REVIEW ARTICLE

A Comparative Study of Two Bangladeshi Medicinal Plants "*Spilanthes acmella* L. and *Wedelia trilobata* L."

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ABSTRACT

Phytochemical screening of the ethanolic extracts of S. acmella L. (local name: Kurchi) and W. trilobata L. (local name: Singapore daisy) revealed that phytochemical groups like carbohydrate, saponins, gum, steroids, tannins, alkaloids and flavonoids are present. Pharmacological interest of these compounds coupled with the use of this plant in traditional medicine tend to investigate S. acmella L. and W. trilobata L. for determining antioxidant, analgesic, cytotoxic, antimicrobial potentials. The antioxidant activity of ethanolic extract of S. acmella L. and W. trilobata L. were evaluated by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay. In DPPH scavenging assay, the IC50 value S. acmella L. and W. trilobata L. were found to be 70µg/ml approximately and IC50 = 45µg/ml approximately which were comparable to the standard ascorbic acid (IC50 value was15µg/ml). The in-vitro anti-microbial activity of the ethanol extract of S. acmella L. and W. trilobata L. investigated. In case of Escherichia coli, Shigella dysenteriae, standard drug Ciprofloxacin (30µg/µl) shows zone of inhibition of 22mm, 22mm respectively whereas S. acmella (500 µg/disc) extract showing zone of inhibition of 13mm & 12mm. In case of Escherichia coli, Shigella sonnei, Streptococcus agalactiae, Enterococcus faecalis, W. trilobata L. (500 µg/disc) shows zone of inhibition of 16mm, 16mm,14mm, and 14mm respectively whereas Ciprofloxacin (30µg/µl) shows zone of inhibition of 22mm, 22mm, 22mm, and 23mm respectively. Using brine shrimp lethality bioassay, general toxicity of the both extracts was elucidated that serves as an indicator of toxicity. The extract of W. trilobata L. showed toxicity in the brine shrimp lethality assay (LC50 = 20µg/ml & LC90 = 80µg/ml). Where the extract of S. acmella L. showed toxicity in the brine shrimp lethality assay (LC50 = 10µg/ml & LC90 = 40µg/ml). Effect of ethanolic extracts of S. acmella L. and W. trilobata L. were tested for analgesic activity by using acetic acid induced writhing in mice. At the dose of 500 mg/kg& 250 mg/kg body weight, the S. acmella L. extract showed a greater writhing inhibition 76.2% & 56.2% respectively. On the other hand, at the dose of 500mg & 250 mg/kg body weight, the ethanolic extract of W trilobata L. which showed writhing inhibition 46.9% & 20% respectively.

Keywords: ***Phytochemical group test, Antioxidant activity, antimicrobial activity, cytotoxicity, analgesic activity***

1. INTRODUCTION

Bangladesh belongs to a large amount of tropical and sub-tropical forest containing wide variety of medicinal plants possessing ethnomedicinal significance. These medicinal plants including herbs may be asset if we can isolate compound containing pharmacological activities. The prime interest of this project was to evaluate pharmacological activity of the two herbal plants *S. acmella* L. and *W. trilobata* L. especially antioxidant, antimicrobial and cytotoxic activities. This study is focused on traditional use of these plants. *W. trilobata* L. is traditionally used as healing of wound [1]. On the other hand, *S. acmella* L. is familiar as toothache plant as it is traditionally used in against pathogenic organisms [2, 3]. So I have decided to conduct antimicrobial assay of *W. trilobata* L. & *S. acmella* L. A number of studies on different effects of ethanolic extract of these plants have been already performed in various parts of the world to investigate the effects of *S. acmella* L. and *W. trilobata* L. But nothing much

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done about cytotoxic activity of *S. acmella* L. and *W. trilobata* L. Since there was few evidences of research regarding cytotoxic activity, antimicrobial property of ethanolic extract of *S. acmella* L. and *W. trilobata* L. [4, 5] that's why, I was interested to conduct antioxidant, antimicrobial and cytotoxic tests on these two plants that were available in Khulna region. As these plants are readily available in everywhere in our country, this study finally may contribute significantly in national economy through identifying potential source of drug.

2. MATERIALS AND METHODS

2.1 Sample collection and extraction

Collection: Whole plants of *S. acmella* L. and *W. trilobata* L. collected from Khulna University, Khulna, Bangladesh on 10/10/2013.

Extraction: I used cold extraction method. First, I took about 250 gm of power of both plants in separate container and saturated in 1000 ml 80% ethanol. Then, containers were stored for about two weeks with random stirring. Then filtered the ethanol containing materials using cotton and subsequently Whatman filter paper. After filtration, the both extracts were dried. The crude extracts were estimated and 8 grams and 10 grams of *S. acmella* L. and *W. trilobata* L. subsequently found.

