Allelopathic Activity of Some Medicinal Plants Against Plant pathogenic Bacteria (*Erwinia carotovora*)

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Abstract:

The in depth use of chemicals in plant protection has given rise to concerns about pesticide residues in the environment. Allelopathic plants provide better alternative for this purpose due to being cost-saving, simple to use, eco-friendly, economical and safe. investigation on plant pathogens has not been given an adequate attention. The present investigation was meted out to guage the allelopathic effect of three medicative plants to seek out a suitable alternative to artificial antibiotics for the management of plant diseases caused by bacteria.

Aqueous extracts of three plant species (*Artemisia herba alba*, *Pistacia atlantica* and *Juniperus phoenicea* which from Al-Jabal Al-Akhdar region (Libya) ,were screened against plant pathogenic bacteria *(Erwinia carotovora subsp. Carotovora)* in comparison to the antibiotic Streptomycin (positive control) under laboratory conditions. Antibacterial activities was evaluated for different concentrations of different extracts for each plant studied.
Results showed varying in levels of the inhibition were observed in response to the tested extracts at the concentrations of 20, 40, 80 and100 %. The differences in their inhibition depend on to the type of plant and concentration of extract .

The effectiveness of the extracts against tested bacteria could be arranged by the following order; *Artemisia herba alba* >*Pistacia atlantica* > *Juniperus phoenicea* .the study gave encouraging results for the use of these plant extracts in the control of tested bacteria.

The results indicate that the rate of inhibition increases with increased concentration in all transactions ,and that it increases the diameter of the inhibition of bacteria by increasing the concentration of the extract and that the highest inhibition of *Erwinia carotovora* bacteria was obtained using the *Artemisia herba alba* extract.

Plant extracts resulted in antibacterial activity is potential to use in the management of plant diseases as an alternative to antibiotics. Further phytochemical analysis is required to identify the bioactive compounds responsible for antibacterial activity.

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

Introduction :

Biological management of plant pathogens and plant diseases 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 (Inderjit and Mukerji, 2006). Allelopathy has been defined is a physiological process with ecological implications (Reigosa *et al*., 2006).

The International Allelopathy Society defined allelopathy as any process it involves secondary metabolites produced by plants, microorganisms, viruses and fungi which effect on the growth and development of agricultural and biological systems (Cheng and Cheng, 2015).

It may also include the substances effect on growth and development of nearby plants through both inhibitory and stimulatory biochemical interactions. (Rice,1974; Rice,1984;Tang,1986).

Allelopathy as an ecological phenomenon, has been defined as any direct or indirect effects of one plant, including micro-organisms, on another through the release of phytochemical compounds (allelochemicals) into the environment, (Rice, 1984; Weir *et al*., 2004; Inderjit, *et al*., 2006).

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 (Parvez *et al*., 2004) .

Allelochemicals are produced by any organ of the plant and are mainly secondary metabolites.

plant secondary metabolites are usually associated with plant defense against herbivores and pathogens, these distinctive compounds may be linked to wide range of ecological functions. (Hussain and Reigosa, 2011) .
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 (Balasundram *et al* 2006). some secondary metabolites are considered as natural pesticides against pathogens, bacteria, fungi, insects, and weeds. (Soltoft *et al* 2008),

merge allelopathy into agricultural management systems could scale back the employment of pesticides, reduction environment/soil pollution, and reducing the risk of auto toxicity dangers, increase crop growth and enhancing productivity (Sodaeizadeh and Hosseini, 2012).

Among the massive diversity of plant pathogens, that include viruses, bacteria, fungi, nematodes, and insects, nearly a hundred and fifty are bacterial species cause diseases to plants that limit the crop production and caused crop losses .the use of agrochemicals to manage bacterial diseases should be minimized due to the increased risk of its harmful residues (Kannan and Bastas, 2015).
The current and future trend towards the use of cheap and safe insecticides from natural plant extracts instead of harmful and expensive industrial chemicals has become the current control of these lesions at present time. (Danahap and Wonang, 2016).

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 . (Hassawi and Kharma, 2006).

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 (Al-Idrissi *et al*., 1996).

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. (Al-Idrissi *et al*., 1996 and El-Barasi *et al*., 2013).

In addition, such plants produce a remarkable diverse array of over low known and high 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. (Hegazy et al., 2011; Attitalla et al., 2010 and Fatope et al., 2000).

In the current study, three of these plants with medicinal properties used in folk medicine were selected in Libya they are ***Artemisia herba alba*** ,***Pistacia atlantica*** and ***Juniperus phoenicea*** . To test its inhibitory activity for the growth of plant pathogenic bacteria.

