Journal of Research in Biology

FicusPress

An International Online Open Access Publication group

OPEN ACCESS

Original Research paper

Evaluation of Antibacterial activity of Medicinal Plants on Fish Pathogen *Aeromonas hydrophila*

Authors:
Abdul Kader Mydeen KP . Haniffa MA.

Institution:
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, INDIA.

Corresponding author:
Abdul Kader Mydeen KP

Email:
abdulmicro2000@yahoo.com

Phone Number:

ABSTRACT:

This study evaluated the antimicrobial potency of aqueous extract of three common medicinal herbs, *Azadirachta indica* (Neem leaves), *Solanum torvum* (Sundakai fruit coat) and *Curcuma longa* (Turmeric,rhizome) against the in vitro growth of pathogenic bacterium *Aeromonas hydrophila* isolated from infected fresh-water fish*, Channa striatus* was assessed by disc diffusion assay.

Determination of minimum inhibitory concentration (MIC) for each aqueous herbal extract was done on Muller Hinton agar swabbed with 0.5 ml of overnight culture of A*. hydrophila* (10^6cfu/ml). After incubation period, zones of inhibition around herbal extracts incorporated discs were measured.

The strongest antibacterial activities among all plant species were obtained by the aqueous extract of *A. indica* with inhibition zone of 18 mm against *A. hydrophila. S. torvum* demonstrated moderate (11 mm) and *C. longa* marked weak (8 mm) inhibiting activity against *A. hydrophila*.

Keywords:
Azadirachta indica, *Solanum torvum*, *Curcuma longa, Aeromonas hydrophila,* anti-bacterial activity.

Web Address:
http://jresearchbiology.com/Documents/B000001.pdf.

Article Citation:

Abdul Kader Mydeen KP and Haniffa MA.
Evaluation of Antibacterial activity of Medicinal Plants on Fish Pathogen *Aeromonas hydrophila.* Journal of research in Biology (2011) 1: 1-5

Dates:
Received: 20 Jan 2011 **Accepted:** 30 Jan 2011 **Published:** 05 Feb 2011

Journal of Research in Biology

INTRODUCTION

Aquaculture has been a growing activity for the last twenty years worldwide and this impressive development has been attended by some practices potentially damaging to animal health (Naylor and Burke, 2005). The large-scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Alcaide *et al*., 2005; Cabello, 2006). The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment (Muniruzzaman and Chowdhury, 2004). Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture (Direkbusarakom, 2004). Since ancient times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio, 2005) and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture (Abutbul *et al*., 2005). In addition, plant-derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field (Punitha *et al*., 2008). *Aeromonas hydrophila*, the most common bacterial pathogen in freshwater fish, has been recognized to be the aetiological agent of several distinct pathological conditions including tail rot, motile *Aeromonas* septicemia (MAS) and epizootic ulcerative syndrome (EUS) as a primary pathogen (Roberts *et al.,* 1992). EUS is a globally distributed disease and has become an epidemic affecting a wide variety of wild and cultured fish species (Roberts *et al.,* 1992) especially in Southeast Asia including Pakistan (Lilley *et al.,* 1997) and India (Vishwanath *et al.,* 1997). The ability of herbs to inhibit activity of bacteria having potential interest as fish pathogens has been documented (Bansemir *et al*., 2006; Dubber and Harder, 2008).

Therefore, the objective of the present study was to evaluate the antibacterial activity of aqueous extracts of *Azadirachta indica* (neem, leaves) *Solanum torvum* (sundakai, fruit coat) and *Curcuma longa* (turmeric, rhizome) on most frequently isolated bacterium in aquaculture industry, *A.hydrophila.*

MATERIALS AND METHODS

Plant material and extraction

A. indica, *S. torvum* and *C. longa* were collected from Agriculture college campus, Killikulam, Tirunelveli, India. They were surface-sterilized separately with 0.1% mercuric chloride (w/v) solution for 10 min and washed thoroughly in running tap water for 10 min, followed by shade drying for about 10 days until weight constancy was achieved (Hobbs,1994). Each sample was finely powdered in an electric blender. For aqueous extraction, twenty grams from each powdered plant sample were extracted with 200 ml water at 80°C in a waterbath for 12 hours and then filtered. Water was evaporated using a lyophilizator.

Antibacterial Assay

The disc diffusion assay (Kirby-Bauer Method) was used to screen the herbal extracts for antibiotic activity (Prescott *et al*., 1990). *Aeromonas hydrophila* isolated from infected freshwater fish, *Channa striatus* (Figure:1). Pure culture of *A. hydrophila* was grown on Tryptic Soy Agar (TSA, Hi media) plate and incubated for 2 days at 37°C. 3-4 colonies were transferred into culture tube containing 5 ml sterile Tryptic Soy Broth (TSB, Hi media) and was incubated for 24 hours at 37°C. All extracts were sterilized by filtering through a 0.22 µm filter (Millipore). To determine minimum inhibitory concentration (MIC), different concentrations of each herbal extract was prepared (10ppm, 20ppm, 30ppm, 40ppm and 50ppm) and were impregnated on 5 mm sterile Whatman No.1 filter paper disc. Then discs were placed on Muller Hinton agar (Hi-media) and plates were swabbed with 0.5 ml of overnight culture of A. *hydrophila* (10^6cfu/ml). Oxytetracycline and unloaded disc were used as a positive and negative control respectively (Lenette *et al.,* 1985). Each antibacterial assay was performed in triplicate. The diameter of the inhibition zone (mm) was measured (Jin *et al*., 1996) after 16 to 18 h of incubation at 37°C in an incubator and the MIC for each herb was observed and recorded (Table:1). Inhibition zones morethan 11mm were stated as "strong", from 9 to 11 mm as "moderate" and lessthan 9mm as "weak" activities.

RESULTS

The present study was attempted to find out the antibacterial activity of *A. indica, S. torvum* and *C. longa* at different concentrations against the fish pathogen *A. hydrophila.* The results of antibacterial activity of herbs against *A. hydrophila* are summarized in **Table 1.** *A. indica, S. torvum* and *C. longa* exhibited zone of inhibition against *A. hydrophila*. The strongest antibacterial activities among all plant species were obtained by the

Table: 1 Antibiotic sensitivity of *Aeromonas hydrophila* against Herbs (Values are mean ± SD).

Name of the herb	Concentration	*Aeromonas hydrophila* (10^6 cfu/ml)
		Zone of inhibition in mm
Azadirachta indica	10 ppm	0
	20 ppm	5 ± 0.8
	30 ppm	10 ± 1.1
	40 ppm	13 ± 0.9
	50 ppm	18 ± 1.2
Solanum torvum	10 ppm	0
	20 ppm	0
	30 ppm	4 ± 0.6
	40 ppm	7 ± 0.8
	50 ppm	11 ± 0.9
Curcuma longa	10 ppm	0
	20 ppm	0
	30 ppm	0
	40 ppm	3 ± 0.6
	50 ppm	8 ± 1.2

aqueous extract of *A .indica* with inhibition zone of 18 mm against *A. hydrophila. S. torvum* demonstrated moderate (11 mm) and *C. longa* marked weak (8 mm) inhibiting activity against *A. hydrophila*. Herbal discs of *A indica* (10ppm), *S. torvum* (10 and 20ppm) and *C. longa (*10 and 20 ppm), didn't produce zones of inhibition against *A. hydrophila.* Positive control (Oxytetracycline) showed antibacterial activity to *A. hydrophila* and there was no inhibition with negative control.

DISCUSSION

Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be one approach because most of them are safe, cost less and effective against a wide range of antibiotic resistant microorganisms. Seyyednejad *et al.,* (2008) showed that ethanolic extract of *Prunus mahaleb* inhibited the growth of various species of Gram positive and Gram negative bacteria. Aqueous extract of the neem is reportedly possessing anti-inflammatory, antimicrobial and immunomodulatory activities (Van Der Nat *et al.,* 1987). Wafaa *et al.,* (2007) reported the antibacterial and antifungal activities of the native and chemically modified extracts from neem seeds, seed-hulls and leaves.

In the present study, *A. hydrophila* showed 18 mm zone of inhibition at a concentration of 50 ppm against neem extract. Similarly, Dhayanithi *et al*., (2010) showed *Enterobacter sp* and *Escherichia coli* isolated from marine fish*, Amphiprion sebae* showed 15 mm zone of inhibition against neem extract. In the present study, *A. hydrophila* was susceptible to extract of *S. torvum* fruit coat and produced zone of inhibition of 11mm. Similarly, Sivapriya *et al*., (2010) stated that extracts of *S. torvum* fruit coat exhibited significant antibacterial activity against *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium* and *Pseudomonas sp and* these extracts were as potent as standard antibiotics, chloramphenicol and streptomycin. Therefore *S. torvum* fruit coat may be yet another source of natural antibiotic. Wafaa *et al.,* (2007) confirmed that native extracts of *A. indica* leaves with concentration of 20 ppm are inhibitory to *Staphylococcus aureus, E. coli, Candida albicans, Aspergillus niger* and *Penicilium citrinum*. The aqueous *A. indica* leaf extract has been tested against *A. hydrophila* infection in common carp, *Cyprinus carpio* and the results showed that this plant could effectively control *A hydrophila* infection in *C.carpio* (Harikrishnan *et al.,* 2003). Similarly, Junaid *et al.,(2006)* evaluated antimicrobial efficacy of water, hexane and methanolic extracts of fresh leaf of *Ocimum gratissimum* against *A. hydrophila, Salmonella typhimurium, Escherichia coli, Yersinia enterocolitica,* and *Bacillus cereus* were determined using the agar gel diffusion method. Results obtained revealed that the water extracts of the fresh leaves of *O. gratissimum* was most potent, inhibiting all isolates with diameter zones of inhibition ranging from 5 mm to 18 mm, followed by hexane extract of the fresh leaves with zone ranging from 6mm to 14 mm whereas methanol extract of the fresh leaf showed no inhibitory effect on all isolates.

Amal *et al.,* (2007) evaluated the antimicrobial activity of indigenous Jordanian plant extracts of *Hypericum triquetrifolium*, *Ballota undulata*, *Ruta chalepensis*, *Ononis natrix*, *Paronychia argentea* and *Marrubium vulgare* against pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and

Pseudomonas aeruginosa and reported that all these extracts had shown promising antimicrobial activity on all isolates. From this study, it was proved that among the three herbs, extract of *A. indica* was very effective against *A.hydrophila*. Heavy antibiotics used in aquaculture need to be reduced and replaced with alternative processes for treating fish diseases to avoid the emergence of antibiotic resistance in pathogenic and environmental bacteria (Sorum and L'Abee-Lund, 2002; Cabello, 2006). The herbal plants may be used as potential and promising source of pharmaceutical agents against fish pathogens in the organic aquaculture. The screening results of our study confirm the possible use of medicinal herbs as a source of antimicrobial agent.

ACKNOWLEDGEMENT

The authors thank to Rev. Dr. Alphonse Manikam S.J., Principal St. Xavier's College, Palayamkottai, Tamilnadu, India for providing necessary facilities.

REFERENCES

Abutbul S, Golan-Goldhirsh A, Barazani O, Ofir R and Zilberg D. 2005. Screening of desert plants for use against bacterial pathogens in fish. Isr. J. Aquacult.- Bamid., 57(2):71-80.

Alcaide E, Blasco MD and Esteve C. 2005. Occurrence of drug-resistant bacteria in two European eel farms. Appl. Environ. Microbiol., 71:3348–3350.

Amal G, Al-Bakri and Fatma U. Afifi (2007). Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. Journal of Microbiological Methods,Volume 68, Issue 1, January 2007, Pages 19-25.

Bansemir A, Blume M, Schröder S and Lindequist U. 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252:79–84.

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ. Microbiol., 8(7):1137-1144.

Dhayanithi NB, Ajith Kumar TT and Kathiresan K. Effect of neem extract against the bacteria isolated from marine fish Journal of Environmental Biology July **2010**, 31, 409-412 (2010).

Direkbusarakom S. 2004. Application of medicinal herbs to aquaculture in Asia. Walailak Journal of Science and Technology, 1(1):7–14.

Dubber D and Harder T. 2008. Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. Aquaculture, 274:196–200.

Harikrishnan R, Nisha Rani M and Balasundaram C. 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for Aeromonas hydrophila infection. *Aquaculture*, vol. 221, no. 1-4, p. 41-50.

Hobbs C. Echinacea: A literature review. Special Supplementt to HerbalGram 1994, 30, 33-48.

Jin LZ, HO YM and Jalaludin S. 1996. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. *Lett. in Appl. Microbiol*. 23, 63 – 71.

Junaid SA AO Olabode, FC Onwuliri, AEJ Okwori, SE Agina. (2006) The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates.African Journal of Biotechnology. Vol 5, No 22 ISSN: 1684-5315.

Lenette EH, Balows A, Jr. Hausler WA and Shadomy H.J. Manual of clinical microbiology (Eds.) 4th Edn. American Society for Microbiology, Washington **(1985)**.

Lilley JH, Phillips MJ and Tonguthai K. 1997. A review of epizootic ulcerative syndrome (EUS) in Asia. Aquatic Animal Health Research Institute and Network of Aquaculture Centres in Asia-Pacific, Bangkok.

Muniruzzaman M and Chowdhury MBR. 2004. Sensitivity of fish pathogenic bacteria to various medicinal herbs. Bangladesh Journal of Veterinary Medicine, 2 (1):75-82.

Naylor R and Burke M. 2005. Aquaculture and ocean resources: raising tigers of the sea. Annual Review of Environmental Resources, 30:185–218.

Prescott LM, Harley JP and Klein DA. 1990. Microbiology, Wm. C. Brown Publishers, Dubuque, IA, USA.

Punitha SMJ, Babu MM, Sivaram V, Shankar VS, Dhas SA, Mahesh TC, Immanuel G. and Citarasu T. 2008. Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. Aquacult. Int., 16:511–523.

Rios JL and Recio MC. 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol., 100:80- 84.

Roberts RJ, Frerichs GN, Miller SD. Epizootic ulcerative syndrome, the current position. In: *Diseases in Asian Aquaculture. I. Fish Health Section.* Edited by Shariff M., Subasinghe R.P., and Arthur J.R., Asian Fisheries Society, Manila, **1992**, pp. 431-436.

Seyyednejad M, Ebrahimzadeh H and Talaie A. 2001. Carbohydrate content in olive zard c.v. and alternate bearing pattern.J.int.sugar,103(1226):84-87.

Sivapriya M, Dinesha R, Harsha R, Gowda SST and Srinivas L. Antibacterial activity of different extracts of Sundakai (Solanum torvum) fruit coat,International Journal of Biological Chemistry 5 (1):65-67, **2010**.

Sørum H and L'Abée-Lund TM. 2002. Antibiotic resistance in food-related bacteria – a result of interfering with the global web of bacterial genetics. Int. J. Food. Microbiol., 78:43–56.

Van Der Nat MG, Van Der Sluis KTD and Labadie RP. 1991. Ethnophormo-cognostical survey of A. indica Juss (Maliaceae). J Ethnopharmacol., 35:1– 24.

Vishwanath TS, Mohan CV, Shankar KM. Mycotic granulomatosis and seasonality are the consistent features of epizootic ulcerative syndrome (EUS) in fresh and brackishwater fish of Karnataka, India. Asian Fish Sci 1997, 10, 149-154.

Wafaa A, Helmy, Hassan Amer MA, Nefisa and EL-Shayeb. Biological and anti-microbial activities of aqueous extracts from neem tree (*Azadirachta indica* A. Juss., Meliaceae). J.App. Sci. Res., 3, 1050-1055 (**2007**).

Journal of Research in Biology

Journal of Research in Biology

FicusPress

An International Online Open Access Publication group

OPEN ACCESS

ORIGINAL PAPER

Evaluation of changes in proximate composition of Bacteriocin supplemented Prawn

Authors:
Karthick Raja Namasivayam S, Sivasubramanian S and Prakash P.

Institution:
Department of Biotechnology, Sathyabama University, Chennai 600119, Tamil Nadu, India.

Corresponding author:
Karthick Raja Namasivayam S,

Email:
biologiask@gmail.com.

ABSTRACT:

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of bacteria. The present study deals with the effect of various form of bacteriocin derived from Lactobacillus brevis as crude culture supernatant, ammonium sulphate precipitate and dialysed product on the changes of proximate composition such as moisture, protein, total ash ,total sugar fat and microbial analysis. These were analysed by seeing total bacterial , fungal, yeast, spore formers, coliform and anaerobes counts in cooked prawn. No effect on proximate composition was observed in all forms of bacteriocin treated food. No microbial growth was recorded but distinct reduction in protein, fat and total sugar, moisture, total ash were observed in non - bacteriocin supplemented food which suggest the possible use of bacteriocin as food preservative for processed food product.

Keywords:
Bacteriocin, *Lactobacillus brevis,* proximate composition.

Web Address:
http://jresearchbiology.com/Documents/RA0001.pdf.

Article Citation:
Karthick Raja Namasivayam S, Sivasubramanian S and Prakash P.
Evaluation of changes in proximate composition of Bacteriocin supplemented Prawn.
Journal of research in Biology (2011) 1: 6-14

Dates:
Received: 18 April 2011 /**Accepted:** 20 April 2011 /**Published:** 28 April 2011

Journal of Research in Biology

INTRODUCTION

One of the concerns in food industry is the contamination by pathogens, which are a frequent cause of food borne diseases. Over the past decade, recurrent outbreaks of diarrhea, combined with the natural resistance of the causative agents, contributed to its status as a hazard. In the recent years the food industry faced the need of increasing the possibilities for better conservation and of the food products (Neysens 2002). Today, the conservation is commonly performed by sterilization or by adding sugar, salt, organic acids or by smoking. However, some of these compounds change the taste quality and the appliance of others is not healthy. For improving the quality of the products the approach by which chemicals are added must be ceased and the sterilization must be avoided as far as possible. A new protection is required, which is healthy and natural. .Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality. (Corr *et al.,* 2007).

The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservation (De Martinis *et al.*, 2001). Antagonistic properties of lactic acid bacteria (LAB) allied to their safe history of use in traditional food fermented products make them very attractive to be used as biopreservatives. (Caplice and Fitzgerald 1999). Antibiotics are at present restricted for use in foods and feeds, and bacteriocins are an interesting group of biomolecules with antimicrobial properties that may represent a good alternative (Jack *et al.,* 1995). The bacteriocins produced by food grade starter bacterial cultures, could be applied as preservatives. Bacteriocins produced by lactic acid bacteria have attracted increasing attention, since they are active in a nanomolar range and have no toxicity. In the present study bacteriocin preparation from Lactobacillus breivis was evaluated against proximate composition of cooked prawn under laboratory condition.

MATERIALS AND METHODS

Isolation of *Lactobacillus brevis*

The *Lactobacillus brevis* was isolated from curd sample by serial dilution technique with modified Lactobacillus agar (Hi - media, Mumbai, India). The bacteriocin culture was identified based on morphological and biochemical characters. The pure culture was maintained on modified Lactobacillus slant.

Bacteriocin production

250 ml of modified Lactobacillus brevis was prepared, sterilized by autoclaving, after sterilization 1 ml of Lactobacillus brevis culture was inoculated and kept under shaking at 37^0 C for 24 hours. After incubation the media was centrifuged at 10,000 rpm for 10 mins. The supernatant was collected in sterile test tubes and used for anti bacterial activity against indicator strain adapting well diffusion assay.

Partial purification:

The collected supernatant was saturated with different concentration of ammonium Sulphate 20, 40 , 60 and 80 % , kept at 4^0 C for 6 to 12 hours. After incubation, the tubes were centrifuged at 10,000 rpm for 10 mins and the collected precipitate was dissolved in Tris buffer and dialysed against 0.1M Tris HCL buffer.

Evaluation of Bacteriocin on cooked Prawn Proximate composition

Sample preparation and treatment:

About 100 g of well cooked prawn was taken in a pre sterilized, air tight, plastic container. 10 ml of filter sterilized supernatant (crude), 10 ml of ammonium sulphate precipitate and 10 ml of dialysed product were added separately. Three replications were maintained for each treatment. One set of treated containers at respective treatment was kept at 30^0 C while the other was maintained at refrigeration without freezing for 15 days.

Daily observation was recorded for pH changes and off flavor etc. After the 15 days the following parameters such as mositure, protein, total ash, total sugar and microbial analysis were carried out by standard method (APHA 1989).

Microbial Analysis:

The respective bacteriocin treated samples were incubated in 37^0 C and 55^0C for 10 days. After 10 days it was analyzed for Total plate count (TPC), Spore count, Anaerobic growth, and coliform growth.For total plate count , plate count agar was used. For spore count dextrose tryptone agar was used. For determining Anaerobes, dextrose tryptone broth was used and for coliforms Mac konkey broth was used.10g of the sample was weighed accurately, then transferred into the pestle and mortar, macerated wll, and it was transferred into the conical flask, containing 90 ml Ringer's solution which gives 1:10 dilution.From the above dilution 1ml of the sample was inoculated into the

sterile petriplates aseptically and the respective media were poured , mixed well, allowed for setting and incubated at both 37^0 C and 55^0 C. For spore count, the diluted sample was kept in the boiling water both for 15min (to analyze the heat resistant spore) and then inoculated to the petriplates. Dextrose tryptone broth was inoculated with one ml of the diluted sample and 1ml of the sterile liquid paraffin to maintain anaerobic sample and 1ml of the sterile liquid paraffin to maintain anaerobic conditions. These tubes were also incubated at 37^0 C and 55^0 C.

RESULTS:

Bacteriocin activity against Indicator Organism

The crude bacteriocin prepared from the cultured filterate of Lactobacillus brevis shows distinct anti microbial activity against indicator organism. A clear zone of 30.0mm was observed.

The effect on changes of Proximate composition and Microbial analysis of cooked prawn after the bacteriocin treatment.

