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REGULAR ARTICLE

# ANTIMICROBIAL ACTIVITY OF SELECTED INDIAN MEDICINAL PLANTS

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## SUMMARY

The traditional medicine still plays a important role in the primary health care, three Indian medicinal plants *Aegle marmelos, Solanum nigrum* and *Cassia fistula* were extracted by soxhlet extraction method. These three plant materials were subjected to preliminary phytochemical screening activity against Gram negative organism of *Escherichia coli* (NCIM: 2065) and gram positive organism of *Staphylococcus aureus* (NCIM: 2079) and they were compared with control drug Penicillin at different concentrations at 0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml by disc diffusion method. At higher concentration of 2.5mg/ml *Cassia fistula* exhibits maximum zone of inhibition of about 30.9mm against *Staphylococcus aureus,* and is considered as susceptible. Such that zone of inhibition not found in *Aegle marmelos* and *Solanum nigrum,* and are considered as resistant. In case of *Escherichia coli*, *Solanum nigrum* exhibits maximum zone of inhibition of about 30.1mm such zones are not found in *Aegle marmelos* and *Cassia fistula,* and are considered as resistant and control drug penicillin shows less activity compared to the plant extract *Aegle marmelos*, *Solanum nigrum*, and *Cassia fistula*

**Keywords**: Agar diffusion method, *Escherichia coli*, *Staphylococcus aureus*, *Aegle marmelos*, *Solanum nigrum*, *Cassia fistula.*
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## 1. Introduction

Plant derived drugs remains important resource especially in developing countries, to combat serious disease. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). And natural products play on important role in drug development programmes in the pharmaceutical industry (Baker et al., 1995).

There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999 and Ignacimuthu et al., 1998).

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (Samy

et al., 1998; Hamil et al., 2003; Motsei et al., 2003).

The development of drug resistance in human pathogens against commonly used antibiotics has necessitate the search for new antimicrobial substance from other sources. Screening of medicinal plants for antimicrobial activities and phytochemical is important for finding potential new compounds for therapeutic uses.

## 2. Materials and Methods

***Collection of plant materials, Bacterial strains and Growth conditions***

The plant materials *Aegle marmelos, Solanum nigrum* and *Cassia fistula* were collected in and around Gudiyatham, Vellore District, Tamil Nadu. And they were identified and authenticated by the Chief Botanist, Tamil Nadu Aromatic Medicinal Plants Corporation Limited (TAMPCOL), Arignar Anna Siddha Medical College and Hospital Campus, Chennai, Tamil Nadu, India. The following cultures *Escherichia coli*: NCIM 2065, and *Staphylococcus aureus* NCIM 2079 were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. Cultures of these bacteria were checked for purity by doing Gram staining and biochemical test and they were grown in nutrient broth at 37˚C and maintained in nutrient agar slants at 2-8˚C.

***Selection of Reference antibiotic***

Reference antibiotic Penicillin was obtained from authorized medical shop Chennai. The purity of the antibiotic is 99.8%

***Alcoholic extraction of plant materials***

The plant materials were dried in shade and powdered in a mechanical grinder. The powder of *Aegle marmelos, Solanum nigrum* and *Cassia fistula* were initially defatted with petroleum benzene at (60 - 80˚C) followed by 1000 ml of ethanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whattman filter paper (No. 1) and then concentrated in vaccum and dried at 45˚C, for ethanol elimination. The extract were kept in a sterile bottle under refrigerated condition about 2-8˚C for further analysis.

***Dilutions and Inoculum preparations***

The dried plant extracts of *Aegle marmelos, Solanum nigrum, Cassia fistula* and antibiotic Penicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 0.5, 1.0, 1.5, 2.0, 2.5mg/ml. (Class A glassware were used for dilution purpose). The inoculum of *Escherichia coli* and *Staphylococcus aureus*, were prepared in nutrient broth medium and kept incubation at 37˚C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8˚C for analysis.

***Procedure for performing the Disc Diffusion test (Bayer et al., 1966)***

The required amount of Mueller-Hinton plates is prepared as per manufacturer instructions, (Himedia) and autoclaved at 121˚C for 15 minutes. And they were allowed to cool under Laminar air flow (Class 100). Aseptically transfer about 25 ml of media into each sterile Petri dishes and allowed to solidify. A readily prepared sterile cotton swab (Himedia) was dipped into the turbid culture suspension. The dried surface of Muller-Hinton agar plate was inoculated by streaking two more times rotating the plate approximately 60˚ each time. The lid may be left aside for 3-5 minutes to dry the excess surface moisture content.

The readily prepared sterile discs (Himedia) were loaded with different concentrations of about 0.5, 1.0, 1.5, 2.0 and 2.5mg/ml of plant extract of *Aegle marmelos, Solanum nigrum, Cassia fistula* and antibiotic penicillin into each separate disc of about 100µl. The discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to permit good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone arou00nd the disc using zone reader (Hi media).

## 3. Results and Discussion

In this study, the results shows that three extract from the aerial parts of *Aegle marmelos*, *Solanum nigrum* and *Cassia fistula* posses antimicrobial activities against screening of the tested organism at different concentration such as 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml.