2.2 Animals Used

Young Swiss-albino mice age range between 4-5 weeks, their average weight was about 20-30 gm.

2.3 Bacterial species

Different species of gram positive and gram-negative bacteria were used for the assessment of antimicrobial assay of both extracts.

2.4 Producing of Brine shrimp

To evaluate the cytotoxic property, saline water was taken in a tank in which shrimp eggs were provide. Under proper temperature, shrimp eggs kept for 24 hours to hatch the eggs as well as to mature as nauplii. Then these nauplii were used for the evaluation of cytotoxic activity.

2.5 Drugs

- Vincristine
- Ciprofloxacin
- Diclofenac Na

2.6 Phytochemical Tests

Phytochemical investigation was done to identify the presence of phytochemical groups such as carbohydrate, gum, steroids, saponins, tannins, alkaloids [6] and flavonoids [7]. Wagner's, Mayer's, Dragendroff's reagents were used for alkaloids, Libermann-Burchard reagent and sulphuric acid for steroids, Ferric Chloride, Potassium Dichromate for tannins, Molish reagent and Sulphuric acid for gum. All these experiments were carried out for ethanol extract of *S. acmella* L. and *W. trilobata* L.

2.7 Evaluation of antioxidant activity

2.7.1 Qualitative test

For this test, 0.1 gm of each plant extracts in a test tube was taken and then diluted properly with ethanol. With the help of a fine capillary tube each sample is applied on the TLC plates. A small amount of ascorbic acid was taken in a test tube & diluted properly with ethanol & was applied by capillary tube on TLC plate in the same way. Then chromatogram was developed on the TLC plate by ascending technique. Different ration of three solvent like Hexane, Chloroform, Acetone were kept in three jars to identify the various groups of compounds in both the plant extracts. When chromatogram is complete, the TLC plate was sprayed using 0.02 % DPPH ethanolic solution with the help of a spray gun. After drying the plates, three plates from three solvent systems were observed visually under UV light and various regions were marked. After spraying DPPH solution on the chromatogram, the pink color of DPPH solution was turned into yellow. This showed that antioxidant compounds were present in the plant extracts [8].

2.7.2 Quantitative test

For evaluation of the antioxidant activities of the both extracts 2, 2diphenyl-1-picrylhydrazyl (DPPH) assay was followed. Comparing with standard ascorbic acid, antioxidant properties of the ethanolic crude extracts determined by the ability to scavenge DPPH. Then, 3 ml of 0.004% DPPH solution was taken in each test tube by pipette. After that the test tubes were kept in dark for thirty minutes to end the reactions. DPPH also added on the blank sample that was prepared with ethanol. After thirty minutes, absorbance of each test tube containing both extracts were measured by UV spectrophotometer at 517 nm. % of inhibition was calculated as- % inhibition = [(Blank absorbance - Sample absorbance) / Blank absorbance] X 100. IC_{50} was calculated from % inhibition versus concentration graph [9].

2.7.3 Determination of antimicrobial activity

Disk diffusion technique was used for the assessment of antimicrobial susceptibility [14]. Sample impregnated discs, standard antibiotic discs (Ciprofloxacin discs) and negative control discs (blank discs) were placed gently on the 10 agar plates. The discs size should not be closer than fifteen millimeters to the brink of the plate. The agar plates were then reversed and kept in refrigerator for about 2 hours at 4°c. After that the plates were incubated at 37°c for 18-24 hours. After that, the diameter of zone of inhibition that found on the disc is measured.

2.7.4 Assessment of cytotoxic activity

To conduct this test, 32 mg of dried ethanolic extracts of *W. trilobata* L. & *S. acmella* L. were taken in 10 ml volumetric flask. Then with the addition of emulsifier Tween-80, both of the extracts were dissolved. Finally, volume was adjusted by saline water. For each extract, the final concentrations of extracts were 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 µg/ml prepared. In case of Standard solution, different concentrations were prepared: 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0.039 µg/ml. As control, same volumes of Tween (as same as sample test tubes) were taken in the ten test tubes. Then, with the help of a pipette 10 living shrimps were placed to each of the test tube [15]. After 24 hours test tubes were observed for the survived nauplii.

2.7.5 Assessment of analgesic activity

The well-established method acetic acid induced writhing in mice was followed to evaluate the analgesic property of the ethanolic extracts of *S. acmella* L. and *W. trilobata* L. [16]. Thirty animals were separated into six groups named as group-I, group-II, group-III, group-IV, group-V and group-VI. Each group of mice has been administered control, positive control and the two different doses of each extract. With the help of a feeding needle, test samples, control and Diclofenac-Na were given to mice orally. After thirty minutes interval, 0.7% acetic acid solution at a dose of 15ml/kg was administered intraperitoneally to each of the animals. Five minutes later, number of writhing was taken for 15minutes. Then total number of writhing given by each of the mice were counted those gave in 15minutes. If half writhing occurs, in that case two half writhing was counted as a full writhing.

