Allelopathic Activity of Some Medicinal Plants Against *Erwinia carotovora*

ABSTRACT

Many medicinal plants ,exhibit allelopathy to biological management of plant pathogens by reducing their regeneration. This process involving secondary metabolites produced by plant influence the growth and development of agricultural and biological system. This research was to determine the allelopathic potential of aqueous extracts of different medicinal species (*Artemisia herba alba, Pistacia atlantica* and *Juniperus phoenicea*) against plant pathogenic bacteria (Erwinia carotovora subsp. Carotovora) in comparison to the antibiotic Streptomycin (positive control) . Effect of water extracts evaluated for different concentrations of different extracts for each plant studied was examined under laboratory conditions in petri dishes. The results showed that all the extracts significantly inhibited growth of tested bacteria. The differences in their inhibition depend on to the type of plant and concentration of extract. However, *Artemisia herba alba* extracts had greater inhibitory potential. Based on the study results, aqueous extracts of three plant species (*A. herba alba, P. atlantica and J. phoenicea*) showed a negative allelopathic effects on plant pathogenic bacteria (Erwinia carotovora subsp. Carotovora).

Key words: Allelopathy, plant pathogenic bacteria, Medicinal plants, Inhibition zone, Disc diffusion Method, Aqueous extract .

1. INTRODUCTION

Biological management of plant pathogens and is critical in agriculture. There is great incentive to find biologically active natural products from higher plants to act as bactericides, insecticides ,fungicides and herbicides that are better than artificial agrochemicals, and more safe environmentally and a healthily . Allelopathy offers beautiful environmentally friendly different to pesticides in agricultural pest management [1]. Allelopathy has been defined is a physiological process with ecological implications [2]. It may also include the substances effect on growth and development of nearby plants through both inhibitory and stimulatory biochemical interactions [3,4,5]. These chemicals vary among plant species and organs and are released into the environment by different processes; root exudation, volatilization, leaching, and tissue decomposition in soil [6]. Phenolic compounds are one in every of the biggest group of secondary metabolites, consisting of four main groups divided in keeping with the number of phenol rings and the structural parts that bind those rings, including flavonoids, phenolic acids, tannins, saponins, cinnamic acid coumarins, terpenoids, quinones, and lignans [7]. Some secondary metabolites are considered as natural pesticides against pathogens, bacteria, fungi, insects, and weeds. [8]. The current and future trend towards the use of safe to environment pesticides from natural plant extracts instead of harmful and expensive industrial chemicals[9]. Some medicinal plants have the ability to eliminate antibiotic- resistance bacterial

species, because they effect on a large number of microbes as well as safe and low cost[10]. Al-Jabal Al-Akhdar region (Libya) has highest species diversity and having distinct environmental characteristics associated with evergreen forest and it has environment similar to other region in Southern Europe [11]. The number of plant species reach up of 1100 species from the total of plant species in Libya (2000 species) with about 75 species of plants that grow only in AL-Jabal AL Akhdar and have been served for as basis of traditional medicinal systems for thousands of years [11,12]. In addition, such plants produce a remarkable diverse array of over low known and high molecular mass natural products which are 5,00,000 as secondary metabolites , which can be used as an alternative from of health care as well as screening for active compound that have significant effects against human and plant pathogens[13,14]. *Artemesia herba-alba* Asso. (Fam. Asteraceae.), which commonly known as the desert wormwood, is a dwarf semi shrub growing widely in Al-Gabal Al-akhdar in Libya and in the Middle East. The plant is a perennial, strongly aromatic herb, with many basal, erect and leafy stems covered by woolly hairs [15]. It is widely used as folk medicine and in particular for common uses such as relief of coughing, intestinal disturbances, colds and muscle tensions by the local population in different countries [16]. Artemisia forms the plants whose allelopathic ability is proved between different species. In this genus, a wide range of active biological compounds are produced which included artemisinin, tannin, flavonoids, sesquiterpene lactone thymol, carvacrol, terpenoids and other secondary metabolites such as coumarin, camphor and bornyl acetate which their toxicity for some other plants, and antimicrobial activities are proved [17,18]. *Juniperus phoenicea* (Fam. Cupressaceae) is a shrub or a small tree which is believed to be originated in northern lands bordering the Mediterranean Sea from Portugal to Palestine and also considered as native to North Africa and mainly found in Libya, Algeria, Morocco and Canary Islands [19]. Previously, from the genus Juniperus some terpenoids have been isolated [20], neolignans [21] and flavonoids [22].The species of Juniperus is considered as an important medicinal plant largely used in traditional medicine. The anti-inflammatory activity of some diterpenoids of Leaves juniperus have been published [20]. Leaves juniperus have high content of α- pinene, Δ3-carene, limonene, terpinolene and the α-terpinyl acetate [23]. [24] reported that *J. phoenicea* extracts have antibacterial properties. *Pistacia atlantica* (Fam. Anacardiaceae) is a species of flowering plants. Traditionally used for stomach aches, dyspepsia and throat infections Various types of compounds like terpenoids, phenolic compounds, fatty acids, and sterols have been identified from different parts of Pistacia species. According to previous researches, wide pharmacological activities had been showed from various parts of Pistacia species such as anti-inflammatory, antitumor, antioxidant, antimicrobial, antiviral and their effects in gastrointestinal disorders improvement [25]. The objective of the present work was to evaluate the allelopathic potential aqueous extracts for three Libyan folk medicinal plants (*Artemisia herba alba ,Pistacia atlantica* and *Juniperus phoenicea*) against plant pathogenic bacteria (Erwinia carotovora subsp. Carotovora).

