Chemical components of different solvent extracts of *Asclepias curassavica* L. and antibacterial effect of the extracts on tomato pathogens

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Abstract. The use of environmentally friendly and sustainable agricultural methods in the control of diseases and pests is of great importance. In both conventional and organic agricultural production systems, the utilization of various plant extracts as part of integrated pest management has gained significance in recent years. The chemical constituents of various solutions derived from the flowers, leaves, and roots of the *Asclepias curassavica* L. plant were investigated, along with the possibilities of utilizing these solutions in the control of tomato plant pathogenic bacteria. As a result of the analysis, acetic acid in 60% and 80% ethanol solutions, as well as acetic acid methy ester in 60% and 80% methanol solutions, were detected in the flowers, leaves, and roots. The effects of *A. curassavica* flower, leaf, and root extracts, prepared using three different solvents (water, methanol, and ethanol), were investigated on eight different pathogenic bacteria (*Agrobacterium tumefaciens, Clavibacter michiganensis, Dickeya zeae, Pectobacterium caratovorum, Pseudomonas phaseolicola, Pseudomonas tomato, Pseudomonas viridiflava,* and *Xanthomonas euvesicatoria*) that cause diseases in plants. Several main compounds such as acetic acid, acetic acid. methyl ester, Furfural, 2-Furanmethanol, 4H-Pyran-4-one. 2.3-dihydro-3.5-dihydroxy-6-methyl-, Glycerin, Benzo furan. 2.3-dihydro- and 5-Hydroxy methyl furfural were identified as analyzed by GC-MS with different concentrations of ethanol and methanol solutions used for the flower, leaves and root of *A. curassavica* plants. The flower extract prepared with 80% ethanol exhibited a higher inhibition zone (ranging from 1.5 mm to 5.3 mm) in all pathogens, compared to other applications. The successful suppression effect of *A. curassavica* flower extracts on this disease is promising, especially in organic farming areas. Additionally, since it is environmentally friendly and sustainable, it can be included in integrated control methods to prevent the loss of productivity caused by diseases.

Key Words: *Asclepias curassavica*, components, *Clavibacter sp, Pseudomonas sp, Xanthomonas sp*

1. Introduction

Secondary metabolites (alkaloids, terpenoids and phenolics) found in medicinal and aromatic plants contain bioactive substances important for people to lead a healthier and higher quality life. In addition, due to the increasing antibiotic resistance problem in bacteria, secondary metabolites; As biomaterial within the scope of natural and new antimicrobial substances, are also significant candidates with strong antimicrobial activity in the control of pathogenic microorganisms [12]. Instead of the use of traditional and commercial crops against diseases and pests, the use of plant products including allelochemicals derived from plants, are highly effective, environmentally friendly and less expensive. Since raw extracts are easy to prepare, they can be integrated into plant protection strategies to enhance the global discovery of medicinal plants [6].

Silk bush or silk grass (*Asclepias curassavica* L.) is a semi-bush plant belonging to the genus '*Asclepias*' of the oleander family (Apocynaceae or Asclepiadaceae). It is grown as an ornamental plant because of its beautiful flowers. It is also called a 'blood flower'. Its homeland is South America. The leaves of the silk bush are like oleander, evergreen. Although it is a perennial plant, it is grown as an annual in cold winter places. The flowers are clustered, star-shaped, yellow red in color and slightly fragrant. If the climatic conditions are favorable, it blooms all year. Some species of *Asclepias* (*Asclepias tuberosa*) are cultivated as 'medicinal plants'. Silk bush attracts butterflies and bees. Its seeds are quite light and silky-haired, flying around, so it has been considered invasive in some countries. Therefore, the spindle-shaped seed capsules are torn off without opening. It was used to make vegetable silk in the past. For this reason, it was called silk bush or silk grass [29].

The leaf and stem of *A. curassavica* contain fixed oils, flavonoids, phenols, kinins, tannins, terpenoids, sugars, xanthoprotein, saponins and steroids. Flavonols, flavonol glycosides, amino acids, carbohydrates and triterpenes were isolated in the plant *A. curassavica*. Moreover, cardenolides, calactin, calotropin, calotropagenin, coroglaucigenin, asclepin, asclepain CI, asclepain CII, asclepin (asclepiadin), uscharidin, uzarin, uzarigenin, korotoxigenin, asclepogenin, curasclepin, asclepain CI, asclepain CII, uscharidin, uzarin, uzarigenin, korotoxigenin, asclepogenin, curassavogenin, curclejoozarin, isolate has been done. Among the polyphenols obtained from this plant are quercetin, kaempferol, rutin, and isorhametin. [3]. Alonso-Castro et al. (2021) reported that ethanol extract from the leaves and stem of *A. curassavica* could be

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considered as slightly toxic and evaluated the antiparasitic activity against *Trichomonas vaginalis*, the in vitro and in vivo anti-inflammatory effects, the antinociceptive and sedative activities and the effects of motor coordination of *A. curassavica*.

The antibacterial activity of *A. curassavica* was investigated against *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Escherichia coli* and *Klebsiella pneumoniae*. Methanol extract was found to exhibit growth inhibition on all microorganisms tested, except *Proteus vulgaris*. Petroleum ether showed activity against three of the five organisms tested but, in comparison, less activity than the methanolic extract. A weak response was obtained with the ethyl acetate extract, which showed activity against only two microorganisms (*Staphylococcus aureus* and *Bacillus subtilis*). No antibacterial activity was found for chloroform and hexane extracts. Among all tested organisms, *Proteus vulgaris* was found to be resistant and remained unaffected. It was determined that *Klebsiella pneumoniae* showed a moderate inhibitory zone with three extracts, and the effect of Petroleum ether root extract against *Escherichia coli* was very pronounced. Among the various leaf and root solvent extracts tested against different bacteria, it was emphasized that root extracts showed better inhibitory effects than leaf [15].