Non - Bacteriocin supplemented prawn:

The protein, total ash, total sugar, moisture, fat are found to be decreased in control (non - bacteriocin supplemented prawn) both in refrigerated and non - refrigerated sample. And are presented in **Table 1,2 and 3.**The protein content in control refrigerated and non - refrigerated was found to be 20.11% and 21.8% after 5 days. After 10 days it was reduced to 15% in control non - refrigerated and 21.6% in refrigerated sample. After 15 days, in control non - refrigerated sample 11.2% and in control refrigerated sample it was 21.4%.The Moisture content in control refrigerated and non - refrigerated were found to be 60.5% and 58.9% after 5 days. After 10 days it was reduced to 58.4% in control non - refrigerated and 56.5% in refrigerated sample. After 15 days, in control non - refrigerated sample 55.5% and in control refrigerated sample it was 50.3%

The total ash in control refrigerated and non - refrigerated were found to be 1.6% and 1.8% after 5 days. After 10 days it was reduced to 1.4% in control non - refrigerated and 1.7% in refrigerated sample. After 15 days, in control non - refrigerated sample 1.2% and in control refrigerated sample it was 1.4%..The total sugar content in control refrigerated and non - refrigerated were found to be 4.2% and 5.4% after 5 days. After 10 days it was reduced to 3.5% in control non - refrigerated and 5.2% in refrigerated sample. After 15 days, in control non - refrigerated sample 2.5% and in control refrigerated sample it was 5.0%. The fat content in contol refrigerated and non - refrigerated were found to be 2.5% and 2.4% after 5 days. After 10 days it was reduced to 2.2% in control non - refrigerated and 2.4% in refrigerated sample. After 15 days, in control non - refrigerated sample 1.7% and in control refrigerated sample it was 2.3%.

Table 1.Changes in Proximate composition of Crude bacteriocin supplemented prawn at different time ntervals (%)

S. No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
1.	Control (Non - refrigerated)		**5**	**10**	**15**
			60.5	58.4	55.5
		Moisture	20.11	15	11.2
		Protein	1.6	1.4	1.2
		Total Ash	4.2	3.5	2.5
		Total Sugar	2.5	2.2	1.9
		Fat			
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71	70.5	69.8
		Protein	22.0	21.7	21.5
		Total Ash	1.7	1.5	1.2
		Total Sugar	5.4	5.4	5.1
		Fat	2.3	2.1	1.8
4.	Test refrigerated	Moisture	70.4	69	67
		Protein	22.0	21.8	21.7
		Total Ash	1.7	1.5	1.3
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.1	1.9

Evaluation of Ammonium sulphate precipitate on proximate composition on prawn at different time intervals:

The significant difference could be observed on all the test parameters in bacteriocin treated food under refrigerated and non- refrigerated, than on non - bacteriocin supplemented food.

Protein

The protein content in Bacteriocin treated sample under refrigeration was found to be 22.0% and without refrigeration was also found to be the same after 5 days. After 10 days there was slight degradation in protein content which was found to be 21.7% but in refrigerated bacteriocin treated sample there found to have less change compared to the non - refrigerated bacteriocin treated sample and the values were recorded as 21.8%. After 15 days, there was not much degradation in the protein content in refrigerated as well as non - refrigerated sample, the values were recorded as 21.5% and 21.7%

Total Ash:

The Total Ash content in Bacteriocin treated sample under refrigeration was found to be 1.7% and without refrigeration was also found to be the same after 5 days. Even after 10 days, there were no change in the values, both in bacteriocin treated refrigerated and non - refrigerated sample, values were noted as 1.5%. After 15 days, There were no negotiable change in the total ash of bacteriocin treated non - refrigerated sample when compared to the referigerated sample. The values were recorded as 1.2% and 1.3%

Total Sugar

The Total sugar in bacteriocin treated under refrigerated and non - refrigerated sample were found to be same after 5 days and the value was recorded as 5.4%. There were no considerable change in non - refrigerated bacteriocin treated sample, the value was recorded as 5.4%. The Total sugar content in refrigerated bacteriocin treated sample was well maintained, the values were recorded as 5.4% after 10 days. There was a further change found in non - refrigerated bacteriocin treated sample and the value was recorded as 5.1% whereas in bacteriocin treated refrigerated sample, it was recorded as 5.3% after 15 days.

Moisture

After 5 days,in bacteriocin refrigerated sample there was retention in the moisture content and the value was 70.4 % and in bacteriocin non – refrigerated sample there was not much retention and the value was found to be 71%.After 10 days there was more retention found in bacteriocin treated refrigerated sample and the value was and in bacteriocin treated non – refrigerated sample the value was maintained as 70.5% and in bacteriocin treated refrigerated, the value was recorded as 69%.After 15 days, the bacteriocin treated non – refrigerated sample was still

Table 2..Changes in Proximate composition of Ammonium sulphate precipitated bacteriocin supplemented prawn at different time intervals (%)

S.No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
			5	10	15
1.	Control (Non - refrigerated)	Moisture	60.5	58.4	55.5
		Protein	20.11	15	11.2
		Total Ash	1.6	1.4	1.2
		Total Sugar	4.2	3.5	2.5
		Fat	25	2.2	1.9
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71	70.8	70.5
		Protein	22.2	22	21.8
		Total Ash	1.6	1.4	1.3
		Total Sugar	5.4	5.3	5.2
		Fat	2.3	2.1	2
4.	Test refrigerated	Moisture	71.1	70.8	70.5
		Protein	22.3	22	21.9
		Total Ash	1.6	1.5	1.3
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.3	2.2

maintained as 69.8% and in bactericoin treated refrigerated sample there was still more retention and the value was found to be 67%.

Fat

The Fat content was found to be slightly degraded with a very minute difference in non - refrigerated bacteriocin treated samples as 2.3% and for refrigerated bacteriocin treated sample was 2.4% after 5 days. The fat content started to degrade more after 10 days in both refrigerated and non - refrigerated bacteriocin treated sample. Both the values were recorded as 2.1%. After 15 days, both the bacteriocin treated refrigerated and non - refrigerated samples were found to be still more degraded. The values were recorded as 1.8% and 1.9% for non - refrigerated and refrigerated bacteriocin treated samples.

Evaluation of Ammonium sulphate precipitate on proximate composition on prawn at different time intervals:

There were no distinct difference on proximate composition observed in ammonium sulphate precipate (Table 2).

Protein

The Protein content in Bacteriocin treated sample under refrigeration was found to be 22.3% and without refrigeration was found to be 22.1%. After 10 days, the protein content was found with significant difference both in Bacteriocin treated refrigerated and non - refrigerated sample and the values were recorded with the same value as 22%. After 15 days, the protein has found to be slightly degraded in both the bacteriocin treated refrigerated and non - refrigerated sample and the values were found to be 21.9% and 21.8%.

Total Ash

In Bacteriocin treated sample under refrigeration, it was found to be 1.6% and without refrigeration was also found to be the same after 5 days. After 10 days there was no significant difference in Bacteriocin referigerated and non - refrigerated sample.The values were recorded as 1.5% and 1.4%.After 15 days, there seen a slight degradation in Bacteriocin treated sample, both refrigerated and non - refrigerated. The values were recorded as 1.3% and 1.3%

Total Sugar

After 5 days, the Total sugar in bacteriocin treated sample under refrigeration and non - refrigeration were found to be same and is noted as 5.4%. After 10 days, there was a significant change in bacteriocin treated non - refrigerated sample whereas in bacteriocin treated refrigerated samples, it was maintained, the values are recorded as 5.3% and 5.4%. After 15 days, the Total sugar content was found to be the same in both bacteriocin treated samples. The values were recorded as 5.2% in bacteriocin treated refrigerated sample ad 5.3% in bacteriocin tread non - refrigerated sample.

Table 3. Changes in Proximate composition of Dialysed product bacteriocin supplemented prawn at different time intervals (%)

S. No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
			5	10	15
1.	Control (Non - refrigerated)	Moisture	60.5	58.4	55.5
		Protein	20.11	15	11.2
		Total Ash	1.6	1.4	1.2
		Total Sugar	4.2	3.5	2.5
		Fat	2.5	2.2	1.9
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71.1	70.9	70.6
		Protein	22.2	22	21.8
		Total Ash	1.7	1.7	1.5
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.2	2.1
4.	Test refrigerated	Moisture	70.8	70.7	70.6
		Protein	22.3	22.1	22
		Total Ash	1.8	1.8	1.7
		Total Sugar	5.4	5.4	5.4
		Fat	2.4	2.3	2.3

Moisture

After 5 days, the bacteriocin treated refrigerated and non – refrigerated samples was found to be same with small difference in the values as 71.1% and 71%. After 10 days, both bacteriocin treated refrigerated and non – refrigerated samples were found to be the same and the value was 70.8%. After 15 days, the bacteriocin treated refrigerated and non – refrigerated samples were found with small difference and their values were recorded as 70.6% and 70.5%.

Fat

After 5 days, the fat content was found to be slightly degraded in bacteriocin treated non refrigerated sample and in refrigerated bacteriocin sample the fat content was maintained. The values were recorded as 71% and 71.1% . After 10 days, there was still more changes in bacteriocin treated non - refrigerated sample and in refrigerated bacteriocin treated sample, there was no change. The values were same and recorded as 70%. After 15 days, there was further degradation in bacteriocin treated non - refrigerated sample and in refrigerated bacteriocin treated sample was found with negotiable changes. The values were recorded as 2% and 2.2%.

Evaluation of Dialysed form of bacteriocin on proximate composition on prawn at different time intervals:

There was not much difference on proximate composition observed in Partially purified product.

Protein

After 5 days, there was not much change in protein content in both bacteriocin treated non - refrigerated and refrigerated samples. The values were recorded as 22.2 and 22.3%. After 10 days, the protein content was maintained without little degradation both in bacteriocins treated non - refrigerated and refrigerated sample. The values were recorded as 22.0% and 22.1%.After 15 days, the protein content was maintained in both bacteriocin treated non - refrigerated and refrigerated samples and the values were recorded as 21.8% and 22%.

Total Sugar

After 5 days, the total sugar content was found to be maintained without degradation in bacteriocin treated non - refrigerated and refrigerated sample. The value is recorded as 5.4% for both the samples. After 10 days, there were no degradation found in total sugar content in both bacteriocin treated non - refrigerated and refrigerated samples. The value was recorded as 5.4% for both the samples. After 15 days, there was still no much change in the values, for bacteriocin treated refrigerated sample it was 5.4% and 5.3% for bacteriocin treated non - refrigerated sample.

Total Ash

After 5 days, the total ash content was found to be nearly similar in bacteriocin treated refrigerated and non - refrigerated samples. The values were recorded as 1.7% and 1.8%.After 10 days, the total ash content was found to be maintained in both the bacteriocin treated refrigerated and non - refrigerated samples. The values were recorded as 1.7% and 1.8%. After 15 days, the total ash content was found to be with slight difference in bacteriocin treated non - refrigerated samples but in bacteriocin treated refrigerated sample there was no change inferred. The values were recorded as 1.7% and 1.8%

Moisture

After 5 days, the Moisture content in bacteriocin treated non – refrigerated and refrigerated sample has no significant difference . The values were found to be 70.8% for bacteriocin treated non – refrigerated and 71.1% for bacteriocin treated refrigerated sample. After 10 days, the moisture content in the bacteriocin treated non – refrigerated sample has very slight difference when compared to refrigerated bacteriocin supplemented sample. The values were recorded as 70.9% for non – bacteriocin treated sample and 70.7% refrigerated bacteriocin treated sample. After 15 days, the moisture content in bacteriocin treated non – refrigerated sample has considerable change when compared to bacteriocin treated refrigerated sample. The values were recorded as 70.6% and 70.6%

Fat

After 5 days, in Bacteriocin treated refrigerated and non – refrigerated samples there were no change and the values were found to be the same and recorded as 2.4%. After 10 days, there was a significant change observed both in refrigerated and non – refrigerated bacteriocin treated sample and the values were recorded as 2.3% for refrigerated bacteriocin treated sample and 2.2% for non – refrigerated sample. After 15 days the values were maintained in both the samples and the value was revealed as 2.3% for bacteriocin treated refrigerated sample and for bacteriocin treated non – refrigerated sample was 2.1%

Microbial Analysis:

Significant difference was observed in bacteriocin supplemented food than the control. The microbial growth was recorded in all the tested time

Table 4. Microbial Analysis in Crude Bacteriocin supplemented prawn at different time intervals

Treatment	Microbial Count (cfu/G.)														
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform		
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Control (Non - Refrigerate)	10.1 x10^2	15.1x10^4	25.1x10^6	7x10^3	14.1x10^4	17.2x10^2	2.5X 10	3X 10	46X1O^2	-	-	-	+	+	+
Control (Refrigerated)	-	1.4x10^1	11.3x10^1	-	-	3.1x10^1	-	-	-	-	-	-	+	+	+
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 5. Microbial Analysis in Ammonium Sulphate precipitate supplemented prawn at different time intervals

Treatment	Microbial Count (CFU/g.)														
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform		
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Control (Non - Refrigerate)	10.1 x10^2	15.1x10^4	25.1x10^6	7x10^3	14.1x10^4	17.2x10^2	2.5X 10	3X 10	46X1O^2	-	-	-	+	+	+
Control (Refrigerated)	-	1.4x10^1	11.3x10^1	-	-	3.1x10^1	-	-	-	-	-	-	+	+	+
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Journal of Research in Biology

Table 6. Microbial Analysis in Dialysed product Bacteriocin supplemented prawn at different time intervals

Treatment	Microbial Count (CFU/g.)															
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform			
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	
Control (Non - Refrigerate)	10.1×10^2	15.1×10^4	25.1×10^6	7×10^3	14.1×10^4	17.2×10^2	2.5X 10	3X 10	$46X1O^2$	-	-	-	+	+	+	
Control (Refrigerated)	-	1.4×10^1	11.3×10^1	-	-	3.1×10^1	-	-	-	-	-	-	+	+	+	
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	

period in non - bacteriocin supplemented food (**Table 4, 5 and 6**).

In control non - refrigerated:

The bacterial count at 5, 10 and 15 days were found to be 10.1×10^2, 15.1×10^2, 25.1×10^6 Cfu/gThe fungal count at 5, 10, and 15 days were found to be 7.0×10^4, 14.1×10^4, 17.2×10^5 Cfu/ gThe spore formers count for 5, 10, 15 days were found to be 2.5X 10, 3×10^1, 4.6×10^2 Cfu/g.Nil count was recorded in anaerobes for 5, 10 , 15 days respectively.The coliform was found to be positive on 5, 10, 15 days respectively.

Control refrigerated

The bacterial count at 5, 10 and 15 days were recorded as 0, 1.4×10^1 and11.3×10^1 Cfu/g. The fungal count at 5, 10 and 15 days were found to be 0,0 and 3.1×10^1 Cfu/gNil count was recorded in spore formers count for 5, 10 and 15 days respectivelyNil count was recorded in anaerobes for 5, 10 and 15 days respectively. The coliform was found to be positive on 5, 10, 15 days respectively. In bacteriocin supplemented food products (both in refrigerated and non - refrigerated) no growth were recorded in all the tested time period at bacteriocin (crude, ammonium sulphate precipitate, partially dialysed product) supplemented food products.

DISCUSSION

Biopreservation systems such as bacteriocinogenic LAB cultures and/or their bacteriocins have received increasing attention, and new approaches to control pathogenic and spoilage microorganisms have been developed. Some lactic acid bacteria (LAB) demonstrated antagonism towards pathogenic and spoilage organisms.Although bacteriocins are produced by many Gram-positive and Gram-negative species, those produced by LAB are of particular interest to the food industry, since these bacteria have generally been regarded as safe (Joshi *et al*., 2005).

Bacterial fermentation of perishable raw materials has been used for centuries to preserve the nutritive value of food and beverages over an extended shelf life.The production of bacteriocins by LAB is advantageous for survival of the producing bacteria in a competitive ecological niche; therefore, they could be exploited by the food industry as a tool to control undesirable bacteria in a food-grade and natural manner, which is likely to be more acceptable to consumers.

From this study it is clear that. the bacteriocin supplemented prawn, retained its original proximate and nutrient composition at

tested time interval under refrigerated and non - refrigerated conditions. This study will be helpful to use exploit bacteriocin as a food preservative to prevent the microbial spoilage without other conventional preservation methods.

REFERENCES

APHA. 1989. Compendium of methods for the microbiological examination of food. American public Health Assaociation (APHA), Washington DC 234.

Caplice E and Fitzgerald GF. 1999. Food fermentations: role of microorganism in food production and preservation. *International Journal of Food Microbiology* 50:131-149.

Corr C, Li Y, Reidel C, Toole P, Hill C and Gahan C. 2007. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Journal of Applied Microbiology 86:1023-1041.

De Martinis ECP, Públio MRP, Santarosa PR and Freitas FZ. 2001. Antilisterial activity of lactic acid bacteria isolated from vacuum-packaged Brazilian meat and meat products. *Brazilian Journal of Microbiology* 32:32-37.

Jack RW, Tagg JR and Ray B. 1995. Bacteriocin of Gram-Positive bacteria. *Microbiology reviews and Molecular Biology reviews* 59:171-200.

Joshi VD, Sharma S and Rana S. 2005. Production, Purification, Stability and Efficacy of Bacteriocin from isolates of Natural Lactic Acid Fermentation of Vegetables. *Journal of Food Technology And Biotechnology* 44:435-439.

Neysens P, Messens W, Gervers D, Swings J and Vuyst D. 2002. Biphasic kinetics of growth and bacteriocin production with *Lactobacillus amylovorus DCE* 471 occur under stress conditions.*Journal of general Microbiology* 149:1073-1082.

Journal of Research in Biology

FicusPress

An International Online Open Access Publication group

OPEN ACCESS

Original Research paper

Preliminary Phytochemical Analysis and Antimicrobial Activity of Some Weeds collected from Marathwada Region

Authors:
Dhole JA[1], Dhole NA[2], Lone KD[3] and Bodke SS[1].

Institution:
[1]Department of Botany, Yeshwant Mahavidyalaya, Nanded-431605.
[2]School of Life Sciences, S.R.T.M. University, Nanded-431606.
[3]Rajarshi Shahu College of Pharmacy & Research, Tathwade, Pune-411033.

Corresponding author:
Dhole JA

Email:
jyotikrishnalone@gmail.com.

ABSTRACT:

The aqueous and ethanolic extracts of selected weeds such as *Portulaca oleracea* L. (Portulaceae), *Cardiospermum helicacabum* L. (Sapindaceae), *Euphorbia hirta* L. (Euphorbiaceae), *Crotalaria retusa* L. (Fabaceae) and *Euphorbia heterophylla* L. (Euphorbiaceae) were screened for phytochemical analysis and antimicrobial activity. The antimicrobial activities were tested against two gram-positive bacteria (*Bacillus subtilis*, *Staphylococus aureus*), one gram-negative bacterium (*Pseudomonas aeruginosa)* and a mould *Aspergillus niger* by agar diffusion method. The phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, steroids and flavonoids are investigated from plant samples. Tannins, steroids and flavonoids were absent in the *Cardiospermum helicacabum* L. Remarkable antibacterial activity was observed in the aquous and ethanolic (root and leaves) extracts of *Portulaca oleracea* L. while *Cardiospermum helicacabum L.* showed no results in same experimental conditions as compared with standard antibiotics. The weed *Euphorbia hirta* L possess significant antifungal activity as compared to nystatin used as a standard antifungal agent.

Keywords:
Weeds, Phytochemical analysis, Antimicrobial activity, Crude extract.

Web Address:
http://jresearchbiology.com/Documents/RA0002.pdf.

Article Citation:
Dhole JA, Dhole NA, Lone KD and Bodke SS.
Preliminary Phytochemical Analysis and Antimicrobial Activity of Some Weeds collected from Marathwada Region.
Journal of research in Biology (2011) 1: 19-23

Dates:
Received: 19 Apr 2011 /**Accepted:** 20 Apr 2011 /**Published:** 03 May 2011

Journal of Research in Biology

Journal of Research in Biology

INTRODUCTION:

The weeds are common, dominant and easily available in almost all the crop fields. These are produced in very huge quantity and competing to crops for water, nutrients and light. The total yield losses per year caused by weeds were more than 24 % (Newman, et al., 1973). But many phytochemicals from these plants were investigated for various preparations of novel natural remedies against many chronic diseases. The ethanopharmacological data provides an alternative approach for discovery of antimicrobial agents, particularly those medicinal plants that have a history of traditional use and have potential source with significant pharmacological and biological activities (Ambasta, 1992). The main objective of the present investigation was to search preliminary phytochemical analysis and evaluation of in-vitro antimicrobial activities using crude extracts of some common weeds. The detailed therapeutic usage of the selected the plants were summarized as below.

1. *Portulaca oleracea* L. (Family: Portulaceae) *Portulaca oleracea* L contains many biological active compounds and also it is used as a good source of nutrients. This plant is used as antihelminthic, antidiarrhoeal and a bactericidal in bacillary dysentery. Fresh leaves were used externally for maturing of abscesses while seeds are effective as a refreshing agent and diuretic. Plant is very effective for wound healing and also used as anti-inflammatory treatment. (Leung and Foster, 1996).

2. *Cardiospermum helicacabum* L. (Family: Sapindaceae) The entire plant has medicinal properties and it is useful in the treatment rheumatism, fever, nervous disorder and in piles. The leaves are useful in the treatment of amenorohoea (William, 2005).

3. *Euphorbia hirta* L. (Family: Euphorbiaceae) A prostrate ascending annual herb, the whole plant possesses medicinal property. A decoction of plant powder is very useful in the treatment of kidney disorders, dysentery, asthma and curing many diseases of urenogenital tract. In many villages, leaves are chewed and spitted on wounds for prevention of infection, it acts as an antiseptic agent. (Ratnam, 2006).

4. *Crotalaria retusa* L. (Family: Fabaceae)In ayurveda, *Crotalaria retusa* L. is very useful in kapha and vata. It is bitter, astringent, expectorant, and specially the leaves are useful for curing ptyalism, dyspepsia, fever and cardiac diseases. The seeds are powdered and boiled in milk and are said to be very useful for increasing body strength and life span (Prajapati, 2003).

5. *Euphorbia heterophylla* L. (Family: Euphorbiaceae). The leaves are used in traditional practices as antigonorrheal, laxative, migraine and have potential to eliminate wart (Rodriguez, 1976).

