ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro Experimental Studies of Some Selected Weed Species for Their Antibacterial Activity.

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ABSTRACT

A weed is commonly defined as a plant that grow out of place and is competitive, persistent and pernicious (James, *et al.,* 1991). Weeds have been a part of civilization and many ancient documents speak of humans battling weeds in the crops they grow. Weeds are also found to be resistant to most of the microbial disease when compared to the crops which shows disease symptoms. The resistance nature and their sustenance towards the microbial disease made us to have an interest to know the potency behind (Anonymous, 1948-76, Chopra, R.N. S.L. Nayar & I.C. Chopra 1956). But they serve as an important elements in any type of ecosystem/s like forest ecosystem, aquatic ecosystems, grass lands and desert ecosystems etc. as sand binders (*Salichornia* sp.), fodder *(Cynodon dactylon, Chloris gayana)* to the cattle and other domestic animals. They can also serve as NWFPs (Non-wood Forest Produce/s) in utilization for roofing, cordage and other domestic purposes by tribals in various parts of Andhra Pradesh, India (Rama Rao, N 1988, Bharath Kumar., R.2000). It is with this background, the present study it has been taken up to detect the preliminary screening of antimicrobial activity of various weed species. All the data is recorded in this paper. The results are mostly conformity of the medicinal uses and they are discussed in detail in this article belongs to the first hand information and sparse information is available related to its other uses as per literature sources.

Keywords: Antibacterial activity, weed species, medicinal & economic uses.

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ISSN: 0975-8585

INTRODUCTION

Vignan's University (VU) (formerly Vignan's Engineering College is a premier institution affiliated to Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh). It is having the splendid avenue, imposing buildings and sprawling playgrounds, and the verdure in and around the campus. The college is a virtual haven of rural quiet and idyllic beauty. Since its inception in 1997, VU has been striving to promote high quality standards in technical education & research for the aspirants of Engineering Studies [18].

TOPOGRAPHY

Vignan's University is located in the serene environs of Vadlamudi on the Guntur- Tenali highway, about 14 km from Guntur and 11 km from Tenali. The nearest railway station Tenali is located on Chennai – Kolkata trunk line.

Brief enumeration and description of habit & habitat of selected weed species for their antibacterial activity:

Scientific Name: ***Axonopus fissifolius*** (Raddi) Kuhlm. (POACEAE)
Ln.: Carpet grass, Mat grass

DISTRIBUTION & USES

It is a rhizomatous, stoloniferous perennial pasture grass. It forms dense mats that are 15-30 cm high but the flowering culms may reach as 60-75 cm. Carpet grass is a summer-growing perennial believed to have originated from the Southern USA, the West Indies or Central America It is now found in many tropical and subtropical regions of America, Africa, Asia and the Pacific Islands[1,10]. It is often referred to as a good cover crop and soil erosion controller for shaded sloping fields. It is much valued in shaded orchards of Hawaii. It improves soil structure, and increases water infiltration rates and soil water capacity. It is useful to get relief from cold, infections, skin diseases and having the antimicrobial & antioxidant properties[8,10,12].

Scientific Name: ***Cynodon dactylon*** (L.)Pers. (POACEAE)
Ln.: Bermuda grass, Durva grass

ISSN: 0975-8585

A fine to robust stoloniferous perennial, mostly with rhizomes. Rhizomes can penetrate 40-50 cm in clay soil and 70-80 cm in sand. Foliage dense, 10-40 cm tall (rarely to 90 cm); leaf blades glabrous or sparsely pubescent, often glaucous, with minutely scabrous margins[6,10]. The stems are slightly flattened, often tinged purple in colour.It is originated in Turkey and Pakistan, but has been introduced to all tropical (India) and subtropical, and some temperate regions of the world[1,6]. It is used in permanent pastures for grazing or cut-and-carry, and for hay or pellets and silage production. It is beneficial to wounds, piles, eczema, urticaria, injuries, eye problems, skin rashes, constipation, indigestion, constipation, mental debility, diabetes, epilepsy, vaginal problems, menstrual problems, gynecological problems[1,15].

