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DOSE RESPONSE CURVE OF VARIOUS PLANT EXTRATS ON G+ve AND G-ve BACTERIAL ISOLATES

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ABSTRACT

The plant extracts are being significantly used in the treatment of various infectious diseases and have a greater impact in Unani medicines. This presentation explores the effects of test plant extracts of garlic and ginger juice and aqueous extracts of coriander and mint prepared in ethanol, methanol and acetaldehyde with the comparison of chloramphenicol and gentamycin antibiotics as control. Our observation revealed that, garlic juices at 25 µl concentrations are effective on *S. aureus* and *E. coli* whereas ginger showed maximum activity on *Ps. aeruginosa, S. aureus* whereas 20 µl concentration revealed antibacterial activity against *E. coli* respectively. The aqueous extracts of coriander and mint extractsprepared in ethanol has greater antimicrobial activity against *Ps. aeruginosa* and *E. coli* respectively as compared to *Staphylococcus aureus* and *Streptococcus pyogenes.*

Spectroscopic observations showed antibacterial activity at 20 and 25 µl concentrations of mint and coriander0.121 and 0.118against *E. coli, Pseudomonas aeruginosa* and *Pseudomonas aeruginosa, E. coli* as compared to other strains at A_{600}respectively.

Key words: Herbal medicines, G-ve and G+ve bacteria, antimicrobial effects

INTRODUCTION

Central Asian countries Pakistan, China, Srilanka, India are rich in Unani medicines produced through different herbal plants that are significant for the development of new medicines with less side effects (Hamarneh 1999; Srivastava & Kumar 2000). Antimicrobials dietary substances are used in food for two main reasons such as to control natural spoilage processes and to prevent microbial contamination. Human nutrition significantly has preservative and medicinal properties (Mukherjee & Wahile 2006; Noor et al., 2011). Today many diseases are known to have unknown mortality in the globe and are eliminated by the use of herbal treatment (Bakht & Shafi 2012; Draughon 2004; Kumar 2008).

Various diseases are being under trials to be treated by plant scientists, pharmacists, microbiologist and others (Sakata et al., 2009; Wadud et al., 2007) and also in search of the substances that could be more efficient against the infectious diseases (Kumar 2008; Jain et al., 2009; Hanzi et al., 2008). This work represents the use of dietary plant extracts that have antimicrobial effects on G+ve and G-ve bacteria such as Ginger/*Zingiber officinale*, Mint/ *Mentha arvensis* (Barbosa et al., 2009) and Coriander/ *Coriandrum sativum* (Angienda et al., 2010).

MATERIALS AND METHODS

Preparation of herbal extracts: The preparation of extracts was done by the modified method of Jain et al., (2009). The ginger and garlic were purchased, washed with water, dried and cut into small pieces separately and later kept in little warm water for 18-24 hours and finally boiled to get 25 ml juice of the ginger and garlic suspension separately. Leaves of Coriander and Mint were plug off and kept them in tray to dry in open environment for drying for seven days then homogenized fine powder was obtained; 60-80 mesh by Meinzer II Sieve shaker using a commercial electric grinder (WK-802). The powdered material was stored in air tight sterilized bottles in refrigerator at 4°C for further use. Known amount (30gm) powder of each plants sample was extracted by soxholet apparatus and powdered extracts were allowed for preparation of ethanol, methanol and acetaldehydes.

1- Antibiotic Assay (Control): Discs were prepared by Whattman's No.1 filter paper discs of 6 mm diameter and soaked in hot distilled water. The distilled water was removed and the discs were kept in hot air oven for 2 hours at 80°C. Commercially available antibiotic gentamycin (10µg) and chloramphenicol (30µl) were used as control. Antibiotic assay was performed by soaking discs for 10 minutes and later placed in laminar chamber according to (Nostro et al., 2000; Baris et al., 2006). Impregnated discs were placed on the surface of pre-inoculated test cultures of *Staphylococcus aureus, Streptococcus pyogenes, E. coli* and *Pseudomonas aeroginosa*. These plates were kept for incubation over night at 37ºC to determine zone size against control antibiotics.

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS

1- **Disc diffusion method:** Test cultures were examined on two ethanolic and methanolic extracts of mint and coriander and two juices of garlic and ginger by Kirby-Bauer disc diffusion method

(Nostro et al., 2000; Baris et al., 2006; Basri and Fan 2005). The Whattman's filter paper discs were placed separately in eachextract (0.2 ml approximately) of garlic, ginger juice and mint and coriander extracts dissolved in ethanol, methanol, acetyaldehyde and distilled water for 10 minutes, placed on Mueller Hinton agar plates and incubated overnight at 37°C.

