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RESEARCH ARTICLE

# Anti-microbial efficacy of Artemisia nilagirica and Cocculus hirsutus against Methicillin Resistant Staphylococcus aureus

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Abstract: ***Background***: Antibiotic resistance among those bacteria is a growing concern. MRSA, or Methicillin-resistant Staphylococcus aureus, is a serious type of bacteria resistant to antibiotics in the penicillin family. Staphylococcus aureus is usually found on the skin and about 1/3 of the population has this bacteria in their noses. These infections typically show up as skin infections, like abscesses or boils. Clinical manifestations of MRSA include abscess or invasion by lymphatic, blood, and major organs. Hence, there is a need to eradicate these resistant bacteria from the oral flora, as there is resistance against various antibiotics; there is a need for several natural compounds which may help in the eradication and prevention of biofilm formation. ***Aim:*** The aim of the study to evaluate the antimicrobial effects of these two plants against Methicillin Resistant Staphylococcus aureus .***Materials And Method***: Fresh leaves of Artemisia nilagirica and Cocculus hirsutus were collected from Nilgiri district, Tamil Nadu, India. Plant leaves were cleaned with deionised water and dried in the shade for a week. The powder was extracted and stored in an airtight container at 4°C. Solvent systems used for the extraction were methanol. A portion of the powdered plant samples was soaked in the conical flask containing solvent, wrapped with aluminium foil and placed in a shaker for 48 hours at 120-130 rpm. After 48 hours, the solvent was evaporated and extract was stored at 4°C and MIC was carried out.***Results:*** The MIC tests of A. nilagirica, C. hirsutus and organic solvent extracts against Methicillin Resistant Staphylococcus aureus were carried out using the micro dilution technique. The concentration of the extracts used were 40 µg/ml. There was no inhibition of growth of S. aureus found with both the plants.***Discussion:*** Recent studies have shown that A. nilagirica, showed inhibitory activity for gram-positive and gram-negative bacteria except for, Enterococcus faecalis and Staphylococcus aureus which predicts that these plant extracts didn't have antimicrobial action against Staphylococcus aureus .***Conclusion*** : There were no antimicrobial effects of A. nilagirica and C. hirsutus against MRSA.

Keywords: Antimicrobial, Artemisia nilagirica, Cocculus hirsutus, MIC, MRSA.

## INTRODUCTION

Antimicrobial resistance among common bacteria is a growing concern in the world and has led to the death of millions of peoples (Murray et al. 2022). And it is estimated to grow further due to continued use of antimicrobial in inadvertent manner and non-compliance with guidelines on antimicrobial use for treatment of infections. Furthermore, it has been observed that microbes have been evolving genetically to develop resistance to antimicrobial drugs. This created a huge challenge to clinicians and put a burden on individuals and health services.

Antibiotics derived from the fungus or bacteria are basically the metabolites or secondary products which help the pathogen to grow(Clardy, Fischbach, and Currie 2009). One of the ways microbes can grow is to compete for energy sources or create an environment conducive to them only. This is achieved by eliminating the other competing microbes by producing the metabolites or secondary metabolites which can be lethal to them. One of the commonly known metabolites is penicillin. The penicillin was first antimicrobial developed in 1928 by Alexander fleming. He derived penicillin from the fungus Penicillium notatum. Similarly, many of antimicrobial drugs currently present in the market are derived from fungus or bacteria present in the environment. However, besides the above mentioned factor involved in antibiotic resistance development, recently quorum sensing (QS) mechanisms have been suggested to play a major role(Zhao, Yu, and Ding 2020). QS, thereby, makes microbe derived antimicrobials ineffective against other microbes. Besides, they can have several side effects besides resistance. As a result, the search for newer microbes has extended to natural sources other than pathogens.

Several ancient texts have written about the herbal preparation for treatment of wound and infection in ayurveda, greek and roman treaties. These herbal plants have an inherent defence mechanism to defend against pathogens through release or formation of metabolites or natural biochemical compounds (Kaur et al. 2022; Agrios 1988), It includes flavonoids, polyphenol oxidase, phytoalexins, melatonin etc. which protect plants against pathogens. Flavonoids, is the important

class of phenolic compounds, with antimicrobial activity (Shamsudin et al. 2022; Tsuchiya et al. 1996). It is reported to have antimicrobial Methicillin resistant Staphylococcus aureus (MRSA). (Tsuchiya et al. 1996). Flavonoids have been found to inhibit the growth of pseudomonas aeruginosa by inhibiting the QS receptors (Paczkowski et al. 2017). Further, flavonoids have little effect on humans (Lee, Kang, and Cho 2007). Give n these advantages, a flavonoid-containing plant appears to be a promising source for the development of antimicrobial drugs.