Table 1. Antibacterial activity of different concentration of extract of *Aegle marmelos*, *Solanum nigrum* and *Cassia fistula* on *Escherichia coli*, *Staphylococcus aureus* using Penicillin as a control drug by agar diffusion method

| S. No. | Microorganism | Concentration (mg/ml) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|---|
| | | | Plant material | | | Antibiotic |
| | | | *Aegle marmelos* | *Solanum nigrum* | *Cassia fistula* | Penicillin |
| 1. | *Staphylococcus aureus* | 0.5 | 17.4 | 19.3 | 19.6 | 17.2 |
| | | 1.0 | 21.1 | 20.5 | 21.9 | 20.4 |
| | | 1.5 | 23.4 | 22.1 | 24.5 | 23.5 |
| | | 2.0 | 26.1 | 24.0 | 27.9 | 26.1 |
| | | 2.5 | 29.0 | 25.7 | 30.9 | 28.9 |
| 2. | *Escherichia coli* | 0.5 | 16.3 | 18.2 | 15.2 | 12.5 |
| | | 1.0 | 18.2 | 21.3 | 17.9 | 14.0 |
| | | 1.5 | 20.1 | 24.4 | 21.3 | 16.0 |
| | | 2.0 | 22.7 | 26.2 | 23.4 | 18.0 |
| | | 2.5 | 25.2 | 30.1 | 26.9 | 22.0 |

In Table -1 the ethanolic extract of *Aegle marmelos*, *Solanum nigrum* and *Cassia fistula* were subjected for antimicrobial activity against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The ethanolic extracts posses good antimicrobial activity when compared with control drug Penicillin. At 0.5 mg/ml concentration *Cassia fistula* exhibits maximum zone of inhibition about 19.6mm when compared with *Solanum nigrum*, *Aegle marmelos* exhibits 19.3mm, 17.4mm and control drug penicillin exhibits 17.2mm. The concentration was further increased for 1.0mg/ml, *Cassia fistula* exhibits 21.9mm, *Aegle marmelos* and *Solanum nigrum* exhibits 21.1, 20.5 mm and penicillin exhibits 20.4mm. At 1.5 mg/ml concentration the maximum zone was exhibited on *Cassia fistula* about 24.5mm and *Aegle marmelos* exhibits minimum zone of inhibition of about 23.4mm, Penicillin exhibits about 23.5mm, *Solanum nigrum* exhibits lowest zone of inhibition about 22.1mm. The concentration of plant extract was further increased for 2.0mg/ml *Cassia fistula* exhibits about 27.9mm *Aegle marmelos* and control drug penicillin exhibits same zone of inhibition of about 26.1mm,*Solanum nigrum* exhibits lowest zone of inhibition about 24.0mm. At higher dilutions about 2.5mg/ml concentration *Cassia fistula* exhibits good antimicrobial activity of about 30.9mm, *Aegle marmelos*, exhibits minimum zone of inhibition of about 29.0mm and control drug penicillin exhibits 28.9mm and *Solanum nigrum* exhibits lowest zone of inhibition.

From the above results *Cassia fistula* shows good antimicrobial activity against gram positive organism of *Staphylococcus aureus* which exhibits maximum zone of inhibition when compared with *Aegle marmelos* and *Solanum nigrum* and control drug penicillin show less activity when compared with plant extracts

The dilutions were subjected on *Escherichia coli*. At 0.5 mg/ml concentration, *Solanum nigrum* exhibits maximum zone of inhibition about 18.2mm, *Aegle marmelos* and *Cassia fistula* exhibits minimum zone of inhibition about 16.3mm, 15.2mm and control drug penicillin exhibits 12.5mm. The concentration was further increased for 1.0mg/ml *Solanum nigrum* exhibits maximum zone of inhibition about 21.3mm, *Aegle marmelos* and *Cassia fistula* exhibits 18.2, 17.9mm and control drug penicillin exhibits 14.0mm. At 1.5 mg/ml concentration *Solanum nigrum* exhibits 24.4mm and *Cassia*

*fistula* and *Aegle marmelos* exhibits 21.3, 20.1mm and control drug penicillin exhibits 16.0mm. Further concentration was increased for 2.0mg/ml *Solanum nigrum* exhibits 26.2mm *Cassia fistula* and *Aegle marmelos* exhibits 23.4 and 22.7mm control drug penicillin exhibits 18.0mm.

At higher dilutions of about 2.5 mg/ml, *Solanum nigrum* exhibits good antimicrobial activity of about 30.1mm zone and *Cassia fistula* exhibits 26.9mm and *Aegle marmelos* exhibits 25.2mm and control drug penicillin shows lowest zone of inhibition about 22.0 mm with less activity. According to the results *Solanum nigrum* exhibits very good antimicrobial activity against gram negative *Escherichia coli*, when compared with *Aegle marmelos*, and *Cassia fistula*, control drug penicillin exhibits lowest zone of inhibition.

## Conclusion

The existing antibiotics become resistant to the infectious diseases and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanism of activities for new and re-emerging infectious disease

In this endeavor, traditional herbal medicines must perforce to be granted the benefit of modern science and technology to serves further global needs. The drugs derived from herbs have the possibility of using in medicine because of its good antimicrobial activity as well as less toxic effects, when compared to control drugs. A novel drug should not contain any toxic side effects, Hence the present study aimed to focus the antibacterial effects of above mentioned plants on *Escherichia coli* and *Staphylococcus aureus*

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