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ANTIOXIDANT AND CHOLINESTERASE INHIBITORY ACTIVITY OF *GREWIA HIRSUTA* VAHL. LEAVES

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ABSTRACT: Objectives: *Grewia hirsuta* Vahl. is a shrub explored for medicinal applications. It has anti ageing, boosting immunity, prevents loss and grey hair *etc*. Ethnopharmacological data suggests that the plant is extensively used as a nerve tonic and to enhance the cognitive properties. The basis of these medicinal uses seems to be antioxidant and anti cholinesterase activity. In order to establish the scientific basis between medicinal uses and its mechanism of action, antioxidant and anti cholinesterase activity investigations were carried out. **Methods:** Evaluation of free radical scavenging and anti cholinesterase activity was carried out using DPPH (1, 1- diphenyl-2-picryl hydrazyl), hydroxyl radical scavenging and ferric reducing method using ascorbic acid as a standard. Cholinesterase inhibitory potentials were measured by Ellman's method against Rivastigmine. **Results:** The inhibitory concentration of hydro ethanolic extract of *Grewia hirsuta* (HEEGH) (IC_{50} 27.25) in DPPH. In reducing power assay (IC_{50} 65.32) and Hydrogen peroxide scavenging activity (IC_{50} 98.28). The inhibitor concentration of acetylcholinesterase by standard (Rivastigmine) was (IC_{50} 14.40µg/ml), and the extract was (IC_{50} 133.4µg/ml), whereas, butyrylcholinesterase (IC_{50} 96.46µg/ml). **Conclusion:** HEEGH possesses the significant concentration-dependent inhibitory action as an antioxidant and anti acetyl cholinesterase and butyrylcholinesterase activity. This activity might be due to the presence of phenols, flavonoids, tannins and specific amino acids in significant amount.

INTRODUCTION: The use of herbal medicine as an antioxidant is an interesting research, as synthetic drugs fall short of resolving the oxidative stress. Oxidative stress is a root cause of manifestation of many diseases like Diabetes (type 1 & 2), Cancer, Alzheimer's, Asthma *etc* [1].

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The free radicals are produced in the body by the electron chain reaction, when glucose is used to make energy. The oxygen molecule (O_2) is split into two singlet oxygen atoms; one of the singlet oxygen's is used in the mitochondria to carry out phosphorylation and subsequent energy production.

The other singlet oxygen is a free radical and is capable of damaging of vital biomolecules like proteins and nucleic acid. Naturally glutathione and other antioxidant molecules are present which scavenge the released free radical and prevent the formation of free radicals [2]. In case, free radical of

proteins and nucleic acids go uninterrupted by antioxidants, this will lead to damage of proteins and nucleic acids rendering them non-functional. In order to prevent and protect the oxidative stress there should be adequate antioxidant molecules. The imbalance of free radicals and antioxidants result in the manifestation of the diseases like diabetes, cancer, cell aging *etc* [3]. It is logical to supplement antioxidant when natural antioxidant falls short of body requirement. Antioxidant supplements include vitamin C, vitamin E, beta-carotene, and other related carotenoids, along with the minerals selenium and manganese. The short & long-term memory is attributed to cholinergic transmission in the brain. The free radical generated in the system gets neutralized when it meets an appropriate antioxidant.

Naturally occurring antioxidants like glutathione and other antioxidant molecules usually take care and offer neuro protection on the oxidative stress condition [4]. If the naturally occurring antioxidants are over powered by natural antioxidants is the oxidative stress over powers antioxidant present in the system. The free radicals come, out and a chain reaction happens leading to widespread conversation of free radical molecules in and around the biomolecules in the neuron is rendered functionless when they convert into free radicals. The major cause of cholinesterase enzyme which was rendered functionless leads to high concentrations of acetyl choline. Thus, leading to damage of neuronal damage of cholinergic neurons [5].

Grewia hirsuta Vahl. is very popular in folklore and Ayurveda as Nagabala. It is a shrub grown up to 1 meter tall, leaves are simple and soft arranged alternately. Flowers are white in color and turn to brown when dried. It is found in India, Bangladesh, Srilanka *etc*. It contains proline, phenylalanine, isoleucine, lysine, glutamic acid, serine and beta sitosterol as phytoconstituents significantly. Root is used to increase sperm count and quality in Ayurveda and Siddha system of medicines. Root is also used to treat heart diseases, controls high blood pressure and in the early stages of tuberculosis. Leaves and fruits are used as expectorants, carminative, abortifacient, and galactagogue. It is also used in splenic enlargement, piles, rheumatism pain in joints and in the breasts [6]. Variety of properties and uses creates curiosity to further explore scientifically and find out the secrete for its genesis of pharmacological and therapeutic applications. In the literature, there is mention of strong antioxidant properties which were reported by Varsha Hutke *et al,* 2002 [7]. As it finds application in ENT there is every reason of involvement of parasympathetic apparatus. So, it was decided to its enzyme inhibition like acetyl cholinesterase and butryl cholinestrase activity [6].

