



## **Antimicrobial Activities of Mint Lemonade Plant Extracts from Salt and Heavy Metal Stressed Plants**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors QA, AM and MAJ managed the analyses of the study. Author QA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Aim of present research was to evaluate the anti-bacterial, anti-fungal, anti-oxidant and multi-stress activities of a medicinal plant *Mint Lemonade* plant extract. Fresh mint was purchased from the nearest market. The samples were cleaned with distilled led water and its leaves, stem and roots were separated them all. The material was dried in shade at room temperature (24°C). The three types of extracts were prepared for each sample of mint plant viz, ethanol, n-hexane and water. The antibacterial and antifungal activities of ethanolic, n-haxene and aqueous mint extract was determined on *Escherichia coli*, *Bacillus cereus*, *Pseudomonas*, *Aeromonas hydrophila*, *Aspergillus niger*, *Aspergillus flavous* and *Rhizopus stolonifer* respectively. From the results of the antibacterial and antifungal activities it was clear that the aqueous extract showed no inhibitory effect on test these microorganisms. In ethanolic extract of antibacterial activity *Escherichia coli* showed the maximum zone of inhibition with diameters of 3.90 cm at 50 µl dose. *Bacillus cereus* showed the zone of inhibition diameter 4.9 cm. *Pseudomonas* showed the maximum zone of inhibition in diameter 3.86mm. *Aeromonas hydrophila* expressed the maximum zone of inhibition in diameter 3.86mm. The n-hexane extracts antibacterial activity of *E. coli*, *Bacillus cereus*, *Pseudomonas* and *Aeromonas hydrophila* showed the zone of inhibition 3.86 cm, 4.5 cm, 7.90 mm and 4.71 cm respectively, while the positive control showed inhibition zone about 0.0 cm. The

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antifungal activity in n-haxene extract showed the maximum result in *Aspergillus flavous* 18 cm of the area of inhibition. In ethanol extracts antifungal activity showed the maximum result in *Aspergillus flavous* 4.8 cm of the zone of inhibition. The inhibitory action of mint plant extracts indicated that the plant may be used as potential antibacterial agent.

**Keywords:** Antibacterial activity; methanol; antifungal activity; extract; defatted.

## 1. INTRODUCTION

Most of medicinal and aromatic plants contain secondary metabolites which play vital role in the protection of the plants as antiviral, antibacterial, insecticides and antifungal. Essential oils extracted from these plants are complex of natural compounds that reduces foodborne pathogens and hence, decrease the use of synthetic and semisynthetic antimicrobial compounds [1] due to their side effects such as carcinogenesis [2]. Herbal compounds such as phenolic compounds that are found in these plants act as antioxidant and radical scavengers [3-6]. Plants with antioxidant properties are very beneficial to human health. There are 220 genera and 3300 species in the family of Labiatae which is extensively used due to their natural antioxidant properties for food, cosmetic and other purposes [7-11] because these plants are rich in natural phenolic compounds. Mentha is one of the important members of this family which have eighteen species and eleven hybrids, most of them contain high valued oil that have great economic importance [12-15]. Mint has its worldwide uses in the preparation of pharmaceutical, perfumery and confectionary products along with traditional medicinal treatments. They are also used in food industry as food additives and furthermore, they enhance the taste of food due to their olfactory properties [16-18]. The aerial parts of Mint are used as carminative, stomachic, tonics, anti-inflammatory and antispasmodic compounds in Algerian folk medicine [19,20]. Furthermore, Mint is cited as primary antioxidants that scavenge the ROS (reactive oxygen species) attack on biological and food systems [21-22].

Citrus genus is widely used fruit crop due to its natural antioxidant properties [23]. Phenolic compounds such as essential oils, dietary fiber, carotenoids, vitamins and minerals along with ascorbic acid, are the most important elements of citrus which contributes to its healthy properties [24,25]. Lemon juice contains citric acid which is used in organic and environment friendly foods as preservative and acidifier due to its natural antioxidant properties. Some of the important

