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PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT POTENTIAL OF TWO AMARANTHACEAE PLANTS: *ACHYRANTHES ASPERA* LINN AND *CHENOPODIUM ALBUM* LINN

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Keywords:

Achyranthes aspera Linn, *Chenopodium album* Linn, flavonoids, saponins, antioxidants

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ABSTRACT: Antioxidants are renowned protective molecules that are significant in the management of many chronic diseases. They can support the free radical mechanism to quench the cellular stress to prevent oxidative damage and improve existing therapy by playing a vital role in protection through antioxidant activities. The family Amaranthaceae is a well-known family for various health benefits. The selected plants *Achyranthes aspera* Linn and *Chenopodium album* Linn are also cited in the modern and traditional literature for their diversified health applications and pharmacological properties. So, the current work aims to screen the total phenolic, flavonoid, and saponin content of fractions made with solvents with various polarities and to screen their detailed antioxidant potential through DPPH assay, NO reducing assay, reducing power assay, and total antioxidant activity. It is inferred from the results that the methanol, chloroform, and aqueous fractions were rich in antioxidant components that are statistically significant with the reference standards.

INTRODUCTION: Antioxidants provide ameliorating effect against various lifestyle diseases. Since, chronic usage of synthetic drugs can severely damage a crucial organ such as the liver and kidney [1, 2]. Natural antioxidants are comparatively innocuous than synthetic ones with less side effects and suitable for long-term therapy. It can be perceived from the huge literature available for the protective effects of a natural antioxidant such as tannins, flavonoids and other polyphenolic compounds [3, 4].

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Achyranthes aspera Linn (Uttareni; Amaranthaceae) is an important herb in the Indian traditional systems of medicine with diuretic and hepatoprotective properties and also for malaria, diarrhoea, asthma, hypertension and diabetes [5].

Pharmacologically, it is proved to possess antimicrobial 16, anti-inflammatory [7], immuno-modulatory [8], anticancer [9], antinociceptive [10], thrombolytic [11], antidiabetic [12], hepatoprotective [13], nephroprotective [14], antidepressant [15], spermicidal [16], anti-allergic [17], *etc.* Phytochemicals such as D-glucuronic Acid and its β-D-galactopyranosyl ester [18], hentriacontane, 10-tricosanone, 10-octacosanone & 4-tritriacontanone [19], oleanolic acid& its glycosides, p-benzoquinone, spathulenol, nerol, α-ionone, asarone and eugenol *etc.* [20-22] *Chenopodium album* Linn (Vastukam; Amaranthaceae) is a famous ayurvedic herb that is having a long history

of treating various ailments. Traditionally it is used as anthelmintic, cardiotonic, carminative, digestive, diuretic, and laxative. It is also useful in peptic ulcers, dyspepsia, laxative, aphrodisiac, tonic flatulence, strangulation, pharyngopathy, splenopathy, opthalmopathy and general debility [23]. Various pharmacological activities were reported for *C. album,* such as antipruritic, analgesic [24], anti-inflammatory [25], anticancer [26], hepatoprotective [27], antibacterial [28], anthelminthic [29], antiulcer [30], *etc.*

In virtue of the above findings, our current investigation is aimed to determine the total phenolic, flavonoid, saponin content of various fractions of aerial parts of *Achyranthes aspera* and Chenopodium album along with their detailed antioxidant screening in four *in-vitro* models *viz.,* DPPH assay, NO reducing assay, reducing power assay and total antioxidant activity.

MATERIALS AND METHODS:

Plant Material: Aerial parts of *Achyranthes aspera* Linn and *Chenopodium album* Linn were collected from the Hyderabad rural areas and authenticated by Dr. P.V. Prasanna, Scientist "G", Botanical survey of India, Hyderabad, Telangana.

Extraction: Two kilograms of the fresh plants were shade dried at a temperature 25-30 ℃ for 7 days. The dried leaves were powdered in a pulverizer.

The powdered plant material was subjected to Soxhlet extraction using petroleum ether, chloroform, Ethyl acetate, Methanol, and water to get the respective extracts.

The extracts were evaporated to dryness with a rotary evaporator and lyophilized to get powder. The percentage of yield was calculated using the following formula [31]: -

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

Where, W_1 = weight of the crude extract residue obtained after solvent removal, W_2 = weight of plant powder packed in the extractor

Phytochemical Screening: The preliminary phytochemical screening of all the extracts of *Achyranthes aspera* Linn and *Chenopodium album* Linn were performed according to the standard procedures [32].

Total Phenolic Content Estimation: Phenolic compounds possess high free radical scavenging activity with antimutagenic, antitumor, and other pharmacological properties. This leads to the development of natural antioxidant compounds from plants33.

Preparation of Standard Gallic Acid for Calibration Curve: Concentrations ranging from 25-100 μg/mL of standard gallic acid solution was prepared by dissolving pure gallic acid in methanol; 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7% Sodium carbonate were also added to make a final volume of 10 mL followed by the 30 minutes incubation at 40 °C. The coloured solution thus obtained was measured at 760 nm using a UV-visible spectrophotometer, and a calibration curve was plotted for the average values of the results obtained in triplicates.

