Inhibitory activity of organic solvent extracts of *Murraya koenigii* **on** *Staphylococcus aureus*

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Abstract-*Staphylococcus aureus* is one of the most in famous and widespread bacterial pathogen. This pathogen can cause a wide variety of diseases, ranging from moderately severe skin infections to fatal pneumonia and sepsis. Treatment of *Staph. aureus* infections is complicated due to antibiotic resistance. There has been ongoing and increasing interest in the extraordinarily high number of toxins and other virulence determinants that *Staph. aureus* produces and how they impact disease. In the past few decades, a more dangerous form of *Staph.aureus* has emerged. This form is known as Methicillin-Resistant *Staph.aureus* and usually referred to by the acronym MRSA. Keeping in view of the problem of Antimicrobial Resistance(AMR).This study was designed to explore antibacterial activity of a common kitchen herb *Murraya koengii* against pathogenic gram positive *Staphylococcus aureus*.Four different plant extracts were prepared using solvents- Methanol, Hexane, Aqueous and Ethanol+Aqueous (1:1) and tested against *Staphylococcus aureus* by using Kirby bauer Disc diffusion method. Methicillin antibiotic disc was used as the positive control.

Keywords: *Murraya koenigii*, Antibiotic resistance, MRSA, Antimicrobial activity, Methicillin

Introduction

Unnecessary use of antibiotics in today's world is one of the major causes for the rising emergence of multi drug resistant pathogenic strains that do not respond to the usual line of treatment. Therefore, the need to search for new antimicrobials remains unchallenged. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicines such as natural or herbal medicines is increasing because they may have fewer side effects or toxicity owing to their natural sources (Anita Joshi et al, 2009).

Curry leaves (*Murraya koenigii*) belongs to the Rutaceae family, and is native to India and the Southeast Asian Region.It is a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. Curry leaves are natural flavoring agents with a number of important health benefits. They contain several medicinal properties such as anti-diabetic, antioxidant, antimicrobial, anti-fungal, anti-inflammatory, anti- carcinogenic and hepato-protective properties. The various notable pharmacological activities of the plant include activity on heart, anti-diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative

property, cytotoxic activity, antidiarrheal activity, phagocytic activity(Bhandari PR, 2012 and Disegha GC., et al 2014). These leaves are widely used in Asian cuisines for flavouring foods. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying. Curry leaf is also used in many traditional cultures namely Indian. Ayurvedic and Unani prescriptions (Suman Singh et al, 2014).

Staph. aureus bacteremia has been noted to account for a greater number of deaths than that caused by acquired immune deficiency syndrome (AIDS), tuberculosis, and viral hepatitis combined (Klevens RM, Morrison MA, Nadle J, et al.) Other *Staph. aureus* infections, such as moderately severe skin infections, including furuncles, abscesses, and wound infections, are usually not life-threatening but may be accompanied by significant morbidity and pain. Due to their frequency they represent a considerable public health burden (McCaig LF, McDonald LC, Mandal S, et al.)

Staph. aureus infections are particularly problematic due to frequently occurring antibiotic resistance in *Staph. aureus* isolates, among which methicillin-resistant *S. aureus* (MRSA) are the most important clinically (Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al.). Infections by MRSA are accompanied by increased mortality, morbidity as compared to those caused by methicillin-sensitive *S. aureus* (MSSA) (Ippolito G, Leone S, LauriaFN, et al.)The rates of methicillin resistance among clinical isolates varies greatly by country, ranging from single-digit rates in Scandinavian countries to over 50% for example in the U.S. and China (Stefani S, Chung DR, Lindsay JA, et al).While hospital-associated MRSA infections are on the decline in the U.S., Europe, China, and many other countries, likely due to increased hygiene and surveillance measures, they are still on the rise in poorly developed countries, for example in Africa (Falagas ME, Karageorgopoulos DE, Leptidis J, et al).

Material and Methods

The plants used in this study were obtained from Himalaya Wellness Company, Dehradun. The curry leaves were shade dried for 3 days and powdered using an electric blender.

Methanol extraction: The methanol extract was made by, adding 50 gms of curry leaves powder in 250 ml of methanol (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Ethanol extraction: The ethanol extract was made by, adding 50 gms of curry leaves powder in250 ml of ethanol (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Hexane extraction: The extract was made by, adding 50 gms of curry leaves powder in250 ml of hexane (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Hydro-alcoholic extraction: 50gms of curry leaves powder in 250 ml of solvent containing ethanol and aqueous in the ratio 1:1(w/v). Flask containing samples were kept on rotary shaker for 24 hours.

After completion ofshaking the extracts were filtered using whattmanfilterpaper. The filtered extracts were collected and concentrated on waterbath at 60°-80°c to make the final volume of the curry leaf extracts for the experiment.

