



Study of Antioxidant and Antimicrobial Screening of *Amaranthus spinosus* and *Achyranthes aspera* Leaves Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The current study aims to evaluate the physiochemical, phytochemical, and antioxidant properties of *Achyranthes aspera* and *Amaranthus spinosus* leaf extracts using a water and methanol solvent system. In India, these plants play an important role in primary healthcare as therapeutic remedies. Traditional healers claim that addition of *Achyranthes aspera* would enhance the efficacy of any drug of plant origin. The antimicrobial activity of the prepared extracts was determined by using disc diffusion method. Both plants had a rich amount of valuable ingredients that are beneficial for health the physiochemical and phytochemical parameters and this can be useful to identify the drug and to establish its quality and purity.

Keywords: *Achyranthes aspera*; *Amaranthus spinosus*; antimicrobial activity.

1. INTRODUCTION

For many centuries and even today plants have provided mankind with remedies for many diseases. In India there are 47000 plant species of which 15000 are reported to have medicinal properties. These plants play a major role in

primary healthcare as therapeutic remedies in India. It is further claimed that these plants have been the bases of treatment and cure for various diseases in India [1].

The Amaranthaceae family includes *Achyranthes aspera* Linn and *Amaranthus spinosus* Linn.

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Achyranthes aspera Linn is a perennial stiff erect plant that grows to a height of 1000 metres. Square stems, elliptic ovate or widely rhombate leaves, 5-22 cm long, 2.5 cm wide, and adpressed pubescent. The inflorescences are 8 - 30 cm long and include several solitary, 37 mm broad white or crimson blooms. Summer is when the flowers bloom. The main root is long and thick, slightly ribbed, and yellowish brown in colour; the secondary and tertiary roots are slightly ribbed, yellowish brown in colour; the odour is mild, and the taste is slightly sweet and mucilaginous; the stem is yellow brownish, erect branched, cylindrical hairy, and about 60 cm tall. Subcylindrical, truncates at apex, rounded at base, black, and gleaming seeds. The plant may be found all throughout India, up to a height of 3000 feet [2].

In English, *Achyranthes aspera* L. is also known as "Prickly chaff flower." Traditional healers revere the plant, which is used to treat asthma, bleeding, facilitating delivery, boils, colds, coughs, colic, debility, dropsy, dog bites, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bites, snake bites, and skin diseases, among other ailments. Traditional healers believe that adding *Achyranthes aspera* to any plant-based medication will improve its efficacy.[3]

Amaranthus spinosus Linn. (*A. spinosus* L.) is believed to originate from South and Central America and then introduced into various regions of Africa specially south tropical African countries such as Zimbabwe, Botswana, Malawi, Zambia and Namibia. The plant is also widely distributed in waste places, roadsides and fields in Bangladesh, Ghana, Cambodia, Philippines, Maldives, Japan, Sri Lanka, Myanmar, Indonesia, Australia and India [4]. *A. spinosus* L. grows annually as an erect perennial herb with many branches. Stems are hard, terete or obtusely angular and greenish to purple in colour. Leaves are alternate, have bitter taste with a characteristic odour. Flowers are numerous, appear throughout the year. Fruit is ovoid shaped. Seed is shiny, black or brownish-black in colour. [5,6].

A. hypochondriacus L. is known in India as "rajgeera" (King's grain) and is frequently popped for use in confections known as "laddoos," which are comparable to Mexican "alegria." Amaranth seeds are consumed in Nepal as a gruel known as "sattoo" or processed into a flour for making chappatis [7]. Amaranth, a heritage of the Atecs,

Mayas, and Incas, is an under-utilized plant with a potential economic value owing to the range of uses and advantages it may bring to growers, processors, and consumers [8].

Amaranth grain is a gluten-free pseudo-cereal that may be found in morning cereals, pancakes, soup, breads, cookies, gluten-free meals, extruded snacks, and confections [9]. South Americans grind it to make light-colored flour or parch it to make gruel or porridge. The grain is popped and tastes like nutty-flavored popcorn as a snack. It's also good when combined with honey [10]. Amaranth flour may be utilised to partially replace ordinary maize flour in extruded snack manufacture, according to Ljubica et al[11]. Xaene et al. have discovered that a combination of instant whole Amaranth and rice may be utilised to make extruded flours for use in beverage formulations [12]. Rosa et al. claim that defatted Amaranth flours may be used to make extruded snacks [13].

