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LC-MS studies and antioxidant potential of the root extracts of *Achyranthes aspera* Linn

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Abstract

Achyranthes aspera L. (Amaranthaceae) is an erect, annual herb. It is an herbaceous roadside weed that grows in various parts of India and possesses several types of pharmaceutical properties. This investigation was conducted to identify the bioactive compounds that confer antioxidant properties to the extracts from its biomass. According to the results, the root-ethyl acetate (R-EA) extracts of A. aspera showed the highest antioxidant activity and exhibited 66.78±1.65 scavenging of DPPH radicals by 500 µg extract. This extract was subjected to thin layer chromatography (TLC). According to the results, root-ethyl acetate revealed fourteen bands. Among all solutions of root biomass, the solution obtained from the second band (Rf=0.16±0.02) exhibited the maximum percentages of antioxidant activity (25.11±4.05 %). LC-MS analysis of this solution showed the presence of a few compounds have been reported to possess antioxidant properties in different plants.

Index Terms: *Achyranthes aspera* L.; antioxidant activity; DPPH assay; TLC; LC-MS.

1.Introduction

Antioxidants protect cells against the damaging effects of reactive oxygen species (ROS). ROS such as superoxide anion (O2−), hydrogen peroxide (H2O2), and hydroxyl radical (HO•) are generated due to the partial reduction of oxygen as a part of aerobic metabolism in the body. In small doses, ROS are beneficial, play physiological roles, and are involved in signaling processes [13]. However, ROS overproduction can induce an inflammatory response, and inflammatory mediators can induce oxidative stress [4]. An imbalance between antioxidants and ROS can lead to ROS pathological quantities that can trigger coronary heart disease, Alzheimer's disease, and cancer. Therefore, using plants that contain antioxidants decreases the risk of such diseases in the human body. Plants are rich in secondary metabolites that possess antioxidant activities such as phenolics, flavonoids and anthocyanins. Natural phenolic antioxidants can control various pathologies induced by oxidative stress [11] and several antioxidant-rich natural products have protective effects against inflammation [26]. Moreover, antioxidants act as anti-aging, anticancer and anti-diabetic agents.

Achyranthes aspera L. (Amaranthaceae) is a procumbent or erect, annual herb. It is a herbaceous roadside weed that grows in various parts of India [20]. Since ancient times, nature has been the ultimate reservoir of medicinal agents, and many modern drugs have been isolated from natural sources [7]. Medicinal plants contain numerous phytochemicals that confer beneficial and medicinal properties to maintain health and cure or reduce the symptoms of diseases. Approximately 25% of modern pharmacopeia drugs are derived from plants, and many others are synthetic analogs built on natural compounds isolated from plants [22].

Medicinal plants are a rich source of secondary metabolites. Phytochemicals play diverse roles in plant life and protect plants through disease resistance, protection against various stresses, and defense mechanisms [12]. These bioactive components include alkaloids, amines, steroids, glycosides, phytoestrogens, carotenoids, phytosterols, glucosinolates, terpenoids, and flavonoids [14]. Most of these secondary metabolites have shown bioactivities, including anti-diabetic and antioxidant activities [10].

In the DPPH radical scavenging assay, the ethyl acetate and aqueous extracts of whole plants of *A. aspera* were shown to have the highest antioxidant potential with IC50 values of 0.96 and 0.76 µg per ml, [2]. The leaf, stem, and inflorescence of *A. aspera* extracted in different solvents have different antioxidant and antibacterial activities depending on the concentration and assay duration [1]. The roots and inflorescence of *A. aspera* contain tannins (up to 1262.50 mg/50 g d.w.), flavonoids (up to 1023.81 mg/ 50 g d.w.), and proanthocyanidins (up to 600 mg /50 g d.w.), which are important antioxidants of plant origin [18]. Hence, the present investigation was undertaken to determine the potential antioxidant activity of A. *aspera* root extracts using the DPPH assay.

