International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

SCREENING OF SOME MEDICINAL PLANTS FOR THEIR ANTIMICROBIAL ACTIVITIES

TILAK RAJ[1], PUNIT KUMAR[1], RADHA RATHEE[2] KASHYAP KUMAR DUBEY[1]*

[1]Microbial Biotechnology Laboratory, University Institute of Engineering and Technology, Maharshi Dayanand University, Rohtak, Haryana, India, [2]Department of Zoology, A. I. Jat H. M. College, Rohtak, Haryana, India
Email: kashyapdubey@gmail.com

Received: 21 Oct 2015 Revised and Accepted: 30 Mar 2016

ABSTRACT

Objective: Our objective of this research work was to investigate antimicrobial activity of five species of traditionally used medicinal plants namely *Adhatoda vasica, Artemisia annua*, *Cordia oblique*, *Croton bonplandianum* and *Euphorbia milli* against different strains of bacteria and fungi which are known to cause various types of infectious diseases.

Methods: Organic extracts of these plants leaves (dry) were prepared, and antimicrobial sensitivity of these organic extracts (Hexane, chloroform, acetone, and methanol) against selected bacterial and fungal strains were performed by disc diffusion assay method and Resazurin-based Microtitre Dilution Assay method.

Results: Among these plants, *Cordia oblique* (chloroform extract) and *Croton bonplandianum* (Hexane extract), which showed superior antimicrobial activity in the primary screening test. *Croton bonplandianum* showed the maximum yield (7.3%) and *Adhatoda vasica* showed minimum yield (0.57) of plant extract. Chloroform extracts of *Cordia oblique* and hexane extract of *Croton bonplandianum* showed very good antimicrobial activity (MIC 0.37 mg/ml) against *Staphylococcus aureus* and *Klebsiella pneumoniae*. With this *Artemisia annua* (chloroform extract) showed very remarkable antifungal activity (MIC 0.37 mg/ml) against *Aspergillus niger***.** Among the different fractions (Hexane, chloroform, acetone and methanol) tested for antimicrobial activity, the non-polar fractions were more active than the polar fractions.

Conclusion: In this study all the tested plants *Adhatoda vasica, Artemisia annua*, *Cordia oblique*, *Croton bonplandianum* and *Euphorbia milli* showed antimicrobial activity against at least one strain of bacterium and fungus. This might justify their claimed uses in the treatment of various infectious diseases.

Keywords: Antimicrobial activity, Crude plant extracts, Organic extracts, Medicinal plants

INTRODUCTION

Microbial infection is a worldwide problem, and huge numbers of antimicrobial agents or antibiotics are available in the market to combat these microbial infections. Several antibiotics have undesirable side effects that lead to exploration of novel antimicrobial agents mainly derived from plant extracts with the goal to discover new chemical structures which overcome the above disadvantages [7]. Although these agents are very effective and broad spectrum but the development of resistance in microbes against these drugs making the microbial infections exacerbate. This appearance of antibiotic drug resistance in pathogenic bacteria has created immense clinical problems in the treatment of infectious diseases. The patients infected with resistance stains are more likely to die and survivors have to spend significantly large time in hospitals and have delayed recuperation time. Thus, researchers are making their efforts in the development of new types of drugs showing effectiveness against resistant strains with minimum toxicity to the patients.

During the past decade, the traditional systems of medicine have become increasingly popular keeping in mind their safety. Natural products proved to be an important source of lead compounds in the development of new drugs [8]. According to the recent survey the conducted in many developing countries, a large part of the population is dependent profoundly on the traditional practitioners and medicinal plants to meet their primary health care needs [9]. World Health Organization (WHO) also noted that a larger part of the world's population depends on traditional medicine for primary healthcare. Plants used for traditional medicine consists an extensive range of substances that can be used to treat chronic and infectious diseases as well.

The medicinal value of a plant lies in some chemical substances that produce a definite action on the human body. Medicinal and aromatic plants are widely used as medicines and constitute a major source of natural organic compounds [10]. Screening of natural products from plants provides the chance to discover new molecules of the unique structure with high activity and selectivity [11]. The most crucial of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [12]. These medicinally active compounds from plants have lead to the discovery of new medicinal drugs which have proficient protection and treatment roles against various diseases, including cancer and Alzheimer's disease [13]. Development of such antimicrobial drugs by exploiting such plant metabolites is one of the potent approaches. It is believed that such metabolites will be a source of new drugs.

