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SYNERGISTIC EFFECT OF ANTI-OXIDANT ACTIVITY OF EXTRACT OF *ACHYRANTHES ASPERA* & *CALOTROPIS GIGANTEAN* LEAF

Rohtash Singh[1]*, Mangal Sain Hooda[1], Krishna Mohan[2] and Neenalchal Trivedi[2]

[1]Janta College of Pharmacy, Butana, Soniapat-131001(Haryana), India.

[2]Teerthanker Mahaveer College of Pharmacy, TMU, Moradabad-244001 (U.P).

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***Corresponding Author**
Rohtash Singh
Janta College of Pharmacy,
Butana, Soniapat-
131001(Haryana), India.

ABSTRACT

Achyranthes aspera & *Calotropis gigantean* plant are used in folklore medicines for a number of ailments. The freshly collected leaves of above plants were screened. Shade dried & firstly defatted with petroleum ether & then ethanol respectively and the leaf extracts were subjected to various physicochemical studies i.e. physical analysis, determination of extractive values, % yield & identification of different phytoconstituents. The percentage yield was (5.1%) for *Calotropis gigantean* & (5.25%) for *Achyranthes aspera* Obtained with ethanol. The obtained extracts separately and in combination were used to carry out antioxidant. *Achyranthes aspera* leaf extract exhibited antioxidant activity when subjected to the test like DPPH and H_2O_2 RSA. The obtained results (IC50) were: for DPPH RSA (39.24µg/ml) and H2O2 RSA (22.37µg/ml. *Calotropis gigantean* leaf extract exhibited antioxidant activity when subjected to the test like DPPH and H_2O_2 RSA. The obtained results (IC50) were: for DPPH RSA (36.18µg/ml) and H_2O_2 RSA (21.25µg/ml). Combination of *Achyranthes aspera* & *Calotropis gigantean* leaf extract exhibited. Antioxidant activity when subjected to the test like DPPH and H_2O_2 RSA. The obtained results (IC50) were: for DPPH RSA (27.95µg/ml) and H_2O_2 RSA (19.27µg/ml).

KEYWORDS: *Achyranthes aspera* & *Calotropis gigantean,* H_2O_2, DPPH.

INTRODUCTION

Antioxidant: The body possesses several defence mechanisms (collectively referred to as antioxidant system) to control or destroy the free radicals generation, besides bringing out repairs to the damaged biomolecules.

Free radicals

Biologically important are superoxide radical, hydrogen peroxide radical & nitric oxide, etc.

Oxygen metabolites

Reactive oxygen species (ROS) were considered to play a causal role in tissue injury resulting in organ dysfunction and disease like atherosclerosis, inflammation, rheumatoid arthritis and other autoimmune diseases etc.

Classification of antioxidant

Antioxidants are classified as natural & synthetic:

Natural antioxidants

These are substances that at low concentrations, prevents or retard the oxidation of easily oxidisable biomolecules like lipids, proteins & DNA. Normally antioxidants are defined as substances which counteract free radicals and thus preventing oxidative damage. Two major types of natural antioxidants are enzymatic as well as non-enzymatic.[1]

Enzymatic

These types of antioxidant includes many types of enzymes like primary enzymes, as superoxide dismutase, catalase, glutathione peroxidise and secondary enzymes as glutathione reductase & glucose-6-phosphate dehydrogenase.

Types of antioxidant

Ascorbic acid (AA)

Vitamin C plays a vital role in human beings. Other name of vitamin C is ascorbic acid and its mechanism at the cellular level is not yet clear. Vitamin C is necessary for the synthesis of collagen and protein play the important role connective functions in the all body. Ascorbic acid required for the synthesis of hormones and other neurotransmitters. Ascorbic acid required for the metabolism of many types of amino acid & Vitamin C is also involved in the detoxification of harmful substance in the liver. It is required for the blood level of immunity. It reacts with histamine as an antioxidant and peroxide minimised inflammatory symptoms.[2,3]