Crude extracts of petroleum ether, chloroform and methanol and two pure fractions obtained from the methanol extract of *A. curassavica* roots were tested for their antimicrobial properties and the crude chloroform extract was found to be effective against *Pseudomonas solanacearum* and *Escherichia coli*. They found that the crude extract of methanol was more effective against *Clavibacter michiganensis* than the other extracts. It was stated that chloroform extract showed an inhibition zone of 13 mm, 19 mm and 13 mm against *Helminthosporium oryzae, Aspergillus niger* and *Fusarium oxysporum*, respectively, while petroleum ether extract and methanol extract did not show any inhibition zone [7]. Root extracts of *A. curassavica* obtained by cold percolation and soxhelet method were investigated in vitro against four bacterial species (*Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*) and two fungal species (*Candida albicans* and *Aspergillus niger*). The MIC value of *Asclepias curassavica* root extracts was found to be 3.06 mg/ml and the bacterial concentration was found to be 100 mg/ml. Ethanol and acetone extracts showed good anticandidal effects [16].

Several bacterial pathogens; *Agrobacterium, Clavibacter, Dickeya, Pectobacterium, Pseudomonas* and *Xanthomonas* genus, cause economically important bacterial disease of tomatoes and yield losses in marketable fruits. The disease management practices in commercial nurseries, fields or greenhouses are quite difficult. This is because current disease management strategies are limited, as the pathogens have acquired resistance to antibiotics and copper-based compounds in Turkey [21]. The pathogens are seed borne and disease-resistant tomato cultivars are not commercially available. Therefore, it is essential to develop alternative disease control strategies [8].

It has been determined that the effect of *A. curassavica* plant extracts on bacteria that cause disease in plants has not been investigated before. This situation reveals the value of the study. However, its antibacterial effect on *Staphylococcus, Bacillus, Corynebacterium* species and *Escherichia coli* different from bacteria that cause disease in humans is mentioned (22). It has been determined that the plant extract of *A. curassavica* has antifungal activity on *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Phomopsis vexans* and *Fusarium oxysporum* which are fungal agents that cause diseases in plants [4]. These data indicate the need for new research on this subject.

The study aims to determine the chemical component of *A. curassavica* plant extracts, their antibacterial effects against eight different pathogenic bacterial species in tomatoes, and to investigate the possibilities of using *A. curassavica* in the control of seed-borne inoculum in tomatoes, within the scope of bilateral collaboration between the Field Crops and Plant Protection departments.

2. Material and Methods

In the Department of Field Crops of the Faculty of Agriculture, Cukurova University the seeds of *A. crussavica* were sown in viols containing seedling soil on 2.9.2021. When the plants were 10 cm tall, in the first week of January, they were grown in greenhouse conditions by transferred as one each in two kg pots. Harvested on 13.4.2022 when the plants full-bloomed.

The antimicrobial effects of flowers, leaves and roots extracts of *A. curassavica* prepared with three different solvents (water, methanol and ethanol) against eight different pathogenic bacteria (*Agrobacterium tumefaciens, Clavibacter michiganensis, Dickeya zeae, Pectobacterium caratovorum, Pseudomonas phaseolicola, Pseudomonas tomato, Pseudomonas viridiflava* and *Xanthomonas euvesicatoria*) were investigated by in vitro petri dishes.

2.1. Preparation of plant extracts

A. curassavica plants were harvested at full bloom and separated into leaf, flower and root parts. It was washed to remove soil in the root parts. Then the plant's parts were dried in a drying cabinet at 60℃ for 48 hours. The dried samples were ground and stored in glass jars at +4℃ in the dark to be used in experiments

2.2. Antibacterial Effect Analysis in vitro petri experiments

Asclepias curassavica flower, leaf and root extract was prepared with water, methanol and ethanol solvents, and its antimicrobial effect on eight different plant pathogenic bacteria was investigated by in vitro petri dishes using the paper diffusion disc method. Applications were:

1. Root + pure water
2. Leaf + pure water
3. Flower + pure water
4. Root + Methanol (60%)
5. Leaf + Methanol (60%)
6. Flower + Methanol (60%)
7. Root + Methanol (80%)
8. Leaf + Methanol (80%)
9. Flower + Methanol (80%)
10. Root + Ethanol (60%)
11. Leaf + Ethanol (60%)
12. Flower + Ethanol (60%)
13. Root + Ethanol (80%)
14. Leaf + Ethanol (80%)
15. Flower + Ethanol (80%)

Pathogenic bacteria isolates grown in King B medium for 48 hours were prepared in a spectrophotometer at 600 nm wavelength at 0.2 measurement value, and dilution series were prepared from the suspension. 100 µl was taken from each batch and spread with a spatula drigalski instead of King B medium in triplicate. And after growing at 25°C for 48 hours, colony counts were made and the pathogen populations were adjusted to 10^7 cells/ml and used [20, 28]. 100 µl of the pathogen suspension was added to the King B medium and spread with a spatula drigalski. After the petri dishes dried (approximately 3 hours), an 18mm chamber sterile paper disc was placed on three separate points of the petri dishes, equidistant from each other. 10 µl of each *Asclepias curassavica* extracts were taken and dropped onto the paper disc. Sterile water was used as negative control and streptomycin antibiotic (0.2gr/liter) was used as positive control. The experiment was set up using three petri dishes, three paper discs in each petri dish. After the petri dishes were incubated for 48 hours at 25℃, the inhibition zones (inhibition) formed around the paper discs were measured in mm and [1].

2.3. GC-MS analysis

A. crussavica flower, leaf and root extracts prepared with two different solvents (ethanol, methanol) at two different ratios (60, 80%) were analyzed by GC-MS technique. Chemical component analysis was determined by Agilent Brand 7890B GC, 7010B MS system. In the analysis, 1 uL of leaves, roots and flowers dissolved in different ratios (60,80%) of ethanol and methanol were injected into a DB-Wax (60 m x 0.25 mm i.dx 0.25 µm, J&W Scientific-Folsom, USA) capillary column. The injection temperature was 250 °C, the column temperature was maintained at 40 °C for 4 minutes, then increased by 3 °C per minute to 90 °C, then by 4 °C per minute to 130 °C, and after 4 minutes at this temperature, the temperature was increased by 5 °C per minute to 240 °C and maintained at this temperature for 8 minutes. He was used as the carrier gas. The electron energy is 70 eV and the mass range is 30-600 m/z. NIST14L was used as a library.

