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GC-MS analysis, antimicrobial, antioxidant, phytochemical, and phytotoxic activities of *Elaeagnus angustifolia* L.using different extracts

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Abstract

This study aimed to assess the phytotoxic, phytochemical, antioxidant, and antibacterial properties of *Elaeagnus angustifolia*, a therapeutic plant. As solvents, distilled water, methanol, and ethanol were used to make the plant extracts. Using the DPPH free radical scavenging activity, the antioxidant activity was ascertained, and the IC_{50} was calculated. The ethanolic extract of *Elaeagnus angustifolia* had 0.90±0.16 total flavonoid components, whereas the methanolic and aqueous extracts were found to contain 0.78±0.16 total phenolic contents. At 1.5 mg/ml, the antioxidant findings of *Elaeagnus angustifolia*'s methanolic extract showed 0.78±0.18 percent inhibition and SCV 49.10%, while the ethanolic extract showed 0.54±0.12 percent inhibition with 64.28% SCV. The best-result-oriented *Elaeagnus angustifolia* extract underwent phytochemical analysis using the Gas Chromatography-Mass Spectrometry (GC-MS) method. The findings showed that a variety of compounds were present, with the most common ones being acetone (1.18%), ethyl acetate (38.95%), isobutyl acetate (2.71%), Phytol (3.57%), isoquinoline,1-[(3,4-diethoxyphenyl)methyl].n-Hexadecanoic acid (5.61%), 9-Octadecenoic acid, 1,2,3-propanetriyl ester (10.72%), Bis(2-ethylhexyl) phthalate (3.48%), Squalene (1.40%), Octadecanoic acid (1.78%), n-propyl acetate (4.09%), 2,2-Dimethyl-3-(3,7,16,20-tetramethyl (1.12%) and 1,6,10,14,18,22-Tetracosahexaen-3-ol (1.195%). In order to determine the phytotoxic potential of *Elaeagnus angustifolia* leaf and stem extracts, a growth bioassay was performed against Lemna minor. The current study demonstrated that in order to create efficient management strategies that drastically lessen the influence of the infections on both human health and the environment.

KEYWORDS:

Phytochemistry, antioxidant potential, GC-MS analysis, phytotoxic activity, *Elaeagnus angustifolia*

1.0 INTRODUCTION

One of the substances thought to be essential to human survival on Earth is the plant kingdom. They may provide for all of an individual's and an animal's essential needs, including food, fuel, medicine, linen, clothes, shelter, gums, lubricants, and more [1]. From the standpoint of both medicine and the ecosystem at large, they are enormous riches. Every type of plant, from prickly bushes to towering evergreen trees serve humans in some capacity. Aromatic and medicinal plants have long been used as therapeutic agents and are very important. [2]. The evolution of plant medicine has followed the path of life on Earth. Plants have been used medicinally for as long as humans have existed. These plant resources have been employed globally to cure a variety of ailments since the invention of modern medications. From a worthless wild plant to a medicine used to cure the illness [3].

According to the World Health Organization (WHO), 121 active compounds are now in use and 252 prescriptions are deemed vital and crucial. These

pharmaceuticals and medicines, which make up 25% of all approved medications, are derived from medicinal plants. Antibacterial substances that are effective against natural infections can be found in medicinal plants in large quantities. Many medicinal plants with significant antibacterial potential that are rich in antibacterial chemicals may be found in Pakistan. [4]. Over the decades, this plant has played a vital role in producing diverse medicines. Drugs isolated from medicinal plants worldwide are predominantly favored over synthetic alternatives in the field of medicine. The therapeutic components of these plants, specifically fresh green leaves and stems, determine the quality and quantity of phytochemicals present. Such phytochemicals encompass various constituents like proteins, gums, mucilage, carbohydrates, pectins, various glycoside forms, phenolic compounds, tannins, resins, lipids, fixed and volatile oils, and diverse alkaloids, among others [5].