In this article, we have investigated the antimicrobial property of plant extracts of five medicinal plants (*Croton bonplandianum, Adhatoda vasica, Artimisia annua, Euphorbia milli* and *Cordia obliqua*) against bacterial and funal reference strains. These selected plants are reported for their pharmaceutical value. *Croton bonplandianum* belongs to the family *Euphorbiaceae* has been used to cure hepatopancreatic, ringworms and skin diseases. Its leaves are vested with medicinal value and maintain the blood pressure [1]. *Adhatoda vasica* member of family Acanthaceae has traditional medicinal history mentioned in Ayurveda. *Vasica* is the remedy for the treatment of respiratory associated problems [2]. *Artimisia annua* belongs to family Compositae and traditionally it was used in Chinese medicine in Quing Hao used to alleviate fever. Artemisinin extracted from *Artimisia annua* used against *Plasmodium falciparum* [3]. *Euphorbia milli* belongs to family *Euphorbiaceae* was also known as crown of thorn. Its milky latex is used to treat abdominal edema, constipation and sprains relive [4]. *Cordia obliqua* belongs to the family *Moraginaceae* and ripen fruits of *Cordia obliqua* are found effective in treatment the lungs diseases and oral sore [5].

MATERIALS AND METHODS

Plant material

Five plants were selected based on their use in the conventional system of medicines for their antimicrobial potential are given respectively as *Adhatoda vasica, Artemisia annua*, *Cordia oblique*, *Croton bonplandianum* and *Euphorbia milli***.**

Preparation of crude extract

The five medicinal plants were selected and identified which were available in a botanical garden in the campus of Maharshi Dayanand University Rohtak, Haryana, India (30.73 °N76.78 °E). The plant leaves were air dried under shade for two weeks and then oven dried at 42 °C for 18-24h. The dried plant material was grinded to powdered form with a mortar and pestle. The powder was weighed (40 g for each plant sample), and the Soxhlet's method was used for extract preparation. The four solvents (200 ml for each): hexane, chloroform, acetone and methanol were used in ascending order of their polarity. The solvents were filtered and evaporated by vacuum distillation at 35 °C using Rotary Vacuum Evaporator. The residual solid was weighed and referred as the crude extract.

Pathogens and reference strain

Bacterial strains *Escherichia coli* (MTCC 433), *Klebsiella pneumoniae* (MTCC 3384), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737) and *Pseudomonas aeruginosa* (MTCC 1688) were procured from Microbial Type Culture Collection (MTCC), Microbial Technology, Chandigarh, India. Fungal strain; *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigates* were available in the university. Antimicrobial activity of prepared leaves extracts was investigated against these strains.

Culture of pathogens

The bacterial strains were grown and maintained in Luria broth. During growth bacterial strains were always used; a set of graphs of killing/viability curves for each strain of bacterial species were prepared. A final concentration of 5×10^6 Colony Forming Unit (CFU)/ml was used for antimicrobial study [14].

Antibacterial evaluation of various plant extracts

The antibacterial activity of isolated plant extracts against selected bacterial and fungal strains was studied by Disc Diffusion assay and Resazurin-based Microtitre Dilution Assay (MDA).

Resazurin-based microtitre dilution assay

The resazurin solution was prepared by dissolving 300 mg resazurin powder in 50 ml of sterile distilled water. Resazurin-based MDA was carried out in 96 well plates under aseptic conditions. A volume of 100 µl of test materials in 10% (v/v) DMSO or sterile water (usually a stock concentration 12 mg/ml for crude extracts) added into the first row of the plate. 50 µl of nutrient broth and 50 µl of normal saline was added to all the wells of the plate. Serial dilutions were performed with the help of a multichannel pipette so that each well had 100 µl of the test material in serially descending concentrations. 10 µl of resazurin indicator solution was added in each well. Finally, 10 µl of bacterial suspension was placed in each well to achieve a concentration of 5×10^6CFU/ml. Each plate was wrapped loosely with cling film to make sure that the bacteria did not become parched. Tetracycline was used as positive control in each plate. Experiments were performed in triplicate and placed in an incubator at 37 °C for 18–24 h. The change in colour was then studied visually. Any colour change from purple to pink or colorless was recorded as positive. The lowest concentration at which colour change occurred can be taken as the minimum inhibitory concentration (MIC) [14].