The purpose of this study was to evaluate physiochemical, phytochemical, and antioxidant content of *Achyranthes aspera* and *Amaranthus spinosus* leaf extracts, as well as to detect the presence of natural therapeutic agents, particularly those related to the control of microbes that cause diseases in humans, using a water and methanol solvent system.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Medicinal plants were selected by the help of local herbal healers and the fresh leaves of selected medicinal plants, were collected from Akshat Nurseykarond Bhopal (M.P), during March to June, 2020. These were further identified by Dr. Saba Naaz, HOD Department of Botany, Saifia Science College Bhopal, and specimens have been submitted and preserved in the Department of Botany, The Specimen voucher no. (*Achyranthus aspera* is 196/Saif/Sci/Clg/Bpl) and (*Amaranthus spinosus* 197/Saif/Sci/Clg/Bpl) dated 17/6/2020. Because fully grown leaves have a higher metabolism than young leaves, the matured leaves from the plants were chosen. The specimens were dried at room temperature and kept at 4°C in polyethylene bags.

2.2 Preparation of Plant Extract

The plants' substance was extracted hydroalcoholically by suspending 100 grammes

of powders from both plants in 1000 ml of methanol and water (80:20, methanol: water, v/v). At 37°C, the extraction was allowed to sit for 72 hours. The extracts were filtered through cotton wool first, then Whatman filter paper No. 1 (125 mm), before being dried in a rotary evaporator. They were placed in sterilised bottles and stored in the refrigerator until needed.

2.3 Phytochemical Analysis

Standard phytochemical screening protocols were used to look for tannins, alkaloids, glycosides, flavonoids, and phenolics[14].

Qualitative analysis of phytochemicals

alkaloids: The extracts were evaporated to dryness, and the residues were heated with 2 percent hydrochloric acid in a boiling water bath, cooled, filtered, and treated with Mayer's reagent. After that, the sample was examined for yellow precipitation or turbidity [14].

Flavonoids: To 4 ml of extracts, 1.5 ml of 50 percent methanol was added. Fill with magnesium filings and a few drops of corrosive hydrochloric acid when they've warmed up. Flavonoid presence is indicated by a pink or red tint[4].

Tannins: A quantity of the extract was diluted 1:4 with distilled water, then a few drops of a 10% ferric chloride solution were added. The presence of tannins is indicated by a blue or green tint[15].

Saponins: The methanol extract was heated in modest amounts. The mixture was filtered, and 2.5 mL of the filtrate was added to 10 mL of distilled water in a test tube, shaken vigorously for 30 seconds, and the foaming observed [14].

Glycosides: Fehling's reagent was added to a methanol extract and heated for 2 minutes. The presence of glycosides is indicated by a brick red colour.

2.4 Quantitative Analysis

Total phenolic contents (TPC) and total flavonoid contents (TFC): The Folin-Ciocalteu reagent technique was used to quantify total phenolic contents (TPC), and Gallic acid was employed as the Gallic acid Equivalent (GAE). The total flavonoid content (TFC) in leaf extracts was measured using a modified technique, with

Quercetin serving as a standard (Quercetin Equivalent) (QE) [16].

Antioxidant activity

DPPH radical scavenging assay: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically. The percent inhibition was calculated as:

$$\% \text{ inhibition} = \frac{\text{normal activity} - \text{inhibitory activity}}{\text{Normal Activity}} \times 100 \%$$

A sample is the absorbance of test samples, whereas A blank is the absorbance of the control reaction (which contains all reagents except the test sample). The plot of percentage against concentration was used to produce IC50 values, which reflect the concentration of Selected plants that induced 50% inhibition.