nutrients such as citric acid, vitamin C, flavonoids and minerals including calcium, potassium, phosphorus, iron, zinc and sodium are found in lemon that act as natural antioxidant [26]. Lemon is rich with its most essential antioxidant nutrient vitamin C which is soluble in water that hunt reactive oxygen species [23]. Flavonoids are another important constituents of lemon which contain auspicious features such as anti-inflammatory, anti-proliferative, anti-atherogenic, anti-allergic, anti-tumor, cardio protective, anti-coagulant and anti-tumor activity other than antioxidant properties [27]. Hesperidin is an important flavonoid that has vasoprotective and venotonic activities that means they reduce the microvascular permeability and increase its resistance along with anti-oxidant, analgesic and anti-inflammatory properties [28-30]. During a research on the anti-oxidant activity of freeze and dried lime juice, Patil et al. perceived the inhibition of pancreatic cancer cell proliferation because of the anti-tumor activity of flavonoids and limonoids (cause bitterness of citrus fruit) [31]. During an investigation, experimental group of Rats were fed with a diet that contain 10mg carcinogenic benzopyrane and limonoids for the period of 18 weeks and the other control group were only fed on benzopyrane and they reported 40% less oncogenesis in experimental group than control group. They further concluded that limonoids contains anti-carcinogenic activities against skin cancer caused by dimethylbenzilantrase. In preclinical studies of mammary carcinogenesis, d- Limonene were found anti-carcinogenic compound in citrus peel oil [32]. These features have opened gateway towards a new era for processing companies and producers of Citrus fruit in food industry [33-35].

Mints are identified for their crusty fresh flavors and are used in cooking, cookery free of charge and herbal medicines. *Menthapiperita* a medicinally imperative belong to the relations Lamiaceae [36,37] and frequently acknowledged as peppermint is a mixture of *M. spicata* L. (spearmint) and *Mentha aquatica*. The curative parts are the important lubricate extracted beginning the above ground parts of the high pointplace in the ground, and the intactplace in

the ground. *M. piperita* is a returning 50-90 cm elevated, on average quadrangular and an archetypal constituent of the mint relatives [38]. The frequently divided branch is repeatedly published or traces purple excluding now and again they are aged- tomentose. The plants are violet are pinkish having forged spikes with abundant not immediately obvious bracts and once in a blue moon abide seeds [14,16]. The place in the ground is by and large hygienic and broadens by resources of candidate. A large amount genus in the mint relatives are twelve-monthly or rosemary approximating perpetual, and an only some species are bushes. The unfavourable effect of chemical products especially antibiotic led to the use of natural products like phytochemicals to improve the effectiveness of feed utilization and growth routine of poultry [23-27]. The use of phytochemicals as feed additives is gaining significance due to their antimicrobial and stimulatory effects on digestive system [30,36,37]. They contain herbs spices or plants that are used to remain the gut microflora of poultry normal, which is a prerequisite for cost efficient and ecofriendly poultry rearing [39]. It has been estimated that there are 250,000-500,000 variety of plants on earth [15]. Plant coloring is a broad appearance worn to allocate a huge amount of molecules. On top of the source of their element construction they can be group of pigments on 5 folds i.e. tetrapyrroles (e.g. chlorophyll), carotenoids (e.g.  $\beta$ -carotene), phenolic compounds. Considerable amounts of carotenoids are in close proximity in unmarked tea vegetation, excluding this charge is genuinely decreased all through tea processing, important to different dilapidation harvest [17-20]. In a Japanese revision on green tea there were perceived 38 carotenoids out of which 6 were mysterious [27-30]. In digestion, IBS, appetite pain in kids and reaction of condition later than surgery. The author of the analysis originates that mint work beside detrimental microbes, regulates influence relaxation, and helps be in charge of inflammation. Mint is cheering rosemary that public have used for thousands of days to facilitate pacify and sadden appetite or indigestion. An evaluation has shown that placebo-controlled learning hold the utilization of peppermint grease as an assortment of gastrointestinal situation. An unusual assessment from the identical time review 12 randomized tryout have proscribed that originates of peppermint grease was not dangerous but found helpful involvement for soreness indication in adults through IBS. Mint

plant surrounds an antioxidant and anti-inflammatory representative called rosmarinic acid. It has been found from study on rat's creatinine rosmarinic acid determined indication of asthma when compared incrementally of meeting that do not attain a complement. The mint place in the ground relatives provides a succession of deposit compounds that have anti-allergenic property.

The present study was conducted to success the antimicrobial activities of mint plant extracts from plants grown under salt and heavy metal stress for evaluation of differences generated after stress of salt and heavy metals.

