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Antioxidant And Antibacterial Activity Of Root And Seed Extracts Of Achyranthes Aspera

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Abstract

Achyranthes aspera L., a common weed belonging to Amaranthaceae family, possessing many Ayurvedic, Unani-Tibbi, Homeopathic, Siddha, Naturopathic, medicinal propertiesis widely distributed throughout the tropical world. The objective of the present study is to extract and quantify the polyphenols and flavonoids besides evaluating their antioxidant and antimicrobial activity against eight bacterial reference strains;Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Proteus vulgaris (ATCC 6380), Salmonella typhimurium (ATCC 25241), Salmonella paratyphi (ATCC 9150), Shigella sonnei (ATCC 25931) and Klebsiella pneumonia (ATCC 27736). The polyphenols and flavanoids of the extracts from both the tissues were quantified separately by Folin-Ciocalteu and Colorimetric methods respectively. The total content of antioxidants of these extracts was assessed separately by the method of Phospho-molybdenum assay, while the antioxidant activity of these extracts was evaluated by a) 1,1-Diphenyl-2-picryl- hydrazyl (DPPH) and b) lipid peroxidation methods. Further, the antibacterial activity of these extracts was evaluated separately by using agar well diffusion assay against the above mentioned eight bacterial reference strains. All the extracts showed the presence of phytochemicals; tannins, terpenoids, steroids, flavonoids and carbohydrates. The polyphenol and flavonoid content of the extracts was found to decrease in the order of seed ethanol > root ethanol>seed aqueous>root aqueous whereas the antioxidant capacity of the extracts was found to decrease in the order of seed ethanol > root ethanol>seed aqueous>root aqueous. The seed ethanol and root ethanol high DPPH scavenging activity and lipid peroxidation activity compared to seed and root aqueous. The result of this study proves that extracts are good source of antioxidants and antimicrobial agents to use them as a broad spectrum of investigation in medicines.

Keywords: Achyranthes aspera, ethanolic and aqueous extracts, antioxidant, antibacterial activity.

Introduction

Since ancient times till date different plant products are being used in traditional medicinal system for treatment of numerous diseases throughout the world [1].The World Health Organization (WHO) reported that 80% of population from African and Asian countries uses herbal medicine for primary health care [2]. Of late, European countries and United States are evincing interest in the use of herbal medicine even in the treatment of most dreaded diseases as scientific evidence about the effectiveness of these herbal products is increasing. The annual global export value of pharmaceutical plant drugs in 2011 accounted for over US$ 2.2 billion [3]. Plant synthesizes a variety of chemical compounds performing defense mechanism [4].Plant Chemical compounds are very effective against different diseases in human beings without any harmful side effects. Ethno-botany (the scientific study of the relationships that exist between peoples and plants) is recognized as an effective way to discover future medicines [5]. In 2001, 122 compounds were derived from ethno-medical studies which are now used as modern medicines. Herbal remedies including digitalis, quinine and opium are being used from many years [6]. Since ancient history till date herbs are being used to treat diseases among non- industrialized societies which are more affordable than expensive modern pharmaceuticals and moreover which are causing microbial resistance due to drugs [7]. Therefore, action must be taken to control the use of antibiotics and continue studies to develop natural drugs. Plants represent a rich source of antimicrobial agents [8]. The antimicrobial properties of plants have been investigated by a number of researchers worldwide [9]. The process of Oxidation which naturally occurs in the body results in the formation of free radicals like Hydrogen peroxide H_2O_2, Hypochlorous acid (HOCl) and Ozone (0_3). These free radicals play a crucial role in the normal aging process and neurodegenerative disorders by damaging the cell membranes, proteins, fats, and genetic material in the cell [10]. Natural oxidants, namely phenols and flavonoids in plants can prevent the oxidative damage caused by free radicals [11, 12]. Plants produce variety of antioxidants which are potent and control the oxidative stress with

site specific mechanism. Therefore, these natural oxidants are effective. They protect the human body from free radicals unlike the drugs which are toxic [13-16].Natural oxidants are more beneficial than synthetic oxidants which are used in the pharma and food industries. Many chronic diseases such as cancer, cardiovascular and neurological disorders can be prevented by natural oxidants. Thus, antioxidant properties of plants have perspective application in health care [17, 19]**.** To avoid the side effects of antibiotics, the scientists are probing many plant families viz., Bignonaceae, Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, Amaranthaceae etc to identify new antimicrobial agents from the extracts of these plants for the treatment of various diseases. Scientific investigations into Achyranthes aspera revealed it to be one of the important medicinal herbs [20] as it is anti-microbial, hypoglycemic, cancer chemo-preventive, hepato-protective, analgesic**,** anti-pyretic, hypo-lipidemic,nephron-protective, diuretic, immune-modulatory and antifungal etc. Achyranthes aspera, contains triterpenoid saponins rich oleanolic acid, aglycon, ecdysterone, an insect moulting hormone and long chain alcohols [21]**.**

In the present investigation, sincethe Achyranthes aspera posseses medicinal properties two types of tissues, the root and seed, in two different solvents, ethanol and water, were evaluated for the differences in the antioxidant and antibacterial activity.