Statistical analyzes were made with the data obtained according to the inhibition zone measurements, and the difference between the applications was determined by the LSD multiple comparison test in the ANOVA statistical program, according to the $P<0.05$ significance level [9].

3. Results and Discussion

3.1. Chemical components of *Asclepias curassavica*

The chemical constituents of *A. curassavica* plant flowers, leaves and roots using different ratios (60, 80%) of ethanol and methanol solutions were analyzed by GC-MS technique. The analysis of solutions of the *A. curassavica* revealed the existence of a high chemical diversity ranging from 32 to 37 compounds identified by different plants parts and which represent 81.26 to 99.41 % of the solutions of ethanol and methanol (Table 1, 2 and 3).

Major components in 60% and 80% ethanol solutions of flower were acetic acid (14.73 and 16.59%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.35 and 10.21%), Glycerin (5.86 and 5.71%) and 5-Hydroxymethyl furfural (13.62 and 21.68 %), respectively (Table 1). Similarly, major components in 60% and 80% methanol solutions of flower were acetic acid (18.58 and 19.02%), 5-Hydroxymethylfurfural (14.58 and 9.95 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(10.50 and 5.77%) and Glycerin (6.67 and 6.39%), respectively (Table 1).

In leaves solutions, major component in 60% and 80% ethanol and methanol solutions were acetic acid (16.48-17.72 and 24.39-18.22%), respectively. Glycerin and Benzofuran. 2.3-dihydro- components came in the second places. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- followed these compounds (Table 2).

Table 1. The chemical constituents of flowers of *Asclepias curassavica* plant in different ratios (60-80%) of ethanol (E) and methanol solutions (M).

Components	RT	FLOWER			
		%60 E	%80E	%60M	%80 M
Acetic acid. methyl ester	25.95	4.09	3.92	5.99	6.19
4,5-Dihydro-2-methyl imidazole -4-one	29.12	0.46	-	-	0.64

Oxalic acid, butyl cyclobutyl ester	29.46	1.34	1.82	1.70	2.03
Acetic acid	**30.60**	**14.73**	**16.59**	**18.58**	**19.02**
Furfural	30.66	2.77	5.30	3.07	-
Acetic anhydride	30.86	1.93	-	3.55	3.87
Formic acid	31.98	4.19	2.84	2.67	3.75
2-Furancarboxaldehyde, 5-methyl-	35.46	0.87	1.30	0.65	-
4-Cyclopentene-1,3-dione	35.92	0.90	0.96	0.83	0.91
Butanoic acid. 4-hydroxy-	38.21	0.87	0.80	0.84	0.96
2-Furanmethanol	38.90	4.90	5.36	4.35	3.67
1.2-Cyclopentanedione	42.88	1.33	1.40	1.51	1.45
3,3-Diacetyl-2,3,4,5-tetrahydro-2-oxofuran	45.44	0.89	1.41	1.07	1.04
Benzyl alcohol	46.21	0.85	0.72	1.38	-
2H-Pyran-2.6(3H)-dione	49.21	1.06	-	1.26	0.62
Phenol	49.51	0.54	1.39	1.07	0.94
Furaneol	50.29	2.31	2.41	1.51	1.39
(S)-(+)-2-Amino-3-methyl-1-butanol	51.56	2.04	3.17	2.31	3.29
Pentanal/ Cyclopropyl carbinol	51.90	1.34	0.90	1.31	1.92
2,4-Hexanedione	52.25	0.83	1.25	0.88	0.82
Pentanal. 2.4-dimethyl	53.47	1.47	1.82	1.93	2.54
2-Methoxy-4-vinylphenol	54.17	2.70	2.05	2.84	2.38
4H-Pyran-4-one. 2.3-dihydro-3.5-dihydroxy-6-methyl-	**55.70**	**8.35**	**10.21**	**10.50**	**5.77**
Glycerin	**56.35**	**5.86**	**5.71**	**6.67**	**6.39**
2(3H)-Furanone. 3-butyldihydro-	58.00	1.31	-	-	5.18
Benzofuran. 2.3-dihydro-	**58.07**	**7.53**	**2.82**	**6.39**	**6.28**
Dihydroactinidiolide	58.22	2.61	0.68	-	1.37
2(3H)-Furanone, 5-heptyldihydro-	60.30	0.73	-	-	-
5-Hydroxy methylfurfural	**60.49**	**13.62**	**21.68**	**14.58**	**9.95**
Dodecanoic acid, 3-hydroxy-	62.03	1.42	1.74	0.66	0.71
Thiophene-3-ol, tetra hydro-, 1,1-dioxide	62.95	1.00	1.03	0.99	1.36
Total (Identificated)		94.84	99.28	99.09	94.44

Table 2. The chemical constituents of leaves of *Asclepias curassavica* plant in different ratios (60-80%) of ethanol (E) and methanol solutions (M).

Components	RT	LEAF			
		%60 E	%80E	%60 M	%80 M
Piperazine. 1-methyl-	23.17	0.79	1.15	0.99	-
Pyrazine. methyl-	23.76	-	0.76	0.89	0.59
Acetic acid, methyl ester	25.95	6.91	6.70	11.97	6.90
2-Propenoic acid. ethenyl ester	29.34	0.72	0.98	1.42	1.13
Acetic acid	30.60	**16.48**	**17.72**	**24.39**	**18.22**
Acetic anhydride	30.86	2.23	3.13	2.59	2.36
Hex-2-yn-4-one, 2 methyl-	31.51	0.57	-	0.98	-
Formic acid	31.98	1.02	3.34	-	3.27
Propanoic acid	34.05	0.72	1.12	1.24	0.94
2-Furancarboxaldehyde, 5-methyl-	35.46	0.60	-	0.76	-
Dimethyl Sulfoxide	36.21	0.66	1.17	-	1.00
Butanoic acid. 4-hydroxy-	38.21	1.53	3.78	1.33	1.73
2-Furanmethanol	38.90	1.83	2.43	1.09	2.66
4(H)-Pyridine, N-acetyl	41.16	1.21	0.94	0.72	0.81
1.2-Cyclopentanedione	42.88	0.61	0.61	0.72	0.68
Methyl salicylate	43.29	-	0.68	-	0.68
Heptanoic acid	45.28	0.58	0.73	-	0.76
Z-3-Methyl-2-hexenoic acid	45.41	0.74	1.11	-	1.03
Benzyl alcohol	46.21	2.26	1.26	2.94	0.94
2-Dodecenoic acid	48.31	0.63	0.78	-	0.74
2H-Pyran-2.6(3H)-dione	49.21	1.54	0.88	-	1.08
Phenol	49.51	0.7	0.88	2.9	0.7

