STUDY OF ANTIOXIDANT AND ANTIMICROBIAL SCREENING OF *AMARANTHUS SPINOSUS* AND *ACHYRANTHES ASPERA* LEAVES EXTRACTS

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.

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]

Achyranthes aspera Linn and Amaranthus spinosus Linnbelongs to the family Amaranthaceae.Achyranthes aspera Linn is a perennial stiff erect herb, 2.0 m high is growing up to 1000 m in height. Stems are square, leaves elliptic ovate or broadly rhombate, 5-22 cm long, 2.5 cm broad, and ad pressed pubescent. The inflorescences are 8 - 30 cm long, with many single, white or red flowers, 37 mm wide. Flowering time is in summer. The main root is long cylindrical thick; secondary and tertiary roots present slightly ribbed, yellowishbrown in color; odor is slight, the taste is slightly sweet and mucilaginous; the stem is yellow brownish, erect branched, cylindrical hairy about 60 cm high. Seeds are subcylindrical, truncates at apex, rounded at base, black, and shining. The plant is distributed throughout India up to an altitude of 3000 ft[2]

Achyranthes aspera L. also known as “Prickly chaff flower” in English. The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in

facilitating delivery, boils, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite, and skin diseases etc. Traditional healers claim that addition of Achyranthes aspera would enhance the efficacy of any drug of plant origin [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]

In India, A. hypochondriacus L. is known as "rajgeera" (the King's grain) and is often popped to be used in confections called "laddoos" which are very similar to Mexican "alegria". In Nepal, Amaranth seeds are eaten as gruel called "sattoo" or milled into a flour to make chappatis[7]. Amaranth, a legacy of the Atecs, Mayas, and Incas, continues to be an under-exploited plant with a promising economic value due to the variety of uses it can have and the benefits it can provide to producers, processors, and consumers [8].

Amaranth grain is a pseudo-cereal and gluten-free used in breakfast cereals, pancakes, soup, breads, cookies, gluten-free foods, extruded snacks and as an ingredient in confections [9]. South Americans parch or cook it for a gruel or porridge, or mill it to produce light-colored flour. As a snack, the grain is popped and tastes like a nutty-flavored popcorn. It can also be mixed with honey [10]. Ljubica et al. havereported that Amaranth flour can be used to partially replace regular corn flour for extruded snack manufacturing [11]. Xaene et al. also reported that mixture of instant whole Amaranth and rice can be used to produce extruded flours to be used in formulations of beverages [12]. According to Rosa et al. extruded snacks can be manufactured from defatted Amaranth flours [13].

The aim of the present study was to evaluate physiochemical, phytochemicals, and antioxidant and to detect the presence of natural therapeutic agents, especially those related to control the microbes that cause diseases in human beings from Achyranthes

aspera and Amaranthus spinosus leaf extracts with water and methanol solvent system.

MATERIALS AND METHODS

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* is 197/Saif/Sci/Clg/Bpl) dated 17/6/2020. The matured leaves from the plants were selected because there is a maximum metabolism in fully matured leaves as compared to young leaves. Specimens were dried at room temperature and stored in polyethylene bags at 4°C.

Preparation of plant extract

Hydroalcoholic extraction of the plants material was carried out by suspending 100 grams of the powders of both the plants in 1000 ml of methanol and water (80:20, methanol: water, v/v). The extraction was allowed to stand for 72 hours at 37°C. The extracts were filtered first through cotton wool, then through Whatman filter paper No.1 (125 mm) and were dried using a rotary evaporator. They were transferred into sterile bottles and kept in refrigerator until used.

Phytochemical analysis

Phytochemical screening for the presence of Tannins, alkaloids, glycosides, flavonoids, and phenolic was performed using standard procedures [14].

Qualitative analysis of phytochemicals alkaloids: The extracts were evaporated to dryness and the residues were heated on a boiling water bath with 2% Hydrochloric acid, cooled, filtered and treated with the Mayer's reagent. The sample was then observed for the presence of yellow precipitation or turbidity [14].

