Determination of antibacterial potential of leaves, stem and roots of ocimum basilicum l. against three pathogenic strains of bacteria i.e. escherichia coli, pseudomonas aeruginosa and klabsiella pneumoniae

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Abstract- The present investigation focused on Determination and Comparatative analysis of extracts made from Leaves, Stem and Roots of *Ocimum basilicum* L. against three human Pathogenic Strains of bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa* and *Klabsiella pneumoniae* was conducted in 2022-2023 at Gulzar Group of Institutions. Antimicrobial efficacy was tested by using Agar Disc Diffusion method and Agar Well Diffusion method. It was concluded that extract of leaves, stems and roots of *Ocimum basilicum* L. dipped in 70% ethanol and 70% methanol respectively and kept on shaker for 24 hours and dried part showed higher antimicrobial potential in comparison to leaves, stems and roots of *Ocimum basilicum* crushed to powder form and dipped in ethanol

Keyword- *Ocimum basilicum*L., Agar Disc Diffusion, Agar Well Diffusion, Antimicrobials Potential.

I. INTRODUCTION

Ocimum basilicum L. (Sweet basil) is an aromatic plant in the family Lamiaceae which is native to India. It is also called "King of Herbs" and "Royal Herbs". It is cultivated for religious and medicinal purpose and also for its essential oil. It is a tender annual aromatic plant with spicy odour and flavour. It grows 12-18 inches tall and colour can range from green to purple. Ocimum basilicum L. is use to treat several pain related disease such as rheumatologic disease, back pain and migraine (Zareba et al 2009).

Disc diffusion method was used to determine antimicrobial activity of South African medicinal plants. The large zone of inhibition large the antimicrobial property. (Van et al 200). Edible fruits, garden plants and medicinal plant's extractions show antioxidant activities against xanthine oxidase, tyrosine and lipooxygenese (Chen et al 2009). Chemical and biochemical antimicrobial compounds derived from plants as secondary metabolites have activity against a range of pathogens which cause food spoilage (Tiwari et al 2009).

Ocimum basilicum plant extract posseses antimicrobial activity against tested microbes so they can have used in treatment of infection caused by microbes (Nascimento et al 2009). Plants have been used for thousands of years to flavor and conserve food to treat health disorder (Silva et al 2009). Ocimum basilicum L exhibit hire nutritional value (Wul et al 2016) and Therapeutic efficiency because of hire flavonoids content (Tshilanda et al 2016).

Ocimum basilicum L, commonly known as a sweer Basil, is naturally distributed in the East Anatolia region in Turkey.Basil, which is popularly culinary herb and its essential oil had been used extensively for many years in flavouring of meat and sausage.Basil oil has wide application in perfumery as well as in dental and oral product (Suppakul et al 2003). In this experiment Disc diffusion method and Agar well diffusion metod were employed for screening of extracts made from Leaves, Stem and Roots of Ocimum basilicum L. against three human Pathogenic Strains of Escherichia coli, Pseudomonas aeruginosa and Klabsiella pneumoniae.

II. LITERATURE REVIEW

(Swain et al 1968) investigated that some of the pharmaceutical currently available to physicians are derived from plants .Example of such drugs include aspirin, quinine and opium. (Silva et al 2012) observed that medicinal plant is any plant which posses one or more organs containing phytochemicals that can be used for medicinal purpose. Phytochemicals are natural bioactive compounds found in plants which provide defense against cardiovascular disease (Vasanthi et al 2012). Lachowic et al (1998) tested the antimicrobial activity of essential oil which was obtained by hydrodistillation methods from Ocimum basilicum L. Dubey et al (1989) studied that the essentiall oil of Ocimum basilicum L.at a dose of 1.5ml/L completely inhibit the mycelia growth of 22 species of fungi including the mycotoxining producing strain of Aspergillus flavus.

III. MATERIAL AND METHODS

The present study "Determination and Comparatative analysis of extracts made from Leaves, Stem and Roots of Ocimum basilicum L. against three human Pathogenic Strains of Escherichia coli, Pseudomonas aeruginosa and Klabsiella pneumoniae" was conducted

in 2022-2023 at Gulzar Group of Institutions, Khanna. Disc diffusion method and Agar well diffusion method were used. Three strains of pathogenic bacteria i.e Escherichia coli, Pseudomonas aeruginosa and Klabsiella pneumoniae were obtained from post graduate institute of medical and research (PGIMER Chandigarh). These strains were selected because they routinely cause infections in human through food, water and contaminated surface. These strains were revived in papton water and bacterial inocumlums was evenly spread on LB Agar plates (Luria Bertain). Discs were prepared from watman no. 1 filter paper of size 5mm. These discs were sterilized by placing them in a autoclave at 1210 C temperature and 15 p.s.i pressure for 15-20 minutes.

