

# Antibacterial Protection of Polyphenols from Curry Leaves (*Murraya koenigii*): A Research Study

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**Abstract:-** The intention of the present study is to find the efficiency of the anti-bacterial effect of polyphenols of Curry leaves (*Murraya koenigii*) plant leaves against the pathogen *staphylococcus aureus*. The materials involved in this study include *Murraya koenigii* plant leaves, micro-organism *staphylococcus aureus* in the bacterial type culture collection, agar, and blood-agar plates. At 10% concentration, of Polyphenols of *Murraya koenigii* leaves had zero anti-bacterial activity, while between 20 to 25% concentrations revealed high activity against the bacteria. Thus, increased in the anti-bacterial activity was promising as the concentration augmented from 20 to 25%. The results acquired from this study points that polyphenol of Curry leaves (*Murraya koenigii*) had antibacterial property against *Staphylococcus aureus* when obtained at appropriate concentration.

**Keywords:-** Curry Leaves Plant, *Murraya Koenigii*, *Staphylococcus Aureus*, Anti-Bacterial Effect, Polyphenols

## I. INTRODUCTION

The traditional usage of plants / herbs / vegetables and its products in the form of extracts have staged a vital role in the remedy of numerous pathologies<sup>[1]</sup>. Drugs owing to their origin in plants possess medicinal value are referred to as the herbal groups<sup>[2]</sup>. The use of such traditional medicines in the form of Ayurveda or Chinese practice has a staunch foundation in Asia, which is now widely followed in developed nations in the rest of the world<sup>[3]</sup>. In a very recent report, the World Health Organization reported that over 76% of the population in the world depend predominantly on traditional remedies that encompass the custom of herbal extracts or their dynamic ingredients<sup>[4]</sup>. Edible, spices and herbal / medicinal plants are becoming promising and popular alternative for microbial diseases and conditions due to they are inexpensive, easily available and tend to have negligible side effects than available synthetic drugs<sup>[5]</sup>. It

was studied to find Tomato plant axillary green shoots resulting from pruning and aerial biomass and mushrooms were characterized for their composition in chlorophylls and phenolic compounds and results were more promising<sup>[6-7]</sup>.

A study was conducted to evaluate the antioxidant and antimicrobial activities of *Auricularia* and *Termitomyces* extracts against the extracts was tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, MRSA, *Candida albicans*, and *Candida parapsilosis*. The hot water extract more potent activity when compared to other extracts<sup>[8]</sup>. Researchers reported that, extracts of edible plants from different countries like China, Japan, Thailand and Yemen for antibacterial activity when they were screened against *Bacillus cereus* and *Staphylococcus aureus*<sup>[8]</sup>. In the same way, the nanoemulsions of *Thymus daenensis* was analyzed for its antibacterial activity and results revealed a noticeable antimicrobial activity against selected microorganism *S. aureus*<sup>[9-11]</sup>. It was reported that, partially purified proteins from Turmeric rhizome showed antimicrobial activities. It was also reported by the same researchers that, plant extracts from shown antioxidant and antimicrobial activities<sup>[12-14]</sup>.

In this study, we focused on antibacterial activity against human pathogenic bacteria *S. aureus* by polyphenol enriched extract of *Murraya koenigii* leaves.

## II. MATERIALS AND METHODS

The present *in-vitro* study was piloted to study the antibacterial efficiency of different concentrations of polyphenols extract of Curry leaves (*Murraya koenigii*) against *S. Aureus*.

Curry leaves are washed thoroughly with water and rinsed in 0.5%  $\text{KMnO}_4$  for five minutes and again washed in

double distilled water to remove if any microbes present. Further, leaves were shade dried, powdered, sieved and stored in a dry glass container for further use. Polyphenol extraction was done by mixing 25g of Curry leaves powder was mixed with 250mL of methanol, followed with Soxhlet extractor for 72 h. Later, the excess methanol solvent was evaporated. In the same way, the extraction was done with other solvents like hexane, chloroform, ethyl acetate and butanol to obtain hexane, ethyl acetate, chloroform-butanol and residual methanol fractions, respectively. Finally, all crude extracts were mixed, filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure<sup>[15]</sup>.

#### A Proximate analysis

The extract was subjected to phytochemical analysis to check the presence of bioactive compounds by using standard protocols<sup>[16-19]</sup>.

The protein estimation was carried according to Bradford's method<sup>[20]</sup> using BSA as standard and absorbance was read at 535nm. Total phenolics was determined according to the method of Folin Ciocalteu reaction<sup>[21]</sup> using gallic acid as a standard and absorbance was read at 750 nm. Ascorbic acid estimation was carried out according to Sadasivam S., Manickam[20] and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done according to Dubois method<sup>[22]</sup> and the absorbance was read at 520 nm. Flavonoids estimation was done according to Cheon et al<sup>[23]</sup> by using Quercetin as a standard and the absorbance was measured at 415 nm. In the above analysis, standard curve was used to compare.

