

Antimicrobial efficacy of Indian Medicinal Plants as alternative source of antimicrobial compounds against Drug Resistant Pathogenic Microorganisms

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Abstract- New sources of antimicrobial compounds need to be identified and improved methods should be developed to combat rising multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their antimicrobial efficacy to exploit it in modern phytomedicine and combinational therapy. In the present study alcoholic extracts of *Rosmarinus officinalis* was screened for their antimicrobial efficacy against drug resistant bacteria and pathogenic yeast. The extract of *Rosmarinus officinalis* showed promising action against one or more drug resistant bacteria as well as against *Candida albicans*.

Key words: Antimicrobial activity, MDR bacteria, *Rosmarinus officinalis*, pathogenic yeast.

Introduction

Medicinal plants have been found good source of therapeutic and novel compounds. Targeted

screening of a large diversity of medicinal plants is expected to yield novel biological activities including group of multidrug resistant bacterial pathogens. Bacteria have evolved numerous defenses against anti-microbial agents and drug resistant pathogens are on the rise and such bacteria have become a global health problem. Nearly twenty years ago over 90% *Staph aureus* strains were reported β -lactamase positive. Strains of β -lactamase resistant *Staphylo-coccus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000). The production of β -lactamase is recognized as one of the main mechanism of bacterial-resistance to β -lactamase antibiotics.

Numerous compounds have been included in the list of β -lactamase inhibitors and some of these have shown potential clinical usefulness based on their synergistic-effects when

they are combined with β -lactamase-labile antibiotics. Many β -lactamase were found to be resistant to β -lactamase inhibitors. Similarly multidrug resistant problem is common in members of family Enterobacteriaceae specially *E.coli*, *Salmonella*, *Shigella* and several other humans and animal pathogen like *Haemophilus influenza*, *Campylobacter*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.*, 2001; Tonkic *et al.*, 2005).

A large portion of the world population especially in the developing countries depends on the traditional-system of medicine for a variety of diseases. The world health organization (WHO) reported that 80% of the world's population rely chiefly on traditional medicines and major part of the traditional therapies involve the use of plant extracts or their active constituents (WHO 1993).

According to an estimate about 119 secondary plant metabolites are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angio-sperms have been investigated chemically and that 74% of pharmacologically active plant derived components were

discovered after following upon ethanobotanical use of plants (Eloff, 1998). The plants are valuable in the three basic ways:

- (1) they are used as source of direct therapeutic agent.
- (2) As a source of new bioactive metabolites including antimicrobial, antihelminthic and antiprotozoan etc.
- (3) they serve as raw material base for elaboration of more complex semisynthetic chemical compounds.

According to a report published in the 'Journal of the American medical association', more than 630 million visit are made to alternative practitioners each year in the U.S. also more than 15 million adults take herbal remedies while taking other medication (Hoffman, 2004).

Concerted efforts have been made all over the world to explore the various biological and specific pharmacological activities and their active compounds all over the world. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic micro-organisms. However, targeted screening with improve strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy.

It is expected that plant extract

showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.*, 1998). In the recent years plants have been screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli*, *Shigella dysenteriae* and *Candida albicans*. The selection of medicinal plant was based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.*, 1992; Ahmad *et al.*, 1998; Mehmood *et al.*, 1999).

The recent development in the phytopharmacology is development of multicombinational drug against multidrug resistant bacteria. This has been possible due to interaction among plant extracts (Phytochemicals) and with other chemotherapeutic agents that may be synergistic or additive in their interaction. The development of these drugs has grown a new future in the area of phytopharmacology and medical practices.

At present multi drug therapy or combinational antibiotic therapy is in use. However its efficacy may be severely hindered against several MDR bacteria. Therefore, there is an increased need to develop novel drugs against multi drug resistant bacteria. One possible

approach is to screen/unexplored Indian medicinal bioactive plant extracts for their potential to be used against multi drug resistant bacteria.

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected *Rosmarinus officinalis* on the basis of their traditional uses, ethanopharmacological data and local availability. The present screening programme has been planned to identify most effective plants with broad spectrum activity against drug resistant microbial pathogens *in vitro*.