MATERIAL AND METHODS

Plant Materials and Preparation of Plant Extracts

The weeds were collected from various crop fields from Nanded district of Marathwada region (MS) India. The plants were identified and authenticated by a taxonomist Dr. S. S. Bodke, Head, Dept. of Botany, Yeshwant Mahavidyalaya, Nanded using "Flora of Marathwada" (Naik, V.N., 1998). The voucher plant specimens were deposited in the herbarium section of Department of Botany, Yeshwant Mahavidyalaya, Nanded (MS). The herbarium voucher specimens no. are *Portulaca oleracea* L. (412A), *Cardiospermum helicacabum* L. (413A), *Euphorbia hirta* L. (414A), *Crotalaria retusa* L. (415A) and *Euphorbia heterophylla* L. (416A). The collected plants were shade dried and made fine powder. Sequential extraction was carried out using Soxhlet extraction apparatus in (water, ethanol) for six hours. Obtained extract were filtered, evaporated and stored in refrigerator for further experiments.

Preliminary Phytochemical Analysis

The selected plants were analyzed for

Table 1: Preliminary phytochemical analysis of selected weeds

Sr. No	Name of the Weeds	Preliminary Phytochemical Analysis					
		Alkaloids	Saponins	Tannins	Cardiac glycosides	Steroids	Flavonoids
1	*Portulaca oleracea L.*	+	+	+	+	+	+
2	*Cardiospermum helicacabum* L.	+	+	-	+	-	-
3	*Euphorbia hirta* L.	+	+	+	+	+	+
4	*Crotalaria retusa* L.	+	+	+	+	+	+
5	*Euphorbia heterophylla* L	+	+	+	+	+	+

(+ Present, - absent)

preliminary phytochemical analysis such as alkaloids, saponins, tannins, steroids and flavonoids according to standard protocols (Jigna et al., 2007).

Microorganisms used

In present investigation two gram-positive bacteria [*Bacillus subtilis* (MTCC-2415), *Staphylococcus aureus* (MTCC-96), one gram-negative organism *Pseudomonas aeruginosa* (MTCC-2488) and one mould *Aspergillus niger* (MTCC-1781)] were included. All the microbial cultures were purchased from Institute of Microbial Technology, Chandigarh, India.

Antimicrobial Activity

The screening of antimicrobial activity of crude extracts was carried out by measuring the zone of inhibition using agar diffusion method (Sahoo et al., 2006). The sterile disc were loaded aseptically with the various concentration of crude extracts (water and ethanolic) 100, 250, 500, 750µg/ml. Streptomycin (30µg), Tetracycline (30 µg) were used as standard antibiotics (a positive control) and appropriate controls were prepared for corrections using respective solvents.

For antifungal activity potato dextrose agars plates were used. Nystatin (30µg) was used as a standard antifungal agent.

RESULTS

The phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, steroids and flavonoids are present in all selected plants except *Cardiospermum helicacabum* L. which showed positive results towards alkaloids, saponins and cardiac glycoside (**Table 1**).

The antimicrobial activities of five weed species (root and leaves) extracts were assayed in

Table 2: Profile of antimicrobial activity (root extracts) of selected weeds

Sr. No	Name of the weeds	Test organisms	Zone of Inhibition (mm)							
			Aqueous extract (µg/ml)				Ethanolic extract (µg/ml)			
			100	250	500	750	100	250	500	750
1	*Portulaca oleracea* L	*B. subtilis*	29	32	37	39	26	29	30	31
		S. aureus	23	31	35	47	31	34	37	40
		P. aeruginosa	23	31	33	35	29	32	44	46
		A. niger	09	12	13	14	05	10	11	13
2	*Cardiospermum helicacabum* L.	*B. subtilis*	NR	NR	NR	NR	NR	NR	NR	NR
		S. aureus	NR	NR	NR	NR	NR	NR	NR	NR
		P. aeruginosa	NR	NR	NR	NR	NR	NR	NR	NR
		A. niger	NR	NR	NR	NR	NR	NR	NR	NR
3	*Euphorbia hirta* L.	*B. subtilis*	06	08	11	13	12	16	21	24
		S. aureus	05	07	09	12	11	13	19	21
		P. aeruginosa	08	10	13	14	21	25	26	28
		A. niger	20	23	24	27	21	24	27	32
4	*Crotalaria retusa* L	*B. subtilis*	15	17	21	29	15	18	21	23
		S. aureus	04	07	09	14	07	11	19	21
		P. aeruginosa	31	33	43	45	37	39	42	43
		A. niger	NR	NR	NR	NR	03	05	06	09
5	*Euphorbia heterophylla* L	*B. subtilis*	10	12	13	14	03	08	11	13
		S. aureus	06	07	10	13	NR	NR	NR	NR
		P. aeruginosa	14	18	26	31	14	17	23	28
		A. niger	08	12	14	16	10	11	13	15
6	Standered drug		**A**	**B**	**C**		**A**	**B**	**C**	
		B. subtilis	31	ND	ND		30	ND	ND	
		S. aureus	ND	34	ND		ND	32	ND	
		P. aeruginosa	ND	19	ND		ND	20	ND	
		A. niger	ND	ND	18		ND	ND	19	

[*B. subtilis* – *Bacillus subtilis*, *S. aureus* - *Staphylococus aureus*, *P. aeruginosa* - *Pseudomonas aeruginosa*, *A. niger* - *Aspergillus niger*]
A-Streptomycin (30µg/ml), B-Tetracycline (30µg/ml), C- Nystatin (30µg)
NR-No results under experimental conditions, ND- Not determined
The results presented are mean values of n=2.

vitro by agar diffusion method against three bacterial species (two gram-positive and one gram-negative bacterium) and one fungal species. The antimicrobial activity of root extracts of different weed species are summarized in (**Table 2**). The highest antibacterial activity was observed in *Portulaca oleracea* L. at the concentration of 750 µg/ml of aqueous extract against *Staphylococus aureus* having zone of inhibition (47 mm) as compared to standard antibiotics Tetracycline. While in case of ethanolic extract of *Portulaca oleracea* L. showed maximum antimicrobial activity against *Pseudomonas aeruginosa* showing zone of inhibition (46 mm). The aqueous and ethanolic extracts of *Cardiospermum helicacabum* L. showed no results under the similar experimental conditions. The aqueous extract of *Crotalaria retusa* L. showed no results against *Aspergillus níger* while in ethanolic extracts of *Euphorbia hirta* L. showed maximum antifungal activity against *Aspergillus níger* at 750 µg/ml having zone of inhibition (32 mm) as compared to standard reference compound of Nystatin. Remaining selected plants exhibited considerable antimicrobial activity.

The summary of antimicrobial activity (leaves extracts) of selected weeds are shown in Table 3. Among the selected weeds aquous extract of *Portulaca oleracea* L. showed maximum antimicrobial activity against *Bacillus subtilis* at 750 µg/ml showing zone of inhibition (35mm). The ethanolic extract of *Euphorbia heterophylla* L. showed no results against *Staphylococus aureus* in similar conditions. All other aquous and ethanolic (leaves) extracts exhibited considerable antimicrobial activity.

Table 3: Summary of antimicrobial activity (leaves extracts) of selected weeds

Sr. No.	Name of the weeds	Test organisms	Zone of Inhibition (mm)							
			Aqueous extract (µg/ml)				Ethanolic extract (µg/ml)			
			100	250	500	750	100	250	500	750
1	*Portulaca oleracea* L	*B. subtilis*	20	24	31	35	21	22	24	25
		S. aureus	14	17	18	20	22	26	27	29
		P. aeruginosa	14	15	20	25	07	14	21	24
		A. niger	04	07	12	14	03	07	10	12
2	*Cardiospermum helicacabum* L.	*B. subtilis*	NR	NR	NR	NR	NR	NR	NR	NR
		S. aureus	NR	NR	NR	NR	NR	NR	NR	NR
		P. aeruginosa	NR	NR	NR	NR	NR	NR	NR	NR
		A. niger	NR	NR	NR	NR	NR	NR	NR	NR
3	*Euphorbia hirta* L.	*B. subtilis*	05	09	12	13	12	15	16	18
		S. aureus	06	09	12	13	08	13	16	24
		P. aeruginosa	03	07	11	13	03	05	09	11
		A. niger	10	13	15	16	12	15	25	27
4	*Crotalaria retusa* L	*B. subtilis*	20	24	25	27	22	23	26	29
		S. aureus	22	26	30	31	15	17	21	24
		P. aeruginosa	16	19	21	23	12	14	17	20
		A. niger	14	23	24	27	05	07	11	15
5	*Euphorbia heterophylla* L	*B. subtilis*	20	24	25	27	13	14	17	18
		S. aureus	22	26	30	31	NR	NR	NR	NR
		P. aeruginosa	16	19	21	23	13	15	17	20
		A. niger	14	23	24	27	05	09	12	13
6	Standered drugs		**A**	**B**	**C**		**A**	**B**	**C**	
		B. subtilis	29	ND	ND		31	ND	ND	
		S. aureus	ND	33	ND		ND	32	ND	
		P. aeruginosa	ND	18	ND		ND	20	ND	
		A. niger	ND	ND	19		ND	ND	20	

A-Streptomycin (30µg/ml), B-Tetracycline (30µg/ml), C- Nystatin (30µg)
NR-No results under experimental conditions, ND- Not determined
[*B. subtilis* – *Bacillus subtilis*, *S. aureus* - *Staphylococus aureus*, *P. aeruginosa* - *Pseudomonas aeruginosa*, *A. niger* - *Aspergillus niger*]
The results presented are mean values of n=2.

DISCUSSION

The potential source of antimicrobial agents developing from plants species is an alternative strategy for the production of safe and standardization of a phytomedicines against harmful microbes. The plant based antimicrobial agents have enormous therapeutic potential while they don't have any major side affects to the human beings (Lwu et al .,1999). The plant *Portulaca oleracea* possesses several phytochemicals and has significant antifungal properties against *Aspergillus* species (Bongoh et al., 2000).

The phytochemicals like alkaloids, saponins, flavonoids and phenolic compounds present in plants are responsible for many biological activities (Rabe, 2000). The ethanopharmacological exploration of plant species derived antimicrobial agents is needed for the production of safe and standardization of therapeutic drugs against harmful microbes. *Portulaca oleracea* L., *Crotalaria retusa* L. *Crotalaria speciosa* L. and *Euphorbia hirta* L. extract possess a broad spectrum of antimicrobial activity against a panel of bacteria responsible for the most common bacterial diseases.

CONCLUSIONS

Further research is necessary to determine the active phytochemicals ingredients contributes for antimicrobial activity from weed species and also to determine their minimum inhibitory concentration (MIC) values required for demonstrating the antimicrobial activity.

ACKNOWLEDGEMENTS

The authors are very thankful to the university Grants Commission (UGC) New Delhi for providing the financial assistance in the form of Rajiv Gandhi National Fellowship. Thanks to the Principal and Head, Department of Botany, Yeshwant Mahavidyalaya, Nanded for providing all necessary facilities.

REFERENCES:

Ambasta SP. 1992. The useful plants of India. Publications and Information Directorate. CSIR, New Delhi, India.

Bongoh, Moochang and Jun Hwang. 2000. Detection of antifungal activity in Portulaca oleracea by a single cell bioassay system" Phytotherapy research 14(5):329-332.

Jigna Parekh, Sumitra V, Chanda. 2007. In vitro Antimicrobial activity and Phytochemical Analysis of Some Indian Medicinal plants, Turk. J. Biol 53-58.

Leung AY and Foster S. 1996. Encyclopedia of Common Natural Ingredients used in food, Drug and cosmetic, 2nd Ed. New York, John Wiley & Sons. Inc.

Lwu MW, Duncan AR, Okunji CO. 1999. New antimicrobials of plant origin. In: Janick J. ed. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press 457-462.

Naik VN. 1998. The Flora of Marathwada Amrut Prakashan, Aurangabad, M.S. (India). Vol I and II.

Newman FI and Andrews RC. 1973. Allelopathy among some British grassland species II. Influence of soaked exudates on phosphorus uptake.J.Ecol.65:399-411.

Prajapati ND, Kumar U.2003. Agro's Dictionary of Medicinal plants. Agrobios (India), Jodhpur 96.

Rabe Tandvan Staden J. 2000. Isolation of an antimicrobial sesquiterpenoid from Warbugie salutaris. J. Ethnopharcol 93:171-174.

Ratnam RK and Martin P. 2006. Ethnomedicinal plants.Agrobios (India) publication, Jodhpur 227.

Rodriguez E, Twers GHN, Mitchell JC. 1976. Biological activites of sesquiterpene Lactones. Phytochemistry 15:1573.

Sahoo S, Kar DM, Mohapatra S, Raut SP and Dash SK. 2006. Antibacterial activity of Hybanthus enneaspermus against selected UTI pathogens. Ind. J. Pharmacol. Soc 68(5):653-655.

William D, Warden CJH and Hooper D. 2005. "Pharmacographia india" A History of the principal Drug of Vegetable Origin. Srishti publication, New Delhi 366-367.

Journal of Research in Biology

FicusPress

An International Online Open Access Publication group

OPEN ACCESS

Original Research paper

Allelopathic effect of aqueous leaf extract of *Parthenium hysterophorus* L. on seed germination and seedling emergence of some cultivated crops

Authors:
Dhole JA, Bodke SS and Dhole[1] NA.

Institution:
Department of Botany, Yeshwant Mahavidyalaya, Nanded-431605.
[1] School of Life Sciences, S.R.T.M. University, Nanded-431606.

Corresponding author:
Dhole JA

Email:
jyotidhole.2008@rediffmail.com.

ABSTRACT:

Allelopathic effect of aqueous extract of *Parthenium hysterophorus* (L). were studied on seed germination and seedling emergence of some cultivated crops like *Triticum aesticum* (L)., *Zea mays* (L)., *Sorghum vulgare* PERS., *Gossipium hirsutum* (L). and *Glycine max* (L).. Seed germination *of Zea mays* (L). was completely inhibited at 2% leaf extract of *Parthenium hysterophorus* (L). and in *Triticum aestivum* (L) it was gradually reduced the seed germination up to the concentration of 10 %. While in *Sorghum vulgare* PERS. it was inhibited at 6 %. At 4 % aqueous extract the maximum inhibition occurred in *Glycine max* (L). *and Gossypium hirsutum* (L). The aqueous extract of *Parthenium hyterophous* L. were having strong inhibitory effect on the growth of root and shoot development in the cultivated crops except *Triticum aesticum* (L). While on *Sorghum vulgare* PERS., it has shown moderate inhibitory potential.

Keywords:
Cultivated crops, waste weed leaf biomass, Allelopathy, seed germination, Seedling emergence.

Web Address:
http://jresearchbiology.com/Documents/RA0003.pdf.

Article Citation:
Dhole JA, Bodke SS and Dhole NA.
Allelopathic effect of aqueous leaf extract of *Parthenium hysterophorus* L. on seed germination and seedling emergence of some cultivated crops.
Journal of research in Biology (2011) 1: 15-18.

Dates:
Received: 19 Apr 2011 /**Accepted:** 20 Apr 2011 /**Published:** 03 May 2011

Journal of Research in Biology

Journal of Research in Biology

INTRODUCTION

The weeds are commonly dominant, unwanted, undesirable and plant that compete with cultivated crop for water, nutrient and sunlight and another several reasons such as, high growth rate, high reproductive rate and produce harmful allelopathical effect of cultivated crops. (Qasem and Foy 2001).Allelopathy is known as the direct or indirect hazardous or beneficial effects of one plant on the plants through the production of several phytochemicals into the environment (Brown et al. 1991). The allelopathic effect is one of an important mechanism for successful establishment of spreading of weeds (Ridenour and Callaway 2001). This allelopathy has been suggested as a mechanism for the impressive success of invasive plant by control virtual monoculture and may control the ability of particular spreading weed species to become dominants in particular crop plant communities (Hierro 2003, Kanchan and Jayachandra 1979).

Parthenium hysterophous L. is native to tropical and subtropical America. This plant species is very invasive invader in large extent and it is threatened grassland ecosystem of Australia and India. This weed possesses many hazardous substances and it is very harmful to the surrounding flora, animals and also to human health. It has been already invaded in most of the useful field areas in urban and village. To explore allelopathic potential of *Parthenium hysterophorus* L. we have studied the effect of aqueous extract of leaves using different concentrations on seed germination and seedling growth of five cultivated crop species. The *Parthenium hysterophorus* L. is growing together with these crops and it is more common and dominant plant species.

MATERIALS AND METHODS

Species characters

Parthenium hysterophorus L., family: Asteraceae, Common name – Gajar gawat.

The plant is erect, profusely branched, leafy herbs. Stem hairy, fistular, striate, pubescent, leaves sessile, radical and alternate, hairs are present in both the side, pinnately divided, pubescent on both surface, inflorescence corymbase cymes, involucral bracts are present, two type of flowers are present –male floret on either side, marginal florets female, fertile with white,3-dentate. Fruit achene's, obovate. This plant is native of tropical America, naturalized and now has become a troublesome weed throughout the region, flowers and fruits produces in September to December.

Collection of plant Materials:

This weed has collected from Nanded district and plant species identified by using "The Flora of Marathwada". Herbarium was prepared and stored in Herbarium Section in the Department of Botany, Yeshwant Mahavidyalaya, Nanded (M.S.). From the collected weed, leaves were separated and dried in shade, finely made powder using Mixer grinder. Ten gram of leaf powder was taken in Soxhlet extraction apparatus and extracted in distilled water for six hours. Finely this extract was evaporated and stored in refrigerator for further experiments.

Bioassay:

Different concentrations of aqueous extract of *Parthenium hysterophorus* L. (2, 4, 6, 8, and 10%) were made. One hundred uniform seeds of wheat were kept for germination in sterilized Petri dishes containing double layered blotting paper and seeds were treated with different concentrations of aqueous extracts (2 to 10 %). Each treatment had 3 replicas (total number of tested seeds + 100X 3=300) and one treatment was run as control with distilled water. The Petri-dishes were maintained under laboratory condition for 7 days. Equal volume of distilled water was added in the dishes when moisture content of the blotting paper declined. After 7 days number of germinated seeds were counted and the root and shoot length were measured.

Same procedure was followed to evaluate allelopathic effects of *Parthenium hysterophorus* L. on seed germination and seedling emergence of other selected crop plant i.e. *Zea mays* (L)., *Sorghum vulgare* PERS., *Gossipium hirsutum* (L). and *Glycine max* (L).

RESULTS AND DISCUSSION

Germination:

The aqueous extract of *Parthenium hysterophorus L.* were studied on the seed germination of five crop plants and results are summarized in **Table No.1**. As concentration of plant extract increases, the percentage of seed germination get inhibited as compared to control in *Triticum aesticum L.* There were maximum inhibition in 10 % aqueous extract in wheat. In *Sorghum vulgare* PERS. the effect of aqueous extract at concentrations from 2 to 6 % seed germination shows inhibitory action. In Zea *mays L., Gossypium hirsutum L.*, and *Glycine max L.* showing inhibition at the concentration of 2 to 4 %

Table No.1 Effect of aqueous extract of *Parthenium hysterophorus* L. on selected crop Plants

Sr. No.	Crop Plant species	Percent seed germination					
		Aqueous extract of *Parthenium hysterophorus* L					
		2 %	4%	6%	8%	10%	control
1	*Triticum aesticum L.*	93	80	53	19	10	95
2	*Sorghum vulgare* PERS.	66	20	04	-	-	83
3	*Zea mays L.*	52	-	-	-	-	100
4	*Gossypium hirsutum* L.	63	04	-	-	-	100
5	*Glycine max L.*	50	04	-	-	-	93

The results are presented are mean value N=3

while in 6 to 10 % extracts no results were shown at the same experimental conditions.

Seedling growth:

The effect of aqueous extracts of *Parthenium hysterophorus* L. were examined for root, shoot growth and results are summarized in **Table No. 2**. Among all the plant species the concentration of extract increases the root and the shoot length reduces as compare to control (distilled water). The control showing 15.1 and 10.3 cm for root and shoot length respectively. In *Triticum aesticum* L. the root length showing in range of 7.3 to 0.9 cm at the concentration of 2 to 10 % while for shoot length having results in range of 9.7 to 0.5 cm at the concentration of 2 to 4 %. In *Sorghum vulgare* PERS., *Zea mays* L., *Gossypium hirsutum* L. and *Glycine maxL.* were showing inhibitory action at 2 to 6 % and there were no results at 6 to 10 % aquous extract of *Parthenium hysterophorus* L. in the same experimental conditions.

The study demonstrated that leaf extract of *Parthenium hysterophorus* L. exhibited significant inhibitory effects on seed germination and seedling growth in selected plant species. Some earlier work have also reported that the *Parthenium hysterophorus* L. reduces root and shoot length of *Zea mays* L. and *Glycine max* L.(Bhatt et al 1994). Due to the presence of allelochemicals in aqueous extract of *Parthenium hysterophorus* L. showing inhibitory effect on different plant species (Rajan 1973) and the scientist Kanchan in 1975 was the first to reported that presence of plant growth inhibitors in *Parthenium hysterophorus* L. was the reason for alleopathic effect. This plant releases the number of allelochemicals to surround such as phenolic acids, sesquiterpene lactones especially parthenin (Kanchan 1975, Swminathan *et al.,* 1990). Studies also shown that the phenolics compounds found in leaf of *Parthenium hysterophorus* L. have inhibitory effect on nitrogen fixing and nitrifying bacteria (Kanchan and Jayachandra 1979).Present results showed that high concentrated aqueous extract of leaves of *Parthenium hysterophorus* L. inhibited seed germination and seedling emergence of selected crop plants.

CONCLUSION:

The *Zea mays* L. were more sensitive to inhibitory effects of the leaf extract of *Parthenium hysterophorus* L. at 2 to 4 % seed emergence while seed germinations were completely inhibited at 2%

Table No. 2 Effect of aqueous extract on root and shoot length of selected crop plant Seeds.

