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Study on Antioxidant Activity of *Grewia asiatica* berries and *Crataegus oxyacantha* leaves

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ABSTRACT

Collective results from our study concludes that *Grewia asiatica* berries and *Crataegus oxyacantha* leaves present a trending therapeutic strategy for prevention of dementia by ameliorating deficits in learning and memory through different modeof actions due to the presence of flavonoids containing phytochemicals. Based on phytochemical tests and percentage yield the chloroform and methanol extract were subjected to estimation of phytochemicals like flavonoids and phenolic content determination. In our study methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* possessed highest total flavonoid and phenolic content. After estimation of phytochemicals in vitro anti-oxidant effect of plants were estimated by DPPH and reducing power assay.

Key Words-: Antioxidant,DPPH,Cognitive

INTRODUCTION

Cognitive disorders involving dementia in India must be addressed, prevented and overcome because this disease being chronic requires lot of efforts, time and money. Earlier almost ten years ago dementia is known among only rare medical specialists. “Senility” was considered inevitable for anyone who lived long enough. But as understanding of the brain functioning has been expanded, scientists are now being able to ascertain factors causing dementia. Among the different types of dementia, Alzheimer’s disease is the most widespread one; however other disorders of brain also do cause dementia. Almost 100 years have been passed since the identification of Alzheimer’s disease for the first time, now almost seventy years have been lapsed before it was crowned as the most common type of dementia.

Ayurveda: A Current Trending Therapeutic Strategy for Dementia Treatment

Ayurveda is the traditional system of Indian Medicine about 5000 years old. Ayurveda has remedy for almost every disease. The drugs used in dementia have been mentioned in the Ayurveda texts (Ven Murthy et al., 2010). Ayurveda contains a number of *Rasayana* herbs that elicit a major role in increasing memory and intellect and rejuvenation of mental well-being (Manyam, 1999). These kind ofherbs are known as *Medhya Rasayanas* (Singh and Mishra, 2004; Puri, 2003; Govindarajan *et al*., 2005). Many drugs reported to be Medhya in Ayurveda have been fathomed using modern medicine tools and have

shown to enhance cognition by increasing cholinergic function (Das et al., 2002; Joshi and Parle. 2006; Vasudevan and Parle, 2006). *Withania somnifera*, a drug commonly used in Ayurveda as Rasayana, is known to enhance NMDA activity in Hippocampus CA1 cells (Bhattarai et al., 2013).As per literature many plants are being shown to possess nootropic effect like Shilajit (Jaiswal and Bhattacharya 1992), *Bacopa monnieri* (Singh and Dhawan 1997), Lawsonia *inermis* (Iyer et al. 1998), *Clitoria ternatea* (Rai et al. 2000), Red ginseng (Lee et al. 2000) and *Albizzia lebbeck* (Uneet al. 2001). Saponins like bacoside a and b isolated from Brahmi and ginsenoside Rb, Rb1 obtained from *Panax ginseng* are the phyto-constituents responsible for increasing functions related to cognition (Ying et al., 2003).

The Traditional systems of Medicine of other countries also have several herbs having cognition enhancing activity. Galantamine is an alkaloid that is obtained synthetically from *Galanthus woronowii* (Amaryllidaceae), *Galanthus caucasicus* and other similar genera i.e. *Leucojumaestivum* (snowflake), *Narcissus* (daffodil) and *Lycoris* including *Lycoris radiata*is used to treat dementia (Olin and Schneider, 2012).

MATERIALS AND METHODS

Collection and Authentification of Plants

Fruits of *Grewia asiatica* L. commonly known as phalsa were purchasedfrom local market of Saharanpur and Delhi in the month of May and leaves of *Crataegus oxyacantha* were collected from botanical garden of Glocal University in the month of June (Fig. 14 a and b).

