Available online at http://jddtonline.info

RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT CONCENTRATIONS OF *CHENOPODIUM ALBUM* LEAVES EXTRACT

***Jyoti Parkash[1], Kannubhai Rameshbhai Patel[2]**

Research Scholar, Department of Pharmaceutical Science, JJT University, Jhunjhunu-333001, Rajasthan, India[1]

Shri B.M. Shah College of Pharmaceutical Education and Research, Modasa-383315, Gujarat, India[2]

**Corresponding Author's Email address- parkash981@yahoo.com*

ABSTRACT

Different concentrations of *Chenopodium album* leaves extract were examined against *B.subtillus, S. aureus, P.aurogenosa* and *E. coli*. Against *B.subtillus*, 100% concentration of extract has shown maximum zone of inhibition 11.0 mm (Table 1). Against *S.aureus*, 100% conc. of extract has shown maximum zone of inhibition 13.0mm. Against *P. auerogenosa* 75% conc. of extract has shown maximum zone of inhibition i.e. 9.75 mm after 8 hours.100% conc. of *Chenopodium* leave extract shows maximum zone of inhibition against *E. coli*. To compare the bacterial inhibition potentiality of crude extract of *Chenopodium album*, it is very necessary to compare with the standard antibiotic regimens. *S. aureus* and *B.subtillus* (gram positive) has been screened against tetracycline, ofloxacin, Azithromycin, Piperacillin, and against *P. auerogenosa* & *E. coli* (gram negative), Nitrofurantoin, Gentamycin, Cefotaxime and Norfloxacin were used. The zone of inhibition is recorded maximum in Norfloxacin i.e. 19 mm and minimum in Gentamycin i.e. 12 mm against *P. auerogenosa*. Against *E. coli*, the maximum zone of inhibition is recorded in Norfloxacin i.e. 18mm & minimum 11mm in Cefotaxime. Against *S.aureus*, 18mm zone of inhibition is observed in ofloxacin and azithromycin and minimum in tetracycline. However, against *B. subtillus* maximum zone of inhibition is 20 mm in Azithromycin and minimum in Piperacillin i.e. 16mm.

Key words- *Chenopodium album*, antibiotic, gram positive, gram negative, maximum zone of inhibition

INTRODUCTION

Ayurveda is believed to have originated over 6000 years ago. Ayurveda consists of two words Ayur and Veda. Ayur meaning life and Veda means knowledge or science. Thus Ayurveda describes the methods of remain healthy and to treat diseases[1]. Ayurveda pharmacopoeia is comprise of more than 1200 species of plants including cinnamon and cardamom etc, nearly 100 minerals including sulpher, arsenic, lead and copper sulphate and over 100 animal products including milk, bones and gall stones[2]. Mankind has known about the therapeutic effects and benefits of herbal drugs from thousands of years. In the ancient years, whole plants were used as drugs. Herbal drugs are nature's gift to treat no. of diseases of men and animals[3]. Approximately 80% of the world inhabitants relay on traditional medicines for their primary health care. India is known as an emporium of medicinal plants[4].

In 1970 the Indian medical central council act aim to standardize qualification for Ayurveda and provide accredited institutes for its study and research and it was passed by Parliament of India[5]. In India over 100 institutes conduct courses in traditional ayurvedic medicine. Central Council of Indian Medicine (CCIM) monitors and inspects institutes for higher education in Ayurveda.

Herbs as Traditional Medicines

The diverse culture of India is a rich source of traditional medicines, many of which are of plant origin. Herbal medicine is also called as botanical medicine or phytomedicine. Herbal medicines refers to the use of any plant's seeds, berries, roots, leaves, bark or flowers for medicinal purpose[6]. Herbalism is becoming main stream and research show value of herbs in the treatment and prevention of disease. There is still a great interest in medicinal herbs all over world. The main reason for this is that herbs contain compounds of therapeutic efficacy and they are more natural and more acceptable to patients than synthetic drugs[7].

Practitioners from herbal medicine usually use unpurified plant extract. This unpurified plant extracts contain several different constituents. They claim that these can work together synergistically so that the effect of whole constituents is greater than the total effect of its individual constituents[8]. Toxicity is also reduced when whole herbs are used instead of isolated ingredients.