		3		9	3
Furaneol	50.29	1.29	1.73	2.33	1.30
Mannosamine	51.45	0.69	0.94	-	0.98
Pentanal	51.90	0.99	1.36	0.83	1.13
Octan-2-one, 3,6-dimethyl-	52.23	0.67	0.59	-	0.58
Pentanal. 2.4-dimethyl	53.47	1.51	1.59	1.59	1.51
2-Methoxy-4-vinyl phenol	54.17	**5.01**	**2.65**	**4.67**	**2.80**
4H-Pyran-4-one. 2.3-dihydro-3.5-dihydroxy-6-methyl-	**55.70**	**8.25**	**7.25**	**0.86**	**8.94**
Glycerin	56.35	**10.72**	**12.16**	**3.20**	**11.74**
1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	57.76	0.64	0.54	-	0.65
Benzofuran. 2.3-dihydro-	58.07	**17.19**	**6.46**	**14.52**	**7.85**
R-Limonene	58.22	0.98	1.13	0.76	1.37
Melezitose	60.08	1.12	1.34	4.21	-
5-Hydroxy methyl furfural	60.49	3.20	4.79	3.02	7.46
Dodecanoic acid, 3-hydroxy-	62.03	1.61	0.55	1.15	0.88
2'.4'-Dimethoxy acetophenone	62.48	0.97	2.05	1.04	1.51
Total (Identificated)		97.20	94.59	93.20	94.95

As in flower and root parts, the main components in ethanol and methanol solutions were acetic acid and 5 hydroxy methylfurfural (Table 3). However, unlike the others, Ethanone, 1-(2-hydroxyphenyl)- and Ethanone, 1-(2-hydroxy-4-methoxyphenyl)- compounds were also prominent in root samples. These two compounds were detected only in root samples (except 80% methanol).
In general, although similar compounds were detected in 60% and 80% ethanol and methanol solutions of the flower, leaf and root parts of *A. curassavica* plant, there were some differences. As mentioned above, besides Ethanone, 1-(2-hydroxyphenyl)- and Ethanone, 1-(2-hydroxy-4-methoxyphenyl)- compounds, Undecanal, 2-methyl- and Octan-2-one, 3,6-dimethyl- detected in root solutions. Tetradecanoic acid was detected only in root+60% ethanol solution. Some compounds (Dimethyl Sulfoxide, 4(H)-Pyridine, N-acetyl, Methyl salicylate) found in leaf and root solutions were not found in flower solutions. Similarly, some compounds (3,3-Diacetyl-2,3,4,5-tetrahydro-2-oxofuran, (S)-(+)-2-Amino-3-methyl-1- butanol, Thiophene-3-ol, tetra hydro-, 1,1-dioxide) found in flower and root solutions were not found in leaf solutions (Tables 1, 2, 3).
In generally, major components all solutions of each part of plant were acetic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Glycerin, Benzofuran 2.3-dihydro- and 5-Hydroxymethyl furfural. These components have different uses areas. 4-6% diluted acetic acid solution is directly used as a flavoring agent for foods and also as food preservatives [30]. 4H-pyran4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) consists flavonoid fractions has received much attention for its antimicrobial and antibacterial activities and a potential source for the development of drugs for the control of microbial diseases [24]. The most remarkable among the components was furfural, which is found in flower and root solutions, is a naturally occurring furan aldehyde with many commercial uses, e.g., industrial manufacturing, food flavoring, fragrance in personal care products, pesticide [25].

Table 3. The chemical constituents of root of *A. curassavica* plant in different ratios (60-80%) of ethanol (E) and methanol solutions (M).

Components	RT	Root			
		%60 E	%80E	%60 M	%80M
Pyrazine, methyl-	23.76	-	-	-	0.59
Acetic acid, methyl ester	25.95	5.15	11.66	6.75	5.60
2-Propenoic acid, ethenyl ester	29.34	1.26	1.41	1.36	1.13
Acetic acid	30.60	**16.91**	**24.33**	**19.71**	**18.00**
Furfural	30.66	2.93	-	2.55	-
Acetic anhydride	30.86	1.72	3.18	1.98	4.00
Formic acid	31.98	3.93	4.23	4.16	2.27
Propanoic acid	34.05	-	-	-	0.94
2-Furancarbox aldehyde, 5-methyl-	35.46	0.62	-	-	0.76
Dimethyl Sulfoxide	36.21	-	-	-	1.00
Butanoic acid, 4-hydroxy-	38.21	0.73	1.05	0.91	0.99
3-Furanmethanol	38.90	4.55	3.12	4.81	4.80
4(H)-Pyridine, N-acetyl	41.16	-	-	-	0.81
1,2-Cyclopentanedione	42.88	0.82	1.06	1.12	0.68
Methyl salicylate	43.29	-	-	-	0.68
Ethanone, 1-(2-hydroxyphenyl)-	44.14	**6.01**	**4.96**	**7.46**	-