Figure-14: (a) *Grewia asiatica (*b) *Crataegus oxyacantha*

Evaluation of *In-vitro* Antioxidant Activity

DPPH Radical Scavenging Assay

Free radical scavenging activity of petroleum ether, chloroform and methanol extract of fruits of *Grewia asiatica* and leaves of *Crataegus oxyacantha* was calculated using methanolic solution of 1, 1-Diphenyl-

2-picrylhydrazyl. This method is most commonly used for determination of antioxidant activity of extracts.

Reagents and Chemicals 1, 1-Diphenyl-2-picrylhydrazine , 0.1mMMethanol

Procedure

0.1mM DPPH solution (4mg/100ml) in methanol was prepared by dissolving 22.2 mg of DPPH in 1000ml of methanol. Different dilutions of all three extracts i.e.petroleum ether, chloroform and methanol extract of *Grewia asiatica* and *Crataegusoxyacantha* were prepared in methanol at a concentration of 100, 200, 300, 400 and 500µg/ml.To 2ml of test samples prepared as above, 1ml of DPPH solution was added. Mixture was incubated at room temperature in dark for 10 min. Blankcontains all reagents except extract. Absorbance of all samples were noted at 515 nmagainst blank using UV-Visible spectrophotometer.

% Inhibition was calculated using formula: Percentage inhibition= $[(A_{C\ 515nm} - A_{t515nm})/ A_{C515nm}] \times 100$ Where A_c is the absorbance of the blank control And A_t is the absorbance of the test sample

Curve for % Inhibition v/s different concentration of extracts of both plants were plotted and using line of regression, IC_{50} values were calculated for all extracts of both plants. IC_{50} values represent the concentration of sample required to scavenge 50% of DPPH free radicals. All determinations were performed in triplicate and average of readings were taken (Gulcin et al., 2006; Jain &Jain. 2011).

Reducing Power Assay

The reducing power of petroleum ether, chloroform and methanol extract of *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves were estimated using the method by Oyaizu. 1986.

Reagents and Chemicals

- Potassium ferricyanide (1%w/v)
- Phosphate buffer (0.2 M, pH 6.6)
- Trichloroacetic acid solution (10%w/v)
- Ferric chloride (0.1%w/v)

Preparation of Extract Solution

A stock solution of 1000µg/ml was prepared by dissolving 100 mg of respective extracts of both plants in 100 ml of methanol. Different dilutions (100 to 500 µg/ml) were prepared by dissolving stock solution with methanol.

Procedur

Different dilutions of all three extracts i.e. petroleum ether, chloroform and methanol extract of *Grewia*

asiatica fruits and *Crataegus oxyacantha* leaves at a concentration of 100, 200, 300, 400 and 500µg/ml were prepared. To 0.5 ml of different concentrations of sample prepared as above, 0.5 ml of phosphate buffer(0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%w/v) was added. Reaction mixture was incubated at 50° C for 20 min. After cooling, 1.5 ml of trichloroacetic acid solution (10% w/v) was added to terminate the reaction. To the solution 0.5 ml of ferric chloride (0.1% w/v) was added and absorbance was measured at 700 nm. Three readings were recorded and average of three readings were taken. Curve of absorbance versus concentration was plotted. Increased absorbance of reaction mixture indicated increase in reducing power.

Result and Discussion

In vitro Antioxidant Potential

DPPH Radical Scavenging Assay

DPPH method is the most commonly employed method for determining the antioxidant activity of any extract. The ability of the antioxidants to quench the free radicals is directly proportional to the concentration of plant extract, thus decrease inabsorbance was observed. Percentage inhibitions of DPPH radical by petroleum ether, chloroform and methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* at concentrations of 100, 200, 300, 400 and 500 µg/ml were measured asgiven in Table 13and Table 14respectively. It was found that with increase inconcentration, the percentage inhibition of DPPH radical by extracts of both plants increases. Among pet. Ether, chloroform and methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* highest percentage inhibition was elicited by methanol extract of both plants (Fig. 17 and Fig. 18). IC_{50} of chloroform and methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* were found to be 385.31µg/ml, 134.84µg/ml, 470.34 µg/ml and 314.85 µg/ml respectively.