Materials and Methods

Plant material

Roots and seeds of Achyranthes aspera collected from Central Research Institute of Unani Medicine (CRIUM), Hyderabad, were cleaned, shade dried, ground to coarse powder and stored separately in air-tight containers at 25 C for further use.

Microbial cultures

Eight bacterial reference strains; Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus vulgaris ATCC 6380, Salmonella enterica paratyphi (Salmonella paratyphi) ATCC 9150 , Salmonella typhimurium ATCC 25241, Klebsiella pneumonia ATCC 27736 and Shigella sonnei ATCC 25931 obtained from Hi-Media (Mumbai) and their pure cultures were maintained on nutrient agar slants for the entire study. All the isolates were sub-cultured at regular time period and stored at 4 C as well as sub cultures are stored at -80 C by making their suspension in 10% glycerol (glycerol stock).

Chemicals

Sodium carbonate, Sodium nitrate, Aluminium chloride, Sodium hydroxide,Sulphuric acid, Sodium phosphate, Ammoniummolybdate, Potassium chloride, Hydrochloric acid, trichloroacetic acid were purchased from Merck, India, Mueller Hinton broth (MHB) Mueller Hinton Agar (MHA) purchased from Hi-Media, India. Standard drugs1,1-Diphenyl-2-picryl-hydrazyl(DPPH), Folin-Ciocalteu, Gallic acid, Querecetin, Ascorbic acid, Fenton's reagent, thiobarbituric acid, butylated hydroxytoluene (BHT), Chloramphenicol reagent were purchased from Sigma-Aldrich chemicals co. (St. Louis O., USA). Analytical grade solvents like Ethanol Methanol were used in this study.

Preparation of plant extracts

Ethanol extracts of root and seed were prepared by Soxhlet extraction method. The root and seed powdered samples of 50gm of each were uniformly packed into two separate thimbles and run in Soxhlet extractor. The samples were extracted separately in ethanol till the solvent in the siphon tube of the extractor became colorless in about 48 hours. Later , the extracts were filtered with the help of Whatmann filter paper 4 followed by the evaporation of solvent in Rotary evaporator at 50-60 ^{0}C under reduced pressure to get the syrupy consistency. Later, the extracts were kept in refrigerator at 4 C. The stock solution, 100 mg/mL of the dried extracts were prepared from which different concentrations were prepared and used in the experiments

root and seed aqueous extracts of Achyranthes aspera were prepared separately by soaking 50 g of each dried powder in 500 mL of distilled water for 48 h at room temperature. The extracts were filtered using Whatmann filter paper No:4 and concentrated in the Rotatory evaporator at 50-60 ^{0}C under reduced pressure leaving dark green residue of seed and light yellow residue of root which were then stored in airtight container. The stock solution, 100 mg/mL of the dried extracts were prepared from which different concentrations were prepared and used in the experiments

Preliminary Phytochemical Screening

Standard procedures consisting of Tannins, Terpenoids, Steroids, Alkaloids and Flavonoids were used for phytochemical studies [22, 23] the experiment were done in triplicates

Determination of total phenolic content

The amount of total soluble phenolics in ethanol and aqueous solvents of root and seed extracts was determined according to Folin-Ciocalteu method [24] with slight modifications. Samples of 5µl of extract and 495µl of water were mixed with 100 µl of Folin-Ciocalteu reagent were taken in to test tubes, vortexed for 5 min, allowed to stand for 10min at 40º C for development of colour and kept for incubation for 10 min. Later, 300 µL of 20% Na_2CO_3 solution was added and the volume was made up to 1 mL by adding distilled water. The mixture was incubated in dark for 2 h and the absorbance was recorded at 765 nm using a UV-Vis spectrophotometer against blank. The total phenolic content was estimated as Gallic acid equivalents (mg GAE)/gram of dry weight (dw). The experiment was replicated thrice.

Determination of total flavonoid content

Total flavonoid content was estimated by Colorimetric method [25] by mixing 5 µL of the extract with 495 µL of distilled water and 30

μL of 5% $NaNO_2$ solution in test tubes and were kept for incubation for 10 min at 40º C. Later, at room temperature, 60 μL of 10% $AlCl_3$ solution, 350 μL of 1 M NaOH and 60 μL of water were added to make up the final volume to 1 mL. Samples were further incubated for 20 min at room temperature and the absorbance was recorded at 510 nmusing a UV-Vis spectrophotometer against blank. The total flavonoid content was measured as Querecetin equivalents (mg QE)/gram of dry weight (dw). Triplicate analysis was done for the experiment.