2. MATERIALS AND METHODS

2.1 Tested microorganisms:

Plant pathogenic bacteria(Erwinia carotovora subsp. Carotovora). Which infects variety of vegetables including carrots, potatoes, cucumbers, onions and tomatoes, it was obtained from Laboratory of bacterial plant diseases Department of prevention, Faculty of Agriculture Omar EL- Mukhtar University, Elbyda , Libya.

2.2 Plant Material Collection

Plant materials of species belonging to 3 botanical families included in this study were collected from Al-Jabal Al-Akhadar of Libya, during October 2018. (Table 1)

Table 1. Selected plant species

Plant species	Family	Local name	Part used
Artemisi aherba alba	Asteraceae	Sheah	Leaves &steams
Juniperus phoenicea	Cupressaceae	Araar	Leaves &steams
Pistacia atlantica	Anacardiaceae	Battom	Leaves &steams

Artemisia herba alba (Asteraceae) from Sosa region (125m) which considered as littoral zone near to the Mediterranean Sea. While *Juniperu phoenicea* (Cupressaceae) and *Pistacia atlantica* (Anacardiaceae) from Shahat region which represents (450 m) above the sea level. The plants were classified and authenticated according to [26] as well as herbarium at Department of Botany, Faculty of Sciences , Omar EL-Mukhtar University, Elbyda , Libya.

2.3. Preparation of Plant Material and Extracts

After the collection of the plants, they were dried in a shady place at room temperature for ten days. The dried aerial parts (leaves and stems) were ground into powdered form then 50 g of the powder were diluted into 500 ml of distilled water, next to that, this mixture was left on shaker for 24 h in room temperature at speed of 120 rpm four-folded cotton fabric was used as a filter to separate rough solid particles from solution. The contents were then filtered with Whatman No.1 filter paper and then it was centrifuged with the speed of 2000 rpm for 15 minutes [27]. Three concentrations of solutions were prepared based on volume/volume percent(v/v. %), except for the basic solution 100 % which was weight was a weight/ volume percent (w/v. %) [28]. Four concentrations were prepaid (20, 40, 80 and 100%) in addition to the antibiotic Streptomycin as a positive control.

2.5 Bactericidal activity

Bactericidal activity of plant aqueous extracts was assessed by monitoring the phytotoxicity using disc diffusion Technique developed by [29]. suspension of the test microorganism was first spread on solidified culture medium (nutrient agar) of petri dishes. Filter paper disks (Ø= 5 mm) were afterwards soaked with 100 µL of aqueous extract and placed on the inoculated plates. Standard antibiotics streptomycin (25 µg/disk) were used as positive control. After incubation at 28 ° C± 2 ° C for 24

h, the diameter of the inhibition clear zones or halos around the disks was measured in millimeters when observed. Tests were carried out in triplicate.

Statistical Analysis :

Statistical analysis was performed using a computer run program (Minitab software). One way ANOVA followed by Tukey,s HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard Division (SD). P-value below 0.05 was considered to be statistically significant.

Results and discussion :