2- **Spectroscopy:** Two concentrations of the test ext-racts with greater zone of inhibition (20 and 25µl) were prepared in Mueller Hinton broth containing test cultures separately. The culture suspensions were incubated for 24 hours at 37°C and observed at A_{600} (Noor et al., 2011). All experiments were performed in triplicate and their mean values were reported according to (Basri and Fan 2005; Noor et al., 2011).

RESULTS

Two antibiotic discs chloramphenicol (30µg) and gentamycin (10µg) were used as control that revealed maximum zone of inhibition 22 and 23 mm on the growth of *E. coli* and *Staphylococcus aureus* respectively (Table-1). Garlic and ginger juices were diluted at 05, 10, 15, 20, 25 (µl) concentrations that revealed maximum zone of inhibition 21, 20, 22, 20 and 18, 17, 20, 22 mm at 20, 25, 25, 20 and 25, 25, 20, 25 µl (Table-2, Fig.1) whereas test extracts prepared in ethanol, methanol and acetaldehyde were also tested by disc diffusion method that revealed 18, 20, 21, 22 mm zone of inhibition of *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Pseudomonas areuginosa* respectively at 25, 25, 20, 25 and 25, 20, 20, 25(µl) concentrations of ethanolic extract of *Mentha arvensis* and *Corriandrum sativum* extracts that indicates the maximum inhibition as compared to other extracts respectively (Table 3-4, Fig. 2-3).

Spectroscopy of two concentrations (20 and 25 µl) having greater antimicrobial activity was done at 600 nm. The observations of both garlic and ginger juice showed greater antimicrobial activity 25 µl concentration on the growth of *E. coli* as compared to other test strains (Fig. 4). Ethanolic extracts of mint and coriander showed maximum growth inhibition 0.121, 0.118 and 0.111, 0.103 of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at A_{600}as compared to other strains and extracts prepared in methanol and acetaldehyde respectively (Fig. 5-6).

Table-1: Effect of Chloramphenicol and Gentamycin (Control) antibiotics on the growth of test strains

Test strains	Chloramphenicol	Gentamycin
	Zone size (mm)	
Staphlycoccus aureus	18	23
Streptococcus pyogenes	15	19
Escherachia coli	22	14
Pseudomonas areuginosa	21	11

Table-2: Effect of test extract *Allum sativum L.* (Garlic) juice and *Zinger officianale L.* (Ginger) at various concentrations (µl) on the growth of test bacteria.

Test strains	Zone size (mm) at various concentrations (µl)									
	Garlic					*Ginger*				
	05	10	15	20	25	05	10	15	20	25
Staphlycoccus aureus	13	15	18	20	21	08	11	15	18	21
Streptococcus pyogenes	09	11	14	18	20	12	14	15	17	19
Escherachia coli	11	14	18	20	22	11	14	18	20	20
Pseudomonas areuginosa	11	13	16	17	20	14	16	19	21	22

Table-3. Effect of *Cooriandrum sativum L.* on the growth of test strains

Test strains	Zone size (mm) at various concentrations (µl)														
	Ethanol					*Methanol*					*Acetaldehyde*				
	05	10	15	20	25	05	10	15	20	25	05	10	15	20	25
Staphylococcus aureus	08	10	12	15	18	06	07	10	12	12	04	06	07	10	10
Streptococcus pyogenes	10	15	18	20	20	04	07	11	14	16	06	08	10	13	15
Escherachia coli	11	14	18	21	21	09	12	14	16	18	04	07	9	13	14
Pseudomonas aeruginosa	12	15	18	20	22	11	12	14	16	19	07	10	13	15	15

Table-4: Effect of *Mentha arvensis L.* on the growth of test bacteria

Test strains	Zone size (mm) at various concentrations (µl)														
	Ethanol					*Methanol*					*Acetaldehyde*				
	05	10	15	20	25	05	10	15	20	25	05	10	15	20	25
Staphlycoccus aureus	11	14	18	21	18	06	08	11	13	16	09	10	12	13	13
Streptococcus pyogenes	08	10	13	13	17	09	12	14	16	17	06	07	09	10	11
Escherachia coli	12	14	18	21	21	09	11	13	18	20	04	06	09	12	15
Pseudomonas areuginosa	11	13	15	18	21	08	11	14	16	16	07	09	12	13	16

