00	–

Table 7. Efficacy of *P. juliflora* stem bark, seed and leaf extracts against cotton aphids for lethal concentration LC95 at the smallest time (12 h) and for lethal time LT95 at the smallest concentration (1%) after treatments.

Extraction methods	Parts	In percentage (%)	In hours
		LC95	LT95
Hydrodistillation	Seeds	-	-
	Stem bark	13.65	3.50
	Leaves	5.38	3.12
Soxhlet extraction	Seeds	6.42	4.06
	Stem bark	4.74	4.40
	Leaves	3.80	2.20
DCM extraction	Seeds	HS	HS
	Stem bark	4.18	4.06
	Leaves	HS	HS
MeOH extraction	Seeds	3.45	3.12
	Stem bark	4.33	3.50
	Leaves	4.60	3.75

HS= highest mortality can be recorded less than 1% concentration (highly significant)

investigated against cotton aphid (*Aphis gossypii* Glover). Insect mortalities were evaluated using dosage-dependent bioassays and the results were recorded after 12, 24, 48, 72 and 96 h. All measurements were done in triplicate and the mean ± standard deviation (SD) values were calculated. Statistical analyses indicated that all of the extracts showed dependence between mortality and concentration ($P < 0.0001$). The calculated mean mortalities of cotton aphids are presented in Table 5.

The result shows on Figure 1 and 2 the mean mortality rates of cotton aphids at the lowest concentration. The DCM extracts of the leaves and seeds caused death to a greater extent compared to other extracts. In this study, the highest insecticidal activity at the lowest

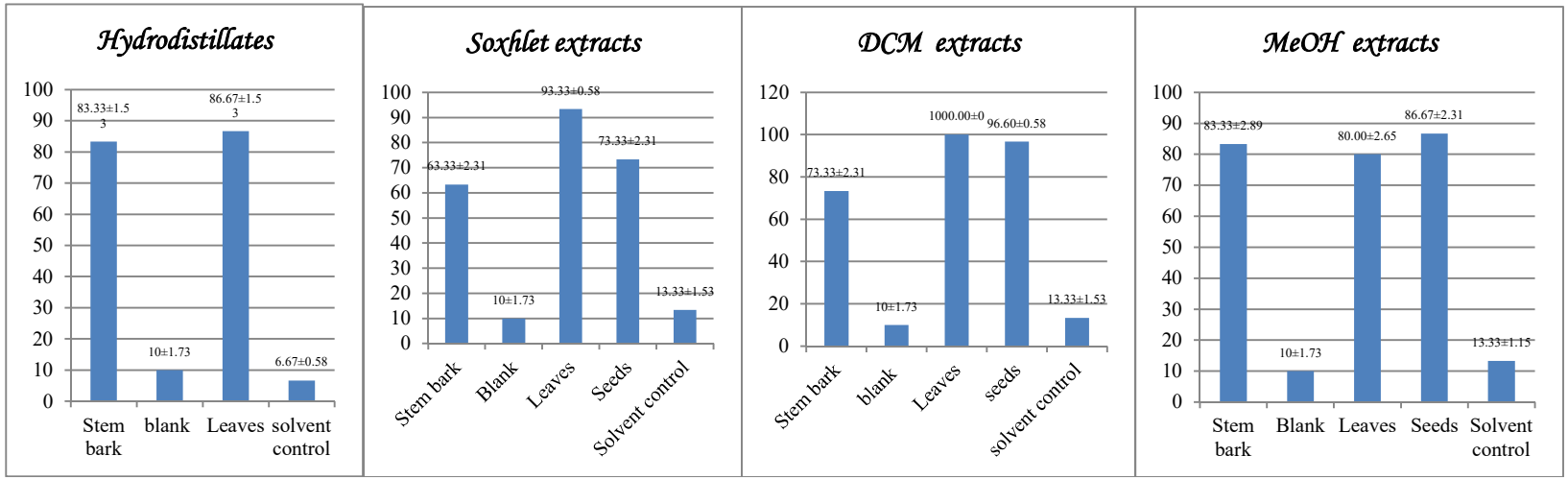

Figure 1. Mean mortality rates of cotton aphids exposed to different extracts at lowest concentration (1%) after 12 h.