Treatment	*Triticum aesticumL*	*Sorghum vulgare* PERS.	*Zea mays* L.	*Gossypium hirsutum* L.	*Glycine max L.*
Root length (cm.)					
Control	15.1	10.5	18.5	16.2	8.2
2%	7.3	5.2	1.7	1.2	0.9
4%	5.2	3.4	-	0.6	0.3
6%	2.1	1.2	-	-	-
8%	1.7	-	-	-	-
10%	0.9	-	-	-	-
Shoot length(cm.)					
Control	10.3	8.5	16.1	12.4	5.4
2%	9.7	4.9	1.2	0.9	0.5
4%	4.8	2.5	-	0.4	0.5
6%	1.8	0.9	-	-	-
8%	1.2	-	-	-	-
10%	0.5	-	-	-	-

The results were presented in the mean value of n=3

concentration of plant extract. *Sorghum vulgare* PERS., *Zea mays L., Gossypium hirsutum* L., *Glycine max L.* seed germination was inhibited completely at 8 to 10 % extract. The extracts showing strong inhibitory effect on root and shoot elongation in *Zea mays* L., *Gossypium hirsutum* L. and *Glycine max* L. at 6 to 10 % aqueous extract. The *Parthenium hysterophorus* L. have potential to inhibit the seed germination and seedling emergence of different plants due to the presence of allelochemicals. In future this plant can be exploited as a good source of natural weedicide to control invasive plant species.

ACKNOWLEDGEMENT

The authors are very thankful to the university Grants Commission (UGC) New Delhi for providing the financial assistance in the form of Rajiv Gandhi National Fellowship. Thanks to the Principal and Head, Department of Botany, Yeshwant Mahavidyalaya, Nanded for providing all necessary facilities.

REFERENCES

Qasem JR, Foy CL. 2001. Weed Allelopathy, its ecological impacts and future prospects: a review. J. Crop Prod. 4:43-119.

Brown PD, Morra JM, McCaffery JP and Auld Willaims DL. 1991. Allelochemicals produced during glucosinolate degradation in soil. J. Chem. Ecol 17:2021-2034.

Ridenour WM and Callaway RM. 2001. The relative importance of allelopathy in interference : the effects of invasive weed of native bunchgrass. Oecologica 126:444- 450.

Hierro JL and Callaway RM. 2003. Allelopathy and exotic plant invasion. Plant and soil 256:29-39.

Stephan WA, Sowerby MS. 1996. Allelopathic potential of the weed, *Parthenium hysterophorus* L. in Australia. Plant Protection Quarterly.13:194-197.

Bhatt BP, Chauhan DS and Todaria NP. 1994. Effect of weed leachates on germination and radicle extension of some food crops. Indian journal of plant physiology 37:177-179.

Rajan L. 1973. Growth inhibitors from *Parthenium hysterophorus* L. Current Science 42(20):729-730.

Kanchan SD. 1975. Growth inhibitors from *Parthenium hysterophorus* L. Current science 44:358-259.

Swaminathan C, Rai RS and Sureshi KK. 1990. Allelopathic effects of *Parthenium hysterophorus* L. on germination and seedling growth of a few multipurpose trees and arable crops. The international tree crops journal 143-150.

Kanchan SD and Jayachandra 1979. Allelopathic effect of *Parthenium hysterophorus* L. I. Exudation of inhibitors through roots. Plant and soil 53:27-35.

Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

Antibacterial evaluation and phytochemical analysis of certain medicinal plants, Western Ghats, Coimbatore

Authors:
Doss A, Parivuguna V, Vijayasanthi M and Sruthi Surendran

Institution:
Department of Microbiology, RVS College of Arts and Science, Sulur, Coimbatore, Tamilndau, South India.

Corresponding author:
Doss A

Email:
androdoss@gmail.com

ABSTRACT:

The antibacterial effect of some selected Indian medicinal plants were evaluated on bacterial strains *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi*. The solvents used for the extraction of plants were petroleum ether, chloroform ethanol and methanol. The in-vitro antibacterial activity was performed by disc diffusion method. The most susceptible gram positive bacteria were *S. aureus* while the most susceptible gram negative bacteria were *E.coli*. The most active antibacterial plant was *P.maximum*. The significant antibacterial activity of active extracts were compared with the standard antimicrobials Ciprofloxacin (10mcg/ml). The results obtained in the present study suggest that *P.maximum* can be used in treating disease caused by the test organisms.

Keywords:
solvents, medicinal plants, microorganisms, antimicrobial activity, Phytochemicals.

Web Address:
http://jresearchbiology.com/Documents/RA0008.pdf.

Article Citation:
Doss A, Parivuguna V, Vijayasanthi M and Sruthi Surendran.
Antibacterial evaluation and phytochemical analysis of certain medicinal plants, Western Ghats, Coimbatore.
Journal of research in Biology (2011) 1: 24-29

Dates:
Received: 28 Apr 2011 /**Accepted:** 29 Apr 2011 /**Published:** 03 May 2011

Journal of Research in Biology

Introduction

Antimicrobials of plant origin have enormous therapeutic potential and have been used since time im-memorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics. Positive response of plant based drugs (less/no side effects) might lies in the structure of the natural products which reacts with toxins and / or pathogens in such a way that less harm is done to other important molecules or physiology of the host. It is because of this reason that drug designing studies nowadays have come up, as new field of research (Sharma and Kumar, 2009).

Chromolaena odorata (L.) King & H.E.Robins.

C. odorata is a big bushy herb with long rambling (but not twining) branches; stems terete, pubescent; leaves opposite, flaccid-membranous, velvety-pubescent, deltoid-ovate, acute, three-nerved, very coarsely toothed, each margin with 1-5 teeth, or entire in youngest leaves; base obtuse or subtruncate but shortly decurrent; petiole slender, 1-1.5cm long; blade mostly 5-12cm long, 3-6cm wide, capitula in sub-corymbose axillary and terminal clusters; peduncles 1-3cm long, bracteate; bracts slender, 10-12mm long; involucre of about 4-5 series of bracts, pale with green nerves, acute, the lowest ones about 2mm long, upper ones 8-9mm long, all acute, distally ciliate, flat, appressed except the extreme divergent tip; florets all alike (disc-florets), pale purple to dull off-white, the styles extending about 4mm beyond the apex of the involucre, spreading radiately; receptacle very narrow; florets about 20-30 or a few more, 10-12mm long; ovarian portion 4mm long; corolla slender trumpet form; pappus of dull white hairs 5mm long; achenes glabrous or nearly so. The seeds of Siam weed are small (3-5mm long, ~1mm wide, and weigh about 2.5mg seed-1. *C. odorata* had the antipyretic, antibacterial and anti-spasmodic properties.

Panicum maximum Jacq.

A tufted perennial, often with a shortly creeping rhizome, variable 60-200 cm high, leaf-blades up to 35 mm wide tapering to fine point; panicle 12- 40 cm long, open spikelets 3-3.5 mm long, obtuse, mostly purple red, glumes unequal, the lower one being one-third to one-fourth as long as the spikelet, lower floret usually male. Upper floret (seed) distinctly transversely wrinkled. Ethanolic leaf extract of *P.maximum* showed anti-diabetic activity. It has antibacterial activity against clinically important microbial pathogens.

Barleria lupulina Lindl.

Erect shrub; stems and leaves glabrous; spines 3 in lower axils, 2 deflexed ca 1-2 cm long, 1 shorter and upright. Leaves narrowly obovate, spine -tipped; lamina 3.5-9 cm long, 0.8-1.2 cm wide; petiole 2-3 mm long. Inflorescence a terminal spike with overlapping bracts; bracts broadly ovate, 15 mm long, shortly mucronate, green with purple upper half, very shortly pubescent all over, cup-shaped-glandular at base; bracteoles lanceolate, ca 5.5 mm long, sparsely glandular. Calyx segments spine-tipped, pubescent, lanceolate; outer 10 mm long, inner ca 8 mm long. Corolla yellow, finely eglandular pubescent outside; tube ca 3 cm long; lobes ca 1 cm long. Longer stamen filaments ca 2 cm long; shorter stamens fertile. Style ca 3 cm long, glabrous. Capsule not seen. Leaves are used to treat snake bites, dog bites, swelling due to fall or assault boils, bleeding wounds and rheumatism. The present study was undertaken to investigate the effects of aqueous and organic extracts of *Chromolaena odorata*, *Panicum maximum* and *Barleria lupulina.*

MATERIALS AND METHODS

Plant collection

Fresh plant parts of *Barleria lupulina*, *Panicum maximum* and *Chromolaena odorata* were collected from Western Ghats, Coimbatore, Tamilnadu, India. The taxonomic identities of plants were confirmed by Dr. V. Sampath Kumar, Scientist, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India and the voucher specimen of the plants have been preserved at RVS College Microbiology Laboratory. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight bottles at 4°C.

Preparation of crude extracts

About 100 g of dried plant material was extracted with 200 ml of Petroleum ether kept on a rotary shaker for 24 h. There after, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume $1/5^{th}$ of the original volume (Sasikumar *et al*., 2005). It was stored at 4°C in airtight bottles for further studies. Same conditions were applied for chloroform, methanol and ethanol extracts. It was stored at 4°C in airtight bottles for further studies.

Journal of Research in Biology

Phytochemical Components

This was carried out according to the methods described by Trease and Evans (1997). Quatification phytochemical analysis of the crude powder of three plants for the identification of phytochemicals like as a tannins, alkaloid, steroid, phenols and terpenoid, flavonoid etc.

Bacterial Strains

Microorganisms were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Amongst seven microorganisms were investigated, two Gram-positive bacteria are *Staphylococcus aureus,*? while five Gram-negative bacteria are *Proteus mirabilis* MTCC 425, *Escherichia coli* MTCC 2961, *Pseudomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. All the microorganisms were maintained at 4^0C on nutrient agar slants.

Antibacterial Activity

The antimicrobial assay was performed by agar disc diffusion method for solvent extract (Bauer *et al*., 1996). The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^6 CFU/ml) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, controls were maintained where pure solvents are used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

Minimum Inhibitory Concentration (MIC)

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacteria (Muhamed Mubarack *et al*., 2011). Different concentrations of plant extracts ranging from 0.125 - 8 mg/ ml^{-1}concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria (10^5 CFU ml^{-1}) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract showing reduction in turbidity when compared with control was regarded as MIC of that extract.

RESULTS AND DISCUSSION

Different solvents and water extracts tested at 100 mg/ml concentrations against six important microbial pathogens are presented in **Table 1**. Among five solvents (Petrolum ether, benzene, chloroform, methanol and water) tested against six microbial pathogens, benzene, methanol and water extracts recorded significant antibacterial activity against all the test pathogens. Antibacterial activity was not observed in petroleum ether and chloroform extracts against all the pathogens. Among benzene, methanol and water extracts, methanol extracts recorded significant antibacterial activity followed by water and benzene. *Staphylococcus aureus* found highly susceptible to methanol extract, where as *Klebsilla pneumonia* and *Pseudomonas aeruginosa* are less susceptible to methanol extract. Methanol extract exhibited similar antibacterial activity against, *E.coli*, *Salmonella typhi* and *Proteus mirabilis*. Antibacterial activity of water extract varied greatly among the different test pathogenic bacteria. Highest antibacterial activity was observed against *Staphylococcus aureus* followed by *E.coli* even though antibacterial activity was observed against other pathogens, also it was not found significant. Of the three candidate plants in this study *Panicum maximum* showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction.

Table 1. Antibacterial activity of *Barleria lupulina*

Microorganisms	Conc. (mg/ml^{-1})	Extracts					Synthetic drug
		P.ether	Ben	Chl	Met	Water	
S.aureus	100	-	-	-	16	-	21
E.coli		-	-	-	12	-	15
S.typhi		-	-	-	-	-	20
P.mirabilis		-	-	-	-	-	23
K. pneumonia		-	-	-	-	-	16
P. aeruginosa		-	-	-	-	-	14

Journal of Research in Biology

Table 2. Antibacterial activity of *Chromolaena odorata*

Microorganisms	Conc. (mg/ml^{-1})	Extracts					Synthetic drug
		P.ether	Ben	Chl	Met	Water	
S.aureus	100	-		-	15	-	21
E.coli		-	-	-	10	-	15
S.typhi		-	-	-	11	-	20
P.mirabilis		-	-	-	9	-	23
K. pneumonia		-	-	-	10	-	16
P. aeruginosa		-	13	-	-	-	14

Table 3. Antibacterial activity of *Panicum maximum*

Microorganisms	Conc. (mg/ml^{-1})	Extracts					Synthetic drug
		P.ether	Ben	Chl	Met	Water	
S.aureus	100	-	-	-	19	9	21
E.coli		-	-	-	14	8	15
S.typhi		-	-	-	13	-	20
P.mirabilis		-	12	-	11	-	23
K. pneumonia		-	-	-	10	-	16
P. aeruginosa		-	-	-	10	-	14
Synthetic drug							21

Minimum Inhibitory Concentration (MIC) of the active extracts is shown in **Table 4**. *P.maximum* and *B. lupulina* showed the strongest antibacterial activity with MIC values of 0.125 mg/ ml^{-1}, followed by *C. odorata* (MIC of 0.250 mg/ ml^{-1}). Available literature results indicate a strong activity when MIC values are between 0.05-0.50 mg/ ml^{-1}, moderate activity in values between 0.6-1.50 mg mL^{-1} and weak activity above 1.50 mg/ ml^{-1} (Diaz *et al*., 2009). In conformity to the existing trend, *P.maximum* and *B. lupulina* showed strong activity, *C. odorata* displayed moderate activity.

Phytochemical analysis of all the plant extracts revealed that alkaloids are generally present in petroleum ether extracts. Tannins were found in Petroleum ether & Chloroform extract and steroids in methanol extract. Flavonoids were found in benzene and methanol extracts (**Table 5**). All plant parts synthesize some chemicals by themselves, to perform their physiological activities. In our present study, the investigated plants have exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites are due to the presence of chemical substances that

Table 4. Minimum Inhibitory Concentrations of *Barleria lupulina*, *Chromolaena odorata* and *Panicum maximum*

Medicinal Plants	Extracts	Minimum Inhibitory Concentrations (mg/ml)					
		S.aureus	*E.coli*	*S.typhi*	*P.mirabilis*	*K. pneumonia*	*P. aeruginosa*
B. lupulina	P.ether	-	-	-	-	-	-
	Ben	-	-	-	-	-	-
	Chl	-	-	-	-	-	-
	Met	0.250	0.500	-	-	-	-
	Water	-	-	-	-	-	-
C. odorata	P.ether	-	-	-	-	-	-
	Ben	-	-	-	-	-	0.500
	Chl	-	-	-	-	-	-
	Met	0.250	1.0	1.0	4	1.0	-
	Water						
P. maximum	P.ether	-	-	-	-	-	-
	Ben	-	-	-	0.500	-	-
	Chl	-	-	-	-	-	-
	Met	0.125	0.250	0.250	1.0	1.0	1.0
	Water	4	4	-	-	-	-

Table 5. Phytochemical screening of *Barleria lupulina*, *Chromolaena odorata* and *Panicum maximum*

Medicinal Plants	Phytoconstituents	Extracts				
		P.ether	Ben	Chl	Met	Water
B. lupulina	Alkaloids	+	-	+	-	-
	Tannins	+	-	-	-	-
	Steroids	-	-	-	+	-
	Saponins	-	-	+	-	-
	Flvonoids	-	+	-	+	-
C. odorata	Alkaloids	+	-	-	-	-
	Tannins	-	-	+	-	-
	Steroids	-	-	-	-	-
	Saponins	-	+	-	-	-
	Flvonoids	+	-	-	+	-
P. maximum	Alkaloids	+	+	-	-	-
	Tannins	-	+	-	-	-
	Steroids	-	-	-	+	-
	Saponins	-	-	-	-	-
	Flvonoids	-	-	-	-	-

produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building (Kubmarawa *et al*., 2008).

Wynn (2001) describes today's traditional medicine, as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. With the traditional knowledge in the background, potential plants can be prospected to reach the active fraction or molecule (s) which can be further formulated. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf extracts of the plant *P.maximum*.

ACKNOWLEDGEMENTS

The 1st and 2nd researchers are grateful to University Grant Commission (UGC) for the financial support given to the present study under the Major Research Project programme entitled A Study of Ethno-veterinary Medicinal Plants and *in vitro* antimicrobial activities against Bovine Mastitis isolated bacterial pathogens [Sanction No. F. No. 35-121/2008 (SR) dt.20 March 2009]. The researchers are thankful to the management of RVS Educational Trust for their encouragement and support.

REFERENCES

Bauer AW, Kirby WMM, Sherris JC. 1996. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45:493-496.

Diaz MAN, Rossi CC, Mendonca VR, Silva DM, Ribon ADOB, Aguilar AP and Munoz GG. 2009. Screening of medicinal plants for antibacterial activities on Staphylococcus aureus strains isolated from bovine mastitis. Brazilian J.Pharmacog.

Kubmarawa D, Khan ME, Punah AM and Hassan 2008. Phytochemical Screening and antibacterial activity of extracts from *Pakia Clapperotoniana keay* against human pathogenic bacteria. Journal of Medicinal Plants Research 2 (12):352-355.

Muhamed Mubarack H, Doss A, Dhanabalan R and Venkataswamy R. 2011. Activity of some selected medicinal plant extracts against Bovine Mastitis Pathogens. Journal of Animal and Veterinary Advances 10(6):738-741.

Sasikumar JM, Pichai Anthoni Doss A, Doss A. 2005. Antibacterial activity of *Eupatorium glandulosum* leaves. Fitoterapia 76(2):240-243.

Sharma B and Kumar P. 2009. Extraction and Pharmacological Evaluation of some extracts of *Tridax procumbens* and *Capparis decidua*. International Journal of Applied Research in Natural Products 1(4):5-12.

Trease GE & Evans WC. 1997. *Pharmacognosy*,

Ed. XIV, Mac Millian Publisher Ltd., London. pp. 568-578.

Trease GE and Evans WC. 1997. Pharmacognosy. Ed.XIV, Mac Millian Publ., Ltd., London 568-578.

Wynn GS. 2001. Herbs in Veterinary Medicine. Alternative Veterinary Medicine. http:// www.altvetmed.com/articles/herbs.html as retrieved on 9 Oct 2001 21(47):38.

Journal of Research in Biology

Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

Habitat preservation is a concern for conserving of *Heliotropium rariflorum* Stocks. in the forest of North Gujarat Region (NGR), Gujarat, India.

Authors:
Rajendra Kumar S[1], Joshua J[2], Sunderraj SFW[3] and Kalavathy S[4].

Institution:
[1] Water, Sanitation and Hygiene Institute, Kodiakanal Tamil Nadu.
[2] Foundation for Ecological Security (FES), Udaipur, Rajasthan.
[3] Gujarat Institute of Desert Ecology (GUIDE), Bhuj – Kachchh, Gujarat.
[4] Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu.

Corresponding author:
Rajendra Kumar S

Email:
meen_rajendrakumar@yahoo.co.in

ABSTRACT:

The present study deals with the status, distribution and conservation of habitats of *Heliotropium rariflorum* – a threatened plant in the forest area of North Gujarat Region. It is tall under shrub; the distribution is exclusive to specific habitat and substratum. Survey was conducted from May 2005 to Dec 2006. A probable list of locations of the species in the study area was prepared based on the literature, reports, thesis and informal interviews with the local people. Using the above information random transects were located and surveyed in two phases.

A total of 631 individuals of *H. rariflorum* were recorded from NGR. Distributions of *H. rariflorum* are restricted into two main habitats; Thorn and Scrub Forest. The sandy soil substratum was found to be the most suitable one for *H. rariflorum*. The major threats to this plant were recorded as cutting and grazing, while most of the individuals are facing indirect threats in the form of habitat dryness.

Keywords:
Conservation, Threatened plants, Gujarat, Encroachment.

Web Address:
http://jresearchbiology.com/Documents/RA0007.pdf.

Article Citation:
Rajendra Kumar S, Joshua J, Sunderraj SFW and Kalavathy S.
Habitat preservation is a concern for conserving of *Heliotropium rariflorum* Stocks. in the forest of North Gujarat Region (NGR), Gujarat, India.
Journal of research in Biology (2011) 1: 30-37

Dates:
Received: 27 Apr 2011 /**Accepted:** 29 Apr 2011 /**Published:** 11 May 2011

Journal of Research in Biology

INTRODUCTION

The modern man's intervention in the forest environment has speeded up the process of degradation and losses. It leads to the fragmentation of forest areas into isolated patches, due to which species with wide and contiguous distribution gets separated and isolated into small sub-populations. Thus, a taxon is considered to be rare/endangered or endemic when its area of distribution is significantly smaller than the average taxa of the same rank in an area or region (Costa, 1997). These taxa face a high risk due to their low population, limited geographic distribution and disturbance (Vischi *et al.,* 2004). Hence, the habitats of these species must be protected from biotic and environmental degradations (Cox, 1993) in order to impede the species extinction process. Condit *et al.* (1998) proposed to understand and study the threatened species and their habitats, in order to make sound decisions for protecting those species and managing their habitats. An, attempt was made to study the status and conservation of *Heliotropium rariflorum* in North Gujarat Region.

It is a tall under shrub with an erect stem which is stiff and rigid and the whole plant has appressed hairs all over the surface (Shah, 1978). Leaves are linear appressed and hairy (Shah, 1978) alternate suborbicular or orbicular emarginated and mucronate. Inflorescence are terminal spikes paniculately arranged sessile slender elongate and bracteate. The bracts are long stalked or unstalked lanceolate hairy and scattered along the rachis.

The colour of the flower is white bracts are 5 -10 mm long, leaf like lanceolate hairy scattered along the rachis. Calyx is penta-partite and hairy tube is scarcely long and the segments are long linear-lanceolate and ciliate. Corolla is long and hairy outside tubes are broad cylindrical with hairs in the throat lobes are long ovate and subacute. The stigma is a very depressed cone sudcapitate but pointed at apex. The fruits are nutlets four lobed scrabid and hairy (Shah, 1978). Fruit lobes are projecting above the persistent above the persistent with spreading calyx segments densely hispid with appressed hairs (Bhandari, 1990).

H. rariflorum is widely distributed in India, Afghanistan, Sudan and Pakistan. In India it is mostly reported in Gujarat, Rajasthan and Punjab states (Bhandari, 1978; Shah, 1978; Bhandari, 1990). A survey shows that, this plant was recorded from three districts of Gujarat and the maximum numbers were found in Kachchh (GEC, MSU and GUIDE, 2002). The status of this plant is "**Intermediate (I)"** (WCMC, 1994), but habitat destruction is a main threat of this plant.

The purpose of evaluation of selected species is to prevent its degradation from native habitat. An important tool for this purpose is the determination of the degree of threat of the taxa to which a special significance is attributed.