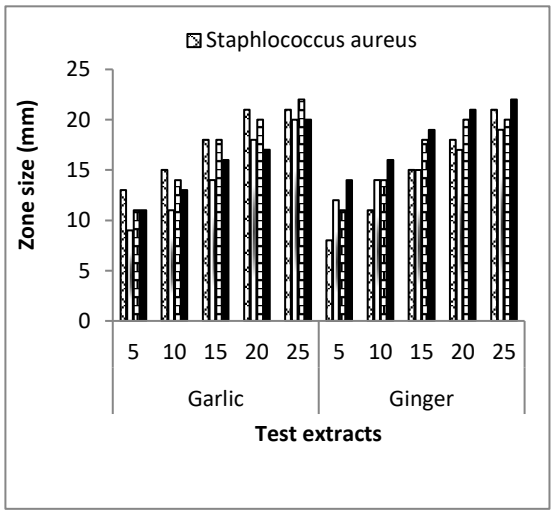

Figure-1: Effect of test extract *Allum sativum L.* juice and *Zinger officianale L.* at various concentrations (µl) on the growth of test strains.

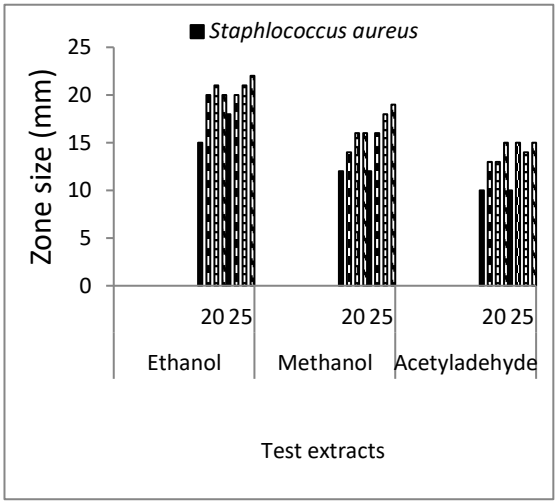

Figure-3: Effect of *Mentha arvensis L.* on the growth of test bacteria

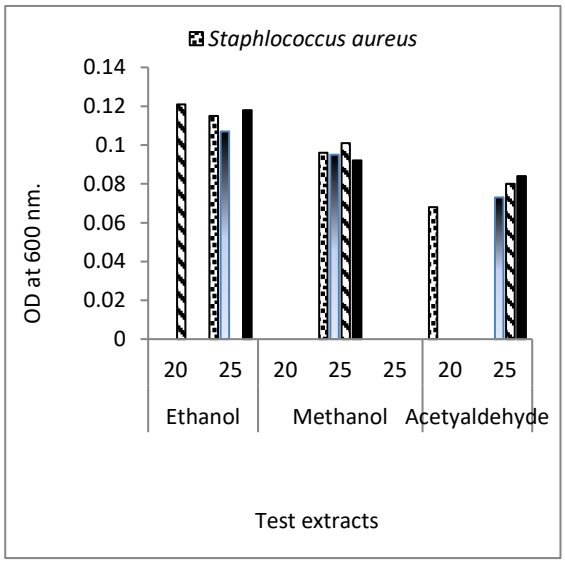

Figure-2: Effect of *CorriandrumsativumL.*on the growth of test strains

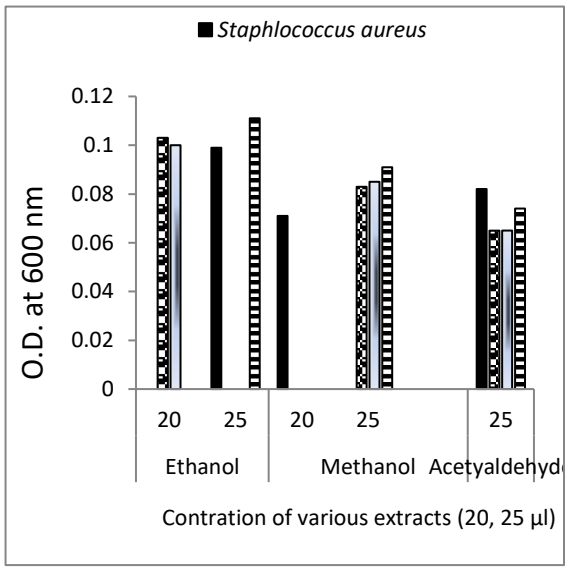

Figure-4. Antimicrobial activity of garlic and Ginger juice at 25 (µl) concentration test strains at 600 nm

