# Phytochemical Screening, Pharmacognostic Evaluation and Biological Activity of Amaranthus spinosus L

## Abstract

Herbal medicines possess a great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. People living in rural areas of Nepal depend largely in the herbal medicines for the treatment. The present study concerns with the study of pharmacognostic characteristics, phytochemical constituents and the biological activity of Amaranthus spinosus L. The hexane, chloroform, ethanolic and aqueous extract of its aerial parts were subjected to preliminary phytochemical analysis and detected saponin, carbohydrate, tannin, protein, glycoside, flavonoid and phenol as phytoconstituents. The transverse section of the stem and leaves showed characteristic vascular bundle tissues. All the extracts were used for testing the antimicrobial activity and brine shrimp lethality. No extract showed the antimicrobial activity and ethanolic extract showed LC50 value of 31.62 ppm.

Hexane, chloroform and water extracts were less toxic with LC50 values 236.88 ppm,194.98 ppm and 320.71 ppm respectively.

Keywords: Antimicrobial activity, brine shrimp lethality phytochemical analysis, vascular bundle.

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## Introduction:

Herbal medicines possess a great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. People living in rural areas of Nepal depend largely in the herbal medicines for the treatment. This shows the importance of plants in the health care system. Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources [1].

Amaranthus spinosus Linn. (Spiny amaranth- English; Ban lunde- Nepali; Tanduliyah- Hindi) has its own traditional use. The tender leaves are eaten as vegetable by the Tamangs of Kathmandu valley. Decoction of leaf and root is taken for intestinal disease at Kathmandu valley. Root juice is taken with cold water in the morning to treat painful urination and is also taken with warm water before going to bed to break and dissolve gravel and to pass it out along with urine. In Central part of Nepal, tender leaves are eaten as vegetable[2-4]. Young shoot is taken as vegetable after proper boiling by the Tamangs of Kathmandu valley. Decoction of leaves and root is also taken for intestinal disease at Kathmandu district. Root paste is applied on boils to remove pus and root juice is recommended in case of fever. Crushed leaves and roots are applied to skin infections, wounds and rheumatic areas.

In the eastern parts of Nepal, decoction of roots with warm water is taken orally by the Satars (a indigenous caste in Terai area of Nepal) of Morang and Jhapa districts to check excessive bleeding at post delivery stage 1 [5].

# Original Article

## Material and Methods

**Collection of Plant Material:** The plant material of Amaranthus spinosus was collected from Kathmandu valley at their flowering season and authenticated as Amaranthus spinosus at National Herbarium and Plant Laboratory, Godavari, Lalitpur, Ministry of Forest and Soil Conservation, Government of Nepal.

**Extraction:** The aerial parts were shade-dried in laboratory of National Model College for Advanced Learning, Khushibu, Kathmandu. They were randomly sampled, foreign organic matter (FOM) were removed and grinded with a grinder to obtain the powder. A sieve of 500 micron was used for sieving powder. 400 g of sieved powder was extracted in soxhlet's extractor using solvent of increasing polarity starting from n-Hexane (nonpolar) to Chloroform, Ethanol and Water. The extracts were concentrated by rotatory evaporator and they were stored at 4°C for phytochemical and biological evaluation.

**Phytochemical Screening:** Phytochemical screening was performed using standard procedures[6,7 ]. By using different specific reagents, the presences of main groups of natural products were detected in n-Hexane (nonpolar) to Chloroform, Ethanol and Water extracts.

a. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

i) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a cream precipitate indicates the presence of Alkaloids.

ii) Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

iii.) Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

b. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

i.) Molisch's Test: Filtrates were treated with 2 drops of alcoholic a - naphthol solution in a test tube and 2 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of crbohydrates.

c. Detection of cardiac glycosides

i.) Killer-killani test: To 0.5 gm of extract diluted to 5ml with distilled water and add 2 ml of glacial acetic acid and containing one drop of ferric chloride solution. This was underplayed with 1 ml of conc. sulphuric acid. Brown ring at the interface indicates the presence of a deoxysugar characteristic of cardenolides.

d. Detection of saponins

i.) Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

e.) Detection of phytosterols

i.) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.

f.) Detection of proteins and aminoacids

i.) Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins.

