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PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND FREE RADICAL SCAVENGING OF *Acyranthes Aspera* & *Calotropis Gigantea* BOTH PLANTS

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ABSTRACT

In the present study the Preliminary Phytochemical Investigation and free radical scavenging of *Acyranthes Aspera* & *Calotropis Gigantea* both Plants was studies.

Phytochemical investigation and free radical scavenging of *Acyranthes Aspera* & *Calotropis Gigantea* both plants ethanolic leaves extract. These both plants are used in traditional medicine for a number of ailments. *A. Aspera* & *C. Gigantea* both are a persistent plant with juicy, thick, and stout stem. Both plants leaves are smile and broad and panicle. Rhizomes are formed under the soil; send up derived shoots near the parent plant.

The present study was concluded that the presence of phytochemical in the both plant ethanolic leaves extracts *A. Aspera* & *C. Gigantea* and both plants exhibited antioxidant activity when subjected to the radical scavenging tests. The *A. Aspera* results (IC_{50}) for DPPH free radical scavenging assay were found to be (153.29µg/kg), and H_2O_2 radical scavenging test to be (117.12µg/ml) and The *C. Gigantea* results (IC_{50}) for DPPH free radical scavenging assay were found to be (135.33µg/kg), and H_2O_2 radical scavenging test to be (97.10µg/ml). The results of the present study also revealed that preliminary phytochemical screening of ethanolic leaves extracts

have Tannins, phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids and fats.

Keywords: *Acyranthes Aspera*, *Calotropis Gigantea*, DPPH and Hydrogen Peroxide, Preliminary Phytochemical Investigation, Ascorbic acid, Anti-oxidants

INTRODUCTION (*Achyranthes aspera*)

Since nature has been a source of therapeutic (medicinal) chemicals for centuries, an impressive number of new or novel drugs have been derived from natural sources **[1]**. Recently it is seen that there have been phenomenal rise in the preparation and usage of plants derived health products in both developed and developing countries, which results in ensuring an exponential growth of herbal products globally. In an effort to identify all medicinal plants used globally the WHO has identified more than 22,000 species **[2]**.

According to a WHO survey, 80% of the population in developing nations uses traditional herbal medicine as their main source of healthcare **[3]**.

Exploration of the chemical components of plants and pharmacological screening may give the foundation for developing leads for the development of new agents. The very important life-saving drugs that are now a part of modern medicine were also provided to us by herbs **[4]**.

Because of prominent cumulative and permanent side effects of contemporary medications there has been a noticeable shift in recent years towards medicinal herbs. However, the natural reservoir and the related traditional knowledge are increasingly in danger due to urbanization, overpopulation, and continuing exploitation of these herbal reserves **[5]**.

Currently, in order to identify and develop novel drug agents, many plants are evaluated based on their traditional uses. *A. aspera* is one of the several plants whose medicinal uses are being investigated which is commonly known as Rough chaff tree (English) and Chirchira (Hindi). In this paper, author intends to provide information of the chemical constituents present in various parts of *A. aspera* as well as the various pharmacological actions, which results in various uses of *A. aspera* for the treatment of various diseases.

Plant profile

Figure 1: Plant of *Achyranthes aspera*

Figure 2: Leaves of *Achyranthes aspera*

Geographical distribution

Up to a height of 2100 m, it grows as a weed on road sides, field boundaries, and waste areas across India. It is also found in the Australia, Bangladesh, South Andaman Islands, America, Ceylon, Africa and Tropical Asia **[6, 7]**.

Traditional uses

Achyranthes aspera has been documented in Ayurveda and Chinese medicine **[8-10]**. In "Nighantus" the plant's medicinal characteristics as a digestive aid, purgative, cure for internal organ inflammation, itch, piles, enlarged cervical glands and abdominal enlargements has been described. Ashes of the whole plant were used by Hindus to prepare caustic alkaline preparations **[11-13]**. Both European and Indian Physicians are familiar with the plant's diuretic properties. In cases of general an asarca and renal dropsy, the plant's decoction is employed as a diuretic **[14]**. In the Philippines, the herb is used to treat toothaches, gastrointestinal issues, and dysentery **[15]**.

The plant is used to cure asthma, dyspepsia, bronchitis, flatulence and menstrual disorders as well as it is used as an expectorant, revulsive, anodyne, depurative, anthelminitic, sudorific and stomachic **[16]**. The roots are utilised to cure cough, abdominal tumours and stomach **[17]**.

The tribal people of Andhra Pradesh's Chitoor district use this plant to cure epilepsy and the Payasam Kheer made from its seed and milk is effective remedy for brain disease **[18]**.

The plant is used as diuretic, astringent, purgatives, a remedy for colic, dropsy, piles, skin eruptions **[19]**, It is also used to treat fractured bones **[20]**, whooping cough, respiratory problems **[21]**, asthma **[22]** and leucoderma **[23]** and laxatives **[24]** antidote for snake bites **[25]**. The inflorescence is utilised for hydrophobia and cough **[26]**. Hydrophobia is treated using fruit. The seeds

are used for gonorrhoea, insect bite, hydrophobia, cough, especially whooping cough, additionally, they have cathartic, purgative, and emetic properties **[27]**.