2.Methodology

Collection of plant material

Fresh *Achyranthes aspera* L. plants were collected in August from the campuses of Savitribai Phule Pune University and Fergusson College, Pune. The collected plant specimens were identified using the Cooks' Flora of Presidency of Mumbai and the identification was authenticated from the Botanical Survey of India, Western Circle, Pune. Roots of the plants were separated and washed in running tap water and cut into small pieces. The fresh biomass was layered on sheets of filter paper and dried in the shade at room temperature for one week. The dried biomass was powdered using a mechanical grinder and the powders were stored at -20 °C in a polythene zipper bag.

Extraction of phytochemicals

Phytochemicals from the powdered root (R) biomass were extracted in a Soxhlet extractor separately in ethanol (Et), ethyl acetate (EA), acetone (Ac), distilled water (DW), and methanol (Me). Twenty grams of biomass was wrapped in Whatman No. 1 filter paper and extracted for five h in 190 mL of solvent. The extract was suction filtered using a Whatman No. 1 filter paper disc placed in a Buchner funnel. The extract was evaporated to dryness in a 300 mL beaker situated in a hot water bath. The dried residue was measured using an electronic balance (CAS-250, Contech Instruments Ltd., India). The residue was suspended in 5.0 mL of solvent for nine h, after which, it was centrifuged at 5000 rpm. The clear supernatant was collected in glass vials and stored at -20 °C for further use. Thus, five types of root extracts R-EA, R-Ac, R-Et, R-Me, R-DW were prepared. Each of these extracts was diluted with the solvent used for extraction to yield a working solution with concentration of 500 µg/mL.

In vitro antioxidant assay

DPPH based antioxidant assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is an oxidant having an odd electron in its structure. Its purple color is changed to yellow-colored diphenylpicrylhydrazine when it is in contact with an antioxidant that can release a hydrogen atom or electron.
The DPPH radical scavenging assay [5] was used to evaluate the antioxidant potential of the extracts. The 3.0 mL test reaction mixture consisted of one mL extract containing 500 µg dissolved residue and 1.0 mL 1 mM DPPH prepared in methanol. The solvent used for extraction was used to make the or a 3.0 mL volume. The control was set with 1.0 mL of 1 mM DPPH mixed with 2.0 mL methanol. The spectrophotometer (BioEra, India) was standardized using a 3.0 mL mixture of extract containing 500 µg dissolved residue mixed with 1.0 mL methanol and the extraction solvent to make a final volume of 3.0 mL.
The reaction mixtures were incubated in the dark at room temperature for 20 min, and the absorbance was measured at 517 nm. Each experiment was performed in triplicates.
The percentage scavenging of DPPH was calculated according to the following formula:
% DPPH radical scavenging= [(Ac-At)/Ac] × 100
Where Ac is the absorbance of the control mixture and at is the absorbance of the test mixture
A series of 100 to 500 µg/mL of each extract was prepared separately and treated to assess DPPH scavenging potential as described earlier. The IC50 value of each extract was calculated from the dose-response curve. IC50 values were used to express the ability of the extracts to scavenge 50% of DPPH. The term "IC50," which suggests the extract's concentration needed to scavenge 50% of DPPH radical, was calculated by plotting the dose-response curve using Microsoft Excel software.

DPPH scavenging activity of ascorbic acid

Ascorbic acid is a strong antioxidant that is used as a standard antioxidant substance in most antioxidant assays. A series of ascorbic acid concentrations were prepared in the range of 0 to 10 µg per mL and tested for its antioxidant activity as described in 6.2.2. A dose-response curve plotted in the MS Excel program was used to calculate the IC50 of ascorbic acid, i.e., the concentration of ascorbic acid required to scavenge 50% of DPPH.

Estimation of phenolics

Total phenolics were estimated by Swain and Hillis's (1959) method, and gallic acid (100 µg/mL) was used as a standard phenol to estimate the phenolic content in the extracts. A series of concentrations from 0 to 100 µg gallic acid was made, and to each test tube, 7.0 mL DW, 0.5 mL Folin Ciocalteu's phenol reagent, and 1.5 mL 20% Na2CO3 were added. The contents were thoroughly mixed, and the absorbance was recorded at 760 nm on a spectrophotometer. The blank was set with DW. The amount of extract containing 100 µg dissolved residue was used to estimate phenolic content and the extracts were treated in the same way as the standard gallic acid solution.