**Pharmacognostic study:** Macroscopical and microscopical features were studied.

**Macroscopical characterization:** Macroscopical studies of leaf and stem were done by naked eye as well as magnifying glass. Shape, color, taste, odor and other characteristic features of leaf and stem were determined and recorded.

### Microscopical characterization

Softening and Sectioning: The samples were softened in cold water for 6 hour. After that, the leaves as well as stem through the internode were cut transversely with the help of stainless steel blade.

**Permanent Mounting:** After 24 hours of fixing, the specimens were dehydrated with graded series of ethyl alcohol. The section was stained with phloroglucinol –hydrochloric acid (1:1) and mounted in Canada balsam to prepare the permanent slide.

**Observation and Photography:** At first, the slide with section was placed under the low power of microscope to observe the whole view. After this the whole section is fixed under the high power of microscope to observe the detailed tissues system. Finally, the sections were photographed with Canon camera.

## Antimicrobial Evaluation

Bacterial Strains: Four bacterial strains were used to assess the antibacterial activities of the test samples, two Gram-positive and two Gram-negative bacteria. The Gram-positive bacteria were Staphylococcus aureus (ATCC 25923), Bacillus cereus (clinical isolate) and two Gram-negative bacteria were Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 14028). All these microorganisms were obtained from Microbiology Laboratory of National Model College for Advanced Learning, Khushibu, Kathmandu.

Disc Diffusion Method: Antimicrobial activity of each extract were carried by disc diffusion method. Bores were made on the medium using sterile borer and 50 micro litre of each extract was added to respective bore and 50 microlitre of the standard antibiotic (0.01 % Ciprofloxacin) was taken as standard. The Petri plates seeded with organisms, containing extracts and the standard were kept in refrigerator at 40°C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion the Petri plates were incubated at 37° ± 1°C for 24 hours in a BOD incubator and zone of inhibition was observed and measured manually using a scale.

Brine Shrimp Bioassay: Samples were prepared by dissolving 10 mg of each crude extract in 1ml of DMSO to give stock solutions. Four different concentrations namely 1000ppm, 500ppm, 250ppm and 125ppm of each extract were prepared by serial dilutions from the stock solution in artificial sea water in respective test tubes. An aliquot of each concentration (10ml) was transferred, in triplicate, into clean test tubes. Ten shrimp nauplii (Artemia salina) were transferred to each test tube. DMSO in seawater was used as negative control. The test tubes were then incubated for 24 hours at 27°C.

After 24 h the numbers of survivors in each triplicate were counted against illuminated background and percentage of death at each dose level and control was calculated using following formula:

$$\% \text{ mortality} = \frac{\text{numberof death shrimps}}{\text{number of death+ alive shrimps}} \text{X } 100$$

Those nauplii which did not show any movement were considered dead.

The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the naupulli (LC50) was determined from the graph using MS-Excel.

## Result

### 1.Extractive value:

The extractive value for the powdered drug powder was higher for extraction with ethanol, followed by chloroform extract, hexane extract and aqueous extract respectively.

Weight of the dried powder of aerial part of the plant=130 gm

Table 1: Extractive value

| S.N | Solvents | Wt of extract (gm) | % yield |
|---|---|---|---|
| 1 | Hexane | 2.017 | 1.55 |
| 2 | Chloroform | 2.898 | 2.22 |
| 3 | Ethanol | 6.879 | 5.29 |
| 4 | Aqueous | 1.9 | 1.46 |

### 2. Phytochemical Screening

The phyto-constituents of different extracts of aerial parts of Amaranthus spinosus were as follows. Hexane extract showed the presence of proteins, glycosides, flavonoid and phenol. Chloroform extracts showed the presence of tannin, glycoside, flavonoid and phenol. Ethanolic extracts showed the presence of saponin,

protein, flavonoid, phenol, cardiac glycoside. Aqueous extract showed the presence of carbohydrate, protein, flavonoid and phenol. The results were found to be similar as mentioned in the literature . The result is given in Table 2.