## 2. MATERIALS AND METHODS

### 2.1 Work Place

All experiments are done University of Lahore laboratories of biotechnology and plant laboratories.

### 2.2 Sample Collection and Preparation

Mint plant was collected from Lahore, Punjab, Pakistan from two different places, one from near university of Lahore and other from Valencia town Lahore. This plant is famous for its antibacterial and antifungal activity. Washed with spigot water for point to get rid of unnecessary material and rinsed with distilled led water. Complete research effort was completed at the chemical biology and microbiology research laboratory at the institute of Molecular Biology and Biotechnology (IMBB) and plants Biotechnology Laboratory of Centre of research in Molecular Medicine (CRIMM), the University of Lahore.

### 2.3 Stress Activity of Mint Plant

Plant stress is a situated anywhere in the plant which increases in non-ideal expansion situation that augment the complete weight on it. The personal property of stress can lead towards the deficiencies in expansion, production of yields, permanent hurt or even death of plant if the stress exceeds the plant acceptance limits. First of all we took seeds of mint then we grow them in six different pots and after some days we added different chemicals in the soil to keep them in stress condition. We added NaCl and Zinc sulphate in three different pots, Zinc 1 Molar in 1<sup>st</sup> pot and added 0.5 Molar in 2<sup>nd</sup> pot and

added 0.75 Molar in 3<sup>rd</sup> pot and NaCl 1 Molar in 1<sup>st</sup> pot and added 0.5 Molar in 2<sup>nd</sup> pot and added 0.75 Molar in 3<sup>rd</sup> pot. For the other three pots we prepared this media by adding NaCl chemicals in three jars with 200ml distilled led water. On other day of adding chemicals cut the shoots after that measured its shoot length, total leaves and width. After that we measured total weight. Then we have added 100 ml solution cut them after 2-3 days and again measured them. The plant parts were used to extract phytochemicals for antimicrobial activities.

## 2.4 Preparation of Plant Extract

A total no of three types of extracts were prepared for each sample of mint by using three organic solvents: ethanol, n- hexane and water.

## 2.5 Aqueous Extraction

Electronic balance is used to weight the 20 grams mint which was soaked in 150 ml of distilled led water in sterile 250 ml conical flasks. After continuous stirring of one minute the opening of conical flask were covered with the aluminum foil and parafilm was used to make air-tight. The flasks were left in shaker incubator at 37°C on 150 rpm for 48 hours.

## 2.6 Ethanol Extraction

Electronic balance is used to weight the 20 grams mint which was soaked in 150 ml of absolute ethanol in sterile 250 ml conical flasks. After continuous stirring of oneminute the opening of conical flasks were covered with aluminum foil and then,parafilmwas used to make air-tight. The flasks were left in shaker incubator at 37°C on 150 rpm for 48 hours.

## 2.7 N-hexane Extraction

Electronic balance is used to weight the 20 grams mint which wassoaked in 150 ml of n-hexane in sterile 250 ml conical flasks. After continuous stirring of one minute theopening of conical flasks were covered with aluminum foil and then,parafilmwas used to make air-tight with parafilm strips. The flasks were left in shaker incubator at 37°C on 150 rpm for 48 hours. After 48 hours the extracts were filtered in 100ml of conical by using Whatman grade 1 filter papers. The filtrate was then heated in water bath at 56°C until solvent evaporated completely. Afterword, it was used for the evaluation of antibacterial activity.

## 2.8 Rotary Evaporator

Rotary Evaporator is a methodused in organic chemistry to get rid of a solvent from samples of different concentration by evaporation. The revolving evaporator or rotovap was invented in 1950 by the chemist Lyman C. Craig. The chiefutilize therotovap is to dehydrate and disinfect taster for lower stream submission. We have used DMSO as solvent during rotatory.

## 2.9 Chemicals and Reagents

All chemicals were used in this experiment or reagent study analytically and given as n-hexane, distilled led water.

## 2.10 Microorganism's Strains

The microorganisms were used as follow *Aspergillus niger*, *Aspergillus flavous*, *Rhizopus stolonifer*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas* and *Aeromonas Hydrophila*. All strains were taken from the microbiology research laboratory.

## 2.11 Storage and Preservation of Extracts

The prepared extracts were stored in autoclaved 25 ml McCartney bottles at 4°C before use. The bottles were carefully and labeled correctly prior to storage.