Disc diffusion assay

The disc diffusion test performed in sterilized Petri plates of 10.0 cm diameter. The disc of the sample placed on the surface of the agar plates already inoculated with bacterial culture. The plates incubated at 37 °C and examined at 48 h for the zone of inhibition around the discs [15].

Qualitative phytochemical analysis

The extracts obtained from all the five plants were subjected to various phytochemical analysis tests to identify various bioactive constituents present in these plants [16].

RESULTS

Organic extracts of selected plants were prepared and analyzed. The preliminary phytochemical screening of all the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoid and phytosteroids (table 1). The yield of the organic extract obtained of the different plant was calculated, and It was found that highest percentage yield of hexane extract was obtained in *Croton bonplandianum* (1.1%) and lowest in *Adhatoda vasica* (0.57%). Highest percentage yield of chloroform extract was obtained in *Croton bonplandianum* (4.9%) and lowest in *Adhatoda vasica* (1.27%). Highest percentage yield of acetone extract was obtained in *Croton bonplandianum* (7.3%) and lowest in *Artemisia annua* (1.07%). And in methanol extract, highest was obtained in the *Artemisia annua* (6.1%) and lowest yield in *Croton bonplandianum* (1.6%) (fig. 1).

Table 1: Qualitative phytochemical analysis of different plants extracts

S. No.	Plants name	Presence of phytochemicals in plant extracts of various serial solvents			
		Hexane	Chloroform	Acetone	Methanol
1	*Artemisia annua*	S/t, T	S/t, T,A	S/t, T	S/t, Sp
2	*Cordia oblique*	S/t, T,A	S/t, A, Sp	S/t, Sp	S/t, A, T
3	*Croton bonplandianum*	S/t, A, T	S/t, T,F	S/t, T	S/t, Sp
4	*Euphorbia milli*	S/t, Sp, A	A, Sp	S/t, Sp	S/t, Sp
5	*Adhatoda vasica*	S/t	A, F	S/t	A, F, Sp

(P = Phenol; S/t = Sterol/terpene; A = Alkaloid; T = Tannin, F = Flavanoids; Sp = Saponins)

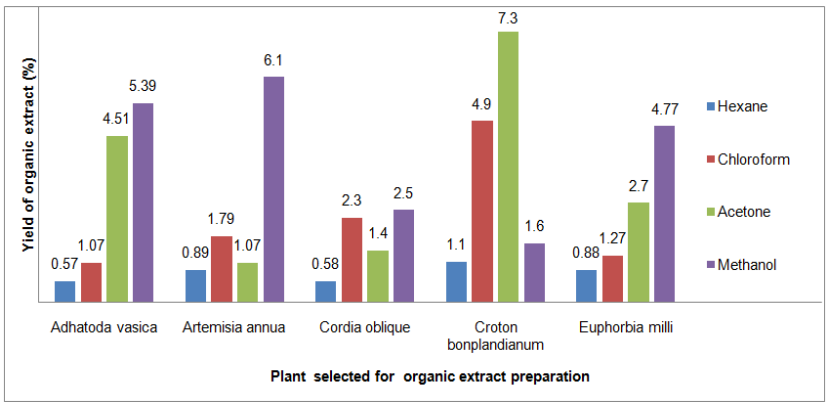

Fig. 1: Percent yield of the crude plant extracts

During organic extract preparation process twenty types of organic extracts of five plants were obtained and tested for their antimicrobial potential against five selected bacterial strains and three selected fungal strains. Antibacterial activity of standard drug (positive control) tetracycline was also conducted in against these selected bacterial strains, and MIC of tetracycline was obtained in the range 0.005-0.078 mg/ml against selected bacterial strains (fig. 2). Antibacterial activity (MIC) of isolated organic extracts was conducted against bacterial strains, and it was compared with positive control (tetracycline).