The use of traditional medicine is increasing day by day in every country. The goal of "health for all" cannot be achieved without traditional medicine. In United state, products such as Ginkgo, Echinacea, Garlic and many others are advertised widely as safer, more natural and healthier alternatives to conventional medicines[9]. According to recent surveys and studies, 15% to 40% consumers have used herbal medicine to cure many diseases. In last 25 years in United States, due to increasing cost of prescription medicines, combined with an interest in reusing to natural or organic remedies, has led to an increase in use of herbal medicines. Approximately 70% of German physians prescribe plant based medicines to the patients[10].

Bacterial resistance and resistance alternatives

There is urgent need to work on drugs obtained from plants and discover new antimicrobial compounds with diverse chemical structure and novel therapeutic effect because the incidence of new and re-emerging infectious diseases is increasing day by day[11]. Another big concern is the development of resistance to the antibiotics in current clinical use

The indiscriminate use of antimicrobial drugs by patients for the treatment of infectious disease is the major cause for multiple drug resistance. Other than this problem, antibiotics produce adverse effects on the host, including hypersensitivity reaction, immune suppression and allergic reactions. This situation forced scientists to think of other alternatives like new antimicrobial substitutions from other sources especially medicinal plants. Several scientists work on the antimicrobial activity of different herbal extracts in different regions of the world. Approximately one-half of all deaths in tropical countries occur due to infectious diseases. Infectious disease mortality rates are also increasing in developed countries, such as the United States[12]. Various medicinal plants and their extracts have been used for years in daily life to treat disease all over the world. Medicines derived from herbs have made significant contribution towards human health. According to the World Health Organization, medicinal plants are the best source to obtain a variety of drugs. Therefore, chemical constituents from such plants should be investigated to obtain a thorough knowledge about their properties, safety and efficacy.

MATERIALS AND METHODS

Collection and processing of plant materials

The plants were collected from local market of Nangal during September-October. The impurities from plant were removed and leaves were separated from the plant. The leaves of plant were shadow dried at room temperature and powdered by mechanical grinder. The powder was sieved by 20 mm and 40 mm sieve and intermediate was used for extraction.

Preparation of extract

The powdered plant material (250 gm) was extracted with methanol (3 Lts) by cold extraction (Maceration and Percolation) method at room temperature. The methanolic extract was concentrated to half of the volume at 40-50^{o}C temperature.

Antibacterial activity

The effect of Chenopodium album extract was studied on both gram positive (*P. auerogenosa* and *S. aureus)* and *gram negative (B .subtilis* and *E. coli)* bacteria by Disc Diffusion method.

Culture medium

Nutrient broth was used as culture media for the activation of microorganisms. All the culture media were prepared and treated according to the manufacturer guidelines.

Method

All the required apparatus and material were sterilized in autoclave and placed in a laminar airflow cabinet under pathogen free conditions. Test organisms were collected from department of microbial standard stock. By streaking with loop microorganisms were included in nutrient broth and incubated at 35^{o} C for twelve hours. Nutrient agar media was prepared and poured in Petri plates and kept for drying. Swap cotton was dipped in broth having microbial growth and gently squeezed against the inside of the tube to remove excess fluid. Inoculated the dried surface of agar plate by streaking the swap over the entire sterile agar surface[13]. Repeated this procedure to more time and rotated the plate 60^{o} each time to ensure and even distribution of inoculums. Replaced the plate top and allow three to five minutes but no longer than 15 minutes for any excess surface moisture to be absorbed before applying the test and antibiotic discs. Discs were dipped in drug of different concentrations (25%, 50%, 75% and 100%) and air dried in laminar airflow before this step. Placed the appropriate discs (drugs and antibiotics) evenly on the surface of the agar plate by using sterilize forceps. Inverted the plates and placed them in an incubator at 35^{o} C within 15 minutes after discs were applied. After 6-8 hrs of incubation, examined each plate at an interval of half an hour and measure the diameter of zones of complete inhibition including the diameter of the disc. Mean of every two hours was taken upto 12 hours.

Statistical Analysis

Results are expressed as the mean value ± standard error of mean (S.E.M).