Estimation of flavonoids

Total flavonoids were estimated using the method of Balbaa et al. (1974), and rutin (100 µg/mL) was used as a standard flavonoid. A series of concentrations from 0 to 100 µg rutin was made, and to each test tube, 1.5 mL 95% methanol, 10% methanolic aluminum chloride, and 2.9 mL DW were added. The contents were mixed thoroughly, and the tubes were incubated at 30 ºC in the dark. After the incubation period, the absorbance was recorded at 420 nm on a spectrophotometer. The blank was set with DW. Finally, the absorbance was plotted against the amount of rutin to draw a standard curve.

The volume of extract containing 100 µg dissolved residue was used to estimate flavonoid content and the extract was treated in the same way as the standard rutin solution.

Thin layer chromatography (TLC) separation of extracts

Five types of extracts prepared from root biomass were tested for antioxidant activity. The extract from the root biomass that showed the highest antioxidant activity was separated on a preparatory TLC plate for the separation of phytochemicals

The pre-coated silica plates (TLC Silica Gel 60 F254, Merk, India) were utilized for the separation. A preparative TLC plate of 200 mm × 200 mm was used to separate 350 µg dissolved residue in the best solvent system toluene: ethyl acetate: acetic acid (18:2:0.5) identified from the separation on analytical plates. Two such plates were simultaneously developed in one chromatography chamber. At the end of separation, the solvent front was marked, and the Rf value of each visible band was calculated.

Elution of separated bands

The TLC plates were air-dried, and each band was scraped off from the plate by using a scalpel, and the powder of two identical bands from two plates was pooled and suspended in 500 µL of ethyl acetate for eight h. The suspension was then centrifuged to eliminate the silica powder, and the clear supernatant was stored in glass vials. The solution thus prepared from each band was subjected to antioxidant testing using DPPH assay. The solution with the highest percent inhibition was subjected to LC-MS analysis.

Among the five extracts, the R-EA extract showed the highest DPPH radical scavenging activity. When subjected to TLC separation as mentioned earlier, R-EA, produced 14 distinct bands on a preparatory TLC plate. The Rf values of these bands ranged between 0.12±0.01 to 0.71±0.02. The antioxidant activity shown by the extracts prepared from these 14 bands is presented in (Table 2). According to the result, among 14 bands of R-EA extract, the R-EA-B2 and S-EA-B1 showed the highest (25.11±4.05%) and lowest (3.00±1.54%) percentage scavenging of DPPH radicals. Following the R-EA-B2 was subjected to the LC-MS analysis. From the data on LC-MS analysis, phytochemicals with the known antioxidant activity were searched in the literature.

3.Results

Phenolic and flavonoid content in the extracts

Out of ten different extracts of root biomass, the R-EA extract showed the highest contents of phenolics and flavonoids. Hundred µg R-EA extract was equivalent to 4.83±0.97 µg gallic acid (phenolics) and 2.69±0.59 µg rutin (flavonoids). The R-EA extract was thus superior over all other extracts for the phenolics and flavonoid contents. Among the root extracts, the phenolic content in the R-EA extract was about eight times higher than the lowest content observed in the R-DW extract. As for flavonoid content, the R-EA extract had almost 18 times higher flavonoid content than the R-Ac extract that showed the lowest flavonoid (0.15±0.02 µg) content in terms of rutin equivalence with 100 µg extract.

To summarize, the R-EA extract was superior over all other extracts in having the highest contents of phenolics and flavonoids in terms of gallic acid and rutin equivalence, respectively, per 100 µg extract.

On the other hand, phenolics and flavonoids are known to be potent antioxidant substances. In all five root extracts prepared from *A. aspera*, phenolics and flavonoids were present at varying concentrations (Table 1). In general, the levels of phenolics and flavonoids did not follow any pattern concerning the polarity of the solvents used for extraction.

