

# Antioxidant Potential and Brine Shrimp Lethality bioassay of *Spilanthes acmella* Flower Extract



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## ABSTRACT

The current research study has been carried out to explore the antioxidant activity and brine shrimp lethality bioassay of different fractions from the flower extract of *Spilanthes acmella*. Besides, this experiment was also assessed to find out the proximate analysis and phytochemical screening by following the perfect protocol. To fractionate by soxhletion using sequential extraction techniques powdered flower of the plant were treated with different solvents including n-hexane, chloroform, ethanol and water. For the evaluation of antioxidant activity, total antioxidant capacity determination, determination of total phenolic content and total Flavonoids contents by aluminium trichloride method were used. In addition, ascorbic acid and gallic acid was used as a standard antioxidant compound in these studies. Concerning the proximate analysis, moisture content, total

ash value, acid insoluble ash and water soluble ash value were found 8.6%, 3.76%, 3.30%, 3.20% respectively. To evaluate cytotoxicity, the brine shrimp lethality bioassay was used. For phytochemical screening different extract of those solvents were utilized that disclosed the presence of alkaloids, flavonoids, phenolic compounds, Tannins, amino acids on different fractions but the absence of reducing sugar and saponins. The results of all assay showed that all the extracts of *Spilanthes acmella* flower possess significant antioxidant activity. In brine shrimp lethality bioassay, ethanol extract of flower effect to brine shrimp nauplii and exhibiting highest toxicity having  $LC_{50}$  value 1.20  $\mu\text{g/ml}$  as compared to standard dimethyl sulfoxide ( $LC_{50}$  1.31  $\mu\text{g/ml}$ ). These evaluations suggest that *Spilanthes acmella* flowers might be a better source of antioxidants and possess important cytotoxic effect.

**Key words:** *Spilanthes acmella*, Antioxidant, Proximate Analysis, Phytochemical Screening, Lethality Bioassay

\*Correspondence to:

Cite This Article:

## INTRODUCTION

Human body gradually faces various diseases from early to the old stage of life because of the oxidation reaction. In our body unstable free radicals gradually created because of natural biological and chemical process which is also called oxidative stress is the main culprit of cell damage. This cell damage is directly related to decay, diseases and death of human civilization.<sup>1</sup> Early brains had to think about the disease and its treatment when people are at the dawn of intellect, since disease, attrition and death was always consistent with life. For decades, medicinal plants are the most ancient and most successful ways of treatment of sickness and wounds. When they want to cure from different kind of illness then they start using plants as a medicine, as a consequence, they get outstanding therapeutic tools against illness.<sup>2,3</sup> Free radicals which are dangerous species created during combustion biological process. The antioxidant is reducing agents suppresses the human cell damage induced by reactive oxygen species and try to minimize the stress in human body.<sup>4</sup> It is important factor to balance between Reactive Oxygen Species and the antioxidant potential activity of human body for maintaining a good health. During the disease attack, the use of medicinal supplements which are prepared from various medicinal plants certainly is used due to imbalance condition between free radicals

and inherent antioxidant potential of the human body. Now many of the pharmacist, doctors, and researchers or scientists have attention an appreciation of antioxidants extracted from various alternative plants for the contribution to the indigenous systems of the world for the maintenance of the population health. Moreover, anti-reducing agents present in plant species protect the cellular defense and health system against oxidative spoil.<sup>5</sup>