Determination of total antioxidant activity (TAA)

The total antioxidant activity of both the root and seed extracts of A. aspera was evaluated by phospho-molybdenum method [26] by dissolving 2.5 μL of extracts in 97.5 μL of distilled water and 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixtures were vortexed and incubated in a thermal block at 95 C for 90 min and the tubes were cooled at room temperature. Absorbance was recorded at 695 nmby using Ascorbic acid (AA) as standard and the total antioxidant activity was expressed as milligrams of ascorbic acid equivalents (mg AAE/g) of dry weight.The experiment was replicated thrice.

Radical scavenging activity of antioxidants by DPPH Method

The radical scavenging activity of the plant extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) [27] was determined by UV spectrophotometer at 517 nm. DPPH solution (0.004% w/v) was prepared in 95% methanol. Various concentrations of extracts were mixed with 900 μL of DPPH solution in test tubes, incubated in dark for 30 min and later the absorbance was recorded at 517 nm against Methanol (95%) DPPH solution as blank and ascorbic acid (AA) as standard. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation:

$$\text{Scavenging activity (\%)} = \frac{100 \times (\text{absorbance of control} - \text{absorbance of sample})}{\text{Absorbance of control}}$$

Triplicate analysis was done for the experiment

Determination of inhibition of Lipid peroxidation

Lipid peroxidation inhibitory activity of Achyranthes aspera extracts was carried out according to the standard protocol [28].

The goat liver lobes purchased from the local slaughter house were dried between blotting paper, to remove blood, and cut into small pieces. They were homogenised in a glass Teflon homogenising tube with 0.15 M KCl. The homogenate was centrifuged at 800 g for 15 min at 4 C and the supernatant was used for the thiobarbutaric acid assay.

Different concentrations of Achyranthes aspera extracts were mixed with the liver microsome preparation and by adding/without adding Fenton's reagent (50 μL of 10 mM $FeCl_3$; 10μL of 2.5 mM H_2O_2) in phosphate buffer (0.2 M, pH7.4) and final volume was made up to 1mL by distilled water and kept for incubation at 37 C for 30 min. After incubation, 2 mL of ice-cold HCl (0.25 N) containing 15% tri-chloro acetic acid, 0.5% thiobarbituric acid and 0.5% butylated hydroxytoluene (BHT) was added to the reaction mixture and heated at 100 C for 60 min. Later, the reaction mixture was put in an ice bath for 10 min, centrifuged at 1000 g for 10 min and the extent of lipid peroxidation was subsequently monitored by the formation of thiobarbituric acid reactive substances (TBARS) as pink chromogen in the presence or absence of extracts and the standard (ascorbic acid). The absorbance of the supernatant was recorded spectro-photo-metrically at 532 nm. The decline in the formation of pink chromogen in the pretreated reactions was considered as inhibition of lipid peroxidation. The percentage of inhibition of lipid peroxidation was calculated by comparing the results of the test samples with those of controls as per the following formula:

$$\text{Inhibition of lipid peroxidation(\%)} = \frac{(\text{Control Absorbance} - \text{Test Absorbance}) \times 100}{\text{Control Absorbance}}$$

Triplicate analysis was done for the experiment

Evaluation for anti-bacterial activity of extracts

Anti-Bacterial activity of the extract was determined by Agar diffusion assay [29]. Bacterial strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 24 h at 37 C. After the incubation period, 0.1ml of the inoculums was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator at 37 C; wells were made using sterile 6mm cork borer in the inoculated MHA plate. The wells were filled with 150μl of the extracts (re-suspended in respective solvents). The concentration of stock extracts were 100 mg/ml. The inoculated plates were incubated at 37 C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotic Chloramphenicol (30mcg/disc). The photograph was taken in U.V-Visible documentation system.The experiment was replicated thrice.