INTRODUCTION (*Calotropis gigantean***)**

From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. Since the beginning of civilization, human beings have worshiped plants and such plants are conserved as a genetic resource and used as food, fodder, fibre, fertilizer, fuel, febrifuge and in every other way , *C. gigantea* is one such plant **[28]**.

In ancient ayurvedic medicine the plant *C. gigantea* is known as “Sweta Arka” and Caotropis procera as “Raktha Arka”. Both of them are often similar in their botanical aspects and also have similar pharmacological effects **[29]**.

The plants Calotropis gigantean are referred to as "Sweta Arka" and "Raktha Arka," respectively, in traditional ayurvedic medicine. Both of them frequently share botanical characteristics and pharmacological effects **[30]**.

The plants Calotropis gigantea and Caotropis procera are referred to as "Sweta Arka" and "Raktha Arka," respectively, in traditional ayurvedic medicine. Both of them frequently share botanical characteristics and pharmacological effects **[31]**.

All over India, this plant is found. In Hindi, it is commonly known as arka. India, a tropical nation, has access to the richest natural resources and the knowledge from antiquity to use them wisely. To be accepted by modern medicine, these therapies must first undergo a scientific evaluation to determine their active principles and comprehend how they work pharmacologically **[32]**.

Many clinically relevant medications were discovered as a result of the hunt for novel pharmacologically active substances from natural resources like plants, animals, and microorganisms **[33]**.

Common wasteland weed *C. gigantea* is also referred to as giant milk weed. Bangladesh, Burma, China, India, Indonesia, Malaysia, Pakistan, Philippines, Thailand, and Sri Lanka are the original home countries of this plant. C. gigantea is widely accessible in India and utilized for a variety of medical conditions in the country's traditional medical system **[34]**.

Plant profile

Figure 3: Plant of *Calotropis gigantea*

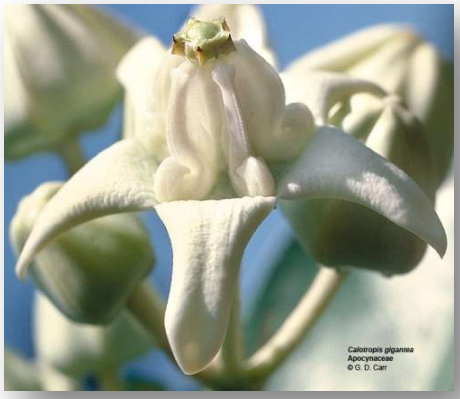

Figure 4: Flower of *Calotropis gigantea*

Therapeutic uses: -

The leaves extract of the plant is anthelmintic and leucoderma, tumours, ascites, and disorders of the abdomen; the plant is purgative, anthelmintic, alexipharmic, and treats leprosy, leucoderma, ulcers, tumours, piles, and diseases of the spleen, the liver, and the abdomen. The leaves are used to treat wounds and paralysed or painful joints and swellings. When dealing with sporadic fevers, the tincture made from the leaves is utilised as an antiperiodic.

Tumors, rat bites, inflammation, and good in ascites. The milk is laxative, purgative, bitter, and treats piles. The root bark is diaphoretic and treats syphilis and asthma. Sweet, bitter, anthelmintic, analgesic, astringent, and curative, the flower **[8-9]**.

Anti-oxidants

Living tissue has a control mechanism to keep reactive oxygen species (ROS) in balance. When ROS are generated in vivo i.e. (neurodegenerative disorders), many antioxidants play important role in our life. Their relative importance depends upon which ROS are generated, how and where they are generated, and which target oxidative damage (cell injuries) is considered. Antioxidants inhibit the production of reactive oxygen species by direct scavenging, decrease the amounts of oxidants in and around the cells, prevent ROS from reaching their biological targets, limit the propagation of oxidants such as the one that occurs during lipid per-oxidation, and oxidative stress, thereby preventing the aging phenomena **[38]**.

MATERIAL AND METHODS

Preparation of extracts:

Both plants leaves of *A. Aspera* & *C. Gigantea* was collected and reduced to small size after drying in shade for one month or till dried and crushed to form coarse powder. The powdered drug (250 gm) was subjected to continuous hot extraction with the help of soxhlet

apparatus using according polarity pet. Ether, ethanol. Each plant material was dried in hot air oven at 50^0C for an hour. After the effective extraction, the solvents were distilled off, the both extracts were then concentrated on water bath to become dried. The obtained extract was weighing and stored in an air tight container**. [Lin *et al*.,]**

Phytochemical Investigation [39-40]

Test for Alkaloids

Mayer's test: To 2-3 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* filtrate, few drops of Mayer's reagent were added along the side of the test tube. Formation of white or creamy precipitate might indicate the presence of alkaloids.

Wagner's test: To 1-2 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate might indicate the presence of alkaloids.

Dragendorff test: To 1-2ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* solution filtrate, few drops of Dragendorff's reagent were added in a test tube. Formation of red precipitate might indicate the presence of alkaloids.

Hager's Test: To 1-2 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow colour precipitate might indicate the presence of alkaloids.

Test for flavonoids

Lead acetate test: Few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was treated with few drops of lead acetate solution and then observed yellow precipitate might indicate the presence of flavonoids.

Shinoda test: Take it the few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea*, and add 5 ml (95%) of ethanol. Then, the mixture was treated with few fragments of magnesium turning, followed by dropwise addition of concentrated HCl. Formation of pink colour might indicate the presence of flavonoids.