Determination of antimicrobial activity: Antibacterial activity of extracts of the

Murrayakoenigii was evaluated by the Kirby bauer Disc diffusion method. Nutrient agar media was prepared and inoculated with *Staph.aureus.* Inoculated media was poured in sterile petridish and kept until plates get solidified. The sterile disc wereplaced in extracts separately (40-50 µl microliter in each disc).After that Sample impregnated discswere placed on nutrient agar plates, using a sterile forcepand the plates were incubated at 35-37°c for 24 hours. The inhibition zone around each disc was measured in millimeters (mm). The standardMethicillin antibiotics disc used as positive control to test the curry leaves microbial inhibition activity.

Results and Discussions

The antibacterial effects of curry leaves (*Murraya koenigii*) extracts as tested on test strain samples and compared with known antibiotics are described with inhibition zones measured in millimeters.

Table -1

Extracts	**Zone of Inhibition** *Staphylococcus Aureus* **(Standard Atcc) Culture**	**Zone of Inhibition** *Staphylococcus Aureus* **(Clinical Isolates)**
Methanol	15 mm	11 mm
Hexane	10 mm	NA
Aqueous	10 mm	NA
Ethanol + Aqueous	16 mm	18 mm
Methicillin	25 mm	15 mm

Results of this study is as shown in table-1, in case of standard culture of *Staph.aureus* the highest zone of inhibition of 16 mm was found in hydro-alcoholic extract, followed by other extracts methanol (15 mm), hexane (10 mm), aqueous (10 mm). 25 mm zone inhibition towards Methicillin(Plate-1and 5). While Clinical isolate of *Staph aureus* showed highest zone of inhibition(18mm) towards Ethanol+Aqueous extract of *Murraya koengii* Plate-1 and 2.While no or very small zone of inhibition against Methicillin antibiotic.

Zones of Inhibition

Staphylococcus aureus **(Standard ATCC culture)**

Plate-1

Plate-2

Plate-3

Plate-4

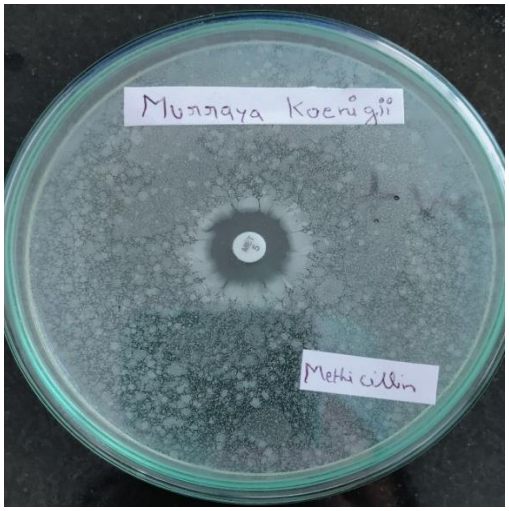

Plate-5

Zones of Inhibition

Staphylococcus aureus **(Clinical isolates)**

Plate-1

Plate-2

Indiscriminate use of antibiotics has led to the rising emergence of multi drug resistant pathogenic strains of bacteria causing diseases that challenge regular treatment protocols. Currently, in addition to antibiotics and chemically synthesized drugs,

the trend to look out for alternative medicine in nature is increasing as the natural resources are less toxic and less deleterious to the overall health of human beings (Anita Joshi et al, 2009) In this context curry leaves have displayed immense potential as that natural alternative, especially with its antimicrobial property. Curry leaf extracts have demonstrated inhibition zone against *Staphylococcus aureus*. Subsequently, fully susceptible bacteria exhibited a large zone of growth inhibition around the disc; less sensitive (intermediate sensitive) isolates exhibited a smaller inhibition zone and resistant bacteria indicated no clear zones or grew up to the disc edge (Mohar Singh A et al, 2011&(Rajendran MP et al, 2014)).

Conclusion

Murrayakoenigii (Curry leaves) extracts have demonstrated antibacterial effects particularly on *Staphylococcus aureus*, as compared to antibiotics such as methicillin in our study. The hydro-alcoholic extract (ethanol + aqueous) of curry leaves were found to be most effective than other extracts against clinical isolate which is highly resistant towards antibiotic methicillin. *M.koenigii* has the potential to develop plant based antimicrobial drug it may be the best natural alternative to antibiotic therapy for *Staphylococcus aureus*. Therefore, curry leaves could be used as a natural remedy for the prevention of bacterial infections. Indeed this phenomenal plant may serve as a useful resource in the food industry and clinical medicine. Detailed research study is required on *Murrya koengii* to further explore its antibacterial activity and its future role as an alternative plant based drug against pathogenic Staphylococci.

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