Scientific Name **: *Gomphrena serrata*** L. (AMARANTHACEAE)
= Gomphrena decumbens Jacq.

Ln.: Globe amaranthus, Tella pogada banthi

Decumbent herbs, stems hispid. Leaves opposite, 4 x 1 cm, obovate, obtuse, tomentose. Spikes 2 x 1 cm, oblong, supported by 2 basal bracts. Flowers many, densely packed; sepals 5, outer ones lanceolate, 7 mm long, strongly aristate, white-cottony hairy. Degraded deciduous forests and scrub jungles[1,6]. It is native to South Brazil, Mexico, Central America and the West Indies and is naturalized in the Southeast of the US and in India.

The leaf extracts of *G.serrata* having natural blood coagulatory properties. The whole plant plant extracts are used to treat for cardiovascular problems and diabetes. It is also useful for extraction of natural colorants and antioxidants[8,14,15].

Scientific Name: ***Chloris gayana*** Kunth. (POACEAE)
Ln.: Rhodes grass

This is a perennial grass which can reach one half to nearly three meters in height and spreads via stolons. This species is naturalized in all mainland states and territories, and also on Lord Howe Island and

Norfolk Island [10, 11]. It is most common and widespread in eastern Australia. Naturalized beyond its native range in Africa and Asia in other parts of the world [7, 14]

Rhodes grass may have the significant properties of drought tolerance and salt tolerance, it is widely grown in drought prone regions. It can be very helpful to farmers and NGOs in terms of sustainable agricultural development. It is an effective supplement feed for goats and cattle [13, 14, 17].

MATERIALS AND METHODS

Vignan's University has campus with a good number of plants. It includes landscaping gardens, exotic elements and natural forest elements, includes rare and endemic categories of trees, shrubs, herbaceous members, climbers and a good number weed species plants like *Axonopus fissifolius* (Capet grass)*,Cynodon dactylon*(Bermuda grass), *Chloris gayana*(Rhodes grass) *and Gomphrena serrata*(Globe amarathus). An inventory experimental study were conducted on selected most promising plant species which are having utilization of domestic, commercial samples. Methodology was adopted for the above mentioned studies are as per standard literature sources [2-4].

The present work was conducted in Department of Biotechnology, Microbiology lab Vignan's University, Vadlamudi to determine the antibacterial activity of plant samples (extracts) of *Axonopus fissifolius* (Capet grass)*,Cynodon dactylon*(Bermuda grass), *Chloris gayana*(Rhodes grass) *and Gomphrena serrata*(Globe amarathus) against two selected bacterial strains viz., *Bacillus cereus* & *E.coli* (bacterial species) for antibacterial activity of plant extracts[3,5,13].

Preparation of plant extract samples:

The selected weed species are collected from both in university campus and Vadlamudi environs of Guntur. They are washed with tap water and chopped into small pieces (1-2 cm in size) shade dried in separate vessels for 1&2 days. They were made in to fine powder by using pulverization [5,17]. These powdered samples are subjected to successive solvent extraction. The extraction was performed using the following solvents acetone, methanol, ethanol & hot water respectively in the first stage of project i.e. just for preparation of samples. In further stages we used combinations of these solvents[2,9].

The Combinations of solvents were as follows:

Single Combination	Dual Combinations
Ethanol	Methanol+ n-hexane
H.Water	Methanol+ Acetone
Methanol	Methanol+ Ethanol
	Methanol + H.Water

Preparation of Media and Screening of Antimicrobial Activity:

Antimicrobial screening was done by the standard procedures. The suitable culture media was prepared by dissolving the below mentioned ingredients for the respective microorganisms. The contents were autoclaved at 15 lbs for 15 min. Microorganisms taken are *Bacillus cereus* & *E.coli* (bacterial species)[3,7].