Only a tiny portion of the about 250,000–500,000 plant species have been investigated for their phytochemical and pharmacological properties, despite the enormous quantity of therapeutic plants [3]. Because of increased confidence in plant-derived medicines and worries about the side effects and expensive expense of allopathic medications, there is a growing interest in using medicinal plants as possible therapeutic agents worldwide [6]. Consequently, it is essential for finding new, potent medications as well as to improve the quality of those that already exist. Herbal remedies have been shown in several investigations to have a variety of antifungal and antibacterial effects. Because they are inexpensive and safe for the environment, plant extracts reduce the hazards associated with synthetic chemicals while also having very little influence on the environment [7].

The objective of this study was to assess the in vitro antimicrobial, antioxidant, phytochemical analysis, and phytotoxic activities of various extracts of *Elaeagnus angustifolia*. Additionally, GC-MS analysis was conducted to identify various compounds present in the *Elaeagnus angustifolia* plant.

2.0 MATERIALS AND METHODS

2.1. Plant material

The *Elaeagnus angustifolia* plant was gathered from the district of Abbottabad, Khyber Pakhtunkhwa, Pakistan. The research activities were carried out in the Microbiology Laboratory at Hazara University, Mansehra, Pakistan. To prepare the plant material, it was first chopped into small pieces and then subjected to shade drying. Once dried, the material was finely ground into powder using an electric grinder. The resulting powder was then soaked in ethanol, methanol, and distilled water for 2 weeks at room temperature, with occasional stirring. Subsequently, the ethanolic, methanolic, and water-soluble components were separated by filtration using filter paper. The ethanolic and methanol extracts underwent concentration under a vacuum at 40 °C utilizing a rotary evaporator. The aqueous extract, on the other hand, was concentrated by allowing the filtrate to stand in a water bath at 40 °C for 10 days. In both instances, a crude extract with a blackish-green appearance was obtained, and these extracts were employed as test samples in the subsequent experiments detailed below.

2.2 Extracts preparation

The specimen underwent a 48-hour soaking process utilizing methanol, ethanol, and distilled water as solvents, with Whitman filter paper No 1 employed for filtration. Subsequently, the filtrate was subjected to vacuum conditions using a rotary evaporator until a viscous crude extract was acquired. The filtrate was further evaporated at a consistent temperature of 50 degrees using a vacuum rotary evaporator, resulting in the formation of a sticky mass of crude extract. To facilitate biochemical analysis, the crude extract was dissolved in Dimethyl sulfoxide (DMSO) to achieve the final working concentrations.

2.3. Methods of Qualitative phytochemical analysis

The following accepted procedures were used to evaluate the leaf and stem extracts for the presence of bioactive components.

2.3.1. Test for Alkaloid

After mixing the plant extract with 1% v/v HCL, heating it, and filtering it. This filter is currently being used for tests [8].

a. Mayer's Test

Mayer's reagent (potassium iodide with magnesium chloride in water) is used to treat the filtrate. Alkaloids are present when a yellow-colored precipitate forms.

b. Hager's Test

Hager's reagent (saturated picric acid solution) is applied to the filtrate. Precipitate with a yellow colour forms when alkaloids are present.

2.3.2. Test for Carbohydrates

Following a 5 millilitre distilled water dissolve, the plant extract is filtered. Carbohydrate content was determined using the filtrates [9].

a. Molisch's Test

In a test tube, the filtrate is treated with two drops of an alcoholic naphthol solution. Gently tilt the test tubes and use a dropper to carefully pour the concentrated sulfuric acid along the test tube's sides. The presence of carbohydrates is indicated by the formation of a violet colour at the interface or junction of two liquids.

b. Benedict's test

Benedict's reagent (sodium carbonate, sodium citrate, and copper sulphate solution) is applied to the filtrate. The combination is then heated to a boiling point for five minutes and allowed to cool. Carbohydrates are shown by the orange-red precipitate.

2.3.3. Phenols and Tannin Test

The powdered leaf sample is heated in a test tube with 20 milliliters of distilled water, and it is then filtered. The 1-4 0.1% v/v dips When ferric chloride is applied to a filtered sample, brownish green or blue colorations are seen, which suggests the presence of tannins or phenols [10].