To conserve these threatened plant species, three basic informations are needed which include collection of field data to understand the area specific population status, distribution and existing threats, which would help to plan appropriate conservation and management strategies for their long term conservation rather than allowing depletion and local extinction. This needs to be achieved with the following objectives:

To assess the status and distribution of *H. rariflorum* in North Gujarat Region.

To assess the existing threats to *H. rariflorum* and

To prepare a conservation plan for a plant and its habitats.

Study area

The North Gujarat region (NGR) lies between 23° 35' 13.0" to 24° 30' 57.0" N and 72° 10' 28.0" to 73° 24' 47.0" E and falls under three administrative districts *viz.* Banaskantha, Sabarkantha and Meshsana. It extends to about 8.7% (1638 km^2) of the total forest cover of Gujarat state (18,868.28 km^2) and includes protected areas *viz.* Jessore Sloth Bear Wildlife Sanctuary (JSBWS), Balaram Ambaji Wildlife Sanctuary (BAWS), Taranga hill and Vijaynagar forest.

Forest was the most predominant land use type of the study area covering 1638 km^2, followed by agriculture land use largely in the valleys. Third major land use is rocky barren surface, while mining areas cover over 15 km^2. Only 8 km^2 areas are in the form of water bodies or wetlands (Joshua *et al.,* 2007). Although major forest types are found in the study area, they have been classified into two major sub-groups *viz.* 5A - Southern Tropical Dry Deciduous Forest and 6B - Northern Tropical Thorn Forest (Champion and Seth, 1968). Zone 5A is further subdivided into various sub zones with different forest types. The Sabarkantha district alone holds conspicuous cover like Dry Teak Forest (C1a), Dry Forest (C1b) and Dry Bamboo Breaks (E9). While Banaskantha district owns two forest covers e.g. *Aegle marmelos* forest (E6) and *Acacia catechu* forest (IS2). These two districts together comprise many forest types such as Southern Dry Mixed Deciduous Forest (E5) and Secondary Dry

Journal of Research in Biology

Deciduous Scrub Forest (2S1). In addition to this, Banaskantha district together with Meshsana district and the adjoining areas of Little Rann of Kachchh (LRK) form the Saline Alkaline Scrub Savannahs (E8). The 6B zone, found in northern part of Banaskantha district, is formed by Desert Thorn Forest (C1), Desert Dune Scrub (IS1), Ravine Thorn Forest (C2) adjacent to Sabarmati River and scattered scrubs forest dominated by *Cassia auriculata*, and *Zizphus nummlaria*. (DS1). Rann Saline Desert Thorn Forest (dominated by *Prosopis juliflora*) and Scrublands (E3) occur in Sabarkantha, Banaskantha and Meshsana districts (Singh, 1998).

The dominant soil of this region is classified as alluvial sandy soil. This soil is a mixture of sandy and coarse particles. Sandy loame and black soil are distributed in Banaskantha and Sabarkantha districts. In Meshana, 90% of the area is covered by light sandy soil and at some patches where sandy soil is mixed with black soil where the cultivation is possible. The pure sandy soil usually distributed in the forest region of Meshana districts, mainly Taranga hill and Abarkantha forest, have good natural thorn forest (Chavan and Lal, 1984), which is also a home for threatened plants.

METHODS

Species inventory of the above mentioned threatened plant was carried out based on the forest map developed by Joshua *et al.,* (2005). The survey was carried from May 2005 to Dec 2006. A probable list of locations of the species in the study area was prepared based on the literature, reports, thesis and informal interviews with the local people following Vischi *et al.* (2004).

Using the above information random transects were located and surveyed in two phases. A total of 123 transects were developed and surveyed in the first phase. For the second phase, few patches were identified, where good numbers of *H. rariflorum* was recorded from the previous of studies.

Searches were made for the *H. rariflorum* along the entire diagonal length of belt transect within a width of 10-15 m. Along these transects whenever a targeted species was located, circular plot (8m radius) was used to enumerate its abundance. Other parameters *viz* associated species, macro and micro habitat parameters (habitats, terrain, slope, substrate, soil type and other related environmental information) and site specific threats were also noted.

RESULTS AND DISCUSSIONS

A total of 631 individuals of *H. rariflorum* were recorded from NGR. Distributions of *H. rariflorum* are restricted into two main habitats; 405 plants from Thorn Forest (TF) and 226 plants from Scrub Forest (SF) (Table 1).

The sandy soil substratum was found to be the most suitable one for *H. rariflorum,* an overall 96.4% of plants (608 individuals) were reported from this soil type. Rest of the individuals, 14 and 9 from loamy coarse and gravel substratum respectively (**Table 1**).

Earlier surveys showed on these plants were recorded from scrub forest, and coastal area with sandy soil, open rocks habitat with gravel substratum (Bhandari, 1978; GEC, MSU and GUIDE, 2002).

A total of 75 species were found to be growing along with *H. rariflorum*. The associated tree species were *Zizyphus mauritiana*, *Maytenus emarginata, Acacia raddiana* and *Balanites aegyptiaca,* shrubs were *Crotalaria burhia*, *Acacia jacquemontii*, *Leptadenia pyrotechnica* and *Cassia auriculata,* climbers were *Mukia maderaspatana*, *Ipomoea nil*, and *Citrullus colocynthis* and ground layer *Aristida adscensionis* subsp. *adscensionis*, *Indigofera cordifolia*, *Tephrosia purpurea*, *Cenchrus setigerus* and *Borreria stricta* (**Table 2**).

The major threats to this plant were recorded as cutting and grazing, while most of the individuals are facing indirect threats in the form of habitat dryness. The major % of *H. rariflorum* was observed from sandy soil. This is an unique habitat and represented in patches in NGR. Usually these kinds of soil type, poor retaining moisture. Hence, most of the time, habitat was in dry conditions. Heavy shower on this soil type will also causes soil erosion andis recorded as a major threats to this habitat (**Figure 1**).

Table 1: Abundance and distribution of *Heliotropium rariflorum* Stocks. in the North Gujarat Region (NGR)

Species Distribution		
Forest type	**Abundance**	**Relative %**
Thorn Forest	405	65
Scrub Forest	226	35
Total	631	100
Substratum	**Abundance**	**Relative %**
Sandy soil	608	96
Loamy coarse	14	3
Gravel	9	1
Total	631	100

Table 2: Species association with *Heliotropium rariflorum* Stocks.in the North Gujarat Region (NGR).

Sl. No	Scientific Name	No	Relative %
	Tree layer		
1	*Maytenus emarginata* (Willd.) D. Hou	2	0.10
2	*Acacia raddiana* Savi.	1	0.05
3	*Acacia senegal*(L.) Willd.	1	0.05
4	*Zizyphus mauritiana* Lam.	3	0.15
5	*Balanites aegyptiaca (L.) Del.*	1	0.05
	Shrub layer		
6	*Acacia jacquemontii* Bth.	11	0.55
7	*Cassia auriculata* L.	10	0.50
8	*Kirganelia reticulata* (Poir.) Baill.	1	0.05
9	*Crotalaria burhia* Buch-Ham. ex Bth	65	3.25
10	*Heliotropium rariflorum* Stock.	28	1.40
11	*Leptadenia pyrotechnica* (Forsk.) Decne.	11	0.55
12	*Hibiscus ovalifolius* (Forsk.) Vahl	7	0.35
13	*Pupalia lappacea* (L.) Juss.	4	0.20
	Climber layer		
14	*Ipomoea nil* (L.) Roth	2	0.10
15	*Mukia maderaspatana* (L.) M. Roem.	7	0.35
16	*Citrullus colocynthis* (L.) Schrad.	1	0.05
	Ground layer		
17	*Indigofera cordifolia* Heyne ex Willd.	348	17.38
18	*Tephrosia purpurea* (L.) Pers.	123	6.14
19	*Borreria stricta* (L. f.) Schum.	70	3.50
20	*Evolvulus alsinoides* (L.) L. var. *alsinoides*	66	3.30
21	*Crotalaria medicaginea* Lam.	36	1.80
22	*Cassia tora* L.	34	1.70
23	*Justicia simplex* Don	32	1.60
24	*Curcuma pseudomontana* Grah.	31	1.55
25	*Amaranthus viridis* L.	30	1.50
26	*Bergia ammannioides* Roxb. ex Roth	29	1.45
27	*Peristrophe paniculata* (Forsk.) Brumm.	20	1.00
28	*Indigofera linifolia* Retz. var. *linifolia*	17	0.85
29	*Commelina albescens* Hassk.	17	0.85
30	*Sida cordata* (Burm. f.) Borss	16	0.80
31	*Justicia procumbens* L.	16	0.80
32	*Gnaphalium luteo-album* L. subsp. *affine* (D. Don) Koster	15	0.75
33	*Xanthium strumarium* L.	14	0.70
34	*Boerhavia diffusa* L.	13	0.65
35	*Convolvulus microphyllus* (Roth) Sieb. ex Spr.	12	0.60
36	*Leucas stricta* Bth.	10	0.50
37	*Achyranthes aspera* L. var. *argentea* Hook. f.	10	0.50
38	*Aerva persica* (Burm.f.) Merrill	9	0.45

Journal of Research in Biology

39	*Tephrosia villosa* (L.) Pers.	8	0.40
40	*Corchorus aestuans* L.	8	0.40
41	*Cardiospermum halicacabum* L.	7	0.35
42	*Ocimum canum* Sims	7	0.35
43	*Euphorbia parviflora* L.	6	0.30
44	*Blepharis repens* (Vahl) Roth	6	0.30
45	*Acanthospermum hispidum* DC.	6	0.30
46	*Phyllanthus virgatus* Forst. F.	5	0.25
47	*Commicarpus chinensis* (L.) Heimerl	4	0.20
48	*Cassia holosericea* Fresen.	4	0.20
49	*Commicarpus verticillatus* (Poir.) Standl.	3	0.15
50	*Indoneesiella echioides* (L.) Sreem.	3	0.15
51	*Ocimum basillicum* L.	2	0.10
52	*Tridax procumbens* L.	2	0.10
53	*Cassia pumila* Lam.	2	0.10
54	*Vernonia cinerea* (L.) Less.	1	0.05
55	*Ipomoea eriocarpa* R. Br.	7	0.35
56	*Aristida adscensionis* L. *subsp. adscensionis*	459	22.93
57	*Cenchrus setigerus* Vahl	92	4.60
58	*Dactyloctenium aegypticum* (L.) P. Beauv.	50	2.50
59	*Cynodon dactylon* (L.) Pers.	49	2.45
60	*Cenchrus ciliaris* L.	29	1.45
61	*Brachiaria reptans* (L.) Gard. & C. E. Hubb.	26	1.30
62	*Sporobolus helvolus* (Trin.) Th. Dur. et Schinz	17	0.85
63	*Heteropogon contortus* (L.) P. Beauv. ex. R. & S.	12	0.60
64	*Acrachne ramosa* (Heyne ex R. & S.) Ohwi	12	0.60
65	*Cymbopogon martinii* (Roxb.) Wats	10	0.50
66	*Aristida funiculata* Trin. & Rupr.	8	0.40
67	*Echinochloa colonum* (L.) Link	7	0.35
68	*Eragrostis poaeoides* P. Beauv.	5	0.25
69	*Paspalum distichum* L.	5	0.25
70	*Brachiaria ramosa* (L.) Stapf	3	0.15
71	*Eragrostis ciliaris* (L.) R. Br. var. *ciliaris*	3	0.15
72	*Setaria tomentosa* (Roxb.) Kunth.	2	0.10
73	*Setaria verticillata* (L.) P. Beauv.	2	0.10
74	*Pennisetum pedicellatum* Trin.	1	0.05
75	*Cyperus triceps* (Rottb.) Endl.	6	0.30

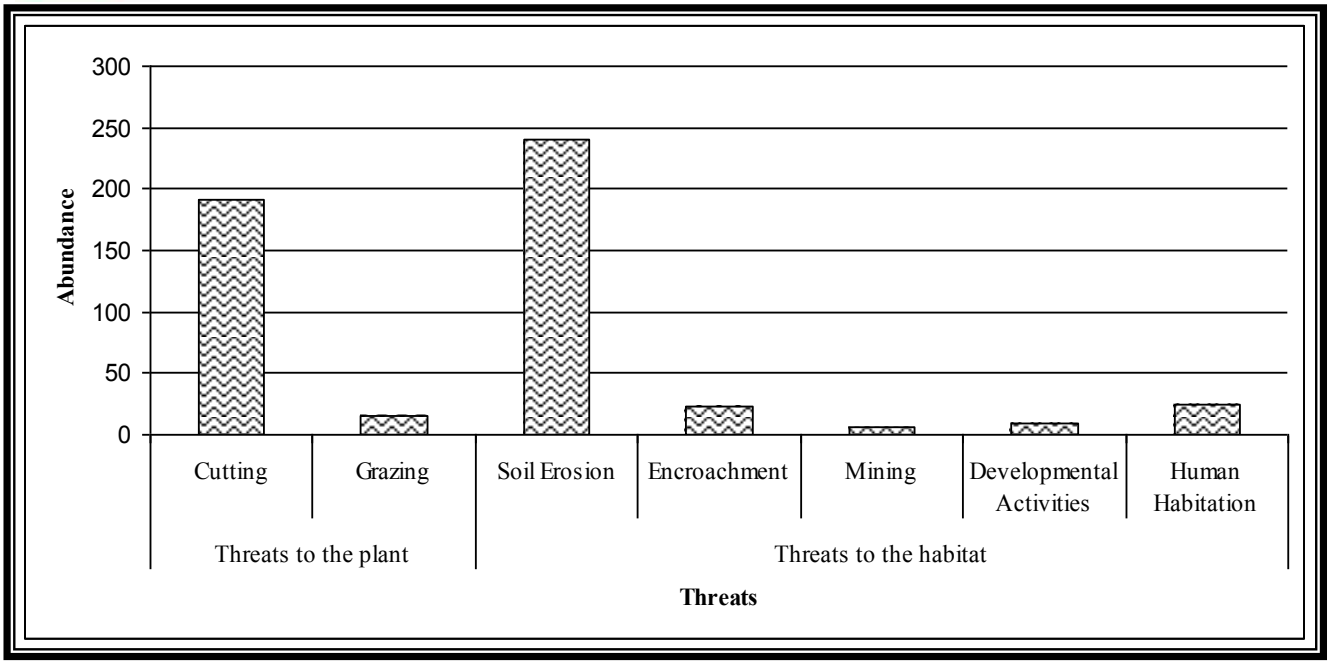


Figure 1: Threat factors to *Heliotropium rariflorum* Stocks. in the North Gujarat Region (NGR).

Site prioritizations

During our survey, 11 sites of *H. rariflorum* were recorded with different range of populations (Map 1). A total of 631 individuals were classified under different ranges 1-25, 26-50, 51-75, 76-100 and >101. The sites fall under this classes are 5, 1, 1, 2, and 2 and conservative rank were given as 5, 4, 3, 2 and 1 respectively (**Map 1**).

Conservation measures

As majority of the threatened plants species were found in Thorn Forest with Sandy substratum, more attention have to be paid to preserve this micro and macro habitat.

Awareness among the people on ecological importance of these plant species must be created.

Large patches with threatened plants to be designated as Preservation Plot.

Conservation measures like soil and moisture conservation are to be taken up, to reduce soil erosion, a main cause of habitat loss.

ACKNOWLEDGEMENT

The authors wish to acknowledge the constant encouragement, supports and facilities provided by Gujarat Institute of Desert Ecology (GUIDE), Bhuj, Kachchh and Foundation for Ecological Security (FES), Anand, Gujarat for successful completion of this work. Many thanks are due to Dr. V. Gokula, M.Sc., M. Phil., Ph.D., Associate Professor, Department of Zoology, National College, Tiruchirappalli for the valuable suggestions and the review of this paper.

REFERENCES

Bhandari MM. 1990. Flora of Indian Desert, MPS Report, Jodhpur. 141-142.

Bhandari MM. 1978. Flora of Indian Desert, MPS Report, Jodhpur, Revised Editions. 222-223.

Champion HG and Seth SK. 1968. Revised Forest types of India. Government of India Publications, New Delhi.

Chavan SA and Lal B. 1984. Biodiversity Status of Important Ecosystems of Gujarat. Pp 66-107. In: Kotwal PC and Banerjee S (eds.). Managed Forests and Protected Areas. Agro Botanic Publications and Distributors, Bikanar, Rajasthan, India.

Condit JH, Tracey JG and Webb LJ. 1998. Compensatory recruitment, growth and mortality as factor maintaining rain forest tree diversity. Ecological Monograph 5:141-164.

Costa M. 1997. Biogeografia In: Izco J, Barreno E, Bruguez M, Costa M, Devesa J, Fernandez F(eds.). Botanica McGraw Hill Interamericana, Madrid, Spain. 683-687.

Cox GW. 1993. The Ecology of Extinction. Pp.13-22. In: Cox GW (ed.). Conservation Ecology. W.C. Brown Publishers, Dubuque, Iowa.

GEC GUIDE and MSU. 2002. Conservation of Rare and Endangered Biodiversity of Gujarat. A final Report by Gujarat Ecological Commission, Gujarat India. 428.

IUCN. 2000. IUCN Red List of Threatened Species. Species Survival Commission (SSC), International Union for Conservation of Nature (IUCN), Gland, Switzerland.

Joshua J, Sundarraj SFW, Joshi PN, Das SK, Rajendrakumar S, Kala HKP, Menon JM and Mewada T. 2005. Assessment of Biodiversity and Preparation of Conservation Plan for the Forests of North Gujarat Region, Gujarat. First Progress Report submitted to: E.I Division, Ministry of Environment and Forests, CGO Complex, Lodi Road, New Delhi. Gujarat Institute of Desert Ecology (GUIDE), Bhuj, Kachchh, Gujarat. 45.

Joshua J, Sunderraj SFW, Rajendrakumar S, Kala HKP, Manojkumar P, Muthuandavan L. 2007. Assessment of Biodiversity and Preparation of Conservation Plan for the Forest of North Gujarat Region. A Final Report Prepared by Gujarat Institute of Desert Ecology, Bhuj, Kachchh, Gujarat. 206.

Shah GL. 1978. Flora of Gujarat State. Volume: I & II. Sardar Patel University Vallabh Vidhynagar, Gujarat, India. 1074.

Singh HS. 1998 Wildlife of Gujarat: Wildlife and Protected Habitats of Gujarat State. Published by Gujarat Ecological Education and Research Foundation. 123.

Vischi N, Natale E and Villamil C. 2004. Six endemic plant species from central Argentina: an evaluation of their conservation status. Biodiversity and Conservation. 13:997-1008.

WCMC. 1994 Status report as of 24 November 1994. Gujarat. Printout from plant database BG-BASE. World Conservation Monitoring Center (WCMC).

Journal of Research in Biology

Map 1: Distribution of *Heliotropium rariflorum* Stocks. in the North Gujarat Region (NGR)

Journal of Research in Biology

FicusPress

An International Online Open Access Publication group

OPEN ACCESS

Original Research paper

Seasonal and relative abundance of butterflies in a scrub jungle habitat of Peraiyur Taluk, Madurai District, Tamilnadu.

Authors:
Alagumurugan C, Pavaraj M and Rajan MK.

Institution:
Post-graduate and Research Department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi – 626 124.

Corresponding author:
Pavaraj M

ABSTRACT:

The present investigation has been aimed to explore the biodiversity of butterflies in a open land habitat of Peraiyur Taluk. Totally 65 species of butterflies belonging to 8 Families were of order Lepidoptera were identified. The family Nymphalidae and Pieridae contribute more number of species with 15 and 14 respectively and followed by Lycaneidae (13 species), Papilionidae (9 species), Danaidae (6 species), Hesperiidae (5 species), Satyridae (2 species) and Acreidae (1 species).

Keywords:
Butterflies, Abundance, Sessional Occurrence, Scrub jungle habitat.

Web Address:
http://jresearchbiology.com/Documents/RA0004.pdf.

Article Citation:
Alagumurugan C, Pavaraj M and Rajan MK.
Seasonal and relative abundance of butterflies in a scrub jungle habitat of Peraiyur Taluk, Madurai District, Tamilnadu.
Journal of research in Biology (2011) 1: 44-50

Dates:
Received: 21 Apr 2011 /**Accepted:** 23 Apr 2011 /**Published:** 11 May 2011

Journal of Research in Biology

INTRODUCTION

India harbors about 1,501 species of butterflies in which 285 species are found in the southern part of India of which 45 species are endemic to south India (Thomos, 1984). Most Lepidopteran species evidently visited a limited range of nectar plants, which may have implications for species conservation and selecting "butterfly plants" for gardening (Tooker *et al*., 2002). Butterflies are generally fairly, readily and identifiable insects. There is a relatively good taxonomic knowledge of the group and they are also sensitive to environment changes in microsite and biotope characteristics (Ehrlich and Raven, 1964). Butterflies are important natural resources as they help in pollination, a key process in natural propagation, important ecological indicators, as they are closely associated with plants both as adults and as larvae, an enhances the aesthetic value of the environments by their exquisite wing colours. Hence, there is increasing global interest in conserving and managing butterflies (New *et al*., 1995). Studies on butterflies in any area would help us to understand the status of the ecosystem and increasing human pressure on natural resources which require a reliable accounting of biodiversity (Kremen, 1992). The different life stages of the butterflies are exposed to a wide range of environmental influences including variation in temperature, humidity, light and disturbances (Murphy *et al.,* 1990). Hence, the present investigation has been carried out to explore the seasonal and relative abudance of butterflies in a scrub jungle habitat of Peraiyur Taluk, Madurai District, Tamilnadu.

MATERIALS AND METHODS

Study area

The study on the biodiversity of butterflies in a scrub jungle habitat was carried out in Salichandhai village. This village is located in between Peraiyur and Kumarapuram. This habitat includes weeds such as, *Leucas aspsera, Murraya paniculata, Lantana camera* and *Tridex procumbens etc*. The following shrubs are also found in this habitat. They are as follows *Calotropis* Sp., *Acacia* Sp., *Wendlandia thyrsoidea etc.* The most dominant vegetation in this habitat is *Zizypus sps, Cassia sps* and *Acacia's* Sp.