*Spilanthes acmella* (*S. acmella*) is a well-known medicinal plant and it is a species of flowering herb in the family Asteraceae. This plant is commonly known as 'tooth-ache' plant obtainable in tropics, subtropics rear specially all over the India and African subcontinent.<sup>1</sup> It has been considered that different chemical compounds including spilanthal, sitosterol-O-D glucoside, stigma sterol, lauric acid, palmitic acid as their methyl esters, n-hexadecanoic acid, myristic acid etc. are available in the *Spilanthes acmella*.<sup>6</sup> At the ancient time, the poor and tribal people used this plant as a folklore remedy for toothache and gum infections. Moreover, in religious festival, different offertory of this plant with "Ajeng Dues" used to offer in Dobur Uie.<sup>7,8</sup> Literature revealed that it has various pharmacological activity, which include antifungal, antipyretic, local anesthetic, aphrodisiac, analgesic, pancreatic lipase inhibitor, antimicrobial,

antinociception, bioinsecticide, diuretic, toothache relieve and anti-inflammatory effects.<sup>9-14</sup> Therefore, the present study was to explore the antioxidant potentiality, proximate analysis and brine shrimp lethality bioassay of fractions from the *S. acmella* flowers.

## MATERIALS AND METHODS

### Instruments and equipment

Laboratory glassware i.e. test tube, conical flask, measuring cylinder, volumetric flask, beaker, funnel, pipette, digital balance machine (AGN 220C, AXIS, Poland), soxhlet extractor, Oven dryer (UM 400, Memmert GmbH, Germany), UV-Vis spectrophotometer (UV 1650 PC, Shimadzu, Japan), A pH (PHS 25, Clida Instrument), Rotary evaporator etc.

### Collection of plant

The flowers of *S. acmella* were collected from Noakhali, Bangladesh, 2017. The collected flowers were separated from unnecessary or unexpected materials or plant parts. Then the plant parts are air dried under shade for about 4 weeks after cutting small pieces. The dried pieces (flowers) were then oven dried for 24 h at a temperature below 40°C. The oven dried flowers parts were ground into powder with a fine mesh and the resulting flower powder was stored in a container in which there is no moisture and temperature is perfectly maintained until different investigation commenced.

### Proximate analysis

Proximate analysis is a reliable old technique that implemented to evaluate the quality of food, which are very much essential for nutrition analysis, for purity and quality of herbal drugs. For the evaluation of proximate analysis, the moisture content, total ash value, acid insoluble ash value and water-soluble ash value of a substance were investigated in the prepared samples using standard protocols.<sup>15</sup>

### Determination of Moisture Content

In a crucible precisely weighed 5 gm. of powdered of *Spilanthes acmella* flowers were taken. Then it was kept in a hot air oven at the temperature limit 105 – 110°C so that free moisture is eliminated easily. The moisture content was then calculated in percentage by following reported methods.

### Determination of Total Ash Value

Exactly weighed 5 gm. of powdered *Spilanthes acmella* flowers were taken in a dried silica crucible. Incineration is performed at 450°C temperature,

due to the elimination total amount of carbon and then cooled. The weight was taken and the total ash value in percentage was calculated.

### Determination of Acid Insoluble Ash Value

The total ash value was obtained by boiling with 25 ml of 2 N HCl for 5 minutes, filtered and the residue matter was collected on ash less filter paper. Then, it was washed with water, ignited in silica crucible for 15 min at a temperature below 450°C, cooled and residual weight received. The percentage of acid insoluble ash was calculated according to proper way of method.

### Determination of Water Soluble Ash Value

The ash was boiled in distilled water for few minutes, then filtrate the ash containing water to collect the insoluble matter in it. Then, it was ignited to constant weight at a temperature not exceeding 450°C, cooled and weighed the obtained residue. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample.

### Preparation of Extract

An effective and easy method is hot extraction in which Soxhlet extractor is used where the probable sample material has a partial soluble in a solvent and the contamination is insoluble.<sup>16</sup> For conventional extraction of solid samples (*S. acmella* flowers), solvent extraction procedure was implemented with different solvent for extract preparation. In this process, solvents are used for dissolving desired substance from the solid samples (dried flower powder) and the undesired substance does not dissolve, then remaining marc further extracted with next solvent.<sup>17</sup> Hot solvent extraction was used for extract preparation of dried flower powder of *S. acmella* (about 150 g) with n-hexane, chloroform, ethanol (600 ml) and water. Moreover, these solvents were used on the basis of polar and non-polar component extraction from dried sample at 60 to 80°C by using Soxhlet apparatus for 8 h. Aqueous extract of the sample was prepared by maceration for 3 days with distilled water and filtrate the residue. The various solvents extract were evaporated at rotary evaporator, then dry at very low temperature. After concentrating the extracts by evaporating the extra solvents, weighed sample extracts were stored for the assessments.<sup>5</sup>