The objective of the present work was to evaluate the allelopathic potential of three Libyan folk medicinal plants aqueous extracts against plant pathogenic bacteria.

Materials and methods:

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. Plant species screened for Allelopathic activity.

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 ***Juniperus 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 Jafri and EL- ghadi (1978). as well as herbarium at Department of Botany, Faculty of Sciences , Omar EL-Mukhtar University, Elbyda , Libya.

Tested microorganisms:

plant pathogenic bacteria ***(Erwinia carotovora subsp. Carotovora)*** (Family: Enterobacteriaceae), The bacterium infects a variety of vegetables and plants including carrots, potatoes, cucumbers, onions, tomatoes, lettuce and ornamental plants like iris..
It was obtained from Laboratory of bacterial plant diseases Department of prevention , Faculty of Agriculture Omar EL- Mukhtar University, Elbyda , Libya.
The bacterial strains were maintained on nutrient agar (NA: Peptone 5g, Beef extract 3g, NaCl 8g).

Plant Material Preparation
After the collection of the plants, they were dried in a shady place at room temperature for ten days.

Preparing the aqueous extracts of plants
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(Bajalan et al., 2013).
Next to that, this mixture was left on a Shaker for 24 hrs in room temperature at speed of 120 rpm (Ghorbani et al., 2008).
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 (Bajalan et al., 2013).

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. %) (Elshatshat, 2010).
Four concentrations were prepaid (20, 40, 80 and 100%) in addition to the antibiotic Streptomycin as a positive control.

Bactericidal activity

The Bactericidal activity of plant aqueous extracts was evaluated by disc diffusion Technique developed by Bauer et al. (1966).
Briefly, this method is based on the diffusion of water extract from a filter paper disc through the solidified culture medium (nutrient agar) of Petri dishes.
Whatman filter paper (No2) was used to prepare small discs (5 mm in diameter), then sterilized at 121 °C for 15 min in an autoclave.
then it was poured the culture medium (nutrient agar) into the Petri dishes (three dishes /concentration).Then processing a bacterial suspension from colony (24 hours old) (10^8 cfu /ml) and performed three dilutions (1000 time),taking from the last dilution 250µl by plastic pipette and distributed regularly in Petri dishes.
The discs of Whatman filter paper were separately impregnated with extract (serial of dilution of aqueous extract ranging from 20,40,80 and 100%) for 5 minutes and left to dry for 1-2 hrs at room temperature, four discs of each plant extracts were placed on the surface of the Petri dishes which Containing the bacterial suspense.
In the same way, tablets were immersed in sterile distilled water as a test and placed on the surface of the dishes.
and under the same laboratory conditions, an antibiotic experiment (streptomycin) was performed with the concentration **(0.004) %** for comparison .
all dishes are placed in the incubator at a temperature of 28 ° C± 2 ° C for 24 hours, (three replicated plates were used for each treatment).
The antimicrobial plant extracts activity was detected as a result of clear inhibition zone ,The diameter of inhibition zone created by each disc was measured (in mm) using a ruler.
Antibacterial activities of different concentrations of different extracts for each plant studied were assessed, and was recorded the lowest concentration of the sample that inhibits visible growth giving clear zones of inhibition after 24 h incubation.

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.
It was found that inhibition zone (IZ) with a diameter greater than 5mm indicated there Antibacterial activity . (Palombo and Semble 2001).
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, with varying degrees of inhibition of different concentrations of different extracts for each plant studied, from 20% to100% among all the aqueous extracts obtained from the three studied plants.
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 (Saleem *et al*., 2010) proved that aqueous extracts could contain anthocyanins, tannins, saponins, terpenoids, polypeptides, and lectins.
Terpenoids derived from plants have antibacterial activity, (Drewes *et al*., 2005; Mathabe *et al*., 2008) . Tannins are water soluble polyphenols found in almost all plants. It is known to possess various biological effects with antifungal and antibacterial effects. (Lanchoti Fiori *et al*., 2013; Scalbert, 1991).
In a study carried out by Mohammed and Abdu EL-Salam,(2013) 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, and diameters of the inhibition zone of bacteria increases by increasing the concentration of the extract.
This is similar to the results obtained in our current study, as shown in table (2) which explained that the rate of the inhibition increased with increased concentration in all transactions ,and that it increases the diameter of the inhibition of bacteria by increasing the concentration of the extract and that the highest inhibition of *Erwinia carotovora* bacteria was obtained by using the *A. herba alba* extract. the diameters of the inhibition zone was 8.33 mm, 10 mm,12 mm, 13.33 mm, followed by *P. atlantica*, with 7.67 mm, 10 mm, 10.67 mm, 12 mm , then *J. phoenicea* extract with a diameters of 7 mm, 9 mm, 10.67 mm, 11.67 mm, for concentrations of 20, 40, 80, 100% respectively, compared to 15 mm, when using the antibiotic at concentrations of 0.004, as indicated in the table (2).