Flavonoids: 1.5 ml of 50% methanol was added to 4 ml of the extracts. After warming add magnesium fillings followed by a few drops of concentrated hydrochloric acid. A pink or red color indicates the presence of flavonoid [4].

Tannins: A portion of the extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution were added. A blue or green color indicates the presence of tannins [15].

Saponins: A small quantity of the methanol extract was boiled. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of the distilled water in a test tube and shake well for about 30 seconds and observed for frothing [14].

Glycosides: In a methanol extract Fehling's reagent was added and boiled for 2 minutes. A brick red coloration indicates the presence of glycosides.

Quantitative analysis

Total phenolic contents (TPC) and total flavonoid contents (TFC): Total phenolic contents (TPC) were determined using the Folin-Ciocalteu reagent method and Gallic acid was used as Gallic acid Equivalent (GAE). The total flavonoid contents (TFC) in the leaf extracts was determined following the modified procedure and Quercetin was used as standard as QuercetinEquivalent (QE) [16].

Antioxidant activity

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

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

A blank the absorbance of the control reaction (containing all reagents except the test sample), and A sample is the absorbance of test samples. IC_{50} values, which represents the concentration of Selected plants that caused 50% inhibition, were calculated from the plot of percentage against concentration.

***Determination of antioxidant activity in linoleic acid system*:** The antioxidant activity of the selected plant extracts materials were also determined by measuring the oxidation of linoleic acid 5 mg of selected plants extracts were added separately to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M Sodium Phosphate buffer (pH=7). The mixture was made up to 25 mL with distilled water and incubated at 40°C up to 360 hours. Extent of oxidation was measured by peroxide value applying Thiocyanate method. Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of an aqueous solution of Ammonium Thiocyanate (30% w/v), 0.2 mL of

sample solution and 0.2 mL of ferrous chloride (FeCl2) solution (20 mM in 3.5% HC1; v/v) added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a spectrophotometer. A negative control contained all reagents with exception of extracts. Synthetic antioxidants ButylatedHydroxytoluene (BHT) (also we can use ascorbic acid) was used as positive control. The maximum per oxidation level was observed at 360 h (15 days) in the sample that possesses no antioxidant component percent inhibition of linoleic acid oxidation was calculated with the following equation:

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

***Determination of reducing power*:** The reducing power of the selected plant extracts were determined according to the spectrophotometric method [17]concentrated extract (0-10.0 mg) was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50°C for 20 min. Then 5 mL of 10% Trichloro Acetic Acid was added and the mixture centrifuged at 980 g for 10 min at 5°C in a refrigerated centrifuge . The upper layer of the solution (5.0 mL) was decanted and diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%), and absorbance read at 700 nm using a spectrophotometer . All samples were analyzed thrice and the results averaged.

Antimicrobial activity Microbial strains:*Staphylococcus aureus* and *Escherichia coli*, together with two pathogenic fungi (*Fussariumsolani* and *Rhizopusoligosporus*) were used as microbial strains. The organisms were bring from J.P. hospital, Bhopal. These bacterial and fungal strains were cultured at 37°C and 28°C overnight in an incubator.

***Disc diffusion method*:** The antimicrobial activity of the prepared extracts was determined by using disc diffusion method [18]. The inoculated extracts were then examined for inhibition zones (in mm) by zone reader, which indicates antimicrobial activity. The discs (6 mm in diameter) were impregnated with 20 µg/ disc, sample extracts (20 µg/ disc) and placed on inoculated agar.Ciprofloxacin (20 µg/disc) and Fluconazole (20 µg/disc) were used as positive reference for bacteria and fungi, respectively [18].

RESULTS AND DISCUSSION

The study was carried out on the plant samples revealed the presence of medicinally active constituents. The percentage yield of *Amaranthus spinosus* and *Achyranthes aspera* were investigated and summarized in tables 1. Alkaloids, tannins, saponins and glycosides were present table 2.