### Preliminary Phytochemical Screening

Phytochemical screening of freshly prepared n-hexane, chloroform, ethanol and aqueous extracts from *S. acmella* flowers sample was carried out to identify the secondary metabolites such as alkaloid, flavonoid, reducing sugar, saponin,

phenolic compound, tannin, and amino acid using the standard methods.<sup>5,18</sup>

### Antioxidant potential

Three complex methods are used to investigate the antioxidant potential of the examined extracts of flower of *S. acmella* are total antioxidant capacity determination, determination of total phenolic content and total flavonoid content determination.

### Total antioxidant capacity determination

Phosphomolybdate tactics designed by Prieto et al.<sup>19</sup> was applied to explore the total antioxidant capacity of the plant's flower extracts by using ascorbic acid as a standard with slight modification. The content of reagent solution is 1 ml in which it was prepared from 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate for this investigation and later this was mixed with an aliquot of 0.1 ml of sample solution. For analysis, 0.1 mL (45% ethanol) was used in place of sample as blank. A capped tube in a water bath at 95°C for 90 mins must be kept in incubated carefully. After that the samples were cooled at normal temperature and determined the absorbance at 695 nm against a blank. The total antioxidant activity was identified with standard as sample absorbance at 695 nm.

### Total phenolic content determination

The phenolic concentration of plant's extracts of flower was determined by spectrophotometric method.<sup>20</sup> Firstly, Ethanolic and water solution were made from these to extract respectively with concentration of 1 mg/ml. To prepare a calibration curve, 0.5 ml aliquots of concentration ranging from 500 µg/ml to 3.9 µg/ml gallic acid solutions were mixed with 2.5 ml (75 g/L) sodium carbonate and 2.5 ml Folin-Ciocalteu reagent (diluted ten times with water) which is a combination of phosphomolybdate and phosphotungstate reduced the polyphenols containing ethanolic and water solution of the extract (concentration 1 mg/ml), thereby producing blue colored complex. The sample was there after incubated at 45°C temperature in a thermostat for 45 min. The absorbance was measured using spectrophotometer at 765 nm against reagent blank. After plotting the absorbance in ordinate vs. the concentration in abscissa a linear property was obtained to determine the samples gallic acid equivalent from the absorbance detected which was expressed as mg of GAE per g of sample extract.

### Total flavonoid content determination

To calculate the amount of whole flavonoid species, quercetin is usually used as a reference in which

this total flavonoid values are disclosed as quercetin equipotential. These flavonoids quantity was investigated by aluminium trichloride method in which a complex flavonoid-aluminium is formed.<sup>21</sup> The plant's extricates (0.5 ml of 1:10 g/ml) in ethanol were separately blended with 1.5 ml of ethanol and 0.1 ml of 10% aluminium chloride. Subsequently, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water also conjoined in that mixer. The temperature should be maintained at room temperature for 30 min and the absorbance of the mixture was kept at 415 nm. The calibration curve was made by preparing Quercetin solution at concentration 0, 0.25, 0.50, 0.75, 1.00 mg per ml in ethanol. Total flavonoids content was determined as mg of Quercetin equivalent per gram.

