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Synergistic activity of *Curcuma longa* L. and *Piper nigrum* L. against pharyngitis causing microorganisms

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Abstract

Pharyngitis is a sudden and very often repetitively occurring process caused by the microorganisms. It is one of the most prevalent health issues. Several diseases caused by bacteria have been addressed using the medicinal capabilities of several spices and herbal plants. Turmeric, a spice known for its therapeutic benefits, has attracted the attentions of medical and scientific researchers, as it is the primary source of the polyphenol curcumin. Piperine, the main active ingredient in black pepper, has been demonstrated to improve bioavailability by 2000% when supplemented with curcumin in a complex. Curcumin combined with enhancing agents provides multiple health benefits. It assists in the treatment of oxidative and inflammatory diseases. The aim of the study was to test the synergistic antibacterial activity of *Curcuma longa* L. and *Piper nigrum* L. Antimicrobial activity of *C. longa* and *P. nigrum* were determined using minimal inhibitory concentration (MIC) and antibiofilm assay. The cytoxicity was assessed using MTT against microorganisms that cause pharyngitis. The ethanolic extracts of turmeric and black pepper had the most potent antibacterial action, inhibiting all of the microorganisms tested.

1. Introduction

Pharyngitis is an infection of the oropharyngeal mucous membranes. The most common cause is a bacterial infection. Antibiotics are used to treat pharyngitis, but they can have a variety of side effects, including gastrointestinal symptoms, skin allergies, liver toxicity, anaphylactic response, and neuropsychiatric issues (Boyarchuk *et al*., 2021). Researchers and the natural health product sector have paid close attention to natural antibacterial agents as replacement of synthetic analogues. As a result, new natural antibacterial drugs produced from plants have been tested against a variety of infectious microorganisms (Sandip *et al.*, 2021; Abebe *et al*., 2020).

Turmeric (*Curcuma longa* L.) is a member of the Zingiberaceae family, which could be located in the tropics of Asia, Africa, and Australia. It offers a wide spectrum of therapeutic properties. Curcumin, phenolics, terpenoids, and flavonoids are the most common compounds found in its rhizomes. Turmeric extract includes a substantial amount of curcumenoids and has a high level of antioxidant activity (Ahmad *et al*., 2020). Curcumin is the major curcumenoid in turmeric, a famous Indian spice with a variety of therapeutic benefits. Curcumin is an antioxidant with almost the same potency as vitamins C, E, and β-carotene (Abd El Hack *et al*., 2021).

In oriental regions, black pepper (*Piper nigrum* L.) is a popular ingredient. Ascorbic acid, β-carotene, camphene, lauric acid, linalyl acetate, myristicin, palmitic acid, terpinen-4-ol, piperine, and feruperine are only a few of the antioxidants found in black pepper (Lee *et al*., 2020). All of the phenolic amides found in black pepper have antioxidant activity that is significantly higher than that of the naturally occurring antioxidant, α-tocophenol, and feruperine has antioxidant activity comparable to that of the synthetic antioxidants bulylated hydroxyl anisole and butylated hydroxytoluene (Zhang *et al*., 2021). At low concentrations, piperine, the major bioactive element of black pepper, acts as a hydroxyl radical scavenger. Piperine has also been proven to promote curcumin's antioxidant action (Shilpa *et al*., 2021).

Plants contain phenols, quinones, flavones, tannins, terpenoids, and alkaloids, all of which have antibacterial properties. Antiadhesive properties, or compounds that inhibit the microbe from adhering to the host tissue, are also present in some spices and culinary herbs, avoiding the initial infection point (Wojno *et al*., 2021). Turmeric and pepper, for example, may increase immune cell activity. When utilised in a combination source, all spices have a higher level of antibacterial activity. As a result, combining curcumin with piperine enhances its health outcomes. Curcumin and piperine together provide a stronger anti-inflammatory, digestive, pain-relieving, and cancer-fighting action (Meghwal *et al*., 2021).

The present study was formulated to analyse the synergistic property of ethanolic extracts of turmeric and black pepper against different bacterial strains causing pharyngitis.

2. Materials and Methods

2.1 Sample preparation

The powdered sample of turmeric and pepper was mixed with 90% of ethanol. The extract was filtered through Whatman no. 1

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filter paper and concentrated using a rotary evaporator. The crude extract was collected and allowed to dry at room temperature and stored.