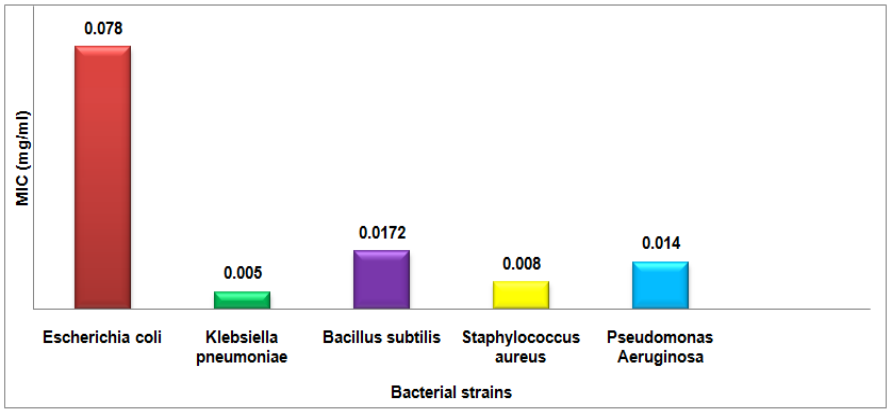

Fig. 2: MIC of standard drug tetracycline against bacteria. Experiments were carried in triplicate and each data point is mean value

The antibacterial activity of isolated plant extracts was conducted against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The screening of plants extracts for antibacterial activity showed that in *Adhatoda vasica* MIC of the organic extract against selected bacterial strain is in the range of 1.5 mg/ml to 3.0 mg/ml. In *Artemisia annua,* MIC of the organic extract against selected strain is in the range of 0.75 mg/ml to 6.00 mg/ml. In *Cordia oblique,* MIC of the organic extract against selected bacterial strain varies between 0.37 mg/ml-6.00 mg/ml. In *Croton bonplandianum,* MIC of organic extract varies between 0.37 mg/ml-6.00 mg/ml and in *Euphorbia milli*, it was in the range of 1.5 mg/ml to 6.0 mg/ml (table 2). It was found out that different organic extract of same plant exhibit different antibacterial activity against the similar bacterium. Out of data obtained it was revealed that chloroform extract of *Cordia oblique* showed potential against *Staphylococcus aureus* at 0.37 mg/ml (MIC) and hexane extract of *Croton bonplandianum* had good potential against *Klebsiella pneumonia* at 0.37 mg/ml (MIC). Some bacterial strains while methanol extract of *Artemisia annua* was ineffective against most of the tested bacterial strains (table 2).

Table 2: Activity of plant extract against bacterial strains

Plant name	Solvent name	MIC (mg/ml) of plant extracts in various serial solvents				
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***E. coli***	***Klebsiella pneumonia***
Adhatoda vasica	Hexane	1.50	1.50	3.00	1.50	3.00
	Chloroform	6.00	6.00	1.50	1.50	1.50
	Acetone	1.50	1.50	3.00	6.00	3.00
	Methanol	3.00	1.50	3.00	6.00	6.00
Artemisia annua	Hexane	6.00	6.00	1.50	3.00	1.50
	Chloroform	1.50	3.00	3.00	3.00	0.75
	Acetone	1.50	3.00	3.00	3.00	3.00
	Methanol	-	-	-	-	6.00
Cordia oblique	Hexane	6.00	1.50	0.75	1.50	1.50
	Chloroform	**0.37**	**1.50**	**0.75**	**0.75**	**1.50**
	Acetone	6.00	6.00	1.50	3.00	1.50
	Methanol	3.00	3.00	6.00	3.00	0.75
Croton bonplandianum	**Hexane**	**1.50**	**3.00**	**1.50**	**1.50**	**0.37**
	Chloroform	6.00	1.50	1.50	1.50	_
	Acetone	6.00	1.50	1.50	1.50	_
	Methanol	1.50	3.00	6.00	1.50	1.50
Euphorbia milli	Hexane	6.00	6.00	1.50	1.50	6.00
	Chloroform	1.50	0.75	1.50	6.00	6.00
	Acetone	_	6.00	_	3.00	6.00
	Methanol	_	6.00	6.00	_	_