Study period

The study was extensively carried out from May 2007 to April 2008 and it was divided into pre-monsoon (June, July and August), monsoon (September, October and November), early post-monsoon (December, January and February) and late post-monsoon (March, April and May).

Methodology Adopted

The butterflies were observed from 07.00 hrs to 16.00 hrs. The butterflies were observed at a distance of 2m. Some butterflies could not be identified accurately upto the species level in the field as they were difficult to locate and identify in the field because of their smaller size and cryptic coloration (Lycaenidae and Hesperiidae). The butterfly species were identified by using standard references such as, the field guide "Some south Indian Butterflies" (Gunathilagaraj *et al.,* 1998) and 'India – a lifescape butterflies of peninsular India (Kunte, 2000). The phenology of some butterflies were also studied based on the observations.

RESULTS AND DISCUSSION

The list of butterflies observed in the scrub jungle habitat was presented in **Table 1**. Totally 65 species of butterflies belonging to 8 Families were identified. The family Nymphalidae and Pierdae contribute more number of species with 15 and 14 respectively and these two families are followed by Lycaneidae (5 species), Satyridae (2 species) and Acraeidae (1 species). The distribution of butterflies is dependant upon the availability of their food plants. Migrants move out of their breeding areas if they do not find suitable food plants source in the new localities as they will fail future generation (Uniyal, and Mehra, 1996). The abundance of butterfly species population in scrub jungle area may be due to the availability of ample food, optimum climate and a serene atmosphere (Ravindra *et al.,* 1996). The relative abundance of butterflies in present scrub jungle habitat was calculated and presented in the **Table 2**. This habitat comprises 65 species of butterflies under 8 families. Among these 8 families Nymphalidae and Pierdae were found to be the most dominant members with (23.07%) and (21.53%) respectively. These two families were followed by Lycaenidae (19.69%), Papilioniae (13.84%), Danaidae (9.23%) and Hesperiidae (7.69%). The minimum number of species were also found in this habitat from the families such as, Satyridae (3.07%) and Acraeidae (1.53%). The seasonal occurrence and abundance of butterflies observed in the scrub jungle were presented in **Table 3**.

During pre monsoon period (June 2007 to August 2007) 87 butterflies belonging to 30 species were observed. Among these butterflies, *Catopsilia*

Table 1. Shows the occurrence of butterflies in scrub jungle habitat of Peraiyur Taluk.

S.No	Family	Zoological Name	Common Name
1.	Acraeidae	*Acraea violae*	Twansy castor
2.	Danaidae	*Danaus chrysippus chrysippus*	Plain tiger
3.	”	*Tirumala limniace exoticus*	Blue tiger
4.	”	*Euploea core core*	Common Indian crow
5.	”	*Idea malabarica*	Malabar tree
6.	”	*Parantica aglea agela*	Grassy blue tiger
7.	”	*Tirumala septentrionis dravidarum*	Dark blue tiger
8.	Hesperiidae	*Hasora chromus chromus*	Common banded owl
9.	”	*Lambrix salsala luteipennis*	Chestnut Bob
10.	”	*Suastus gremius gremius*	Indian balm Bob
11.	”	*Spialia galba galba*	Indian skipper
12.	”	*Ampittia dioscorides dioscorides*	Bush hopper
13.	Lycaenidae	*Azanus ubaldus*	Bright babul blue
14.	”	*Castalius rosimon rosimon*	Common pierrot
15.	”	*Pseudozizeeria maha*	Pale grass blue
16.	”	*Zizeeria karsandra*	Grass blue
17.	”	*Jamides alecto*	Metallic cerulean
18.	”	*Zizina otis*	Lesser grass blue
19.	”	*Rapala manea*	Slate flash
20.	”	*Leptotes plinius*	Zebra blue
21.	”	*Rapala jarbus*	Slate flash
22.	”	*Jamides celeno*	Common cerulean
23.	”	*Chilades pandava*	The plain cupid
24.	”	*Freyeria trochylus*	Grass jewel
25.	”	*Spindasis vulcanus*	Common silver line
26.	Nymphalidae	*Precis atlites*	Grey pansy
27.	”	*Precis iphita iphita*	Choclate pansy
28.	”	*Polyura athamas athamas*	Common nawab
29.	”	*Precis almana almanac*	Peacock pansy
30.	”	*Hypolimnas misippus*	Danaid egg fly
31.	”	*Junonia lemonias*	Lemon pansy
32.	”	*Pantoporia hordonia*	The common lascar
33.	”	*Euthalia aconthea maridionalis*	Baron
34.	”	*Ariadne merione merione*	Common castor
35.	”	*Neptis hylas varmona*	Common sailer
36.	”	*Vindula erota*	Crusier
37.	”	*Argyreus hyperbius hybrida*	Indian fritillary
38.	”	*Moduza procris undifragus*	Commander
39.	”	*Hypolimns bolina jacintha*	Great egg fly
40.	”	*Ariadne ariadne*	Angeled castor

41.	Papilionidae	*Graphium doson*	Common jay
42.	”	*Pachilopta aristolochiae aristolochiae*	Common rose
43.	”	*Papilio polytes polytes*	Common mormon
44.	”	*Papilio polytes romulus*	Malabar Raven
45.	”	*Papilio polymnestor*	Blue mormon
46.	”	*Pachliopta hector*	Crimson rose
47.	”	*Papilio helenus*	Red helan
48.	”	*Papilio demoleus*	Lime butterfly
49.	”	*Troides minos*	Southern bird wing
50.	Pieridae	*Catopsilia crocale*	Common emigrant
51.	”	*Catopsilia florella*	African emigrant
52.	”	*Delias eucharis*	Common jezebel
53.	”	*Hebomoia glaucippe*	Great orange tip
54.	”	*Anaphaeis aurota*	The pioneer
55.	”	*Catopsilia pomona*	Lemon emigrant
56.	”	*Catopsilia pyranthe*	Mottled emigrant
57.	”	*Pareonia valeria hippie*	Common wanderer
58.	”	*Cepora nerissa nerissa*	Common gull
59.	”	*Colotis etrida*	Little orange tip
60.	”	*Eurema hecabe simulate*	Common grass yellow
61.	”	*Colias nilagiriensis*	Nilgiri clouded yellow
62.	”	*Ixias marianne*	White orange tip
63.	”	*Ixias pyrene*	Yellow orange tip
64.	Satyridae	*Melanitis leda leda*	Common evening brown
65.	”	*Mycalesis perseus typhlus*	Common bush brown

pomona of family Pierdae was found to be a dominant member (5 numbers) followed by *Delias eucharis* (5 numbers), *Tirumala limniace exoticus* (4 numbers), *Chilades pandave* (4 numbers), *Pachliopta hector* (4 numbers) and *Catopsilia florella* (4 numbers). During monsoon period (September 2007 to November 2007) 181 butterflies belonging to 65 species were observed. Among these butterflies, *Papilio polytes polytes* of Papilionidae was found to be a dominant member (8 numbers) followed by *Papilio romulus* (6 numbers) *Delias euchrias* (6 numbers), *Tirumala limniace exoticus* (5 numbers), *Euploea core core* (4 numbers), *Moduza procris undifragus* (4 numbers), *Papilio polymnestor* (4 numbers), *Colotis etrida* (4 numbers) and *Ixias Marianne* (4 numbers). During early post monsoon (December 20007 to February 2008) 156 butterflies belonging to 58 species were observed. Among these butterflies, *Papilio demoleus* of family Papilionidae was found to be a dominant member (5 numbers) followed by *Chilades pandava* (4 numbers), *Jamides alecto* (4 numbers), *Euthalia aconthea meridionalis* (4 numbers), *Pachliopta hector* (4 numbers), *Papilio helenus daksha* (4 numbers) and *Iixias marine* (4 numbers).

Table 2. Shows relative abundance of butterflies in the scrub jungle habitat of Peraiyur Taluk.

S.No	Family	Number of Species	Relative Abundance
1.	Acraeidae	01	1.53%
2.	Danaidae	06	9.23%
3.	Hesperiidae	05	7.69%
4.	Lycaenidae	13	19.69%
5.	Nymphalidae	15	23.07%
6.	Papilionidae	09	13.84%
7.	Pieridae	14	21.53%
8.	Satyridae	02	3.07%
	Total	65	100%

Journal of Research in Biology

Journal of Research in Biology

Table 3. Shows the seasonal occurrence of butterflies in the scrub jungle habitat of Peraiyur Taluk.

S.No	Family and species of Butterflies	Pre Monsoon	Monsoon	Early Post Monsoon	Late Post Monsoon
	I. Acraecidae				
1.	*Acraea violae*	-	2	2	2
	II. Danidae				
2.	*Danaus chrysippus chrysippus*	2	3	2	-
3.	*Euploea core core*	3	4	2	-
4.	*Idea malabarica*	-	3	2	1
5.	*Parantica agela agela*	-	2	2	-
6.	*Tirumala limniace exoticus*	4	5	3	-
7.	*Tirumala septentrionis dravidarum*	-	2	2	-
	III. Hesperiidae				
8.	*Ampittia dioscorides dioscorides*	3	1	1	-
9.	*Azanus ubaldus*	-	2	2	-
10.	*Hasora chromus chromus*	2	2	2	2
11.	*Lambrix salsala luteipennis*	2	2	1	1
12.	*Spialia galba galba*	1	1	-	-
13.	*Suastus gremius gremius*	1	2	-	-
	IV. Lycanidae				
14.	*Castalius rosimon rosimon*	3	1	3	3
15.	*Chilades pandava*	4	-	4	2
16.	*Freyeria trochylus*	-	2	1	-
17.	*Jamides alecto*	-	3	4	-
18.	*Jamides celeno*	-	2	3	2
19.	*Leptotes plinius*	-	2	3	-
20.	*Rapala jarbus*	-	2	-	-
21.	*Rapala manea*	-	3	-	-
22.	*Spindasis vulcanus*	-	2	3	3
23.	*Zizeeria karsandra*	-	2	3	-
24.	*Pseudozizeeria maha*	2	2	-	-
25.	*Zizina otis*	-	3	2	-
	V. Nymphalidae				
26.	*Argyreus hyperbius hybrida*	-	2	2	-
27.	*Ariadne ariadne*	2	2	-	-
28.	*Ariadne merione merione*	3	3	3	-
29.	*Euthalia aconthea meridionalis*	3	2	4	-
30.	*Hypolimnas bolina jacintha*	-	2	2	-
31.	*Hypolimnas misippus*	-	3	2	2
32.	*Juonia lemonias*	3	3	2	-
33.	*Moduza procris undifragus*	-	4	3	-
34.	*Neptis hylas varmona*	2	3	4	2
35.	*Pantoporia hordonia*	-	3	3	-
36.	*Polyura athamas athamas*	-	2	2	-
37.	*Precis almana almanac*	2	3	3	3
38.	*Precis atlites*	3	2	3	-
39.	*Precis iphita iphita*	3	3	4	2
40.	*Vindula erota saloma*	-	2	3	-

	VI. Papilionidae				
41.	*Graphium doson*	-	4	3	3
42.	*Pachliopta hector*	4	6	4	-
43.	*Papilio polytes romulus*	3	7	3	2
44.	*Papilio crino*	1	3	4	-
45.	*Papilio demoleus*	3	4	5	-
46.	*Papilio helenus daksha*	-	3	4	3
47.	*Papilio polymnestor*	-	4	3	3
48.	*Papilio polytes polytes*	-	8	4	1
49.	*Troides minos*	-	3	3	-
	VII. Pieridae				
50.	*Anaphaeis aurota*	2	3	-	2
51.	*Catopsilia florella*	4	2	3	3
52.	*Catopsilia crocale*	-	2	2	-
53.	*Catopsilia pomona*	5	3	3	3
54.	*Catopsilia pyranthe*	3	2	-	2
55.	*Cepra nerissa nerissa*	-	3	2	2
56.	*Colias nilagiriensis*	3	3	2	-
57.	*Colotis etrida*	-	4	3	-
58.	*Delias euchrias*	5	6	2	4
59.	*Eurema hecabe simulate*	-	4	3	-
60.	*Hebomoia glaucippe*	-	1	4	3
61.	*Ixias marianne*	-	4	3	2
62.	*Ixias pyrene*	-	2	4	-
63.	*Pareronia valeria*	-	3	2	2
	VIII. Satyridae				
64.	*Melanitis leda leda*	-	1	2	-
65.	*Mycalesis perseus typhlus*	2	2	1	-
	Total no. of Individuals	**87**	**181**	**156**	**60**
	Total no. of Species	**30**	**65**	**58**	**26**

During late post – monsoon period 60 butterflies belonging to 26 species were observed. Among these butterflies, *Delias euchrias* of family Pieridae was found to be a dominant member (4 numbers) followed by *Castalius rosimon rosimon* (3 numbers), *Spindasis vulcanus* (3 numbers), *Pracis almana almanac* (3 numbers), *Graphium doson* (3 numbers), *Papilio helenus daksha* (3 numbers), *Papilio polymnestor* (3 numbers), *Catopsilia florella* (3 numbers), *Captopsilia Pomona* (3 numbers) and *Hebomoia glaucippe* (3 numbers). The quality of water has been reported to affect the abundance of butterflies. The males however, often crowd in hundred besides river or mineral springs. Possibly they were attracted by dissolved salts; those found near rivers usually congregate at drinking place where animals have urinated on the sand (Mathews and Carrington, 1970). The butterflies as most others Lepidoptera, show distinct patterns of habitat association. The nature of vegetation, sunshine availability of water etc., are the factors that determine the survival of a given species in a particular habitat (De Vries, 1987).

ACKNOWLEDGEMENT

The authors express the profound thanks to the Management and Head of the Department of Zoology. Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi for providing facilities to carry out this work.

REFERENCES

DeVries PJ. 1987. The Butterflies of Costarica and Their Natural History, Princeton University Press, Princeton 10-15.

Ehrlich PR, Raven PR. 1964. Butterflies and plants a study in co-evolution, *Evolution* 18:566-608.

Gunathilagaraj K, Perumal TNA, Jayaram K

Ganeshkumar M. 1998. Some South Indian Butterflies, Nilgiri Wildlife Environment Association, Udhagamandalam.

Kremen K. 1992. Assessing the insulator properties of species Assemblages for natural area monitoring. *Ecol. Appl* 2:203-217.

Kunte K. 2000. Butterflies of Penisular India. University press, Hyderabad, India. 254.

Mathews LH, Carrington R. (Eds). 1970. The Brilliant World of Insects.In: **The Living World of Animals.** The Reader's Digest Association Ltd., London. 27-38.

Murphy DD, Freas KE, Weiss SB. 1990. An environment meta population approach to population viability analysis for a threatened inverstbrate, *Conserv. Biol* 4:41-51.

New TR, Pyle RM, Thomas JA, Thomas CD, Hammond PC. 1995. Butterflies conservation management, *Ann. Rev. Entomol.,* 40:57-83.

Ravindra M. Viswanathan S, Ram GM. 1996. Checklist of butterfly species of Osmania University Campus, Hyderabad, *Zoo's Print* XI (10):5.

Thomos JA. 1984. The conservation of butterflies in temperate countries. Past effects and lessons for the future, In: *The Biology of butterflies* (Eds. Van – Wright, R.I. Ackery), Royal Entomological Society, London. 45-57.

Tooker JF, Reagel PF, Hanks LM. 2002. Nectar sources of day flying Lepidoptera of central Illionis, *Eotomological Society of America,* 95(1); 84-96.

Uniyal VP Mehra BS. 1996. Preliminary observation on the diversity of butterflies (Lepidoptera: insecta) in high sltitude grazing pasture in Great Himalayan National Park, *Zoo's Print,* XI(9):7-11.

Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

Screening of *Dahlia pinnata* for its Antimicrobial Activity

Authors:
Sharad Bissa, Avinash Bohra and Bohra A.

Institution:
Faculty of Science, Mahila PG Mahavidyalaya Jodhpur-342001 (India).

Corresponding author:
Sharad Bissa

Email:
bissasharad@yahoo.co.in

ABSTRACT:

The demand for more and more drugs from plant sources is continuously increasing. The present study deals with the antibacterial activity of different plant part (Root, stem, leaf and flowers) extracts of *Dahlia pinnata*. The antibacterial activity of both fresh and dried plant parts were determined in aqueous, alcohol, chloroform and petroleum ether extracts using agar disc diffusion method against *E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Agrobacterium tumefaciens*. *Dahlia pinnata* possessed highest antibacterial activity in its chloroform extract of dried leaves against *Enterobacter aerogenes*.

Keywords:
Dahlia pinnata, Antibacterial activity, *E. coli*, *S. typhi.*

Web Address:
http://jresearchbiology.com/Documents/RA0006.pdf.

Article Citation:
Sharad Bissa, Avinash Bohra and Bohra A.
Screening of *Dahlia pinnata* For Its Antimicrobial Activity.
Journal of research in Biology (2011) 1: 51-55

Dates:
Received: 27 Apr 2011 /**Accepted:** 29 Apr 2011 /**Published:** 12 May 2011

Journal of Research in Biology

Journal of Research in Biology

INTRODUCTION

In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. According to National Health Experts, 2000 different plants are used for medicinal preparations for both internal and external use in India alone. *Dahlia pinnata* belongs to the family asteraceae and is a perennial growing to 1m. It is in flower from June to October. The flowers are hermaphrodite (has both male and female organs) and are pollinated by Insects. The flower petals are used in salads. Root - cooked and used as a vegetable. A bitter flavour. A sweet extract of the tuber, called 'dacopa', is used as a beverage or as a flavouring. It is mixed with hot or cold water and sprinkled on ice cream. Its naturally sweet mellow taste is said to combine the characteristics of coffee, tea and chocolate. The root is rich in the starch inulin. Whilst not absorbed by the body, this starch can be converted into fructose, a sweetening substance suitable for diabetics to use. An orange dye is obtained from the flowers and seed heads. The aim of the present study is to determine the antibacterial activity of various extracts of *Dahlia pinnata* against some pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh plant parts were collected from many residential gardens, local nurseries and farm houses, at different localities, in Jodhpur, during their growing seasons. Their identity was confirmed by Botanical Survey of India, Jodhpur, from the literature available on exotic plants and also from literature available in Department of Botany, J.N.V,University, Jodhpur. The voucher specimens were deposited in herbaria of the Department of Botany, J.N.V.University, Jodhpur(Raj.), India.

Preparation of Plant Extracts:

From Fresh Plant Parts:

25 g of fresh plant parts, viz. Stem, Leaves, roots and flowers were washed for 3-4 times with tap water and distilled water, then surface sterilized with 90% alcohol. Subsequently, the plant materials were grounded in 100 ml of distilled water, ethanol, chloroform and petroleum ether separately for aqueous, alcoholic extracts, chloroform extracts and petroleum ether extracts, respectively. The macerates were kept for 24 hours at room temperature to evaporate the solvents. The macerates were squeezed through double layered muslin cloth and filtered through filter paper. After filteration, aliquot was centrifuged at 10,000 rpm for 20 minutes. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 micron disposable filters. The extracts were diluted to get a concentration of 50 mg per ml and were used for the *in vitro* studies.

From Dried Plant Parts:

The selected plants were thoroughly washed and then dried under shade at 28± 2^0C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried plant powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether (60^0C–80^0C), Chloroform (61^0C), Ethanol (78.5^0C) and Aqueous (80^0C) as solvents and the extracts were dried. The dried extracts were stored in a refrigerator at 4^0C. Finally, concentration of 5 mg per disc was loaded on each disc.

Preparation of Inoculum:

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient Agar Medium and were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh Nutrient Agar broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria.

Antimicrobial Susceptibility Test:

All the plant extracts were screened against five pathogenic bacterial strains. The tested organisms were *E.coli* (MTCC No. 729), *Salmonella typhi* (MTCC No.734), *Klebsiella pneumoniae* (MTCC No.109), *Enterobacter aerogenes* (MTCC No. 111) and *Agrobacterium tumefaciens* (MTCC No. 431), obtained from IMTECH, Chandigarh, India. The disc diffusion method (Bauer *et al*, 1966) was used to test the antimicrobial activity of the plant extracts. 20ml of sterilized nutrient agar medium for *E.coli*, *S.typhi, K.pneumoniae, E.aerogenes and A.tumefaciens* were poured into each sterile petridish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated

Journal of Research in Biology

with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) soaked in 0.1 ml of the plant extract (In case of fresh extract) are loaded with 5 mg/ disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37^0C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

RESULTS

The present study was aimed to screen both the fresh and dry plant part extracts of *Dahlia pinnata* against five pathogenic bacteria. **Table 1** illustrates antibacterial activity of fresh plant part extracts (Root, Stem, Leaves and Flowers) and **Table 2** illustrates antibacterial activity of dried plant part extracts (Leaves and Flowers). The plants extracts responded to bacteria as follows:

E.coli:

Fresh root extract was found to be effective against this bacterium. Fresh leaf extracts showed some antibacterial activity with maximum in chloroform extract. Dried leaves and flower extracts showed significant antimicrobial activity. Chloroform and petroleum ether extract of dried leaves exhibited inhibition zones of 7mm and 8mm respectively. Dried flower also showed inhibition zone of 5mm in aqueous extract and 6mm in alcoholic extract.

S.typhi:

No significant antibacterial activity was recorded in fresh plant part extracts against *S.typhi*. Fresh leaves inhibited the growth of bacteria to some extent in aqueous, alcoholic and petroleum ether extracts. Again in dried plant parts, there was no significant activity observed, except inhibition zone of 6mm in the chloroform extract of dried leaves.

K.pneumoniae:

Fresh plant part extracts were not able to inhibit the growth of tested bacteria. Maximum zone of inhibition was recorded in the petroleum ether extract of flower (5mm). Dried leaves extract exhibited antibacterial activity in alcoholic and petroleum ether solvents. Dried flower inhibited bacteria in chloroform and petroleum ether extracts.

E.aerogenes:

Fresh root and stem extracts exhibited moderate antibacterial activity against *E.aerogenes*, Similar results were recorded in fresh flower extracts. Dried leaves inhibited the growth of bacteria to significant extent as revealed by the inhibition zones of 6mm, 7mm, 11mm and 9mm in aqueous, alcoholic, chloroform and petroleum ether extract srespectively.