Estimation of Total Phenolic Content: The total phenolic content of all the extracts of *A. aspera* and *C. album* were estimated by the Folin-Ciocalteu method as described by Singleton *et al.,* (1965) with slight modifications. Various concentrations of the extracts ranging from 25-100 μg/mL were prepared, and the total phenolic content was estimated as described above. The total phenolic content of the extracts was expressed as mg of gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) [20].

% NO radical scavenging activity = $[(A_{control} - A_{sample}) / A_{sample}] \times 100$

Total Flavonoid Content Estimation: The flavonoids content for *A. aspera* and *C. album* were determined by the $AlCl_3$ method using quercetin as standard. 125 μL of the extract solution is added to 75 μL of a 5% $NaNO_2$ solution. After 6 min, 150 μL of 10% $AlCl_3$ was added, followed by 5 min of incubation. 750 μL of 1M NaOH solution was added to this mixture, diluted the final volume to 2500 μL with distilled water, and incubated for 15 min to get the pink colour. The absorbance was measured at 510 nm. The total flavonoid content was expressed as μg of quercetin equivalents per mg dry matter (μg QE/ mg dry weight) using the calibration curve. All the experiments were run in triplicate. The mean values and standard deviations were calculated [34].

Total Saponin Content Estimation: The total saponins content of various extracts of *A. aspera* and *C. album* were determined using the method of Hiai *et al.,* [35]. Add 250 mL of vanillin reagent of 8% w/v to the 250 mL aliquot of plant extract (1000 µg/ml) and add 2.5 mL of 72% v/v of sulfuric acid slowly to the walls of the test tube, heat it on a water bath for 10 min at 65 ºC and then cooled in ice-cold water bath for 3 to 4 minutes before reading the absorbance at 544 nm against the blank. The total saponin content was expressed as diosgenin equivalents in milligram per gram of the extract using a standard curve generated with diosgenin (ranging from 20 to 80 µg/ml).

***In-vitro* Antioxidant Activity:**

DPPH Antiradical Capacity: The free radical scavenging activity of various extracts of A. aspera and *C. album* were determined by Ilahi *et al.* [36] A stock solution of DPPH was prepared in methanol by dissolving 33mg of DPPH in 1L of methanol, the initial absorbance of stock solution is 0.493, and 5 ml of this stock solution was mixed with 1 ml of plant extract at different concentrations (125, 250, 500, 1000 µg/ml). After 30 min, the absorbance was measured at 517 nm and compared with standard Ascorbic acid. Scavenging activity was expressed as the percentage inhibition and calculated using the following formula:

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / (A_{control}) \times 100$$

Whereas control and sample indicate the absorbance of the DPPH solution and the reaction mixture, respectively.

The effective dose of plant extract needed to neutralize 50% of the DPPH radical solution (IC50) was obtained from a plot comparing percent inhibition to extract concentration.

Nitric Oxide Scavenging Activity: Nitric oxide radical scavenging activity was determined by the method reported by Garrat [37]. Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, which interacts with oxygen to produce nitrite ions, which the use of the GriessIllosvoy reaction can determine. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract of various concentrations and the mixture was incubated at 25 °C for 180 min. From the above solution, 0.5 ml was taken out, and add 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid). The solution was incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) were added and incubated at room temperature for 30 min; the absorbance was measured at 540 nm using a spectrophotometer. The nitric oxide radical scavenging activity was calculated.

Reducing Power: Reducing power of the *A. aspera* and *C. album* were determined by Oyaizu method with slight modifications38. 0.75 mL plant extract of various concentrations(125, 250, 500, 1000) were mixed with 0.75 mL of phosphate buffer (0.2 moles, pH 6.6) and 0.75 mL of potassium hexacyanoferrate (1%, w/v), the solution was incubated at 50°C in a water bath for 20 min.

The reaction was stopped by adding 0.75 mL of 10%Trichloro Acetic Acid (TCA) and then centrifuged at 3000 rpm for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/v) for 10 min. The absorbance was measured at 700 nm using UV visible Spectrophotometer. Higher the absorbance of the reaction mixture indicates greater the reducing power.

Total Antioxidant Activity: Total antioxidant activity for *A. aspera* and *C. album* Linn. was determined by the phosphomolybdenum method. 0.1 ml of various concentrations of plant extracts (25-200 µg/ml) were added to 1 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate).

After an incubation of 90 minutes at 95 °C, samples were cooled to room temperature, and the absorbance of the mixture was measured at 695 nm by using UV Visible spectrophotometer (Labindia 3000+). Ascorbic acid was used as standard. 0.1 ml of methanol was used as blank [39].

RESULTS AND DISCUSSION:

Percentage Yield: The percentage yield with all the extracts was calculated and depicted in **Table 1**. The methanol yielded more quantity, followed by extraction with water. *C. album* is found to yield more extract than *A. aspera*.