Nitric oxide

It is used as therapeutic drug (nitroglycerine & amyl nitrate) for the treatment of angina pectoris. NO acts as a vasodilator, relaxation of smooth muscles, platelet aggregation,

adhesion. It is act as a bactericidal action of macrophages and messenger molecule of the nervous system (neurotransmitter).[4]

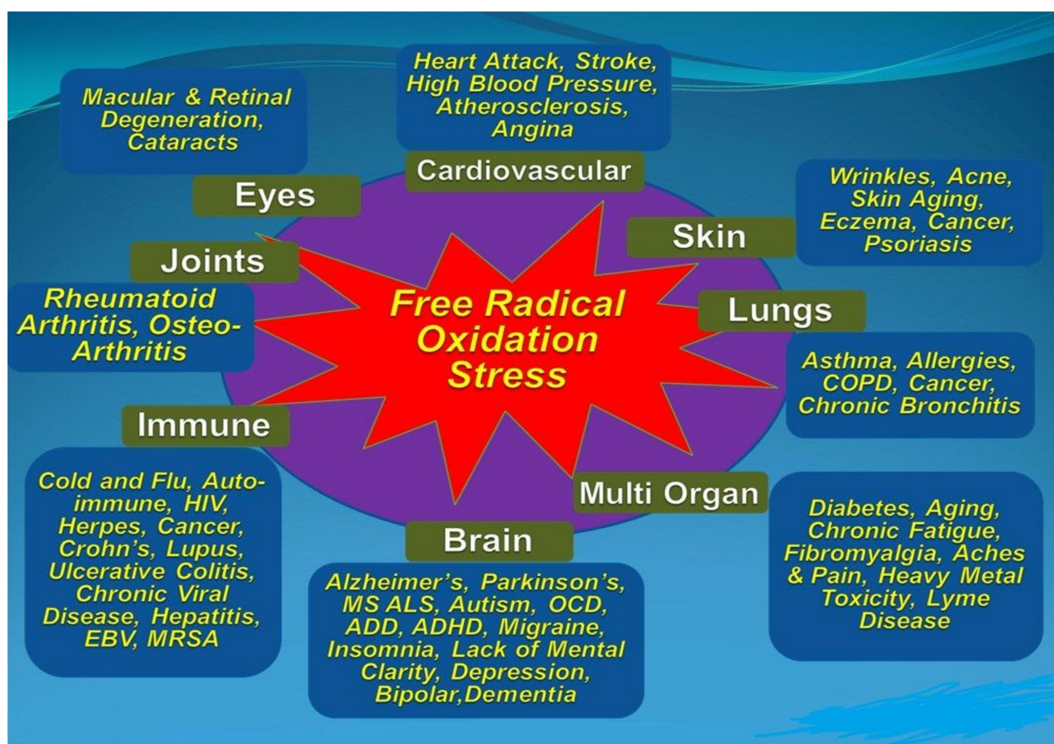

Fig 1: Function of free radical oxidation stress.

Plant profile

Fig 2. Plant of *Achyranthes aspera.*

Fig 3. Plant of *Calotropis gigantea.*

Table 1. Classification of both plants.

Achyranthes aspera[5]		*Calotropis gigantea*[6]	
Kingdom	Plantae	**Kingdom**	Planatae
Subkingdom	Tracheobinota	**Subkingdom**	Tracheobionta
Super Division	Spermatophyta	**Superdivision**	Spermatophyta
Division	Mangoliophyta	**Division**	Magnoliophyta
Class	Mangoliophsida	**Class**	*Dicotyledones*

Subclass	Caryophyllidae	**Sub class**	Asteridae
Order	Caryophyllales	**Series**	Bicarpellatae
Family	Amaranthaceae	**Order**	Gentianales
Genus	*Achyranthes*	**Family**	Apocynaceae
Species	*Aspera*	**Subfamily**	Asclepidiaceae
Common name	Latjira, Chirchira	**Genus**	*Calotropis*
		Species	*Calotropis gigantean*
		Common name	Madar

Geographical Source of *Achyranthes aspera*

The plant is found on road sides, field boundaries & waste places as a weed throughout India up to an altitude of 2100 m including in South Andaman Islands. The plant is also widespread in Baluchistan, Ceylon, Tropical Asia, Africa, Australia & America.[6]

Chemical constituents

The plant contains carbohydrates, phenolic compounds, oil & fats, saponins, flavonoids, alkaloids, tannins & polysaccharides. The main constituents are ecdysterone achyranthine, betaine, vanillic acid, syringic acid, *p*-coumaric acid.[7]

Uses[8]

The plant is used as anti-asthmatic, immunostimulant, hypolipidemic, antifertility, antiinflamatory, anti-dandruff, diuretic, anti snake venom & in renal disorders.