Table 1. Antibacterial activity of fresh plant part extract of *Dahlia pinnata*

Plant Part	Plant Extracts	Zone of Inhibition (mm)				
		E. coli	*Salmonella typhi*	*Klebsiella pneumoniae*	*Enterobacter aerogenes*	*Agrobacterium tumefaciens*
Root	Aqueous	3	-	-	-	-
	Alcoholic	2	-	-	2	-
	Chloroform	-	-	-	4	3
	Pet. ether	4	-	-	3	4
Stem	Aqueous	-	2	-	3	-
	Alcoholic	-	-	-	2	3
	Chloroform	4	-	-	3	-
	Pet. ether	6	4	3	5	3
Leaves	Aqueous	-	4	-	-	6
	Alcoholic	3	3	2	-	4
	Chloroform	**7**	-	-	2	2
	Pet. ether	4	6	4	3	**9**
Flower	Aqueous	2	-	2	-	3
	Alcoholic	3	-	3	2	5
	Chloroform	-	3	-	5	4
	Pet. ether	-	4	5	6	5

Journal of Research in Biology

Table 2. Antibacterial activity of dried plant part extract of *Dahlia pinnata*

Plant Part	Plant Extracts	Zone of Inhibition (mm)				
		E.coli	***Salmonella typhi***	***Klebsiella pneumoniae***	***Enterobacter aerogenes***	***Agrobacterium tumefaciens***
Leaves	Aqueous	-	-	-	6	-
	Alcoholic	-	2	4	7	-
	Chloroform	7	6	-	**11**	4
	Pet. ether	8	4	6	**9**	7
Flower	Aqueous	5	-	-	-	-
	Alcoholic	6	2	-	-	4
	Chloroform	5	4	3	-	5
	Pet. ether	4	4	5	4	7

***A.tumefaciens*:**

Fresh root extract was found to be effective against this bacterium. Fresh leaves extract exhibited significant antibacterial activity with inhibition zones of 6mm in aqueous extract and 9mm in petroleum ether extract. Similarly, fresh flower extract also proved to be toxic to *A.tumefaciens*. In case of dried plant parts, dried leaves showed inhibitory in chloroform and petroleum ether extracts. In dried stem, inhibition zones of 4mm, 5mm and 7mm were exhibited by alcoholic, chloroform and petroleum ether extracts respectively.

DISCUSSION

The quest for plants with medicinal properties continues to receive attention as scientists survey plants, particularly of ethnobotanical significance, for a complete range of biological activities, which range from antibiotic to antitumor. Thus far, plants have provided western medicine with an abundance of drugs and treatments for a variety of health problems (Lewis & Elvin-Lewis, 1977; Bruneton, 1999). Whitley (1985) studied the medicinal and nutritional properties of *Dahlia* species and reported antibiotic compounds concentrated in the skin of tubers. Rai and Acharya (1999) reported the antimycotic property of *Dahlia pinnata* against *Fusarium oxysporum*. Rai and Acharya (2000) investigated the fugitoxic potential in essential oils of *Dahlia pinnata*. In the present work antibacterial activity of *Dahlia pinnata* was also tested against some pathogenic bacteria. Fresh root extract inhibited the growth of *E.coli* and *E.aerogenes*. Fresh stem extracts were effective against *E.aerogenes* whereas fresh leaf extract was found to inhibit the growth of *A.tumefaciens*. In the same way fresh flower extracts also reduced the growth of *A.tumefaciens*. In case of dried plant parts, leaves extract was effective against *E.aerogenes*. Dried flower extracts exhibited antimicrobial activity against *E.coli* and *A.tumefaciens*. Plants belonging to Asteraceae family have been shown to posses high antibacterial as well as antifungal properties (Kasim *et al*, 2011, Wijaya *et al*, 2011 and Thorat *et al*, 2010).

CONCLUSION

The present study reveals that plant parts of *Dahlia pinnata* posses significant antibacterial activity and can be explored for novel antimicrobial agents. Disease control by herbal drugs do not have side effects on body, consequently great efforts have been exerted on the identification of herbal drugs to suppress various diseases. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

REFERENCES:

Bauer AW, Kirby WMM, Sherris JC and Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. The American Journal of Clinical Pathology 45(4):493-496.

Bruneton J. 1999. Pharmacognosy, Phytochemistry, Medicinal Plants. Second Edition. Lavoisier Publishing, France 1119.

Kasim S, Ferro VA, Odukoya OA, Drummond A, Ukpo GE, Seidel V, Gray AI and Waigh R. 2011. Antimicrobial agents frm leaf of *Struchium sparganophora* (Linn.) ktze, Asteraceae. Journal of Microbiology and Antimicrobials 3(1):13-17

Lewis WH and Elvin-Lewis MPF. 1977. Medical

Botany. Plants Affecting Man's Health. John Wiley & Sons, New York 515.

Rai MK and Acharya D. 1999. Screening of some Asteraceous plants for antimycotic activity. Compositae News letter 34:37-43.

Rai MK and Acharya D. 2000. Search for fungitoxic potential in essential oils of Asteraceous plants. Compositae News letter 35:18-23.

Thorat RM, Jadhav VM, Gaikwad DP and Jadhav SL. 2010. Phytochemical and pharmalogical potential of *Eclipta alba*: A Review. International Research Journal of Pharmacy 1(1):77 -80.

Whitley GR. 1985. The medicinal and nutritional properties of *Dahlia* spp. J. Ethnopharmacol, 14 (1):75-82.

Wijaya S, Nee TK, Jin KT, Hon LK, San LH and Wiart C. 2011. Antibacterial and antioxidant activity of *Synedrella nodiflora (L.)* Gaertn (Asteraceae). Journal of Complementary and Integrative medicine, 8(1):13.

Journal of Research in Biology

Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

Comparison of different parameters of invasive and non invasive sampling methods for microsatellite genotyping: a case study from Red Junglefowl and its application for other Galliformes

Authors:
Mukesh[1,2], Merwyn Fernandes[1], Mandhan RP[2] and Sathyakumar S[1].

Institution:
1. Wildlife Institute of India, P.O. Box 18, Chandrabani, Dehradun - 248 001, Uttarakhand, India.
2. Department of Biotechnology, Kurukshetra University, Kurukshetra- 136119, Haryana, India.

Corresponding author:
Sathyakumar S

Email:
ssk@wii.gov.in

Web Address:
http://jresearchbiology.com/Documents/RA0017.pdf.

ABSTRACT:

In studies dealing with genetic and disease prognosis, biological sampling is a prerequisite. Earlier, blood and muscle samples were taken after live captures. These invasive procedures are of considerable risk when sampling endangered species. Therefore, non invasive samples like shed feathers/hairs, faeces and hatched or predated egg shells are the alternative source for genetic study. We collected 18 Red Junglefowl feather samples (plucked feathers n= 6; shed feathers n=12) across its northwestern distribution range in India. The DNA yield was compared with other sample types *i.e*. blood, egg shell and faeces (n=6 for each sample type). We found a gradient in DNA yield as blood (770 µg/ml) > plucked feathers > shed feathers > egg shell = faeces (30 µg/ml). DNA extracts from feathers were amplified for four microsatellite loci. Three samples (17%) for all 4 microsatellite loci, four samples (22%) for 3 and 2 loci each, and seven samples (39%) for 1 locus were successfully genotyped. We found that the successful amplification of multilocus genotypes were dependant on the condition of the starting material and the type of sample. There are few studies that have compared the quality and quantity of DNA being produced through varying degree of invasiveness. None have considered effort (time) and the cost involved in procuring the samples from field and processing samples in lab. We scaled all the aspects of cost and efforts from 1 (low) to 10 (high) and here presenting a comparative analysis for the efficacy of invasive and non-invasive sampling methods.

Keywords:
Invasive and non-invasive sampling, feathers, genotyping and Red Jungle fowl.

Article Citation:
Mukesh, Merwyn Fernandes, Mandhan RP and Sathyakumar S.
Comparison of different parameters of invasive and non invasive sampling methods for microsatellite genotyping: a case study from Red Junglefowl and its application for other Galliformes.
Journal of research in Biology (2011) 1: 38-43

Dates:
Received: 06 May 2011 /**Accepted:** 11 May 2011 /**Published:** 18 May 2011

Journal of Research in Biology

INTRODUCTION

Red Junglefowl (RJF) [*Gallus gallus*] is a common pheasant species widely distributed in a variety of habitats within India (Ali and Ripley, 1983). They are ground dwelling and nesting birds preferring a mosaic of habitats with good undercover, where they prefer to breed during the summer months from March to June (Johnsgard, 1986).The RJF is widely distributed and its five subspecies are spread from the Indian subcontinent eastwards across Myanmar, South China, Indonesia to Java. In India, two sub-species occur, the type specimen are *Gallus gallus murghii* and *Gallus gallus spadiceus* (Ali and Ripley, 1983; Fernandes *et al.*, 2008 and 2009). While the former is found in the north and central part of India, extending eastwards to Orissa and West Bengal, the latter is confined to the north eastern parts of India. The species is present in 205 Districts in 21 range states in India. Of the 255 PAs that occur within the RJF distribution range in India, 190 PAs (31 NPs and 159 WSs) have reported presence in their area. There were concerns regarding issues of hybridisation of wild RJF with domestic chicken, leading to genetic endangerment of the species (Brisbin, 1995). In order to investigate these concerns, we have been collecting different sample types through various methods and extracting genomic DNA (g DNA). Less invasive methods such as plucked feather (Taberlet and Bouvet, 1991 and Fiona *et al.*, 2008) and non invasive methods which use faecal droppings and eggshells have increased over time (Segelbacher *et al.*, 2001 and Bush *et al.*, 2005). Though, there is constant limitation with the DNA yield with these sample types but are importance as being a vital source of genomic DNA. Earlier, Bush *et al.*, (2005) has investigated the quality and quantity of DNA being extracted through various degree of invasiveness in temperate region but there are no studies within the tropical system. Therefore, we had collected different sample types using various methods of invasive and non-invasive sampling techniques and compared them for some essential parameters *viz.* effort and cost involved in getting samples from field and processing them in lab and yield of DNA from each sample type.

MATERIALS AND METHODS

Sample collection and DNA extraction

We sampled six sites within the northwestern distribution range of RJF from February to May 2008 in the States of Jammu and Kashmir (n= 2), Himachal Pradesh (n= 1), Haryana (n= 1) and Uttarakhand (n= 2). Live trapping was attempted using leg-hold noose (n= 10) (Bub, 1991 and Ramesh *et al.*, 2008) in combination with fall net (n= 2). From the live trapped bird, blood was drawn from brachial vein (n= 6) and stored in DNA zol BD (Mackey *et al.,* 1996). A single feather was plucked (n= 6) and preserved in 70% ethanol (www.gallus.forestry.uga.edu). Shed feathers (n= 12) of RJF that were collected during field surveys were stored in plastic zip lock bags. Incidental collections of hatched eggshells (n= 2) and faecal droppings (n= 3) were made. A tissue sample that was collected from a predated RJF specimen outside the study area was used as a comparative reference sample during the analysis. The g DNA was extracted from individual feather using Qiagen DNeasy tissue kit (Qiagen, Germany) following manufacturer's protocol with the following alterations: (i) addition of 100 mg/ml DTT solution in the lysis buffer, (ii) digestion was performed overnight at 55^0C in a shaking water bath and addition of ice chilled ethanol for better precipitation, and (iii) DNA was finally recovered in 30-40 µl of elution buffer and stored at -20^0 C. DNA zol BD based extraction protocol was used to extract DNA from blood while for tissue and eggshells the Qiagen DNeasy tissue kit (Qiagen, Germany) was used following manufacturer's protocol. The DNA isolated from all the above sources were quantified using UV-Visible spectrophotometer (GeneQuant *Pro*, Amersham Biosciences) and compared across different sources.

Microsatellite Genotyping

Individual samples extracted from feathers were genotyped twice for increasing the efficiency of the results with four (CA) n dinucleotide microsatellite loci *viz.,* MCW-0295, MCW-034, LEI-111 and LEI 192 (Sharma., 2006). PCR was carried out in a 10 µl reaction volume in an Applied biosystem thermal cycler (2700 and 2720) and the reaction mixture consisted 1 X PCR Buffer (50 mM KCl, 10 mM Tris-HCl), 1.5 mM $MgCl_2$, 200 µM of each d-NTP, 1.25 µg BSA, 4 *p*-mole of each primer, 0.5 unit of Taq DNA polymerase, 50 to 80ng of gDNA. PCR profiles consisted of 2 min initial denaturation at 94^0C, followed by 35 cycles of denaturation at 94^0C for 45 sec, annealing at specific temperature (MCW 295- 55^0.0C, MCW-111-64.9^0C, LEI-192- 62^0.0C, MCW-034- 64.9^0C) for 45 sec and extension at 72^0 C for 2 min with a final extension for 10 min at 72^0 C. PCR products were resolved on 2% agarose gel containing

Journal of Research in Biology

ethidium bromide (4µl/100ml). To avoid cross contamination during pre and post PCRs, all pipetting were carried out using aerosol resistant filter pipette tips in separate rooms.

Parameters set for Analysis

For analysis, we compared across sampling methods with time (effort to obtain and to process a sample) and costs involved in field and lab. All the aspects of cost and effort were scaled from 1 (low) to 10 (high). For the effort in the field, the minimum and maximum time taken for a successful sample, *i.e.,* live-trapped (blood/ plucked feather), shed feather, faecal droppings and collection of hatched egg shells while operational costs that include personnel and field expenditure were summed and then scaled. This also includes collection of faeces when the trapped bird defecated and opportunistic collection of hatched egg shells. Travel costs to different field sites were not included in the analysis. We averaged effort and costs across sites for collection of field samples. Similarly laboratory efforts included processing of a sample for microsatellite genotyping (DNA extraction, gel electrophoresis, PCR amplification and genotyping) and the cost included personnel and the approximate expenditure for processing one sample for genotyping but not including infrastructure and equipment costs of the lab.

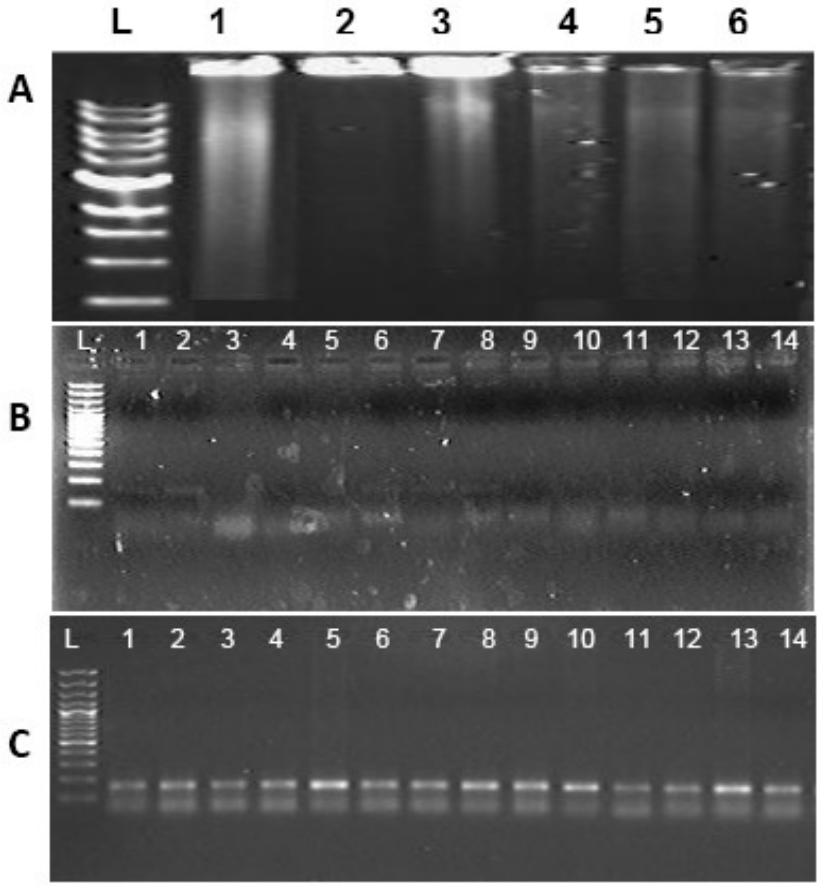


Figure 1 Gel Electrophoresis of extracted DNA and amplified PCR products

A - Gel Electrophoresis (0.8% agarose) of extracted DNA (L- 1Kb DNA ladder, Lane- 1 DNA from blood sample, Lane- 2, 3 DNA from tissue, Lane - 4 DNA from plucked feather, Lane-5 DNA from shed feather, Lane-6 DNA from egg shell membrane)

B - Gel Electrophoresis (2% agarose) of amplified PCR products from Feathers samples (I PCR) (L- 100 bp DNA ladder, Lane 1-10 Plucked feathers, Lane 11-14 Shed feathers)

C - Gel Electrophoresis (2% agarose) of amplified PCR products from Feathers samples (II PCR) (L- 100 bp DNA ladder, Lane 1-10 Plucked feathers, Lane 11-14 Shed feathers).

Journal of Research in Biology

Table 1 A comparison of genetic sampling methods with some essential parameters

Technique	Type of Sample	Field effort (min) [1]	Field cost (₹) [2]	Lab effort (min) [3]	Lab cost (₹) [4]	Average Score (1 to 4) (%)	DNA concentration (µg/ml) # [5]	Efficacy Rank
Invasive (Live capture)	Blood	1120 (5)	1633 (5)	3315 (1)	723 (1)	32.5	770	1
	Plucked Feathers	1120 (5)	1633 (5)	1035 (7)	763 (3)	52.5	67	2
Non-Invasive	Shed Feathers	240 (1)	700 (1)	1275 (9)	891 (7)	45	34	3
	Eggshell *	Nil	Nil	1515 (10)	1001 (10)	Nil	30	-
	Faeces*	Nil	Nil	1515 (10)	1001 (10)	Nil	30	-

Figures in parenthesis denote scores (1 = low and 10 = high), Denoted effort and costs are calculated for one sample.

*** Field effort and cost not considered as the collection of these samples types were incidental during the study and such samples are usually very difficult to obtain.**

 # the values presented in the Table is the mean of six samples of each sample type.

RESULTS AND DISCUSSION

All the sample types were extracted successfully for g DNA but there was a gradient in the DNA yield (mg/ml) (**Table 1**) *i.e.* blood (770 µg/ml) > plucked feathers (67 µg/ml) > shed feathers (34 µg/ml) > egg shell (30 µg/ml) = faeces (30 µg/ml). DNA concentration (µg/ml) values presented in table are the mean of six samples of each sample types to reduce the error during quantification through spectrophotometer. The extracted DNA was visualized on gel for quality assessment (**Figure 1- A**). For genotyping with four microsatellite loci, we followed the multiple tube approach and a second or third PCR for shed feathers in order to obtain visible gel bands that could be scored (Gagnex et al., 1997; Kohn et al., 1999 and Morin *et al.,* 2001) (**Figure 1 B & C**). In genotyping all DNA extracts of shed (n=12) and plucked (n=6) feathers, three samples (17%) for all 4 microsatellite loci, four samples (22%) for 3 and 2 loci each, and seven samples (39%) for 1 loci were successfully genotyped (**Table 2**). The remaining samples (n=11) yielded either no PCR product (n=6) or showed weak gel bands (n=5) and therefore were excluded from further analysis. Comparison of invasive and non invasive sampling methods with various sample types has been shown in Table 1 considering time and effort as parameters for field and lab.

Although all sample types yielded gDNA, there was a large gradation in the concentrations of DNA from invasive and non invasive methods, which are in accordance to many studies (Taberlet and Bouvet, 1991; Fiona *et al.,* 2008; Segelbacher and Steinbrück *et al.,*2001 and Bush *et al*., 2005) (Table 1 Column 5). In terms of quality and quantity of g DNA obtained, blood was considered to be better when compared to feather follicle and egg membrane (Fig 1A and Table 1). The lower yield of gDNA from shed feather, faeces and eggshell could be due to enhanced microbial activity causing decay since sampling was undertaken during post winter and summer seasons. We found that samples with low DNA quality have

Table 2 Amplification success of Microsatellite loci from Feathers (Shed *vs.* Plucked feathers)

No. of loci amplified	No. of samples (%)	Type of Feather Samples	
		Shed feathers	Plucked feathers
1	7 (39)	4	3
2	4 (22)	3	1
3	4 (22)	3	1
4	3 (17)	2	1
Total	18	12	6

a higher probability of amplification failure and need a multiple tube approach to get scorable bands on gel. Various methods address these problems, including the 'multitubes approach' (Navidi *et al*. 1992 and Taberlet *et al*. 1996). In fact Jason *et al.* (2005) stated the locus size effects on amplification success, allelic dropout and error rates in non-invasive genotyping studies while considerable amplification success was also achieved by few researchers using non-invasive samples (Kohn *et al*., 1995; Gerloff *et al*., 1995 and Vidya and Sukumar, 2005). Comparison of different sampling methods in the field suggests that samples obtained through non-invasive methods were much efficient than invasive methods (Table 1, Columns 1 & 2). Though the field efforts were primarily focussed on gathering information on the ecology of the species through observations, incidental finds of faecal droppings, eggshells and a predated RJF were collected to supplement information and subsequently used for extracting DNA. As sampling was undertaken during the breeding season, it could have facilitated the availability of shed feathers and egg shells. The field identification of the shed feathers for RJF was possible because of its distinguishable features (Morejohn, 1968). However, for field identification of shed feathers for other galliformes species, advanced techniques (Sivakumar *et al.,* 2007) would be required for confirmation. Similarly, hatched eggshells were collected from where breeding observations were made. Though the cost and effort in field were higher in case of invasive methods, it yielded better results with low cost and effort in the lab. Whereas in case of non invasive methods, the cost and effort were lower in field and higher in lab for shed feather samples due to consequent increase in the cost and effort for processing samples to obtaining g DNA.

CONCLUSION

Based on this study, we may conclude that there is a certain trade-off in deciding which sampling method is the most appropriate for galliformes. Invasive sampling method is suitable for the common or abundant species. The efforts in field may increase manifold for species that are shy, elusive, inhabit dense habitats, and have limited distributional range or low in abundance. Hence, in order to sample these species, efforts could be made to obtain shed feathers though cost and effort increases in the lab and quantity of DNA may be low.