Increasing the efficiency with increasing the concentration is may be due to the effect of the extract on the membrane permeability of the cell membrane and the work of enzymes transferring (Transferase) of cell bacteria.
The effectiveness of the extracts of the selected plants is attributed to the presence of phenolic compounds that have a high inhibitory effect on the bacteria. Mounir Tilaoui, *et al* (2015). 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(Jones, G. A,*et al* 1994).

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.

IZ (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 for the allelopathic activity of *Artemisia hrba alba* are consistent with many previous studies including those referred to by(Abdel-Rahman et al., 2017) 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.
Artemisia judaica extract showed the highest level of antibacterial activities against all the tested bacterial strains also recorded the best result of minimum inhibitory concentrations.
Dababneh (2008).which reported that *Artemisia hrba alba* possesses great anti-bacterial effect of pathogenic bacteria using disc diffusion method .
Hassawi and Kharma (2006) stated that Artemisia sp. extract is anti-diabetic, antitumor, antifungal against *Candida albicans* and bactericidal.

It was pointed out by (Halbuda *et al*., 2014) that the water extract of *Artemisia 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 and flavonoids are present in these species According to Wright (2002) . (Sellami *et al*.2010) confirmed the presence of alkaloids, sesquiterpene , coumarins and acetylenes.

Our results showed the presence of antimicrobial effects of the aqueous extract from the leaf of the *Pistacia atlantica* and this corresponds to some of the previous research(Panahi *et al*., 2013and Roozegar *et al*., 2016).

The antibacterial activity of the extracts of *Pistacia atlantica* may be due to the presence of certain antimicrobial secondary metabolite, the leaf extract is rich in phenol compounds and antibacterial properties. (Farzangi *et al* 2013).

Leaf extracts of *P. atlantica* proved to be very efficient against plant pathogenic fungi and bacteria. (Rhouma *et al*., 2009).

P. atlantica extracts contain polyphenols, which have a high level of antimicrobial activity against plant pathogens. Several studies have suggested that polyphenols inhibit the growth of microorganisms. (Mila *et al*., 1996).

Our results agrees with that obtained by Ekweny and Elegalam(2005) who reported that juniper phoenicea inhibit growth E. coli.

Also, Malu *et al*., (2009) reported that *juniper phoenicea* extracts have antibacterial properties and could be used for the treatment of bacterial infections.

Bouzouita *et al*. (2008) found that *J. phoenicea* oil shows inhibition effect against *Klebsiella oxytoca, Lactobacillus plantarum*, *Saccharomyces cereviseae* and *Geotrichum candidum.*

terpenoids have been isolated from the genus Juniperus according to (Barrero *et al* 2006 and Mansouri *et al* 2010)

and flavonoids according to (Nakanishi, 2005). The species of Juniperus important medicinal plants used extensively in traditional medicine as Juniperus leaves have anti-inflammatory activity. (Mansouri *et al* 2010).

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. (Barbour *et al*., 2004 and Entezari *et al*., 2009).

The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids/ terpenoids, phenols etc. have been reported in the various plants extracts (Aswal *et al*., 1984; Abraham et al., 1986; Chopra et al., 1992)

which may be responsible for the antibacterial properties of the plants.

leaves contain several types of water-soluble polyphenols which have a high antioxidant and antimicrobial potential (Barotto *et al*., 2003; Ljubuncic *et al*., 2005).

Based on these results, the antibacterial activities seem attributable to flavonoids, these compounds have been associated with antimicrobial effects in various studies using plant extracts (Nwaogu *et al*., 2007; Newze *et al*., 2004).

Conclusion:

In conclusion, the obtained results showed that the aquatic extracts of some medicinal plants that grow wild in the eastern region of Libya showed allelopathic activities, which proved effective in reducing the growth of bacteria responsible for changes in some vegetables . 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. To date, this type of studies has been conducted only a limited number of them to test commercially grown commercial plant compounds available to the agro-industry. While some of these compounds have a role in activating the mechanisms of plant protection. Therefore, we need to deepen our knowledge about the mechanisms of plant water 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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