Geographical source of *Calotropis gigantean*

The plant is a native of India, Bangladesh, Burma, China, Indonesia, Malaysia, Pakistan, Philippines, Thailand & Sri Lanka.[9]

Chemical constituents[10]

The plant contains cardenolide, triterpinoids, alkaloids, resins, anthocyanins including proteolytic enzymes in latex. Plant also contains flavonoids, tannins, sterol, saponins, cardiac glycosides. Amyrin, amyrin acetate, ß-sitosterol, urosolic acid and cardenolides likes calactin, calotoxin, calotropagenin, uscharin, uzarigenin, voruscharin. Proceroside, syriogenine, uscharidin.

Uses

The plant is widely used as an anti-asthmatic, anti-inflammatory, antihelmintic, anti cancer, antiviral, anti-diarrheal.

MATERIAL AND METHODS

Collection & Authentication of plant leaf

The leaves of *Achyranthes aspera* & *Calotropis gigantean* were collected from "Mohabbatpur" village of Barabanki, Utter Pradesh, India. **Authentication:** The leaves of plant Achyranthes aspera & Calotropis gigantean were authenticated by a Senior Botanist Dr. D.C kasana; head of deportment of botany, I.P College of science, Bulandshahr (U.P), India.

Preparation of extract

Leaves of plant *Achyranthes aspera & Calotropis gigantean* were collected, reduced to small size after drying in shade for ten days and crushed to form coarse powder. The powdered drug (5000 gm) was subjected to continuous hot extraction with the help of soxhlet apparatus using petroleum ether & ethanol successively. Each time before extracting with the next solvent the plant material was dried in hot air oven at 50^0C for an hour. After the effective extraction, the solvent were distilled off, the extract were then concentrated on water bath to become dried. The obtained extract with each solvent was weighed and stored in an air tight container.

The percentage yield was determined using the formula:

$$\text{Percentage yield (W/V)} = \frac{\text{wt of extract obtained}}{\text{weight of crude drug taken}} \times 100$$

In-Vitro Antioxidant Evaluation

Hydrogen Peroxide radical Scavenging Assay[11,12]

The ability of the leaf extract 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 (1-50µg/ml). 1 ml of each solution of ascorbic acid was mixed with 2.4ml 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 test solutions**

Various concentrations of the leaf extracts were prepared in distilled water to give solutions of varying concentrations (1-50 µg/ml). 1 ml of each solution of plant leaf extract was mixed

with 2.4ml of 0.1 M phosphate buffer and 600 µl 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 0.1 M phosphate buffer solution was mixed with 600 µl of 40 mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230nm.

Percentage hydrogen peroxide radical scavenging activity of plant leaf extract and ascorbic acid were calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{dy\text{Ac } 230 \text{ nm} - \text{At } 230 \text{ nm}}{dx\text{Ac } 230 \text{ nm}} \text{x } 100\text{nm}$$

Where,

Ac = Absorbance of control (0.1 M phosphate buffer solution and H_2O_2).

At = Absorbance of ascorbic acid/ plant leaf extract.

DPPH free Radical Scavenging Assay[13,14,15]

The DPPH assay of leaf extract was determined according to **Pin Der Duh *et. At.,* 1995.** Antioxidants react with DPPH, a stable free radical, which gets reduced to DPPH-H. Consequently, the absorbance gets decreased.

- **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 230nm using phosphate buffer as blank.