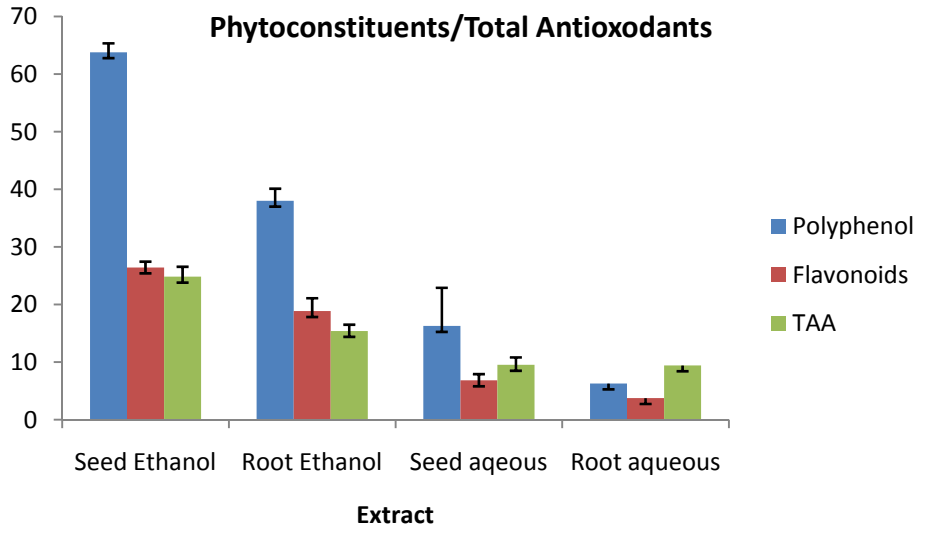
Results and Discussion

Table1: Total Polyphenols, Flavonoids and Antioxidant activity of Achyranthes aspera.

Extract	Polyphenol [a]	Flavonoids[b]	Total Antioxidant Activity[c]
Seed Ethanol	63.79±1.57	26.43 ± 1.03	24.82±1.75
Root Ethanol	38.01±2.12	18.85±2.26	15.4±1.11
Seed Aqueous	16.27±6.65	6.82±1.101	9.52±1.31
Root Aqueous	6.29±2.7	3.75±2.29	9.43±1.52

a: gallic acid; b: quercetin; c: ascrobic acid equivalents mg/g dw plant material respectively; Results represented in means ± standard deviation.

Graph 1. Total Phenolic and Flavanoid content and Total antioxidant activity of SE[1], RE[2], SA[3]& RA[4] Extracts



1 Seed Ethanol Extract; 2 Root Ethanol Extract; 3 Seed Aqueous Extract ; 4 Root Aqueous Extract;

Total phenolic content

Total phenolic content of ethanolic Seed and Root extracts of A. aspera were found significantly. The total phenol content in the Root and Seed extracts (Root Ethanol extract R E, Root Aqueous extract RA, Seed Ethanol extract S E and Seed Aqueous extract S A) was expressed as gallic acid equivalent (GAE). Seed extract in Ethanol had 63.79mg GAE/g dry weight and Root ethanol had38.01mg GAE/g dry weight Seed aqueous extract had 16.27 mg GAE/g dry weight, Root Aqueous had 6.29mg GAE/g dry weight hence polyphenol content which was higher in Seed Ethanolic extract (Table 1 & Graph 1).

Total flavonoid content

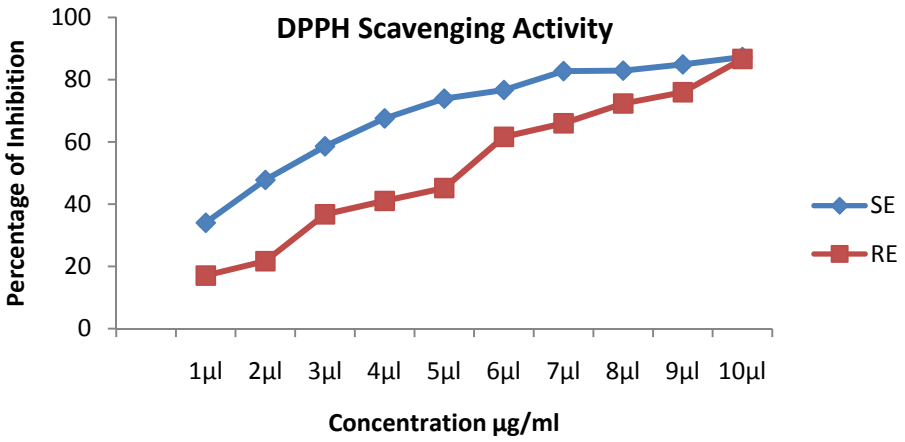
The total flavonoid content was expressed as quercetin equivalents. Seed Ethanolic extract had highest flavonoids content of 26.43mg QE/ml which was higher than Root Ethanolic extracts of 18.85mg QE/ml, whereas Seed Aqueous extract contains 6.82mg QE/ml Root Aqueous extract contains 3.75mg QE/ml, which was lesser than (Table 1 & Graph 1).