IV. RESULTS AND DISCUSSIONS

On the basis of result it can be concluded that sweet basil stem and roots also exhibit antimicrobial property which is normally used in traditional medicine and in food preservation (Roberto et al 2006). Hence stems and roots could became a potential source of traditional medicine and also modern drugs can be synthesized from stem and roots.

After the sterilization of glassware to be used preparation of LB media was done.

Table 1 Composition of LB media:

Sr. No.	Composition	Quantity (g/per litre)
1.	Tryptone	10.0
2.	Yeast Extract	5.0
3.	Sodium chloride	10.0
4.	Agar	15.0
5.	Distilled Water	1 litre

The pH of media was set at 7.0 with the help of 1 N NaOH and volume finalized to 1 liter

Preparation of Extracts

After preparation of LB media two types of extracts was prepared i.e. from dried and powered part (used for Agar well method) and from plant parts (used for disc diffusion as well as for agar well method) dipped in methanol (70%) and ethanol (70%) for 24 hours. For preparation of extracts from dried plant parts (stem, root and leaves|), the oven dried plant parts were crushed to powder form. These powdered parts were weighted and added to different solvent viz., ethanol (70%), methanol (70%).

Preparation of medium for revival of bacterial strain

Peptone water was prepared for the revival of bacterial strain

Table 2 Composition of peptone water

Ingredients	gm/l
Peptic digest of animal tissue	10.0
Sodium chloride	5.0
Final pH	7.2+0.2 -
Final volume	1 liters

Determination of antimicrobial potential

Following two methods was used to determine antimicrobial potential of different extract prepared from Ocimum basilicum L. against three human Pathogenic Strains of Escherichia coli, Pseudomonas aeruginosa and Klabsiella pneumoniae.

1. Disc Diffusion method
2. Agar well method

Disc Diffusion method

Discs were prepared from whatman No 1 filter paper of size 5mm.The bacterial inoculum was spread uniformly on LB agar media plates. Different plates were spread with different test organisms. The discs were impregnated with different plant extract. Inhibition of bacterial growth was observed as zone around the disc known as zone of inhibition. The result were recorded by measuring these zones diameters in millimeters.

Disc diffusion method			
Average diameter of Zone of inhibition of growth (in mm)			
Name of extract	Escherichia coli	Pseudomonas Aeruginosa	Klebsiella Pneumoniae
BL1	13	8	12.5
BL2	-	-	6
BS1	9	8	10.5
BS2	7.5	-	10
BR1	7	-	11
BR2	-	-	-

BL1 Basil leaves dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.
BL2 Basil leaves dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use.
BS1 Basil stem dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use
BS2 Basil stem dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use
BR1 Basil root dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use
BL1 root leaves dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use agar well method

The bacterial inoculums was evenly spread on LB agar plates using sterilized spreaders. Different plates were prepared foe different organisms.5mm diameter equally spaced well were made in the plates using micropipette tip 20-30 µl of the plant extract were added to each well. Inhibition of bacterial growth was observed as zone around the disc known as zone of inhibition. The result were recorded by measuring these zones diameters in millimeters

Agar well diffusion method			
Average diameter of Zone of inhibition of growth (in mm)			
Name of extract	Escherichia coli	Pseudomonas Aeruginosa	Klebsiella Pneumoniae
BL1	13.5	11	14
BL2	6	6	6.5
BL3	8	6.5	11
BL4	6	-	8
BS1	13	8	14
BS2	9.5	-	9
BS3	-	-	7
BS4	-	-	5.5
BR1	8	6	11
BR2	8	-	9
BR3	-	-	7
BR4	6	-	8

BL1 Basil leaves dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BL2 Basil leaves dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use.

BL3 Dried basil leaves crushed to powder form, added 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BL4 Dried basil leaves crushed to powder form, added 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use.

BS1 Basil stem dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BS2 Basil stem dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use.

BS3 Dried basil stem crushed to powder form, added 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BS4 Dried basil stem crushed to powder form, added 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use

BR1 Basil root dipped in 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BR2 Basil root dipped in 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use.

BR3 Dried basil root crushed to powder form, added 70% ethanol, kept on shaker for 24 hours, filtered and vortexed before use.

BR4 Dried basil root crushed to powder form, added 70% methanol, kept on shaker for 24 hours, filtered and vortexed before use

V. CONCLUSIONS

In nutshell, it can be said that direct extract made from leaves, stem and root of Ocimum Basilicum.L and dipped in ethanol shows higher antimicrobial potential in comparison to leaves, stems and roots of Ocimum basilicum crushed to powder form and dipped in ethanol. Moreover Ocimum Basilicum.L extract showed good antimicrobial potential against Klebsiella Pneumoniae than against Escherichia coli and Pseudomonas aeruginosa.

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