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BASAVAPRABHU KORE ARTS, SCIENCE AND COMMERCE COLLEGE,
CHIKKODI – 591201 District – Belagavi (Karnataka state, India)
(ACCREDITED AT 'A' GRADE BY NAAC WITH CGPA OF 3.26 IN THE THIRD CYCLE)

## Department of Zoology (2019 – 20)

# PROJECT WORK COMPLETION CERTIFICATE

This is to certify that following six B.Sc Final year students have undertaken the project entitled "***Analysis of antimicrobial activity of Curcumin***" in-partial fulfillment of the syllabus of Rani Channamma University, Belagavi during the year 2019-20. Following six students have successfully completed the said project under the guidance of Dr Sridevi I Puranik.

| Sl. No | Gender | Name of the student | Fathers name | Roll Number | Exam Reg. Number |
|---|---|---|---|---|---|
| 1 | Miss. | Chaitra Mukkannavar | Gopal | 166 | S1715634 |
| 2 | Miss. | Sandhya Kambar | Shivanand | 180 | S1715745 |
| 3 | Miss. | Priyanka Halijol | Raju | 176 | S1715712 |
| 4 | Miss. | Varsha Karagar | Kallappa | 191 | S1715832 |
| 5 | Miss. | Vachana Kolkar | Ramachandra | 190 | S1715827 |
| 6 | Miss. | Meghashree Gundaalle | Gajanan | 171 | S1715682 |

**Dr Sridevi I Puranik**
PROJECT GUIDE

**Dr N R Birasal**
HEAD
DEPARTMENT OF ZOOLOGY

**Prof U R Rajput**
PRINCIPAL
KLES'S Basavaprabhu Kore
Arts, Science and Commerce College
CHIKODI - 591 201

# Project Team Members

| Sl. No. | Gender | Name of the Student | Fathers Name | Roll Number | Exam Seat Number |
|---|---|---|---|---|---|
| 1. | Miss. | Chaitra Mukannavar | Gopal | 166 | S1715634 |
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| 6. | Miss. | Meghashree Gondakalle | Gajanan | 171 | S1715682 |

# ACKNOWLEDGEMENT

In the present world of competition there is a race of existence in which those are having will to come forward succeed. Project is like a bridge between theoretical and practical working. With this willing, it is great pleasure for us to undertake this project. We feel highly doing the project entitled "**Analysis of antimicrobial activity of Curcumin"**.

We would like to express our special thanks of gratitude to our professor and even our project guide **Prof. Mrs. Sridevi I. Puranik**, Assistant Professor Department of Zoology. This project would not have completed without her enormous help and worthy experience. Whenever we were in need, she was there behind us.

We are very grateful to **Dr. N. R. Birasal,** Head of the Department of Zoology as well as our beloved Principal **Shri. U. R. Rajput** who gave us the golden opportunity to do this wonderful project.

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Although, this report has been prepared with utmost care and deep routed interest, even then we accept respondent and imperfection.

# CONTENT

# INTRODUCTION

Food borne diseases have always been a major concern in both developing and developed countries. *Campylobacter jejuni*, *Staphylococcus aureus*, *Salmonella*, *Escherichia coli*, *Streptococci*, etc are some of the major bacterial species that causes food borne diseases. *E. coli* are the most commonly found bacterium in the human intestinal tract. Under normal conditions, its presence is conducive to digestive processes (Abbas *et al.*, 2019). But when present in excess it causes diseases.

With increasing use of drugs, microorganisms are attaining resistance to commonly used antibiotics, which leads to downfall of effectiveness of conventional medicines and therefore, search for new antimicrobial agents has become necessary. Traditional medicines have been used for many centuries by a substantial proportion of the population of India. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in developing countries like India, plants are the main medicinal source to treat infectious diseases. Approximately 20% of the plants found in the world have been subjected to pharmacological or biological test, and a substantial number of new antibiotics introduced in the market are obtained from natural or semi-synthetic resources (Ahmadi, 2010)**.**

The active ingredients of plants against microorganisms are mostly some of the secondary metabolites (i.e. alkaloids, glycosides etc) that are present in abundance in herbs and spices commonly used in Indian food preparations. Herbs are small plants used by human being for various purposes like medicines, food supplements for imparting flavour or scant, and as a part of offerings to God since beginning of civilization.

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger), root (turmeric), and other plant parts. The importance of spices can be found not only in the flavouring, but also in their medicinal, preservative and antioxidant properties. Being plants, the natural food stuffs, spices appeal to consumers who tend to question the safety of synthetic additives **(**Akram *et al.*, 2010)**.**

Antimicrobial properties of the spices have been documented in ancient literature and the interest continues to the present.

Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Here, various antimicrobial agents are used. Antimicrobial may be anti-bacterial, anti-fungal or anti-viral. They all have different modes of action by which they act to suppress the infection.

An anti-microbial is an agent that kills micro-organisms or inhabits their growth. Antimicrobial medicines can be grouped according to the micro-organisms they act primarily against. Antimicrobial agents are of various classes. Some of the class includes; beta lactam, cephalosporins, quinolones, tetracyclines, macrolides, sulphonamides, aminoglycosides, etc. These different classes act in a different way and on different kind of bacteria.

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature **(**Kocaadam and Şanlier, 2017). Because of the side effects and the resistance that pathogenic micro-organisms build against antibiotics, many Scientist have recently paid attention to herbal extracts and biologically active compounds isolated from plant spices used in herbal medicines.

**Figure 1: Spice samples - Curcumin roots and Curcumin powder**

Turmeric is a spice that comes from the root Curcuma longa, a member of the ginger family, *Zingaberaceae*. It is brightly yellow and has been used as colouring agent in food (McLean, 2018). In India, it has been used for centuries as a spice and a food preservative, and also for its various medicinal properties. In Ayurveda, turmeric has been used for various

purposes and through different routes of administration. It has been used topically on the skin for wounds, blistering diseases such as pemphigus and herpes zoster, for parasitic skin infections, and for acne. It has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a blood purifier **(**Rahmani *et al.*, 2018).

In the last few decades there has been considerable interest in the active compounds in turmeric called curcuminoids. The major curcuminoid is called curcumin (diferuloyl methane), which makes up approximately 90% of the curcuminoid content in turmeric, followed by demethoxy curcumin and bismethoxy curcumin. The curcuminoids give turmeric its bright yellow colour. The antibacterial activity of curcumin bioconjugates has been tested particularly for β-lactamase producing microorganisms.

*Escherichia coli* are the most commonly present bacteria in the human intestine, which helps in preventing the entry of pathogenic microorganisms. *E. coli* are non-pathogenic in normal conditions, but if present in excess, will become causative agent of various diseases like urinary tract infection, diarrhoea, vomiting etc. With increasing resistance of microorganisms to antibiotics, there is a shift of choice from allopathic to Ayurvedic and naturopathy, where herbs and spices are very common ingredients of medicines (Shahid *et al.*, 2020).

Herbs and spices are used in Indian recipes as they impart aroma and flavour to it. Most of the studies performed to check sensitivity of extraction of the active component(s) with some organic solvents. In the present study, turmeric spice is selected, its extract is made using distilled water and tested for its antimicrobial effect against *E. coli* the most common intestinal non-pathogenic organism. The turmeric tested was able to inhibit *E. coli* growth and it was found to be most effective against *E. coli.*

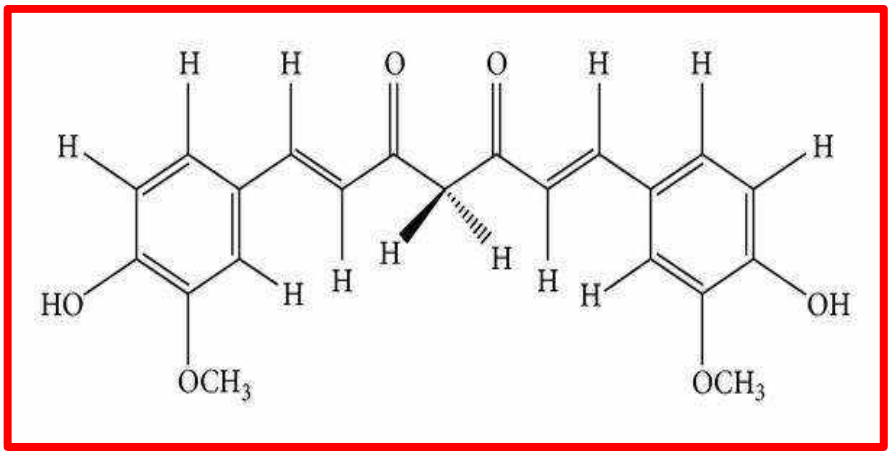



**Figure 2: Chemical structure of Curcumin.**

Curcumin, a yellow pigment present in the Indian spice turmeric, has been linked with suppression of inflammation; angiogenesis; tumorigenesis; diabetes; disease of the cardiovascular, pulmonary, and neurological systems, of skin, and of liver; loss of bone and muscle; depression; chronic fatigue; and neuropathic pain.

The utility of curcumin is limited by its colour, lack of water solubility, and relatively low *in vivo* bioavailability. Because of the multiple therapeutic activities attributed to curcumin, there is an intense search for 'super curcumin'. In present study, anti-microbial activity of turmeric natural dye against *E. coli* bacteria was measured by disk diffusion method. Turmeric natural dye showed good inhibitory activity against *E. coli* with a zone of inhibition 7 mm to 15mm. The incorporation of turmeric dye with natural fibre will help to produce value added handicrafts.