2.3.4. Test for Flavonoids

A test tube was filled with two millilitres of plant extract, two millilitres of sodium hydroxide, and two millilitres of diluted hydrochloric acid. The mixture produced a yellow colour, which suggests the presence of flavonoids in the plant extract [11].

2.3.5. Test for Quinones

For the quinones test, one milliliter of plant extract and one milliliter of sulfuric acid were added to the test tube. The red colouring indicates the presence of quinones [12].

2.3.6. Test for Glycoside

wo milliliters of plant extracts were mixed with 3 mL of chloroform and shaken, resulting in the separation of chloroform layers. To this, 10% ammonia solution was added. The presence of a pink color indicates the presence of glycosides [13].

2.4. Legal's Test for Cardiac Glycosides

Dil. HCl is used to treat the plant extract, and sodium nitroprusside in pyridine and sodium hydroxide are added to the solution. The presence of cardiac glycosides is indicated by the formation of a pink to blood-red coloration [14].

2.4.1. Test for Phenols

Two milliliters of water, a few drops of 10% ferric chloride, and one milliliter of plant extract were mixed. The blue-green color is indicated by the presence of phenols [15].

2.4.2. Test for Coumarins

One milliliter of plant extract was combined with one milliliter of 10% NaOH. A yellowish color indicates the existence of coumarins [16].

2.4.3. Test for Paleobotanies

One milliliter of plant extract and two drops of 2% HCL were applied to a test tube for paleobotanies. The reddish hue is indicated by the existence of Paleobotanies [17].

2.4.4. Test for Anthraquinone

After treating the plant extract with ferric chloride solution, it is submerged in a bath of boiling water for five minutes. After the mixture had cooled, benzene was added. Two millilitres of ammonia solution were added after the benzene layer had been separated. Anthraquinone glycosides are present when the ammonical layer begins to form a rose-pink hue [18].

2.4.4. Test for Oil Investigation

On filter paper, powder is applied for the oil test. There is likely oil present if a sticky material starts to accumulate on the filter paper [19].

2.5. Antioxidant activity

The process was carried out to scavenge DPPH-free radicals [20]. The procedure involved dissolving 24 mg of DPPH in 100 ml of methanol to form a DPPH solution. To create stock solutions in methanol, 1 mg/ml of plant samples are made. These stock solutions are then diluted to concentrations of 0.5, 1 and 1.5 mg/ml. Sample

solutions were mixed 1:1 with DPPH solution, and the mixture was incubated for 30 minutes at 23 °C. A spectrophotometer was used to detect the absorption at 517 nm, with ascorbic acid serving as a standard [21]. The following equation was used to calculate the percentage of scavenging activity.

Scavenging effect % = control absorbance - sample absorbance/control absorbance x100.

2.6. Antibacterial activity

The antibacterial activities were performed using a modified method, as clearly demonstrated in the literature [22], widely employed for testing activities. A loopful of bacteria was isolated from the solution and added to 1 milliliter of saline. Following the disc placement, all experiments were carried out on Mueller Hinton agar surfaces that had been previously infected for both gram-positive and gram-negative bacteria using 10 microliters of liquid medium MHA containing various crude solvent extracts in discs with a diameter of 9 mm. There was also a control with a known solvent and no plant extract. Positive controls included the common antibiotics streptomycin (30 mg/disc) and chloramphenicol (30 mg/disc). After that, the plate was incubated for 24 hours at 37°C, during which time clear zones began to develop around the discs.

2.7. Antifungal activity

The fungal strains used are *Penicillium*, *M.citri* and *Rhizopus stolonifer* for antifungal activities followed by [23].

The Media preparation for fungal growth

After dissolving 39gPDA in 1 liter of pure H2O, autoclave the mixture for 15 minutes at 15 pressure (121 oC). Pour into sterile Petri plates after cooling to room temperature so that it solidifies. For thirty minutes, it was kept at room temperature to solidify.

Agar well diffusion method

The process of AWD (Agar well diffusion) is pursued and characterize. Micropipette is used; one hundred micro-litters of various cultures of fungal in SDW were incorporate on the surface of plate of agar and stretch to use an antiseptic injection hoop, made of each culture plate. The raw form is75µl of and various chunks of selected plants were added. Depending on the fungal growth the plates were then nurturing at 37°C, then the final result shown in 24 hours. The zones which are cleared around each wall were estimated in millimeter, the plant extract showing response showing across the strains of given fungus. Such result was triplicated and standard deviation was calculated.

