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"ANTIBACTERIAL, ANTIOXIDANT, PHYTOCHEMICALS EVALUATION OF FIVE INDIAN MEDICINAL PLANTS"

Rahul More[1]*, Govind Sanap[2], Ayesha Siddiqui[1], Shailaja Dhutekar[1], Shital Patil[1], Vrushali Diwan[1], Maya Lokare[1], Aakash Ingle[1] and Priya Maske[1]

[1]*Department of Microbiology, Dayanand Science College, Latur-413512 (MS), India.

[2]Department of Zoology, Khare Dhere Bhosale College, Guhagar- 415703 (MS), India.

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***Corresponding Author**
Dr. Rahul More
Department of Microbiology, Dayanand Science College, Latur-413512 (MS), India.

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of the patients in hospitals has suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. The problem of microbial resistance is growing and the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patients. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to world health organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.[1] Therefore such plants should be investigated to better understand their properties, safety and efficiency. The use of plant extracts and phyto-chemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare System. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. About 61% of new drugs developed between 1981 and 2002 were based on

natural products. And they have been very successful, especially in the areas of infectious diseases and cancer. Recent trends, however, show that the discovery rate of active novel chemical entities is declining. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. Plants are in rich in secondary metabolites such as tannins, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The present study was aimed to evaluate antimicrobial, antioxidant and phytochemical analysis of five Indian medicinal plants frequently used in Ayurveda.[2]

KEYWORD: Disk diffusion, MIC, DPPH, OH, Phytochemicals.

MATERIALS AND METHODS

MATERIALS

Bacterial strains

Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli.

Plants

Beriberis aristata (Family: Berberidaceae, Part used: leaves)

Tinospora cardifolia (Family: Menispermaceae, Part used: leaves)

Nigella sativa (Family: Ranunculaceae, Part used: leaves)

Myristica fragrans (Family of plant: Myristicaceae, Part used: Fruit)

Trachyspermum copticum (Family: Apiaceae, Part used:leaves)

METHODS

Extraction of plant samples

Authenticated plant samples were shade dried, powered and subjected to subsequent extraction with water. Samples were boiled for 2 hrs at 55^0C. and filter. The filtrate were then evaporated on rotator till it becomes powered.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of alkaloids, flavonoids, terpenoids, anthraquinones and saponins from selected plant samples were carried out using standard methods described previously.[3]

Test for Alkaloids

The individual plant extract (0.5 g) of each plant was diluted to 10 mL with aqueous HCL (10% in water), boiled and filtered. To 5 mL of the filtrate, 2 mL of dilute ammonia and 5 mL of chloroform was added and shaken gently to extract the alkaloids. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Each of the half portions was added to Mayer's reagent and Draggendorff's reagent. Formation of creamish and reddish brown precipitate respectively, confirmed for presence of alkaloids.

Test for Flavonoids

A part of individual extract 0.5g was heated with 10 mL of ethyl acetate over a steam bath for 30 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for Terpenoids

To 0.5 g each of the plant extract, 2 mL of chloroform was added. 3 mL of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for Anthraquinones

The each plant extract (0.5 g) was boiled with 10 mL of sulphuric acid and filtered. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipetted into another test tube and 1 mL of diluted ammonia was added. The resulting solution was observed for color changes.

Test for Saponins

To an individual plant extract (0.5g), 5 mL of distilled water added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. This was then mixed with 3-4 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Disc Diffusion method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method to assess the presence of antibacterial activities of the plant extracts. Three bacterial cultures were used to lawn agar plates evenly by sterile swabs. The discs which had been impregnated with a series of plant extracts were placed on the agar surface. Three test

plates comprises four discs and three test plates comprises three discs. The standard antibiotic disc was penicillin. The plates were then incubated at 37^{o}C for 18 to 24 hours. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using calipers and recorded.[4]

Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by using Microtitre plate method and the cut off points and procedure was adopted as mentioned in the CLSI guidelines. The Minimal Inhibitory Concentration (MIC) assay was performed to determine the concentration of the extract that inhibits the growth of bacteria in vitro.[5]

Hydroxyl radical assay (OH)

The OH radical scavenging activity was demonstrated with Fenton reaction. Briefly, the typical reaction mixture contained 60 µL of $FeCl_2$ (1 mM), 90 µL of 1-10 phenanthroline (1 mM), and 2.4 mL of phosphate buffer (0.2 M, pH 7.8), 150 µL of H_2O_2 (0.17 M) and 1.5 mL Of individual plant extract (1 mg/mL). The reaction was started by adding H_2O_2. After 5 min incubation at room temperature, the absorbance was read at 560 nm. Ascorbic acid (1 mM) was used as reference.