Table 13: Percentage Inhibition of DPPH Radical Scavenging Activity by *Grewia asiatica* Extracts at Different Concentrations

S.No.	Concentration (µg/ml)	% Inhibitions by *Grewia asiatica* Extract		
		Pet. ether	Chloroform	Methanol
1	100	12.59	32.06	45.80
2	200	23.28	40.26	56.75
3	300	24.04	41.79	60.30
4	400	28.43	49.80	68.51
5	500	32.44	59.54	72.13
	IC50		385.31µg/ml	134.84µg/ml

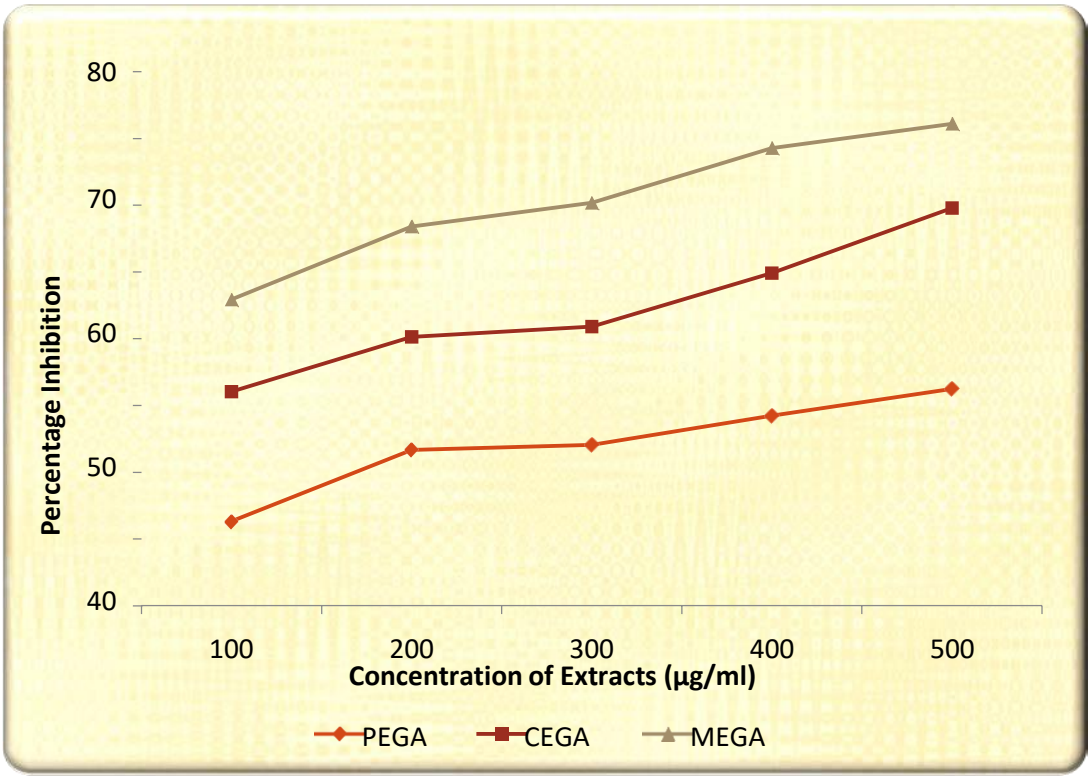

Figure-17: Percentage Inhibition by Extracts of *Grewia asiatica* with IncreasingConcentration in DPPH Scavenging Assay. Where PEGA, CEGA and MEGA Represent Petroleum Ether, Chloroform and Methanol Extract of *Grewia asiatica* Respectively

Table 14: Percentage Inhibition of DPPH Radical Scavenging Activity by *Crataegus oxyacantha* Extracts at Different Concentrations