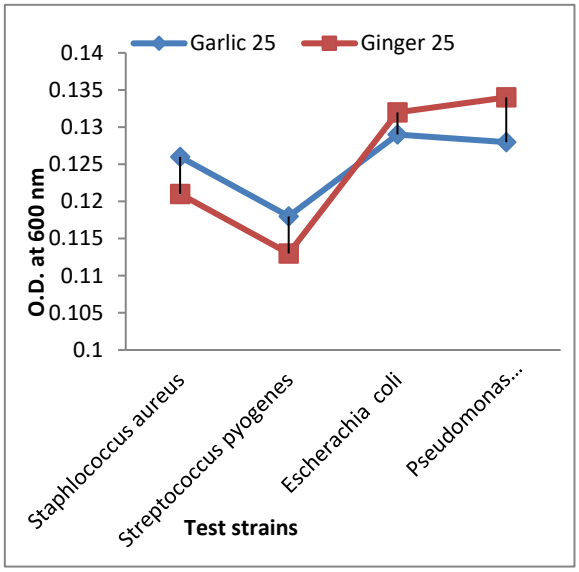

Fig.-5: Antimicrobial activity of *Corriandrum sativum* L. extracts in ethanol, methanol and acetaldehyde at 20, 25 (µl) concentration test strains at 600 nm

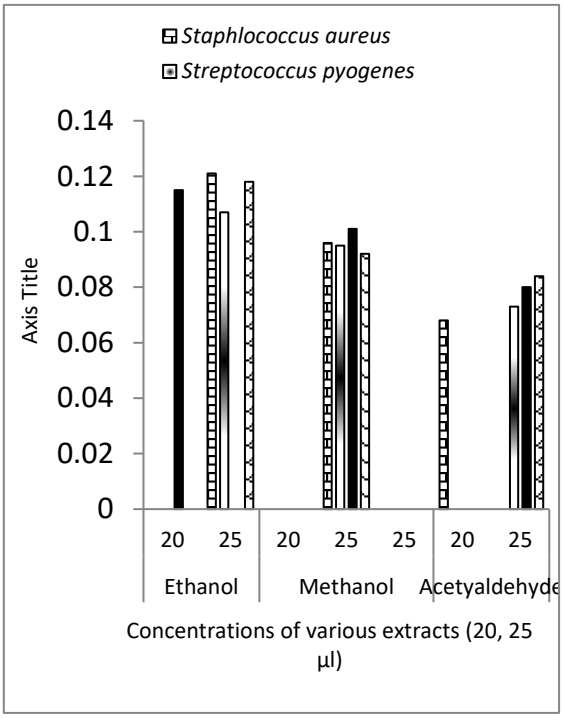

Fig.-6: Antimicrobial activity of *Mentha arvensis* L. extracts in ethanol, methanol and acetaldehyde at 20, 25 (µl) concentration test strains at 600 nm.

DISCUSSIONS

Plant extracts are known to be used since many years as alternative medicine for health assurance (Bauer et al., 1959). This work presents the antibacterial activity of garlic and ginger juices and the plant extracts *Mentha arvensis* and *Corriandrum sativum* dissolved in solvents in order to release the antimicrobial compounds present in the plant extracts. The test strains were sensitive to the test extracts due to high potency of the compounds and the solvents used chemical composition, diffusion into the medium, hydrophobic nature or disturbing cellular structures (Bauer et al., 1966; Bakht & Shafi 2012). Many G+ve and G-ve bacteria are sensitive to ginger especially the colon residing bacteria due to the presence of efficient antibacterial compounds. The effect on gram negative bacteria may be due to the fact that the degree of depolarization and increased permeability in the lipid bilayer may allow the known antibacterial compounds in to the cell. The observation highlights our results in accordance to (Noor et al., 2011; Bakht & Shafi 2012). It has been also known that leakage of ions and other cell constituents also supports the passage of antimicrobial substances that may interact with cellular metabolic mechanisms (Evrendilek and Balasubramaniam, 2011) that indicates the permeability, which impair the respiration, efflux pump activity and membrane potentials of the cell (Bakht & Shafi 2012). Gram positive bacteria may show less sensitivity against the menthol containing (Joshi et al., 2011). On the other hand, mint has greater effect of *E. coli* due their active ingredients may be due to differences of specific compounds and selectivity of action (Jagetiaet al, 2003). More specifically on the growth of particular microorganism the effect of plant extracts may be due to the intrinsic and extrinsic factors (Marino et al, 2011).

CONCLUSIONS

The present study was carried out to identify traditional plants that are effective against human pathogens *Staphylococcus aureus*, *Streptococcus pyogenes*, *E. coli*, *Pseudomonas aeruginosa.* It is concluded that garlic and ginger have has greater effect on *E. coli*, *S. aureus and Ps. aeruginosa, S. aureus respectively* whereas the ethanolic extract of coriander and mint has greater antimicrobial activity against *Ps. aeruginosa, E. coli* and *E. coli*, *Ps. aeruginosa* respectively. It is further concluded that plant extracts prepared in ethanol has more antimicrobial activity than the other extracts. The use of plant extracts and phytochemicals, with known antimicrobial properties, could be of great signifycance in disease treatments.

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