MATERIALS AND METHODS: The leaves of *Grewia hirsuta vahl.* collected from surroundings of Chittoor, Andra Pradesh. The identification and authentication was done by Dr. K. Madhava Chetty, Plant taxonomist (IAAT: 357) belonging to Sri Venkateswara University, Tirupati, Andhra Pradesh. The shade dried leaves were extracted by continuous hot extraction method [8].

Reagents and Chemicals: The reagents and chemicals of analytical grade were used.

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

DPPH Radical Scavenging Assay: DPPH (1, 1-diphenyl-2-picryl hydrazyl) a hydrogen donor was prepared in methanol (1mg/ml). HEEGH were added in the serial dilution of (10, 20,40,60,80 & 100µg/ml). After allowing reaction time of 30 minutes, the optical density was measured at 517 nm and standard curve of ascorbic acid (water soluble hydrogen acceptor) is plotted for estimating the concentration of antioxidant principle of the plant [9-10].

Reducing Power Assay: The reducing power of HEEGH was determined according to method of Oyaizu (Oyaizu, 1986). Aliquots of HEEGH were mixed serial dilutions from 10µg to 100µg/ml. The optical density was measured at 700nm and the concentration was measured by using standard curve ascorbic acid [11-12].

Hydrogen Peroxide Scavenging (H_2O_2) Activity: The scavenging activity of hydrogen peroxide of HEEGH was estimated as described by Keser S *et al* [14]. Briefly, 2ml of HEEGH solution (100µ-600µg/ml) in methanol was added to 4ml of hydrogen peroxide (20 mM) solution in phosphate buffer (P^H 7.4). After 10min, the absorbance was measured at 230nm against phosphate buffer blank solution [13-14].

***In-vitro* Acetyl cholinesterase and Butryl Cholinesterase Inhibition [15]:** The cholinesterase inhibitor activity of the HEEGH was measured as per Ellman's method. Rivastigmine was used as a positive control for both enzymes. The enzyme inhibition (%) was calculated as follows:

$$\% \text{ Inhibition} = (A0-A1) / (A0) \times 100$$

Where, A0 = Absorbance of control; A1 = Absorbance of extract/ standard.

RESULTS:

***In-vitro* DPPH Free Radical Scavenging Activity:** The results indicate both in the standard and HEEGH shows DPPH free radical scavenging activity is dependent of concentration. The percentage of inhibition of standard ranges from 42.24% - 82.25%, HEEGH 28.44%-80.33%. The inhibitory concentration of ascorbic acid (IC_{50} 20.14) and HEEGH (IC_{50} 27.25). All the readings were an average of triplicate sample. (See **Table 1**).

TABLE 1: EFFECT OF HEEGH ON PERCENTAGE INHIBITION OF DPPH

Sl. no.	Concentration (µg/ml)	Standard (Ascorbic acid)	HEEGH
		Percentage inhibition	Percentage inhibition
1.	10	42.24	28.44
2.	20	46.16	37.22
3.	40	62.32	59.17
4.	60	68.42	65.43
5.	80	74.68	70.54
6.	100	82.25	80.33
	IC_{50} (µg/ml)	20.14	27.25

Reducing Power Activity using Potassium Ferricyanide: The results were given in **Table 2**. The results indicate both in the standard and sample shows reducing power activity dependent of concentration. The percentage inhibition of standard ranges from 38.43-73.68% & HEEGH 18.04% - 58.53%. All the readings were an average of triplicate of sample. The inhibitor concentration ascorbic acid (IC_{50} 31.54) and HEEGH (IC_{50} 65.32).

TABLE 2: EFFECT OF HEEGH ON PERCENTAGE INHIBITION IN REDUCING POWER ASSAY

Sl. no.	Concentration (µg/ml)	Standard (Ascorbic acid)	HEEGH
		Percentage inhibition	Percentage inhibition
1.	10	38.43	18.04
2.	20	44.28	24.52
3.	40	53.22	34.43
4.	60	58.19	42.86
5.	80	61.54	49.60
6.	100	73.68	58.53
	IC 50 (µg/ml)	31.54	65.32

Hydrogen Peroxide Scavenging (H_2O_2) Assay: In this assay also both in the standard and test extract shows hydrogen peroxide radical scavenging activity is dependent of concentration. The percentage of inhibition of standard ranges from 2.45% - 65.15%, & HEEGH 1.02% - 45.20%. The inhibitor concentration ascorbic acid (IC_{50} 36.16) and HEEGH (IC_{50} 98.28). All the readings were an average of triplicate sample (See **Table 3**).