Alkaline Reagent Test: ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was treated with few drops of NaOH solution one by one in a test tube. And then, observed yellow colour, and add the few drops of dilute acid, then the colour is less, might indicate the presence of flavonoids.

Tests for Glycosides

Borntrager's Test: To 3 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* and, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, an equal volume of chloroform (10%) was added and shaken well. The solvent layer was separated and then ammonia was added. Formation of pink to red colour in

ammoniacal layer might indicate the presence of glycosides.

Legal's test: To 1 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was dissolved in pyridine. 1 ml of nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour might indicate the presence of cardiac glycosides.

Keller-Killiani test: To 2 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5ml of concentrated H_2SO_4 was added carefully by the side of the test tube. Formation of blue colour in the acetic acid layer might indicate the presence of cardiac glycosides.

Test for Saponins

Froth test: few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam might indicate the presence of Saponins.

Tests for Tannins and Phenolic compounds

Ferric chloride test: Few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour might indicate the presence of phenolic compounds.

Lead Acetate Test: Few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was dissolved in distilled water. To this solution few drops of lead acetate solution were added. Formation of white precipitate might indicate the presence of Phenolic compounds.

Iodine Test: To 2-3 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* and, few drops of dilute iodine solution was added. Formation of transient red color might indicate the presence of Phenolic compounds.

Test for Carbohydrates:

Molisch Test: Few ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* was taken, treated with two drops of alcoholic α-naphthol solution in a test tube and then 1 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Then, observed that violet ring at the junction might indicate the presence of carbohydrates.

Benedict's test: Equal volume of Benedict's reagent and ethanolic leaves extract of *A. Aspera* or *C. Gigantea* were mixed in a test tube and heated on the water bath for 5-10 minutes. The solution appeared green, yellow or red which might indicate the presence of reducing sugars.

Fehling's Test: To 1 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea***,** 1 ml of Fehling's A and 1 ml of Fehling's B solutions

were added in a test tube and heated on a water bath for 10 minutes. Formation of red precipitate might indicate the presence of reducing sugar.

Barfoed Test: 1 ml of ethanolic leaves extract of *A. Aspera* or *C. Gigantea* and barfoed reagent, were mixed in a test tube and heated on a water bath for 2 minutes. Red colour due to the formation of cupric oxide might indicate the presence of monosaccharide.

In-Vitro Antioxidant Evaluation

DPPH free Radical Scavenging Assay [40-41]

The DPPH assay of leaf extract was determined by according **(Pin Der Duh *et al*, 1995)**

Preparation of Standard Ascorbic acid solutions:

Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100 µg/ml). 200 µM solution of DPPH (in methanol) was prepared and 1.5ml of this solution was added to 1.5 ml of a methanolic ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

Preparation of Test solutions:

Various solutions of ethanolic leaves extract of *A. Aspera* or *C. Gigantea*, were prepared in 90% methanol to obtain different concentrations (10-100 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5 ml of a methanolic leaves extract of *A. Aspera* or *C. Gigantea*, solution of different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517 nm.

Preparation of Control solution:

For control, 1.5 ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. The absorbance of the control was taken after 30min against methanol (as blank) at 517 nm.

The antioxidant activity of ethanolic leaves extract of *A. Aspera* or *C. Gigantea*, and ascorbic acid were calculated by using the following formula in terms of % inhibition:

$$\% \text{ Inhibition} = \frac{\text{Ac 230} - \text{At 230nm}}{\text{Ac 230nm}} \times 100$$

Where:- Ac = Absorbance of control, At =Absorbance of ascorbic acid / leaves extract.

Hydrogen Peroxide radical Scavenging Assayss [42-43]

The ability of the ethanolic leaves extract of *A. Aspera* or *C. Gigantea*, to scavenge hydrogen peroxide was determined according to the method of **Ruch *et al* (1989).**

Preparation of Standard Ascorbic Acid solutions:

Different concentrations of the ascorbic acid were prepared in distilled water to give the solutions of varying concentrations (1-100µg/ml). 1ml of each solution of ascorbic acid was mixed with 2.4ml of 0.1M phosphate buffer and 600µl of 40mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230 nm using phosphate buffer as blank.

Preparation of test solutions:

Various concentrations of the ethanolic leaves extract of *A. Aspera* or *C. Gigantea***,** were prepared in distilled water to give solutions of varying concentrations (1-100µg/ml). 1ml of each solution of ethanolic leaves extract of *A. Aspera* or *C. Gigantea***,** was mixed with 2.4 ml of 0.1M phosphate buffer and 600µl of 40 mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230nm using phosphate buffer as blank.

Preparation of Control Solution:

For control, 2.5 ml of 0.1M phosphate buffer solution was mixed with 600µl of 40mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230 nm.

H_2O_2 radical scavenging activity of ethanolic leaves extract of *A. Aspera* or *C. Gigantea***,** and ascorbic acid were calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Ac 230} - \text{At 230nm}}{\text{Ac 230nm}} \times 100$$

Where:- Ac = Absorbance of control (0.1M phosphate buffer solution and H_2O_2)

At = Absorbance of ascorbic acid / leaves extract.

STATISTICAL ANALYSIS

The data of results obtained were subjected to statistical analysis and expressed as regression curve and % Inhibition curve value with help of EXCEL. The data were statically analyzed by Graph pad prism Software version (7.1).