TABLE 1: PERCENTAGE YIELD OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM*

Solvents	%yield	
	A. aspera	*C. album*
Petroleum ether	3.87	1.96
Chloroform	3.35	2.9
Ethyl acetate	0.57	3.4
Methanol	5.15	4.05
Aqueous	5.85	3.58

Phytochemical Analysis: Qualitative phytochemical analysis was carried out for the various extracts of *A. aspera* and *C. album* using standard protocols and revealed the presence of various phytochemicals like alkaloids, glycosides, saponins, tannins, steroids and carbohydrates. Phytoconstituents in various extracts were shown in **Table 2**.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM*

Phytochemicals	*A. aspera*					*C. album*				
	PE	CE	EE	ME	AE	PE	CE	EE	ME	AE
Alkaloids	+	+	+	+	+	-	+	+	+	+
Tannins	-	+	-	+	+	-	+	+	+	+
Steroids	+	-	-	-	+	+	+	-	-	-
Flavonoid s	-	-	+	+	-	-	+	+	+	+
Terpenoids	+	+	-	-	-	+	+	-	-	-
Proteins	-	-	-	-	-	-	-	-	+	+
Carbohydrates	-	-	-	+	+	-	-	-	+	+
Phenols	-	-	-	+	+	-	-	-	+	+
Saponins	-	+	+	+	+	-	+	-	+	+
Glycosides	+	+	-	+	+	-	-	-	+	+

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethyl acetate extract, ME- Methanolic extract, AE- Aqueous extract, '+'indicates presence, '-' indicates the absence of phytochemical

Estimation of Total Phenolic Content: The total phenolic content in various fractions of the whole plant *A. aspera* and *C. album* were determined using the Folin-Ciocalteau method. The results were expressed in terms of milligram per gram equivalents of gallic acid **Table 3**.

A calibration curve was plotted by the absorbance and concentrations (mg/mL) using prepared dilutions.

The regression analysis was performed and the resulting equation was Abs = y = 0.0052x + 0.0123. The coefficient of determination for standard curves was greater than 0.99 (R^2 = 0.9922).

Thus, the calculated straight line could explain more than 99% of the experimental data **Fig. 1**. The total phenolic content of the extracts was calculated and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Where y is the absorbance at 760 nm and x is the total phenolic content in the extracts.

Among the five extracts, methanolic extract (101.22 ± 0.22 & 280.27 ± 0.3) and ethyl acetate extract (15.87 ± 0.25 & 97.07 ± 0.23) has shown the higher and lower amount of phenolic compounds for *Achyranthes aspera* Linn and *Chenopodium album* Linn respectively **Fig. 2**. The concentration of the phenolic compounds was increased with an increase in the dose.

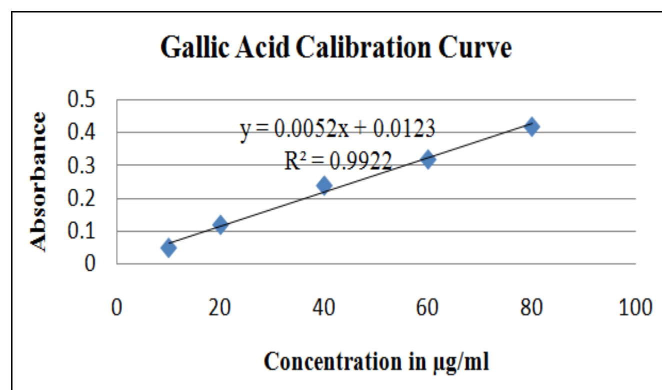

FIG. 1: CALIBRATION CURVE OF GALLIC ACID

TABLE 3: TOTAL PHENOLIC CONTENT OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM* EXPRESSED IN GALLIC ACID EQUIVALENTS

S. no.	Extract	*A. aspera*	*C. album*
1	Petroleum Ether	96.47±0.23	59.53±0.34
2	Chloroform	98.35±0.42	164.13±0.3
3	Ethyl Acetate	15.87±0.25	97.07±0.23
4	Methanol	101.22±0.22	280.27±0.3
5	Aqueous	36.73±0.11	116.87±0.3

Each value in the table is represented as mean ± SD (n=3), mg GAE/gm DW: milligram gallic acid equivalent per gram dry weight, mgQE/gm DW: milligram quercetin equivalent per gram dry weight.

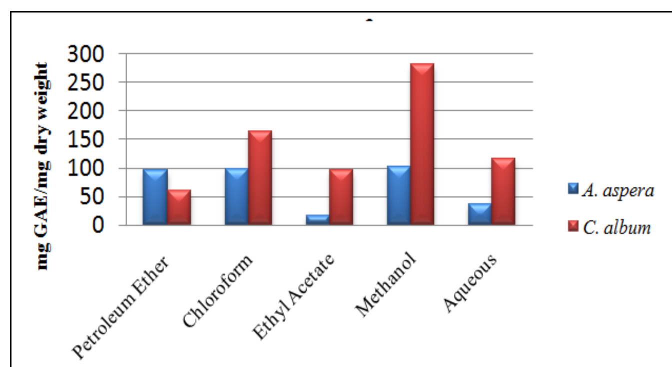

FIG. 2: TOTAL FLAVONOID CONTENT (MG QE/MG DRY WEIGHT) OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM*

Estimation of Flavonoid Content: Flavonoids scavenges the excess free radicals that cause cellular stress. Flavonoids are polyphenolic substances obtained from a large number of plants. They exhibit various biological activities such as antioxidants, antimicrobials, anti-inflammatory, antiallergic and antiviral.