2.8. Phytotoxic Activity

To see if ethanolic and aqueous *Elaeagnus angustifolia* extracts were detrimental to Lemna minor, tests were performed. Phytotoxicity tests were performed in accordance with [6]. Three levels of extracts were used: 10, 100, and 1000 g/mL. on a nutshell, ethanolic and aqueous extracts were placed on distinct Petri dishes. Each Petri plate received 20 mL of E-medium. Then, in each dish, 10 healthy L. minor plants with a rosette of three fronds were inserted. The plates were incubated at 28 °C for 7 days and observed for plant mortality. Growth inhibition was estimated using the formula. % GOI=100- (Number of fronds in plant extract/number of fronds in negative control) ×100.

2.9. GC-MS analysis of plant extracts

A 5975C MSD and HP5MSI separation column with a length of 30 m, ID of 0.250 mm, and film thickness of 0.25 mm was utilized in conjunction with an Agilent 7890C gas chromatograph to conduct GC-MS analysis of ethanolic extracts of *Elaeagnus angustifolia*, which showed the most promising anticancer activity. Using a manually selected retention-indexed GC-MS library, AMDIS was used for compound identification and quantification. NIST17 and Wiley 11 GC-MS spectrum libraries were used for further chemical identification. [24].

2.10. Statistical analysis

One-way Statistics 8.1 software was used to do ANOVA [25]. For three repetitions, mean and standard errors were obtained. The LSD tests were used to compare means at a significant level (5%).

3.0 RESULTS

From the qualitative analysis of leaves and stem of *Elaeagnus angustifolia*, the presence or absence of

Alkaloid, carbohydrates, flavonoids, Phenols, Tannin, Quinones, Anthraquinone, glycosides, Paleobotanies, Coumarins and Oil was investigated. The results of this study are shown in the following **Table-1**. The result of qualitative analysis of leaves and stem of *Elaeagnus angustifolia* shows that Phenols, Fixed oil, Carbohydrates, Cardiac Glycosides, Quinones, Flavonoids and Alkaloids were present in ethanol and methanol extract of leaves of *Elaeagnus angustifolia* were studied. The Fixed oil, Carbohydrates, Flavonoids and Alkaloids were found to be present in aqueous Extract of *Elaeagnus angustifolia*. The result of qualitative analysis of Flavonoids stem extract investigate that Carbohydrates, Fixed oil, Alkaloids and Flavonoids are studied and present in all three extracts i.e ethanol, methanol and distilled water. Cardiac glycosides, Terpenoids, Phenols and Flavonoids are present in ethanol and methanol extract and absent in aqueous extract of *Elaeagnus angustifolia* stem. Bothe the stem and leaves extract of *Elaeagnus angustifolia* show high content of phytochemicals in ethanol and methanol extract as compared to aqueous extract.

Table1: Qualitative phytochemical analysis of *Elaeagnus angustifolia*

Plant Parts	Phytochemicals	Ethanol	Methanol	DistilledWater	Plant Parts	Phytochemicals	Ethanol	Methanol	Water
Stem	Carbohydrates	+	+	+	Leaves	Phenols	+	-	-
	Fixed oil	+	+	+		Anthraquinones	-	-	-
	Saponins	-	-	-		Terpenoids	+	+	-
	Paleobotanies	-	-	-		Tannins	-	-	-
	Quinones	-	-	-		Saponins	-	-	-
	Cardiac glycosides	+	+	-		Fixed oil	+	+	+
	Alkaloids	+	+	+		Coumarins	-	-	-
	Terpenoids	+	+	-		Paleobotanies	-	-	-
	Phenols	+	+	-		Carbohydrates	+	+	+
	Anthraquinones	-	-	-		CardiacGlycosides	+	+	-
	Flavonoids	+	+	+		Glycosides	-	-	-
	Tannins	-	-	-		Quinones	+	+	-
	Coumarins	-	-	-		Flavonoids	+	+	+