2.2 Bacterial culture

Five different bacterial species, namely; *Bacillus subtilis, Streptococcus aureus, Escherichia coli, Pseudomonas aeuroginosa, Klebsiella pneumoniae* which are known for enhancing pharyngitis were selected. All tested strains were obtained from the micro biological laboratories and were sub-cultured on Mueller Hinton Agar slants and were maintained at 4°C.

2.3 Broth microdilution method

For the broth microdilution test 50 µl of each bacterial suspension in suitable growth medium was added to the wells of a sterile 96-well microtitre plate already containing different concentrations (31.5, 62.5, 125, 250, 500 µg/ml) of plant extract in proper growth medium. Control wells were prepared with culture medium with bacterial suspension. The MIC was the lowest concentration where no viability was observed after 24 h on the basis of metabolic activity (McMurray *et al*., 2020).

2.4 MTT assay

The cytotoxicity of the plant extracts was assessed using the selected microorganisms (Awad *et al*., 2019). Overnight culture of each bacterial strain was prepared in nutrient broth and 100 µl of the overnight culture were added to 96 well titre plate and the different concentrations of extracts were added. The cells were incubated for one hour. And added 50 µl of MTT to 100 µl of treated cells of strain and incubated at 37ºC for 3 h. Then 200 µl of acid-propanol was added to it after incubation and left overnight in dark room. The cell viability was noted at 650 nm in a microtitre readerby fixing the control group as 100% viability and the percentage of viable cells were calculated relative to other treatment groups.

$$\% \text{ of cell viability} = \frac{\text{OD of control} - \text{OD of treatment} \times 100}{\text{OD of Control}}$$

2.5 Antibiofilm activity

The effect of extract on biofilm formation of each representative strain was examined using the modified microdilution method (Lahiri *et al*., 2019). Overnight cultures in trypticase soy broth was diluted in the ratio 1:100 in respective fresh medium and allowed to grow for another hour. 100 µl of the diluted strains were added to 96 well titre plate and the different concentrations of plant extracts were added and incubated at 37ºC for overnight. After the incubation, the medium was removed and 100 µl of crystal violet solution was added and incubated at room temperature for 30 min. The dye was removed after staining and the wells were washed thoroughly with distilled water and finally incubated with 95% ethanol for 15 min and read in spectrophotometer at 595 nm. Inhibition mediated reduction of biofilm formation was calculated by the following formula:

$$\% \text{ of cell viability} = \frac{\text{OD of control} - \text{OD of treatment} \times 100}{\text{OD of Control}}$$

2.6 Statistical analysis

All data were represented as Mean ± SD using at least three independent replicates using Microsoft excel.

3. Results

3.1 Evaluation of minimum inhibitory concentration (MIC) for selected bacterial strains

The antimicrobial activity of ethanolic extract was investigated against the pharyngitis causing microbes, namely; *Bacillus subtilis, Streptococcus aureus, Escherichia coli, Pseudomonas aeuroginosa, Klebsiella pneumoniae*. Table 1 shows the MIC obtained for the assays against causative agents of pharyngitis. Turmeric and black pepper afforded MIC value of 0.31 mg/ml against *Bacillus subtilis*, *Streptococcus aureus* and *Pseudomonas aeuroginosa*. *Escherichia coli* and *Klebsiella pneumoniae* exhibited MIC value of 0.62 mg/ml, respectively.

Table 1: Determination of minimum inhibitory concentration using plant extract against pharyngitis causing micro-organisms

S. No.	Test organisms	MIC (mg/ml of turmeric + black pepper extract)
1.	*B.subtilis*	0.31 ± 1
2.	*S.aureus*	0.31 ± 1
3.	*E.coli*	0.62 ± 1.5
4.	*P.aeruginosa*	0.31 ± 2
5.	*K.pneumonia*	0.62 ± 1.5

3.2 Cytotoxicity of plant extract

The ability of the extract to inhibit the growth of microorganisms was tested using the MTT assay. The results of MTT cell viability assay is depicted in Figure 1. When the concentration increased percentage of the bacterial cell death of the microbes, namely; *Bacillus subtilis, Streptococcus aureus, Escherichia coli, Pseudomonas aeuroginosa, Klebsiella pneumoniae* also increased. The synergistic effect of turmeric and pepper reduced the viability of microbe's causing pharyngitis.

