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FREE RADICAL SCAVENGING OF ETHANOLIC LEAVES EXTRACT OF ACHYRANTHES ASPERA & EUPHORBIA HIRTA

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ABSTRACT
In the present study ethanolic leaves extract of free radical scavenging of *Acyranthes Aspera* & *Euphorbia hirta* both Plants was studies. free radical scavenging of *Acyranthes Aspera* & *Euphorbia hirta* both plants ethanolic leaves extract. These both plants are used in traditional medicine for a number of ailments. *A. Aspera* & *E. hirta* 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 were concluded that the presence of phytochemical in the both plants ethanolic leaves extract *A. Aspera* & *E. hirta* and both plant 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 (119.38µg/kg), and H_2O_2 radical scavenging test to be (116.76µg/ml) and The *E. hirta* results (IC_{50}) for DPPH free radical scavenging assay were found to be (125.63µg/kg), and H_2O_2 radical scavenging test to be (126.05 µg/ml).

KEYWORDS: *Acyranthes Aspera*, *Euphorbia hirta*, DPPH and Hydrogen Peroxide, 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

Fig: 1 Plant of *Achyranthes aspera.*

Fig: 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

(*Euphorbia hirta*): Euphorbia hirta Linn. is commonly known as milkweed (Dudhy) and an asthma plant. It is known by the different names in different parts of the world.[28-29] The plant is characterized by the presence of milky white latex, which is more or less toxic. Latices of E. ingens, E. tirucalli, E. mey, and E. triangularis are possible sources of rubber. The plants of this family have been a subject of intense phytochemical examination such as flavonoids, triterpenoids, alkanes, amino acids and alkaloids. E. hirta is used as folklore medicine in the treatment of the gastrointestinal disorder (Diarrhea, amoebic dysentery, intestinal parasitosis, peptic ulcers, etc.), Bronchial and respiratory diseases (Asthma, bronchitis, hay fever, laryngeal spasms, coughcolds) and conjunctivitis.[30-34] Moreover, modern pharmacological investigations revealed that E. hirta and its active constituents possess wide array of pharmacological potential viz, antibacterial, antifungal, antioxidant, anti-inflammatory, antiasthmatic, antitumor, antimalarial, larvicidal, diuretic and antidiabetic activity. It is also noteworthy that E. hirta is used as antidiabetic, anti-inflammatory, antispasmodic and as anticancer curative agent. E. hirta has been used as a medicinal herb in China for long time. Different composition such as crude drug, infusion, lotion, decoction, and powders are also used. The plant of E. hirta plays a major role in traditional medicinal system due to its wide range of biological and pharmacological properties. Keeping in minds the effects of E. hirta in curing skin ulcer and body swelling, the plant was first recorded in 'Ling Nan Cai Yao Lu'. More than 10 books regarding the folk medicinal uses of this plant have also been recorded in China. A comprehensive and updated review is desirable to advance research on E. hirta. Hence we reviewed different studies on this plant in recent years. Thus besides taxonomic detail and ethnobotany, the chemical constituents and pharmacological potential is discussed. As per information available, it is the first composite review on this Kumaun Himalayan invasive plant, which provides a piece of comprehensive knowledge about E. hirta with its pharmacological potential and chemical compositions.

Fig. 3 Euphoria hirta.

Botanical Description: E. hirta Linn. as illustrated in Fig. 3, is a small annual, branched herb reaching up to 70 cm in height, purplish or reddish in colour with an ample amount of latex, and coated with shoot hairs. Leaves are opposite, distichous, and simple, stipules are linear, leaf blades are lanceolate, oblong serrated, long elliptic, acute apex, 3-4 cm in length and 1-1.4 cm in width, and its margin are smooth toothed. The inflorescence monoceious, an axillary or terminal cluster of flowers, is known as cyathium with several cyathia arranged into a cyme. The male and female flowers are in one involucre and both appellate. The flowers are unisexual, male flowers are sessile, bracteoles are linear, fringed, perianth is absent and possesses one stamen, female flowers have small pedicel, the perianth is rimmed, ovary is superior covered with minute hairs, 3- celled, possesses 3- styles, small and the apex is two-fold. The flowering period is usually the whole of the year. The fruit is allomorphic, pistillate, exerted, 3- lobed, truncate base covered with shoot hairs. The seeds are oblong, 4-sided prismatic, wrinkled, and brownish pink in color, capsule 3- seeded, green, and covered with fleshy prickles, seed smooth, hard mottle crustaceous testa with a white caruncle at the top enclosing oily endosperm. The root is distinct and developed primary root (tap root system). E. hirta Linn. Belongs to the family Euphorbiaceae, known as the spurge family of flowering plants. It is the largest family consisting of nearly 300 genera and 5000 species. Euphorbia is the largest genus of the Euphorbiaceae family, comprises about 1600 species.[35-47]