3.1. Antioxidant activity

The antioxidant activity (**Table-2**) showed that the DPPH free Radical scavenging assay of *Bassia indica* were carried out using different concentration of methanol, ethanol and distilled water. The most effective extract was the ethanolic one, which had an IC 50 of 47.94% and a percent inhibition of 0.89±0.02 at 0.5 mg/mL. Methanol and water came in second and third, respectively. The least effective extracts are the water and ethanol, with IC_{50} values of 33.94% and 26.78%, respectively. Overall, the activity in the experiment was concentration dependent and ascorbic acid used as a standard.

3.2. Antibacterial activity

Elaeagnus angustifolia L.'s antibacterial activity data are shown in (Table-3). The antibacterial

activity of leaves against *Salmonella Typha* was greater (24±1, Mean±SD) compared to lesser activities against *Klebsiella pneumoniae* and Staphylococcus aureus. The stem exhibited reduced antibacterial activity against *Listeria monocytogenes* and *Staphylococcus*, but was shown to be more effective against E. Coli.
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Table-2: Antioxidant activity (expressed as IC_{50}) of *Elaeagnus angustifolia* Leaves and stem extract.

The plant parts used	Extract	Concentrations	%inhibition (means± SD)	%SCV	IC_{50}
Elaeagnus angustifolia	Methanolic	0.5mg/ml	0.74±0.35	36.60%	33.94%
		1mg/ml	0.81±0.10	37.5%	
		1.5mg/ml	0.78±0.18	49.10%	
	Distilledwater	0.5mg/ml	0.54±0.12	64.28%	26.78%
		1mg/ml	0.35±0.14	57.14%	
		1.5mg/ml	0.30±0.18	55.35%	
	Ethanolic	0.5mg/ml	0.89±0.02	20.53%	47.94%
		1mg/ml	0.77±0.01	32.14%	
		1.5mg/ml	**0.77±0.26**	**33.93%**	

Table-3: Antibacterial activities of of *Elaeagnus angustifolia*

Bacterial strains	Leaves	Stem	Antibiotics
Escherichia coli	21 ± 2.64^{ab}	26 ± 1.73^{a}	30 ± 2^{ab}
Klebsiella pneumoniae	16.66 ± 0.57^{c}	16 ± 2^{bc}	22.66 ± 3.05^{c}
Listeria monocytogenes	18.33 ± 3.51^{bc}	14.66 ± 1.52^{c}	27 ± 2.64^{bc}
Pseudomonas aureginosa	18.33 ± 1.52^{bc}	19 ± 1^{b}	33.33 ± 3.05^{a}
Salmonella typhi	24 ± 1^{a}	27 ± 3^{a}	27.33 ± 2.08^{bc}
Staphylococcus aureus	16.33 ± 1.52^{c}	14 ± 2.64^{c}	27.33 ± 3.05^{bc}

Moreover, antibiotics showed the higher zone of inhibition against *Pseudomonas aeruginosa*. In
In general, it was observed that the zone of inhibition of antibiotics against bacterial strains was higher than that of leaves or stem extract. Comparing different plant parts, the leaves extract exhibited a greater zone of inhibition against the monocyte genes of *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Listeriae*, while the stem showed a higher zone of inhibition against Salmonella Typha, E. Coli, and *Pseudomonas aeruginosa.*

3.3. Antifungal activity

Antifungal activity of *Elaeagnus angustifolia* at various concentrations (0.30, 0.5, and 01) was Investigated against *M. citri*, *Penicillium* and *Rhizopus stolonifer*. The average reductions in F. *M. citri* growth in response to leaves methanolic extract of *Elaeagnus angustifolia* treatment are shown in the **Table-4.**

All tested concentrations exhibited varied inhibitory activity compared with positive control (Terbisil 0.006) and the untreated experimental control. Inhibition of mycelium growth increased with time and complete growth reduction was in

M. citri, Penicillium and *Rhizopus stolonifer* achieved after 7 days of incubation at 27 ◦C in the case of *Elaeagnus angustifolia* at 0.30, 0.5, and 01 Leaves and stem. Whereas, the highest significant growth inhibition (17.66±2.08** and 14.66±2.51A**) was achieved in the case of leaves and stem at concentration of 0.5, 01and Terbisil 0.006, respectively, compared with control. The lowest insignificant growth inhibition, 11.66±1.52b %, was obtained with *Elaeagnus angustifolia* leaves at 0.30 %.