- **Preparation of test solutions**

Extracts were prepared in distilled water to give solutions of varying concentrations (1-100µg/ml). 1ml of each solution of plant leaf extract 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 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 230nm.

The antioxidant activity of plant leaf extract and ascorbic acid were calculated by using the following formula in terms of % inhibition:

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

Where,

Ac = Absorbance of control.

At =Absorbance of ascorbic acid/ ethanolic leaf extract.

RESULT AND DISCUSSION

Evaluation of antioxidant activity

Hydrogen peroxide radical (H_2O_2) scavenging activity

Hydrogen peroxide radical scavenging of *Achyranthes aspera* & *Calotropis gigantean* leaf extracts and their combination was performed using ascorbic acid as standard solution. Standard inhibition curve for H_2O_2 radical scavenging of ascorbic acid (Fig.4) and inhibition curve of ethanolic extract of leaves of *Achyranthes aspera* & *Calotropis gigantean* including their combination (Fig.7.) were plotted. From these IC_{50} values of percentage inhibition of H_2O_2 radical scavenging of the ascorbic acid and ethanolic extracts of leaves were calculated using regression equation (Table 6.).

Table 2: % Inhibition data of hydrogen peroxide radical scavenging of ascorbic acid

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	10	0.560	0.450	19.64
2	20		0.430	23.21
3	30		0.418	25.35
4	40		0.390	30.35
5	50		0.350	37.5

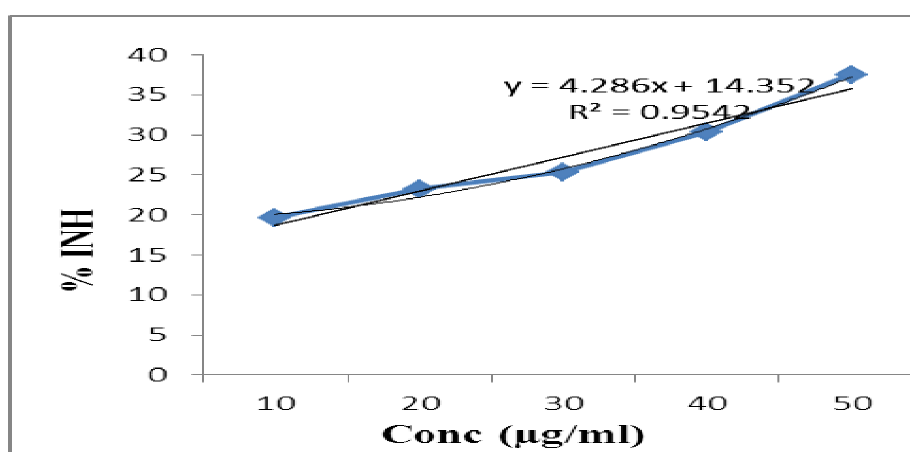

Fig 4: Standard % Inhibition curve of hydrogen peroxide radical scavenging of ascorbic acid.

Table 3: % Inhibition data of hydrogen peroxide radical scavenging of *Achyranthes Aspera* leaf extracts.

S.No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	10	0.56	0.488	12.85
2	20		0.476	15
3	30		0.469	16.25
4	40		0.459	18.03
5	50		0.448	20