Classification: Kingdom

- Plantae Division: Spermatomatophyta
- Class: Dicotyledonae
- Order: Euphorbiales
- Family: Euphorbiaceae
- Genus: Euphorbia
- Species: Hirta

Vernacular Names of E. hirta: E. hirta has diverse synonyms, and vernacular names vary from region to region. In India it is known as Dudhy (In Hindi), Asthma plant (In English), Amampatchairaisi, dugadhika (In Sanskrit), Dudeli (In Gujrat), Dudnali, govardhan (In Marathi), Jhotikhuntian (In Orissa), Daun bijii kacang (In Indonesia), Ambin Janyan, Keremak susu (In Malaysia), Boro kerui (In Bangladesh).[48]

Nativity and Distribution: The plant species E. hirta Linn. is native to Central America. It is cosmopolitan in distribution, widely distributed Throughout tropical or temperate regions of India, Asia, Africa, and Australia. It prefers dry and humid conditions, from sea level up to 2200 meters altitude. It commonly grows in paddy fields, gardens, lowlands, waste places near roadside.[49]

Therapeutic uses: Medicinal Uses: The plant of E. hirta has a widespread traditional use in China and was recorded in Chinese pharmacopeia in the year 1977. The Yao people of China use the whole plant in the treatment of bronchitis. The decoction of dry plant is used externally in burned and scald, whereas freshly crushed leaves are applied to treat skin disease. This plant's decoction or tincture is used to cure asthma, chronic bronchial disorders, and emphysema diseases by the Zhuang people of China.[50] Dai people of China applied E. hirta in the stimulation of milk secretion and also in cessation of cough. It is extensively used in cough, kidney stones abscesses, and bronchial asthma.[51] It is also used traditionally to cure and prevent gastrointestinal disorders, afflictions of mucous membranes, and respiratory system disorders.[52] The cold extract of the leaves of E. hirta is used on a large scale to bathe small babies with skin --infections in Nigeria. The literature of ethnomedicinal plant also indicates that it is commonly known for increasing milk flow in females and different disorders.[53] The decoctions of this plant are also applicable in ear disease and the treatment of sore, boils, and it also has wound healing properties.[54-56] Different parts of this plant are traditionally used to cure the babies from worm infestations and also prove helpful in dysentery, jaundice, gonorrhea, acne, pimples, digestive orders, diabetes, several types of tumors, and in cancers in India.[57-59] The extracts of E. hirta are used against vomiting, diarrhea and as anti-venum against snakebite. It is also used in the treatment of asthma in South Africa. The leaves of E. hirta are mixed with leaves and petals of Datura metal to prepare asthma cigarettes in the Philippines. E. hirta possesses antispasmodic, antidiabetic, anti-inflammatory and anticancer curative properties.[60-62]

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.[63]

MATERIAL AND METHOD

Preparation of extracts: Both plants leaves of *A. Aspera* & *E.hirta* was collecting and reduced to small size after drying in shade for one month or till dry 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. The plant material was dry 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.,]**

DPPH free Radical Scavenging Assay[64-65]

The DPPH assay of leaf extract was determined by according **(Pin Der Duh et. At., 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 *E. hirta* 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 *E. hirta***,** 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 *E .hirta***,** and ascorbic acid were calculated by using the following formula in terms of % inhibition:

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

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 *E. hirta***,** 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 *E. hirta***,** 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 *E. hirta***,** 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 *E.hirta***,** and ascorbic acid were calculated by using the following formula:

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

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 *E.hirta***.**

The results are shown in tables and figure for illustration (Tables 1-8 and fig. 4-11).