RESULT

Preliminary Phytochemical Investigation of leaves extract of *A. Aspera* or *C. Gigantea***.**

The results are shown in tables and figure for illustration (Tables 1-9 and fig. 5-24).

The phytochemical screening of ethanolic leaves extract of *A. Aspera* or *C. Gigantea***,** exhibited that the main components i.e. Tannins, phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids and fats were present in **(Table 1)**.

Table 1: Phytochemical screening of ethanolic leaves extract of *A. Aspera*

S. No	Phytochemical	Test	*A. Aspera*	*C. Gigantea*
1	Alkaloids	Mayer's test	+	+
		Wagner's	+	+
		Hager's	-+	+
		Dragendroff's	+	-
2	Flavonoids	Lead acetate test	+	+
		Alkaline reagent test	+	+
		Shinoda test	+	+
3	Glycosides	Killer killians test	+	+
		Legal's	-	+
		Bortrager's test	-	+
4	Saponins	Froth test	+	-
5	Tannins and Phenolic compounds	Ferric chloride test	+	+
		Lead acetate test	+	-
		Iodine test	+	+
6.	Carbohydrates	Molish test	+	+
		Benedict's test	+	+
		Fehling's reagent test	+	+
		Barfoaed test	+	+

"+" indicating presence of compound, "–" indicating absence of compound

All images shows the primilary phytochemical screening test of ethanolic extract of *A. Aspera*

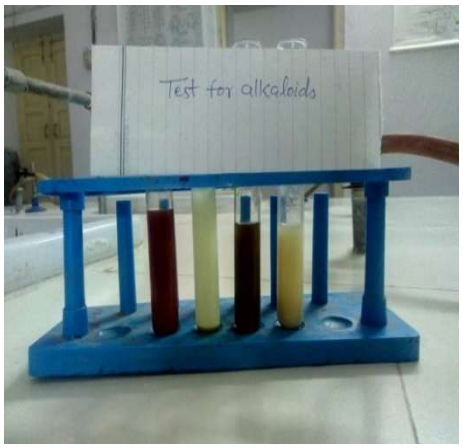

Figure 5: Test of alkaloids

Figure 6: Test of flavonoids

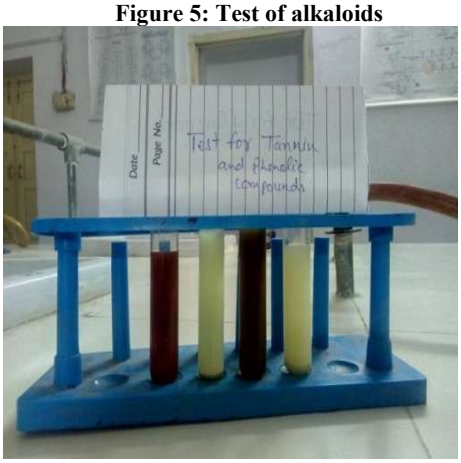

Figure 7: Test of tannins and phenolic compounds

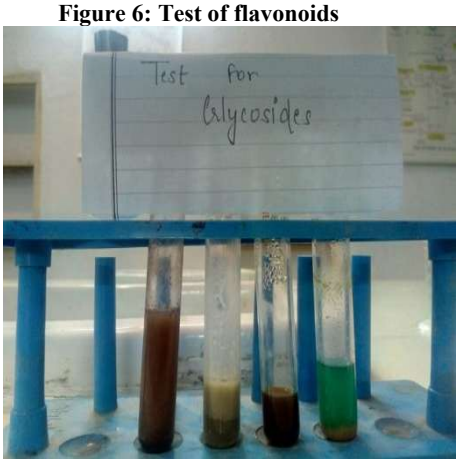

Figure 8: Test of glycosides

Figure 9: Test of saponins

Figure 10: Test of carbohydrates

All images of test of *C. Gigantea* are under the below:

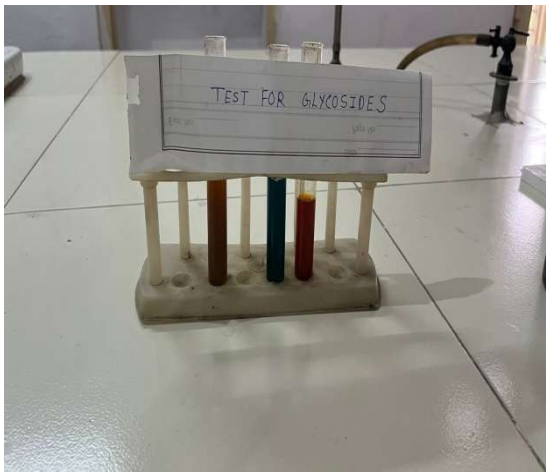

Figure 11: Tests for glycosides

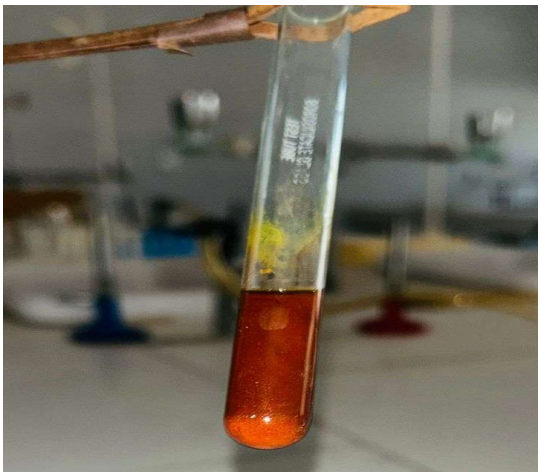

Figure 12: Test of saponins

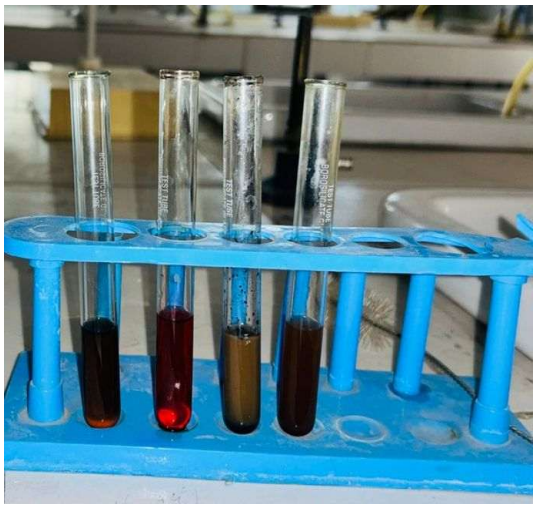

Figure 13: Tests for carbohydrates

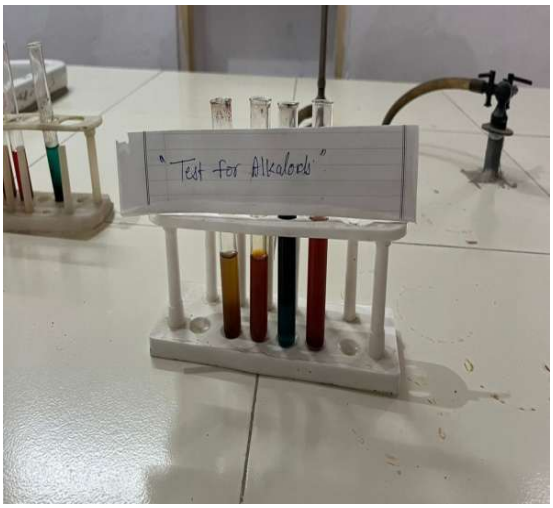

Figure 14: Tests for alkaloids

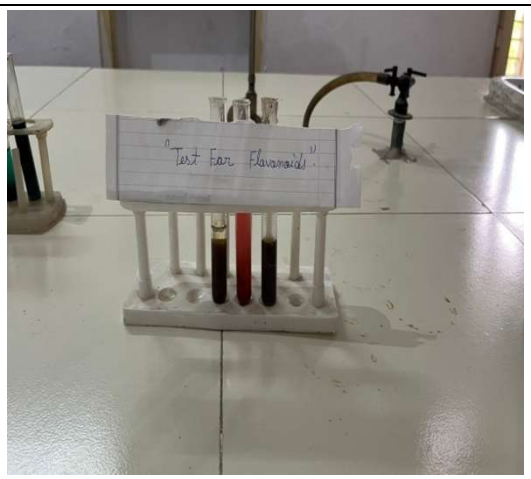

Figure 15: Tests for flavonoids

Figure 16: Test of tannins & phenolic compound

In-Vitro Antioxidant Assay

Antioxidant activity was assessed on the basis of different assay methods i.e. DPPH radical scavenging, hydrogen peroxide scavenging activities **(Table 2, 4, 6, 8) and IC_{50} (Table 3, 5, 7, 9)**.

DPPH Free Radical Scavenging Assay

DPPH (1, 1 diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule.

DPPH assay for ethanolic leaves extract was performed by using Ascorbic acid solution as standard. The absorbance data were recorded against the selected concentrations (1- 10 µg/ml for ascorbic acid and 10- 100µg/ml for fresh ethanolic leaves extract) at 517nm.

The percentage (%) inhibition curves for DPPH free radical scavenging assay of ascorbic acid and ethanolic leaves extract were plotted from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and ethanolic leaves extract were calculated using regression equation.

Table 2: The percentage Inhibition data of DPPH free radical scavenging assay by ascorbic acid and ethanolic extract of *A. Aspera*

S. No.	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.680	0.580	14.7 %	0.615	9.50 %
2.	40		0.520	23.5 %	0.560	17.64 %
3.	60		0.463	31.9 %	0.510	25.00%
4.	80		0.382	43.8 %	0.470	30.88 %
5.	100		0.340	50.0 %	0.410	39.70 %