Total antioxidant activity (TAA)

The results of the antioxidant measurements are summarized in Table 1 & Graph 1. The antioxidant values are expressed in Ascorbic acid equivalents. The significant value of 24.82 mg AAE/g dw was observed in Seed Ethanolic extract, whereas 15.4mg AAE/g dw was found in Root Ethanolic extract. Seed Aqueous extract showed 9.52mg AAE/g dw whereas Root Aqueous extract showed 9.43mg AAE/g dw. The Seed Ethanol extracts of Achyranthes aspera showed reasonably higher antioxidant activity in comparison to the Root Ethanol, Seed Aqueous and Root Aqueous extracts. The extracts of Seed and Root exhibited significant antioxidant activity, thus establishing the extracts as antioxidants.

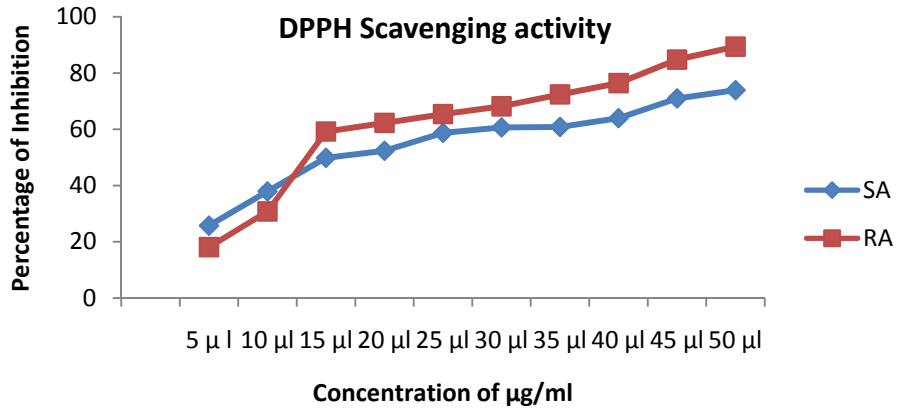
DPPH radical scavenging activity

Graph 2a. DPPH Radical Scavenging Activity of SE[1], RE[2]



1 Seed Ethanol Extract; 2 Root Ethanol Extract

Graph 2b. DPPH Radical Scavenging Activity of SA[1]& RA[2] Extract.



1 Seed Aqueous Extract ; 2 Root Aqueous Extract

Table 2a. DPPH radical scavenging activity of SE[1]&RE[2] Extracts.

Concentration	SE[1] (mean±SD)	RE[2] (mean±SD)
1µl	34±0.7	17.03±0.3536
2µl	47.77±0.754	21.67±0.546
3µl	58.6±0.7128	36.72±1.28
4µl	67.56±0.4613	41.02±0.2532
5µl	73.92±0.5404	45.16±0.3055
6µl	76.71±0.7171	61.6±0.9848
7µl	82.77±0.8219	65.96±0.4086
8µl	82.91±0.7336	72.32±0.8515
9µl	84.9±0.5254	75.95±0.55
10µl	87.29±1.015	86.65±0.6787

1 Stem Ethanol Extract; 2 Root Ethanol Extract

Table 2b. DPPH radical scavenging activity of SA^1 &RA^2 extract.

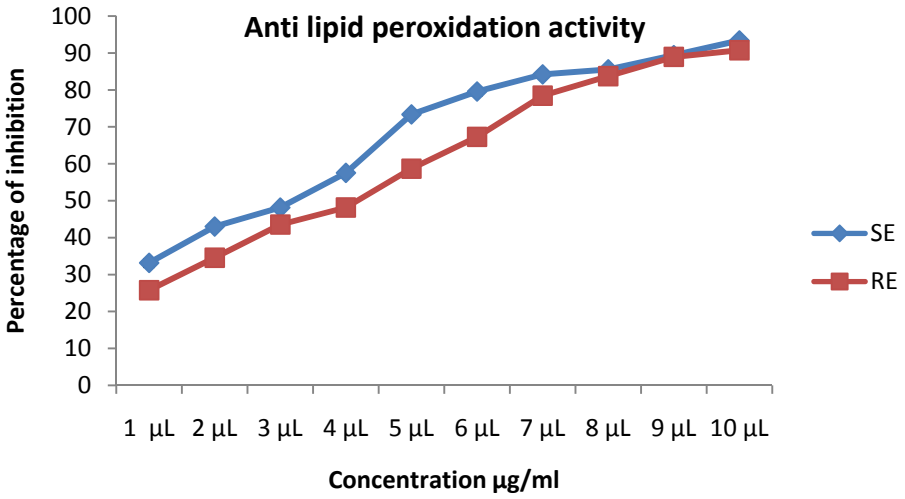
Concentration	SA^1 (mean±SD)	RA^2 (mean±SD)
5 µ l	25.74±0.4866	18.12±0.096
10 µl	37.93±1.15	30.79±0.2173
15 µl	49.84±1.36	59.21±0.569
20 µl	52.36±1.167	62.28±0.151
25 µl	58.74±0.5973	65.41±0.04
30 µl	60.65±0.6726	68.17±0.055
35 µl	60.83±1.0396	72.41±0.224
40 µl	63.94±0.4105	76.43±0.116
45 µl	70.97±0.7124	84.77±0.105
50 µl	73.91±0.7044	89.39±0.4523

1 seed aqueous extract ; 2 Root Aqueous Extract.