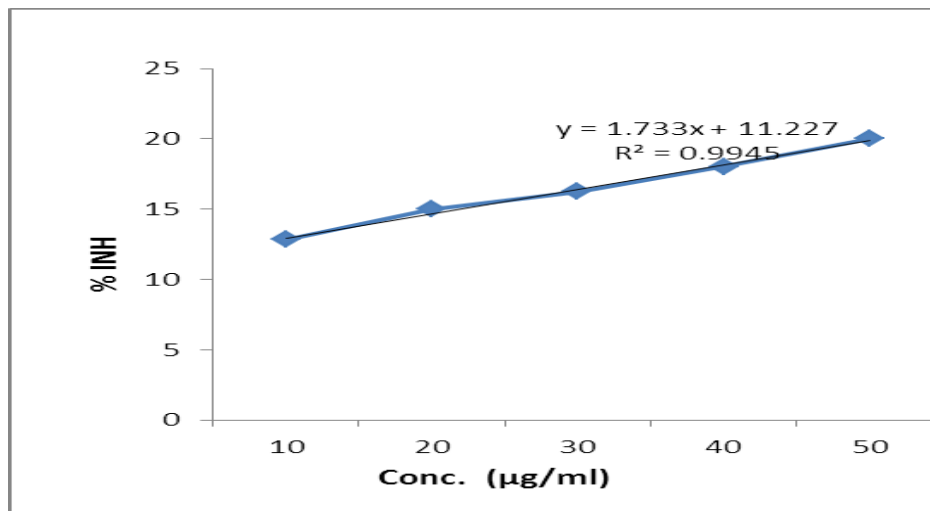

Fig 5: % Inhibition curve of ethanolic extract of *Achyranthes Aspera.*

Table 4: % Inhibition data of hydrogen peroxide radical scavenging of *Calotropis gigantean* leaf extract.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	10	0.560	0.478	14.64
2	20		0.466	16.78
3	30		0.459	18.03
4	40		0.449	19.82
5	50		0.438	21.78

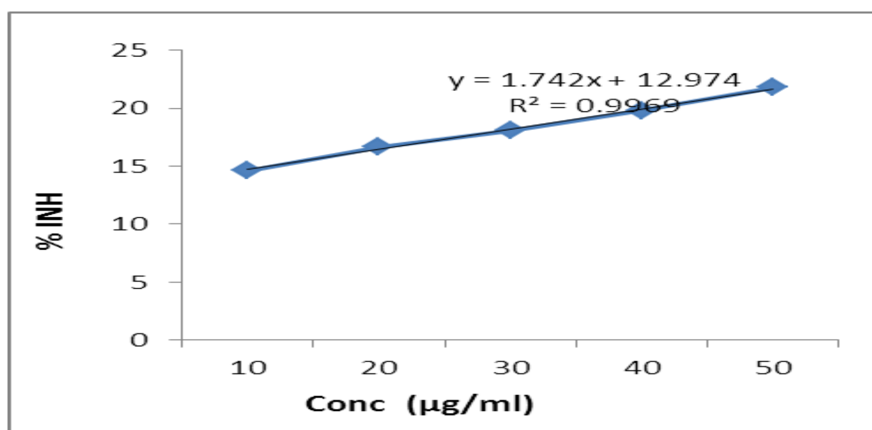

Fig 6: % Inhibition curve of ethanolic of leaf extract *Calotropis gigantean.*

Table 5: Percentage (%) Inhibition data of hydrogen peroxide radical scavenging of *Achyranthes aspera* & *Calotropis gigantean* ethanolic leaf extracts.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	10	0.560	0.474	15.35
2	20		0.462	17.50
3	30		0.454	18.92
4	40		0.440	21.42
5	50		0.432	22.85

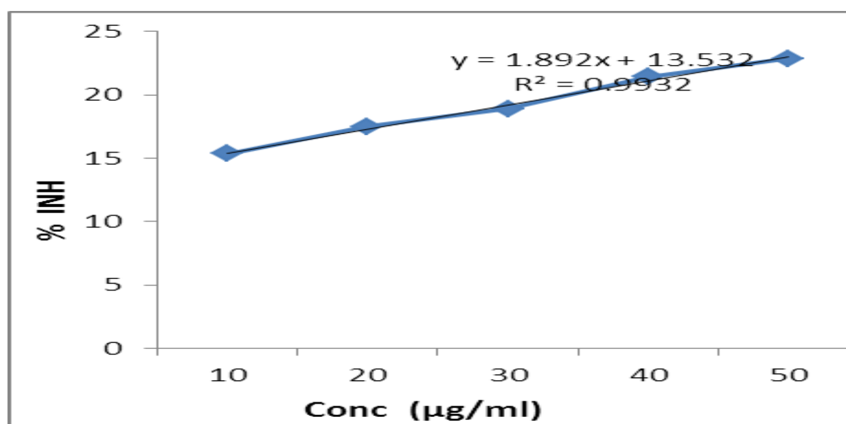

Fig 7. % Inhibition curve of combind ethanolic *Achyranthes aspera* & *Calotropis gigantean* leaf extracts.