3,3-Diacetyl-2,3,4,5-tetrahydro-2-oxofuran	45.44	-	-	-	1.03
Benzyl alcohol	46.21	-	0.84	-	0.94
Tetradecanoic acid	47.67	1.48	-	-	-
2H-Pyran-2,6(3H)-dione	49.21	-	-	-	1.08
Phenol	49.51	0.74	1.03	0.63	0.73
Furaneol	50.29	1.37	2.06	2.02	1.30
(S)-(+)-2-Amino-3-methyl-1-butanol	51.56	2.44	3.47	2.64	0.98
Pentanal	51.90	1.03	2.30	1.59	1.70
Hexan-2,4-dione, enol	52.25	0.85	0.76	0.98	0.58
Undecanal, 2-methyl-/Heptanal, 2-methyl	53.46	1.40	-	2.07	1.50
2-Hydroxy-gamma-butyrolactone	53.48	-	4.01	-	-
2-Methoxy-4-vinylphenol	54.17	0.73	1.14	0.78	0.80
Adrenone	54.96	0.63	-	-	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	55.70	8.16	2.32	6.97	0.50
Ethanone, 1-(2-hydroxy -4-methoxyphenyl)-	56.15	**6.20**	**9.24**	**6.12**	-
Glycerin	56.35	3.91	6.36	3.88	0.70
Octan-2-one, 3,6-dimethyl-	56.90	0.66	-	0.67	0.65
Benzofuran, 2,3-dihydro-	58.07	-	-	-	2.20
Dihydroactinidiolide	58.22	-	-	-	**8.60**
5-Hydroxy methylfurfural	60.49	**20.09**	**10.71**	**18.14**	**8.20**
Dodecanoic acid, 3-hydroxy-	62.03	2.22	-	1.00	0.88
7-Hydroxy-6-methyl-oct-3-enoic acid	62.04	1.07	-	-	1.51
Thiophene-3-ol, tetra hydro-, 1,1-dioxide	62.92	1.16	-	1.11	1.14
Unknown		-	-	-	3.96
Total		98.77	99.24	99.41	81.26

Shelke and Bhot (2019) reported that the GC-MS analysis of various compounds from *Asclepias curassavica* L. leaf and stem extracts were determined and ethanol extract resulted many compounds such as 9,12- Octadecadienoic acid, Benzene, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-(R*,S*) and 1-Monolinoleoylglycerol trimethylsilyl ether. Bihana et al. (2018), in the their study, was shown the presence of 49 compounds in the ethanolic extract of the whole plant of A. curassavica, mainly compounds were (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, card-20(22)-enolide, 9,12-octadecadienoic acid, heneicosane, methyl Commate D, cymarin, 2-methylhexacosane, gamma.- tocopherol, Ergost-5-en-3-ol, 22-stigmasten-3-one, n-hexadecanoic acid (14.31%), 2-hexadecen1-ol(11.14%), 9,12,15-octadecatrienoic acid(7.45%), neophytadiene (6.30%), cytidine (4.60%), squalene (4.51%) and stigmast-5-en-3-ol (4.23%).

In the present study, results of GC-MS analysis were really different some researches [3, 5, 26] because it is known that environmental factors (such as temperature, precipitation, duration and intensity of light, altitude, viewing, drought, salinity, soil nutrients and soil structure) have a great influence on the synthesis and accumulation of secondary metabolites and their constituents [11,18,19).

3.2. Antimicrobial effect of *Asclepias curassavica*

In the study in which the antimicrobial effect of *Asclepias curassavica*, prepared with three different solvents (water, methanol and ethanol) against eight different pathogenic bacteria, was investigated, the inhibitory effect of flower and leaf extracts prepared with 80% ethanol came into prominence. (Figure 1).

Extract of the flower of Asclepias curassavica prepared with 80% ethanol formed inhibition zones between 1.5 mm and 5.3 mm in eight plant pathogens. As seen in Figure 1, Flower+80% ethanol application formed the highest inhibition zone (5.3 mm) against Pseudomonas tomato compared to other solvents, but it was determined to be less effective than the streptomycin antibiotic (21.3 mm). This application also created a higher inhibition zone for pathogens other than *Clavibacter michiganensis* compared to other solvents. Unlike other pathogens, *C. michiganensis* flower formed the highest inhibition zone (6.8mm) in the application of 80% methanol. With the application of pure water, an inhibition zone of 3.0 mm for *C. michiganensis*, *P. tomato* and *X. euvesicatoria* 0.5 mm was formed, but it did not affect other pathogens (Table 4).

Leaf+80% ethanol application showed a 3.7mm inhibition zone against *P. caratovorum*, demonstrating the highest effect in this pathogen. While this application created an inhibition zone of 3.5, 3.2 and 3.0 mm against *Dickeya zeae*, *C. michiganensis* and *A. tumefaciens* pathogens, respectively, it had no effect against *X. euvesicatoria* and *P. tomato* (Table 5).

Root+80% ethanol application formed the highest inhibition zone against Clavibacter michiganensis

(4.8mm). While this application shaped a 1.5mm and 1.0mm inhibition zone against *Dickeya zeae* and *Pseudomonas tomato*, it had little effect on other pathogens. It did not show any effect on *Pectobacterium caratovorum* (Table 6).
When all data were evaluated, flower extract prepared with 80% ethanol was highly effective in all pathogens. The effect of Pseudomonas tomato, in which this application created the highest inhibition zone, in different populations (107, 106, 105) was also evaluated.
The extract of *Asclepias curassavica* prepared with 80% ethanol, both flower and leaf, became more effective as the density of *Pseudomonas tomato* decreased (Table 7).

Table 4. Antimicrobial effect of the flower extracts of *curassavica* prepared with water (W), ethanol (E) and methanol (M solvents on eight different plant pathogenic bacteria.

	Flower+ W	Flower+ 60% E	Flower+ 80% E	Flower+ 60% M	Flower+ 80% M	Streptomisin
Xanthomonas euvesicatoria	0.5b*	0.4d	2.3b	0.2c	0.1e	3.0 e
Agrobacterium tumefaciens	0.0c	0.2d	1.5b	0.0d	0.1e	6.8 d
Pseudomonas viridiflava	0.0c	0.4d	2.0b	0.0d	0.1e	1.8 e
Pseudomonas tomato	0.5b	0.7c	5.3a	0.0d	0.3d	21.3 a
Clavibacter michiganensis	3.0a	2.4a	4.4a	0.0d	6.8a	11.8 b
Dickeya zeae	0.0c	1.8b	2.2b	1.2a	1.3b	8.8 c
Pectobacterium caratovorum	0.0c	1.5b	1.6b	0.0d	0.7c	13.5 b
Pseudomonas phaseolicola	0.0c	1.0c	2.5b	0.4b	0.0e	22.5 a

* According to the LSD multiple test, the means shown with different letters in the same column are different according to p≤0.05.

Table 5. Antimicrobial effect of the leaves extracts of *A. curassavica* prepared with water (W), ethanol (E) and methanol (M solvents on eight different plant pathogenic bacteria.