The radical scavenging effects of the A. aspera extracts were evaluated by using methanolic solution of the 1,1-Diphenyl-2-picryl- hydrazyl (DPPH) free radical, which exhibits a deep purple colour with maximum absorption at 527nm. In this study, all the extracts showed dose response curve of DPPH free radicals scavenging activity, as indicated by the concentration dependent increase in percentage inhibition. The DPPH radical scavenging activity of seed ethanol and root ethanol starts showing an increase in percentage inhibition at100 µg/µl, whereas the seed aqueous and root aqueous showed at 500µg/ µl.

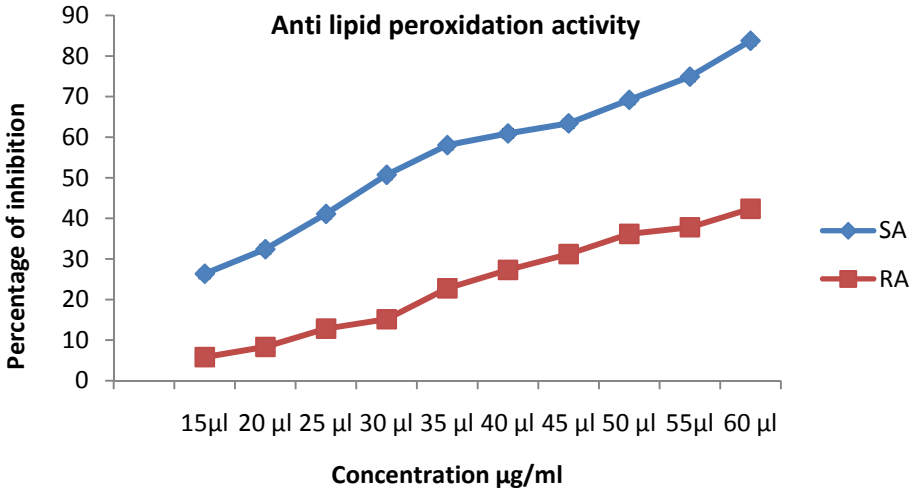
Inhibition of lipid peroxidation

Graph 3a.Lipid Peroxidation Activity of SE^1& RE^2Extracts.



1 Stem Ethanol Extract ; 2 Root Ethanol Extract.

Graph 3b. Lipid Peroxidation Activity of SA[1]&RA[2] extract.



1 Stem Aqueous Extract; 2 Root Aqueous Extract

Table 3a : Lipid Peroxidation activity of SE[2] RE[1] Extracts.

Concentration	SE[1] (mean±SD)	RE[2] (mean±SD)
1µl	33.16±1.005	25.7366±0.9066
2µl	43.02±0.5259	34.533±0.4609
3µl	48.14±0.6773	43.5266±0.8429
4µl	57.54±0.6312	48.15±0.8321
5µl	73.37±0.5887	58.6566±1.2424
6µl	79.57±0.913	67.2733±1.0507
7µl	84.21±0.2535	78.4233±0.686
8µl	85.58±0.3704	83.7±0.6245
9µl	89.41±0.553	88.933±0.5507
10µl	93.41±0.8237	90.7466±0.706

1 Seed Ethanol Extract; 2 Root Ethanol Extract;

Table 3b. Lipid Peroxidation activity of RA[1] & SA[2]Extract.

Concentration	SA[1] (mean±SD)	RA[2] (mean±SD)
15µl	26.33±0.3785	5.8±0.121
20 µl	32.39±0.9296	8.31±0.047
25 µl	41.08±1.0563	12.83±0.1234
30 µl	50.72±0.6009	15.13±0.08
35 µl	58.03±0.7686	22.76±0.0642
40 µl	60.9±0.6082	27.3±0.0351
45 µl	63.41±1.0327	31.19±0.0642
50 µl	69.18±0.9493	36.15±0.01
55µl	74.9±0.4	37.79±0.0378
60 µl	83.75±0.5352	42.35±0.0251

The percentage inhibition of lipid peroxidation was observed with 100µg/µl, in root ethanol, seed ethanol whereas 1500µg/µl was observed in root aqueous and seed aqueous.