Table 6: Hydrogen peroxide radical scavenging IC_{50} of Ascorbic acid & Ethanolic leaf extracts of *Achyranthes aspera*, *Calotropis gigantean* and their combination.

Sample	IC_{50}
Ascorbic acid	**8.31**
Ethanolic leaf extracts of *Achyranthes Aspera*	**22.37**
Ethanolic leaf extracts of *Calotropis gigantean*	**21.25**
Ethanolic leaf extracts of *Achyranthes Aspera*, *Calotropis gigantean*	**19.27**

DPPH Assay

DPPH assay of ethanolic leaf extracts of *Achyranthes aspera* and *Calotropis gigantean* their combination was estimated by using Ascorbic acid solution as standard. The absorbance data (Table 8, 9, 10 & 11) were recorded against the selected concentration (20, 40, 60, 80 & 100).

Table 7: % Inhibition data for DPPH assay of ascorbic acid.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.770	0.675	12.33
2	40		0.652	15.32
3	60		0.639	17.01
4	80		0.612	20.51
5	100		0.572	25.71

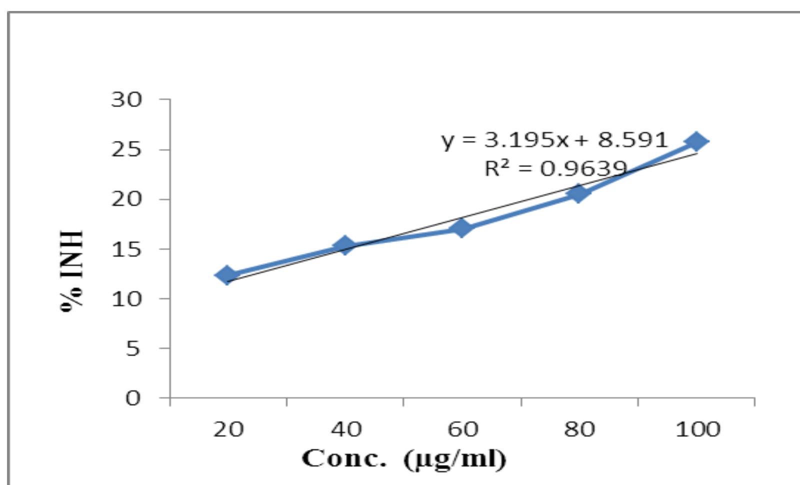

Fig 8: % Inhibition Curve for DPPH assay curve of ascorbic acid.

Table 8: % Inhibition data for DPPH assay of ethanolic leaf extract of *Achyranthes aspera.*

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.770	0.735	4.54
2	40		0.730	5.19
3	60		0.720	6.49
4	80		0.710	7.79
5	100		0.699	9.22

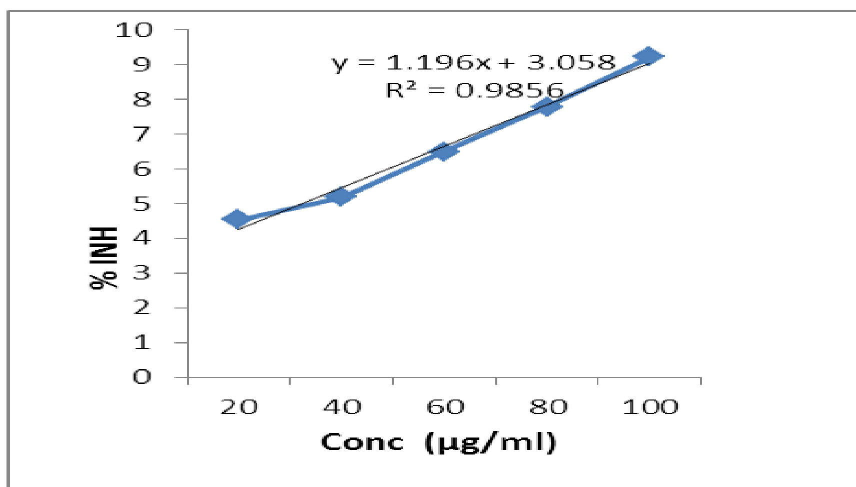

Fig 9: % Inhibition Curve for DPPH assay curve of *Achyranthes Aspera* ethanol Leaf extract.

