A Study on Antioxidant, Antimicrobial and Larvicidal Activity of *D.bipinatta* Leaves

Nivethini.D[1*], Dr.Sumathi.R.K[2]

[1*]*Assistant Professor, Sri Ramakrishna College of Arts & Science for Women, Coimbatore*

[2]*Associate Professor, Sri Ramakrishna College of Arts & Science for Women, Coimbatore*

[1*]nivemicro@srcw.ac.in, [2]sumathimicro@srcw.ac.in

Abstract: *The desire towards herbal medicine has increased tremendously in recent years as the people love to have medicines that have minimum or no side effects. Due to the presence of antioxidants and several phytochemicals present in plants, it scored major consideration and priority towards treatment of diverse disease such as cancer, asthma, cardiovascular disease, etc. the synthetic substances employed in larvicidal activity possess various harmful effects to every forms of life. The groundwork focuses on the ability of D.bipinnata leaves to act against clinical isolates as well as mosquito larvae. The plant extracts were prepared and analyzed for the presence of active components by phytochemical analysis. The antioxidant and antimicrobial activity was evaluated. The plant extracts were also evaluated for its larvicidal activity against mosquito larvae.*

Keywords: ***D.bipinnata,*** **antioxidant, DPPH assay, antimicrobial activity, larvicidal, mosquito larvae**

1. Introduction

Medicinal systems such as Siddha, Unani, Ayurveda, Chinese medicines have fancied people in recent years. The desire towards herbal medicine is increasing tremendously because of their safety, efficacy and minimum side effects [26]. These herbal medicines are natural products obtained from plants that are generally secondary metabolites with medicinal importance [20]. In the interest of natural antioxidants present in plants, it scored major consideration and priority towards the treatment of diverse diseases such as cancer, asthma, stress-affiliated diseases, cardiovascular, neurodegenerative diseases etc. [05]. These gifts from nature are used in several rituals unaware of their actual benefits [16]. *Desmostachya bipinnata* is a perennial grass that is bounteous in distribution all over India [16] and also in Nubia, Egypt and Syria [20]. About three species are found in this genus, *D.bipinnata*, *D.cynosuroides*, *D.pingalaiae* [17]. It is commonly known as sacrificial grass in English due to its usage in Yagnas and religious rites and Darbha in Sanskrit [09]. Apart from the sacred properties, the plant contains several bioactive compounds which are responsible for its medicinal uses. It has been discovered to cure various infections or diseases, and it has also been discovered that the grass can absorb approximately 60% of the radiation, so it is

kept in vessels containing water or food during the eclipse period. The plant exhibits DNA damage protection and antioxidant activity [26]. The plant has been shown to be effective against a variety of microbial infections most notably *H.pyroli* [15]. Antifungal activity was also observed against different fungal species (Pragya *et al*., 2018) as well as found to be effective against tamoxifen-induced hepatotoxicity [10]. Anti-diarrhoeal activity was found and the root extracts of the plant were used in the treatment of intestinal disorders [04]. The plant has the ability to treat urinary disorders [08] and excessive vaginal discharges [19]. *Aedes aegypti* is an anthropophilic domicile mosquito that has been identified as the primary vector of dengue viruses in North America. As the mosquito was investigated to spread chikungunya, zika and yellow fever urban virus, monitoring and controlling the vector became necessary. Several plants, such as *Cinnamon umzeylanicum, Ocimum* sp., *Cuminum cymium, Cedrus* sp. and *Piper* sp. were found to possess pest control properties. In addition, extracts that were prepared from Tanzanian plants have been investigated for their larvicidal activity [01]. Lemon grass and clove essential oils were examined to be effective against *Ae.aegypti* larval stages [22].

2. Materials and methods

2.1 Collection of plant material:

The leaves of *Desmostachya bipinnata* was collected in and around the Coimbatore district. The sample was then rinsed and air-dried. The dried plant material was grounded to a fine powder and used for further studies.

2.2 Preparation of extracts [24 ; 18]:

To prepare ethanol extract 10grams of leaf material of the above mentioned plants were soaked in 100ml of ethanol and kept in an orbital shaker for about 48 hours at 150 rpm. The solvent was removed from the sample through evaporation at room temperature. The remaining extracts were filtered and the filtrate was used for further studies.

To prepare the aqueous extract, 5grams of each plant leaf material were boiled in 100ml of distilled water for few minutes at 100C. The extracts were then filtered through Whatmann filter paper and the filtrates were used for further studies.