The percentage inhibition of lipid peroxidation was observed with 100µg/µl, in root ethanol, seed ethanol whereas 1500µg/µl was observed in root aqueous and seed aqueous.

Phytochemical Screening

Phytochemical Screening of Achyranthes aspera Root and Seed extracts

The phytochemical screening tests are useful in the detection of bioactive and development of drug discovery, further these tests can also be used for quantitative and qualitative estimation and qualitative separation of pharmacologically active chemical compounds.

Phytochemicals Screening of Achyranthes aspera Root and Seed extracts was carried according to Trease GE & Evans WC [22]. We detected all important phytocompounds Tannins, Terpenoids, Steroids, Flavanoids, and Carbohydrates which have medicinal properties.

Table 4: Phytochemical Screening of Achyranthes aspera Root and Seed extracts

Phytochemicals	Seed ethanol	Seed aqueous	Root ethanol	Root aqueous
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	+	+	+	+

Antimicrobial activity

In the present study, the seed and root extracts of A.aspera were evaluated for its antimicrobial activity. The inhibitory effects of the extracts were compared with the standard antibiotics such as Chloramphenicol (25mcg/disc). The antibacterial activity of the ethanolic extracts showed varying degrees of antimicrobial activity against tested microorganisms. Root ethanolic and Seed ethanolic extracts exhibited higher degrees of antimicrobial activity compared to the aqueous extracts (root and seed). Among all the organisms, Klebsiella pneumonia showed maximum sensitivity with diameter of zone of inhibition 14.75mm with Seed ethanolic extract and 13.66mm with Root ethanolic extract. Seed ethanolic extract shows lesser antimicrobial activity against Salmonella typhimurium with 9.25mm diameter of zone of inhibition, followed by Proteus vulgaris with zone of inhibition of 11mm by using Root ethanolic extract and 11.66mm with seed ethanol. Both the root and seed aqueous extracts did not showed any antimicrobial activity. Ethanolic extracts of seed and root showed strong antibacterial activity comparable to that of standard chloramphenicol (25mcg/disc).

Table 5: Antimicrobial activity of Achyranthes aspera Root and Seed extracts

S.no	Organisms	RE mean±S.D	SE mean±S.D	Chloramphenicol(25mc) mean±S.D
1	Salmonella typhimurium (ATCC 25241)	13.12±2.1	9.25±3.304	9±0.3785
2	Escherichia coli (ATCC 25922)	12±0.41	13.7±3.171	8±0.3055
3	Pseudomonas aeruginosa (ATCC 27853)	12.5±0.41	12.6±2.498	8±0.8082
4	Shigella sonnei (ATCC 25931)	11.25±3.3	11.8±1.65	8±0.4163
5	Proteus Vulgaris (ATCC 6380)	11±2.05	11.7±1.028	8±0.2516
6	Klebsiella pneumonia (ATCC 27736)	13.66±2.05	14.75±5.172	19±0.4358
7	Salmonella paratyphi (ATCC 9150)	11.75±0.22	12.7±0.577	9±0.4932
8	Staphylococcus aureus(ATCC 25923)	13±1.41	12.5±0.1	21±0.1527

Discussions

Phytochemical screening by standard procedures showed the presence of Tannins, Terpenoids, Steroids, Alkaloids, Flavonoids.

The present study stated that phenolic contents are partially responsible for antibacterial, antioxidant and antiradical activity [30]. The ethanolic seed and root extracts possess antioxidant properties and could serve as free radical inhibitors or scavenger or, act possibly as primary antioxidants. The results of antimicrobial activity showed that of Seed Ethanolic extract and Root Ethanolic extract had varying degrees of growth inhibition against the microorganisms tested. Free radicals are unpaired electrons which are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability, they are formed inside the system and are highly reactive and damage transient chemical species. Body possess defense mechanisms

against free radicals like preventive and repair mechanisms by nonenzymatic antioxidants like carotenoids, ascorbic acid, phenolic compounds, flavonoids etc which act by different mechanisms like reducing ability, free radical scavenging and quenching of singlet oxygen. Chronic diseases can be prevented either by natural antioxidant provided by the body or supplementing with proven antioxidants