Table 9: Percentage (%) inhibition data for DPPH assay of *Calotropis gigantean* ethanolic leaf extracts.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.770	0.730	5.19
2	40		0.720	6.49
3	60		0.709	7.92
4	80		0.700	9.09
5	100		0.691	10.25

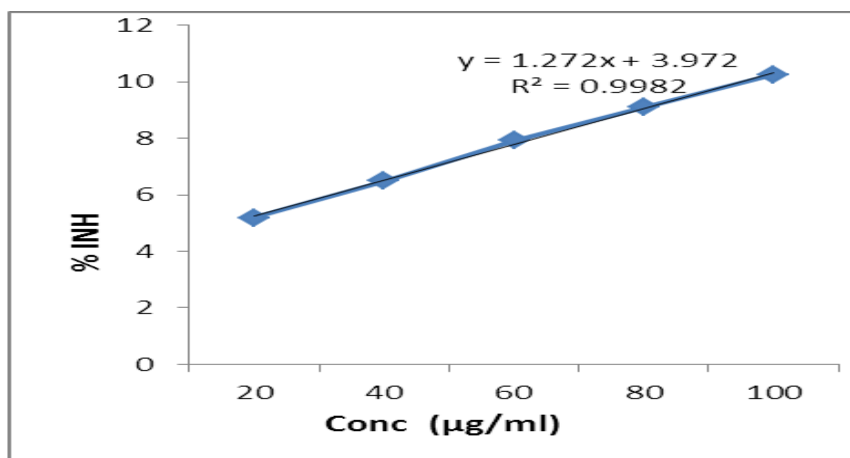

Fig 10: % Inhibition Curve for DPPH assay curve of *Calotropis gigantean* ethanolic leaf extract.

Table 10: % Inhibition data of DPPH assay by combined *Achyranthes Aspera* & *Calotropis gigantean* ethanolic leaf extract.

S. No	Conc.(µg/ml)	Absorbance (control), Ac	Absorbance (test), At	% Inhibition
1	20	0.770	0.709	7.90
2	40		0.700	9.09
3	60		0.691	10.15
4	80		0.675	12.33
5	100		0.661	14.15

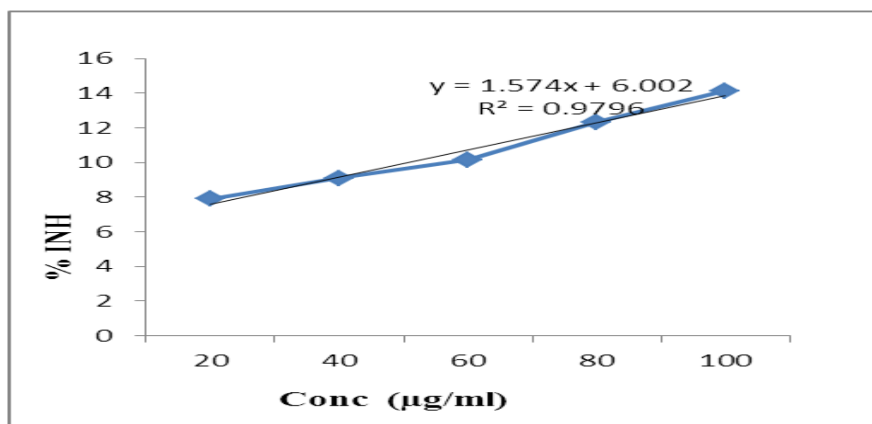

Fig 11: % Inhibition Curve for DPPH assay curve of combined *Achyranthes Aspera* & *Calotropis gigantean* ethanolic leaf extracts.