### Brine Shrimp Lethality Bioassay

The brine shrimp lethality represents an easy and mostly used bioassay guide for testing plant extract lethality for the isolation of antitumor and cytotoxic activity from medicinal plants. Following the reported<sup>22</sup> method with some modification, the lethality of the crude ethanolic and aqueous extract of *S. acmella* was determined. Firstly, *Artemia salina* (the brine shrimp eggs) were sited in 1 L of simulated sea water which is collected from pet shop, 48 h aeration was done at 37°C for brine shrimp nauplii from hatch. In the present study, 100 µl of DMSO was added to simulated sea water in a 5 ml vials as a negative control only and for control groups 10 shrimp naupliis were used. Ten living nauplii were added in each test tube with the help of pasteur pipette. 4 mg of each sample including both aqueous and methanol extracts were taken in two separate vials and samples were thoroughly mixed in the DMSO. 10 test tubes were taken for each sample marked for different concentrations. The vials with samples were under observation for 24 hr and found out that there was number of surviving naupliis. At different concentrations, dissimilar mortality rates were found on each test samples; the 50% concentration mortality data of brine shrimp nauplii (median lethal concentration, LC<sub>50</sub>) were analyzed by a curve of percentage of the shrimps killed against the logarithm of the sample concentration.

## RESULTS AND DISCUSSION

### Proximate analysis

Proximate analysis is an important experiment to know the classes and percentage of nutrients present in the sample. [Figure 1](#) shows the evaluated total ash value, acid insoluble ash, water soluble ash and moisture content present in *S. acmella* samples. The moisture content, total ash value, acid insoluble

**Table 1** Phytochemical compound in the different extracts of *s. acmella*

Plant name	Phytochemical composition	Results of different extracts			
		n-hexane	Chloroform	Ethanol	Water
<i>Spilanthes acmella</i> (flower)	Alkaloid	-	-	+	-
	Reducing sugar	-	-	-	-
	Flavonoid	+	-	+	-
	Saponin	-	-	-	-
	Phenolic compound	-	-	+	+
	Tannin	-	+	-	-
Amino acid	+	-	+	-	

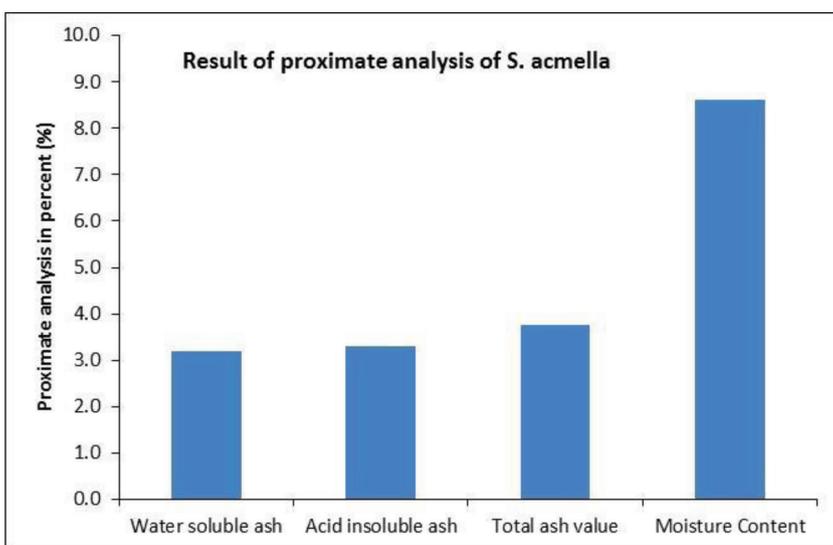
(+) Present, (-) Absent, (++) Significantly present

**Table 2** Total Phenolic and Flavonoid Content of *S. Acmella* Flower

Name of Plant Extract	Total Phenolic content (mg gallic acid equivalent/gm)	Total flavonoid ( mg quercetin/gm)
n-hexane	-	13.5
Chloroform	-	-
Ethanol	71.5	40.8
Water	25.9	-

**Table 3** Effect of extract of *s. acmella* on brine shrimp nauplii

Sample/Extract	LC <sub>50</sub>
Dimethyl Sulfoxide (DMSO)	1.31
n-hexane	-
Chloroform	-
Ethanol	1.20
Water	0.85

**Figure 1** Different amount in percent of proximate analysis of *s. acmella* flower

ash and water soluble ash value were found 8.6%, 3.76%, 3.30%, 3.20% respectively.