2.3 Phytochemical analysis [14; 27; 21; 16]:

The plant extracts were subjected to phytochemical analysis as follows.
2.3.1 Test for flavonoids: To 0.5ml of extract, 5ml of dilute ammonia was added and later 1ml of concentrated sulphuric acid was added. Appearance of yellow colour indicates the presence of flavonoids.
2.3.2 Test for tannins: To 0.5ml of extract, 10ml of distilled water was added and boiled. A few drops of 0.1% ferric chloride was added to the solution. The appearance of brownish green or blue or black colouration indicates the presence of tannins.
2.3.3 Test for phenols (Ferric chloride test): A few drops of ferric chloride solution were added to 0.5ml of extract. Appearance of bluish black colour

indicates the presence of phenols.

2.3.4 Test for alkaloids (Mayer's test): To about 1ml of extract a few drops of Mayer's reagent was added and observed. Turbidity or creamy precipitate indicates the presence of alkaloids.

2.3.5 Test for saponins (Foam test): To 0.5ml of extract, 5ml of distilled water was added and shaken vigorously for about 30 minutes. A thick froth of about 1cm height indicates the presence of saponins.

2.3.6 Test for steroids: To about 0.5ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid was added. Appearance of blue or green colour indicates the presence of steroid.

2.3.7 Test for carbohydrates (Benedict's test): To about 1ml of extract, 2ml of Benedict's reagent was added. Appearance of blue, green, orange or red colour indicates the presence of carbohydrates.

2.3.8 Test for amino acid or protein (Ninhydrin test): A few drops of ninhydrin reagent was added to a small amount of extract and kept in water bath for 2-3 minutes. Appearance of yellow colour indicates the presence of amino acid and bluish black colour indicates the presence of protein.

2.3.9 Test for reducing sugars: To about 0.5ml of extract, fehling's solution A and B was added and kept in boiling water bath for few minutes. Appearance of reddish brown colour indicates the presence of reducing sugars.

2.3.10 Test for terpenoids (Salkowski test): About 2ml of chloroform was added to 0.5ml of extract and 3ml of concentrated sulphuric acid was added along the walls of test tube. Appearance of reddish brown colour at interface indicates the presence of terpenoids.

2.3.11 Test for phlobatannins: About 2ml of 1% aqueous hydrochloric acid was added to 1ml of extract and heated. The presence of phlobatannins can be indicated by deposition of red precipitate.

2.4 Evaluation of antioxidant activity (DPPH ASSAY) [18]:

The DPPH (diphenyl picrylhydrazyl) radical scavenging activity of the plant extracts were investigated by modified method of Mensor *et al*., 2001. Various concentrations of aqueous and ethanolic extracts were prepared along with the standard solution (ascorbic acid). About 0.1mM of DPPH was added to 0.5ml of various concentrations of extracts and shaken vigorously. The mixture was allowed to stand at room temperature for 30 minutes the absorbance was measured at 516nm using UV spectrometer. The antioxidant activity percentage (AA%) was calculated using the formula,

$$\text{AA\%} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} * 100$$

where Abs stands for absorbance.

2.5 Isolation and identification of microorganisms:

The samples were collected from skin infections of hospitalised patients. The organisms were then isolated from the collected samples.

2.5.1 Isolation:

The samples were collected using sterile swabs. The swab cultures were inoculated onto solid media such as Nutrient agar, Mac Conkey agar and Mannitol Salt agar and incubated at 37C for 24 hours. The colonies were then identified for the organism.

2.5.2 Identification:

The identification of the isolates was performed according to their morphological and cultural characteristic by following Bergey's Manual of Systemic Bacteriology. The bacterial colonies on the media were subjected to staining and biochemical tests for their identification.

2.5.3 Gram staining [25]:

The gram staining is a method of staining used to distinguish and classify the bacterial species into two large groups (gram-positive and gram-negative). The gram-positive bacteria retain the crystal violet dye, and thus are stained violet, while the gram-negative bacteria do not retain crystal violet and thus are stained pink. A bacterial smear was made on a clean slide with a sterile loop; air-dried and heat fixed. The smear was then subjected to standard gram staining procedure.

2.5.4 Biochemical tests [12]:

The biochemical tests were performed based on standard procedures. Several tests were performed such as Indole, Methyl Red, Voges Proskauer, Citrate utilization, Catalase, Oxidase, Urease, Hydrogen sulphide production etc. the organism were identified based on the results obtained.