Determination of antioxidant activity in linoleic acid system:

The oxidation of linoleic acid was used to test the antioxidant activity of the chosen plant extracts materials. 5 mg of chosen plant extracts were added to a solution of 0.13 mL linoleic acid, 10 mL 99.8% ethanol, and 10 mL 0.2 M Sodium Phosphate buffer (pH=7) individually. With distilled water, the combination was prepared up to 25 mL and incubated at 40°C for 360 hours. Peroxide value was used to determine the extent of oxidation using the Thiocyanate procedure. 10 mL ethanol (75 percent v/v), 0.2 mL aqueous Ammonium Thiocyanate (30 percent w/v), 0.2 mL sample solution, and 0.2 mL ferrous chloride (FeCl₂) solution (20 mM in 3.5 percent HC1; v/v) were added in that order. A spectrophotometer was used to detect the absorbance at 500 nm after 3 minutes of churning. All reagents were present as a negative control, with the exception of extracts. Antioxidants that are synthesized as a positive control, butylated hydroxytoluene (BHT) (or ascorbic acid) was utilised. In the sample with no antioxidant component, the highest per oxidation level was found at 360 hours (15 days). The following calculation was used to compute the % inhibition of linoleic acid oxidation:

$$100 - \frac{\text{Absorption increase of sample at 360 h}}{\text{Absorption increase of control at 360 h}} \times 100$$

Determination of Reducing Power: The spectrophotometric approach was used to determine the reduction power of the chosen

plant extracts. The [17]concentrated extract (0-10.0 mg) was combined with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0 percent) and incubated for 20 minutes at 50°C. The mixture was then centrifuged at 980 g for 10 minutes at 5°C in a chilled centrifuge with 5 mL of 10% Trichloro Acetic Acid added. The solution's top layer (5.0 mL) was decanted and diluted with 5.0 mL distilled water and 1.0 mL ferric chloride (0.1 percent), and the absorbance was measured at 700 nm with a spectrophotometer. All samples were examined three times, with the averaged findings.

Antimicrobial activity Microbial strains: Microbial strains included *Staphylococcus aureus* and *Escherichia coli*, as well as two dangerous fungus (*Fussarium solani* & *Rhizopus oligosporus*). The germs were transported from Bhopal's J.P. Hospital. In an incubator, these bacterial and fungal strains were cultivated overnight at 37°C and 28°C.

Disc diffusion method: The disc diffusion technique [18] was used to assess the antibacterial activity of the produced extracts. The inoculated extracts were then tested for inhibitory zones (in mm), which indicate antibiotic activity, using a zone reader. The discs (6 mm in diameter) were inoculated with 20 g/ disc of sample extracts and put on inoculated agar. For bacteria and fungi, Ciprofloxacin (20 g/disc) and Fluconazole (20 g/disc) were employed as positive references, respectively[18].

3. RESULTS AND DISCUSSION

The presence of medicinally active components was discovered in the plant samples studied. Tables 1 and 2 show the % yield of *Amaranthus spinosus* & *Achyranthes aspera*, respectively. Table 2 shows the presence of alkaloids, tannins, saponins, and glycosides.

Table 3 summarises the quantitative assessment of the % crude chemical contents in *Amaranthus spinosus* & *Achyranthes aspera*. The % yield and

antioxidant activity of *Amaranthus spinosus* & *Achyranthes aspera* leaf extracts are shown in Table 3. Hydro alcoholic leaf extracts had the greatest DPPH activity, TP, and TF concentration, as demonstrated in Table 3.

Table 4 shows that the extract has a larger % inhibition of linoleic acid oxidation than the reference medication, BHT, implying that it has a significant and stronger antioxidant activity. Furthermore, for the *Amaranthus spinosus* & *Achyranthes aspera*, the percentage inhibition of linoleic acid oxidation was (65%) and (64%) correspondingly. Ferrous ions (Fe²⁺) exist only transiently in the presence of oxygen and phosphate ions (PO₄²⁻) at physiological pH (7.4) before being auto-oxidized to ferric ions (Fe³⁺). By the Fenton reaction, one electron is transferred from iron to oxygen, forming a superoxide radical anion and a hydroperoxyl radical (HO₂[•]).

The high reducing power of the aqueous extract of *Amaranthus spinosus* leaves, which was concentration dependent, suggests that the extract could be effective in reducing the transition state of iron and thus the rate at which super oxide and hydroperoxyl radicals are generated from the metal under physiological conditions. Table No. 5 A substantial link has been shown between total phenolic content and lowering activity in fruits and vegetables [17]. As a result, the extract's reducing capability can be linked to its phenolic concentration.