From the Figure 1, it is clear that low moisture content of the flower of *S. acmella* reveals that it would hinder the growth of microorganism and long life storage property would be high. Moreover, knowing the moisture content of a substance helps to determine if that substance is suitable for a specific use. The ash content of 3.76 % indicates that the flower is rich in mineral elements. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Total ash value determination is important because it indicates the quality and purity of a crude drug.<sup>15</sup>

### Phytochemical analysis

The experimental results of phytochemical screening showed the prominent presence of alkaloid, flavonoid, amino acid, tannin, phenolic compound in the extract of *S. acmella*. On the other hand, no extracts of *S. acmella* has reducing sugar, saponins (Table 1).

### Antioxidant activity

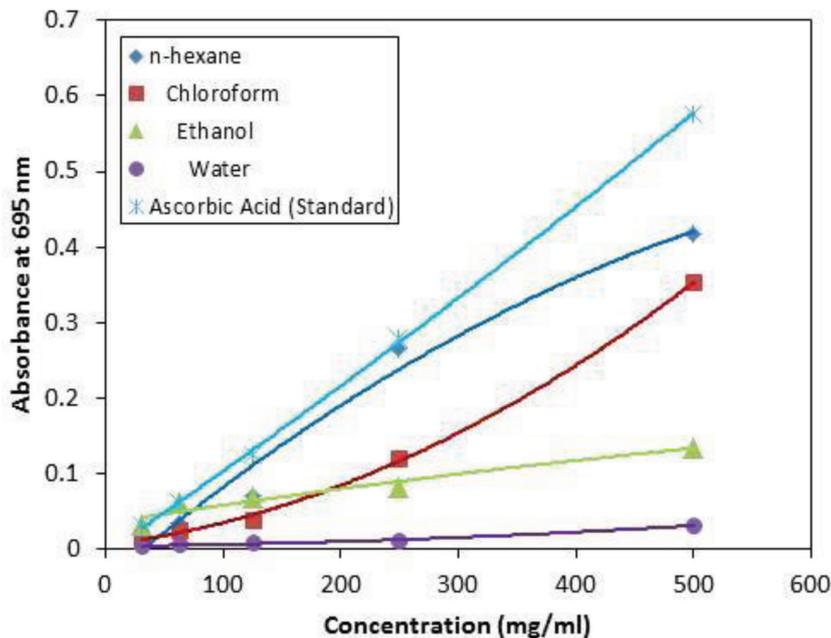
#### Total Antioxidant Activity

The absorbance at 695 nm versus concentration of different extracts and standard curves were shown in Figure 2. Based on the reduction of Mo (VI) to Mo (V), total antioxidant activity (TAC) of the different extract was determined in which maximum absorbance is appeared at 695 nm. The phosphomolybdate method is quantitative, since the total antioxidant capacity of different extract is expressed as ascorbic acid equivalents were shown in Figure 3. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacities (total antioxidant capacity).