2.6 Evaluation of antimicrobial activity [13]:

Antibacterial activity was evaluated by well-diffusion method. The organisms which were isolated from clinical samples were inoculated onto the Muller-hinton agar surface by spreading a standard volume of microbial inoculum using sterile swabs. A hole was made by punching the agar surface aseptically to a diameter of about 6-8mm using a sterile cork borer or tip. About 20µl, 40µl, 60µl and 80µl of aqueous and ethanolic extracts of *D.bipinnata* at specific concentration were introduced into the well. The plates were then incubated at 37C for about 24hours. The extracts get diffused into the medium and thus the microbial growth gets inhibited. A clear zone around the wells, were measured and zones produced by various extracts were compared.

2.7 Evaluation of larvicical activity [01]:

Mosquito larvicidal test was carried out according to WHO (World Health Organisation) guidelines and standard procedures. The screening was done with aqueous and ethanolic extracts of *D.bipinnata* with different dosages ranging from 1mg/ml to 100mg/ml. Twenty-third instar larvae (*Aedes aegypti*) were placed in plastic cups containing distilled water for about 1 hour in order to reduce the stress. Later 0.2ml of stock solution (phosphate buffer) was added to their respective cups to prepare test solutions at concentrations ranging from 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml. A standard number of larvae (20 in each cup) were added and used for performing larvicidal test. A negative control was also prepared that contains 19.8ml of distilled water and 0.2ml of ethanol with 20 larvae each. Each test was performed with 2 replicates of 5 concentrations (800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml, and 50µg/ml) and each sample was tested two times on various days with both replicates. During the test, yeast powder was provided to mosquito larvae after 24hours of monitoring and later monitored for 72 hours.

The experiments were carried out under laboratory conditions 25-28C and a photoperiod of 12 hours light followed by 12hours of dark (12L: 12D).

Mortality was recorded after 12 hours and 24 hours. The larval food (yeast powder) was added to those larvae that are alive and mortality recorded after 48hours. The larvae were considered to be dead when they were morbidund or becomes non-

motile. The number of dead larvae was counted, and the average percentage of mortality was calculated.

Mortality % = X-Y/X * 100

In the above formula,
X-stands for the percentage of survival in the control larvae population
Y-stands for the percentage survival in the treated larvae population

Thus the extracts of collected plant materials were analysed for chemical constituents present in it by phytochemical screening. It was also investigated for its antioxidant, antimicrobial and larvicidal activity.

3. Results and discussion

The plant material was collected and the extracts were prepared. These extracts were subjected to phytochemical analysis and antioxidant activity. The antimicrobial activity of the extract was evaluated against microbes isolated from clinical samples. The microbes were identified by staining as well as biochemical tests. The larvicidal activity was also found and all the results were compared.

3.1 Plant extracts:

The aqueous as well as ethanol extracts of *Desmostachya bipinnata* were prepared using appropriate methods and displayed in plate 1&2 [24; 18].

Plate 1: Aqueous extract

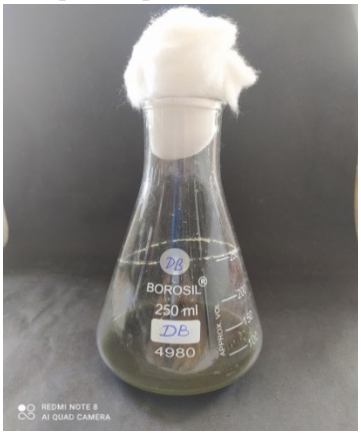

Plate 2: Ethanol extract

3.2 Phytochemical analysis:

Phytochemical screening of the plant extracts (aqueous and alcoholic) showed the presence of various chemical constituents such as phenols, tannins, alkaloids, steroids, etc. and were tabulated (Table 1).

