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ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF METHANOL EXTRACT OF *SPILANTHES CALVA* (DC) LEAVES

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ABSTRACT

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Methanol extract of leaves of *Spilanthes calva* (DC) was examined for their antimicrobial and cytotoxic properties. To determine antibacterial activities, the extract was tested against four gram-positive and six gram-negative bacteria at three concentrations (500, 800, 1000 µg disc⁻¹) through disc diffusion method. The extract showed moderate antibacterial actions and that was increased by increasing the concentration of the sample. The maximum antimicrobial potential was obtained against *Staphylococcus aureus* and no sensitivity was found for *Escherichia coli*. Comparatively gram-positive bacteria demonstrated more susceptibility to the extract than gram-negative bacteria. Cytotoxic property of the sample was done using Brine shrimp lethality bioassay where it did not show noticeable toxicity. So, our present study reveals that the leaves extract of *S. calva* possess considerable antibacterial properties along with lesser amount of cytotoxicity.

INTRODUCTION: Infectious diseases have been, in the past and continue to remain, a leading cause of morbidity, disability and mortality in the world. Approximately 15 million people die each year due to infectious diseases- nearly all live in developing countries ¹. Their control is a constant challenge that faces health workers and public health officials in both industrialized and developing countries.

Only one infectious disease, smallpox, has been eradicated and stands as a landmark in the history of the control of infectious diseases. The International community is now embarked on the path to eradicate poliomyelitis and dracunculiasis (guinea worm infection) by the close of this century. Other infectious diseases, such as malaria and tuberculosis, have foiled eradication attempts or control efforts and are re-emerging as increasing threats in many countries.

Some infectious diseases, such as tetanus, will always be a threat if control measures are not maintained. Drugs that once seemed invincible are losing their effectiveness for a wide range of community-acquired infections, including tuberculosis, gonorrhoea, pneumococcal infections (a leading cause of otitis media, pneumonia, and meningitis), and for hospital-acquired enterococcal and staphylococcal infections ².

Indiscriminate and unconcerned use of antibiotics has led to increased microbial resistance. Consequently, newer agents have been brought in at increased economic costs to the patient but they too have become inefficacious in due course and pose a great threat to human health in world. So, noble, emerging and re-emerging infectious diseases have become a focus for the development of new cost-effective drug in both industrialized and developing countries.

Nature has been a source of medical treatments for thousands of years and today plant based systems continue to play an important role in the primary health care of 80% of the world's population^{3,4}. According to a study by Grifo and colleagues⁵, 84 of a representative 150 prescription drugs in the United States fell into the category of natural products and related drugs. They were prescribed predominantly as anti-allergic/pulmonary/respiratory agents, analgesics, cardiovascular drugs, and for infectious diseases.

The increasing prevalence of multidrug resistance in pathogenic microorganisms, as well as, undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Common side effects are allergic reaction or toxic reactions or mutagenic substances.

Therefore, medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat⁶. As natural products have been elaborated within living systems, they are often perceived as showing more "Drug likeness" and biological friendliness than totally synthetic molecules," making them good candidates for further drug development^{7, 8, 9}. In continuation of this effort, we study antibacterial potential of *Spilanthes calva* and its cytotoxic properties.

The medicinal plant, *Spilanthes calva* DC is also known as marhatitiga (Bangla) or paracress (English) belongs to the family Asteraceae. It is an annual herb which is up to 60 centimeter tall. The leaves are triangular ovate or lanceolate in shape. The roots, flower heads and whole aerial part possess stimulant, sialogogue, and local anesthetic. Tincture of flowers relieves toothache and is useful in infections of throat and paralysis of the tongue¹⁰.

Besides, oil of *S. calva* has been reported in folk medicine in the treatment of inflammation, skin diseases, purgative, diuretic, lithotriptic and dysentery. The plant shows potential antioxidant, antifungal and diuretic activities due to presence of different compounds like saponins, volatile and non-volatile oils¹¹⁻¹³.

But there is no detailed report on antibacterial and cytotoxic activities of the *S. calva*. Hence, the purpose of our present investigation is to evaluate the antimicrobial activity of the plant and establish a scientific report for their folkloric use against infections that may lead to development of new antibiotic in future.

MATERIALS AND METHODS:

Preparation of Extract: Methanol extract of *Spilanthes calva* leaves was used in the present study. The matured leaves were collected in the month of November 2010 from Chittagong, Bangladesh. The leaves was dried under shade and pulverized in a mechanical grinder. The powder was extracted with methanol. The extracted juice was filtered by using a clean cloth and then filter paper. The extract was concentrated first in Rotary vacuum evaporator and then on water bath. The extract was then kept in freeze for antimicrobial and cytotoxic properties.

Antibacterial Assay: The disc diffusion method was used to test antimicrobial activity against ten bacteria¹⁴. The antibacterial activity of methanol extract of *Spilanthes calva* leaves were determined at three concentration (500, 800, 1000 $\mu\text{g disc}^{-1}$). Solutions of known concentration (10 mg/ml) of the test sample were made by dissolving measured amount of the samples in calculated volume of solvent. Dried and sterilized filter paper discs (5.5 mm diameter) were then impregnated with known amounts of the test substances using micropipette.

Discs containing the test material were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard antibiotic discs (Kanamycin 30 $\mu\text{g disc}^{-1}$) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4°C) for 1 h to allow diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37°C for 12 h to allow growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms

and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading was taken.

Brine Shrimp Lethality Bioassay: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds^{15, 16}. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method¹⁵. The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 μ L in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 μ g/ml.

A vial containing 50 μ L DMSO diluted to 5 mL was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{N_t}{N_0} \times 100$$

Where, N_t = Number of killed nauplii after 24 hrs of incubation, N_0 = Number of total nauplii transferred i.e 10.