RESULTS

The antimicrobial activity of different concentrations of *Chenopodium album* was investigated against gram positive (*S. aureus* and *B .subtilis*) and gram negative (*P. auerogenosa* and *E. coli*) bacteria, and compared with standard antibiotics. Results are shown in table no. 1-6. The results show that plant extract has significant antibacterial activity against both gram positive and gram negative bacteria. Table no. 1-4 showed the antibacterial activity of different concentrations of *chenopodium album* leaves extract against gram negative and gram positive bacteria. Table no. 5 & 6 shows the antibacterial activity of standard antibiotics against *S. aureus, B .subtilis, P. auerogenosa* and *E. coli.*

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Table 1: Antibacterial activity of different concentration of *Chenopodium album* against *B. subtillus*

Conc.	[Zone of inhibition (mm)] after					
	0-2hr	2-4hr	4-6hr	6-8hr	8-10hr	10-12hr
25%	7.125± 0.25	7.0 ± 0.41	7.375 ± 0.48	7.5 ± 0.0	7.125 ± 0.25	7.0 ± 0.0
50%	8.25 ± 0.25	8.625± 0.25	8.825 ± 0.25	8. 5 ± 0.57	8.875 ± 0.62	8.5 ± 0.0
75%	8.625± 0.75	8.125± 0.25	9.0 ± 0.00	8.75 ± 0.28	9.0 ± 0.0	9.125± 0.47
100%	9.625±0.98	10.0± 0.28	11.0 ± 0.57	10.625 ± 0.25	8.875 ± 0.25	9.0 ± 0.0

Table 2: Antibacterial activity of different concentration of *Chenopodium album* against *S. aureus*

Conc.	[Zone of inhibition (mm)] after					
	0-2 hrs	2-4 hrs	4-6 hrs	6-8 hrs	8-10 hrs	10-12 hrs
25%	7.75 ± 0.5	8.0 ± 0.0	8.5 ± 0.58	9.25 ± 0.5	8.625±0.47	9.25 ± 0.28
50%	9.5 ± 0.58	10.25 ± 0.5	11.25 ± 0.98	11.75 ± 0.5	11.25 ± 0.5	11.125 ± 0.25
75%	11.75 ± 0.5	11.0 ± 0.0	12.25 ± 0.5	11.5±0.58	11.125±0.25	10.875 ± 0.25
100%	11.75 ± 0.5	12.5 ± 0.577	13.0 ±0.0	12.375±0.47	11.625± 0.25	11.0 ± 0.0

Table 3: Antibacterial activity of different concentration of *Chenopodium album* against *P.aurogenosa*

Conc.	[Zone of inhibition (mm)] after					
	0-2hr	2-4hr	4-6hr	6-8hr	8-10hr	10-12hr
25%	6.75 ± 0.5	7.0 ± 0.0	7.5 ± 0.58	8.0 ± 0.0	8.125± 0.25	8.375 ± 0.25
50%	7.0 ± 0.0	7.5 ± 0.58	8.0 ± 0.0	8.25 ± 0.5	8.625± 0.25	8.75 ± 0.29
75%	7.0 ± 0.0	7.625± 0.25	8.5± 0.58	9.125 ± 0.25	9.75 ± 0.5	9.0 ± 0.0
100%	8.0 ± 0.0	8.375± 0.25	9.0 ± 0.0	9.0 ± 0.0	9.125± 0.25	8.5 ± 0.0

Table 4: Antibacterial activity of different concentration of *Chenopodium album* against *E. coli*

Conc.	[Zone of inhibition (mm)] after					
	0-2hr	2-4hr	4-6hr	6-8hr	8-10hr	10-12hr
25%	4.625±0.25	5.25±0.58	5.625 ± 0.25	6.0 ± 0.0	6.0 ± 0.0	6.25 ± 0.25
50%	5.25 ± 0.28	5.50 ± 0.25	6.125 ± 0.0	6.75 ± 0.50	7.25 ± 0.25	7. 50 ± 0.25
75%	6.25 ± 0.47	6.75 ± 0.5	7.0 ± 0.0	7.5 ± 0.29	7.75 ± 0.25	8.0 ± 0.0
100%	6.0 ± 0.28	6.5 ± 0.29	7.25 ± 0.25	8.25 ± 0.25	9.0 ± 0.0	9.5 ± 0.28