## 2.12 Maintenance and Preservation of Bacterial Sample

The sample (*Pseudomonas aeuginosa*, *Bacillus subtilis* and *Escherichia coli*) of these organisms were spread on freshly Nutrient Agar medium in Petri dishes and these plates were positioned in incubator for 24 hours. The plants were covered with parafilm and then, stored at 4°C until the next use. Before every experiment, these microorganisms were freshly sub cultured for 24-hours and then used. Purity of cultures was preserved by consistent sub culturing.

## 2.13 Antibacterial Activity Test of Plant Extract

*In vitro*, agar well diffusion method was used for the primary screening of the antimicrobial activity of plant extract against the targeted pathogens.

## 2.14 Preparation of Nutrient Agar Medium

2.8 gm nutrient agar and 4 gm nutrient broth were liquefied into a conical flask having 500 ml

of distilled led water. Then it was shaken to mix and liquefied in a hot plate. It was further pasteurized by heating at 121°C for 15 mints and permitted to cool down. After the sterilization the media was poured into sterile Petri dishes and were allowed to solidify (Wayne 2012).

### 2.15 Disc Preparation

An antibiotic was used to analyze the effect of disc to give accurate results. Whatman filter paper was used for antibiotic disc. These discs then prepared by punching the Whitman filter paper and sterilized in hot air oven at 180°C for half an hour. The size of those discs was 6mm and it was impregnated by using micropipettes of 15, 20, 25 and 30 micro liters in concentration.

### 2.16 Disc Application

On the marked area of the Petri dish was fixed with the help of sterile loop. Disc was fixed by following some necessary parameters. The distance of the disc from the sides of the Petri plates was 15mm so that disc cannot combine with each other. The disc to disc distance was 24 mm by this rule we got best results. This process was done in 20 minutes. Then, bacterial plates were incubated for 18 to 24 hours in an incubator and the temperature of incubator was set on 35°C.

### 2.17 Interpretation of Test Results

After 18 to 24 hours the interpretation and susceptibility of the test results were yet to be obtained. By using Venire scale we observed that the area of inhibition.

### 2.18 Antimicrobial Susceptibility Assay

For the antimicrobial activity of different fractions of *Mentha* used for evaluation by different analysis. The dried parts of (1700 ug) were liquefied in 10% DMSO (W/V). By the help of cotton swab the bacterial liquid culture were inoculated in the petri dishes. Sterilized cotton swab was use to the immunization of bacterial culture on ager plates. The plates filled with 20 ml of the Nutrient Ager medium and inoculated with 100 ul of culture after that it allowed to the solidify. The sterile disc (6 mm) were placed on the Petri plates of media and pressed the disc to ensure the interaction in pasteurized laminar air flow cabinet. Different fractions of size 30µl were loaded with suitable positive and negative control and incubated these plates at 37°C for 24 hours

in incubator. The experimentation was done in triplicate. After 24 hours the incubation period was ended and the antimicrobial activity was recorded by measuring the zone of inhibition with the bacterial growth with different fractions. For antimicrobial activity Dimethyle sulfur oxide (DMSO) used for positive control and tetracycline used for negative control to regulate the parts in contrast to particular bacteria. All the result was recorded by mile meter in the form of area of inhibition.

### 2.19 Antifungal Activity of *Mentha*

We have selected the fungi *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus Niger*, *Fusarium* and *moniliforme*. We used the 0.02 thimerosal to prevent the contamination spread to environment. Before using these fungi in laboratory we have had studied about these fungi and we were aware with the health related issues. We adopt safety instructions because safety was and will our First priority. Many growth media were used for the growth of fungi like Sabouraud dextrose agar (SDA), Muller Hinton agar (MHA) and potato agar. Fungi can easily grow in all these media. We prepared autoclaved media and poured it in sterilized Petri plates. We inoculated the media through streaking method with the help of cotton swab. Disc of different concentrations were placed on plates of media which were (50, 100, 150, 200) µg/ml. The plates were incubated for 3 days at the 37°C and observe the zone of inhibition. The inhibition zone measured in mile meter.