	Leaf+ W	Leaf+ 60% E	Leaf+ 80% E	Leaf+ 60% M	Leaf+ 80% M	Streptomisin
Xanthomonas euvesicatoria	0.3 b*	0.5 d	0.0 d	0.0 b	0.0 e	3.0 e
Agrobacterium tumefaciens	0.0 c	0.0 e	3.0 b	0.0 b	0.0 e	6.8 d
Pseudomonas viridiflava	0.0 c	0.4 d	1.8 c	0.0 b	0.0 e	1.8 e
Pseudomonas tomato	0.4 b	0.0 e	0.0 d	0.0 b	0.0 e	21.3 a
Clavibacter michiganensis	0.0 c	2.4 a	3.2 ab	0.0 b	3.4 a	11.8 b
Dickeya zeae	0.0 c	1.6 b	3.5 ab	1.3 a	1.7 b	8.8 c
Pectobacterium caratovorum	1.9 a	1.2 c	3.7 a	0.0 b	0.5 d	13.5 b
Pseudomonas phaseolicola	0.0 c	0.0 e	1.3 c	0.0 b	1.3 c	22.5 a

* According to the LSD multiple test, the means shown with different letters in the same column are different according to p≤0.05

Table 6. Antimicrobial effect of the root extracts of *A. curassavica* prepared with water (W), ethanol (E) and methanol (M solvents on eight different plant pathogenic bacteria.

	Root+ W	Root+ 60% E	Root+ 80% E	Root+ 60% M	Root+ 80% M	Streptomisin
Xanthomonas euvesicatoria	0.5 a*	0.2 cd	0.2 def	0.3 c	0.0 e	3.0 e
Agrobacterium tumefaciens	0.0 b	0.0 d	0.8 cd	0.4 c	0.4 d	6.8 d
Pseudomonas viridiflava	0.0 b	0.4 c	0.5 de	0.0 d	0.2 de	1.8 e
Pseudomonas tomato	0.5 a	0.2 cd	1.0 c	0.8 b	0.3 de	21.3 a
Clavibacter michiganensis	0.0 b	3.7 a	4.8 a	4.0 a	2.8 a	11.8 b
Dickeya zeae	0.0 b	1.4 b	1.5 b	0.0 d	1.2 b	8.8 c
Pectobacterium caratovorum	0.0 b	1.1 b	0.0 f	0.5 c	0.8 c	13.5 b
Pseudomonas phaseolicola	0.0 b	0.5 c	0.4 de	0.0 d	1.3 b	22.5 a

* According to the LSD multiple test, the means shown with different letters in the same column are different according to p≤0.05.

Table 7. Inhibition zone of *Asclepias curassavica* extract prepared with 80% ethanol, both flower and leaf, against

Pseudomonas tomato in different populations

Pathogen	**Pathogen Population**	**Inhibition Zone (mm)**			
		1	**2**	**3**	**Sum**
Pseudomonas tomato	10^7	2,1	1,5	2,0	1,9
Pseudomonas tomato	10^6	2,5	3,2	1,9	2,5
Pseudomonas tomato	10^5	3,1	2,8	2,8	2,9

Like most medicinal plants, *A. curassavica* is of great importance for its antimicrobial effects. Plant extracts that inhibit the growth of pathogenic microorganisms without harming the host plant may have potential applications as therapeutic agents. Therefore, as the result of this study, *A. curassavica* was determined as a promising medicinal plant, which can be used in the biological control of plant diseases regarding its antimicrobial activity.

Within the scope of the study, the antibacterial activity of the flower, root and leaf of *A. curassavica* was examined against *Agrobacterium tumefaciens*, *Clavibacter michiganensis* [22, 7], *Dickeya zeae*, *Pectobacterium caratovorum*, *Pseudomonas phaseolicola*, *Pseudomonas tomato*, *Xanthomonas euvesicatoria*, and *A. curassavica* plant extract was found to have antimicrobial activity. Among various solvent extracts of flower, leaf and root tested against different bacteria, flower extracts showed better inhibitory effects. It was found that the Flower+80% Ethanol extract exhibited growth inhibition on all microorganisms tested [16], however, it showed an effect on six out of eight microorganisms in the leaf extract applied comparatively and on seven microorganisms from the root extract. They found that *A. curassavica* was more effective.