S. No.	Concentration (µg/ml)	% Inhibitions by *Crataegus oxyacantha*extracts		
		Pet. ether	Chloroform	Methanol
1	100	4.96	28.62	26.52
2	200	11.06	34.35	41.79
3	300	23.47	41.79	45.22
4	400	25.13	43.32	61.06
5	500	28.24	53.43	68.70
	IC_{50}		470.34 µg/ml	314.85 µg/ml

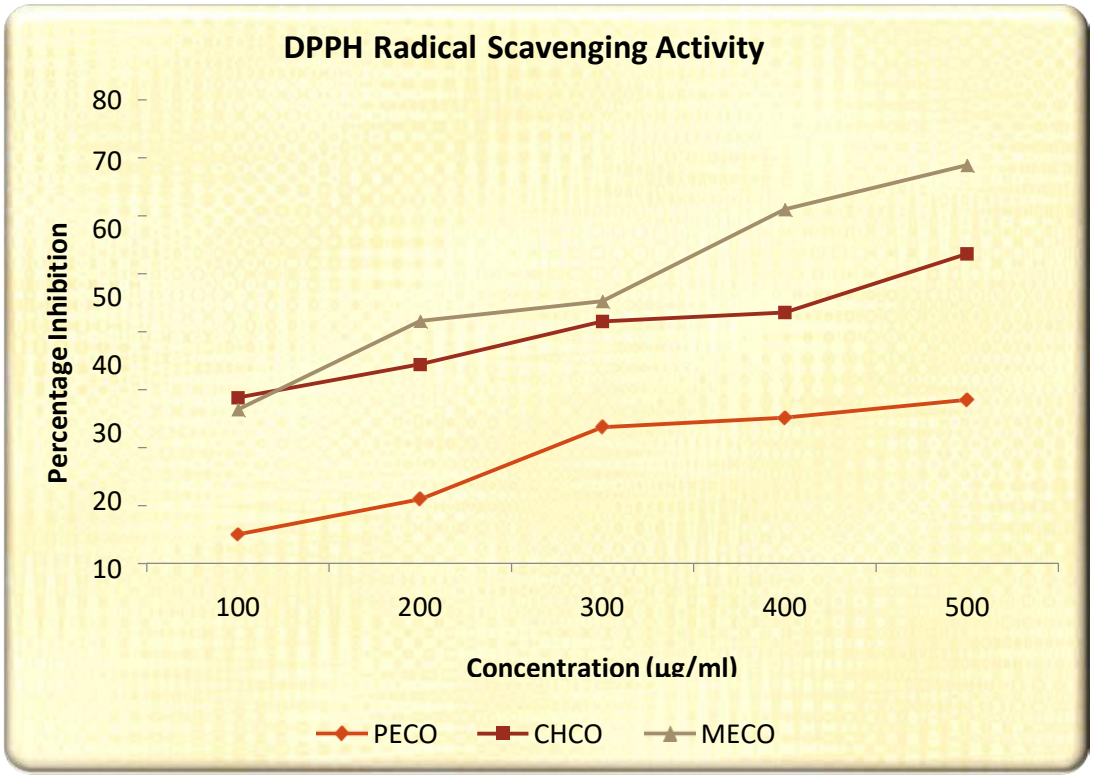

Figure-18: Percentage Inhibition of DPPH Radical Scavenging Activity of *Crataegus oxyacantha*extracts at Different Concentrations. Where PECO, CECO and MECO Represent Petroleum Ether, Chloroform and MethanolExtract of *Crataegus oxyacantha* Respectively

Reducing Power Assay

In reducing power assay reductive capabilities of pet. Ether, chloroform and methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* were measured. It was observed that with increase in concentration of extract reducing power increases(Table 15, Table 16). Highest reducing power was exhibited by methanol extract of both plants as evidenced by graph (Fig.19 and Fig. 20). Reducing power of different extracts of *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves decreases in the following order methanol extract>chloroform extract> pet ether extract.