Table 5: Antibacterial activity of standard antibiotic disc (gram negative) against different bacteria

Name of microorganisms	Name of Standard antibiotics [zone of inhibition(mm)]			
	Nitrofurantoin	Gentamycin	Cefotaxime	Norfloxacin
E.coli	14	17	11	18
P. auerogenosa	15	12	16	19

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Table 6: Antibacterial activity of standard antibiotic disc (gram positive) against different bacteria

Name of microorganisms	Name of Standard antibiotics [zone of inhibition(mm)]			
	Tetracycline	Ofloxacin	Azithromycin	Piperacillin
S.aureus	17	18	18	19
B.subtillus	17	17	20	16

DISCUSSION

In the present study, methanolic extract of leaves of *Chenopodium album* were tested against selected Gram positive and Gram negative bacterial species. The leaf extracts limited the growth of both Gram-positive and Gram-negative bacteria species tested. Antibacterial activity of the drug was determined by 'Agar Disc diffusion method' (Kirby-Bauer Method)[14]. Different conc. of leaf extracts of *Chenopodium album* exhibited significant anti-bacterial activity against all test organisms. 50% Methanolic extract of *Chenopodium album* possess the best antibacterial activity against *S.aureus* exhibits zone of inhibition 13.0 mm at 100% concentration. 100% concentration of *Chenopodium album* shows 12 mm zone of inhibition against *B.subtillus* However all the concentration has shown reduction in zone of inhibition.

Although work is required to be commenced on the broad range of microbial strain and use of specific isolated constituents for further evaluation.

It has been noticed that crude extract of *Chenopodium album* is showing good antibacterial property against gram positive micro flora, but the extract required more differential dosing against wide range of gram positive and gram negative bacteria.

ACKNOWLEDGMENT

The author is thankful to Principal Dr. D.N. Prasad, Shivalik College of Pharmacy, Nangal and Vice-Chancellor, Registrar and Ph.D. Coordinator, JJT University, Jhunjhunu, Rajasthan for providing necessary facilities for my research work.

REFERENCES

1. Kraft K. Complementary/Alternative Medicine in the context of prevention of disease and maintenance of health. *Prev Med*. 2009 May 22
2. Dos Santos-Neto LL, de Vilhena Toledo MA, Medeiros-Souza P, de Souza GA. The use of herbal medicine in Alzheimer's disease-a systematic review. *Evid Based Complement Alternat Med*. 2006;3(4):441-5.
3. Bruneton J. (1995). Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept. Ltd. England, U.K.
4. National Center for Complementary and Alternative Medicine. What is Complementary and Alternative Medicine? Available at http://nccam.nih.gov/health/whatiscam. Last accessed April 8, 2013.
5. binson A, McGrail MR. Disclosure of CAM use to medical practitioners: a review of qualitative and quantitative studies. Complement Ther Med. 2004;12(2-3):90-98.
6. Balunas M. J, Kinghorn A. D. Drug discovery from medicinal plants. Life Sci. 2005; 78:431–41.
7. Butler M. S. The role of natural product chemistry in drug discovery. J Nat Prod. 2004; 67:2141–53.
8. Phillipson, J. D. Phytochemistry and medicinal plants. *Phytochemistry* **2001,** 56, 237−243.
9. Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod*. **2003,** 66, 1022−1037.
10. Koehn, F. E.; Carter, G. T. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov*. **2005,** 4, 206−220.
11. Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z., Global importance of medicinal plants. In *The Conservation of Medicinal Plants,* **1991.**
12. Dev, S. Ancient-modern concordance in ayurvedic plants: Some examples. *Environmental Health Perspectives* **1999,** 107, 783−789.
13. Anonymous, 1996. Pharmacopiea of India (The Indian Pharmacopiea), 3rd Edition. Govt. of India, New Delhi, Ministry of Health and Family Welfare.
14. Murray, P. R., A. C. Niles, and R. L. Heeren. (1987). Comparison of a highly automated antimicrobic 5-h susceptibility testing system, the Cobas-Bact, with two reference methods: Kirby-Bauer disk diffusion and broth micro dilution. J. Clin. Microbiol. 25:2372–2377