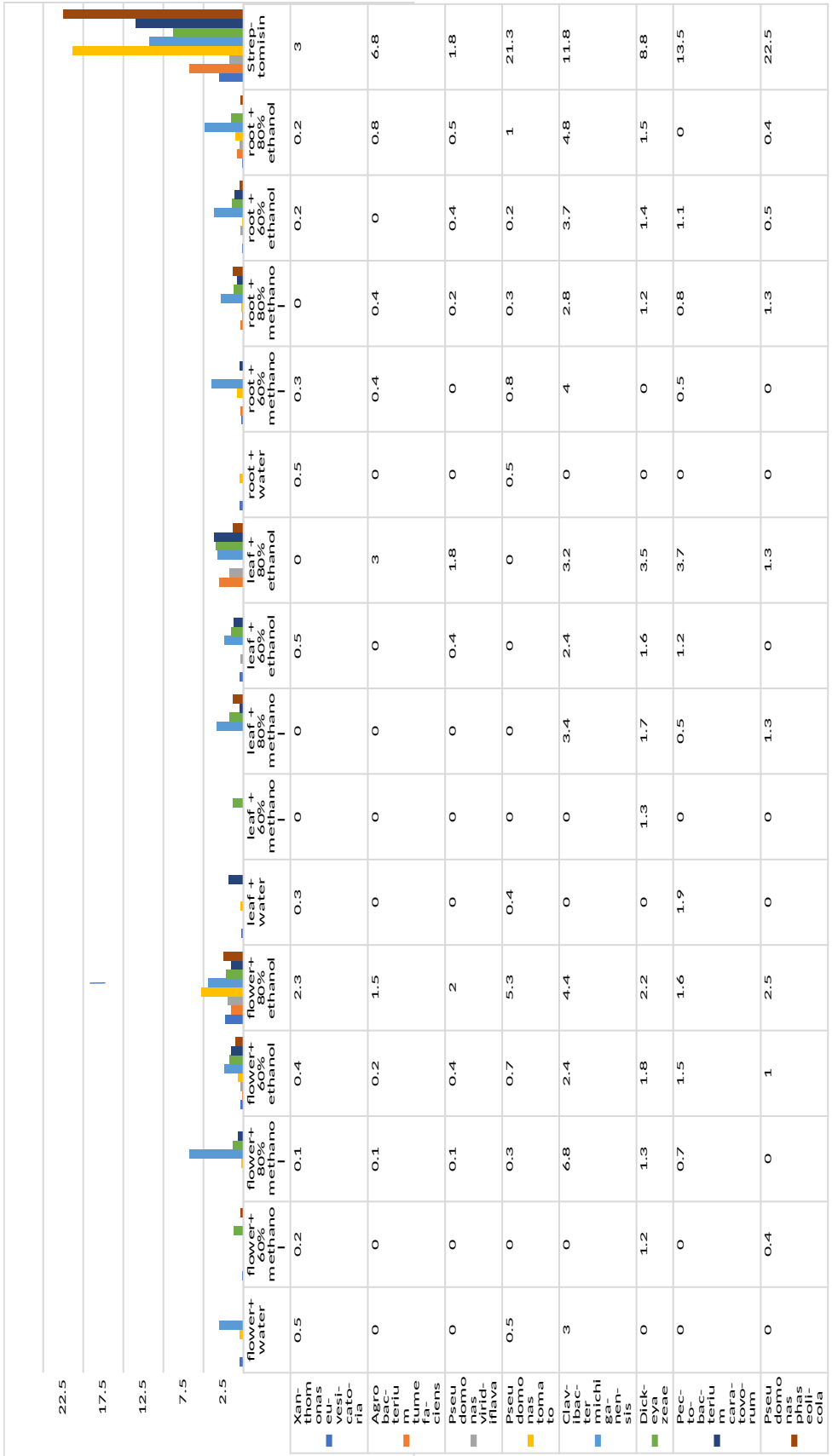

Figure 1. Antimicrobial effect of the extract of flowers. leaves and roots of *Asclepias curassavica* prepared with water, methanol and ethanol solvents against eight different plant pathogenic bacteria.

against *Clavibacter michiganensis* in methanol extract application than other extracts [7] On the other hand, the extracts prepared only with pure water were effective against three microorganisms at a very low level. *A. curassavica* plant extract is successful in terms of its antibacterial effect. Recently, its antibacterial effect on *Clavibacter michiganensis*, *Pseudomonas solanacearum*, *Escherichia coli* [22, 7] and its antifungal effect on *Aspergillus niger* and *Fusarium oxysporum* have been reported [10, 4]. Similarly, various extracts obtained from medicinal and aromatic plants and/or their essential oils serve as natural bactericides, and their use as a part of integrated control in the control of plant bacterial diseases is seen as a promising alternative.

Antibacterial compounds obtained from medicinal and aromatic plants have been evaluated as an alternative to antibiotics and copper in the control of bacterial diseases. Depending on the use of antibiotics, using essential oils within the scope of integrated control is extremely important in preventing the development of resistant forms of bacterial disease agents [1].

4. Conclusion

Medicinal plants are known to produce secondary metabolites with known effects against bacterial pathogens and less adverse effects in comparison to conventional antibacterial agents [23]. Natural products from medicinal plants can be a resource for researching new drugs, especially in organic farming areas. This research revealed that flower extracts of *A. curassavica* have antimicrobial potential that can be purified and used as broad-spectrum antibiotics. In addition, being environmentally friendly and sustainable, it can be included in integrated control methods and the loss of yield caused by plant diseases can be prevented. Since medicinal and aromatic plants will be used as an alternative method to pesticides in the control of bacteria that cause disease in plants, these environmentally friendly practices that do not harm nature will contribute to the preservation of the natural balance of our country. Although some main components were similar in GC-MS results of ethanol and methanol solutions of the flower, leaf and root parts of *A. curassavica* plant, the detection of different components caused differences in the antibacterial effects of the solutions.
In addition, investigating the mechanism of action of the extracts obtained from *A. curassavica* will shed light on future studies, contribute to the country's economy with the potential for commercial preparations, and provide an opportunity to create an industry branch. Culturing the *A. curassavica* plant will increase producer size and provide another economic benefit.

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Author Contribution

SK and YA designed research, SK and EF were grown the plant materials, EF prepared the samples, YA, BPA and EF conducted bioassays, BPA and EF analyzed data, SK, YA, BPA and EF wrote this manuscript. All authors read and approved the manuscript.

Conflicts of Interest The authors declare no confict of interest.

References

1. B.P., Aktepe, K., Mertoğlu, Y., Evrenosoğlu, Y., Aysan, Farklı bitki uçucu yağların *Erwinia amylovora*'ya karşı antibakteriyel etkisinin belirlenmesi. Tekirdağ Zir. Fak. Der.**16** (1),34-41 (2019)
2. A.J., Alonso-Castro, V., Arana-Argáez, E., Yáñez-Barrientos, J. C., Torres-Romero, R. J., Chable-Cetz, K., Worbel, M., del Carmen Juárez-Vázquez, Pharmacological activities of *Asclepias curassavica* L.(Apocynaceae) aerial parts. J of Ethnoph, **281,** 114554 (2021)
3. A.E., Al-Snafi, Chemical constituents and pharmacological effects of *Asclepias curassavica* – a review. Asian J. Pharm. Res. **5**(2), 83-87 (2015)
4. A.E., Al-Snafi, Medicinal plants with antimicrobial activities (part 2): Plant-based review. Sch Acad J Pharm. **5**(6),208-239 (2016)
5. S., Bihana, A., Dhiman, G., Singh, S., Satija, *Gas chromatography-mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of Asclepias Curassavica L.* International Journal of Green Pharmacy (IJGP) Special Issue, Conference Apr-Jun 2018, 104-114
6. R.T., Gahukar, Evaluation of plant-derived products against pest and diseases of medicinal plants: A review. Crop Production. **42,** 202-209 (2012)
7. C., Hemavani, B., Thippeswamy, Evaluation of antimicrobial activity of root extract of *Asclepias curassavica*. Recent Research in Science and Technology, **4**(1), 40-43 (2012)
8. S., Horuz, A,. Ocal, Y., Aysan, Efficacy of hot water and chemical seed treatments on bacterial speck of tomato in Turkey. Fres. Environmental Bull. **27**(5), 3185-3190 (2018)
9. M., Karman, Bitki Koruma Araştırmalarında Genel Bilgiler. Denemelerin Kuruluşu ve Değerlendirme Esasları. T.C. Tar. Bak. Zir. Müc. ve Zir.i Karantina Gnl. Müd.Yay.. 279s (1971)