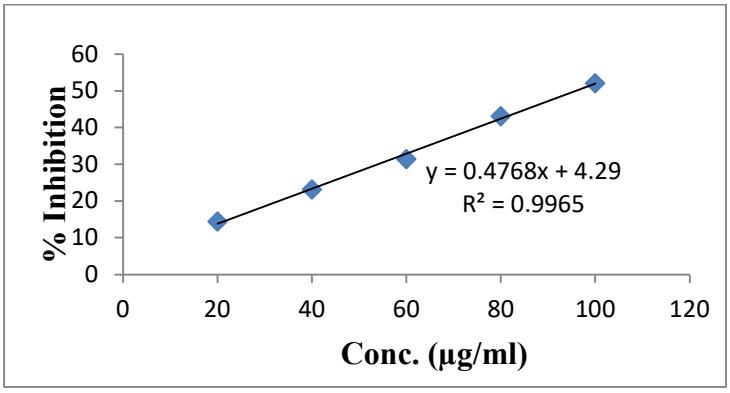

Figure 17: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

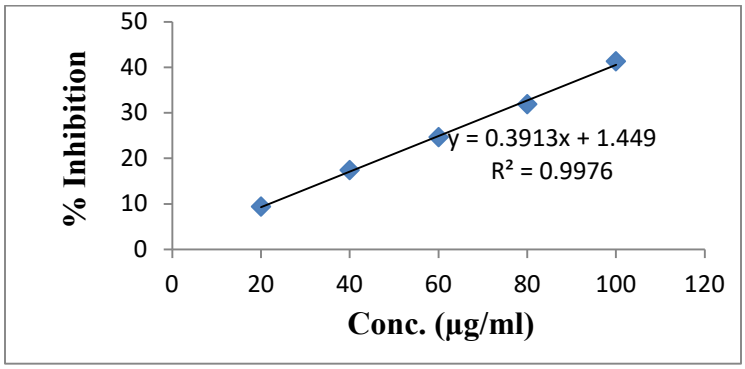

Figure 18: Representing % Inhibition curve and regression curve of leaves extract *A. Aspera* by DPPH assay method

Table 3: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *A. Aspera*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	84.40 µg/ml	y =0.476x + 4.29	R^2 = 0.996	828.2	1,3	<0.0001
ethanolic leaves extract	153.29 µg/ml	y=0.391x + 1.449	R^2 = 0.997	1146	1,3	<0.0001

It was observed that ethanolic leaves extract significant activity in DPPH assay in the concentration range of 10- 100µg/ml. IC_{50} for ascorbic acid was found to be 85.40µg/ml while for ethanolic leaves extract is 153.29µg/ml.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. H_2O_2 radical scavenging of ethanolic leaves extract was estimated by using the ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10-100µg/ml for ascorbic acid and ethanolic leaves extract).

The standard curve for H_2O_2 radical scavenging of ascorbic acid and ethanolic leaves extract were plotted from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and *A. Aspera* were calculated using regression equations.

Table 4: Percentage Inhibition data H_2O_2 of free radical scavenging assay by ascorbic acid and ethanolic extract of *A. Aspera*

S. No.	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.670	0.590	11.94	0.580	13.29
2.	40		0.530	20.89	0.530	20.89
3.	60		0.465	30.59	0.490	26.86
4.	80		0.392	41.49	0.430	35.82
5.	100		0.350	47.76	0.375	44.02

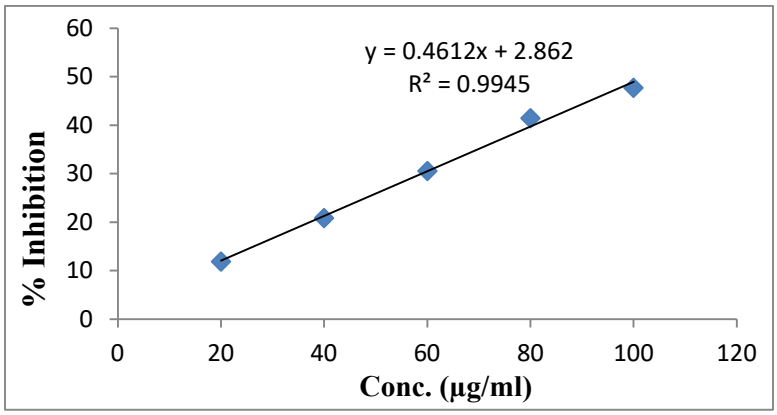

Figure 19: Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method

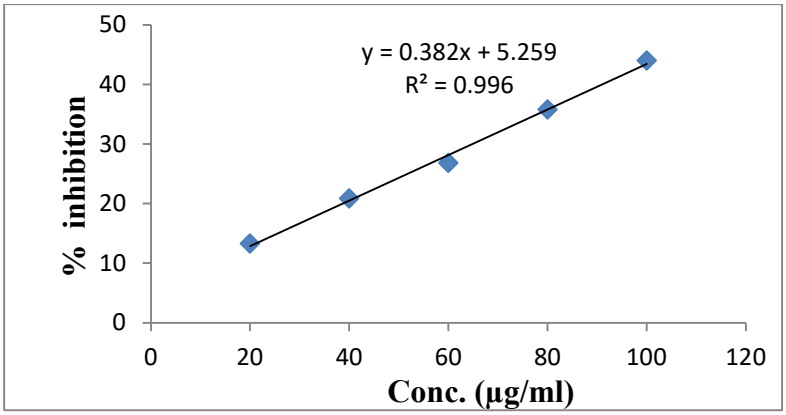

Figure 20: Percentage Inhibition curve and regression curve of leaves extract *A. Aspera* by H_2O_2 assay method

IC_{50} value was calculated by using straight-line equations. In H_2O_2 scavenging assay, it was observed that leaves extract served as a good scavenger of hydrogen peroxide in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 85.40 µg/ml while that for it was found to ethanolic extract of *A. Aspera* be 117.12 µg/ml.

