Antimicrobial potentials of plant extracts against drug resistant bacteria

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DOI 10.51129/ujpah-2022-34-1(6)

Received – 28 May, 2023
Revised – 30 May, 2023
Accepted – 8 June, 2023
Published – 24 June, 2023

Abstract-Antimicrobial resistance (AMR) is a serious and growing threat to human health. The development of new antibiotics is limited and slow. The tradition of synergy in herbal medicine is being used as a source of research ideas. The in vitro findings were that most of the research reported synergy both within plants and between plants and antibiotics. Whole plant extracts and combinations of compounds were shown to be more effective antimicrobials than isolated constituents. New sources of antimicrobial drugs need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern phytomedicine and combinational therapy. In the present study alcoholic extracts of medicinal plants were screened for their antimicrobial efficacy against drug resistant bacteria. The extracts of *Hemidesmus indicus,and Carum copticum,* showed promising action against one or more drug resistant bacteria with MIC ranged from 0.53 mg/ml to 7.80 mg/ml which has indicated their potential to be exploited in antimicrobial therapy and combination drug therapy after careful evaluation *in vivo* model.

Key words: Antimicrobial activity, MDR bacteria, MIC, Antibiotics, Antimicrobial resistance

Introduction

The World Health Organization (WHO) reports that there are internationally high levels of AMR in common bacteria alongside limited understanding and uncoordinated surveillance of AMR (WHO, 2014). There have been just two new classes of antibiotics developed in the last 40 years. The development pipeline is slow and although two new Cephalosporin combinations are expected to be licensed in Europe soon for use in humans, AMR will also emerge for these (O'Neil, 2015). Bacterial mechanisms for resistance are innate but the high correlation between antibiotic use and AMR is clear (ECDPC, 2015). Further research, development of collaborative working, novel approaches to prevent and treat infections and the exploration of possibilities for enhancing immunity (in relation to infection by bacteria) including using prebiotics and probiotics have been recommended (DOH and DEFRA, 2013). Research and approaches for improving human immunity and resilience have been lacking (EUROCAM, 2014). WHO (2012) advises innovation and testing natural products to address AMR.

The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds.

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global helath problem. Nearly twenty years ago over 90% *S. aureus* strains were reported β-lactamase positive. Strains of β-lacatam resistant *Staphylococcus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000).. 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, Campylo-bacter*, *Pseudomonas* aeruginosa, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.,* 2001; Tonkic *et al.*, 2005;)India has one of the world's richest flora with about 120 families of plant comprising 1, 30,000 species. 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 angiosperms 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 pharmaco-logical activities and their active compounds all over the world. 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 dysentriae* 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 (Phytocompounds) 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 request 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 15 plants on the basis of their traditional uses, ethano-pharmocological 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 and to assess synergy with antibiotics *in vitro* in future studies.

Material and methods

Plants material

The authentic plant material was obtained from the Himalaya Wellness Company, Dehradun and identification of the plant samples was further confirmed by the plant taxonomist Dr. Maya Ram Uniyal, Former Jari Buti expert, Govt. of Uttarakhand.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard ATCC Culture strains were obtained from Hi-Media, Mumbai and clinical isolates were collected from Department of Microbiology, Himalaya Wellness Company. Multidrug resistant bacteria *Staphylococci* including methicillin resistant *Staphylococcus aureus* (MRSA), and Gram negative bacteria were also used in our laboratory.

Chemicals and Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt. Ltd., Mumbai, India. The indicator dye p-iodonitro tetrazolium violet were purchased from Sigma Chemical Co., USA. MMS and Sodium azide were purchased from Sisco Reseach Laboratory, India. All the other media/chemicals used were of analytical grade.

Bacterial cultures

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

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 ^{0}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 sterilized 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 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extracts

Minimum inhibitory concentration of plant extracts against test bacterial strains was determined by tube broth dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff 1998). 2 ml of the plant extract was mixed with 2 ml of Muller-Hinton broth (Hi-Media Ltd., Mumbai, India) and serially diluted into the next tube and so on. 2 ml of an actively growing culture of different test strains was added before incubating for over night, at 37 °C. After examining turbidity visually, 0.8 ml of 0.02 mg/ml indicator dye (p-iodonitro tetrazolium violet) was added to each tube and incubated at 37 °C. The tubes were examined for the colour development, after 30 min. Absence of growth was also confirmed by spreading 0.1 ml of broth from such test tube on normal nutrient agar plate.