Quantitative estimation of the percentage crude chemical constituents in *Amaranthus spinosus* and *Achyranthes aspera* were studied and summarized in table 3. Table 3 contains the percentage yield and antioxidant activity of *Amaranthus spinosus* and *Achyranthes aspera*leaf extracts. The highest DPPH activity, TP and TF content was found in Hydro alcoholic leaf extracts as shown in Table 3.

The higher percentage inhibition of linoleic acid oxidation by the extract compared with the reference drug, BHT, suggests a marked and higher antioxidant activity Table 4. Furthermore, the percentage inhibition of linoleic acid oxidation (65 %) and (64 %) obtained for the *Amaranthus spinosus* and *Achyranthes aspera*respectively. At physiological pH (7.4), ferrous ions (Fe2+), in the presence of oxygen and phosphate ions (PO_{24}), exist only transiently before being auto-oxidized to ferric ion (Fe3+). During this process, an electron is transferred from iron to oxygen to form a superoxide radical anion and hydroperoxyl radical (HO_2•) by Fenton reaction.

The concentration dependent, high reducing power of the aqueous extract of Amaranthus spinosus leaves suggests that the extract possessed the ability to be effective, under physiological conditions, in reducing the transition state of iron and consequently, the rate at which super oxide and hydroperoxyl radicals are generated from the metal Table 5. A strong relationship between the total phenolic content and reducing activity in fruits and vegetables has been reported [17]. Therefore, the reducing power of the extract may be attributed to its phenolic content.

Antimicrobial activity

The leaves of *Amaranthus spinosus*and *Achyranthes aspera* extracts showed consider antimicrobial activities in the disc diffusion assay. The quantitative estimation of antimicrobial activity of *Amaranthus spinosus* and *Achyranthes aspera* leaf extracts against food-borne and pathogenic microorganisms are shown in Tables 6 .The trend for antifungal activity was same to that of antibacterial activity except that the efficacy towards fungal strain was not much effective as for bacterial strains.

Table 1: Data showing yields of Hydro alcoholic of powdered leaf of *Amaranths spinosus* and *Achyranthes aspera*

Extracts	*Amaranthus spinosus* % yield (w/w)	*Achyranthes aspera* % yield (w/w)
Hydro alcoholic	5.37%	6.47%

Table 2: phytochemical constituents of hydro alcoholic leaf extracts of *Amaranths spinosus* and *Achyranthes aspera*

Phytochemical constituent	*Amaranthus spinosus*	*Achyranthes aspera*
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 *Amaranths spinosus*and *Achyranthes aspera*

Plants	% age yield*	TPC contents	TFC contents
Amaranthus spinosus	6.3 ± 0.23	2.1 ± 0.8	3.66 ± 0. 10
Achyranthes aspera	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 *Amaranths spinosus* and *Achyranthes aspera*

Plants	DPPH assay* (µg/mL) IC50	Inhibition of per oxidation* %
Amaranthus spinosus	16.21 ± 2.9	62.2 ± 2.9

Achyranthes aspera	19.45 ± 4.84	66.4 ± 3.5

***average of three determinations (mean ± SD)**

Table 5: Reducing power of hydro alcoholic leaf extracts of *Amaranths spinosus* and *Achyranthes aspera*

Concentration mg/ ml	*Amaranthus spinosus*	*Achyranthes aspera*	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 *Amaranths spinosus* and *Achyranthes aspera.*

Plants (Leaves)	Zones of growth inhibition*(mm)			
	S. aureus	*E. coli*	*F. solani*	*R. oligosporus*
Ciprofloxacin	28±0.20	25±0.21	-	-
Fluconazole	-	-	22±0.21	20±0.50
Amaranthus spinosus	25±0.15	17±0.25	18±0.25	9.0±0.32
Achyranthes aspera	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)**

Conclusion: From the research work, it was concluded that the plant *Amaranths spinosus* and *Achyranthes aspera* 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

References:

1.Jadhav, V.M., Kamble, S.S. and Kadam, V.J., 2009. Herbal medicine: Syzygiumcumini: a review. *Journal of Pharmacy Research*, *2*(8), pp.1212-1219.