Experiments were carried in triplicate, and each data point is mean value

Isolated organic extracts were also analyzed for antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigates* (table 3). Fungicidal property of a positive control (amphotericin B) was tested against these fungal strains. It found that MIC of positive control against *Aspergillus niger* was 2.4µg/ml, against *Aspergillus flavus* was 2.6µg/ml and against *Aspergillus fumigates* was 2.5µg/ml. It was analyzed that chloroform extract of *Euphorbia milli* showed best antifungal activity (0.37 mg/ml MIC) against *Aspergillus fumigates* while methanol and acetone extract of *Artemisia annua* showed the least activity (6.00 mg/ml) against *Aspergillus fumigates.* The hexane extract of *Croton bonplandianum* and chloroform extract of *Artemisia annua* and *Cordia oblique* showed best antifungal

activity (0.75 mg/ml MIC) against *Aspergillus flavus* while hexane extract and methanol extract of *Euphorbia milli* showed minimum activity (6.00 mg/ml) against *Aspergillus flavus.* Chloroform extract of *Artemisia annua* showed best antifungal activity (0.37 mg/ml MIC) against *Aspergillus niger* while acetone extract of *Croton bonplandianum* and methanol extract of *Artemisia annua, Cordia oblique, Croton bonplandianum* and *Euphorbia milli* showed the least activity (6.00 mg/ml MIC) against *Aspergillus niger*.

Table 3: Activity of plants extracts against pathogenic selected *Aspergillus spp.*

S. No.	Fungal strain	Plant	MIC (mg/ml) of plant extracts in organic solvents			
			Hexane	Chloroform	Acetone	Methanol
1.	*Aspergillus fumigates*	*Adhatoda vasica*	0.75	3.00	1.50	-
		Artemisia annua	3.00	1.50	6.00	6.00
		Cordia oblique	1.50	0.75	1.50	1.50
		Croton bonplandianum	3.00	1.50	1.50	3.00
		Euphorbia milli	6.00	0.37	1.50	6.00
2.	*Aspergillus flavus*	*Adhatoda vasica*	1.50	1.50	3.00	3.00
		Artemisia annua	1.50	0.75	3.00	3.00
		Cordia oblique	3.00	0.75	1.50	3.00
		Croton bonplandianum	0.75	3.00	1.50	1.50
		Euphorbia milli	6.00	1.50	3.00	6.00
3.	*Aspergillus niger*	*Adhatoda vasica*	1.50	3.00	3.00	3.00
		Artemisia annua	3.00	0.37	1.50	6.00
		Cordia oblique	1.50	1.50	3.00	6.00
		Croton bonplandianum	1.50	3.00	6.00	6.00
		Euphorbia milli	1.50	1.50	3.00	6.00

Experiments were carried in triplicate and each data point is mean value

Antimicrobial activity of organic plants extracts which exhibited good antibacterial and antifungal activity was screened by disc diffusion assay against selected bacterial and fungal strains (table 4). For each study 25 µg organic extract per disc was used, and zone of inhibition was measured. It was find out that hexane extract of *A. vasica* showed maximum zone of inhibition (7.8±0.4 mm) against *P. aeruginosa* and minimum zone of inhibition against *Aspergillus flavus* (6.1±0.7 mm) with this chloroform extract of this plant showed zone of inhibition against *Aspergillus flavus* (6.6±0.7 mm) and zone of inhibition against *E. coli* (6.2±0.6 mm). Chloroform extract of *A. annua* showed a maximum zone of inhibition against *Aspergillus niger* (7.2±0.2 mm) and minimum zone of inhibition against *S. aureus* (6.0±0.8 mm). Chloroform extract of *C. oblique* showed a maximum zone of inhibition against *Aspergillus niger* (7.4±0.3 mm) and minimum zone of inhibition against *S. aureus* (6.2±0.3 mm). Hexanes extract of *C. bonplandianum* showed a maximum zone of inhibition against *K. pneumoniae* (7.5±0.3 mm) and minimum zone of inhibition against *Aspergillus flavus* (6.2±0.6 mm).

Chloroform extract of *E. milli* showed a maximum zone of inhibition against *P. aeruginosa* (7.2±0.4 mm) and minimum zone of inhibition against *S. aureus* (6.4±0.2 mm).

