ANTIMICROBIAL SUSCEPTIBILITY OF VARIOUS NATURAL EXTRACTS ON COLIFORMS

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Abstract

Coliforms are several types of bacteria that are found in the soil, on vegetation or on surface waters. The distribution of resistance to various antibiotics among coliforms in sewage, surface waters, and sea water was investigated. The incidence of resistant strains among isolates varied significantly among the water samples and there is no obvious connection with the water source or the level of pollution. That means higher risk of presence of pathogens. Coliforms were isolated from water samples and plated on Nutrient agar, Mac Conkeys agar plates and incubated at 37°C for 24h and the colony morphology was noted. They were microscopically identified as Gram negative bacilli. A subculture stage confirmation was performed on EMB agar plate and a greenish metallic sheen was identified confirming the presence of coliforms. Antimicrobial activity was done with various aqueous natural extracts of turmeric, cinnamon, cardamom, garlic, ginger and cloves against coliforms. The aqueous extracts of turmeric (1.5cm) showed maximum antimicrobial susceptibility against coliforms followed by cloves (1.4cm), ginger (1.2cm), garlic (0.8cm), cinnamon (0.6cm), and cardamom (0.2cm) respectively. A similar study was done with antibiotics on coliforms, it was identified that norfloxacin (10mcg) showed a zone of inhibition 1.5cm, while gentamycin (10mcg) showed 1.3cm, streptomycin (10mcg) showed 1cm and vancomycin (30mcg) 0.3 cm. Natural products are a major source of new natural drugs and they can be used as an alternative medicine for treatment of various diseases. In comparison to the formulated drugs the herbs and spices have fewer side effects.

Keywords- natural products, coliforms, antimicrobial activity, turmeric, antibiotics.

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pharmacological or biological activity [5]. These are been given more attention due to the increase concerns on chemical preservatives among consumers. There are over 1340 plants with defined antimicrobial activities, and over 30,000 antimicrobial compounds that have been isolated from plants [6]. However, there are increasing reports of nonphenolic oil compounds, which are effective against both Gram-positive and Gram-negative groups, from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemon grass, muscadine seeds, and sage [7-11]. The antimicrobial activity of natural compounds could be influenced by number of factors including botanical source, time of harvesting, stage of development, and method of extraction in addition to the composition, structure, and functional groups of the natural compounds [12]. As a result, a solution to the issue of antimicrobial resistance is a matter of urgent importance. Natural products are viewed as a privileged group of structures which have evolved to interact with a wide variety of protein targets for specific purposes.

II. MATERIALS & METHODS

2.1 Collection of Material
The materials were collected from local markets of Secunderabad, Telangana.

2.2 Preparation of Extracts and antibiotics
The natural products like turmeric, cinnamon, cardamom, garlic, ginger, and cloves were dried and ground to fine powder. 0.5gm of the powder was dissolved in 20 ml of sterile distilled water and vortexed. These extracts were filtered using Whatmann filter paper No 1 and stored at 4°C for further use.

Antibiotic discs of norfloxacin (10mcg), gentamycin (10mcg), streptomycin (10mcg), and vancomycin (30mcg) were also used in the study.

2.3 Microorganisms
The microorganisms used for the study are the coliforms isolated from water samples. The coliforms cultural characters were noted on nutrient agar, mac conkey’s agar and EMB agar.

2.4 Preparation of Culture media
The culture media used for cultivation of coliforms were nutrient agar (peptone 0.5 g, beef extract 0.3 g, sodium chloride 0.5 g, agar 2.0 g, distilled water 100 ml , pH 7), nutrient broth (peptone 0.5 g, beef extract 0.3 g, sodium chloride 0.5 gm, distilled water 100 ml, pH 7), Mac conkeys agar (peptone 20 g, lactose 10 g, bile salts 1.5 g, sodium chloride 5g, neutral red 0.03 g, crystal violet 0.001 g, agar 20 g, water 1 litre, pH 7) Eosin Methylene Blue) pancreatic digest of gelatin 10.0g, lactose 5.0g, sucrose 5.0g, di potassium phosphate 2.0g, eosin y 0.4gm, methylene blue 0.065g, agar 20 g, water 1 litre, pH 7).

2.5 Antimicrobial assay
The antimicrobial assay was performed by an agar well diffusion method with aqueous extracts. For agar well diffusion method, a well was prepared in the nutrient agar plates with the help of a cork-borer (0.6 cm). The culture was spread on the entire plate and 50ul of the test compound was introduced into the well and the plates were incubated overnight at 37°C for 24h. Microbial growth was determined by measuring the diameter of the zone of inhibition. A control was maintained where aqueous solutions were used instead of the extracts [13]. The result was obtained by measuring the zone diameter. The experiments were done in the duplicates and the mean values are presented.

Antibiotic sensitivity test of coliforms was also performed by using four different antibiotics by disc diffusion method. The antibiotics used were norfloxacin (10mcg), gentamycin (10mcg), streptomycin (10mcg), and vancomycin (30mcg).
III. RESULTS AND DISCUSSION

On nutrient agar, the coliforms are large, circular, white, moist, smooth, and opaque. While on mac conkey’s agar, colonies are bright pink due to lactose fermentation and on EMB agar the colonies showed greenish metallic sheen, confirming the presence of coliforms as seen in Fig 1. The microscopic identification confirmed the presence of Gram negative bacilli.

![Figure 1. Coliforms cultural characters on Nutrient agar, Mac Conkeys agar and EMB agar.](image)

It was observed that turmeric showed a zone of inhibition of 1.5cm, cinnamon 0.6cm, cardamom 0.2cm, garlic 0.8cm, ginger 1.2cm, cloves 1.4 cm. All the aqueous extracts of these natural products showed sensitivity against coliforms as seen in Fig 2.

![Figure 2. Antimicrobial assay with various natural products by agar well diffusion method](image)

The present study revealed that turmeric had the most effective antimicrobial activity on coliforms followed by cloves, ginger, garlic, cinnamon, cardamom which is in align with the previous studies.

On exposure with antibiotics on coliforms, it was identified that norfloxacin (10mcg) showed a zone of inhibition 1.5cm, while gentamycin (10mcg) showed 1.3cm, streptomycin (10mcg) showed 1cm and vancomycin (30mcg). 0.3 cm as seen in Fig 3. All the four showed sensitivity to coliforms at varying levels almost as effective as the natural extracts.
IV. CONCLUSION

Natural products are inexpensive, show better patient tolerance and are readily available for low socioeconomic population. It is likely that the search for antimicrobials from natural sources would yield better results than from combinatorial chemistry and other synthetic procedures. In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries.

It is believed that turmeric has the maximum antimicrobial property and women in north apply turmeric on their feet before they enter the waters of ganges. They do this to protect themselves from various water borne infections from the polluted waters of ganges. The present study confirms that turmeric has the best antimicrobial property which inhibits coliforms present in such waters. So people going for holy dips are advised to consume foods with turmeric and use traditional methods like applying turmeric to protect themselves from the acquiring infections. By this we can control the spread of infections transmitted through water. Therefore the present study revealed that natural antimicrobial substances are not only potent against target pathogens but also seems to stand a better chance to overcome the microbial resistance mechanisms.

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