### 2.20 Stress Activity of Ethanol Fraction from *Mint* Plant

Plant stress is a state where the plant is growing in non-ideal growth conditions that increase the demands made upon it. The effects of stress can lead to deficiencies in growth, crop yields, permanent damage or death if the stress exceeds the plant tolerance limits.

## 3. RESULTS AND DISCUSSION

The results given in Tables 1-6 indicated that the morphological behavior of mint plant under treatments of slat and heavy metal. It was found that the plant height was higher under the treatment of ZnSO<sub>4</sub> applications as compared with the treatments of NaCl. The number of leaves per plant was shown higher under the treatment of 0.5 Molar and 0.75Molar ZnSO<sub>4</sub> as

compared with all other treatments of NaCl and ZnSO<sub>4</sub>. The leaf width was also found higher under the treatments of ZnSO<sub>4</sub> as compared with NaCl treatments. The results indicated that the application of NaCl caused adverse effects on mint plant growth as compared with ZnSO<sub>4</sub>. The

better plant performance under heavy metal treatments indicated that the mint plant has tolerance against heavy metals which shows mint may be grown under heavy metal effected soil rather than the salt affected soils [39-47].

**Table 1. First stress zinc 1molar then noted the value of plant length, total leaves and width**

Zinc 1	Length	Total leaves	Leaves 1 width	Leaves 2 width	Leaves 3 width
1Molar	24.5cm	8	1.3cm	0.9cm	1.2cm
1Molar	17cm	7	0.7cm	1.3cm	0.9cm
1Molar	16.5cm	8	2.5cm	0.9cm	2.5cm

**Table 2. Second stress zinc 0.5 molar and noted the values of plant length, total No. leaves and leaf width**

Zinc 2	Length	Total No. of leaves	Leaf 1 width	Leaf 2 width	Leaf 3 width
0.75Molar	28.7 cm	12	2.7 cm	2.4 cm	2.5 cm
0.75Molar	18.8 cm	7	2.4 cm	2.2 cm	1.6 cm
0.75Molar	19 cm	5	2.5 cm	0.9 cm	2.5 cm

**Table 3. Third stress zinc 0.75molar then noted the value of plant length, total leaves and leaf width**

Zinc 3	Length	Total No. of leaves	Leaf 1 width	Leaf 2 width	Leaf 3 width
0.5Molar	25.5 cm	12	1.2 cm	2.4 cm	2.5 cm
0.5Molar	18 cm	7	1.1 cm	2.3 cm	1.8 cm
0.5Molar	13.7 cm	5	1.8 cm	1.5 cm	2.4 cm

**Table 4. First stress NaCl 0.75molar then noted the value of plant length, total leaves and leaf width**

NaCl1	Length	Total No. of leaves	Leaf 1 width	Leaf 2 width	Leaf 3 width
0.75Molar	22 cm	7	1.5 cm	1.9 cm	2 cm
0.75Molar	18 cm	7	1.7 cm	2.5 cm	1.8 cm
0.75Molar	14.7 cm	8	1.8 cm	1.5 cm	2.3 cm

**Table 5. Second stress NaCl1molar then noted the value of plant length, total leaves and width**

NaCl 2	Length	Total No. of leaves	Leaf 1 width	Leaf 2 width	Leaf 3 width
1Molar	22	7	1.2	1.8	3
1Molar	15	9	1.7	2.4	1.6
1Molar	13.7	8	1.9	1.8	2.5

**Table 6. Third stress NaCl0.5molar then noted the value of plant length, total leaves and width**

NaCl 3	Length	Total No. of leaves	Leaf 1 width	Leaf 2 width	Leaf 3 width
0.5Molar	2.5	7	1.6	1.5	3
0.5Molar	15.2	8	1.9	2.8	2.6
0.5Molar	12.7	9	1.4	2.8	1.5

### 3.1 Antibacterial Activity for Ethanol Extraction from Mint

We used four different strains for the antibacterial activity in ethanol fraction: *Escherichia coli*, *Bacillus cereus*, *Pseudomonas* and *Aero Monas hydrophila* with control zone of bacteria. Ethanol extract gave the results of inhibition zone with different concentration 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml against the microbial strains. The zone of inhibition of *Escherichia coli* in the ethanol extraction with the concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml. *Escherichia coli* showed inhibition zone with the different diameters. The higher inhibition zones indicated that the mint plant may be used in medicines for preparation of antibiotics [5,6,8,12].