Table 5: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *A. Aspera*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	84.40 µg/ml	y =0.461x + 2.862	R^2 = 0.994	483.7	1,3	<0.0002
Leaves extract	117.12 µg/ml	y =0.382x +5.259	R^2 = 0.996	178.1	1,3	<0.0001

DPPH Free Radical Scavenging Assay (Calotropis Gigantea)

DPPH (1, 1 diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule.

DPPH assay for ethanolic leaves extract was performed by using Ascorbic acid solution as standard. The absorbance data were recorded against the selected concentrations (1- 10 µg/ml for ascorbic acid and 10- 100µg/ml for fresh ethanolic leaves extract) at 517nm.

The percentage (%) inhibition curves for DPPH free radical scavenging assay of ascorbic acid and ethanolic leaves extract were plotted from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and ethanolic leaves extract were calculated using regression equation.

Table 6: The percentage Inhibition data of DPPH free radical scavenging assay by ascorbic acid and ethanolic extract of *C. Gigantea*

S. No.	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.690	0.610	11.59 %	0.590	14.49 %
2.	40		0.550	20.28 %	0.540	21.73 %
3.	60		0.493	28.55 %	0.500	27.53 %
4.	80		0.432	37.39 %	0.461	33.18 %
5.	100		0.380	44.92 %	0.420	39.13 %

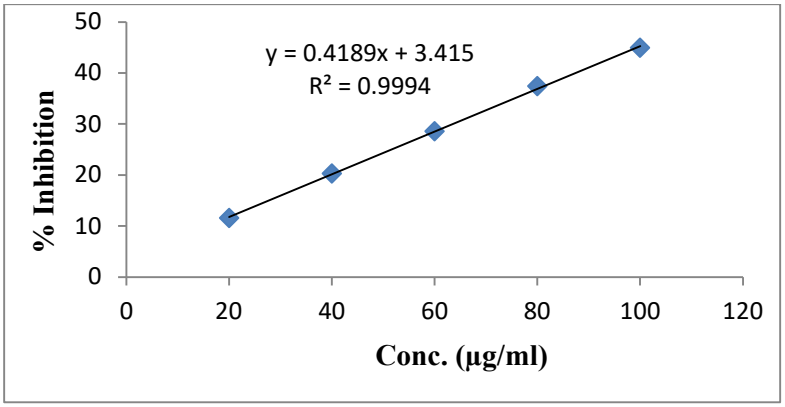

Figure 21: Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method

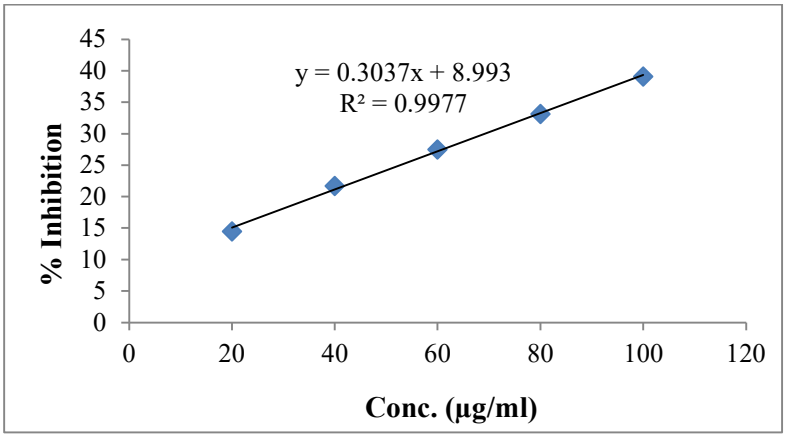

Figure 22: Representing % Inhibition curve and regression curve of leaves extract *C. Gigantea* by DPPH assay method

Table 7: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *A. Aspera*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn & Dfd value	P value
Ascorbic acid	84.40 µg/ml	y =0.418x + 3.415	R^2 = 0.999	928.2	1,3	<0.0001
ethanolic leaves extract	135.33 µg/ml	y=0.303x + 8.993	R^2 = 0.997	1016	1,3	<0.0001

It was observed that ethanolic leaves extract significant activity in DPPH assay in the concentration range of 10- 100µg/ml. IC_{50} for ascorbic acid was found to be 85.40µg/ml while for ethanolic leaves extract is 135.33µg/ml.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. H_2O_2 radical scavenging of ethanolic leaves extract was estimated by using the ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10-100µg/ml for ascorbic acid and ethanolic leaves extract).

The standard curve for H_2O_2 radical scavenging of ascorbic acid and ethanolic leaves extract were plotted from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and *C. Gigantea* were calculated using regression equations.