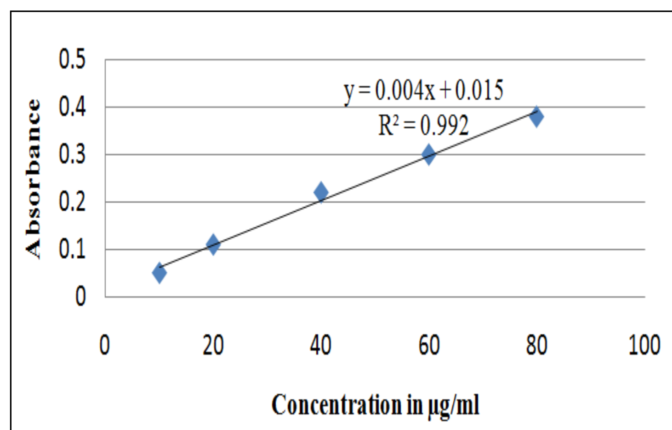

FIG. 3: CALIBRATION CURVE OF QUERCETIN

The total flavonoid content profile of the plant extracts was established through the colorimetric method using $AlCl_3$.

A calibration curve ($y = 0.0047x + 0.0151$, $R^2 = 0.9923$) was plotted using various concentrations of standard quercetin (0–100 µg/mL) and expressed in quercetin equivalents (QE) per gram **Fig. 3**.

The total flavonoid content of the extracts was calculated and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g).

Where y is the absorbance at 760 nm and x is the total flavonoid content in the extracts of *A. aspera* and *C. album* were depicted in **Table 4** and **Fig. 4**.

Among the five crude extracts, chloroform extract of *A. aspera* (67.16 mg/g of QE), methanolic extract of *C. album* (292.25 mg/g of QE) has higher flavonoid content when compared with the other fractions.

TABLE 4: TOTAL FLAVONOID CONTENT IN VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM* EXPRESSED IN QUERCETIN EQUIVALENTS

S. no	Extract	*A. aspera*	*C. album*
1	Petroleum Ether	53.92±0.14	51.83±0.29
2	Chloroform	67.17±0.39	124.33±0.14
3	Ethyl Acetate	24.83±0.38	110.58±0.29
4	Methanol	60.08±0.29	292.25±0.5
5	Aqueous	34.25±0.25	152.08±0.38

Each value in the table is represented as mean ± SD (n=3), mg GAE/gm DW: milligram gallic acid equivalent per gram dry weight

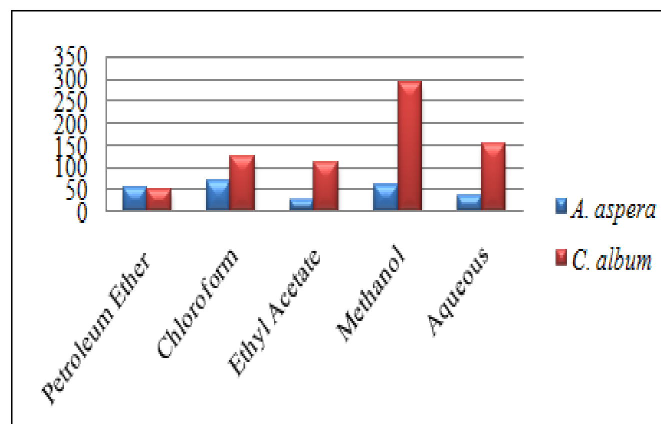

FIG. 4: TOTAL PHENOLIC CONTENT OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM*

Estimation of Saponin Content: The total saponin content of various extracts of *A. aspera* was determined and established by using a vanillin reagent.

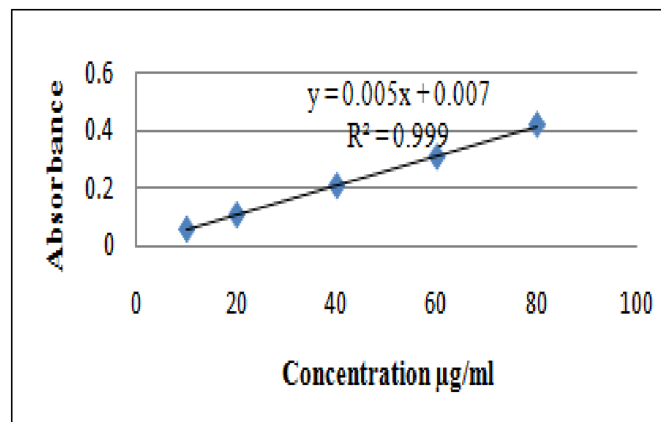

FIG. 5: CALIBRATION CURVE OF DIOSGENIN

Diosgenin was used as a standard compound, and the total saponin content was expressed as mg/g

diosgenin equivalent using the standard curve equation: y = 0.0051x +0.0071, R^2 = 0.9996 **Fig. 5**; where y is the absorbance at 544 nm and x is total saponin content in the extracts of *A. aspera* and *C. album* expressed in mg/g.