# MATERIALS AND METHODS

**Materials:**

Nutrient Agar Medium, Curcumin powder mg/mL.

**Methods**

1. **Preparation of Nutrient agar medium**

   **Composition of Nutrient Broth**

| | | |
|---|---|---|
| Peptone | : | 5 gm |
| NaCl | : | 5 gm |
| Beef extract | : | 3 gm |
| Distilled water | : | 1000 mL |
| pH | : | 7.0 |

**Preparation of Media**

- Accurately weigh the ingredients of nutrient broth and transfer them into a beaker containing 500 mL of distilled water
- Gently, heat the contents with continuous shaking to dissolve them.
- Add, more distilled water to make the volume 1 litre.
- Measure pH of broth, using pH meter and adjust pH to 7 by adding drops of either HCl or NaOH solution.
- Dispense 10 mL broth to each culture tube and put cotton plug to mouth of test tube.
- Tightly cover the mouth of cotton with aluminium foil or paper and tie with a rubber band.
- Transfer, all the broth into test tube and place them inside autoclave basket and sterilise at $121^0$ C for 15 minutes.
- After sterilization, take out the broth tubes and store them in refrigerator for further use.


**Figure 3: laminar air flow and nutrient media in Petri plates**

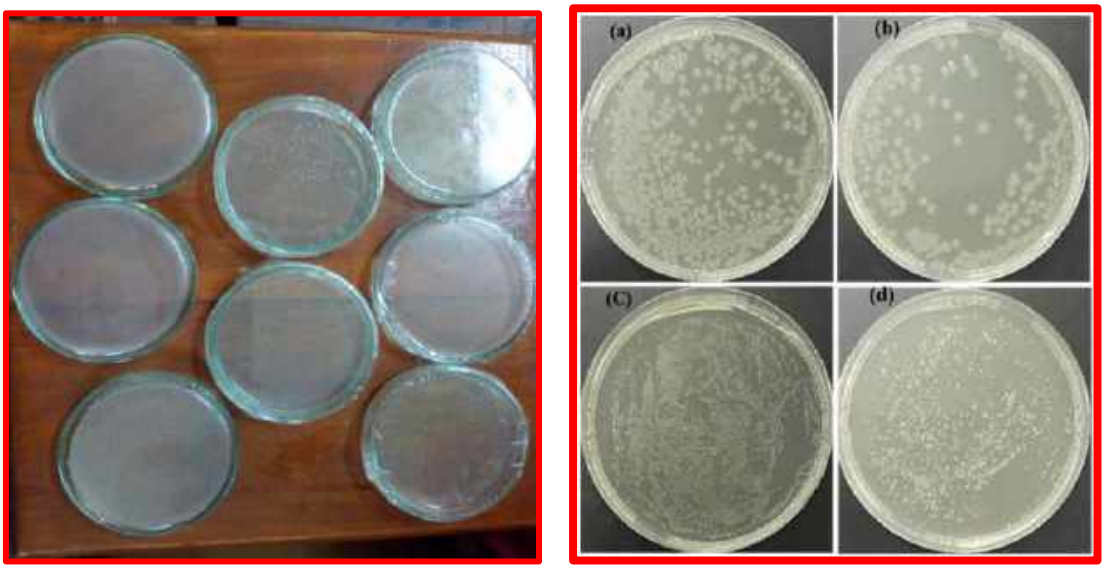
**2. Extraction of turmeric sample**

- The turmeric root was soaked in water and ground with mortar and pestle along with 5ml of distilled water. Then, the sample was centrifuged (3000 RPM, 10 min) and the supernatant obtained was used as an extract.
- The turmeric powder ground with 5mL of distilled water and centrifuged at 3000 RPM for 10 minutes and the supernatant was separated and used as extract and represented as µg/mL.

**Figure 4: Curcumin extract**

### 3. Preparation of *E. coli* inoculum

- Firstly, the nutrient medium was prepared using peptones, beef extract, distilled water. The pH is adjusted to 7.0.
- Then the bacterial strain was streaked over the nutrient medium and kept for incubation at $27^0$ C for 24 hours the growth of the bacterial colony.




**A** **B**

**Figure 05: A) Nutrient media in petri plates, streaked with bacterial strain.**

**B) Bacteria colony developed on nutrient media.**

**4. Paper disc method:**

- The inoculums were spread uniformly in N-agar plates with the help of glass spreader or streaker and left for 5 minutes.
- Pre-sterilised paper discs were dipped into turmeric extract and placed in inoculated plates.
- The plates were incubated for 24 hours at $37^0$ C and size of clear zone developed surrounding each disc.