Compounds present	Aqueous extract	Ethanol extract
Phenol	+	+
Tannin	+	+
Alkaloids	-	+

Saponins	+	-
Flavonoids	-	+
Steroids	-	+
Carbohydrate	+	+
Protein / Amino acid	+	-
Terpenoids	+	+
Reducing agents	+	+
Phlobatannins	-	+

Table 1: Phytochemical analysis of *D.bipinnata*

Ali Esmail Al snafi [03] with his colleagues has conducted experiments and found that *D.bipinnata* contains several compounds such as coumarins, carbohydrates, alkaloids, tannins, flavonoids, etc. Other compounds such as alkaloids, tannins and anthocyanin were also isolated in research conducted by Khatune *et al.*, [11]. The chemical compounds found to be present were similar to those constituents isolated or screened by previous experiments conducted by several researchers. The compounds were found to be responsible for its following antioxidant, antimicrobial and larvicidal properties. The compounds such as phenols, tannins, alkaloids, flavonoids, steroids, carbohydrate and terpenoids were found to be common in all three plant extracts.

3.3 Antioxidant activity:

Antioxidant can be referred as against oxidation. These antioxidants play a vital role in food quality preservation as well as in maintenance of good health [22]**.** The antiradical activity was evaluated by the capacity of antioxidant compound in reducing DPPH radical which can be indicated by decrease in its absorbance at 516nm and the results were observed and interpreted in table 2; figure 1.

Concentration	Aqueous extract	Ethanol extract
25%	17	40
50%	22	55
75%	61	83
100%	76	89

Table 2: Results for DPPH assay of *D.bipinnata*

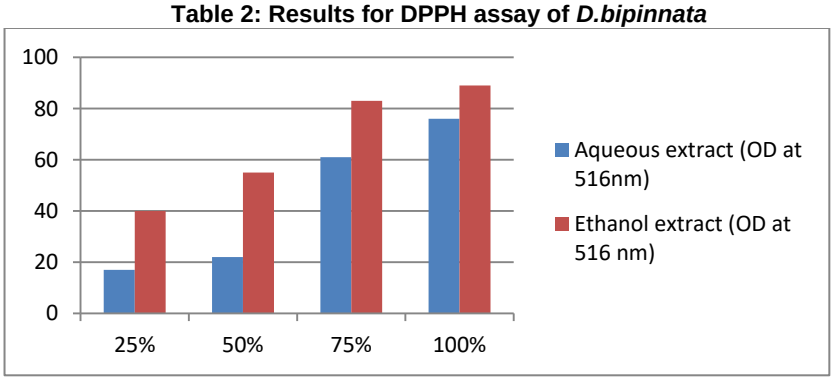

Figure 1: Graph showing increased antioxidant activity with increase in concentration of plant extracts

The antioxidant activity was found to be increasing with the increase in concentration of plant extracts. The aqueous and ethanol extracts of *D.bipinnata* showed DPPH scavenging activity ranging from 17(25%) to 76(100%) and 40(25%) to 89(100%) respectively. The enhancement of antioxidant activity was about 59 and 49 folds in aqueous and ethanol extract respectively.

3.4 Identification of clinical isolates:

3.4.1 Gram staining: Both gram positive and gram negative bacteria were observed under oil immersion objective. The results were displayed in table 3.

Sample	Gram positive/ negative
Sample 1	Gram negative rods
Sample 2	Gram positive cocci
Sample 3	Gram negative rods
Sample 4	Gram negative rods
Sample 5	Gram positive bacilli

Table 3: Results for gram staining of clinical isolates

3.4.2 Biochemical tests:

The biochemical results were observed and tabulated in table 4&5.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Indole	+	-	-	-	-
Methyl Red	+	+	-	-	-
Voges Proskauer	-	+	-	+	+
Citrate utilisation	-	+	+	+	+
Catalase	+	+	+	+	+
Urease	+	+	-	+	+
Oxidase	-	-	+	-	-
Hydrogen sulphide production	-	-	-	-	-
Starch utilisation	-	-	-	-	+
Triple sugar iron	A/A	A/A	Alk/Alk	A/A	A/A
Nitrate reduction	+	+	+	+	-

Table 4: Results for biochemical tests

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Glucose	+	+	+	+	+

Sucrose	-	+	-	+	+
Lactose	+	+	-	+	-
Mannitol	+	+	+	+	+
Gas production	+	-	-	-	-

Table 5: Results for carbohydrate fermentation

Aynalem *et al*., [07] has reported that several organisms such as bacteria, fungi, protozoa and viruses were found in wound infections. Among others the common bacteria reported to be present were *S.aureus, E.coli, P.aeruginosa, K.pneumoniae, S.pyogens, Proteus* sp., *Streptococcus* sp., *Enterococcus* sp. etc. Similarly on biochemical identification and gram staining the bacteria were identified to be *E.coli, S.aureus, P.aeruginosa, K.pneumoniae* and *B.pumilus* respectively.