In the present work, methanolic extracts (88.62 ± 0.01 & 341.2 ± 0.2 mg/g DE) have shown high saponin content for both the plants when compared to other extracts **Table 5** & **Fig. 6**.

TABLE 5: TOTAL SAPONIN CONTENT IN VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM* EXPRESSED IN DIOSGENIN EQUIVALENTS

S. no	Extract	*A. aspera*	*C. album*
1	Petroleum Ether	56.61±0.005	83.46±0.09
2	Chloroform	62.62±0.001	237.26±1.12
3	Ethyl Acetate	25.02±0.001	185.8±0.2
4	Methanol	88.62±0.01	341.2±0.2
5	Aqueous	44.65±0.01	125.06±0.11

Each value in the table is represented as mean ± SD (n=3), mg DE/gm DW: milligram Diosgenin equivalent per gram dry weight.

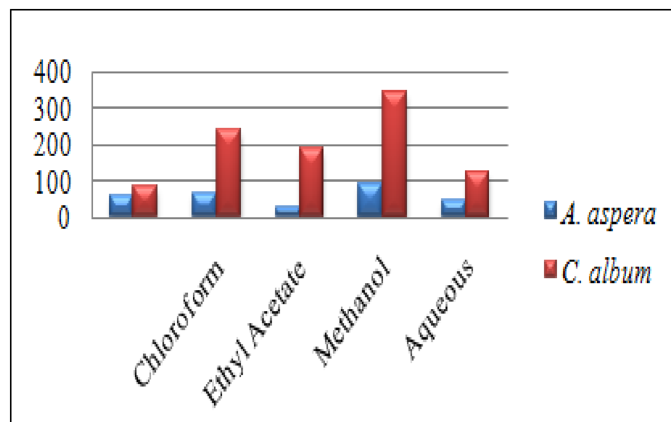

FIG. 6: TOTAL SAPONIN CONTENT OF VARIOUS EXTRACTS OF *A. ASPERA* AND *C. ALBUM*

DPPH Antiradical Capacity: DPPH is a stable free radical with a purple chromophoric group, which upon gaining proton from phenolic compounds it changes its color from purple to yellow. The plant extracts were scavenged for the free radicals in a concentration-dependent manner. A calibration curve (y = 6.7859x+ 0.2233, R^2 = 0.9928) was plotted using various concentrations of standard Ascorbic acid (0–75 µg/mL) and expressed in percentage. DPPH radical scavenging of various extracts of *A. aspera* and *C. album* were compared with standard Ascorbic acid, which was reported in **Fig. 7** & **8**. Among all the various extracts, the percentage inhibition of scavenging activities of the methanolic extract showed high %inhibition of free radical scavenging activity of 68.42% at 1000 µg/mL when compared with the standard ascorbic acid has shown 70.34%inhibition at 1000 µg/mL. The antioxidant activity is mainly due to the presence of the amount of total polyphenolic compounds. IC_{50} values of various extracts of *A. aspera* are mentioned in **Table 8**.

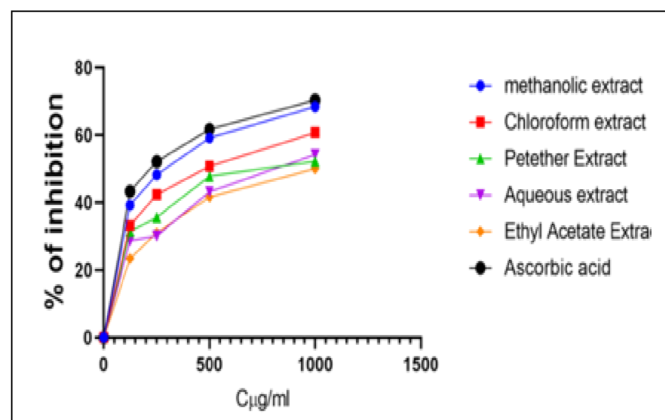

FIG. 7: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF *A. ASPERA*

Similarly, *C. album*. Methanolic fraction has shown maximum scavenging activity followed by chloroform fraction. The lowest radical scavenging activity was shown by the petroleum ether fraction. Scavenging potential was compared with standard Ascorbic acid. IC_{50} values of various fractions of *C. album* were shown in **Table 8**. Lower the IC_{50} higher the antioxidant activity. IC_{50} values of methanolic, chloroform, aqueous, ethylacetate fractions of *C. album* and ascorbic acid are 208.44 µg/ml, 214.96 µg/ml, 234.93 µg/ml, 268.36 µg/ml and 192.002. µg/ml. The result reveals there is no significant difference between the % inhibition of methanolic extract and the reference standard ascorbic acid.