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 1, 3, 5,7) and IC_{50} (Table 2, 4, 6,8).**

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 1: 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	0.700	0.630	10.00	0.615	9.50
2.	40		0.590	15.71	0.560	20.00
3.	60		0.550	21.42	0.510	27.14
4.	80		0.509	27.28	0.470	32.85
5.	100		0.460	34.28	0.400	42.85

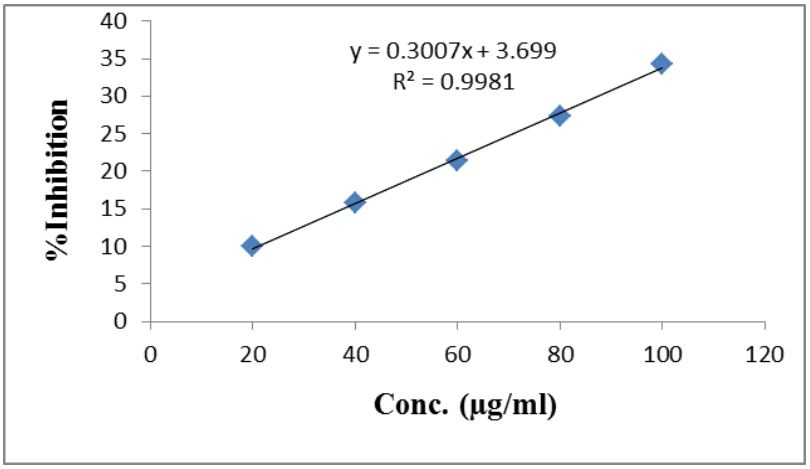

Fig. 4:- Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method.

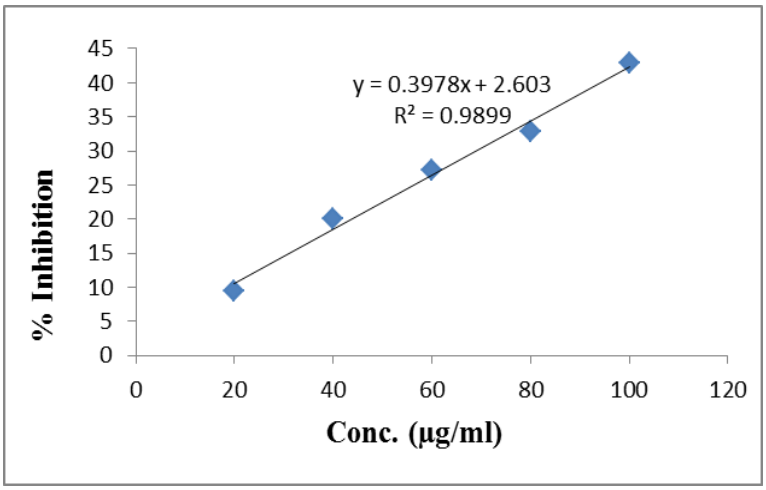

Fig. 5:- Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method.

Table 2: 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	154.33 µg/ml	y =0.300x + 3.699	R^2 = 0.998	728.2	1,3	<0.0001
ethanolic leaves extract	119.38 µg/ml	y=0.397x + 2.603	R^2 = 0.989	814.6	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 154.33µg/ml while for ethanolic leaves extract is 119.38µg/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 3: 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	**0.660**	0.590	10.60	0.575	12.87
2.	40		0.530	19.69	0.530	20.89
3.	60		0.465	29.54	0.490	26.86
4.	80		0.392	40.60	0.430	35.82
5.	100		0.350	46.96	0.375	44.02

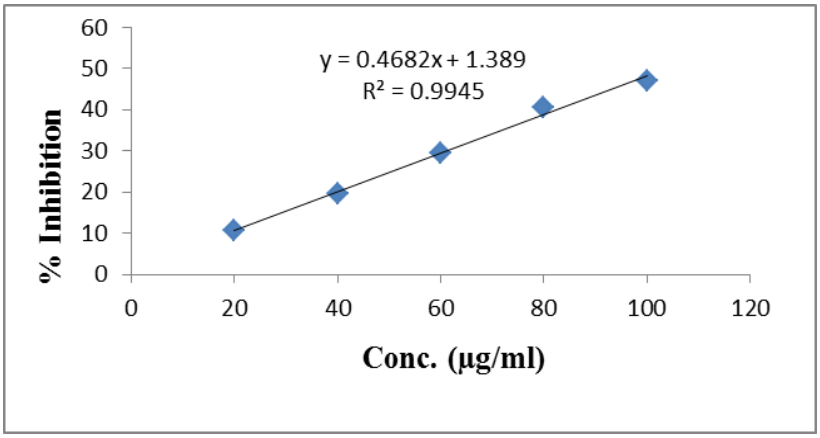

Fig. 6:- Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method.