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Table 1 Phenolics and flavonoids in various root extracts of *Achyranthes aspera*

Extract type	Gallic acid equivalence of 100 µg extract	Rutin equivalence of 100 µg extract	% scavenging of DPPH radicals by 500 µg extract	IC50 for % DPPH scavenging
R-EA	**4.83±0.97**	**2.69±0.59**	**66.78±1.65**	**466.85±11.79**
R-Ac	0.81±0.26	0.15±0.02	44.22±1.42	449.23±13.75
R-Et	1.73±0.08	0.91±0.08	51.91±2.35	450.67±20.21
R-Me	3.84±0.86	1.59±0.04	50.21±1.04	454.33±09.50
R-DW	0.59±0.04	0.21±0.06	13.93±0.45	1712.35±18.45

(Values represent mean±SD. Values followed by similar letters in a column do not differ significantly at p =0.05 as per DMRT performed separately for a group of extracts from root biomass).

Antioxidant status of *A. aspera* extracts

Each of the ten extracts prepared from roots of *A. aspera* showed the potential to scavenge free radicals of DPPH (Table 1). The percent scavenging of DPPH radicals by 500 µg/mL extract ranged from a minimum of 13.93±0.45 % (R-DW extract) to a maximum of 66.78±1.65% (R-EA extract).

According to the results, the R-Et and R-Me extracts showed almost the same levels of antioxidant activities (50-51%). The R-DW extract showed the lowest DPPH scavenging potential, and it was about 80% lower (13.93±0.45%) than the R-EA extract.

The inverse relationship between the antioxidant potential of extract and its IC50 value is evident in Table 1. Thus, the extracts that showed the highest antioxidant potential had the lowest IC50 value. Among the 5 extracts, R-EA extract showed the highest antioxidant potentials, and consequently, showed the lowest IC50 values of 466.85±11.79 µg/mL. Therefore, the R-EA extract of *A. aspera* biomass were separated by TLC.Next, the separated bands on the TLC plates were eluted in the solvent used for extraction as described above. and the extracts thus obtained were subjected to the DPPH free radical scavenging assay as described earlier.

Table 2 Antioxidant potential of TLC bands of R-EA extract of *A. aspera*

Band No. Root/EA	Rf	% Antioxidant activity	Band No. Root/EA	Rf	% Antioxidant activity
B1	0.12±0.01	7.40±2.60	B8	0.44±0.04	9.27±0.67
B2	0.16±0.02	**25.11±4.05**	B9	0.48±0.03	3.83±0.61
B3	0.21±0.03	11.02±0.49	B10	0.50±0.03	9.33±1.21
B4	0.24±0.04	8.12±2.93	B11	0.58±0.03	6.43±1.29
B5	0.30±0.04	11.44±1.94	B12	0.65±0.04	5.98±1.59
B6	0.37±0.05	15.29±1.84	B13	0.68±0.02	6.44±1.94
B7	0.40±0.05	8.12±1.25	B14	0.71±0.02	3.35±0.62

(Values represent mean ± SD. Values followed by similar letters in a column do not differ significantly at p =0.05 as per DMRT).

Statistical analysis

All experiments were performed in three replicates, and the values were expressed as mean ±SD or mean ±standard error (SE) of means.

Antioxidant phytochemicals from the eluted TLC band

LC-MS analysis

LC-MS profiling of TLC R-EA-B2 that showed the highest antioxidant activity were performed on Agilent G6540B Quadrupole Time of Flight (QTOF) binary LC system equipped with Dual Agilent Jet Stream Electrospray Ionization (AJS ESI) and separated on Agilent Zorbax column (50 × 2.1 mm, 1.8 µm) using aqueous 0.1% formic acid (Solvent A) and acetonitrile (Solvent B). A stepwise increasing polarity gradient of solvent A and solvent B was applied at the flow rate of 0.3 mL/min for 30 minutes. The capillary voltage, cone voltage, and fragmentor voltage were 3.5 kV, 45 V, and 150 V, respectively. The gas temperature was set to 325 °C. Electroscopy mass spectra data were recorded in a positive ionization mode. The data was acquired at a scan rate of 2 spectra/sec in the mass range of 60-1700 m/z and analyzed with Mass Hunter Qualitative Software and METLIN database. The class of the putatively identified compounds was deduced from the websites of the metabolomic workbench (www.metabolomicsworkbench.org) and the Lipidomics Gateway (www.lipidmaps.org).