Table 2: Phytochemical Screening of Different Extracts of Amaranthus spinosus

| SN | Phytoconstituents | Extracts | | | |
|---|---|---|---|---|---|
| | | Hexane | Chloroform | Ethanol | Aqueous |
| 1 | Alkaloid | - | - | - | - |
| 2 | Saponin | - | - | + | - |
| 3 | Carbohydrate | - | - | - | + |
| 4 | Tannin | - | + | - | - |
| 5 | Protein | + | - | + | + |
| 6 | Phytosterol | - | - | - | - |
| 7 | Cardiac glycoside | + | + | + | - |
| 8 | Reducing sugar | - | - | - | - |
| 9 | Flavonoid | + | + | + | + |
| 10 | Phenol | - | + | + | + |

Note: Presence + ; Absence –

## 3. Pharmacognostic Evaluation

## 3.1Macroscopical Characters:

Stem are obtusely angular, slightly pubescent and green. The leaves are simple and alternate without stipules. Petiole is approximately as long as the leaf blade. The blade shape is ovate-lanceolate, acute and often slightly decurrent at base, obtuse apex, entire margin, glabrous or slightly pubescent. The inflorescence consists of dense clusters, lower ones are axillary, higher ones often collected in an axillary and terminal spike which is often branched in its lower part. Axiliary clusters are usually armed with very sharp spines up to 2 cm long. Its flowers are unisexual, solitary in the axil of a bract, subtended by 2 bracteoles; bracts and bracteoles scarious, mucronate from a broad base, shorter or as long as the perianth. Male flowers are usually arranged in a terminal spike above the base of the inflorescence, green. Female flowers with superior, oblong ovary.

## 3.2 Microscopical characters

The transverse section of the stem showed the presence of following tissue item

In microscopic studies, leaf was found to be dorsiventral and shows all the typical characteristics of leaf, as lamina shows epidermis, upper palisade and middle spongy parenchyma. Midrib region shows upper and lower epidermis, collenchymas and centrally vascular bundle as phloem surrounds with the xylem.

## 4. Brine Shrimp Lethality Test

The brine shrimp lethality assay showed that the ethanol extracts is mildly toxic. Ethanol extracts showed LC50 value of 31.62 ppm. Hexane extract, chloroform extract and water extracts are non toxic.LC50 values are 236.88ppm,194.98 ppm and 320.71ppm respectively.

Table 3: Brine shrimp lethality assay of ethanol extract

| S.N | Concentration (ppm) | Log concn | Average no. of live nauplii +S.E.M | % Mortality | $LC_{50}$ ppm |
|---|---|---|---|---|---|
| 1 | Control | 0 | 10 +0 | 0 | 31.62 |
| 2 | 1000 | 3 | 0.33 +0.33 | 96.67 | |
| 3 | 500 | 2.69 | 0.33+0.33 | 96.67 | |
| 4 | 250 | 2.39 | 2+0.577 | 80 | |
| 5 | 125 | 2.09 | 3+0 | 70 | |

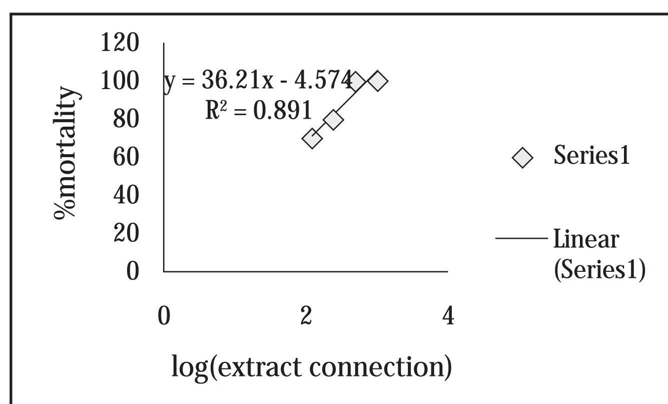

Table 4: Brine shrimp lethality assay of chloroform extract

| S.N | Concentration (ppm) | Log concn | Average no. of live nauplii +S.E.M | % Mortality | $LC_{50}$ ppm |
|---|---|---|---|---|---|
| 1 | Control | 0 | 10 +0 | 0 | 194.98 |
| 2 | 1000 | 3 | 1 +0.0.577 | 90 | |
| 3 | 500 | 2.69 | 3.67+0.33 | 63.33 | |
| 4 | 250 | 2.39 | 3.67+0.88 | 63.33 | |
| 5 | 125 | 2.09 | 6+1.5 | 40 | |