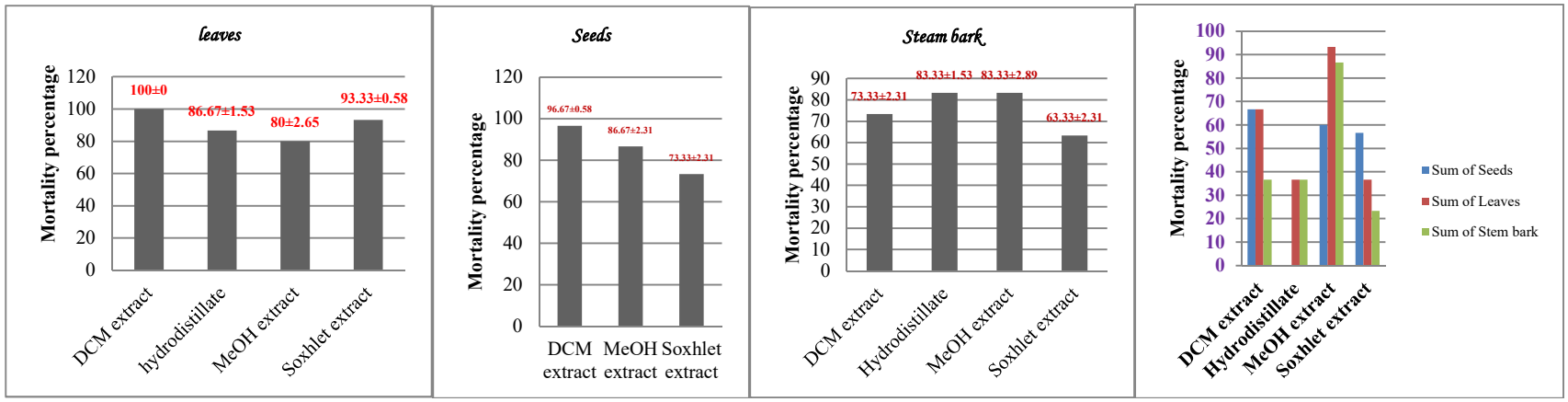

Figure 2. Mean mortality rates of cotton aphids due to extracts from different parts of *P. juliflora* at lowest concentration (1%) after 12 h

concentration (1%) was recorded for the leaf extracts after12 h of treatment. Extracts of the stem bark were found to be the least effective at lower concentrations.

The lethal times LT50 and LT95 were calculated after 12, 24, 48, 72 and 96 h for the lowest concentration (1%) whereas, the lethal concentrations LC50 and LC95 were evaluated at 1%, 2.5%, 5%, 10% and 15% concentrations for the smallest time (12 h) after treatment. The calculated mortality, concentrations and time data were subjected to probity analysis to workout LC50 and LC95 values. Based on probity analysis the calibration curve was obtained. From the calibration curve their slope values for different extracts are shown in Table 7. The linear curve equation of M = yC+b and M = yT+b. where M= Mortality, y= slope of the curve, C= concentration, T = time and b = y-intercept of the curve.

The LC50, LC95, LT50 and LT95 values were obtained through probit analysis (Ashford and Sowden 1970). The LC50 values showed that all extracts of all parts of the plant had high efficacies at the lowest concentration, Dichloromethane extracts of leaves and seeds of the plant were found to be most active. They both resulted in 95% death of the cotton aphids at 1% concentration in 12 h. To the best of our knowledge, this is the first time that insecticidal activities of extracts from different parts of *P. juliflora* were studied against cotton aphids.