Results 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, oflaxacin etc.) in many pathogenic bacteria. In the present

study, clinical isolates of *S. aureus,*. and *E. coli,* were used. These microbial strains are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance **(Table-1)**.

Table-1 Antibiotics resistant pattern of test strains

Name of bacteria	**Strains code**	**Resistant pattern of used strains against antibiotics**
Staphylococcus aureus	SA-03	Cx, M, A. Pn, Cf, Do, Sm, Na
Staphylococcus aureus	SA-08	Cx, M, A, Pn, Cf, Sm,
Staphylococcus aureus	SA-11	Pn, Am, M, S, T, Do, Na, Cu,
Staphylococcus aureus	SA-21	Cx, M, A, Pn, Cf, Do, Sm,
Staphylococcus aureus	SA-22	Sensitive to all drugs
Staphylococcus aureus	SA-28	Pn, Am, Cx, Cf, M, Pc, Kt, T, S,
Staphylococcus aureus	SA-29	Cx, M, A, P,
E.coli	UP-2556	Pn, A, Cx, Do,
E.coli	EC-14	Pn, A, Cx, M, Ce, Cfx, Cep, Cu,
E.coli	EC-20	Pn, A, Cx, M, Ce, Cfx, Cu, Va, T, E,

Pn- Penicillin; A- Ampicillin; Cx- Cloxacillin; Ce- Cephotaxime; Cu- Cefuroxime; Cfx-Cefixime, Cefpodoxime; M- Methicillin; Va- Vancomycin; Nf- Nitrofurantoin; Nx- Norfloxacin; Nv- Novobiocin; Co- Co-trimoxazole; Na- Nalidixic acid; T- Tetracyline; C- Chloramphenicol; Do- Doxycycline and E- Erthromycin.

In the present study, 02 medicinal plants were selected on the basis of their traditional uses in treatment of different disease 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).

Antibacterial activity of crude extracts of the both medicinal plants against Gram positive bacteria (7 distinct isolates of *S. aureus*) and Gram-negative bacteria *E.coli* is presented in **Table-2 and Tabe-3**. Activity of ethanolic curde extracts against Gram positive bacteria showed broad spectrum antibacterial activity **(Table-2)**. On the other hand broad spectrum activity against Gram negative MDR bacteria was exhibited by *C. copticum*, followed by *Hemidesmus indicus* as evidenced from their activity against both test bacteria with fair size of zone of inhibition **(Table-3).** Most potential plant extract was *Carum copticum* followed by *Hemidesmus indicus*. While activity of *Carum copticum, and Hemidesmus indicus* against MDR bacteria are probably reported for the Ist time.

Table -2 Antibacterial activity of plant extracts against Gram positive bacteria

S. No	Scientific Name (Family)	Antimicrobial activity (Radius in mm)							
		SA-03	SA-08	SA-11	SA-21	SA-22	SA-28	SA-29	ATCC 6538*
1.	*Hemidesmus indicus*	21	20	23	22	23	21	19	28
2.	*Carum copticum*	26	24	22	25	26	20	18	30

Table- 3 Antibacterial activity of plant extracts against Gram negative bacteria

S. No	Scientific Name (Family)	Antimicrobial activity (Zone in mm)	
		EC-14	EC-20
1	**Hemidesmus indicus**	22	20
2	*Carum copticum*	25	24

MIC values of *Carum. copticum* varied greatly from 0.53 mg/ml to 5.42 mg/ml against test bacteria. Similarly MIC ranged from 3.35 mg/ml to 7.80 mg/ml for *Hemidesmus indicus* **(Table-4)**. Variation in MIC values might be due to difference in cell well composition and intrinsic tolerance of the test isolates, nature and composition of phytoconstituents. Our antimicrobial screening results also justify the traditional uses of these plants in ailments and localized skin infections caused by *S.aureus, E.coli,etc*.

Table -4 Activity profile of crude plant extracts in terms of Minimum inhibitory concentration (MIC)

S. No	Plant Extract	Yield in mg/100 gm of dry powder	Minimum inhibitory concentration against test microorganisms (mg/ml)								
			SA						EC		
			SA-03	SA-08	SA-11	SA-21	SA-28	SA-29	EC-M	EC-14	EC-20
1	**C. copticum**	6.38	4.6	5.42	4.42	0.53	1.48	4.17	4.14	2.04	2.05
2	*H.indicus*	4.36	6.7	7.80	5.42	3.35	4.34	6.89	6.34	4.56	5.76

Conclusion

This preliminary investigation indicated that potential plant extracts showing broad spectrum antimicrobial activity and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of various plants 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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