Preparation of Sterile Paper Discs:

Using an ordinary office two-hole puncher, paper disks with approximate diameter of 6.3 mm. were punched out one by one from a sheet of blotting paper, the disks were placed in boiling test tubes then autoclaved for 15 minutes at 15 lbs. pressure and allowed to cool.

Medium for Bacterial Species:

Nutrient Broth/Nutrient Agar Medium (NBM/NAM) composition:

ISSN: 0975-8585

Peptone-5gm
Beef extract-3gm
Agar-5 gm
Distilled water-1000 ml
P^H – 7

Microorganisms-Bacteria:

Gram +ve: *Bacillus*:

Bacillus cereus, is an endemic, soil-dwelling, Gram-positive, rod-shaped, motile, beta hemolytic bacterium. Some strains are harmful to humans and cause foodborne illness. It may contaminate food but rarely causes food poisoning.

Gram – ve: *Escherichia coli*:

Escherichia coli, occurs in the lower portion of intestine of humans and warm blooded animals' causes' gastroenteritis, urinary tract infection. It is a Gram-negative bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms).

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination.

The above mentioned microbial cultures are maintained on their respective media in slants at 4 ^{0}C. (as per standard procedures).

Preparation of Test Plates for Antimicrobial Screening Tests:

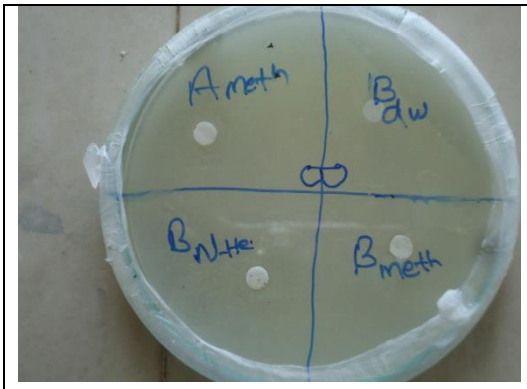
The Nutrient Agar (NA) test plates (Petri dishes) were prepared by pouring about 15 ml of the medium. These test plates were placed under aseptic conditions at 4 °C for 24 hours to control sterility. After solidifying the media (NA). The inoculums (bacteria 24 hrs) Stock cultures were uniformly spread on their respective test plates[2,7]. The filter paper discs were prepared in ethanol, methanol (M) and acetone (A) extracts as taken for control. The filter paper discs are carefully placed the spreaded culture test plates are incubated at appropriate temperature for bacteria at 37 °C for 24 hrs. After the incubation period the test plates are examined for inhibitory zones are recorded in **Test Plates** (Antibacterial Inhibition zone response of *Axonopus fissifolius,Cynodon dactylon,Chloris gayana* &*Gomphrena serrata plant sps.with their Methonal, ht.w & Methonal +n-Hexane extracts subjected with B. cereus & E. coli organisms*). All determinants were made at least in triplicate for each of the test organisms in different extracts was also recorded[18,19].

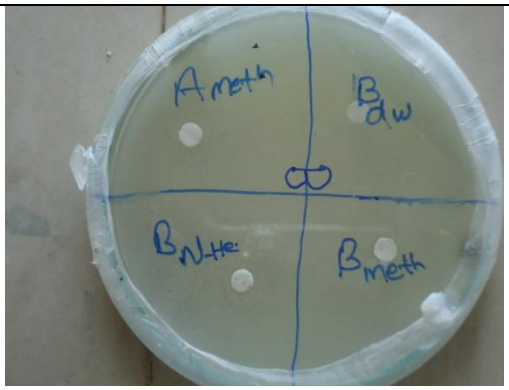
Test Plates:

Axonopus fissifolius (Methonal, Ht.w & Methonal +n-Hexane extracts) *B.cereus*

Cynodon dactylon (Methonal, Ht.w & Methonal +n-Hexane extracts) *B.cereus*

ISSN: 0975-8585





Chloris gayana, Gomphrena serrata (*Methonal, Ht. w, n-Hexane extracts*) *B.cereus*