Out of 36 compounds identified in the R-EA-B2 extract respectively (Table 3), phytol and dihydrosphingosine (Table 3) have been reported possessing antioxidant activity in different investigations. Moreover, the structures and spectra of these antioxidant phytochemicals have been brought in Table 4.

Phytol (C20H40O, RT= 13.50) is a diterpene and a member of the group of branched-chain unsaturated alcohols. A strong antioxidant property of this compound has been reported. It can remove hydroxyl radicals and nitric oxide and prevent the formation of thiobarbituric acid reactive substances (TBARS) in in vitro assay. In addition, it has shown antioxidant properties in terms of scavenging hydroxyl radicals. Mere 0.9 ng/mL of phytol led to an increase in the removal of hydroxyl radicals by 9.66% and 8.62% compared to the 5.4 and 7.2 ng/mL concentrations, respectively. Phytol had also exhibited antioxidant properties in terms of scavenging nitrites and inhibited nitrite production at all the concentrations used [17].

The antioxidant property of phytol through non- and pre-clinical models has also been reported in vitro and in vivo methods where DPPH and ABTS•+ radical scavenging tests were used as in vitro methods, and *Saccharomyces cerevisiae* test was used as in vivo method. At 7.2 µg/ml, phytol was shown to reduce 59.89% and 62.79 % scavenging capacity of DPPH• and ABTS•+, respectively. In the Swiss mouse hippocampus, phytol decreased the NO2 and LP - contents while increasing the GSH, SOD, and CAT activities [6].

Dihydrosphingosine (C18H39NO2, RT= 12.15) had shown the antioxidant property by triacylglycerol (TAG) oxidation with and without α-tocopherol. Three types of TAG from linseed, fish, and soybean oil were oxidized at 50 °C to determine the effect of dihydrosphingosine (d18:0) with or without α tocopherol by using triacylglycerol (TAG) oxidation. Based on the oxygen consumption and total volatile formation, dihydrosphingosine was shown to have antioxidant properties on TAG oxidation in the absence of α-tocopherol. Further, the combination of dihydrosphingosine with α-tocopherol exhibited strong antioxidant properties [24]. Shinde et al. have also concluded dihydrosphingosine to be the most probable antihaemolytic and antioxidant compound [19].

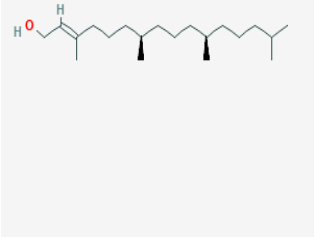
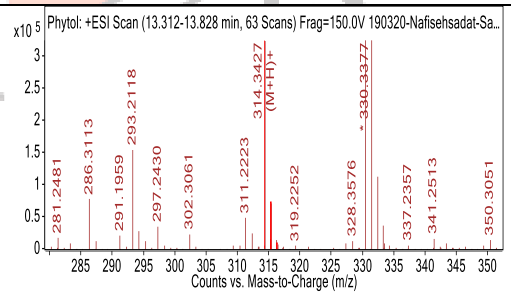
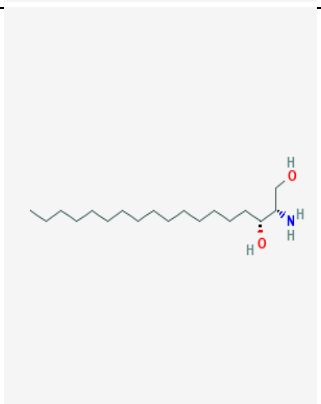
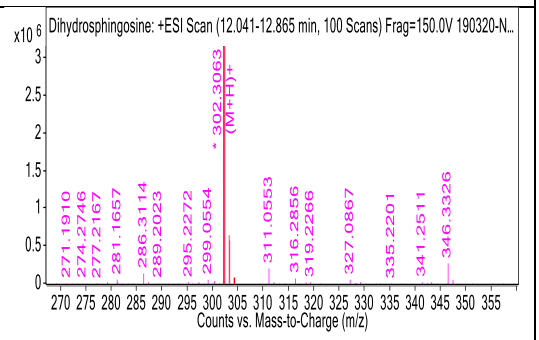
Table 3 Phytochemicals from R-EA-B2 and /S/EA/B4 extracts