#### 3.1.1 Sample 1

This Table 7 shows the quantity needed for antibacterial movement of ethanol division from the shoots of mint plant (sample 1), bacterial strains *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and *Klebsiella pneumoniae* (ATCC 10031). Ethanolic fraction at quantity of 5ul, 10ul and 15ul adjacent to tested bacterial strains showed the inhibition zone. Ethanolic division at quantity of 5ul, 10ul and 15ul alongside *E. coli* showed distance of shyness after 24hrs with distance of 4.08, 5.02 and 5.02cm, and after 48hrs 3.76, 4.71 and 4.91 in diameter respectively. Similarly, table shows the activity of Ethanolic fraction at dose of 5ul, 10ul and 15ul against *Staphylococcus aureus* showed inhibition zone after 24hrs with diameter of 3.90, 4.9 and 4.90cm and after 48hrs 3.86, 4.51 and 4.71 in diameters respectively.

*Klebsiella pneumoniae* which was also found susceptible and showed diameter of 3.9cm, 4.8 and 4.9 after 24 hrs and 3.5, 4.5 and 4.3 after 48 hrs. The results show that in ethanolic extract of shoots of *Mint plant*. Maximum antibacterial activity was found against *E. coli*. The dose of 10ul show more activity than other doses (Table 7). The antibacterial activities have shown the potential of mint plant as fine herbal plant species [14-17].

#### 3.1.2 Sample 2

Antibacterial movement of ethanol portion equipped beginning the shoots of *Mint* (sample 2), touching bacterial strains *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against tested bacterial strains showed inhibition zone. Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against *S. typhi* showed zone of inhibition after 24hrs with diameter of 2.61, 3.76 and 5.75cm, and after 48hrs 3.87, 4.29 and 6.01 in diameter respectively. The antibacterial potential of mint plant indicated that it may be used as potential medicine [8,12].

In the same way, (Table 8) the activity of Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against *Bacillus subtilis* showed zone of inhibition after 24hrs with diameter of 2.9, 3.01 and 4.8cm and after 48hrs 3.2, 3.4 and 5.3 in diameters respectively. *Pseudomonas aeruginosa* which was also found susceptible and showed diameter of 3.01cm, 3.9 and 4.9 after 24 hrs and 3.4, 4.01 and 4.3 after 48 hrs. The results shows that in shoot sample of Table 2 show *S. typhi* was found more sensitive and dose of 10ul illustrate more activity.

**Table 7. Bacterial zone of inhibition under the application of shoot extract sample 1**

Shoot sample 1 Doses	<i>E. coli</i>		<i>S. aureus</i>		<i>K. pneumoniae</i>	
	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs
2.5ul	4.08	3.76	3.9	3.8	3.9	3.5
5ul	5.02	4.71	4.9	4.5	4.8	4.3
10ul	5.02	4.91	4.9	4.7	4.9	4.5

**Table 8. Bacterial zone of inhibition under the application of shoot extract sample 2**

Shoot Sample 2	<i>S. typhi</i>		<i>B. subtilis</i>		<i>P. aeruginosa</i>	
	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs
2.5ul	2.61	3.81	2.9	3.2	3.01	3.4
5ul	3.76	4.29	3.01	3.4	3.09	4.01
10ul	5.75	6.01	4.8	5.3	4.3	4.9

### 3.2 Antibacterial Activity of Mint Leaves

#### 3.2.1 Sample 2

The antibacterial activity of ethanol fraction prepared from the shoots of *Mint* (sample 1), bacterial strain *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and *Klebsiella pneumoniae* (ATCC 10031). Ethanolic fraction at dose of 2.5 ul, 5 ul and 10 ul against tested bacterial strains showed zone of inhibition. Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against *Staphylococcus aureus* showed zone of inhibition after 24hrs with diameter of 4.27, 4.56 and 4.8 and after 48 hrs 4.6, 5.3 and 5.9 in diameters respectively. In the same way, the activity of Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against *Escherichia coli* showed zone of inhibition after 24 hrs with diameter of 3.6, 3.9 and 4.1 cm and after 48 hrs 3.9, 4.1 and 5.3 in diameters respectively. *Klebsiella pneumoniae* which were also founds susceptible and showed diameter of 3.6, 4.1 and 4.8 after 24 hrs and 4.1, 5.2 and 5.9 after 48 hrs. The domino effect shows that *Escherichia coli* are more sensitive than other bacterial strains (Table 9). The antibacterial potential of mint plant indicated that it may be used as potential medicine [8,12,17].