ACKNOWLEDGEMENTS:

We would like to thank the Director, Dean and Research Coordinator, WII, Dehradun and the Forest/Wildlife Departments of the study area. The study was funded by Wildlife Institute of India, Dehradun.

REFERENCES:

Ali S and Ripley SD. 1983. *Handbook of the Birds of India and Pakistan*. Oxford University Press, Delhi.

Brisbin IL Jr. 1995. Conservation of the wild ancestors of domestic animals. Conserv. Biol., 9:1327.

Bub H. 1991. *Bird trapping and bird banding*. Cornell University Press, New York.

Bush KL, Vinsky MD, Aldridge C L, Paszkowski, CA. 2005. Comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (*Centrocercus uropihasianus*), *Conservation Genetics* 6:867.

Fernandes M, Mukesh S, Sathyakumar, Rahul Kaul, Rajiv S Kalsi and Deepak Sharma, 2009. Conservation of Red Junglefowl *Gallus gallus* in India. *International Journal for Galliformes Conservation* 1:94.

Fernandes M, Mukesh, Sathyakumar S, Kaul R and Kalsi, R S. 2008. Conservation of Red Junglefowl *Gallus gallus* in India. Interim Report – Phase I. Wildlife Institute of India, Dehradun, 49.

Fiona E, Hogan, Raylene Cooke, Christopher P. Burridge and Janette A. Norman. 2008. Optimizing the use of shed feathers for genetic analysis, Mol. Ecol. Resources 8:561.

Gagnex P, Boesch C, Woodruff D. 1997. Microsatellite scoring errors associated with non-invasive genotyping based on nuclear DNA amplified from shed hair. *Mol Ecol,* 6:861.

Gerloff U, Schlötterer C, Rassmann K, Rambold I, Hohmann G, Fruth B and Tautz D. 1995. Amplification of hypervariable simple sequence

repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Mol. Ecol.*, 4:515-518.

Jason CB, Elizabeth AA, Russell CVH, Cynthia JM and Susan CA. 2005. Locus effects and sources of error in noninvasive genotyping, *Mol. Ecol.Notes* 5:680-683.

Johnsgard PA. 1986. *The Pheasants of the World*. Oxford University Press, New York. 300.

Kohn M, Knauer F, Stoffella A, der Schröder W and Paabo S. 1995. Conservation genetics of the European brown bear: A study using excremental PCR of nuclear and mitochondrial sequences. *Mol. Ecol.*, 4:95-103.

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM, Wayne RK. 1999. Estimating population size by genotyping faeces. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 266, 657.

Mackey K, Williams P, Seim S, Chomczynski P. 1996. The use of DNAzol® for the rapid isolation of genomic DNA from whole blood. *Biomedical Products Supplement* 13.

Morejohn VG. 1968. Study of Plumage of the four species of the genus *Gallus*. The Condor 70:56.

Morin PA, Chambers KE, Boesch C, Vigilant L. 2001. Quantitative Polymerase chain reaction of DNA from non-invasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol Ecol*, 10:1835.

Navidi W, Arnheim N, Waterman MS. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *Am J Hum Genet*., 50:347–359.

Ramesh K, Sathyakumar S, Rawat GS. 2008. Methods of capture and radio tracking of western tragopan *Tragopan melanocephalus* J.E. Gray 1829 in the Great Himalayan National Park, India, *J Bombay Nat Hist Soc*., 105, 2, 127.

Segelbacher G, Steinbrück G. 2001. Bird faeces for sex identification and microsatellite analysis. *Vogelwarte* 41, 139.

Sharma D. 2006. *Comparative genomic studies on wild and domesticated avian species*. Annual Progress Report, Submitted to the Department of Biotechnology, New Delhi.

Sivakumar K, Goyal SP, Mukherjee SK. 2007. Identification of Galliformes using the Microstructure of Feathers: Preliminary Findings. (IN) Sathyakumar, S., Sivakumar, S., (Eds.). *Galliformes of India. ENVIS Bulletin: Wildlife and Protected Areas*, 10 (1), Wildlife Institute of India, Dehradun, India. 197.

Taberlet P, Bouvet J. 1991. A single plucked feather as a source of DNA for bird genetic studies. Auk, 108, 959.

Taberlet P, Griffin S, Goossens B. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res*, 24:3189-3194.

Vidya TNC and Sukumar R. 2005. Amplification success and feasibility of using microsatellite loci amplified from dung to population genetic studies of the Asian elephant (*Elephas maximus*), *Curr Sci,* 88(3):489-492

Website visited:
http://gallus.forestry.uga.edu/genetics/protocols/pdf/feather.pdf, Accessed on 5th March, 09.

Journal of Research in Biology

Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

Allelopathic effect of aqueous leaf extract of *Parthenium hysterophorus* on germination and seedling growth of the *Gossypium hirsutum*

Authors:
*Gangasuresh P[1],
Ajithal Begam A[4],
Saranya A[4],
Senthil kumar P[2],
Rajkumarbharathi M[3].

Institution:
*Head, [1]P.G. Department of Microbiology, Sri Ram Nallamani Yadava College of Arts & Sciences, Kodikurichi, Tenkasi – 627 811.
[2]P.G.Department of Microbiology, Karpagam University, Coimbatore.
[3]Sri Paramakalyani Centre for Environmental Studies, Manonmaniam Sundaranar University, Alwarkurichi – 627 412.
[4]P. G. Department of Microbiology, JJ College of Arts & Sciences, Pudhukottai.

ABSTRACT:

Weeds are sometimes considered as unwanted plants. Some weeds reduce human efficiency through physical discomfort caused by allergies and poisoning. Weeds such as parthenium (*Parthenium hysterophorus*) and rag weed (Limbrosia species) causes etching, hay fever and other debilitating allergies, which contribute markedly to chronic human illness and suffering. Cotton seeds were treated with different concentrations of leaf exudates of *parthenium hysterophorus* and the biochemical constituents of treated cotton plants were analysed. The protein and phenol were found to increase in treated plants due to the release of protease inhibitors. In addition to this, increase in the activity of Nitrate reductase and peroxidase were observed followed by dthe ecreased activity of catalase. The plants with higher concentration of phenol showed resistance to pest and they were healthy compound to that of controlled plants. The treated plants also showed some morphological variations. Increase in height was noted in 80% treated plants and there were no growth and decrease in 100% treated. This inhibitory effect was due to high concentration of first noted in 80% treated plants. Thus 80% treatment was found to have optimal activity.

Corresponding author:
Gangasuresh P

Email:
gng.suresh@gmail.com.

Web Address:
http://jresearchbiology.com/Documents/RA0013.pdf.

Article Citation:
Gangasuresh P, Ajithal Begam A, Saranya A, Senthil kumar P, Rajkumarbharathi M.
Allelopathic effect of aqueous leaf extract of "*Parthenium hysterophorus*" on germination and seedling growth of the "*Gossypium hirsutum*".
Journal of research in Biology (2011) 1: 56-61.

Dates:
Received: 01 May 2011 /**Accepted:** 04 May 2011 /**Published:** 19 May 2011

Journal of Research in Biology

Journal of Research in Biology

INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another, both crop and weed species, release chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. It is the science that studies any process involving secondary metabolites produced by plants, algae, bacteria, coral and fungi that influences the growth and development of agricultural and biological systems. (IAS, 1966). The biomolecules are called allelochemicals and are produced by some plants as secondary metabolites.

When the allelochemicals are released into the environment, they inhibit the development of neighbouring plants. Allelopathic plants release compounds into the environment through root exudation, leaching by dews and rains, and volatilization or decaying plant tissue (Rice, 1984). In most cases, the compounds inhibit, germination or growth of neighboring plants although sometimes the compounds stimulate their growth.

Allelopathy in crops may act as a biological weed control in the agroecosystem. The genetic improvement of the allelopathic effect in crops is a strategy for biological weed control in breeding programs. In the 1970s, germ plasm assessment was extensively undertaken to detect allelopathic accessions of crops. Accessions with an allelopathic effect have been found in crops such as beet (*Beta vulgaris* L.), lupine (*Lupinus lutens* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), pea (*Pisum sativum* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and cucumber (*Cucumis sativus* L.) reviewed by Rice (1984). A total of 538 accessions of cultivated and wild cucumber were screened by the pot and field test, several accessions inhibited the growth of weeds (Putnam and Duke, 1974). Out of more than 3000 assessions of oat, several were found with a fluorescent microscope to exude a large amount of an allelochemical, scopoletin (Fay and Duke, 1977). One of the most studied aspects of allelopathy is the role of allelopathy in agriculture. Current research is focused on the effects of weeds on crops, crops on weeds, and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture. Weeds are always considered as unwanted plants (Oudhia, 1998). A number of such allelochemicals are commercially available or in the process of large-scale manufacture. For example, Leptospermone is a purported allelochemical in lemon bottlebrush (*Callistemon citrinus*). Although it was found to be too weak as a commercial herbicide, a chemical analog of it, mesotrione (tradename Callisto), was found to be effective.

The present study aims to investigate the seed germination and growth of cotton seeds which were treated with different concentrations of the leaf exudates of *Parthenium hysterophorus* at 20%, 40%, 60%,80%, and 100%. This investigation is proceeded for the future prospect of allelopathy. This may develop the legume crops with high biological N2 functionpotential and least inhibitory effects on components crops in intercropping systems and on succeeding crops in crop relations.
Green herbicides containing Green Allelochemicals are an integral part of eco or organic farming . Use of natural compounds as herbicides or as the chemical basis for the development of new herbicides offers several advantages.

1. The wide array of phytotoxic compounds produced by plants provide many complex chemical structure that are unlikely to be discovered in the traditional synthetic strategies used by pesticides companies.
2. Degradation of natural compounds in the environment proceeds faster than that of synthetic compounds and thus reduces the environment pollution and ground water contamination etc.,
3. The halogenated hydrocarbon which constitute about 60% of the registered herbicides are of environmental concern, while vast majority of natural compounds from plant pose little hazards and therefore, are environmentally safe. The negative (stimulatory) allelopathic effects of weeds on agricultural crops can be used to develop “Green growth promoters”. Many studies conducted at department of Agronomy, Indira Gandhi Agricultural University, Raipur, India) have clearly revealeld that stimulatory allelopathic effects weeds on crops can be utilizied successfully for higher crop production.

Weeds reduces human efficiency through physical comfort caused by allergies and poisoning. Weeds such as Parthenium and rag weed (Ambrosia species) that cause etching, hay fever, and other debilitating allergies contribute markedly to chronic human illness and suffering. So objective of the present study is to investigate the effect of aqueous leaf extract of parthenium hysterophorous on

germination, seedling growth and development of cotton plant (*Gossypium hirsutum*).

MATERIALS AND METHODS

Materials:

***Gossypium hirsutum* (Cotton)**

Viable seeds of cotton were selected . The seeds were surface sterilized with 0.1% Mercuric Chloride for 2 minutes. It was then washed with tap water thrice and distilled water twice.

Parthenium hysterophorus

Pot Mixtures

Red soil, Black soil and dry manure in the ratio 1:2:1.

Aqueous leaf extract of *Parthenium hysterophorus*.

Methodology:

The sterilized seeds were sown in respective pots. The plant samples were taken at 6 ranges from all the treatments corresponding to germination stage. Control was maintained without adding the leaf extract of *parthenium hysterophorus*. Five Treatments were made in remaining five pots. The leaf extract was treated in the seed of *Gossypium hirsutum* in the concentration of 20%, 40%, 60%, 80% and 100%(Picture 2-5). The concentration was made by diluting with water.

Percentage of Germination

Healthy seeds were sown in land and treated with composted mycostraw. The seeds were considered to be germinated only when the radicle emergence was more than 1.0 cm. This was considered as the first day of germination percentage. The germination percentage was calculated using the formula:

$$\text{Germinating percentage} = \frac{\text{Total Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100.$$

The seed germination of Gossypium hirsutum was determined (Picture-1) in the concentration of (20%, 40%, 60%, 80% and 100%).

Parameters Analysis:

Assay of Nitrate Reductase:

5ml of 0.1 M Phosphate buffer (pH 7.5) to the freshly cut leaves, followed by the addition of 1ml of Potassium nitrite and 4 ml of Propanal and incubated in dark for 30 minutes. The constituents omitting plant tissues were kept as control. The 1 ml of sample from test and control solution was taken with different aliquots of potassium nitrite into a series of test tubes. The solution was made upto 1 ml with water. Then 1 ml of 1 % Sulphanilamide and 1 ml of NEDA was added to all the test tubes. After 10 minutes reading was noted at the absorbance of 540 nm.

Assay of Catalase:

100 ml of phosphate buffer from the conical flask was pipetted out and 0.4 ml of substrate into each flask were added. To one of the flask 0.5 ml of the enzyme extract was added and incubated for 15 minutes at room temperature. After 15 minutes 10 ml of 2N H_2SO_4 was added to both control and test flasks. Then the contents against 0.01N $Kmno_4$ one by one was titrated. Difference between these values give the volume of permanganate equivalent to enzymatic activity.

Assay of Peroxidase:

One minute fixed time assay is used to measure peroxidase activity. Two cuvettes were taken for blank and sample. 2.5 ml of aminoanmtipyrine-phenol solution and 2.5 ml of H_2O_2 was added and absorbance was readed. Then 0.1 ml of enzyme extract in the other cuvette was taken for the absorbance. The time was noted exactly for one minute to read the absorbance of the same cuvette.

The activity of the peroxidase can be calculated from the absorbance change.

$$\text{nit / mg} = \frac{\Delta A \text{ / min}}{6.58 \times \text{mg of the sample.}}$$

$$\Delta A = A_{1\text{ minute}} - A_{0\text{ minute}}$$

Where, A = Over all absorbance change

$A_{1\text{ minute}}$ = Absorbance at 510 nm after 1 minutes

$A_{0\text{ minute}}$ = Absorbane at 510 nm after 0 time.

Estimation of Chlorophyll:

1 gram of finely cut and well mixed representative sample of leaf was weighed and grinded to a fine pulp with the addition of 20 ml of 80 % acetone. It was centrifuged and the supernatant was transferred to 100 ml volumetric flask. The residue was grinded with 20 ml of 80 % acetone. Again centrifuged and the supernatant was transferred to the same volumetric flask. This procedure was repeated until this residue becomes colourless. The absorbance was recorded at 645 nm against the solvent (80% acetone) blank.

$$\text{mg chlorophyll a / g tissue} = 12.7\,(A663) - (A645) \times \frac{V}{1000 \times w}$$

$$\text{mg chlorophyll b / g tissue} = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times w}$$

Journal of Research in Biology

$$\text{mg chlorophyll / g tissue} = 20.2\,(A\,645) + 8.02\,(A663) \times \frac{V}{1000 \times w}$$

Where,
A = Absorbance of specific wavelength
V = Final volume of chlorophyll extract on 80 % acetone.

Table-1. Percentage of Seed Germination

Treatment	Percentage of Germination (%)
Control	60
20%	70
40%	80
60%	60
80%	90

Estimation of Protein:

The different aliquots of working standard and sample extract was pipetted out to make upto 4 ml with distilled water. A tube with 4 ml distilled water serve as blank. 5.5 ml of alkaline copper solution was added in all the test tubes and mixed well. It was incubated at room temperature in the dark for 30 minutes. The blue color was developed. Then the color development was readed colorimetrically at 660 nm.

Estimation of Phenol

The different aliquots were pipetted out in the series of test tubes. The volume was made up to 3 ml with distilled water. Then 0.5 ml of Folin – Ciocalteau reagent was added. After 3 minutes 20 % Sodium Carbonate solution was added and mixed thoroughly and kept in the boiling water for one minute. The absorbance was noted at 650 nm.

RESULT

The present study was made to investigate the effect of aqueous leaf extract of *Parthenium hysterophorus* on the biochemical characteristics of Cotton (*Gossypium hirsutum*). The plumules arising from seeds during germination and the leaves during 15th,30th and 45th days taken for the analysis of various parameters (Picture 2-5).

A notable increase in activity was observed in 80% treated plants on all days especially in 30th day. The increase in nitrate in nitrate reductase activity may be due to the nitrate or nitrite released from the root nodules of plants.

The catalase activity was found to be decrease in the treated plants compared to that of control. The catalase activity was decreased on 80% treated plants during the germination and slightly in 20% and 60% treated plants. The activity decreased from 3.5 to 7.1 in 80% treated plants in control. The activity decreased with increased concentration of aqueous extract with some fluctuations.

Table- 2. Effect of Aqueous leaf extract of Parthenium Hysterophorus on Nitrate Reductase (ug of Nitrate formed / 30 minutes)

Treatment	Seed Germination	15th day	30th day	45th day
Control	87.5	92.0	102.5	69.5
20%	89.4	95.0	105.6	104.5
40%	89.5	93.0	106.0	103.5
60%	86.9	93.0	104.5	102.5
80%	98.8	99.5	107.5	105.5

Table – 3. Effect of Aqueous leaf extract of *Parthenium Hysterophorus* on the Catalase Activity (ml of 0.01 N Kmno 4 Consumed / mt / ml / of enzyme).

Treatment	Seed Germination	15th day	30th day	45th day
Control	3.5	6.3	7.2	6.8
20%	5.2	8.5	9.0	9.2
40%	5.5	8.3	9.1	9.0
60%	5.4	8.8	9.5	9.4
80%	7.1	10.2	12.8	13.5

Table -4. Effect of Aqueous leaf extract of *Parthenium Hysterophorus* on Peroxidase activity (units / mg)

Treatment	Seed Germination	15th day	30th day	45th day
Control	0.15	3.5	5.6	2.5
20%	0.30	3.9	7.0	4.1
40%	0.75	4.2	6.8	3.4
60%	0.60	3.7	7.2	3.3
80%	0.91	4.5	8.1	4.5

Journal of Research in Biology

Table – 5. Effect of Aqueous leaf extract of Parthenium Hysterophorus on Chlorophyll

Treatment	Chlorophyll	Seed Germination	15th day	30th day	45th day
Control	a	0.0002	0.0009	0.0004	0.0004
	b	0.0004	0.0007	0.0007	0.0003
	Total	0.0006	0.0016	0.0011	0.0007
20%	a	0.0003	0.0008	0.0005	0.0004
	b	0.0005	0.0007	0.0001	0.0003
	Total	0.0018	0.0015	0.0006	0.0007
40%	a	0.0003	0.0005	0.0005	0.0004
	b	0.0003	0.0003	0.0001	0.0002
	Total	0.0006	0.0008	0.0006	0.0006
60%	a	0.0005	0.0005	0.0003	0.0004
	b	0.0002	0.0003	0.0005	0.0001
	Total	0.0007	0.0008	0.0008	0.0005
80%	a	0.0002	0.0004	0.0002	0.0003
	b	0.0003	0.0005	0.0004	0.0001
	Total	0.0005	0.0009	0.0006	0.0004

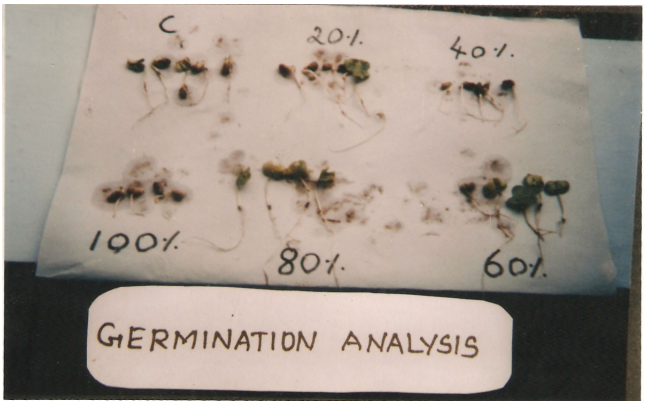
The peroxidase activity was increased in all the treated plants. Votable increase was found in 80% during 30th day analysis. The activity increased from 0.15 to 0.91 in 80% treated plants during seed germination.

The Chlorophyll concentration was increased from 20% - 80% treated plants compared to that of control during the germination and 15th day analysis. Chlorophyll concentration was decreased from 0.0011 in control and finally to 0.0006 in treated plants, and 0.0007 in control and finally to 0.0004 in treated plants in during 30th day and 45th day.

SUMMARY AND CONCLUSION:

In the present study cotton seeds were treated with different concentrations of leaf exudates of *Parthenium hysterophorus* and the biochemical constituents of the obtained cotton plants were analysed. The present study was conducted to investigate the allelopathic effects of *Parthenium hysterophorus* weed on seed germination and seedling growth of *Gossypium hirsutum*. The aqueous leaf extracts of *Parthenium* at 20%, 40%, 60%, 80%, and 100% of concentrations were applied to determine their effect on the seed germination and seedling growth under laboratory conditions. The protein, phenol were found to be increased in the treated plants due to the release of protease inhibitors and other non determined allelochemicals from the root exudates. In addition to this, increased activity of Nitrate reductase activity and peroxidase were observed followed by decreased activity of Catalase.

Inspite of this, the plants with higher concentration of phenol showed resistance to pest and they were healthy compared to that of control



Picture – 1. SEED GERMINATION AT DIFFERENT CONCENTRATION

2 plant growth at 15th day at different concentration

plant. The treated plants also showed some morphological variations. Increase in height noted in 80 % treated plants, and there is no growth and decrease in 100% treated. The inhibitory effect was due to high concentration of first noted in 80% treated plants. Thus 80% treatment was found to have optimal activity. Thus it can be concluded that efficient elimination of some toxic compound, the application of leaf extract of *Parthenium hysterophorus* can be used for the growth and yield of the cotton plants, though the many of works implies. Leaf extracts at the high concentration (80%) greatly promoted root length.

REFERENCES:

Fay PK and Duke WB. 1977. An assessment of allelopathic potential in *Avena* germplasm. Weed Sci. 25:224–22.

International Allelopathy Society (IAS). 1996.

Oudhia P. 1998. Parthenium hysterophorus: A curse for the biological diversity of Chattisgarh plains. Abstract. National Research Seminar on Biochemical changes.

Putnam AR and Duke WB. 1974. Biological suppression of weeds: Evidence for allelopathy in accessions of cucumber. Science 185:370-372.

Rice EL. 1984. Allelopathy. 2nd ed. Academic Press, London.