CONCLUSION

The present study was conducted to evaluate the insecticidal activities of extracts of *P. juliflora* against cotton (*Aphis gossypii Glover*). All extracts of *P. juliflora* showed high percentage mortality at 1% concentration in 12 h against cotton aphid. The LC50 values showed that all extracts of all parts of the plant had high efficacies at the lowest concentration, Dichloromethane extracts of leaves and seeds of the plant were found to be most active. They both resulted in 95% death of the cotton aphids at 1% concentration in 12 h. 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.

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Appendixes

Table 1. Compounds identified from the essential oil of the leaves of *P. juliflora* using GC-MS.

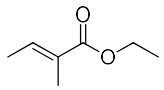
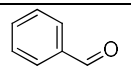
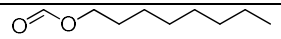
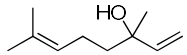
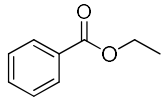
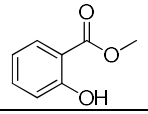
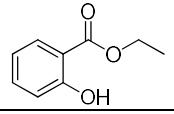
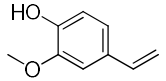
PK	RT (min)	Quality	Compound	Formula	Structure	Area	RA%
1	4.942	97	(*E*)-ethyl 2-methyl-2-butenoate	$C_7H_{12}O_2$		35521208.92	3.49
2	5.256	96	Benzaldehyde	C_7H_6O		11554220.35	1.14
3	6.530	86	Octylformate	$C_9H_{18}O_2$		42932163.03	4.22
4	6.893	96	3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$		44309188.23	4.36
5	7.718	90	Ethyl benzoate	$C_9H_{10}O$		100708143.10	9.90
6	8.007	93	Methyl 2-hydroxybenzoate	$C_8H_8O_3$		87003526.67	8.56
7	8.771	91	Ethyl 2-hydroxybenzoate	$C_9H_{10}O_3$		117073700.30	11.51
8	9.173	95	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$		52557436.11	5.17
9	9.854	98	Tetradecane	$C_{14}H_{30}$	$CH_3(CH_2)_{12}CH_3$	14829434.83	1.46

Table 1. continuation

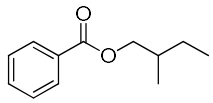
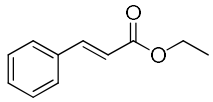
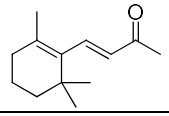
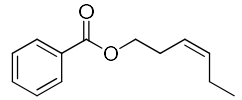
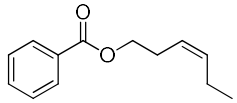
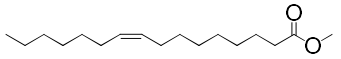
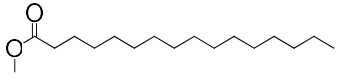
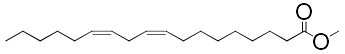
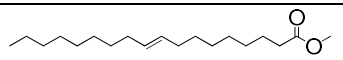
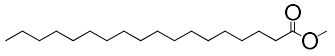
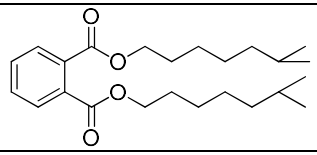
10	10.295	90	2-methylbutyl benzoate	$C_{12}H_{15}O_2$		13988411.78	1.38
11	10.540	97	Ethyl cinnamate	$C_{11}H_{12}O_2$		12220926.01	1.20
12	10.740	95	(E)-4-(2,6,6-trimethylcyclohexenyl)but-3-en-2-one	$C_{13}H_{20}O$		18880129.32	1.86
13	11.435	91	(*Z*)-3-Hexenyl benzoate	$C_{13}H_{16}O_2$		65200540.79	6.41
14	11.529	90	(*E*)-4-hexen-2-yl benzoate	$C_{13}H_{16}O_2$		11054604.55	1.09
15	14.698	99	(*Z*)-Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$		9738101.10	0.96
16	14.945	98	Methyl palmitate	$C_{17}H_{34}O_2$		98898076.96	9.73
17	17.504	99	(9*Z*,12*Z*)-Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$		14195671.50	1.40
18	17.598	99	(*E*)-Methyl octadec-9-enoate	$C_{19}H_{36}O_2$		97136010.36	9.55
19	18.009	99	Methyl stearate	$C_{19}H_{38}O_2$		59742637.40	5.88