Name/R/EA/B2	Type of compound	Empirical Formula	Retention time (min)	m/z	**Name/R/EA/B2**	Type of compound	Empirical Formula	Retention time (min)	m/z
Terephthalic acid	Carboxylic acid	C8H6O4	15.143	49.0233	4,8-dimethyl-dodecanoic acid	Fatty acyl	C14H28O2	8.832	228.2097
Ambelline	Alkaloid	C18H21 N O5	7.778	14.1387	1-hexadecanoyl-sn-glycerol	Glycerolipid	C19H38O4	17.827	330.278
N-Acetylsphingosine	Sphingolipid	C20H39 N O3	15.978	324.29	Dodecyl glucoside	Carbohydrate	C18H36O6	10.554	348.2523
2-Hydroxymyristic acid	Fatty acyl	C14H28 O3	7.854	262.2381	6-deoxyerythronolide B	Polyketide	C21H38O6	14.027	386.2679
4-hydroxy palmitic acid	Fatty acyl	C16H32 O3	9.587	290.2694	2,2,9,9-tetramethyl-undecan-1,10-diol	Fatty acyl	C14H30O2	11.001	230.2256
6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	-	C24H32 O7	11.996	415.212	3-hydroxy-eicosanoic acid	Fatty acyl	C20H40O3	14.066	328.2989
2E,4Z,6Z,8Z-Decatetraenedioic acid	-	C10H10 O4	8.528	177.0551	1alpha,25-dihydroxy-26,27-dimethyl-24a-homo-22-oxavitamin D3 / 1alpha,25-dihydroxy-26,27-dimethyl-24	-	C29H48O4	16.593	460.356

Phytosphingosine	Sphingolipid	C18H39 N O3	11.04	318.3008	Trp Trp Tyr	Amino acid	C31H31 N5O5	18.129	553.2341
***Phytol**	**Diterpenes**	**C20H40O**	**13.50**	**314.28**	Stearaldehyde	Fatty acyl	C18H36 O	12.271	268.2777
***Dihydrosphingosine**	**Amine**	**C18H39 NO2**	**12.15**	**302.3056**	4-oxo-docosanoic acid	Fatty acyl	C22H42 O3	13.481	354.3147
1-Hexadecyl-2-O-methyl-glycerol	Sugar alcohol	C20H42 O3	13.803	330.3371	8,13-dihydroxy-9,11-octadecadienoic acid	Fatty acid	C18H32 O4	10.54	312.2313
1,3-Dicyclohexylurea	Organic compound	C13H24 N2 O	11.1	225.1969	12-oxo-9-octadecynoic acid	Fatty acyl	C18H30 O3	10.971	294.2207
C8-Dihydroceramide	Amine	C26H53 NO3	16.726	428.4109	N-depyridomethyl-Indinavir	-	C30H42 N4O4	19.428	522.3196
N-(3-hydroxy-7Z-tetradecenoyl)-homoserine lactone	Fatty acyl	C18H31 NO4	8.099	308.2229	1R,9S-HYDRASTINE	Isoquinoline	C21H21 N O6	8.645	383.1351
4-hydroxy lauric acid	Fatty acyl	C12 H24 O3	7.914	216.1733	Lys Cys His	Amino acid	C15H26 N6 O4 S	11.408	386.1737
C16 Sphinganine	Sphingolipid	C16H35 NO2	10.863	274.2746	1-Hexadecyl-2-O-methyl-glycerol	Sugar alcohol	C20H42 O3	13.803	330.3138
13-hydroxy-docosanoic acid	Fatty acyl	C22H44 O3	14.22	356.3532					
calcifediol	Sterol lipid	C22H44 O2	15.389	340.3348					
2-Acetyl-1-oleoyl-sn-glycerol	Lipid	C23H42 O5	19.702	398.3031					
Dihydroceramide C2	Carbohydrate	C20H41 N O3	17.162	343.3095					