Recent reports suggest that polyphenols or phenols have antioxidant, antimutagenic and antitumor activities. These phenolic compounds like flavonoids, phenolic acids and tannins are widely present in plants, and have gained importance due to their antioxidant activities [31]. Invitro antioxidant activity of Achyranthes aspera Linn. were measured by selective assays (phenolic and flavanoid content by Folin-Ciocalteu method and Colorimetric method respectively and antioxidant activity by using phosphomolybdenum assay, 1,1-Diphenyl-2-picryl-hydrazyl(DPPH) method, and lipid peroxidation method). The method of extracting polyphenols from the plant materials is an important factor for determination of antioxidant activity. The results suggest that seed ethanol (63.79mg GAE/g dw) and root ethanol (38.01 mg GAE/g dw) had high polyphenol content compared to seed aqueous (16.27 mg GAE/g dw)and root aqueous (6.29 mg GAE/g dw). Seed ethanol had highest flavonoids content of 26.43mg QE/ml, root ethanol showed 18.85mg QE/ml, seed aqueous showed 6.82mg QE/ml root aqueous showed 3.75mg QE/ml and. The highest value of antioxidant activity of 24.82 mg AAE/g dw was observed in seed ethanol whereas the second highest value 15.4 mg AAE/g dw was found in root ethanol. Seed aqueous showed 9.52mg AAE/g dw and Root aqueous showed 9.43 mg AAE/g dw.

The seed and root ethanol extracts of Achyranthes aspera showed reasonably higher antioxidant activity in comparison to the seed and root aqueous extractsThe DPPH radical scavenging activity of seed ethanol and root ethanol showed an increase in percentage inhibition at100 µg/µl, whereas the seed aqueous and root aqueous showed at 500µg/ µl.

DPPH assay is the most widely reported method for screening antioxidant activity of many plant drugs assay on the reduction of methanol solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorbance maxima of 517 nm, which is proportional to the concentration of free radical scavenger added to DPPH reagent solution. DPPH is a stable, nitrogen-centered free radical which produces violet colour in methanol solution. It is reduced to yellow colored product, diphenyl picryl hydrazine, with addition of sample in the concentration dependent manner. DPPH scavenging activity is influenced by the polarity of the reaction medium, chemical structure of the radical scavenger and the pH of the reaction mixture, sample concentration and reaction time.

Phenolic compounds contains a hydroxyl group on an aromatic ring. These phenolic compounds involve in chain oxidation reactions by donation of a hydrogen atom or chelating metals, they are reducing agents and antioxidants.

Flavonols (such as quercetin, myricetin, kaempherol) and flavones (apigenin, luteolin) in plant materials are also antioxidant due to redox properties, free radical scavenging activity and singlet – oxygen quenching activity.

The different concentrations showed levels of antioxidant potential when compared to ascorbic acid. They have many biological activities. This method is based on the reduction of Mo (IV) to Mo(V) complex which shows maximum absorption at 695 nm. Total antioxidant activity by phosphomolybdenum method revealed the total antioxidant content of root ethanol>seed ethanol>root aqueous>seed aqueous, hence this could be a source of antioxidant compounds. Reactive oxygen species, superoxide, hydroxyl radical, singlet oxygen, nitrogen species etc causes oxidative stress and approved to produce tissue damage and aging and is involved in lipid peroxidation of the tissue which damage the tissue and immune response. Nitric oxide release cause damage to tissue, causes vasoconstriction and impairment in normal blood flow. Anti-oxidants compete for oxygen and reduce the production of nitric oxide. Synthetic antioxidants are carcinogenic. In the present study Achyranthes aspera inhibited lipid peroxidation.The percentage inhibition of lipid peroxidation was observed with 100µg/µl, in root ethanol, seed ethanol whereas 1500µg/µl was observed in root aqueous and seed aqueous.

The literature reports showed that there is high correlation between antioxidant activity and phenolics content[32].

Conclusions

The literature reports shows high relationof biological activities related to antibacterial and antioxidant correlated with flavonoid total polyphenol [32]. Reports also suggest that flavonoids are compounds that have medicinal properties, among antimicrobial, antioxidant and antiinflamatory. The extracts with high content of phenols, flavonoids Total antioxidant activity by phosphomolybdenum method showed best antibacterial, radical scavenging and lipid peroxidation activity.Extract with lowest Total antioxidant activity, seed aqueous & root aqueous showed less antimicrobial activityradical scavenging and lipid peroxidation activity. Seed ethanol and Root ethanol extracts with high polyphenol, flavonoid and TAA (Total antioxidant activity) showed best antimicrobial activity therefore polyphenols and flavanoids are responsible for antimicrobialshowed DPPH radical scavenging and lipid peroxidation dose dependent percentage

The above characterisation should be further investigated.

This present investigation suggest that ethanolic extracts of seed and root of Achyranthes aspera showed good results in phenolic compounds, total antioxidant activity and flavonoid content. Antimicrobial activity, DPPH assay and Lipid peroxidation assay were also been observed with the above extracts, therefore it would be worthful to isolate the active components of the extracts for further investigation of effectiveness of this plant in preventing oxidative stress related diseases and medicating the immune system in vivo.

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