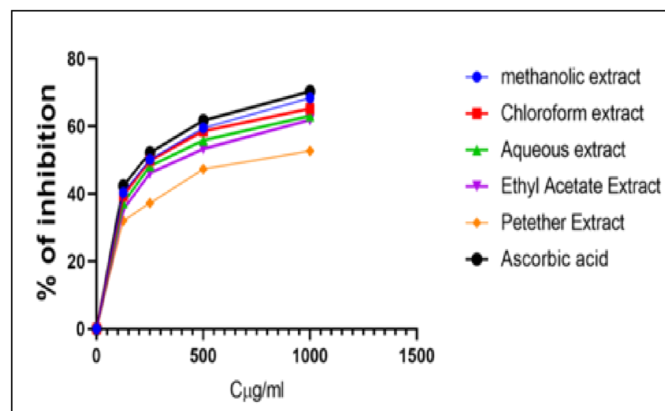

FIG. 8: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF *C. ALBUM*

TABLE 6: IC_{50} VALUES FOR THE VARIOUS EXTRACTS IN DPPH METHOD

S. no.	Extract	*A. aspera*	*C. album*
1	Petroleum Ether	821.01	841.72
2	Chloroform	509.83	214.19
3	Ethyl Acetate	995.72	268.36
4	Methanol	223.58	208.44
5	Aqueous	850.89	234.93
6.	Ascorbic Acid	188.49	192.002

Nitric Oxide Scavenging Activity: Nitric Oxide is a potent chemical mediator for various physiological processes, which plays a vital role in various biological systems. Excess generation of NO in biological tissues alters the structural and functional behavior of many cellular components. Sodium nitroprusside solution in Phosphate buffer is incubated at 25˚C for 2 h results in a linear time-dependent nitrite production.

The various extracts of the *A. aspera* were tested. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. Nitric oxide scavenging activity of various extracts of *A. aspera* was shown in **Fig. 9**. IC_{50} values of NO scavenging activity were shown in **Table 7**.

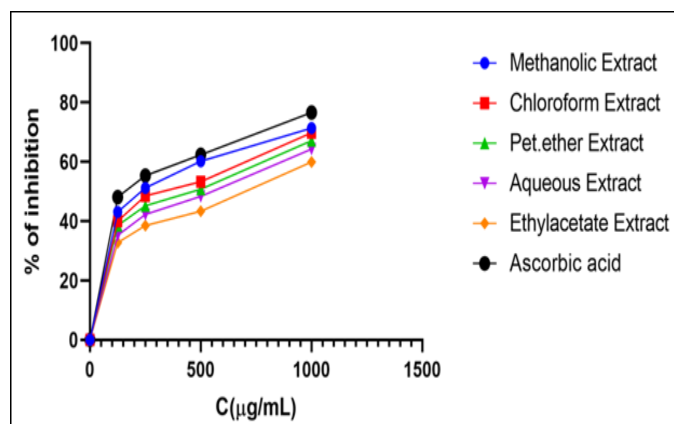

FIG. 9: THE NITRIC OXIDE SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF *A. ASPERA*

Nitric oxide scavenging activity was evaluated for the various fractions of *C. album* and compared with standard ascorbic acid. Methanolic fraction exhibited higher nitric oxide scavenging activity when compared with other fractions. Decreasing order of the nitric oxide scavenging activity of the various fractions of *C. album* is methanolic extract > Chloroform extract > Aqueous extract > Ethylacetate extract > Petroleum ether extract **Fig. 10**. IC_{50} values of various extracts of *C. album* was shown in the **Table 7**.

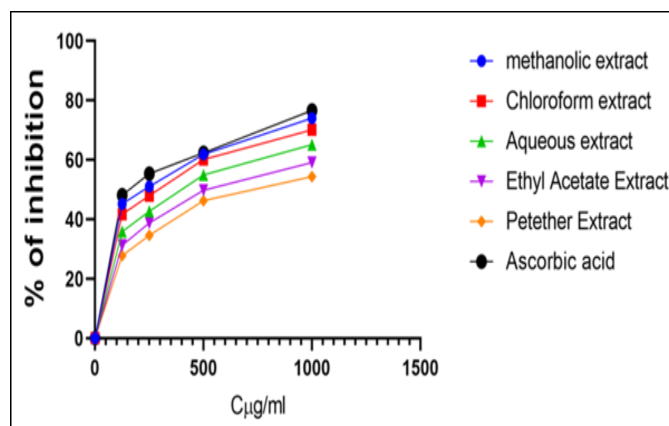

FIG. 10: NITRIC OXIDE SCAVENGING ACTIVITY OF VARIOUS FRACTIONS OF *C. ALBUM*

TABLE 7: IC_{50} VALUES FOR THE VARIOUS EXTRACTS IN NO SCAVENGING ACTIVITY

S. no	Sample	*A. aspera*	*C. album*
1	Petroleum Ether	260.94±0.06	772.19±0.03
2	Chloroform	219.43±0.07	214.33±0.04
3	Ethyl Acetate	803.41±0.06	586.61±0.36
4	Methanol	192.57±0.04	186.33±0.004
5	Aqueous	673.49±0.24	294.62±0.13
6.	Ascorbic acid	162.60±0.002	162.61± 0.01