Table-4: Antifungal activity of standard drugs against test organisms

Fungal strains	Concentration %	Leaves	Stem
M. citri	5	17.66±2.08A	14.66±2.51A
Penicillium	10	11.66±1.52B	14.66±1.15A
Rhizopus stolonifer	15	14.66±0.57AB	17±3A

3.4. Phytotoxic activity

A growth bioassay using *Lemna minor* was used to assess the phytotoxic potential of *Elaeagnus angustifolia* leaf and stem extracts. The examined plant species' leaves and stem growth were significantly suppressed by the methanolic extracts of *Elaeagnus angustifolia*. Depending on the concentration and test plant, the extracts' phytotoxicity levels differed significantly. The growth of tested plant declined with increasing extract concentration. Significant inhibition of the test plant started with extract concentration 1000 µg/ml. At the concentration of 1000 µg/ml of stem, the growth of Lemna minor was more inhibited. When the concentration was decreased to100µg/ml, the Lemna minor were stunted to reduce to 59.28% and 45.64% of leave extract 4.5%, 10.0%, and 90 ± 1.11% of control, respectively. At the same concentration, Lemna minor were reduced to 38.32 % and 49.63% of leave extract at concentration of 10µg/ml and 100µg/ml.

The LD 50 values of the extracts against the test plants ranged from 1.01to 4.39 µg/ml as compared to P/Control (Atrazine100µg/ml) which is 7.87 µg/ml. Comparing LD 50 values, the seedling growth of Lemna minor (LD_{50} = 1.01–4.39 µg/ml respectively. As demonstrated in Tabel-5, the stem and leaves of *Elaeagnus angustifolia* had a great potential against Limna minor. Plant extract from the stem was more phytotoxic than the leaves. The growth of inhibition was 76.38±1.21 in stem extract at 1000µg/ml concentration, and 59.21±1.31 in leaves extract at the same concentration.

Table-5: Phytotoxic potential of the leaf and stem extracts of *Elaeagnus angustifolia*

Samples	% growth inhibition			LD50
	10µg/ml	100µg/ml	1000 µg/ml	
N/Control Methanol	0.00±0.00	0.00±0.00	0.00±0.00	-
P/ Control (Atrazine100µg/ml)	90 ± 1.11	90 ± 1.11	90 ± 1.11	7.87

Leaves	38.32 ± 2.24	49.63 ± 1.39	59.21 ± 1.31	1.01
Stem	45.64 ± 2.76	59.28 ± 2.09	76.38 ± 1.21	4.39

3.5. Gas chromatography-mass spectroscopic analyses of *Elaeagnus angustifolia*

Superlative result-oriented extract of *Elaeagnus angustifolia* was further subjected to GC-MS analysis (**Fig. 1)**. The compounds identified from the extract along with their molecular weight, molecular formulae, retention time and peak areas (%) are presented in **Table-6**. The dominant compounds present in ethanolic extracts in *Elaeagnus angustifolia* were Acetone (1.18%), Ethyl Acetate (38.95%), (20.77%), n-Propyl acetate (4.09%), Isobutyl acetate (2.71%), (3.84%), isoquinoline,1-[(3,4-diethoxyphenyl)methyl]-6,7-diethoxy- (3.36%), Cyclohexanone (1.43%), 1,1-Diisobutoxy-isobutane (2.02%), n-Hexadecanoic acid (5.61%), Phytol (3.57%), 9- Octadecenoic acid, 1,2,3-propanetriyl ester (10.72%), Octadecanoic acid (1.78%), Bis(2- ethylhexyl) phthalate (3.48%), Squalene (1.40%), 2,2-Dimethyl-3-(3,7,16,20-tetramethyl (1.12%) and 1,6,10,14,18,22-Tetracosahexaen-3-ol (1.195%).. Different phenolic compounds were detected from *Elaeagnus angustifolia* plant which proved their medicinal value.