#### B Preparation of cultural media

*Staphylococcus aureus bacteria* obtained from a local Culture Collection and Gene Bank was added to a liquid infused with nutrient broth and incubated at 37°C for a period of 24 hours. The additive culture is then cultured on the nutrient agar plate, and it was passed through an incubation cycle at a temperature of 37°C for a time period of 24 hours.

#### C Well plate method

The anti-bacterial efficiency of different concentrations of *Murraya koenigii polyphenol* extract against *S. aureus* was tested with the help of well plate method. Wells were prepared in Petrie-dishes with the aid of a punch. The wells were packed with the equivalent quantity of *Murraya koenigii polyphenol* extract. The entire process was repeated to test the four different concentrations of extract. The well plates were then incubated at 37°C for a period of 48 hours.

#### D Study process

The wells were intended on the blood agar plates with the aid of a punch consisting of 3mm radius. An equal quantity of each of 10, 15 and 20 and 25% *Murraya koenigii polyphenol* extract was rested onto Petri dishes. The plates were kept at the normal temperature for a period of 1 hour which was then followed by incubation at 37°C for a period

of 48 hours. The zone of inhibition was then examined and noted in millimeters.

#### E Minimum inhibitory concentration (MIC)

By Serial dilution method in the nutrient agar, the minimum inhibitory concentration of isolated extract was determined, with concentrations like 10, 15, 20 and 25µg at a ratio of 1:10. Plates were incubated for 24 h at 37°C. MIC was recorded as the lowest extract concentration demonstrating no visible growth in the broth<sup>[24]</sup>.

### III. RESULTS AND DISCUSSION

The polyphenol extract of Curry leaves was subjected to proximate analysis. It was noticed that, the extract rich with Polyphenols when compared to other phytochemicals.

Table 1 showed the effects of different concentrations of Polyphenol enriched Drum stick plant leaves extracts on *S. aureus*. There was nearly zero zone of inhibition detected with 10% Drum stick leaves extract. Zone of inhibition of 15.0 mm was witnessed with 15% extract and zone of inhibition of 23.0 mm was witnessed with 20 and 25% extract.

Concentration of Drum stick plant leaves extract (%)	Zone of inhibition (in mm)
10	0
15	15
20	23
25	23

The minimum inhibitory concentration of Curry leaves polyphenol extract against staphylococcus was 15.5±0.5 µg at a ratio of 1:10 (w/v)

In our study, the polyphenol extract of *Murraya koenigii* was done as explained in materials and methods. It was analyzed for its antibacterial activity against human pathogenic bacteria by well plate method, where streptomycin was used as positive control. The results showed a promising inhibition of bacterial growth which was compared with standard. In MIC studies, it was observed that the MIC value of 15.5±0.5 µg at a ratio of 1:10 (w/v). The MIC value of extract compared with standard antibiotics, which ranged from 19 to 20 µg (1:10 w/v). Thus the polyphenol extract of Curry leaves is inhibiting the growth of *S.aures* strain and further studies to be done in this direction to find which active polyphenol is responsible for the above.

### IV. CONCLUSION

The anti-bacterial activity of the Curry leaves polyphenol extract was perceived with 10%, 15%, 20% and 25%. Anti-bacterial action augmented as the concentration amplified from 15 to 25%. With the results attained from the study, it can be determined that polyphenol extract of Curry leaves have antibacterial property against *S. Aureus*.

### ACKNOWLEDGEMENT

The authors gratefully thank Adichunchanagiri University for providing facility and opportunity to conduct the above studies at Adichunchanagiri Institute for Molecular Medicine and Sri Adichunchanagiri College of Pharmacy.