Reducing Power: Ferric reducing power assay experiment determines the antioxidant potential of the plant. The plant extract with high reducing power is reported to possess high antioxidant potential. The reducing power assay method is based on the principle that substances that have reduction potential react with potassium ferricyanide (Fe^{3+}) converted to potassium ferrocyanide (Fe^{2+}), which in turn reacts with ferric chloride to form ferric–ferrous complex ions, which are identified by a change in color from yellow to bluish-green and the absorbance is measured at 700 nm.

TABLE 8: REDUCING POWER ASSAY OF VARIOUS EXTRACTS OF *C. ALBUM*

Sample	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Ethylacetate extract	0.05	0.09	0.12	0.13
Aqueous extract	0.12	0.12	0.24	0.35
petroleum ether extract	0.12	0.13	0.27	0.38
Chloroform extract	0.13	0.14	0.32	0.47
Methanolic extract	0.11	0.13	0.76	0.96
Ascorbic acid	0.21	0.22	1.02	1.43

The results for ferric reducing power activity of various extracts of *A. aspera* is compared with

standard ascorbic acid are shown in **Table 8** & **Fig. 11**. 1000 µg/ml of methanolic extract has shown high reducing power when compared with that of other extracts. The reducing power potential of extracts increased with the increase in dose; however, MEAA exhibited low, reducing power when compared to that of ascorbic acid.

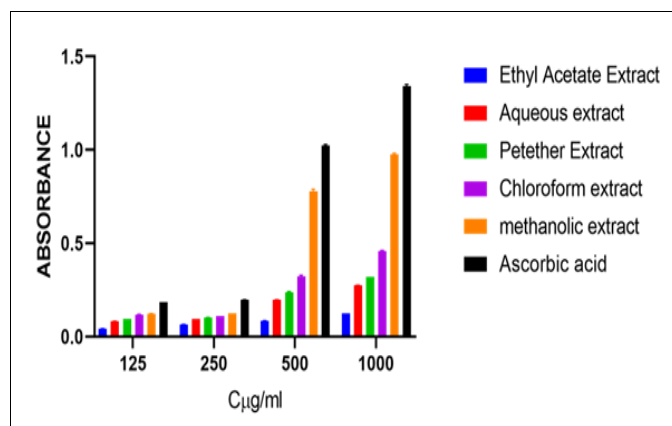

FIG. 11: REDUCING POWER ASSAY OF VARIOUS FRACTIONS OF *A. ASPERA*

Reducing power assay determines the reduction of iron (III) to iron (II). The reductive capabilities of various extracts of *C. album* are compared with standard ascorbic acid, a strong reducing agent. The reducing power of the various fraction of *C. album* was shown in **Fig. 12**. An increase in absorbance indicates an increase in reducing power, and it is found that absorbance is concentration-dependent.

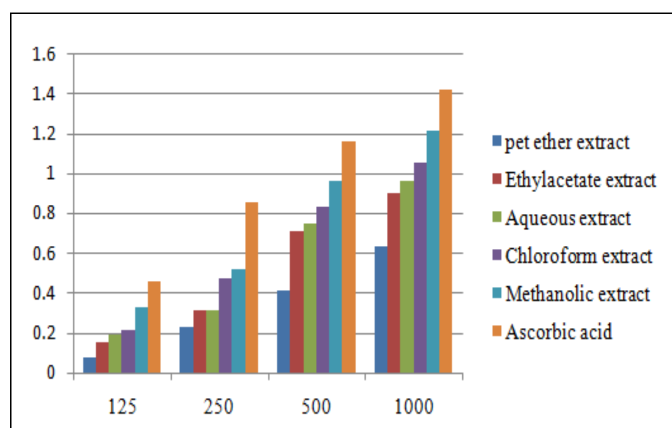

FIG. 12: REDUCING POWER ASSAY OF VARIOUS FRACTIONS OF *C. ALBUM*

Total Antioxidant Assay: The total antioxidant activity of the extracts of the *A. aspera* was screened through phosphomolybdenum assay. The total antioxidant capacity of the extracts was estimated based on the reduction of Mo, and the results found that the extracts were showing increased absorbance in a dose-dependant manner that demonstrates the total antioxidant potential of the plant's **Table 9** & **Fig. 13**. Standard Ascorbic acid that showed highest absorbance (0.911±0.02) that is comparable with the methanol extract (0.891 ± 0.05), followed by aqueous extract (0.816±0.11) and ethylacetate extract (0.812±0.06).