2. Sharma, V. and Chaudhary, U., 2015. An overview on indigenous knowledge of Achyranthes aspera. *J Crit Rev*, *2*(1), pp.7-19.

3. Jain, J.B., Kumane, S.C. and Bhattacharya, S., 2006. Medicinal flora of Madhya Pradesh and Chattisgarh–a review.

4. Joshi, S.K., Sharma, B.D., Bhatia, C.R., Singh, R.V. and Thakur, R.S., 1992. The wealth of India raw materials. *Council of Scientific and Industrial Research Publication, New Delhi*, *3*, pp.270-271.

5. KAYA, M. and Uygur, F.N., 2000. A New Record for the Flora of Turkey Amaranthus spinosusL.(Amaranthaceae). *Turkish journal of Botany*, *24*(6), pp.359-360.

6. Xian, S., 2003. Amaranthus Linnaeus. *Flora of China*, *5*, pp.417-421.

7. Singhal, R.S. and Kulkarni, P.R., 1988. Amaranths–an underutilized resource 125-139.

8. Mburu, M.W., Gikonyo, N.K., Kenji, G.M. and Mwasaru, A.M., 2012. Nutritional and functional properties of a complementary food based on Kenyan amaranth grain (Amaranthus cruentus). *African journal of food, agriculture, nutrition and development*, *12*(2), pp.5959-5977.

9. Emire, S.A. and Arega, M., 2012. Value added product development and quality characterization of amaranth (Amaranthus caudatus L.) grown in East Africa. *African Journal of Food Science and Technology*, *3*(6), pp.129-141.

10. Chávez-Jáuregui, R.N., Cardoso-Santiago, R.A., Silva, M.E.P.E. and Arêas, J.A., 2003. Acceptability of snacks produced by the extrusion of amaranth and blends of chickpea and bovine lung. *International Journal of Food Science & Technology*, *38*(7), pp.795-798.

11. Dokić, L.P., Bodroža-Solarov, M.I., Hadnađev, M.S. and Nikolić, I.R., 2009. Properties of extruded snacks supplemented with amaranth grain grits. *Actaperiodicatechnologica*, (40), pp.17-24.

12. Xaene, M.F.D., Regina, C.D., José, L.R., Maria, C.A., Diego, P.R. and Carlos, W.P., 2008. CHEMICAL CHARACTERISTIC AND COLOR ANALYSIS OF INSTANT FLOURS OF AMARANTH AND RICE USED IN NEW EXTRUDED PRODUCTS. *Das AV Américas*, *29501*, pp.23020-47.

13. Chávez-Jáuregui, R.N., Santos, R.D., Macedo, A., Chacra, A.P.M., Martinez, T.L. and Arêas, J.A.G., 2010. Effects of defatted amaranth (Amaranthus caudatus L.) snacks on lipid metabolism of patients with moderate hypercholesterolemia. *Food Science and Technology*, *30*, pp.1007-1010.

14. Das, S., 2012. Systematics and taxonomic delimitation of vegetable, grain and weed amaranths: a morphological and biochemical approach. *Genetic resources and crop evolution*, *59*(2), pp.289-303.

15. Fransen, M., Crosbie, J. and Edmonds, J.O.H.N., 2001. Physical therapy is effective for patients with osteoarthritis of the knee: a randomized controlled clinical trial. *The Journal of rheumatology*, *28*(1), pp.156-164.

16.Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N., 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, *8*(4), p.96.

17. Hotz, C. and Gibson, R.S., 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of nutrition*, *137*(4), pp.1097-1100.

18. Iheanacho, K.M. and Udebuani, A.C., 2009. Nutritional composition of some leafy vegetables consumed in Imo state, Nigeria. *Journal of Applied Sciences and Environmental Management*, *13*(3).