Table 15: Reducing Power of *Grewia asiatica's* Extracts at Different Concentrations. Values were Expressed as mean±SD. Samples were Taken in Triplicate

S. No	Concentration (µg/ml)	Reducing Power of Extracts of *Grewia asiatica of* Different Concentrations, at 700 nm		
		Petroleum Ether Extract	Chloroform Extract	Methanol Extract
1.	100	0.10±0.011	0.2±0.005	0.28±0.01
2.	200	0.14±0.025	0.25±0.01	0.36±0.01
3.	300	0.22±0.025	0.31±0.01	0.52±0.01
4.	400	0.28±0.005	0.38±0.005	0.6±0.0057
5.	500	0.31±0.01	0.50±0.005	0.81±0.01

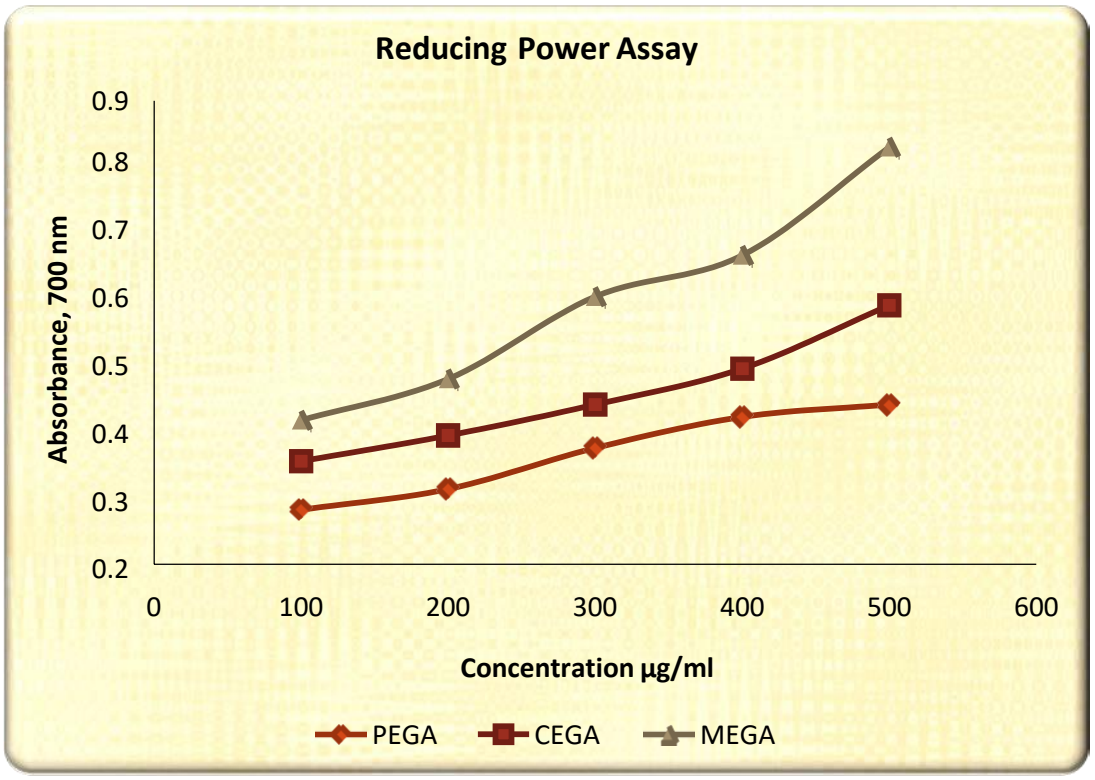

Figure-19: Reducing Power of Different Extracts of *Grewia asiatica* at DifferentConcentrations. Where PEGA, CEGA and MEGA Represents Petroleum Ether, Chloroform and Methanol extract of *Grewia asiatica* Respectively

Table 16: Reducing Power of *Crataegus oxyacantha*'s Extracts at Different Concentrations. Values are Expressed as mean±SD. Samples were Taken in Triplicate