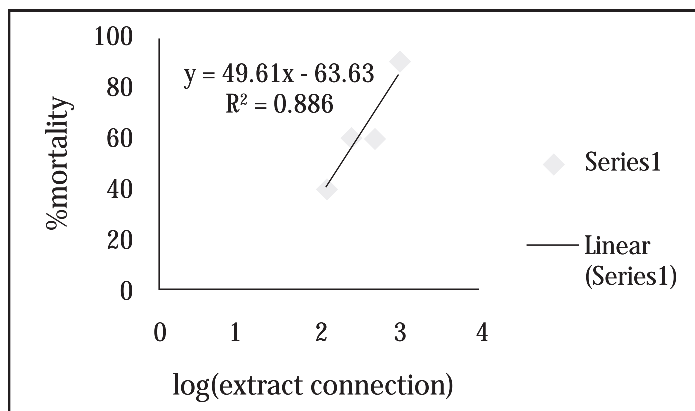


Table 5: Brine shrimp lethality assay of hexane extract

| S.N | Concentration (ppm) | Log concn | Average no. of live nauplii +S.E.M | % Mortality | $LC_{50}$ ppm |
|---|---|---|---|---|---|
| 1 | Control | 0 | 10 +0 | 0 | 236.88 |
| 2 | 1000 | 3 | 1 +0.0.577 | 90 | |
| 3 | 500 | 2.69 | 3.33+1.2 | 66.67 | |
| 4 | 250 | 2.39 | 6+0.577 | 40 | |
| 5 | 125 | 2.09 | 4.33+1.2 | 40 | |

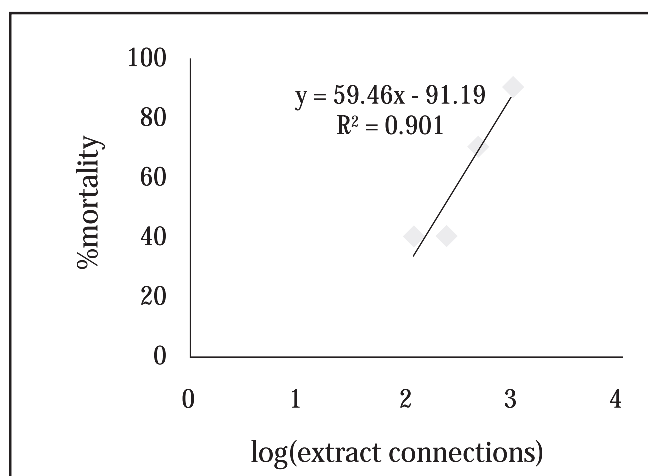


Table 6: Brine shrimp lethality assay of aqueous extract

| S.N | Concentration (ppm) | Log concn | Average no. of live nauplii +S.E.M | % Mortality | $LC_{50}$ ppm |
|---|---|---|---|---|---|
| 1 | Control | 0 | 10 +0 | 0 | 320.71 |
| 2 | 1000 | 3 | 2+ 1 | 80 | |
| 3 | 500 | 2.69 | 3.67+1.8 | 63.33 | |
| 4 | 250 | 2.39 | 7.33+0.33 | 26.67 | |
| 5 | 125 | 2.09 | 7.33+0.33 | 26.67 | |

## Conclusion

The transverse section of the stem and leaves showed characteristic vascular bundle tissues. The phytochecmical screening revealed the presence of different phytoconstituents like carbohydrate, protein, saponin, tannin, cardiac glycoside, flavonoid and phenol. All extracts of plants were used for testing biological activity where no extract showed the antimicrobial properties. The ethanolic extract showed the highest toxicity with LC50 value of 31.62 ppm and the toxicity decreases

## Acknowledgement

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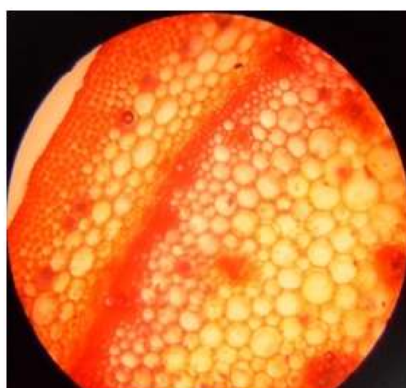
Annex – 1: Picture of Amaranthus spinosa

T.S of stem



T.S of stem