The LC_{50} (Median lethal concentration) was then determined using Probit analysis.

RESULTS:

Antibacterial test: The antibacterial activities of the extract obtained from the plant under study by the diffusion method are shown in **Table 1**. The extract of the plant screened at three concentration (500, 800, 1000 μ g disc⁻¹) against four gram positive and six gram negative bacteria and showed various levels of antimicrobial activities with the different zone of inhibition. Larger zone of inhibition was obtained with the increase concentration of the sample and all tested microorganisms were more sensitive at 1000 μ g disc⁻¹.

So, the antimicrobial activity is directly proportional to the concentration of the extract. The extract showed the most potent inhibition for *Staphylococcus aureus* at all three concentrations whereas no activity was found against *E. coli*. Among six gram negative bacteria, *Salmonella typhi* showed maximum sensitivity to the extract. No zone of inhibition was found for negative control disc.

TABLE 1: ANTIMICROBIAL EFFECT OF METHANOL EXTRACT OF *SPILANTHES CALVA* AND KANAMYCIN

Name of Bacteria	Zone of Inhibition (mm)			
	Kanamycin disc (30 μ g/disc)	500 μ g/disc	800 μ g/dis	1000 μ g/disc
Gram Positive				
<i>Staphylococcus aureus</i>	30	11	13	15
<i>Bacillus subtilis</i>	25	-	7	12
<i>Bacillus cereus</i>	31	6	6.5	8
<i>Bacillus megaterium</i>	30	-	-	9
Gram negative				
<i>Salmonella typhi</i>	28	-	7	13
<i>Shigella dysenteriae</i>	30	-	7	8
<i>Pseudomonas aeruginosa</i>	31	-	7	8
<i>Escherichia coli</i>	22	-	-	-
<i>Klebsiella species</i>	33	-	6	7
<i>Shigella flexneri</i>	31	-	6	7.5

The results of Brine Shrimp Lethality Bioassay:

Following the procedure of Meyer, the lethality of the crude extract of *Spilanthes calva* leaves to brine shrimp was determined on *Artemia salina* after 24 h of exposure the samples, the positive control and vincristine sulphate. This technique was applied for the

determination of general toxic property of the plant extract. From the **Figure 1**, we have observed that the LC₅₀ value of the extract was 167.5 µg/ml and that for standard vincristine sulphate was 9.5 µg/ml. No mortality was found in the control group, using DMSO and sea water.

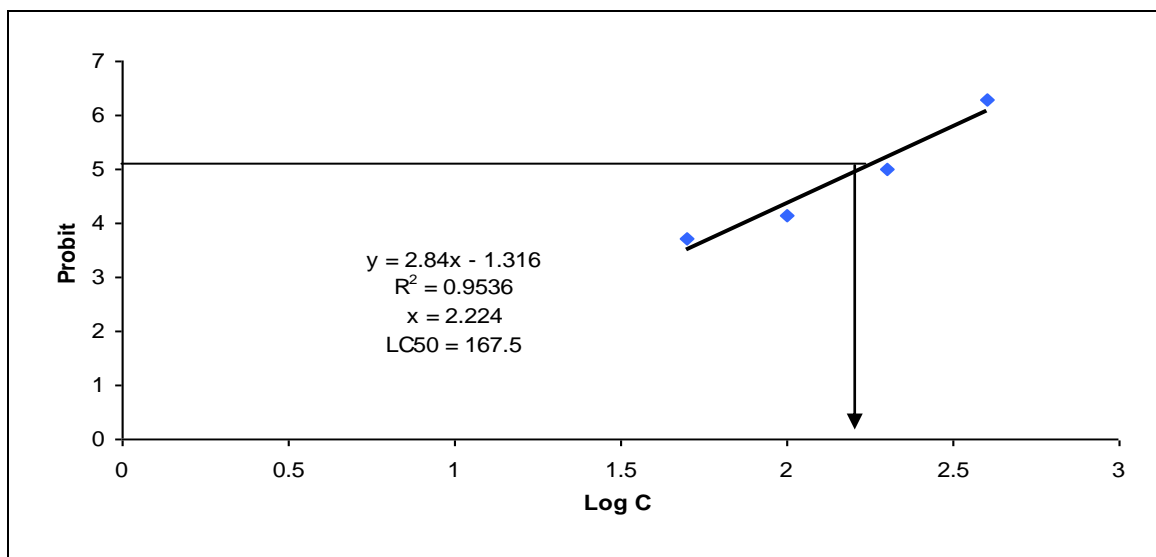


FIG. 1: DETERMINATION OF LC₅₀ VALUE FOR *SPILANTHES CALVA* LEAVES FROM LINEAR CORRELATION BETWEEN LOG CONCENTRATIONS VERSUS PROBIT MORTALITY

DISCUSSION: Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has regained public attention over the past 20 years as this type of medicine is easily accessible in some regions. A postulation denoted that by 2010, at least two-thirds of Americans will opt for alternative therapies¹⁷. The non prescription use of medicinal plants is cited today as an important health problem, in particularly their toxicity to the kidney¹⁸. So, if the plant extract found to show significant antimicrobial activities must take into account acceptable levels of toxicity.

The results indicated that the crude methanol extract of *Spilanthes calva* leaves showed better antibacterial activities towards the gram-positive bacteria at higher concentration while gram-negative bacteria are less sensitive to the extract probably due to permeability barrier provided by cell wall or to the membrane accumulation¹⁹. The extract did not cause any apparent *in vitro* toxicity in brine-shrimp lethality bioassay. But the extract demonstrated antibacterial action at higher concentration that may cause hazardous effect to the patient.

So, further studies are needed to understand the mode of action of the individual compounds after separation from the extract and this may lead to develop a new antimicrobial agent in future.

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