Table 11: DPPH IC_{50} of Ascorbic acid & ethanolic leaf extracts of *Achyranthes Aspera*, *Calotropis gigantean* and their combination.

Sample	IC_{50}
Ascorbic acid	12.96
ethanolic leaf extracts of *Achyranthes aspera*	39.24
ethanolic leaf extracts of *Calotropis gigantean*	36.18
ethanolic leaf extracts of *Achyranthes aspera*, *Calotropis gigantean*	27.95

CONCLUSION

IC_{50} values for standard (Ascorbic acid) and the ethanolic leaf extracts of *Achyranthes aspera* & *Calotropis gigantean* and their combination were 8.31µm/ml, 22.37µg/ml, 21.25µg/ml and 19.27µg/ml respectively which represented the antioxidant potential of the standard and extract. IC_{50} values for standard (Ascorbic acid) and the ethanolic leaf extracts of *Achyranthes aspera* & *Calotropis gigantean* and their combination were 12.96µm/ml, 39.24µg/ml, 36.18µg/ml and 27.95µg/ml respectively which represented the antioxidant potential of the standard and extract.

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REFERENCES

1. Dr. Mark percival (1996) "Antioxidants", clinical nutrition insights. 1: 1-4.
2. José Luis Silencio Barrita, María del Socorro Santiago Sánchez (2013) "Antioxidant Role of Ascorbic Acid and His Protective Effects on Chronic Diseases", intech open science. pp:- 449-484.
3. Danyelle M. Townsend A, Kenneth D. Tew A, Haim Tapiero B (2003) *"The importance* of glutathione in human disease", Biomedicine & Pharmacotherapy., 57: 145–155.
4. U. Satyanarayana, U. Chakrapani, "Biochemistry" Thrird edition (2006), Books and allied (p) ltd., pp:- 366-368.
5. Srivastav S., Singh P., mishra G., Jha K.K., Khosa R.L., (2011) *"Achyranthes aspera*-an important medicinal plant: a review" scholars research library, 1(1): 1-14.
6. Ahamed M, Rana AC & Dixit V.K. Plant review Calotropis species (Ascelpediaceae): A comprehensive review. Pharmacognosy Magazine. 2005; 2: 48-52.

7. Priya, C.l., kumar G., karthik, L., K.V., Rao B., (2012) "Phytochemical composition and in vitro antioxidant activity of achyranthes aspera linn (amaranthaceae) leaf extracts" journal of agricultural technology, 8(1): 143-156.
8. Dey A., (2011) *"Achyranthes aspera L:* phytochemical and pharmacological aspects". International journal of pharmaceutical sciences review and research, 9(2): 10-13.
9. Sarkar S., chakraverty R., ghosh A., (2014) "*calotropis gigantea* Linn.-a complete busket of indian traditional medicine" international journal of pharmacy research and science., 02(1): 7-17.
10. Kumar P.S., Suresh E., kalavathy S., (2013) "Review on a potential herb calotropis gigantea (l.) R. Br." Scholars academic journal of pharmacy (sajp), 2(2): 135-143.
11. Muthukumaran P., Shanmuganathan P., and Malathi C. (2011) "In vitro Antioxidant Evaluation of Mimosa Pudica" Asian J. Pharma. Res.; 1: 44-46.
12. Jayprakasha G.K., Rao L.J., and Sakaroah K.K., (2004) "Antioxidant Activities of Flavidin in different In-Vitro model systems" Bioorganic and Medicinal chemistry, 12: 5141-5146.
13. Duh P.D., Yen G.C. "Anti-oxidative activity of three herbal water extract" food Chemistry, 1997; 60(4): 639-645.
14. Flogel A., Kim D., Chung S., Koo S.I., and Chum O.K. "Journal comparision of ABTS/DPPH Assay. To Measure Antioxidant Capacity in Popular Antioxidant- Rich Foods" Food Comparision and Analysis, 2011; 24: 1043-1048.
15. Brand Williams W., Cuvelier M.E., and Berset C. "Use of a Free Radical Method to Evaluate Antioxidant Activity" Lebennsm-Aiss. U.-Technol, 1995; 28: 25-30.