#### 3.2.2 Sample 1

Antibacterial activity of ethanol fraction prepared from the Bark of *Mint* (sample 2), against bacterial strains *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Ethanolic fraction at dose of 2.5ul, 5ul and 10ul adjacent to tested bacterial strains demonstrate zone of inhibition. Ethanolic fraction at dose of 2.5ul, 5ul and 10ul against *Bacillus subtilis* showed zone of inhibition after 24hrs with diameter of 3.55, 3.87 and 4.29 and after 48hrs 3.7, 4.21 and 5.75 in diameters respectively. In the same way, the activity of Ethanolic fraction at dose of 2.5ul, 5ul

and 10 ul against *S. typhi* showed zone of inhibition after 24 hrs with diameter of 3.31, 3.50 and 4.01 cm and after 48 hrs 3.8, 4.01 and 4.9 in diameters respectively. *Pseudomonas aeruginosa*, which was also found susceptible and showed diameter of 3.4, 3.9 and 4.9 after 24 hrs and 3.9, 4.1 and 5.2 after 48 hrs. The outcome demonstrate that *Bacillus subtilis* show more zone of inhibition under the application of leaves and much more results under 10ul dose (Table 9). The antibacterial potential of mint plant indicated that it may be used as potential medicine [14,17].

#### 3.2.3 Antibacterial activity of mint shoot extract against gram + bacteria

The Mint plant shoot extract tested on gram Positive bacteria by the zone of inhibition method and the observation are illustrated in table. The maximum zone of Inhibition 15 mm measured in case of *Staphylococcus aureus* and the smallest amount district of embarrassment 12mm in case of *Bacillus cereus* at concentration of 35ug/ml. And the highest zone of Inhibition is 10mm observed in case of *Bacillus subtilis* at concentration of 10ug/ml. The antibacterial potential of mint plant indicated that it may be used as potential medicine [15,17,23-28].

#### 3.2.4 Antifungal activity of ethanol root extract in case of pucciniales (rust fungi)

To determine the antifungal efficacy of ethanol root Extract of Mint test on pathogenic fungi Pucciniales (common name rust). The disc diffusion method is used in experiment. The difference concentration of ethanol root extract tested on Inhibit the growth of rust fungi but there is no zone of inhibition observed illustrated in Table 10.

**Table 9. Bacterial zone of inhibition under the application of bark extract sample 1**

Bark sample 1	<i>S. aureus</i>		<i>E. coli</i>		<i>K. pneumoniae</i>	
	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs	After 24 hrs	After 48 hrs
2.5ul	4.97	5.38	3.1	4.9	3.3	3.8
5ul	5.72	6.01	4.8	5.3	4.4	4.9
10ul	6.65	7.51	5.7	6.1	4.9	5.3

**Table 10. Allroot, shoot, barkextracts concentration and zone of inhibition size**

Microbes	10 ug/ml	20 ug/ml	30 ug/ml	35 ug/ml	Control
<i>Pucciniales</i>	0	0	0	0	-



Approximately every civilization and culture has engaged undergrowth in the management of individual condition [5-7]. Other researchers also point towards the anti-bacterial activity, but not much data is accessible [11,14,17]. To find the extent of the antimicrobial activity of this plant, more testing is desirable to be done. The extent of testing has not been this wide increase before and testing of anti-microbial activity of this plant has been done on a partial number of strains [8,17,21]. From the available data of antimicrobial activity of this plant, it is not apparent that what part of the plant show best activity [32,30,36]. The extracts of this plant leaves was used to test the antibacterial properties recently [17,27,36].

#### 4. CONCLUSION

This study conducted to success the antimicrobial activities of mint plant extracts from plants grown under salt and heavy metal stress for evaluation of differences generated after stress of salt and heavy metals. The antifungal activity in n-haxene extract showed the maximum result in *Aspergillus flavous* 18 cm of the area of inhibition. In ethanol extracts antifungal activity showed the maximum result in *Aspergillus flavous* 4.8 cm of the zone of inhibition. The inhibitory action of mint plant extracts indicated that the plant may be used as potential antibacterial agent.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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