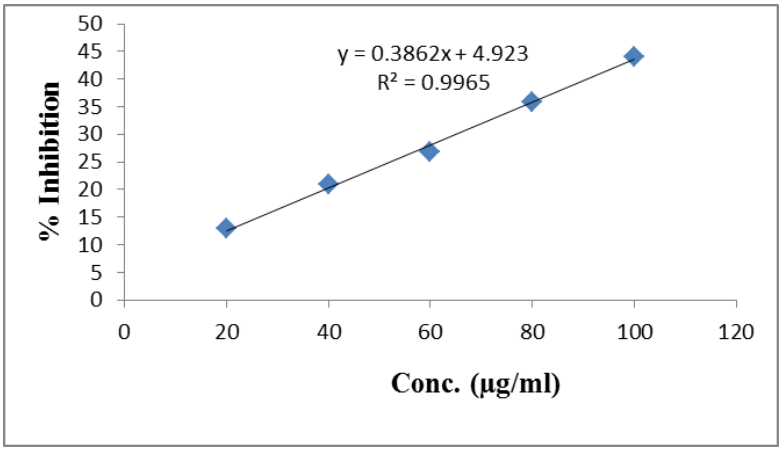

Fig. 7:- Percentage Inhibition curve and regression curve of leaves extract *A. Aspera* by H_2O_2 assay method.

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 103.86 µg/ml while that for it was found to ethanolic extract of *A. Aspera* be 116.76 µg/ml.

Table 4: 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	103.86 µg/ml	y =0.468x + 1.389	R^2 = 0.994	473.7	1,3	<0.0002
Leaves extract	116.76µg/ml	y =0.386x +4.923	R^2 = 0.996	170.1	1,3	<0.0001

DPPH Free Radical Scavenging Assay (*E. hirta*)

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 5: the percentage Inhibition data of DPPH free radical scavenging assay by ascorbic acid and ethanolic extract of *E.hirta.*

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	0.596	13.62
2.	40		0.560	18.40	0.545	21.01
3.	60		0.513	25.65	0.510	27.53
4.	80		0.432	37.39	0.461	33.18
5.	100		0.390	43.47	0.400	42.02

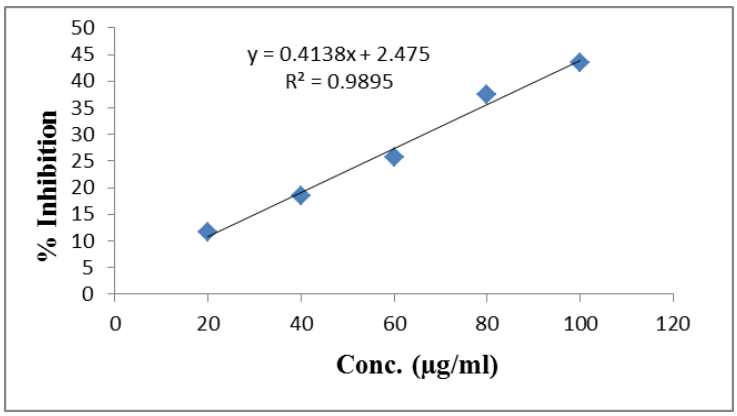

Fig. 8:- Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method.

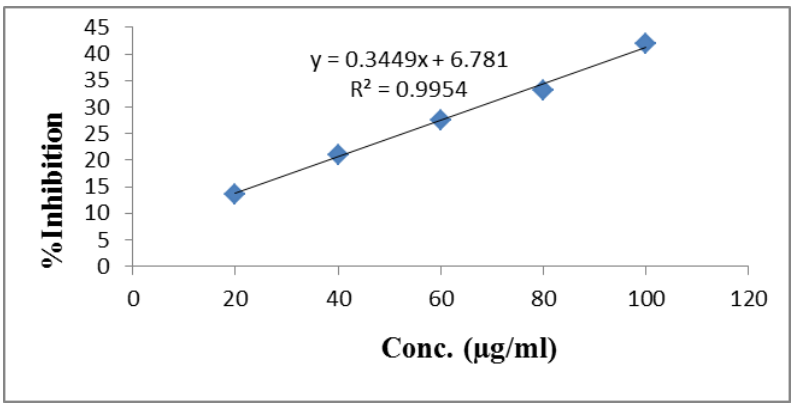

Fig. 9:- Representing % Inhibition curve and regression curve of ascorbic acid by DPPH assay method.

Table 6: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *E. hirta*.