TABLE 9: TOTAL ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *A. ASPERA*

Sample	50µg/ml	100µg/ml	150µg/ml	200µg/ml
Petroleum Ether	0.091 ±0.05	0.186 ±0.03	0.264 ±0.01	0.317 ±0.17
Chloroform	0.118 ±0.13	0.181 ±0.11	0.229 ±0.13	0.315 ±0.16
Ethyl Acetate	0.224 ±0.08	0.418 ±0.02	0.626 ±0.16	0.812 ±0.06
Methanol	0.131 ±0.07	0.397 ±0.03	0.682 ±0.11	0.891 ±0.05
Aqueous	0.242 ±0.17	0.386 ±0.1	0.624 ±0.01	0.816 ±0.11
Ascorbic acid	0.471 ±0.1	0.682 ±0.14	0.813 ±0.01	0.911 ±0.02

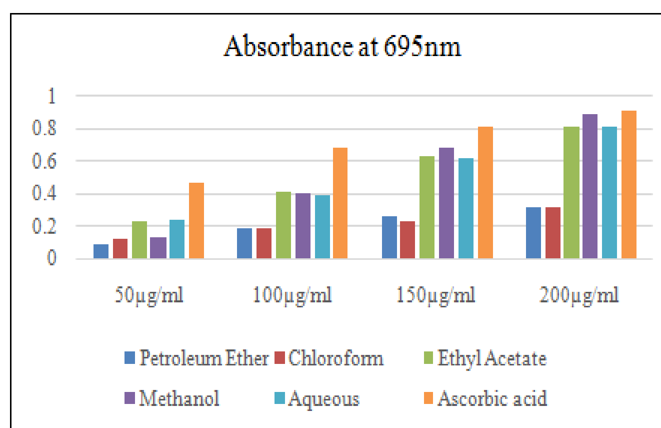

FIG. 13: TOTAL ANTIOXIDANT ACTIVITY OF *A. ASPERA*

TABLE 10: TOTAL ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *C. ALBUM*

Sample	50µg/ml	100µg/ml	150µg/ml	200µg/ml
Petroleum Ether	0.105 ±0.12	0.19 ±0.08	0.29 ±0.14	0.36 ±0.24
Chloroform	0.13 ±0.04	0.20 ±0.14	0.27 ±0.19	0.37 ±0.32
Ethyl Acetate	0.26 ±0.15	0.40 ±0.02	0.67 ±0.34	0.84 ± 0.65
Methanol	0.20 ±0.08	0.41 ±0.01	0.71 ±0.28	0.897 ±0.14
Aqueous	0.19 ±0.14	0.40 ±0.05	0.65 ±0.19	0.82 ±0.04
Ascorbic acid	0.47 ±0.12	0.68 ±0.07	0.81 ±.014	0.91 ±0.63

Similarly, *C. album* extract also showed significant antioxidant activity at the given doses in

comparison with the standard ascorbic acid. *C. album* extract is found to be more antioxidant than *A. aspera* **Table 10** & **Fig. 14**. Methanol extract of *C. album* exhibited (0.897±0.14), best antioxidant activity followed by ethyl acetate extract (0.84 ± 0.65) and aqueous extract (0.82±0.04).

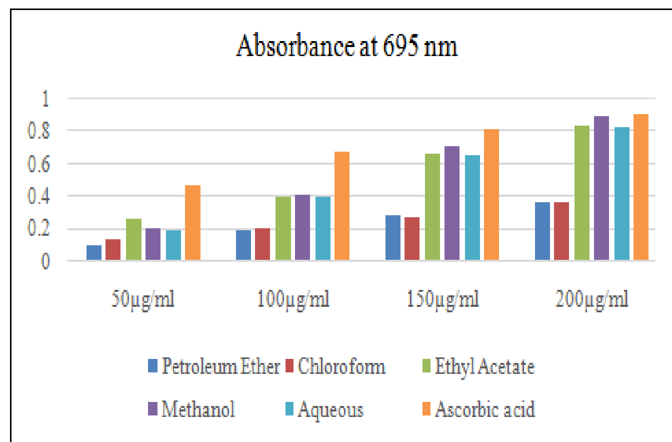

FIG. 14: TOTAL ANTIOXIDANT ACTIVITY OF *C. ALBUM*

SUMMARY AND CONCLUSION: To conclude the results, *A. aspera* and *C. album* were evaluated for various phytochemicals and proved to cherish with various phytochemicals such as alkaloids, glycosides, saponins, tannins, steroids and carbohydrates. Various fractionation of the plants is studied for detailed phytochemical profiling and reported to possess a good amount of phenolic content, flavonoid content and total saponin content. The antioxidant activity of all the fractions of *A. aspera* and *C. album* were screened in various *in-vitro* methods such as DPPH assay, NO free radical scavenging assay, reducing power assay, and total antioxidant assay.

The results suggest that methanolic, ethyl acetate and aqueous extracts for both the plants are having potent antioxidant activity when compared to the standard ascorbic acid. From this study it can be also be concluded that the polyphenolic contents present in the aerial parts of *A. aspera* and *C. album* are responsible for the reported antioxidant activity. Further studies are needed to understand the mechanism involved in the antioxidant activity.

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CONFLICTS OF INTEREST: Nil

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