10. K., Keymanesh, J., Hamedi, S., Moradi, F., Mohammadipanah, S., Sardari, Antibacterial. antifungal and toxicity of rare Iranian plants. IJP-International J.of Pharm. **5**(1), 81-85 (2009)
11. S., Kırıcı, M., İnan*, Effect of different harvesting time on the essential oil content of the rosemary (Rosmarinus officinalis L.) in the Çukurova condition,* Workshop on Agricultural and Quality Aspects of Medicinal and Aromatic Plants 29 May – 1 June. Proceeding Book Adana, Türkiye, S.263-272 (2001)
12. S., Kırıcı, E., Bayram, S., Tansı, O., Arabacı, H., Baydar, İ., Telci, M., İnan, DA., Kaya, A., Özel, *Tıbbi ve aromatik bitkilerin üretiminde mevcut durum ve gelecek*. Türkiye Ziraat Mühendisliği IX.Teknik Kongresi: C. 1:505-528. ISBN-978-605-01-1321-1 (2020)
13. B., Kokoskova, R., Pavela. Effectiveness of plant essential oils on the growth of *Erwinia amylovora* the causal agent of fire blight disease. Pest Tech.**1**(1), 76-80 (2007)
14. B., Kokoskova, R., Pavela, D., Pouvova, Effectiveness of plant essential oils against *Erwinia amylovora, Pseudomonas syringae* pv. *syringae* and associated saprophytic bacteria on/in host plants. J of Plant Path. **93**(1), 133-139 (2011)
15. R., Kumar, R., Mishra. Analgesic and antipyretic activity of extracts of *Asclepias currasavica* Linn. International J of Pharm Tech Research. **4**(1), 306-308 **(**2012**)**
16. RR., Kurdekar, GR., Hegde, SS., Hebbar, Antimicrobial efficacy of Bridelia retusa (Linn.) Spreng. and *Asclepias curassavica* Linn. Indian J of Natural Products and Resources. **3**(4),589-593 (2012)
17. RM., Leão, JVS., Cruz, VM., Ramos, VT., Almeida, PH., Gorni, RS., Camargo, AC., Pacheco, LV., Lima, L C., Forti, Secondary metabolites of *Asclepias curassavica* (Apocynaceae) and its effects on food preference and mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Emirates Journal of Food and Agriculture. **32**(8), 583-590 (2020)
18. H., Maral, S., Kırıcı, Changes within the daytime of essential oil content and composition of zahter (*Thymbra spicata* L.) grown in Ermenek conditions. Turkish J of Agri.-Food Sci. and Tech. **7**(2),13-16 (2019)
19. H., Maral, S., Kırıcı, Some Yield and Quality Properties of Endemic Origanum husnucan-baserii Grown in Different Ecological Conditions. J of Tekirdag Agri. Fac. **20**(2),1-11 (2023)
20. M., Mirik, Y., Aysan, Effect of some plant extracts as seed treatments on bacterial spot disease of tomato and pepper. The J of Turkish Phytopath. **34** (1-2-3) (2005)
21. M., Mirik, Y., Aysan, O., Cinar, Copper-resistant strains of *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) dye in the eastern Mediterranean region of Turkey. J of Plant Pathology. **89**(1),153-154 (2007)
22. C.C., Neto, C.W., Owens, R.D., Langfield, A.B., Comeau, J.S., Onge, A.J., Vaisberg, G.B., Hammond, Antibacterial activity of some Peruvian medicinal plants from the Callejon de Huaylas, J of Ethnopharmacology. **79**(1),133-138 (2002)
23. P. O, Nyalo, G. I., Omwenga, M. P. Ngugi,. Antibacterial properties and GC-MS analysis of ethyl acetate extracts of Xerophyta spekei (Baker) and Grewia tembensis (Fresen). Heliyon 9(3), March, **e14461**:1-12 (2023)
24. O., Ortega-Buitrago, G., Chaves-Bedoya, L.Y., Luz Yineth Ortiz-Rojas, Chemical Composition and Antimicrobial Activity of Ethanolic Bark and Leave Extract of Zanthoxylum Caribaeum Lam. from Norte de Santander, Colombia. Rev. Chim. **72** (4):152-161 152 (2021)
25. N.R., Reed, E.S.C., Kwok, Furfural. In Encyclopedia of Toxicology (Third Edition, ed: Wexler P, Academic Press: Oxford, 685–688 (2014)
26. V, Shelke, M., Bhot, GC-MS Analysis of Bio-active Compounds in Ethanolic extract of Leaf and Stem of Asclepias curassavica L. Int. J. Pharm. Investigation. **9**(2),67-70 (2019)
27. S., Bihana, A., Dhiman, G., Singh, S., Satija, Gas chromatography-mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of *Asclepias curassavica* L. International J of Green Pharmacy (IJGP). **12**(02) (2018)
28. M., Umarusman, Y., Aysan, M., Özgüven, Farklı bitki ekstraktlarının bezelye bakteriyel LEAF yanıklığına (*Pseudomonas syringae* pv. *pisi*) antibakteriyel etkilerinin araştırılması. Tekirdağ Zir. Fak. Der. 16(3):297-314 (2019)
29. E., Yücel, "Ağaçlar ve Çalılar 1 (Trees and Schrubs 1)" Türmatsan. 301 sayfa. ISBN 979-975-93746-2-5. İstanbul, (2005)
30. A., Vidra, A.**,** Németh, Bio-produced Acetic Acid: A Review. Periodica Polytechnica Chemical Engineering. **62**(3), 245-256 **(**2018**)**