S. No.	Concentration (µg/ml)	Reducing power of *Crataegus oxyacantha* extracts at 700 nm		
		Petroleum ether extract	Chloroformextract	Methanolextract
1.	100	0.18±0.005	0.27±0.036	0.31±0.01
2.	200	0.20±0.005	0.35±0.01	0.44±0.01
3.	300	0.23±0.01	0.41±0.01	0.59±0.01
4.	400	0.2±0.01	0.52±0.01	0.72±0.005
5.	500	0.25±0.005	0.71±0.01	0.93±0.01

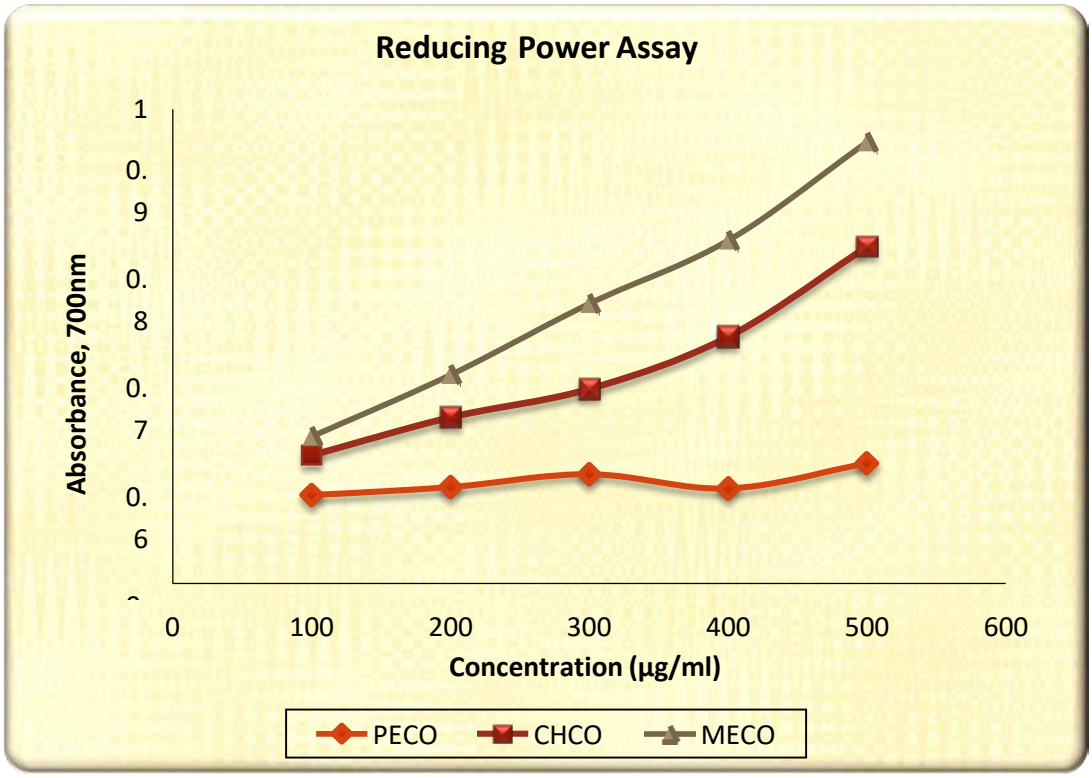

Figure-20: Reducing Power of *Crataegus oxyacantha* Leaves Extract (Where PECO, CHCO and MECO are Petroleum Ether, Chloroform and Methanol Extract of *Crataegus oxyacantha* Leaves Respectively)

Conclusion

Extracts thus obtained were subjected to preliminary phytochemical screening like solubility studies and phytochemical characterization to determine thephytoconstituents present. Based on phytochemical tests and percentage yield the chloroform and methanol extract were subjected to estimation of phytochemicals like flavonoids and phenolic content determination. In our study methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* possessed highest total flavonoid and phenolic content. After estimation of phytochemicals in vitro anti-oxidant effect of plants were estimated by DPPH and reducing power assay.

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