Table 8: Percentage Inhibition data H_2O_2 of free radical scavenging assay by ascorbic acid and ethanolic extract of *C. Gigantea*

S. No.	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.680	0.600	11.76	0.590	13.23
2.	40		0.550	19.11	0.540	20.58
3.	60		0.495	27.20	0.490	27.94
4.	80		0.440	35.29	0.440	35.29
5.	100		0.390	42.64	0.395	41.91

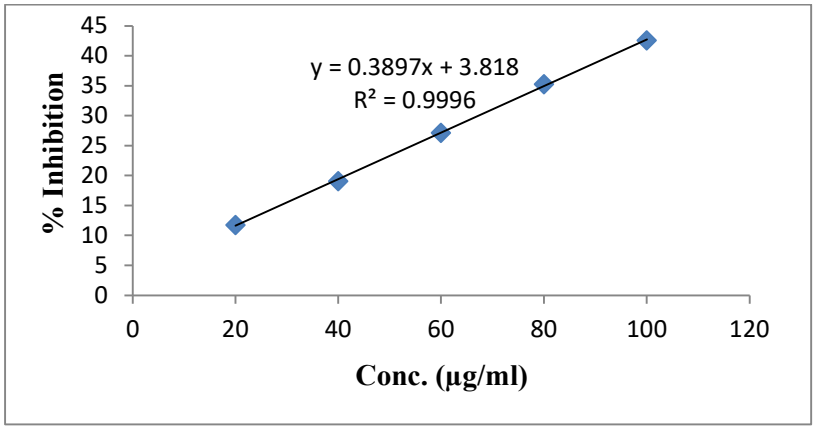

Figure 23: Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method

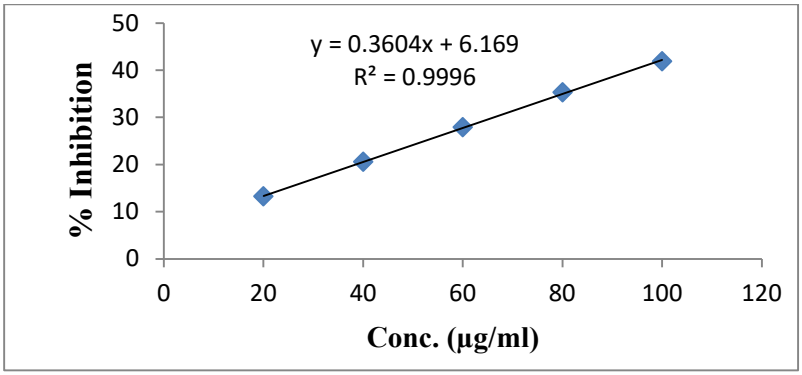

Figure 24: Percentage Inhibition curve and regression curve of leaves extract *C. Gigantea* by H_2O_2 assay method

IC_{50} value was calculated by using straight-line equations. In H_2O_2 scavenging assay, it was observed that leaves extract served as a good scavenger of hydrogen peroxide in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 85.40 µg/ml while that for it was found to ethanolic extract of *C. Gigantea* be 97.10 µg/ml.

Table 9: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *C. Gigantea*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	84.40 µg/ml	y =0.389x + 3.818	R^2 = 0.994	483.7	1,3	<0.0002
Leaves extract	97.10 µg/ml	y =0.360x +6.169	R^2 = 0.996	178.1	1,3	<0.0001

Preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponine, carbohydrates, flavonoids, tannins and phenolic compounds. Moreover, recent studies suggest that DPPH and Hydrogen peroxide is a strong oxidizing agent and can inactive few enzymes directly, usually by oxidation of essential thiol (-SH) groups.

Hydrogen peroxide can cross cell membrane rapidly. Once entered the cell, DPPH and H_2O_2 can react with Fe^{+2}/Cu^{2+} to form hydroxyl radical leading to the origin of many of its toxic effects. The study concluded that leaves extract of both plants exerted higher potential even better than ascorbic towards the hydrogen peroxide radical scavenging activity.

DISCUSSION

Free radicals have been a subject of significant interest among scientists in the past decade and their possible role in human diseases has gained importance now in days. Antioxidants neutralize toxin and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron. These also include related reactive oxygen species (ROS) that leads to free radical generation, causes the cascading chain reaction in biological system. In a normal, healthy organism or human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these ROS during various disease conditions. Lipids, proteins and DNA are all susceptible to attack by free radicals and cellular damage induced by oxidative stress has been implicated in the etiology of numerous diseases. DPPH and H_2O_2 radicals is widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic or crude extract of plants. The ability of both plants ethanolic leaves extract to reduce DPPH and H_2O_2 radicals supports its free radical scavenging activities. Our study indicates the proton donating property may be responsible for free radical scavenging activities of ethanolic leaves extract. Antioxidant compounds for example, sesamol, gallic acid poly-phenols reduce the Fe3+ to Fe2+ and are considered as chain breaking antioxidant for their proton donating activities. Both plants Ethanolic leaves extract reduces ferric ion at pH 7.4 which indicates its proton donating ability and supports its free radical scavenging activity. The reducing capacity of a compound may serve as a significant indicator of its antioxidant activity. It was therefore to be expected that both pants ethanolic leaves extract have antioxidant and radical scavenging ability. This activity is believed to be mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, or decomposing peroxides. Hence present study revealed that antioxidant property of both pants ethanolic leaves extract and the present study also revealed that preliminary phytochemical screening of both pants ethanolic leaves extract were found Tannins,

phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids.

CONCLUSION

Preliminary phytochemical screening of the both pants ethanolic leaves extract was found that Tannins and phenolic compounds, carbohydrate, Saponins, glycosides, flavonoids, alkaloids and both plants also found that anti-oxidant activities i.e. DPPH free radical and hydrogen peroxide scavenging activities.

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Author Contribution statement

Mr. Jitender Kumar conceptualized and gathered the data with regard to this work. Dr. Bhagwati Devi analyzed these data and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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