Chloris gayana, Gomphrena serrata (*Methonal, Ht. w, n-Hexane extracts*) *E.coli*

A. fissifolius, C.dactylon,C. gayana, G. serrata (*Methonal, ht.w & Methonal +n-Hexane extracts*) *B.cereus*

A. fissifolius, C.dactylon,C. gayana, G. serrata (*Methonal, ht.w & Methonal +n-Hexane extracts*) *E.coli*

RESULTS AND DISCUSSIONS

The observations are recorded and they have been categorized into maximum zone of inhibition (cognizable inhibitory zone). Among the 56 samples obtained 5 samples has shown the zone of inhibition i.e. (1 to 5mm), the other samples of 3 has shown the zone of inhibition i.e. (6-10 mm) and the remaining samples of 21 samples has shown the zone of inhibition i.e. (12 -15mm) out of the total 56 samples of individual. The moderate inhibition zone of expression was observed in 8 samples of (E/M/A/n-Hexane extracts) (i.e.1to 5 & 6 to10 mm inhibition zones) [(Table 1 &2) given in separate pages.]

Table1: Samples consists of the extracts of Cognizable Zone of Inhibition obtained from the antimicrobial screening of selected plant extracts against *Bacillus cereus* species.

S. No.	Name of the plant	Name of the organism	1 to 5 mm							6 to 10 mm						12 to 15 mm							
			E	Ht. W	M	M + n-H	M + A	M +E	M+ D. W	E	Ht. W	M	M + n-H	M + A	M +E	M +D W	E	Ht. W	M	M + n-H	M + A	M+ E	M+ D. W
1.	*Axonopus fissifolius*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
2.	*Cynodon dactylon*	*B. cereus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
3.	*Chloris gayana*	*B. cereus*	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+

ISSN: 0975-8585

4.	*Gomphrena serrata*	*B. cereus*	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-

Table2: Samples consist of the extracts of Cognizable Zone of Inhibition obtained from the antimicrobial reening of selected plant extracts against *E.coli* species.

S. No.	Name of the plant	Name of the organism	1 to 5 mm							6 to 10 mm							12 to 15 mm						
			E	Ht. W	M	M +n -H	M + A	M +E	M + D W	E	Ht. W	M	M + n - H	M + A	M + E	M + D W	E	Ht. W	M	M + n - H	M + A	M +E	M + D W
1.	*Axonopus fissifolius*	*E.coli*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+
2.	*Cynodon dactylon*	*E.coli*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
3.	*Chloris gayana*	*E.coli*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
4.	*Gomphrena serrata*	*E.coli*	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+

The present work will focus on the medicinal importance of selected weed species for their antibacterial activity. Out of the four different plant species taken the maximum zone of inhibition was observed with the combination of different solvents like n-Hexane, Methanol, Acetone and Hot Distilled Water were taken as a combination. The maximal zone of inhibition was observed for the combination of solvents as mentioned above. There was a minimal zone of inhibition for the single solvent as the effect was less when compared with the combination of solvents. The maximum zone of inhibition was inhibited in the range of 12-15 mm for *B. cereus* us and *E.coli* species. Therefore these combinations were suggested for further analysis of different weed species for the development of various pharmaceuticals, nutrients for utilization[4,18,20].

ACKNOWLEDGEMENTS

Authors are expressing their gratitude to the Chancellor and Vice-Chancellor for their encouragement. Authors are thankful to the Dean, Engineering and Management (Dean E & M), Vignan's University, Vadlamudi, for providing facilities and encouragement. Authors are expressing their sincere thanks to Head, Department of Biotechnology for providing necessary facilities to carryout fieldwork and laboratory analysis. Authors are also thankful to the Management for extending financial assistance & providing facilities.

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