Sample	IC_{50}	Equation	R^2 value	F value	Dfn & Dfd value	P value
Ascorbic acid	115.07 µg/ml	y =0.418x + 2.475	R^2 = 0.997	968.2	1,3	<0.0001
ethanolic leaves extract	125.63 µg/ml	y=0.344x + 6.781	R^2 = 0.995	997.1	1,3	<0.0002

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 115.07µg/ml while for ethanolic leaves extract is 125.63µg/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 *E.hirta* 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 *E.hirta*.

S. No	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Ascorbic acid) At	% Inhibition	Absorbance (Absorbance of ethanolic extract), At	% Inhibition
1.	20	0.680	0.620	8.82	0.600	11.76
2.	40		0.570	16.17	0.551	18.97
3.	60		0.510	25.00	0.510	27.94
4.	80		0.461	32.20	0.454	33.23
5.	100		0.410	39.70	0.405	40.40

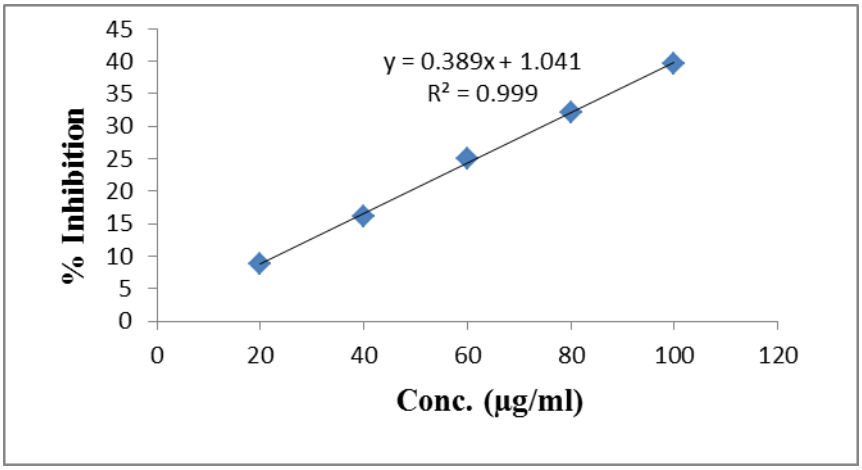

Fig. 10:- Representing % inhibition curve and regression curve of ascorbic acid by H_2O_2 assay method.

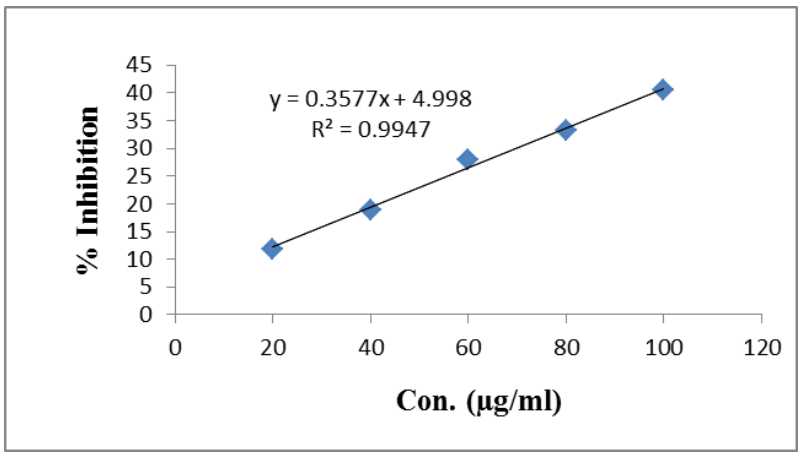

Fig.11:- Percentage Inhibition curve and regression curve of leaves extract *E.hirta* by H_2O_2 assay method.

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 125.85 µg/ml while that for it was found to ethanolic extract of *E. hirta* be 126.05 µg/ml.

Table 8: IC_{50} value and Statistical analysis of ascorbic acid and ethanolic extract of *E.hirta.*

Sample	IC_{50}	Equation	R^2 value	F value	Dfn and Dfd value	P value
Ascorbic acid	125.85µg/ml	y =0.389x + 1.041	R^2 = 0.999	473.7	1,3	<0.0002
Leaves extract	126.05 µg/ml	y =0.357x +4.998	R^2 = 0.994	168.1	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.

CONCLUSION

Preliminary phytochemical screening of the both pants ethanolic leaves extract was found that anti-oxidant activities i.e. DPPH free radical and hydrogen peroxide scavenging activities.

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AUTHOR CONTRIBUTION STATEMENT

Mr. Jitendra Kumar conceptualized and gathered the data with regard to this work. Dr. Anand Singh 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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