Table 4: Antimicrobial activity of best-selected plants extracts by disc diffusion assay (at 25 µg/disc)

Plant name/(Solvent name)	Zone of inhibition in mm by disc diffusion assay							
	Aspergillus sp.			*S. aureus*	*P. aeruginosa*	*B. subtilis*	*E. coli*	*K. pneumoniae*
	fumigatus	*flavus*	*niger*					
A. vasica (hexane)	6.6±0.7	6.1±0.7	_	7.4±0.6	7.8±0.4	_	7.0±0.8	_
A. vasica (chloroform)	_	6.6±0.7		_	_	_	6.2±0.6	_
A. annua (chloroform)	_	6.6±0.2	7.2±0.2	6.0±0.8	6.2±0.6	_	_	6.8±0.2
C. oblique (chloroform)	6.2±0.6	6.7±0.2	7.4±0.3	6.2±0.3	_	6.6±0.3	6.7±0.6	_
C. bonplandianum (hexane)	_	6.2±0.6	_	6.6±0.2	_	_	_	7.5±0.3
E. milli (chloroform)	7.1±0.8	_	_	6.4±0.2	7.2±0.4	6.6±0.2	_	_

Experiments were carried in triplicate, and each data point is mean value±SD. (-) means no activity.

DISCUSSION

The plant leaves extracts in four different solvents viz. hexane, chloroform, acetone, and methanol, were appraised for antimicrobial activity against the reference strains of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *B. subtilis*. The test organisms used in this study are associated with various forms of human infections. A research study shows that *E. coli* is the cause of septicemias and can even infect the gallbladder, meninges, surgical wounds, skin lesions and the lungs, primarily in weak and immunodeficient patients [12]. Furthermore, from a clinical point of view, *K. pneumoniae* is a member of the *Klebsiella* genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection [13, 18-20].

Different solvents have various degrees of solubility for different phytoconstituents [16, 19, 21, 22], these results show that the best antifungal activity shown by *Artemisia annua (*chloroform extract) against *A. niger* having MIC value of 0.37 mg/ml. Similarly, the extract of *Adhatoda vasica* (hexane extract) and *Cordia oblique* (chloroform extract) confirm the admirable bioactivity against *A. fumigates* (MIC=0.75 mg/ml). In addition to this, *Cordia oblique* (chloroform extract) and *Artemisia annua (*chloroform extract) also corroborate the venerable bioactivity against *A. flavus* (MIC=0.75 mg/ml). Furthermore, it was observed that only six out of twenty extracts were found to be endowed with antimicrobial activity at a preset concentration of 25 µg/disc (table 2, 3 and 4) by disc diffusion assay.

These results clearly exhibit that there are differences in the antimicrobial effect of plant groups; which may be due to phytochemicals differences among the various plant species [23-25]. Few plant extracts were revealing the broad range activity possibly due to the presence of several antimicrobial compounds or synergic effects of these compounds.

It is far easier for a pathogenic microorganism to develop resistance against a single purified compound against a variety of compounds

acting simultaneously possibly with different targets to result in antimicrobial activity. The use in antimicrobial therapy of crude extracts, which contain enormous compounds, may act to shrink the incidence or development of resistant microbes [26, 27]. This supports the expansion of efficient herbal goods derived from biologically active plant extracts, rather than pharmaceuticals comprising a single active constituent isolated from the plant extract.

CONCLUSION

The present study reveals that highest antibacterial potential was observed with chloroform extract fraction of *Cordia oblique* against all pathogenic microbes studied. Due to the expansion of multi-drug resistant bacterial and fungal strains, it will be interesting to purify the active fractions for potential lead antimicrobial compounds. Indeed; *Cordia oblique* plant presents a resourceful reservoir of the various bioactive metabolites and can be of possible use in modern medicine. In anticipation, our study could donate the progress of newer and highly active antimicrobial drugs which would rapidly progress to various stages of formulation development and reach the pharmaceutical market, thereby contributing to a reduction in the incidence of infections.