TABLE 3: EFFECT OF HEEGH ON HYDROXYL RADICAL SCAVENGING ACTIVITY

Sl. no.	Concentration (µg/ml)	Standard (Ascorbic acid)	HEEGH
		Percentage inhibition	Percentage inhibition
1.	100	2.45%	1.02%
2.	200	4.98%	2.85%
3.	300	14.25%	6.15%
4.	400	33.44%	29.68%
5.	500	45.68%	32.17%
6.	600	65.15%	45.20%
	IC_{50} (µg/ml)	36.16	98.28

***In-vitro* Acetylcholinesterase and Butryl-cholinestrase Activity:** Anti acetylcholinesterase and butrylcholinestrase activity by Elman's method. The standard curve was plotted against concentration versus percentage inhibition. The HEEGH concentration shows increased inhibition of enzyme activity similarly the standard curve. (See **Table 4** and **Fig. 1 & 2**).

TABLE 4: PERCENTAGE INHIBITION OF HEEGH AND RIVASTIGMINE ON CHOLINESTERASE

	Acetylcholinesterase Activity		Butrylcholinesterase Activity	
Concentration (µg/ml)	**Percentage inhibition of HEEGH**	**Percentage inhibition of Rivastigmine**	**Percentage inhibition of HEEGH**	**Percentage inhibition of Rivastigmine**
Control	0.00	0.00	0.00	0.00
20	10.03	61.25	3.29	60.47
40	16.96	70.42	17.44	72.09
60	23.18	80.62	27.52	80.43
80	30.62	84.95	41.47	85.27
100	43.77	92.91	51.74	94.38
IC_{50} (µg/ml)	133.4		96.46	14.40

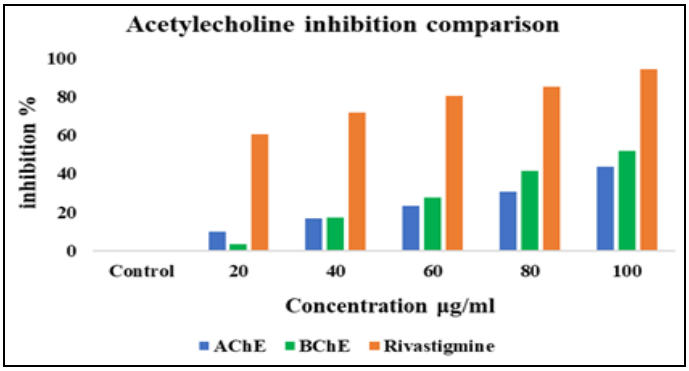

FIG. 1: ACHE & BCHE INHIBITION ACTIVITY COMPARISON OF HEEGH AND RIVASTIGMINE

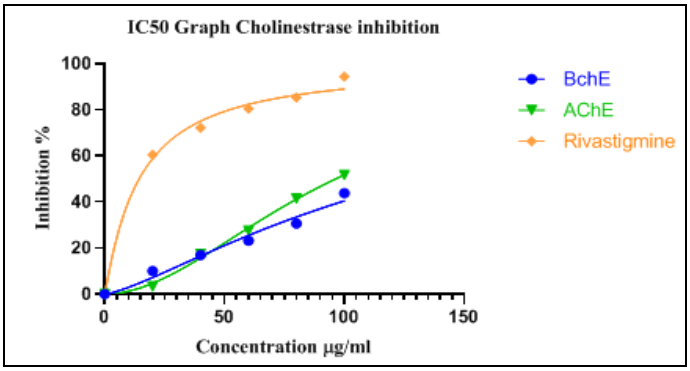

FIG. 2: EFFECT OF HEEGH ON IC_{50} CHOLINESTERASE INHIBITION ACTIVITY

DISCUSSION: Oxidative stress and memory impairment are commonly observed among adults and elderly impairing the quality of life. Oxidative stress disturbs the milieu interieur. Acetylcholine is responsible for quality neurotransmission which is important for short and long term memory. The synthetic drugs available include ascorbic acid and tocopherol for an antioxidant activity, whereas; rivastigmine, galantamine, piracetam *etc*. are as Nootropics [16]. In this paper, we have compared the antioxidant activity by DPPH, reducing power antioxidant assay and hydrogen peroxide assay method. The results indicate that HEEGH has comparable the antioxidant and nootropic activity. The limitation of the study is *in-vitro* methods and further evaluation should be done on animal models and clinical studies. One of the strong evidence is from ethnopharmacological uses of *Grewia hirsuta* reference by traditional healers.

CONCLUSION: There is a need for herbal options in the treatment of oxidative stress and neurodegenerative diseases. Hence, this research proves an alternative antioxidant and nootropic drugs for synthetic drugs. Synthetic drugs are having well established side effects which mandates for Pharmacovigilance studies. *Grewia hirsuta* has been in use time immemorial and may not manifest severe toxicity effects like synthetic drugs.

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