Material and methods

Plants material

The authentic plant material was obtained from the Himalaya Wellness Company, Dehradun an. The identification of the samples was further confirmed by the plant taxonomist in the Department of Pharmacognosy.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard strains were obtained from Microbiologics U.S.A. While Multidrug resistant bacteria include the strains of *Staphylococci* including methi-cillin resistant *Staphylococcus aureus* (MRSA), strains of *E. coli*. Were isolated in our laboratory.

Source of Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt Ltd, Mumbai, India.

Bacterial cultures

Bacterial isolates were obtained from different sources were subjected to antibiotic sensitivity by disc diffusion, method (Bauer et al., 1966).

β -lactamase production

The method described earlier (Ahmad et al., 2008) was used for detection of production of β -lactamase.

Culture Media and Inoculum preparation

Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 °C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 – 10^6 CFU/ml.

Preparation of plant extracts and its fractionation

Plant extract was prepared as described earlier (Ahmad and Mehmood,1998) with a little modification. 800 gram of dry, plant powder was soaked in 2.5 liter of 70% ethanol, for 8–10 days and stirred after every 10 hr using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This alcoholic filtrate was concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of

100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Mehmood,1998) was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 μ l of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol & Ciplox) at 100 μ g/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Phytochemical analysis of plant extracts

Major phytochemicals, in the crude extracts of plants, were detected by standard colour tests and thin layer chromatography, as described elsewhere (Ahmad and Beg 2001).

Result and Discussion

Antimicrobial activity of plant extracts against drug resistance pathogenic bacteria

Multiple drug resistance in pathogenic bacteria has emerged as important problem in many countries of the world. There are now increasing case reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like

fluroquinolone (norfloxacin, ciprofloxacin, ofloxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates and ATCC cultures of *Staph. aureus*, *E. coli*, and *Candida albicans* were used. These microbial strains (clinical isolates) are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance. These findings are in agreement with the reports of previous workers as these strains have been previously tested for their sensitivity to antibiotics (Ahmad and Arina, 2001; Aqil et al., 2005). In the present study, *Rosmarinus officinalis* was selected on the basis of their traditional uses in treatment of different diseases in India and worldwide. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff, 1998; Ahmad et al., 1998).

Antibacterial activity of crude extracts of *Rosmarinus officinalis* against Gram positive bacteria (isolates of *S. aureus*) and Gram-negative bacteria (*E. coli*,) and a yeast (*C. albicans*) is presented in Table 1. Activity of ethanolic crude extracts against Gram positive bacteria showed broad spectrum activity (Table 1). On the other hand broad spectrum activity

against Gram negative MDR bacteria was also exhibited by this plant as evidenced with fair size of zone of inhibition (Table 1). Similarly, anticandidal activity of this plant extracts demonstrated that they exhibit good anticandidal activity (Table 1).

Thus our antimicrobial screening results also justify the traditional uses of this plants in ailments and localized skin infections caused by *Staph. aureus*, *E. coli*, and *Candida albicans*.

This preliminary investigation of this plants open an avenue to evaluate the possible additive, synergistic or antagonistic interaction of this plant extracts in different combinations to obtain enhanced activity of herbal preparations. Although, it will also require an additional data on *in vivo* studies.

Multiple antibiotic therapy is now considered an effective way to control infectious diseases caused by drug resistant bacteria. Phyto-compounds which may have strong activity against antibiotic resistant bacteria is expected to give strong synergistic and additive effect with antibiotics. Considering this known fact we will be tried to see the possible synergistic effect between plant extracts *Rosmarinus officinalis* and antibiotics.

Table-1

S. N o.	Test Microorg anisms	Alcohol ic extract of <i>Rosmarinus officinalis</i>	Antibiotics as positive control	
			<i>Ciplox</i>	<i>Chloramphenicol</i>
Diameter of zone of inhibition(mm)				
1.	<i>Staph aureus(AT CC)</i>	25	38	26
2.	<i>Staph aureus(Clinical isolate)</i>	22	25	R
3.	<i>E.coli(AT CC)</i>	24	40	10
4.	<i>E.coli(Clinical isolate)</i>	22	32	R
5.	<i>Candida albicans ATCC</i>	24	40	16

Conclusion

This preliminary investigation indicated that *Rosmarinus officinalis* showing broad spectrum antimicrobial activity against standard cultures and clinical isolates comparable with chloramphenicol antibiotic and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of this plant may also be exploited in preparation of herbal formulation of improved efficacy and quality.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no

conflict of interest between authors and producers of the product.

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