ACKNOWLEDGEMENT

The author (Tilak Raj) is very thankful to HSCST (Haryana State Council of Science and Technology, Panchkula, India) for providing the financial facility, and Department of Biotechnology (UIET) M D University, Rohtak for providing lab facility.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Divya S, Naveen Krishna K, Ramachandran S, Dhanaraju MD. Wound healing and *in vitro* antioxidant activities of *Croton bonplandianum* leaf extract in rats. Global J Pharmacol 2011;5:159-63.
2. Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica. Int J Herbal 2014;2:88-91.
3. WHO. Guidelines for the treatment of malaria. 2nd ed. Geneva, World Health Organization; 2010.
4. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: Its chemistry, traditional and medicinal uses and pharmacological activities. Pharmacogn Rev 2010;4:58-61.
5. Thirupathi K, Kumar SS, Raju VS, Ravikumar B, Krishna DR, Mohan GK. A review of medicinal plants of the genus cordia: their chemistry and pharmacological uses. J Nat Remed 2008;8:1-10.
6. Srinivasan K, Natarajan D, Mohanasundari C, Venkatakrishan C, Nagamurugan N. Antibacterial, preliminary phytochemical and pharmacognostical screening on the leaves of vicoa Indica (L.) DC. Iran J Pharmacol Ther 2007;6:109-13.
7. Ushimaru PI, Nogueirada Silva TN, Claudio Di SL, Barbosa L, Fernandes junior A. Antibacterial activity of medicinal plants extracts. Braz J Microbiol 2007;38:717-19.
8. Sheeja K, Kuttan G. Activation of cytotoxic T-lymphocyte responses and attenuation of tumor growth *in vivo* by Andrographis paniculata extract and andrographolide. Immunopharmacol Immunotoxicol 2003;29:81-93.
9. Turker AU, Usta C. Biological screening of some Turkish medicinal plants for antimicrobial and toxicity studies. Nat Prod 2008;22:136-46.
10. Mukherjee PK, Kumar Vand Houghton PJ. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytother Res 2007;21:1142-5.
11. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complementary Altern Med 2006;6:39.
12. Kayser O, Kiderlen A, Croft S. Natural products as antiparasitic drugs. Parasitol Res 2003;90:555-62.
13. Fleming RV, Walsh TJ, Anaissie EJ. Emerging and less common fungal pathogens. Infectious Disease Clin North Am 2002;16:915-33.
14. Sarkar DS, Lutfun N, Kumara Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 2007;42:321-4.
15. Chhillar AK, Arya P, Mukherjee C, Gupta P, Yadav Y, Sharma AK. Microwave-assisted synthesis of antimicrobial dihydropyridines & tetradropyrimidin-2-ones: novel compound against aspergillosis. Bioorg Med Chem 2006; 14:973-81.
16. Harborne JB. Plant phenolics, Encyclopedia of Plant Physiology, Secondary Plant Products. Berlin Heidelberg, Springer-Verlag, New York; 1980.
17. Gupta P, Murali P, Murali MV, Faridi MA, Kaul PB, Ramachandran VC, *et al.* Clinical profile of *Klebsiella septicaemia* in neonates. Indian J Paediatr 1993;60:565-72.
18. Black JG. Microbiology: Principles and Application, Prentice Hall NJ; 1996. p. 260.
19. Mastroeni P. Immunity to systemic *Salmonella* infections. Curr Mol Med 2002;2:393-406.
20. Marjorie MC. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
21. Jia HL, Ji QL, Xing SL, Zhang PH, Zhu GL, Wang XH. Chemical composition and antioxidant, antimicrobial activities of the essential oils of *Thymus marschallianus*. *Thymus proximus* Serg. J Food Sci 2010;75:59-65.
22. Li RW, Leach DN, Meyers P, Leach GJ, Lin GD, Brushett DJ, *et al.* Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina* Benth. Phytother Res 2004;18:78-83.
23. Modupe O, Wesley OW, Morufu Elizabeth AO. Analysis of essential oil from the stem of *Chasmanthera dependens*. J Nat Prod 2010;3:47-53.
24. Bayoub K, Baibai T, Mountassif D, Retmane A, Soukri A. Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains. Afr J Biotechnol 2010;9:4251-8.
25. Carballeira NM, Emiliano A, Alonso NH, Ganzalez FA. Facile total synthesis and antimicrobial activity of the marine fatty acids (z)-2-methoxy-6-hexadecenoic acid. J Nat Prod 1998;61:1543-6.
26. Guerrini A, Sacchetti G, Muzzoli M, Rueda GM, Medici A, Besco E. Composition of the volatile fraction of *Ocotea bofo* Kunth (Lauraceae) calyces by GC-MS and NMR fingerprinting and its antimicrobial and antioxidant activity. J Agric Food Chem 2006;54:7778-88.
27. Saravanan VS, Shanmugapandiyan P, Mahesh K, Shalini N, Nagaramya K, Jagadeesh B. Antimicrobial activity of chloroform extract of leaves of *Catharanthus roseus*. Asian J Chem 2012;24:3126-8.