3. Statistical Analysis

Student's *t*- test was used to determine a significant difference between the control group and experimental groups.

4. OBSERVATION AND RESULT

4.1 Phytochemical Tests

The phytochemical screening showed that in the extracts of *S. acmella L.* are carbohydrate, alkaloid, steroid, gum, tannin, flavonoids, and saponins. In case of extracts of *W. trilobata* L., it was revealed that tannin, gum, carbohydrate, and flavonoids are contained in this plant.

4.2 Qualitative Test

After spraying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the extract of *S. acmella* L. *and W. trilobata* L.

4.3 Quantitative test: IC_{50} Determination

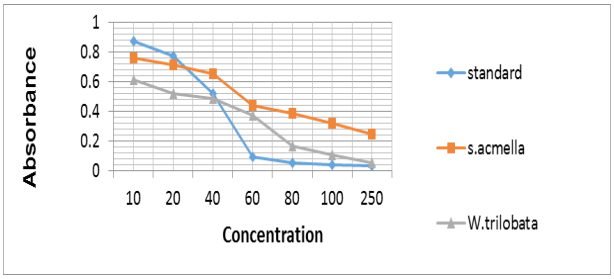


Fig. 1: Absorbance Vs. concentration of ethanolic extract of *Spilanthes acmella* L. and *Wedelia trilobata* L. extract and standard ascorbic acid.

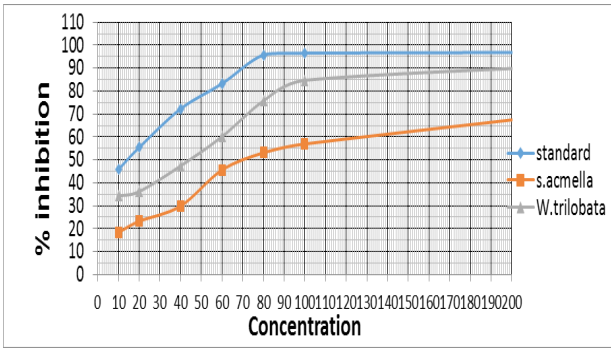
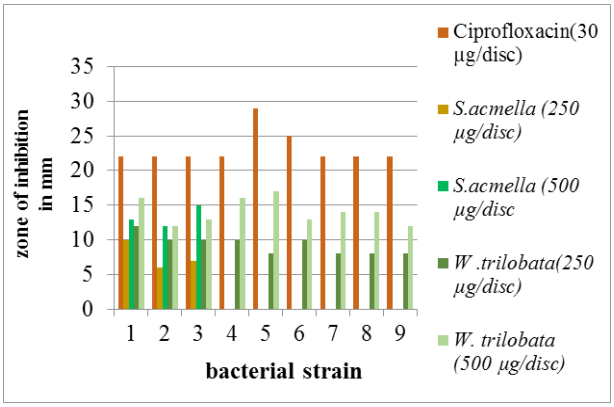


Fig. 2: Comparing with standard ascorbic acid, % inhibition of *S. acmella* L. and *W. trilobata* L. are shown.

In the quantitative assay, *S. acmella* & *W. trilobata* extract displayed a free radical scavenging activity in the DPPH assay ((IC_{50} = 70µg/ml approximately) & (IC_{50} = 45µg/ml approximately) which is comparable to that of ascorbic acid (IC_{50} = 15µg/ml approximately), a well-known standard antioxidant.

4.4 Determination of antimicrobial activity

The ethanolic extract of *W. trilobata* L. and *S. acmella* L. were examined for antimicrobial susceptibility using nine species of both gram-positive and gram-negative bacteria. From above shows that in case of *Escherichia coli, Shigella dysenteriae,* standard drug Ciprofloxacin (30µg/µl) shows zone of inhibition of 22mm, 22mm respectively whereas *S. acmella* (500 µg/disc) extract showing zone of inhibition of 13mm & 12mm. *W. trilobata* L. (500 µg/disc) shows maximum zone of inhibition against *Escherichia coli,Shigella sonnei, Streptococcus agalactiae* , *Enterococcus faecalis* of 16mm, 16mm,14mm and 14mm respectively whereas Ciprofloxacin (30µg/µl) shows zone of inhibition of 22mm, 22mm, 22mm and 23mm respectively .