**Figure 6: Sterilised paper discs in beaker.**

# RESULTS AND DISCUSSION

Traditionally, herbs and spices are part of routine Indian food preparations as they make food appealing by providing better appearance, smell and taste. As mentioned in 'Atharva veda' these herbs and spices have healing, soothing and rejuvenating properties (Srinivasan, 2019).

**(A)** **(B)**

**Figure 07: A) Student performing experiment under the supervision of guide.**
**B) Students performed experiment Infront of the laminar hood.**

The turmeric possessed high activity against *E. coli* bacterial strain. This activity is due to the presence of curcuminoid, a phenolic compound. The antimicrobial property of turmeric has been attributed to the presence of the essential oil, an alkaloid, curcumin and other curcuminoids, turmeric oil, tumerol and veleric acid (Srinivasan *et al.*, 2004).

In the present study it was observed that the turmeric extract possessed significant antibacterial activity against *E. coli* bacteria. The turmeric extract was able to inhibit the growth

of the bacteria tested. It was indicated by an inhibition zone surrounding the paper disc containing the turmeric extract placed in the bacterial colony.

**Figure 8: Petri plates showing inhibition zones surrounding the paper discs.**

Considering the result, we got, it can be suggested that addition of herbs and spices to the food preparations helps to keep a check on the concentration of *E. coli* in the body. Turmeric used as most important food additive with antimicrobial activities in Indian recipes (Vinodhini *et al.*, 2019).

# CONCLUSION

Turmeric is the golden spice and has been used for thousands of years as a medicinal herb to treat inflammation, bacterial infections and digestive issues. Curcumin is the main active ingredient in turmeric. It has powerful anti-inflammatory effects and is a very strong antioxidant. The present study states that the curcumin present in the turmeric, inhibits the growth of disease-causing bacteria. Bacteria is good for some processes, although it can result in ulcers, inflammation of the lining of the stomach, and an increased risk of stomach cancer.

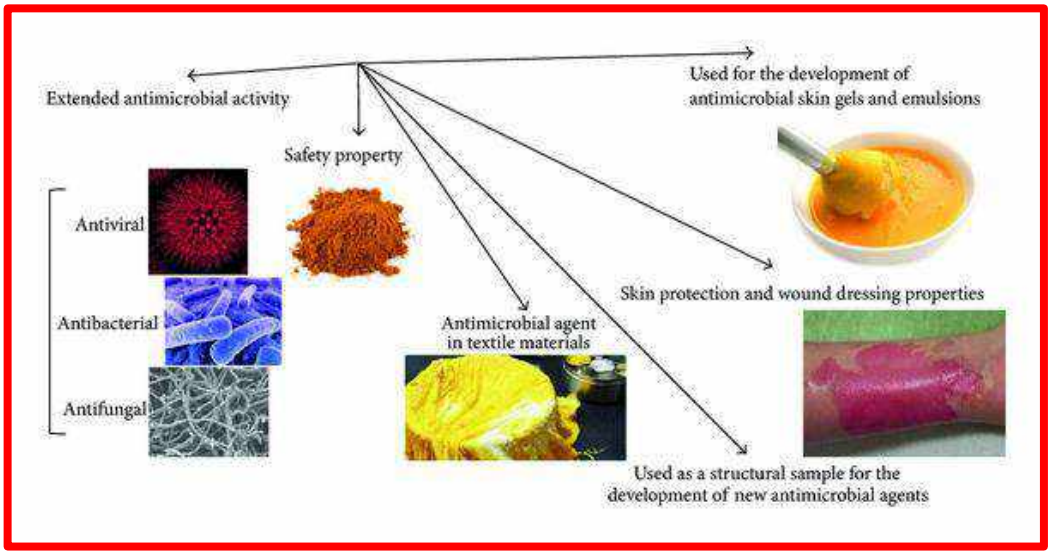



**Figure 9: Application of turmeric.**

"The bacteria hide under the gastric mucous layer where antibiotics do not penetrate effectively. This often leads to recurrent infections and gives rise to resistant strains," says Goycoolea, professor, the school of food science and nutrition in leeds in the UK. Curcumin is a natural anti-inflammatory compound. It helps our body to fight over foreign invaders and also has a role in repairing damage. Without inflammation, pathogens like bacteria could easily take over our body and may kill us. Hence this turmeric spice not only as a dye but also act as antibiotic material. It is strongly recommended in the skin related problems and anciently it was used for the wounds and now too it's continued. Turmeric is highly used by the women in their food items for colour and they apply to skin to enhance their beauty too. These all are possible only by knowing the antimicrobial activity of turmeric spice and its effect on the micro-organisms.

**Figure 10: Turmeric powder.**

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