*Identified phytochemicals with known antioxidant activity

Table 4 LC-MS spectra of antioxidant phytochemicals detected in the R-EA-B2 and S-EA-B4 extracts of *A. aspera*

Name of biomass	Name of the compounds	Structure	Spectrum
Root	Phytol		
Root	Dihydrosphingosine		

4.Discussion

In the present investigation roots of *A. aspera* extracted in solvents of different polarities showed phenolic compounds and substantial antioxidant potential in the DPPH scavenging assay.

The phenolic and flavonoid contents in *A. aspera* differed with the type of plant biomass and solvent used for extraction. The maximum phenolic content was observed in the R-EA extracts of *A. aspera*, and these extracts also showed the highest antioxidant activities. These results corroborate those reported by Sharma et al. [18]. They have shown the maximum phenolic content (400 mg gallic acid equivalent per 50 g tissue) in the ethyl acetate extracts of root and inflorescence (291.67 mg gallic acid equivalent per 50 g tissue). Among the solvents used for extraction in the present investigation, those with a relatively higher non-polar nature (ethyl acetate and acetone) seem to have better extracted the potential antioxidant phytochemicals. Similar results have been reported earlier. The ethyl acetate extracts of leaves, stem, and inflorescence of *A. aspera* were shown to possess excellent antioxidant property in DPPH assay with 20 – 100 µg/ml extract [16]. The ethanol leaf extract of *A. aspera* was shown to have antioxidant activity with IC50 of 7.49 µg/ml, whereas the ascorbic acid standard had IC50 of 11.73 µg/ml in the phosphomolybdinum method [21]. The ethanol and aqueous extracts of leaves of *A. aspera* have shown significant wound healing and antioxidant activities in Wistar rat models [9]. However, better antioxidant activities were observed in extracts prepared from a more non-polar solvent like acetone and ethyl acetate in the present investigation. The methanolic extract of *A. aspera* leaves at 100 µg/mL concentrations was reported to have the maximum antioxidant activity in DPPH radical scavenging and ferric ion reduction assays [15].

Higher antioxidant potential of the methanol extracts of the plant's roots and leaves has been reported from the DPPH scavenging assay [8].In the present investigation, despite a very high antioxidant activity in the selected TLC bands of S-EA, and R-EA extracts, they did not show the presence of any phenolic substances or flavonoids. It seems to be a very strange result. This TLC band showed high potential to scavenge the DPPH free radicals, yet they contained mainly lipids. These results tend to indicate that these phytochemicals are at par with the widely accepted phytochemicals of phenolics and flavonoids classes in having a better DPPH free radical scavenging potential. For example, the GC-MS analysis of a hydro-alcoholic leaf extract of *A. aspera* showed antioxidant components like lupeol, hexadecanoic acid, and ethyl ester [25].

5.Conclusion

In conclusion, most of the earlier studies have shown that *A. aspera* extracts prepared in more polar solvents have better higher antioxidant activities. Further, the extracts that showed higher phenolic and flavonoid contents also showed higher antioxidant activities. Therefore, the results of the present investigation are in line with such earlier reports. However, when the extracts were separated by TLC, the higher antioxidant activities were noted in the fractions containing mostly lipids. This is because lipids being non-polar are better extracted in more non-polar solvents. Therefore, it is reasonable to conclude that the extracts of *A. aspera* that contain more lipids are likely to offer better antioxidant potential.

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