01	*Escherichia coli*
02	*Shigella dysenteriae*
03	*Staphylococcus aureus*
04	*Shigella sonnei*
05	*Shigella flexineri*
06	*Streptococcus pyogenes*
07	*Streptococcus agalactiae*
08	*Enterococcus faecalis*
09	*Staphylococcus epidermidis*

Fig.3: Graphical presentation of zone of inhibition *Wedelia trilobata* L. and *Spilanthes acmella* L.

4.5 Determination of analgesic activity

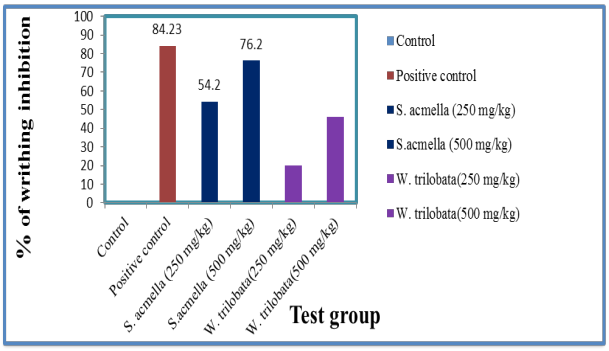


Fig. 4: Effect of *S. acmella* L. and *W. trilobata* L. on acetic acid induced writhing of mice.

The results of the test showed that ethanol extract of *S. acmella* L. exhibited greater inhibition of writhing reflex by 76.2 % & 56.2 % at the dose of 500 mg/kg & 250 mg/

kg body wt. respectively than the ethanolic extract of *W trilobata* L. which showed 46.9 % & 20 % respectively. Whereas, the standard drug Diclofenac Na inhibition was found to be 84.23% at a dose of 25 mg/kg body weight.

4.6 Determination of cytotoxic activity

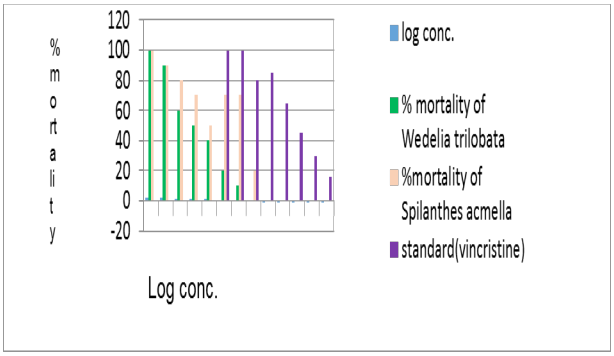


Fig. 5: Graphical presentation of cytotoxic results of *Wedelia trilobata* L. and *Spilanthes acmella* L.

From observation, for crude extract of *W. trilobata* the concentration at which 50% mortality ($LC_{50=}$20µg/ml approx.) & 90% mortality ($LC_{90=}$ 80 µg/ml approx.) of brine shrimp nauplii occurred were obtained. For the crude ethanolic extract of *Spilanthe sacmella*, the concentration at which 50% mortality ($LC_{50=}$10µg/ml approx.) & 90% mortality ($LC_{90=}$ 40 µg/ml approx.) occurred were found by extrapolation. In case of standard drug Vincristine, the (LC_{50}) value & (LC_{90}) value were 5 µg/ml (approx.) & 40 µg/ml (approx.) respectively.

5. Discussion

Recently, because of potentialities of phytochemicals in the therapy of various chronic and infectious diseases, much investigation is going on to find the possible pharmacological activities of plants. In the TLC-based qualitative antioxidant assay using DPPH assay, *S. acmella* L. & *W. trilobata* L. extract showed free radical scavenging properties indicated by the presence of yellow color on a purple background on the TLC plate. Both the ethanol extract of *W. trilobata* L. and *S. acmella* L. shows various zone of inhibition against different micro-organisms in disk diffusion test. Among two plants, it has been shown that *W. trilobata* L. has greater antimicrobial activity than *S. acmella* L. Further study should be carried out to utilize this experiment in the development of antimicrobial agent. The brine shrimp test (BST) is used for testing plant extract lethality which in most cases cogent well with cytotoxic and anti-tumour properties [21]. This assay shows better results in case of *S. acmella* in comparison with *W. trilobata*. This significant lethality of *S. acmella* plant indicates that this plant may contain potent cytotoxic component which requires further investigation. For both sample, the mortality rate of brine shrimp was shown to be better with the increase in concentration. From the analysis, it can be suggested that the ethanol extract of *S. acmella* L. & *W. trilobata* L. might retain analgesic properties that holds the claim about these plants are used as analgesic traditionally. This investigation may serve as a pathway for future research on the biological and pharmacological activities of *W. trilobata* L. and *S. acmella* L.

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