In the present study, the tested bacteria were to be sensitive to tested plant extracts. The preliminary screening of selected plant extracts against the phytopathogen bacteria was done using disc diffusion method. Our results showed that all the aqueous extracts screened against plant bacteria shows significant allelopathic potency ,the in vitro antimicrobial activity of *Artemisia herba- alba*, *Pistacia atlantica* and *Juniperus phoenicea* against phytopathogen bacteria (Erwinia carotovora) was assessed by the diameter of inhibition zone around the discs, we notice the inhibitory effect increased by increasing the concentration of plant extracts. Allelopathic activity of aqueous extracts was illustrated in(Table 2).
These results are consistent with previous research on the containment of aqueous extracts of a number of plants on biologically effective compounds against bacteria and pathogenic fungi, where[30] proved that aqueous extracts could contain anthocyanins, tannins, saponins, terpenoids, and polypeptides. Terpenoids derived from plants have antibacterial activity [31,32]. Tannins are water soluble polyphenols found in almost all plants. It is known to possess antifungal and antibacterial effects [33.34]. In a study carried out by [35] on the extracts of some medicinal plants developing in al-Jabal al-Akhdar area of Libya (*Rosmarinus fficinalis, Datura alba* and *Capparis spinose*) which showed allelopathic activity against bacteria and pathogenic fungi of plant. The effect of the allelopathic was examined using the disc diffusion method which proved to be that the inhibitory effect increased by increasing the concentration of plant extracts. Increasing the efficiency with increasing the concentration is may be due to the effect of the extract on the permeability of bacterial cell membrane and the work of enzymes. The effectiveness of the extracts of the plants is attributed to the presence of phenolic compounds that have a high inhibitory effect on the bacteria[36]. Also found that tannins are toxic to fungi, yeast and bacteria through their association with the wall of these microorganisms, preventing their growth and the effectiveness of protease this is accordance with [37].
In the present study, the highest allelopathic activity is reported in the aqueous extract for *A. herba alba* the diameters of the inhibition zone was 8.33, 10 ,12 and 13.33 mm for concentrations of 20, 40, 80, 100% respectively. Our results for the allelopathic activity of *A. hrba alba* are consistent with many previous studies including referred to by [38] which conducted a study to assess the allelopathic effect of three desert plants (*Artemisia judaica, Asphodelus microcarpus* and *Solanum nigrum*) against plant pathogenic bacteria (Erwinia carotovora, Xanthomonas campestris and Ralestonia solanacearum), using leaf extracts of each plant with different concentrations. *A.judaica* extract showed the highest level of inhibition activities against all the tested bacterial strains also recorded the best result of

minimum inhibitory concentrations, and those referred to by [39] that the water extract of A. herba alba that growing in Libya has an inhibitory effect against bacteria. Chemical studies of Artemisia species indicate that all classes of compounds, especially terpenes, alkaloids, sesquiterpene , coumarins and flavonoids are present in these species according to [40,41].

Table 2. Allelopathic activity of aqueous extracts of the studied plants against plant pathogenic bacteria using agar disc diffusion method, Streptomycin was used as positive control.

Mean diameter of inhibition zone (mm ± SD) in mm					
The plant	**streptomycin**	**20**	**40**	**80**	**100**
	Mean± SD	**Mean ± SD**	**Mean ±SD**	**Mean ±SD**	**Mean± SD**
A. herba alba	**$15^{a} \pm 0$**	**$8.33^{c} \pm 0.47$**	**$10^{c} \pm 0.82$**	**$12^{b} \pm 0$**	**$13.33^{ab} \pm 0.94$**
J. phoenicea	**$15^{a} \pm 0$**	**$7^{d} \pm 0.82$**	**$9^{c} \pm 0$**	**$10.67^{bc} \pm 0.94$**	**$11.67^{b} \pm 0.47$**
P. atlantica,	**$15^{a} \pm 0$**	**$7.67^{d} \pm 0.47$**	**$10^{c} \pm 0.82$**	**$10.67^{bc} \pm 0.47$**	**$12^{b} \pm 0$**

Data are expressed as mean ± SD of three replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same letters mean that there is no significant difference ($p>0.05$).

Our results showed inhibitory activity in the aqueous extract for *Pistacia atlantica* against plant pathogenic bacteria Erwinia carotovora, the diameters of the inhibition zone was 7.67, 10, 10.67 and 12 mm for concentrations of 20, 40, 80, 100% respectively, and this corresponds with some of the previous research [42,43]. The antibacterial activity of the extracts of *P. atlantica* may be due to the presence of certain antimicrobial secondary metabolite, the leaf extract is rich in phenol compounds which have a high level of antibacterial properties against plant pathogens [44,45]. In this study phytotoxicity of *juniper phoenicea* extract on the tested microorganism are shown, the diameters of the inhibition zone was 7, 9, 10.67 and 11.67 mm, for concentrations of 20, 40, 80, 100% respectively, Our results agrees with that obtained by [24,46] who reported that *juniper phoenicea* extracts have antibacterial properties. according to [47,22,20] terpenoids and flavonoids have been isolated from the genus Juniperus. There are many reasons that may lead to varying efficacy of the plant extracts and their allelopathic effect. The difference in efficacy may be due to the stage of plant sample collection, soil nature, other environmental factors, storage conditions, plant part used, extraction method, Extraction, and different sensitivity of strain test. [48,49].

Conclusion:

In conclusion, the obtained results showed that aqueous extracts of some medicinal plants (*Artemisia herba alba* ,*Pistacia atlantica* and *Juniperus phoenicea*) that grow wild in the eastern region of Libya showed allelopathic activities,which proved effective in reducing the growth of plant pathogenic bacteria Erwinia carotovora.

These results obtained from laboratory experiments can be supplemented by other, more comprehensive, open-field studies to assess the practical use of these extracts within an integrated pest management system. Therefore, we need to deepen our knowledge about the mechanisms of plant aqueous extracts against microorganisms. Allelopathy offers safe solutions in pest management; in the long run it would be a luminous direction to proceed in order to develop bactericidal by using the allelochemicals.

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