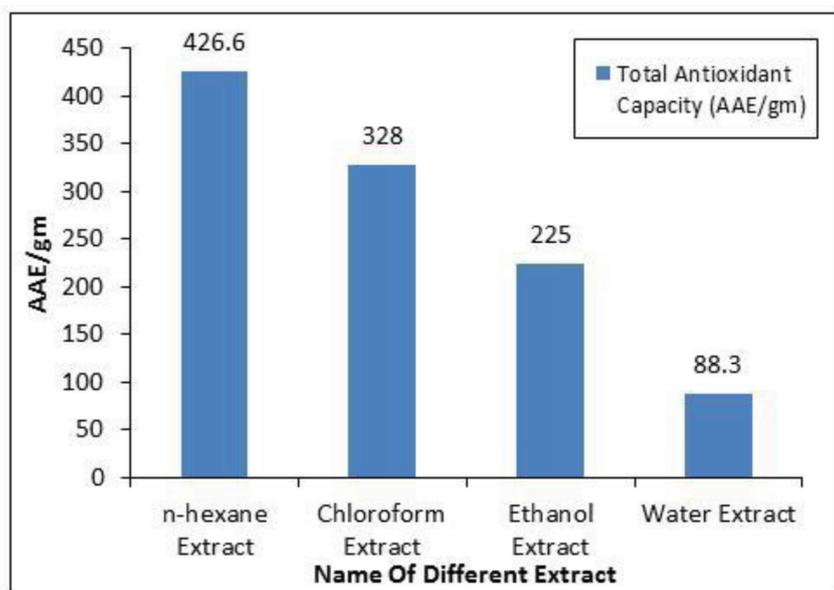
From Figure 3, the antioxidant capacity of various solvent extracts (500 µg/ml) of *S. acmella* flowers were found to increase in this order: water (88.3 AAE/g) > ethanol (225 AAE/g) > Chloroform (328 AAE/g) > n-hexane (426.6 AAE/g). The present study demonstrated that n-hexane extract exhibited the highest total antioxidant capacity for phosphomolybdate reduction.

### Determination of Total Phenolic and Flavonoid Content

Phenol is significant plant ingredients due to their oxygen molecule quencher hydroxyl groups. In the primary phytochemical screening, only ethanol and water extracts exhibited the existence of phenol and so these two extracts were tested for total phenolic content in which total phenolic content of the flower



**Figure 2** Total antioxidant capacity of different extracts of *s. Acemella* flowers with standard



**Figure 3** Total antioxidant capacity of different solvent extracts for 500 ppm at 695 nm of *s. Acemella*

extracts of this plant was expressed as milligrams of gallic acid equivalents (GAE). The phenolic content of ethanol and water extracts (71.5 GAE/g and 25.9 GAE/g) in which ethanol extract show higher concentration. The most conventional method was used to determine the total flavonoid content, i.e. aluminium chloride colorimetric method in which  $Al^{3+}$  forms color that gives an abundant absorbance at 415 nm. The presence of Flavonoid content was found in n-hexane and ethanol in group test results (13.5 & 40.8 mg quercetin equivalent/g of extract) (Table 2).

### Lethality Bioassay

Brine shrimp lethality bioassay is mainly performed for the evaluation of cytotoxicity of *S. acmella* flower. In cytotoxicity study,  $LC_{50}$  values of crude ethanol was found to be 1.20  $\mu\text{g/ml}$ , compared with the negative control DMSO showed  $LC_{50}$  at 1.31  $\mu\text{g/ml}$  concentration (Table 3). Besides water extract showed least amount of this property. So ethanol crude extract showed magnificent antitumor and cytotoxicity activity than water extract.

### CONCLUSION

The flower of plant *Spilanthes acmella*, (Family-Asteracea) was investigated for its physicochemical, antioxidant and phytochemical screening. From the investigation it is easily said that the whole plant of *Spilanthes acmella* is reported to exhibit good medicinal values in traditional system of medicines especially for various diseases. It is easily said from the above discussion the phenolic content of this flower extract demonstrated special anti-oxidative property by which it provide to stop oxidation chain reaction. In addition this extracts also exhibited better high storage life property and high quantity of mineral elements (proximate analysis). Further study, however, is required to detect the active compounds responsible for perceived properties and also identify and poof of the better treatment of cancer or antitumor activity for its cytotoxicity properties.<sup>5</sup>

### ACKNOWLEDGMENT

The authors are highly acknowledged all the provided facilities; specially laboratory support by the Department of Applied Chemistry and Chemical Engineering, Noakhali Science and Technology University.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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