### REFERENCES

- [1]. Makadia N, Vaghasia M, Patel DK, Patel H, Kaur R, Shah J. 2016. Anti-Microbial Effect of Cardamom Extract on *Staphylococcus Aureus*: An Original Research Study. *Int J Oral Health Med Res.* 3(3):28-30.
- [2]. Sahraie-Rad M, Izadyari A, Rakizadeh S, Sharifi-Rad J. 2015. Preparation of strong antidandruff shampoo using medicinal plant extracts: a clinical trial and chronic dandruff treatment. *Jundishapur Journal of Natural Pharmaceutical Products* 10(4): e21517
- [3]. Shankar A, Dubey A, Saini D, Prasad CP. 2020. Role of Complementary and Alternative Medicine in Prevention and Treatment of COVID-19: An Overhyped Hope. *Chinese Journal of Integrative Medicine.* 26(8):565.
- [4]. World Health Organization. Guidance on mainstreaming biodiversity for nutrition and health. 2020
- [5]. Alfei S. 2020. Nanotechnology Applications to Improve Solubility of Bioactive Constituents of Foods for Health-Promoting Purposes. In *Nano-food Engineering Springer, Cham.* 189-257.
- [6]. Añibarro-Ortega M, Pinela J, Ćirić A, Martins V, Rocha F, Soković MD, Barata AM, Carvalho AM, Barros L, Ferreira IC. 2020. Valorisation of table tomato crop by-products: Phenolic profiles and in vitro antioxidant and antimicrobial activities. *Food and Bioproducts Processing.* 1;124:307-19.
- [7]. Bach F, Zielinski AA, Helm CV, Maciel GM, Pedro AC, Stafussa AP, Ávila S, Haminiuk CW. 2019. Bio compounds of edible mushrooms: In vitro antioxidant and antimicrobial activities. *LWT.* 1;107:214-20.
- [8]. Gebreyohannes G, Nyerere A, Bii C, Sbhutu DB. 2019. Investigation of antioxidant and antimicrobial activities of different extracts of *auricularia* and *Termitomyces* species of mushrooms. *The Scientific World Journal.* 1;2019.
- [9]. Moghimi R, Aliahmadi A, Rafati H. Antibacterial hydroxypropyl methyl cellulose edible films containing nanoemulsions of *Thymus daenensis* essential oil for food packaging. *Carbohydrate polymers.* 2017;1:175:241-8.
- [10]. Petrović J, Stojković D, Soković M. Terpene core in selected aromatic and edible plants: Natural health improving agents. In *Advances in food and nutrition research* 2019;90:423-451. Academic Press.
- [11]. Panda SK, Mohanta YK, Padhi L, Luyten W. Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens. *LWT.* 2019;1;113:108246.
- [12]. Dinesha, R., Thammanna Gowda S.S., Harsha R and Leela Srinivas. Antioxidant and antimicrobial activity of partially purified proteins from hot water extract of Turmeric (*Curcuma longa* L). *Pharmacologyonline,* 2010;1:996-1004.
- [13]. Dinesha Ramadas and Leela Srinivas. Antioxidant effects of 28kda Protein from Turmeric (*Curcuma Longa* L). *Asian Journal of Pharmaceutical and Clinical Research,* 2011;4(1):75-79.
- [14]. Adamczak, A.; Ożarowski, M.; Karpiński, T.M. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. *J. Clin. Med.* 2020; 9:109.
- [15]. Hossain MA, Al-Hdhrami SS, Weli AM, Al-Riyami Q, Al-Sabahi JN, Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman, *Asian Pacific Journal of Tropical Biomedicine,* 2014; 4(1):S368-S372.
- [16]. Dinesha, R., Thammanna Gowda S.S., Harsha R and Leela Srinivas. Antioxidant and antimicrobial activity of partially purified proteins from hot water extract of Turmeric (*Curcuma longa* L). *Pharmacologyonline,* 2010 1: 996-1004.
- [17]. Ningappa M.B., Dhananjaya B.L., Dinesha R., Harsha R. and Leela Srinivas, "Potent anti-bacterial property of APC protein from Curry leaves (*Murraya koenigii* L), *Food Chemistry,* 2010, 118, I3,747-750.
- [18]. Sivapriya M, Dinesha R, Harsha R, Gowda SST and Leela Srinivas, Antibacterial activity of different extracts of Sundakai (*Solanumtorvum*) fruit coat, *International Journal of Biological chemistry,* 2011, 5(1):61-67.
- [19]. Chikkanna D, Dinesha R. Amitha R and Subhas Chandrappa Mundasada, Antibacterial activity of Pippali proteins (*Piper longum*), *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology.* 2015, 3(2), 49 - 54.
- [20]. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein. *Analytical Biochemistry.* 1976;72; 248-54.
- [21]. Kujala TS, Ioponen JM, Klika KD, Pihaja K. Phenolics and betacyanins in red beetroot (*Beta Vulgaris*) root: description and effect of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural Food Chemistry,* 2000; 48;5338-5342.
- [22]. Sadasivam S, Manickam A. In. *Biochemical Methods.* New Age Internationals, 1997; 184-186.
- [23]. Dubois M, Gilles, KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugar and related substances. *Analytical Chemistry.*1956; 28;350-356.
- [24]. Cheon BS, Kim YH, Son KS, Chan HW, Kang SS, Kim HP. Effects of prenylated flavonoids and bioflavonoids on lipopolysaccharide-induced nitric oxide production from the mouse macrophage cell line RAW. *Plant Medicine,* 2000;66;596-600.