% radical scavenging activity = 1 – T/C x 100

2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

The electron donation ability of the crude extracts was measured from the bleaching of the purple colored ethyl acetate solution of DPPH. DPPH is stable reagent used in this spectrophotometric assay. Briefly, the assay was performed by mixing of equal DPPH solution in methanol, so that the final volume is made up to 3 mL incubate the samples for 20 min, read the absorbance at 517 nm using (Shimadzu). Ascorbic acid (1 mM) was used as a standard.[6] Percent inhibition was calculated using following formula:

% radical scavenging activity = 1 – T/C x 100

RESULTS AND DISCUSSION

The present study focused on identifying plants for use as potential antimicrobial agents in infectious diseases. The results demonstrated that *Trachyspermum copticum* methanolic extract was highly effective against *S. typhi* and *E.coli* (MIC 31.5 µg/mL) and methanolic extract of *Tinospora cordifolia* showed good inhibitory activity against S. typhi (MIC 31.5 µg/mL). During the drug treatment the patient is highly suffering from the free radical stress

due to consumption of higher doses of drugs. Hence, antioxidant potential of plant samples was studied. The *Myristica fragrans* and *Tinospora cordifolia* found to have best DPPH and OH radical scavenging potential. Difference in antimicrobial activity of the plant may be related to the presence of varied phytochemicals in the respected plant sample.

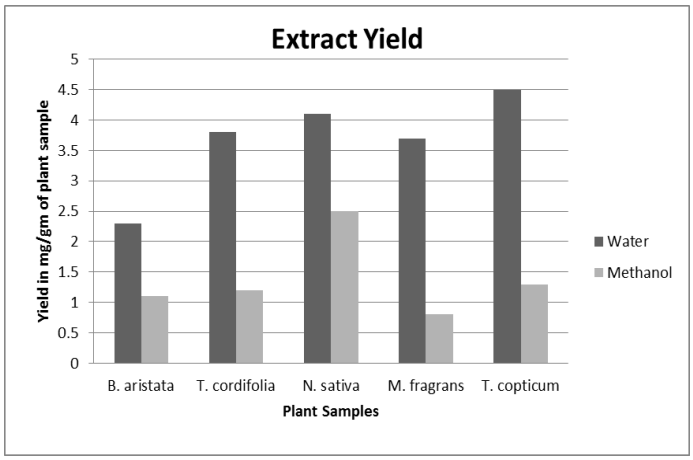

Figure 1. The yield of plant extracts.

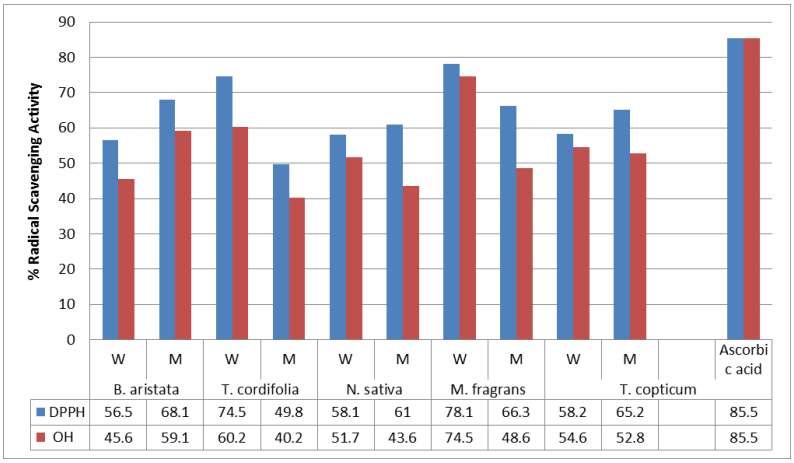

Figure 2. Antioxidant activities of plant extracts.

W-Water, M-Methanol.

Table 1: Antimicrobial potential of the Plant extracts against bacterial pathogens.

Plant sample	Solvent	Bacterial Pathogen	Zone of inhibition (mm)	MIC (µg/mL)
Berberis aristata	W	*P. aeruginosa*	--	
		S. typhi	12	62.5
		E.coli	08	250
	M	*P. aeruginosa*	--	>500
		S. typhi	12	62.5
		E.coli	10	125

Tinospora cordifolia	W	*P. aeruginosa*	--	>500
		S. typhi	--	>500
		E.coli	--	>500
	M	*P. aeruginosa*	14	62.5
		S. typhi	16	31.2
		E.coli	06	500
Nigella sativa	W	*P. aeruginosa*	--	>500
		S. typhi	14	62.5
		E.coli	10	62.5
	M	*P. aeruginosa*	04	500
		S. typhi	02	500
		E.coli	--	>500
Myristica fragrans	W	*P. aeruginosa*	08	250
		S. typhi	06	500
		E.coli	--	>500
	M	*P. aeruginosa*	08	500
		S. typhi	10	250
		E.coli	14	62.5
Trachyspermum copticum	W	*P. aeruginosa*	08	250
		S. typhi	10	250
		E.coli	12	125
	M	*P. aeruginosa*	--	>500
		S. typhi	16	31.5
		E.coli	18	31.5
	Penicillin	*P. aeruginosa*	20	15.6
		S. typhi	22	7.8
		E.coli	18	15.6

W-Water, M-Methanol, -- No Zone

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