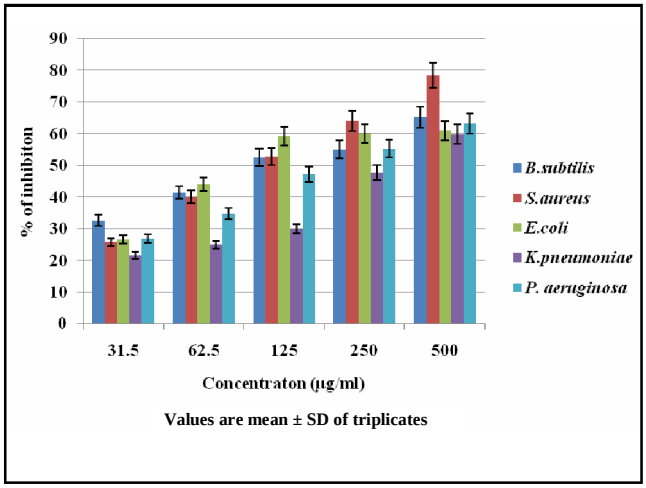

Figure 1: Antimicrobial activity of ethanolic extract by MTT cytotoxicity assay.

3.3 Antibiofilm assay

To investigate whether plant extract reduced biofilm formation, antibiofilm assay was done. Turmeric and pepper were found to exhibit antibiofilm effect against the selected microorganisms. The results are depicted in the Figure 2, shows that the ethanolic extract exhibited antibiofilm activity, by inhibiting the growth of microorganisms. Higher concentration of the extract 500 µg/ml showed maximum percentage of inhibition for all the four bacterial cultures, *Bacillus subtilis, Streptococcus aureus, Escherichia coli, Pseudomonas aeuroginosa, Klebsiella pneumoniae*. It confirmed that the ethanolic extract of turmeric and black pepper caused bacterial cell death of pharyngitis causing microbes.

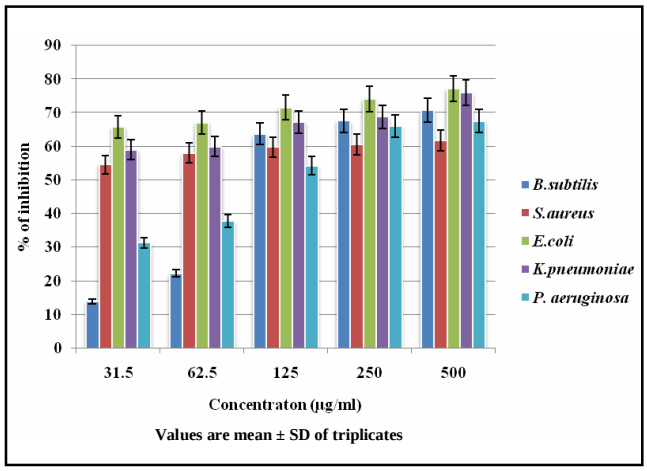

Figure 2: Antibiofilm activity of turmeric and black pepper.

4. Discussion

The development of multidrug resistance strains of key pathogens including *S. aureus, P. aeruginosa*, and *E. coli* has raised interest in medicinal plant research during the last few decades (Abdalhamed *et al*., 2021; Vidya *et al*., 2021; Naba, 2021). Penicillin G, macrolides, lincosamides, tetracycline, and gentamicin are some of the antibiotics that have developed resistance. Antibacterial medicines with broad range efficacy against resistant microbes need to be investigated further (Laws *et al*., 2019).

The antibacterial activity of turmeric and pepper, which are used to treat disorders of the respiratory system, was tested in this study against microbes that cause pharyngitis. Although, several researchers have noted these spices' antibacterial properties, their synergistic action against pharyngitis strains has received less attention. The antibacterial activity of the spices' ethanolic extracts was tested against ATCC bacterial cultures of gram-positive and gram-negative bacteria. The inhibition of biofilm formation is an interesting way to prevent the formation of well-organized attached bacterial biofilms and, thus, pharyngitis. The combined effect showed a cytotoxic effect against the selected organisms

Considering the possible therapeutic applications of our study, additional experiments could be performed on combined effect of turmeric and pepper. If, the combination has a synergistic and/or complementary impact, it could be used as part of a combined treatment for pain management in patients (White *et al*., 2019). Further research is needed to confirm the synergic effect in the treatment of pharyngitis.

5. Conclusion

In conclusion, this research reveals that *C. longa* and *P. nigrum* contain bioactive chemicals that may be responsible for their antibacterial action. Ethanolic extracts of spices is effective in the inhibition of microbes responsible for pharyngitis. More research is needed to separately examine the characteristics of the spices and potentially work toward the formulation of pharmaceuticals employing these extracts for topical, oral, or systemic usage.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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