Artemisia is a genus of plants belonging to the daisy family Asteraceae. Several species of this family have reported to contain a flavonoid and are reported to have antimicrobial activity against several pathogenic bacteria (Ahameethunisa and Hopper 2010; Arokiyaraj et al. 2012); (Kasmiyati et al. 2021; Ewunu, Awoke, and Getachew, n.d.). In addition, it has anti-inflammatory properties (Bhat et al. 2017; Parameswari, Devika, and Vijayaraghavan 2019). Similarly, Cocculus hirsutus also has anti-inflammatory and anit-microbial properties (Gupta et al. 2018).

Therefore, the study was conducted to evaluate the antimicrobial effects of Artemesia nilagirica and Cocculus hirsutus against MRSA.

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# MATERIALS AND METHODS

**Plant material and extraction**

The fresh leaves of Artemisia nilagirica and Cocculus hirsutus were purchased from a local herbal garden. Sufficient amounts of leaves were collected and washed with distilled water. After that leaves were kept for air dry under the room temperature without direct sunlight exposure. The leaves were grinded using the grinder and filtered. The powder was stored at 4 degree celsius for further use.

Methanol as solvent was used for the cold extraction process in soxhlet technique. 5 gm of powdered leaves were added to the beaker containing methanol and covered with aluminium foil. After 48 hours, the extract was scraped, dried, filtered, and kept at 4 degree celsius.

**Estimation of minimum inhibitory concentration (MIC) values**

The MRSA subculture was prepared for an antimicrobial test. Broth microdilution was done followed by distribution into appropriate wells of tubes and minimum Inhibitory Concentration (MIC) was calculated. The MIC value is the lowest concentration of antibiotic at which bacterial growth is inhibited

