ejpmr, 2015,2(2),459-465

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

COMPARATIVE STUDIES AND ANTIMICROBIAL EMPIRE OF TWO MEDICINAL PLANTS FROM INDIA AGAINST CLINICAL ISOLATES OF *STREPTOCOCCUS PNEUMONIAE*

R. Kadhar Nivas*[1], Dr. M. Boominathan[2]

[1]Research Scholar, [2]Head of the Department, Department of Biotechnology, Marudupandiyar Institutions, Bharathidasan University, Thanjavur-613403, Tamilnadu, India.

Article Received on 25/01/2015 **Article Revised on 14/02/2015** **Article Accepted on 05/03/2015**

***Correspondence for Author**

Dr. R. Kadhar Nivas

Research Scholar, Department of Biotechnology, Marudupandiyar Institutions, Bharathidasan University, Thanjavur-613403, Tamilnadu, India.

ABSTRACT

To examine the *in vitro* antibacterial activity of the ethanol extract of fresh leaves of *Allium cepa* and *Ficus bengalensis* and to determine and quantify the phenol compounds of the investigated plant parts. This study was drifting out at the MP Institutions, Bhrathidasan University, Thanjavur, Tamilnadu, India from Sep 2014 to Oct 2014. Clinical strains of Streptococcus Pneumoniae and two ethanol extracts of two plant species were used for the antimicrobial study. Thirty grams of each sample was ground, filtrated, and each filtrate mixed with 100 ml ethanol and placed in a shaker for 48 hours. The ethanol was evaporated from the sample, weighed, and subjected to an antibacterial activity test using the agar diffusion method. The high- performance liquid chromatography was used to identify and quantify the phenols extracts of investigated samples. Ethanol extract of the investigated plant parts showed antibacterial activities against different pathogenic bacteria. Leaf extracts of *Allium cepa* showed the highest antibacterial activity, followed by the leaves of *Ficus bengalensis*. The amount of main phenols detected in *Allium cepa* higher than *Ficus bengalensis.* The ethanol extract of the tested plants could be considered as an alternative source of new antibacterial drugs.

KEYWORDS: HPLC, Antimicrobial Empire, Streptococcus Pneumoniae, Medicinal Plants.

INTRODUCTION

Development of antibiotic resistance among common respiratory pathogens is a major cause of concern worldwide. *Streptococcus pneumoniae* are the most common respiratory

pathogens. Streptococcus pneumoniae (S. pneumoniae) remains a common pathogen and leading cause of morbidity and mortality [1] Transmission of S. pneumoniae occurs as the result of direct person-to-person contact via respiratory droplets and by autoinoculation in persons carrying the bacteria in their upper respiratory tract [2] Many human diseases are known to have been treated with herbal medicine throughout the history of human beings. The increasing evolution of multi drug resistant bacteria, and the recent appearance of strains with reduced susceptibility to antibiotics leads to the emergence of untreatable bacterial diseases.[3,4] In addition, the revival of interest in plant derived drugs is mainly due to the widespread belief that 'natural medicines' are safe and more dependable than the costly, synthetic drugs, many of which are toxic and possess adverse effects. Thus, there is a growing interest to explore the alternative drugs from different plant species that have antimicrobial properties and can be used as antibiotic resources [5, 6]**.** In this respect, *Allium cepa* and *Ficus bengalensis* are applying significant ayurvedic remedy against various microbial diseases. At the present time, the *Allium* family has over 500 members, each differing in appearance, color and taste, but close in biochemical, phytochemical and neutraceutical content [7] *Ficus bengalensis* plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India[8]**.** Recent studies revealed many bioactive compounds from these two medicinal species. Hence, the present study was undertaken evaluate antimicrobial activity of pure fractions from these plants against human pathogenic organism *Streptococcus pneumoniae*.

MATERIALS AND METHODS

Collection of plant species

Allium cepa fresh leaves and *Ficus bengalensis* was collected from various area of Tamil nadu and analysis was carried out at MP Institutions, Bharathidasan University, Thanjavur, Tamil Nadu, and India. The plant materials which were free from disease, and swashed thoroughly 2-3 times with running water and once with sterile distilled water.

Extraction of plant material

Thirty grams of fresh leaves of *Allium cepa* fresh leaves and *Ficus bengalensis* were weighed out and crushed directly by grinder for 15 minutes, and the solution samples were filtered through 2-layered muslin cloth. The filtrates were mixed with 100 ml ethanol, placed in a shaker for 48 hours, and then filtered through Whatman No. 1 filter paper. The ethanol in

each filtrate was evaporated completely, and each extract was weighed and subjected to an antibacterial activity test.

Collection of Organism

Human pathogenic microbes *Streptococcus pneumoniae* were clinical isolates of patients isolated from clinical patients at dental clinics in and around Thiruvarur and Mannarkudi Tamil Nadu, India.

