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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *SYZYGIUM AROMATICUM, LAURUS NOBILIS* AND *CUMINUM CYMINUM* EXTRACTS AND THEIR COMBINATION

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ABSTRACT: Antibacterial agents are essential drugs for human health and welfare. Many pharmaceutical industries have produced a number of antibiotics and resistance to these drugs by microorganisms increased. This development of resistance has necessitated the search for new anti-bacterial agents. Medicinal plants like spices are considered as a good source of natural products for maintaining human health since decades. The use of these plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments. The main objective is to study the antimicrobial activity of combined plant extracts and to screen their anti-bacterial efficacy with the individual plant extracts by using Disc diffusion method against 5 bacterial strains *(Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Proteus vulgaris*). Aqueous extracts like Infusions and Decoctions from the following plants were prepared: *Syzygium aromaticum* (Clove), *Laurus nobilis* (Bay leaf), *Cuminum cyminum* (Cumin) and the comparison is made between individual extracts with the combined mixture of infusions and decoctions. The combination of three infusions was found to be more effective than combined decoction mixture of three spices in case of *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*. Bay leaf infusion was found to be the most promising extract in case of *Pseudomonas aeruginosa* when compared to all individual and combined mixture of infusions and decoctions.

INTRODUCTION: Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [1]. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [2]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain antibacterial drugs with no or less possible side effects.

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Resistance is not easily developed to medicinal plants when compared to allopathic medicines. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants [3]. In particular, extracts from many kinds of oriental spice plants are known to possess antimicrobial effects [4]. Spices are defined as plant substances used to enhance flavor, they include leaves (mint

and coriander), flower (clove), bulbs (garlic, turmeric), fruits (black pepper), stem (cinnamon), and rhizomes (ginger and turmeric). They are used for the purpose of food preservation, appetizer promotion and medicinal purposes [4, 5]. Many spices have been used because of their antibacterial activity which is due to compounds synthesized in the secondary metabolism of plants like isoflavones, anthocynanins and flavanoids [6] etc. From ancient history of Ayurveda many plant extracts are combined in order to get maximum activity [7, 8].

From the literature review, we found that Clove, Cumin and Bay leaf extracts are reported for their individual anti-bacterial activities but the combination of extracts (polyherbal extract) is not done.

The present study is being undertaken with the objective to evaluate anti-bacterial potential of spices like *Syzygium aromaticum* (clove), *Laurus nobilis* (bay leaf) *and Cuminum cyminum* (cumin) *by* using extract preparation methods of infusions and decoctions and also includes a comparative study of individual extracts with the combination mixture of extracts and an attempt is also done to compare the effect of extraction methods like Infusions and decoctions on the bacteria.

MATERIALS AND METHODS: Three medicinal spices of the study are clove flower buds *(Syzybium aromaticum)*, bayleaves (*Laurus nobilis*), and Cumin seeds (*Cuminum* cyminum) were collected from Ayurvedic stores. The collected spices were screened in order to remove any foreign materials present. Infusion and Decoction are prepared for 3 spices separately.

Preparation of infusions [9]: The infusions of bay leaf, cumin, and clove were prepared by taking 10 g of bay leaf, cumin and clove in 100 ml distilled water and left for 24 hours at room temperature with occasional shaking. After 24 hours, respective infusions were filtered.

Preparation of decoctions [10]: All three spices were pulverized to obtain coarse powder. The decoctions were prepared by boiling 10 g each of coarse powder in 100 ml distilled water in a beaker for 20 minutes .The beakers were taken, allowed to cool and filtered to obtain respective decoctions.

Test organisms: The test organisms includes the following Gram positive bacteria: *Staphylococcus aureus* (MTCC740), *Bacillus subtilis* (MTCC441) and Gram negative bacteria: *Pseudomonas aeruginosa* (MTCC424), *Escherichia coli* (MTC C41), *Proteus vulgaris* (MTCC426). All the strains were obtained from Institute of Microbial Technology, Chandigarh, India.

Procedure for Antibacterial activity testing: Disc diffusion method [11] was employed for testing antibacterial activity. Sterilized discs of filter paper (6mm in diameter) were soaked in 1ml of aqueous infusions and decoctions of bay leaf, cumin and clove separately for 1-2minutes and then used for screening. The combination of three infusions was prepared by taking equal ratios of all infusions in a small beaker and mixed properly.

After proper mixing, 1ml of combined infusion mixture was taken and sterilized filter paper disc were soaked for 1-2 minutes and then used for screening. In the similar way combined decoction mixture of three spices was also prepared and this mixture of decoctions was used for screening. The concentration for each disc was 10µl (100discs/ml of aqueous extracts). Mueller-Hinton agar (MHA) (Himedia, Mumbai) was used as test medium and Mueller-Hinton broth (MHB) (Himedia, Mumbai) was used for preparation of innoculum.

A small amount of culture is placed on the tip of an inoculation loop/needle is taken into flask containing MHB. This is carried out in laminar airflow. The flask is plugged with cotton. These flasks were incubated to allow the growth of organisms for 18 to 24 hrs which is used as pure cultures for activity. 20ml of MHA was transferred into each petri plate. The petri plates were left undisturbed for 1-2hrs. 100 µl of each strain pure culture was transferred into petri plates using micro pipette.

The pure cultures were evenly spread with the help of sterile bent glass rod. Previously soaked discs in aqueous infusions and decoctions of bay leaf, cumin and clove and discs in combined infusions mixture and combined decoctions mixture were placed on the surface of inoculated plates with sterile forceps. All plates were incubated at 37°C for 24 hours [12].