# RESULTS AND DISCUSSION

**Figure 1 : Leaves of A)** ***Atremesia nilagirica ; B) Cocculus hirsutus***


**Figure 2: Plant extracts (methanolic)**

**Figure 3: Dried and grinded leaves**

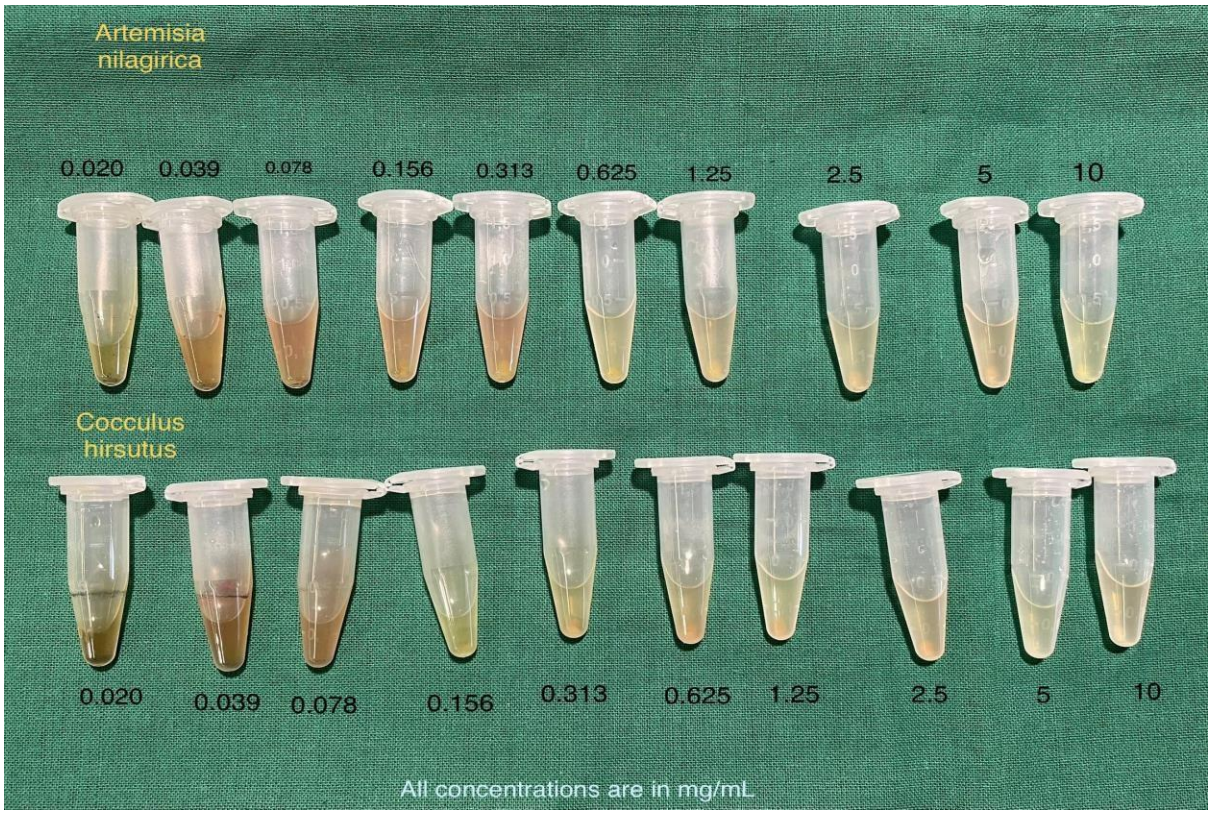


**Figure 4: Serial dilution of plant extract for evaluation of Minimum Inhibitory Concentration need for inhibition of MRSA**

**Table 1: Results of MIC using broth dilution method. Both plant extracts showed antimicrobial activity at higher concentration (10 mg/ml).**

| Sr. No | Concentration | Minimum inhibitory concentration / Growth | |
|---|---|---|---|
| | | ***A nilgarica*** | ***Cocculus hirsutus*** |
| 1 | Control | Growth | Growth |
| 2 | 10 mg/ml | MIC | MIC |
| 3 | 5 mg/ml | Growth | Growth |
| 4 | 2.5 mg/ml | Growth | Growth |
| 5 | 1.25 mg/ml | Growth | Growth |
| 6 | 0.625 mg/ml | Growth | Growth |
| 7 | 0.313 mg/ml | Growth | Growth |
| 8 | 0.156 mg/ml | Growth | Growth |
| 9 | 0.078 mg/ml | Growth | Growth |
| 10 | 0.039 mg/ml | Growth | Growth |
| 11 | 0.0.20 mg/ml | Growth | Growth |

This may be due to the methanol extraction method that may not have significant antimicrobial activity against MRSA. Ahameethunisa et al. (Ahameethunisa and Hopper 2010) reported no antimicrobial activity of methanol extract against *S aureus*. However, in the same study, *A. nilagirica* extract carried out using hexane solvent found to be effective against S. aureus. In another study, methanol extract of A. nilagirica showed moderate activity against patho gens (Arokiyaraj et al. 2012). Similar observation was noted in *C. hirsutus* methanol extracts, which was consistent with other studies (Singh, Patni, and Meena 2015; "Screening Antibacterial Activity of Root Extract of Cocculus Hirsutus (L.) Diels. Used in Folklore Remedies in South India" n.d.).

Present study results consistent with previous studies on extraction methods indicates that the extraction method plays an important role in extracting the compounds that have antimicrobial activity (Martins and da Conceição 2015; "Influence of Extraction Conditions on Chemical Composition and Thermal Properties of Chestnut Wood Extracts as Tannin Feedstock," n.d.; Alam, Sartini, and Alfath 2019). There are several methods of solvent extraction used for assessing the antimicrobial activity of plants. Although, there is no clarity on the best method of extraction. It appears that the nature of compound and plant may decide the solvent method's success in extracting the compound of interest and its antimicrobial activity. For example, the ethanolic extract and crude alkaloidal fraction of *C. hirsutus* reported to show significant antimicrobial activity against the pathogenic bacteria whereas another study showed that the methanol extract has better antimicrobial activity against Gram positive and Gram negative bacteria.

Plants usually have different parts such as leaf, root, barks, stemetc. which can exhibit antimicrobial activity. It is important to identify which part of the plant can yield the maximu m amount of antimicrobial compounds. The leaf extract from *A nilagirica* showed potent activity against *C. albicans, P. aeruginosa, B. subtilis, S. typhi, S. aureus and E. coli* . Similarly, different parts of *C. hirsutus* also show the effective properties. In the present study, leaves extract was tested for anti-microbial activity. Similar observations were seen in Sathish et al. study where they observed antimicrobial activity against E. Coli. In addition, antimicrobial activity was also reported in the root part of C. Hirsutus (Nayak and Singhai 2003). However, there is limited evidence on antimicrobial activity of bark or stem of *C hirsutus* or *A nilagirica*. It would be interesting to see their potential antimicrobial activity

# CONCLUSION

Chirsutus or A nilagirica leaf extract showed antimicrobial activity, however, at higher concentration which may not be preferred. However, further studies involve exploration of compounds from leaf extract . Furthermore, different extraction methods and different parts of the plant could assist in the development of natural antibiotics with potent antimicrobial activity.

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**Conflict of Interest**

The authors declare no potential conflict of interest.

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