Table 1. Compounds identified from the essential oil of the leaves of *P. juliflora* using GC-MS.

20	23.279	99	Tetracosane	$C_{24}H_{50}$	$CH_3(CH_2)_{22}CH_3$	38030476.14	3.74
21	25.418	98	Pentacosane	$C_{25}H_{52}$	$CH_3(CH2)_{23}CH_3$	20424126.65	2.01
22	26.535	91	Bis(6-methylheptyl) phthalate	$C_{24}H_{38}O_4$		50741997.44	4.99
Total						1016740732.00	100.00

PK = Peak number, RT= Retention time in minutes, RA= Relative area

Table 2 . Compounds identified from the essential oil of the stem bark using gas chromatography mass spectrometry (GC-MS).

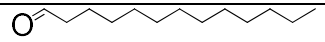
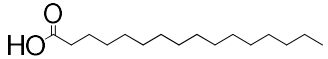
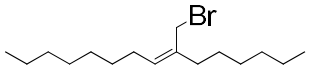
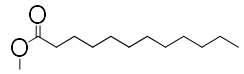
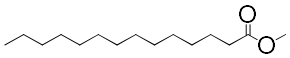
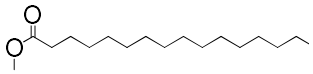
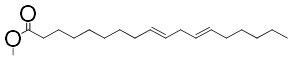
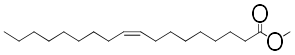
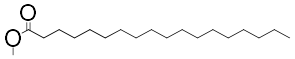
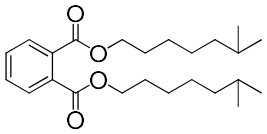
Pk	RT (min)	Quality	Compounds	Structures	Formula	Area	RA %
1	9.057	95	Tridecanal		$C_{13}H_{26}$	7137529.79	2.35
2	13.458	98	Octadecane	$CH_3(CH_2)_{16}CH_3$	$C_{18}H_{38}$	16491665.02	5.43
3	14.629	95	Nonadecane	$CH_3(CH_2)_{17}CH_3$	$C_{19}H_{40}$	23011006.53	7.57
4	15.464	99	Palmitic acid		$C_{16}H_{32}O_2$	68217125.46	22.45
5	16.000	97	Heptadecane	$CH_3(CH_2)_{15}CH_3$	$C_{17}H_{36}$	34197581.50	11.25

Table 2 . Compounds identified from the essential oil of the stem bark using gas chromatography mass spectrometry (GC-MS).

6	16.961	83	(Z)-7-(bromomethyl)pentadec-7-ene		$C_{16}H_{31}Br$	16089017.66	5.29
7	21.292	96	Heneicosane	$CH_3(CH_2)_{19}CH_3$	$C_{21}H_{44}$	32741442.68	10.77
8	23.349	97	Tetracosane	$CH_3(CH_2)_{22}CH_3$	$C_{24}H_{50}$	50165873.31	16.51
9	25.492	96	Pentacosane	$CH_3(CH_2)_{23}CH_3$	$C_{25}H_{52}$	32456210.56	10.68
10	27.678	96	Hexacosane	$CH_3(CH_2)_{24}CH_3$	$C_{26}H_{54}$	23393107.24	7.70
Total						303900559.75	100.00

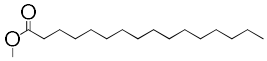
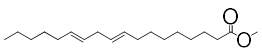
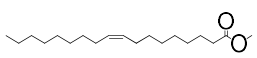
PK = Peak number, RT= Retention time in minute, RA= relative area

Table 3. Fatty acid methyl esters that were identified from the oil obtained from the stem bark of *P. juliflora* by GC-MS.