4.0 DISCUSSION

The presence of phytochemical elements in medicinal plants makes them effective for both treating and curing human ailments. Our result of qualitative phytochemical analysis using three different extracts ethanol, methanol and aqueous which shows that Carbohydrates, Fixed oil, Alkaloids and Flavonoids are presence in all extract as compared to Saponins, Paleobotanies, Quinones , Anthraquinones , Tannins and Coumarins are absent in all three extract in the stem of *Elaeagnus angustifolia*. Our work is link with [26], working on Preliminary Phytochemical Profile, in vitro Antioxidant and Sun Protective Activities of *Alhagi pseudalhagi* and *Elaeagnus angustifolia*. Cardiac glycosides, Terpenoids and Phenols are only present in ethanol and methanol extract of stem of *Elaeagnus angustifolia* while absent in distilled water. [27], same work on *Elaeagnus angustifolia* cultivated in Iraq but their investigated phytochemical different from our result. Phytochemical quantitatively investigated in the leaves of *Elaeagnus angustifolia* using different extract i.e ethanol, methanol and water. [28], perform same work and select leaves for qualitative phytochemistry.

Table-6: Compound identified from methanolic inflorescence extract of *Elaeagnus angustifolia*

S.NO	Names of Compounds	Formula	Mol.Wt (g/mol)	Retention Time (Min)	Peak area (%)
1	Acetone	C_3H_6O	58.08	1.790	3.57
2	1-Propanol, 2-methyl-	$C_4H_{10}O$	74.122	2.708	63.27
3	1-Butanol	$C_4H_{10}O$	74.121	2.903	0.42
4	Cyclohexanone	$C_6H_{10}O$	98.15	7.884	6.60
5	Propanoic acid, 2-methyl-, 2-methylpropyl ester	$C_8H_{16}O_2$	14421	8.368	0.48
6	1,1-Diisobutoxy-isobutane	$C_{12}H_{26}O_2$	202.334	16.025	8.83
7	Cyclohexene,1-(1,1-dimethylethoxy)	$C_{10}H_{18}O$	154.25	16.339	0.31
8	Hexanoic acid, 2-methylpropyl ester	$C_{10}H_{20}O_2$	172.26	17.294	2.75
9	2-Butenedioic acid (Z)-, dibutyl ester	$C_{12}H_{20}O_4$	228.2848	24.775	1.41
10	Cyclopentane carboxylic acid, butyl ester	$C_{10}H_{18}O_2$	170.25	27.213	0.65
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	43.343	1.47
12	Phytol	$C_{20}H_{40}O$	128.1705	46.792	1.00

13	Oleic Acid	$C_{18}H_{34}O_2$	282.47	47.817	7.54
14	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	48.255	0.78
15	Sebacic acid, ethyl hexyl ester	$C_{18}H_{34}O_4$	314.4602	50.892	0.93