3.1 Antimicrobial Activity

In the disc diffusion experiment, extracts from *Amaranthus spinosus* leaves and *Achyranthes aspera* leaves revealed significant antibacterial activity. Tables 6 and 7 reveal the quantitative antibacterial activity of *Amaranthus spinosus* and *Achyranthes aspera* leaf extracts against food-borne and pathogenic microorganisms. Antifungal activity followed the same pattern as antibacterial activity, with the exception that fungal strain efficacy was not as high as bacterial strain efficacy.

Table 1. Data showing yields of Hydro alcoholic of powdered leaf of *Amaranthus spinosus* and *Achyranthes aspera*

Extracts	<i>Amaranthus spinosus</i> % yield (w/w)	<i>Achyranthes aspera</i> % yield (w/w)
Hydro alcoholic	5.37%	6.47%

Table 2. Phytochemical constituents of hydro alcoholic leaf extracts of *Amaranthus spinosus* and *Achyranthes aspera*

Phytochemical constituent	<i>Amaranthus spinosus</i>	<i>Achyranthes aspera</i>
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+

+: Presence of the phytochemical constituent; -: Absence of the phytochemical constituent

Table 3. Percentage yield and Quantitative estimation of hydro alcoholic leaf extracts of *Amaranthus spinosus* and *Achyranthes aspera*

Plants	% age yield*	TPC contents	TFC contents
<i>Amaranthus spinosus</i>	6.3 ± 0.23	2.1 ± 0.8	3.66 ± 0.10
<i>Achyranthes aspera</i>	6.1 ± 0.25	2.6 ± 0.2	4.4 ± 0.30

Values are mean ± SD of samples analyzed individually in triplicate. Total phenolic contents in Gallic acid equivalent. Total flavonoid contents in Quercetinequivalent

Table 4. Percent inhibition of linoleic acid per oxidation of hydro alcoholic leaf extracts of *Amaranthus spinosus* and *Achyranthes aspera*

Plants	DPPH assay* ($\mu\text{g/mL}$) IC ₅₀	Inhibition of per oxidation* %
<i>Amaranthus spinosus</i>	16.21 ± 2.9	62.2 ± 2.9
<i>Achyranthes aspera</i>	19.45 ± 4.84	66.4 ± 3.5

*average of three determinations (mean ± SD)

Table 5. Reducing power of hydro alcoholic leaf extracts of *Amaranthus spinosus* and *Achyranthes aspera*

Concentration mg/ ml	<i>Amaranthus spinosus</i>	<i>Achyranthes aspera</i>	BHT
2	0.08 ± 0.01	0.10 ± 0.01	0.73 ± 0.04
4	0.41 ± 0.01	0.33 ± 0.07	0.95 ± 0.05
6	0.70 ± 0.06	0.69 ± 0.09	1.14 ± 0.06
8	0.88 ± 0.08	1.91 ± 0.12	1.54 ± 0.08
10	1.39 ± 0.03	1.58 ± 0.13	1.80 ± 0.09

*average of three determinations (mean ± SD)

Table 6. Antimicrobial activities of hydro alcoholic leaf extracts of *Amaranthus spinosus* and *Achyranthes aspera*

Plants (Leaves)	Zones of growth inhibition*(mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>F. solani</i>	<i>R. oligosporus</i>
Ciprofloxacin	28±0.20	25±0.21	-	-
Fluconazole	-	-	22±0.21	20±0.50
<i>Amaranthus spinosus</i>	25±0.15	17±0.25	18±0.25	9.0±0.32
<i>Achyranthes aspera</i>	26±0.20	12±0.15	15±0.32	8.0±0.24
Control	28±0.12	19±0.20	20±0.45	17±0.15

*average of three determinations (mean ± SD)

4. CONCLUSION

According to the findings, the plants *Amaranthus spinosus* and *Achyranthes aspera* have a large number of important substances that are helpful to human health. The physiochemical and phytochemical characteristics may be used to identify the medicine and determine its quality and purity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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