Pk	RT (min)	Compounds	Q	RA%	C (ppm)	Formula	Structures
1	10.951	Methyl dodecanoate	98	0.58	0.21	$C_{13}H_{26}O_2$	
2	12.686	Methyl tetradecanoate	98	0.78	0.11	$C_{15}H_{30}O_2$	
3	14.951	Methyl palmitate	98	17.05	8.10	$C_{17}H_{34}O_2$	
4	17.517	(9*E*,12*E*)-methyl octadeca-9,12-dienoate	99	31.54	15.40	$C_{19}H_{34}O_2$	
5	17.613	Methyl oleate	99	19.02	9.09	$C_{19}H_{36}O_2$	
6	18.019	Methyl stearate	99	4.59	1.81	$C_{19}H_{38}O_2$	
7	26.550	Bis(2-ethylhexyl) phthalate	98	26.45	12.83	$C_{24}H_{38}O_4$	
		Total		100.00			

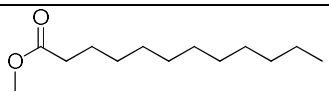
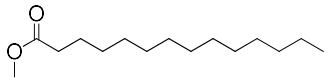
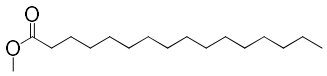
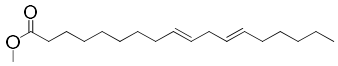
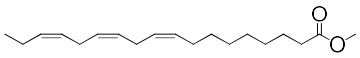
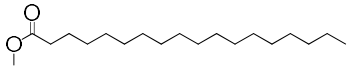
PK = Peak number, Q= Quality, RT= Retention time in minutes, RA = Relative area, C= Concentration

Table 4. Major fatty acid methyl esters identified in seed oil of *P. juliflora* using gas chromatography mass spectrometry (GC-MS).

Pk	RT(min)	Compounds	Q	RA%	C (ppm)	Formula	structure
1	14.953	Methyl palmitate	98	16.15	1.17	$C_{17}H_{34}O_2$	
2	17.518	(9*E*,12*E*)-methyl octadeca-9,12-dienoate	99	51.85	4.86	$C_{19}H_{34}O_2$	
3	17.607	Methyl oleate	99	32.00	2.81	$C_{19}H_{36}O_2$	
Total				**100.00**			

PK = Peak number, Q= Quality, RT= Retention time in minute, RA = Relative area, C= Concentration

Table 5. Fatty acid methyl esters identified by GC-MS from in the oil obtained from the leaves of *P. juliflora.*

Pk	RT (min)	Compound	Q	RA (%)	Formula	C (ppm)	Structures
1	10.953	Methyl dodecanoate	98	0.80	$C_{13}H_{26}O_2$	0.14	
2	12.688	Methyl tetradecanoate	99	1.40	$C_{15}H_{30}O_2$	0.62	
3	14.953	Methyl palmitate	99	12.89	$C_{17}H_{34}O_2$	9.77	
4	17.521	(9*E*,12*E*)-methyl octadeca-9,12-dienoate	99	10.45	$C_{19}H_{34}O_2$	7.82	
5	17.647	(9*Z*,12*Z*,15*Z*)-methyl octadeca-9,12,15-trienoate	84	67.07	$C_{19}H_{32}O_2$	52.92	
6	18.022	Methyl stearate	99	7.38	$C_{19}H_{38}O_2$	5.38	
		Total		**100.00**			

PK = Peak number, RT = Retention time in minute, Q = Quality, RA = Relative area, C= Concentration