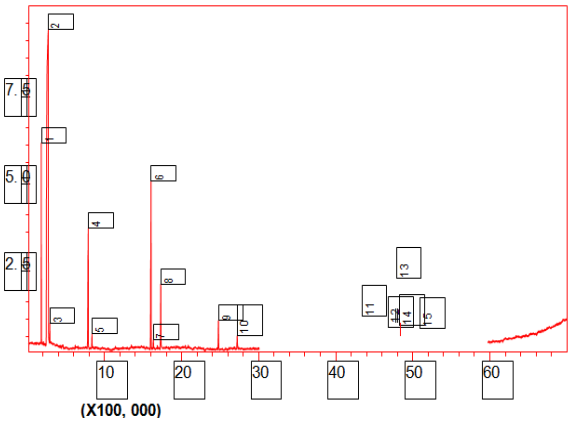

Fig. 1: GC-MS Chromatograms of methanolic extract of *Elaeagnus angustifolia*

The content of Fixed oil, Carbohydrates and v are present in all three extract of *Elaeagnus angustifolia* leaves while Cardiac Glycosides, Terpenoids and Quinones are present in both ethanol and methanol and absent in water. The same plant (*Elaeagnus angustifolia* L.) was used in [29]'s study to extract the peel's aqueous extract on the qualitative characteristics of cold-pressed sesame oil, however their findings do not align with our observations. Using fruits and vegetables strong in antioxidants, which may counteract free radicals, can enhance human health. The *Elaeagnus angustifolia* were tested using various concentrations of methanol, ethanol, and distilled water. The ethanolic extract was the most effective, with a percent inhibition of 0.890.02 at 0.5mg/mL and an IC_{50} of 47.94%, followed by methanol and water. Ethanol and water extracts are the least effective, having IC_{50} values of 26.78% and 33.94%, respectively. The results of DPPH free radical scavenging activity were presented in percent inhibition with IC_{50} values in the current study. Ascorbic acid was utilised as a control. The overall level of activity was concentration dependant. Furthermore, our findings are consistent with plant research, indicating that novel treatments in the form of pure chemicals or standardised extracts are possible. Biological activities revealed information on the potency of plants or their extracts in the treatment of disease, leading to the isolation of chemical compounds for the treatment of chronic and infectious diseases. GC-MS analysis has the capacity to identify chemicals contained in plant extracts. In this study, fifteen major compounds were identified from the ethanolic extract of Elaeagnus angustifolia, while eleven other compounds shared minor peak area. *Elaeagnus angustifolia* methanolic extracts significantly suppressed the development of the tested plant species' leaves and stems. The Phytotoxicity of the extracts varied substantially depending on concentration and test plant. With increasing extract concentration, the growth of the tested plant decreased. The test plant was significantly inhibited at extract concentrations of 1000g/ml. The development of Lemna minor was stopped at a concentration of 1000 g/ml of stem, the same research performed by working on the same plant Russian olive (Elaeagnus angustifolia) as a herbal healer. When the concentration was reduced to 100g/ml, the Lemna minor was stunted, resulting in 59.28% and 45.64% of the leaf extract, respectively, and 4.5%, 10.0%, and 90 1.11% of the control. Lemna minor were reduced to 38.32 % and 49.63% of leave extract at concentration of 10µg/ml and 100µg/ml. The LD_{50} values of the extracts against the test plants ranged from 1.01to 4.39 µg/ml as compared to P/Control (Atrazine100µg/ml) which is 7.87 µg/ml. Comparing LD_{50} values, the seedling growth of Lemna minor (LD_{50} = 1.01–4.39 µg/ml respectively. working on Phytotoxicity assessment of a methanolic coal dust extract in Lemna minor.. While (Tuttle, G2017) investigates effects and management of the invasive Russian olive (*Elaeagnus angustifolia* L.) in a hereogenous riparian habitat, it demonstrates that the stem and leaves of *Elaeagnus angustifolia* demonstrated the

significant potential against Lemna minor. Plant extract from the stem was more phytotoxic than the leaves. The growth of inhibition was 76.38±1.21 in stem extract at 1000µg/ml concentration, and 59.21±1.31 in leaves extract at the same concentration, but in different forms. [30] Investigating the phytotoxicity of zinc oxide nanoparticles mediated by onion peel waste on *Elaeagnus angustifolia* and wheat plant growth. The following study was to evaluate the antimicrobial, antioxidant, phytochemical analysis and phytotoxic activities of different extracts of *Elaeagnus angustifolia* in vitro. In addition, various compounds found in the *Elaeagnus angustifolia* plant were measured by GC-MS analysis.

4.0. CONCLUSION

According to current studies, herbal treatments have the ability to cure human illnesses. This study strongly supports the use of specific safe medications to treat many types of infectious infections. *Elaeagnus angustifolia* ethanol, methanol, and aqueous extracts were found to contain potential botanical components, phytochemical screening, antioxidants, antibacterial, and phytotoxic action. The extract of *Elaeagnus angustifolia* was created utilising gas chromatography-mass spectrometry (GC-MS) technology. The findings confirmed the existence of several chemicals. As a result, additional study is required to discover more components for the development of stable products and to create effective management measures that dramatically limit pathogen impacts on human health and the environment.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

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