3.5 Antibiotic susceptibility test:

Ali *et al* [02] reported that natural antibodies were obtained from plants and the activity was found to be due to phenolic compounds such as flavonoids, phenolic acids, tocopherols, etc. Ogunwenmo *et al* (2007) have proposed that the alkaloids present in plants possess anti-microbial activity. Awoyinka *et al.,* [06] exhibit anti-fungal, anti-parasitic property, etc. Researchers also reported that saponins possess anti-bacterial activity. Researchers have reported that the chemical constituent terpenoids exhibit anti-bacterial, anti-fungal, anti-viral, anti-protozoan activity, etc. The phytochemicals studied to be responsible for antimicrobial activity was found to be present in the plant extracts taken for study.

The antibiotic sensitivity patterns were observed and shown in table 6 & 7.

TEST ORGANISM	**CONCENTRATION**			
	20µl	**40µl**	**60µl**	**80µl**
E.coli	12mm	14mm	15mm	17mm
S.aureus	15mm	17mm	18mm	20mm
P.aeruginosa	12mm	16mm	16mm	19mm
K.pneumoniae	-	12mm	14mm	14mm
B.pumilus	11mm	14mm	17mm	17mm

Table 6: ABST result for aqueous extract of *D.bipinnata*

TEST ORGANISM	**CONCENTRATION**			
	20µl	**40µl**	**60µl**	**80µl**
E.coli	18mm	17mm	19mm	22mm
S.aureus	18mm	21mm	21mm	27mm
P.aeruginosa	15mm	18mm	21mm	25mm
K.pneumoniae	17mm	21mm	21mm	23mm
B.pumilus	16mm	16mm	20mm	24mm

Table 7: ABST result for ethanol extract of *D.bipinnata*

Shakila [19] and colleagues studied that as *D.bipinnata* possess certain bioactive compounds, it exhibits significant antimicrobial activity and it was found to be effective against several organism *A.hydrophila, B.cereus, B.subtilis, P.vulgaris,* etc. Similarly the aqueous as well as ethanol extracts were found to be effective

against several organisms and maximum activity was observed against *S.aureus* at concentration of 80µl.

3.6 Larvicidal activity [01]:

The effect of aqueous and ethanol extract of *D.bipinnata* was studied at different concentrations such as 50µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml against twenty-third instar larvae of *Aedes aegypti* and the activity was observed and tabulated in table 8; figure 2.

Plant extracts used	DIFFERENT CONCENTRATION														
	50µg/ml			100 µg/ml			200 µg/ml			400 µg/ml			800 µg/ml		
	12h	24h	48h	12h	24h	48h	12h	24h	48h	12h	24h	48h	12h	24h	48h
Aqueous extract	0	0	0	0	1	1	1	1	0	3	3	5	5	5	8
Ethanol extract	0	1	2	1	1	3	3	3	4	5	7	9	6	10	14

Table 14: Results for larvicidal activity of *D.bipinnata*

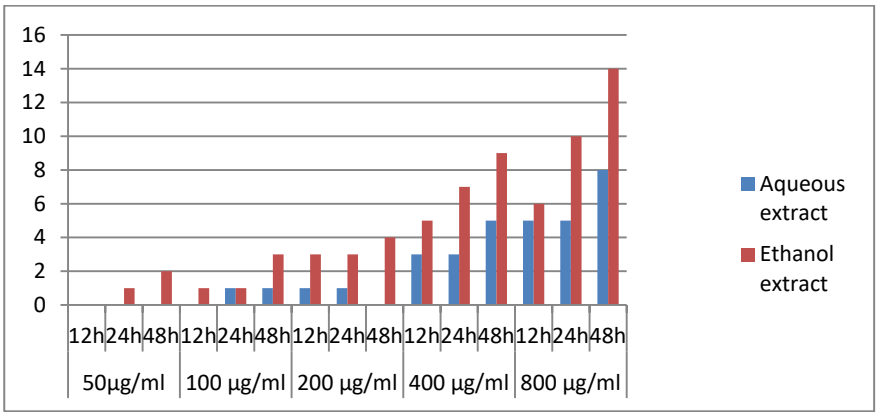

Figure 3: Graph showing increased larvicidal activity of aqueous and ethanol extracts with increased concentration of plant extracts

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