Analysis of Antimicrobial activity

The antimicrobial activity was determined by the agar well diffusion method against different strains of bacteria 100 µl of standardized inoculum of each test bacterium was spread onto sterile Muller-Hinton Agar (Hi-Media). A 9 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 0.1 ml of the ethanol plant extraction. Sterile dimethyl sulfoxide (DMSO) served as negative and gentamicin (10 ug/disc) as positive controls to determine the sensitivity of each bacterial species tested. The plates were kept at room temperature for one hour to allow proper diffusion of the extract into agar and then incubated at 37oC for 48 hours. Nine clinical strains of bacteria and two ethanol extracts of two plant species were used for the antimicrobial study. The tests were performed in triplicate, and the minimal inhibitory concentration (MIC) and diameter of the cleared zones were determined. All tests were lugged out from Sep 2014 to Oct 2014 at the MP Institutions, Bharathidasan University, Thanjavur, Tamil Nadu, India.

Quantization of phenols using by HPLC

A Shimadzu model HPLC system consisting of a solvent delivery module (LC- 10AD) with a double plunger reciprocating pump, UVVIS detector (SPA-10A), column oven (CTO-10A) and 20-ul injection loop were used. The column used was an Apex octadecyl 104 C18 (25x0.4 cm ID) with 5-um packing (Jones Chromatography Limited, Colorado, USA) [9]. Phenolic compounds present in the samples were identified by comparing retention time (Rt) of the standards and by the co-injection. Contents of phenolic compounds were calculated by comparing peak areas of reference compound with those in the samples run under similar elution conditions.[10, 11]

Statistical analysis: The results of the antimicrobial activity were expressed as the means obtained from 3 independent analyses. Analysis of variance was used to compare between

data. All analyses were performed at $p<0.05$ using Minitab version 2000 13.1 (Minitab, State College, PA, USA).[11]

RESULTS

The results of the antimicrobial activity of the ethanol extract of fresh roots of *Allium cepa* fresh leaves and *Ficus bengalensis by* agar well diffusion method revealed that all two extracts showed inhibitory activity against *Streptococcus Pneumoniae*. Shown in Table 1. Among the two plants tested, the ethanol leaf extracts of Allium cepa showed the highest antibacterial activity with a zone of inhibition ranging from 11.87- 19.23 mm at 20mg/ml concentration and MIC value at 10mg/ml. The ethanol extract of the leaves of Ficus bengalensis presented moderate antibacterial activity with respective means between 8.66-13.33 mm at 25mg/ml concentration and MIC value of 12.5 mg/ml. The HPLC scrutiny of the ethanol extract of fresh leave of *Allium cepa* fresh and *Ficus bengalensis* grand the description of five phenolic compounds: Catechin, P-coumaric acid, ferulic acid, cinnamic acid and sinapic acid. From the issue exposed in Table 2, it perhaps certain that the type and amount of phenolic compounds detected varied in terms of the tested plants.

Table 1 – Comparative Studies, Antibacterial activity and MIC values of ethanol extract of fresh leaves of *Allium cepa* and *Ficus bengalensis*, Values are expressed as mean of the two replicates.

Human Pathogenic Bacteria	Allium cepa (MIC)		Ficus bengalensis (MIC)		DMSO	Gentamicin 95% Cl 10µg/disc (95%cl)
	20mg/ml	10mg/ml	25mg/ml	12.5mg/ml		
Streptococcus Pneumoniae	**11.87 (9.43-14.87)**	**18.62 (16.63-23.68)**	**12.19 (10.43-14.45)**	**8.66 (7.4-1.27)**	**NIL**	**35.63 (35.52-36.89)**
Nil-no inhibition zone, DMSO-dimethyl sulfoxide, MIC-minimum inhibitory concentration, Cl-confidence interval at p<0.05						

Table 2 – Constitutions of five phenols in the ethanol extract of fresh leaves of *Allium cepa* fresh leaves and *Ficus bengalensi.*

Analyte	Allium cepa		Ficus bengalensi	
	RT (min)	mg/100g	RT (min)	mg/100g
Catechin	ND	ND	ND	ND
Cinnamic acid	ND	ND	ND	ND
Ferulic acid	13.83	0.028	2.88	8.9
P-coumaric acid	14.45	0.011	11.12	Tr
Sinapic acid	ND	ND	ND	ND
RT-retention time of the peak, Tr-Concentration<0.001mg/100mg, ND-not detected, min-minutes, CI-confidence interval at p<0.05				

DISCUSSION

Medicinal plants play a crucial role in the search for alternative antimicrobial components. According to the World Health Organization, it is estimated that around 80% of the earth's population use some form of herbal medicine in their health care, whereas natural products are a preferable option than synthetic ones[12]. The literature indicates that medicinal plants have secondary compounds that are of great importance in human life in terms of acting as antioxidants, anti-inflammatories, and being involved in the modulation of detoxification enzymes, the stimulation of the immune system, the modulation of steroid metabolism and antimicrobial effects [13]. The results obtained in the present study indicate that the ethanol extract of fresh leave of *Allium cepa* and *Ficus bengalensis* have varied antimicrobial activities to the test organism used, namely *Streptococcus pneumoniae* . The results also show that different plants assayed here possess different levels of antimicrobial activities, that ethanol extracts of fresh leaves of *Allium cepa* exhibited the highest activity, followed by the leaves of *Ficus bengalensis*. The findings support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds [14-16]. However, the literature contains scant information regarding the relationship between the phenolic content and the antimicrobial activity of these two plants.