The diameters of the zones of inhibition appearing around the discs were measured to the nearest millimeter (mm) and recorded [13]. Gentamycin antibiotic was used as standard.

RESULTS AND DISCUSSION:

Infusions: The diameter of zones of inhibitions of all individual plant infusions and combined infusion mixture were given in **table 1**.

Decoctions: The diameter of zones of inhibitions of all individual plant decoctions and combined decoction mixture was given in **table 2**.

Combined Extracts: A comparative study is made on combination of infusions and combination of decoctions. The results are shown in **table 3**.

TABLE 1: COMPARATIVE STUDY OF ZONE OF INHIBITIONS OF ALL INDIVIDUAL PLANT INFUSIONS AND COMBINATION OF INFUSIONS

S. No.	Bacterial strains	zone of inhibition at 10 µl/ml of respective extracts {to the nearest millimeter(mm)}				
		Clove Infusion (a)	Cumin Infusion (b)	Bay leaf Infusion (c)	Combined mixture of all Infusions (a+b+c)	Standard (Gentamycin)
1.	*Bacillus subtilis*	10±0.45	12±0.74	6±0.45	13±0.56	14±0.48
2.	*Escherichia coli*	8±0.12	16±0.44	10±0.54	8±0.35	12.8±0.92
3.	*Proteus vulgaris*	10±0.15	12±0.35	10±0.34	8±0.57	10±86
4.	*Pseudomonas aeruginosa*	6±0.45	8±0.58	18±0.42	14±0.46	10±0.67
5	*Staphylococcus aureus*	8±0.64	15±0.82	8±0.41	10±0.35	11.6±0.83

TABLE 2: COMPARATIVE STUDY OF ZONE OF INHIBITIONS OF ALL INDIVIDUAL PLANT DECOCTIONS AND COMBINATION OF DECOCTIONS

S. No.	Bacterial strains	Zone of inhibition at 10 µl/ml of respective extracts {to the nearest millimeter (mm)}				
		Clove Decoction (a)	Cumin Decoction (b)	Bayleaf Decoction (c)	Combined mixture of all decoctions (a+b+c)	Standard (Gentamycin)
1.	*Bacillus subtilis*	6±0.18	15±0.25	6±0.21	6±0.28	14±0.48
2.	*Escherichia coli*	9±0.44	10±0.70	10±0.55	6±0.35	12.8±0.92
3.	*Proteus vulgaris*	12±0.71	8±0.71	8±0.54	8±0.89	10±86
4.	*Pseudomonas aeruginosa*	12±0.56	6±0.56	11±0.87	12±0.45	10±0.67
5	*Staphylococcus aureus*	10±0.45	6±0.86	6±0.41	10±0.54	11.6±0.83

TABLE 3: COMPARATIVE STUDY OF COMBINATION OF INFUSIONS AND DECOCTION

S. No.	Bacterial strains	Zone of inhibition at 10 µl/ml of combined mixture of extracts {to the nearest millimeter (mm)}	
		Combined infusion mixture	Combined decoction mixture
1.	*Bacillus subtilis*	13±0.56	6±0.28
2.	*Escherichia coli*	8±0.35	6±0.35
3.	*Proteus vulgaris*	8±0.57	8±0.89
4.	*Pseudomonas aeruginosa*	14±0.46	12±0.45
5.	*Staphylococcus aureus*	10±0.35	10±0.54

DISCUSSION: In case of *Escherichia coli* as shown in Table 1, Cumin infusion exhibited maximum effect (16±0.44mm) which is higher than standard. In the case of *Proteus vulgaris*, cumin infusion only (12±0.35mm) exhibited maximum activity than standard. Among all infusions bay leaf infusion is the most promising one in case of *Pseudomonas aeruginosa* (18±0.42mm) and almost two-fold times higher than standard.

Infusion combination mixture exhibited maximum antibacterial potential against *Bacillus Subtilis* (13±0.56mm) when compared to all other individual infusions but the potential is less than standard (table 1).

This helps us to draw conclusion that combined infusion mixture worked only in the case of *Bacillus subtilis* when compared to individual plant extracts.

Cumin decoction (Table 2) exhibited maximum activity against *Bacillus subtilis* (15±0.25mm) which is comparatively higher than standard. All individual decoctions and decoction mixture were found to be not effective against *Escherichia coli* and *Staphylococcus aureus* when compared to the standard. Clove decoction has good inhibitory action towards gram positive bacteria *Proteus vulgaris* (12±0.71mm) which is comparatively higher than standard. The combined decoction mixture has shown highest activity (12±0.45) in case of Pseudomonas *aeruginosa* than standard.

Infusion extraction of combined mixture has shown good antibacterial activity than decoction combined mixture (Table 3) in case of gram positive bacteria *Bacillus subtilis*, gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* 13±0.56, 8±0.35, 14±0.46 respectively. Both infusion and decoction combined mixtures have almost shown equipotent effect against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Proteus vulgaris*.

CONCLUSION: Bay leaf infusion has got highest potent activity towards gram negative bacteria *Pseudomonas aeruginosa* when compared to all individual, combined extracts and standard drug. Cumin infusion has got highest potent activity towards gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*. In case of *Proteus vulgaris* Clove decoction and Cumin infusion are the most promising extracts when compared to all extracts. Cumin decoction has shown maximum activity when compared to all extracts against *Bacillus subtilis.*

From our study, we conclude that the combined infusion mixture was effective than combined decoction mixture. But in some cases the individual extracts shown better activity than combined extracts. This particular Study has a wide scope in future for development of polyherbal drugs with less or no side effects and there are some void in these three drugs which should be filled by research in near future.

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