In this study, most of the antimicrobial activity in ethanol extracts of investigated plants appears to be explainable by phenolic compounds including catechin, *p*-coumaric acid, ferulic acid, cinnamic acid, and sinapic acid. The contents of the main phenols in *Allium cepa* higher than *Ficus bengalensis*. These bioactive components that are naturally occurring in most plant materials have been reported to account for the exertion of antimicrobial activity. The results highlight the strong positive relationship between antimicrobial activity and the phenol content in all the plant extracts examined. The fact that the ethanol extract of the plants studied were active against both clinical and laboratory isolates, is an indication that it can be used as an important source of very potent antibiotic drugs. Investigating the toxic effects of ethanol extracts of these plants could be a limitation of this study.

CONCLUSION

In this study has shown that ethanol extracts from the fresh leaves of *Allium cepa* higher than *Ficus bengalensis* possess antimicrobial properties. It can be suggested that ethanol extracts of these plants are a great potential source of antibacterial compounds that could be used in

the formulation of new antimicrobial drugs of natural basis. The bioactive phenolic substances obtained from these plants can therefore be a promising antibiotic source for the treatment of various bacterial infections. Isolation, characterization, and purification of these phytoconstituents and the determination of their respective antibacterial activities, together with a toxicological analysis should be the future direction for researchers.

REFERENCES

1. Ying QH, Zhang JJ, Yang L, Ding JL, Song XH, Du W, et al. Antimicrobial resistance, serotypes and genotypes of Streptococcus pneumoniae isolates associated with ocular infection in China. Afr J Microbiol Res 2012; 6(1): 160-164.
2. Patrick RM, Kent R. Medical Microbiology. 4th ed. St Louis, USA: Mosby Inc; 2002, p.232-234.
3. Tomoka N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I, et al. Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. J Health Sci 2002; 48: 273-276.
4. Pillar CM, Draghi DC, Sheehan DJ, Sahm DF. Prevalence of multidrug-resistant, methicillin-resistant Staphylococcus aureus in the United States: findings of the stratified analysis of the 2004 to 2005 LEADER Surveillance Programs. Diagn Microbiol Infect Dis 2008; 60: 221-224.
5. Gottlieb OR, Borin MR, de Brito NR. Integration of ethnobotany and phytochemistry. dream or reality? Phytochemistry 2002; 60: 145-152.
6. Narod FB, Gurib-Fakim A, Subratty AH. Biological investigations into Antidesma madagascariense Lam. (Euphorbiaceae), Faujasiopsis flexuosa (Lam.) C. Jeffery (Asteraceae), Toddalia asiatica (L.) Lam. and Vepris lanceolata (Lam.) G. Don (Rutaceae). Journal of Cell and Molecular Biology 2004; 3: 15-21.
7. Benkeblia N, “Antimicrobial activity of essential oil extracts of various onions (Allium
8. cepa) and garlic (Allium sativum)” Lebensm.-Wiss. u.-Technol, 2004; 37: 263–268.
9. Shandavi C.Bhangale, Vikas V. Patil, Vijay R. Pati. “Anti-bacterial Activity of Ficus
10. Bengalensis Linn. Bark on Actinomyces viscosus”, International Journal of Pharmaceutical Sciences, Vol.2 (1), 2010, 39-43.
11. Tasioula-Margari M, Okogeri O. Simultaneous determination of phenolic compounds and tocopherols in virgin olive oil using HPLC and UV detection. Food Chemistry 2001; 74: 369-375.

12. Singh UP, Suman A, Sharma M, Singh JN, Singh A, Maurya S. HPLC analysis of the phenolic profiles in different parts of Chilli (Capsicum annum) and Okra (Abelmoschus esculentus L.) Moench. The Internet Journal of Alternative Medicine 2008; 5: 2.
13. Saad A. Alamri, MSc, PhD, Mahmoud F. Moustafa, MSc, PhD Antimicrobial properties of 3 medicinal plants from Saudi Arabia against some clinical isolates of bacteria. Saudi Med J 2012; Vol. 33 (3): 272-27.
14. Madhuri S, Pandey G. Some anticancer medicinal plants of foreign origin. Current Science 2009; 96: 779-783.
15. Lampe JW. Spicing up a vegetarian diet: chemopreventive effects of phytochemicals. Am J Clin Nutr 2003; 78: 579S-583S.
16. 14.Thankamma L. Hevea latex as wound healer and pain killer. Current Science 2003; 84: 971-972.
17. 15.Narendra N, Gaurav P, Lokesh D. Naveen KJ. Wound healing activity of latex of Calotropis gigantean. International Journal of Pharmaceutical Science 2009; 1: 176-181.
18. 16.Sequeira BJ, Vital MJ, Pohlit AM, Pararols IC, Caúper GS. Antibacterial and antifungal activity of extracts and exudates of the Amazonian medicinal tree Himatanthus articulatus (Vahl) Woodson (common name: sucuba). Mem Inst Oswaldo Cruz. 2009; 104: 659-661.