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Study on Physiochemical & Organoleptic Evaluation of *Grewia asiatica* berries and *Crataegus oxyacantha* leaves

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ABSTRACT

After estimation of phytochemicals in vitro Physiochemical effect of plants were estimated by DPPH and reducing power assay. 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 phytochemicalslike flavonoids and phenolic content determination. In our study methanol extract of *Grewia asiatica* and *Crataegus oxyacantha* possessed highest total flavonoid and phenolic content.

Key Words-: Antioxidant,DPPH,Cognitive

INTRODUCTION

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). 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.*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.

Method

Preparation and Organoleptic Evaluation

Collected and authenticated plant materials i.e. fruit of *Grewia asiatica* and leaves of *Crataegus oxyacantha* were washed with water then allowed to dry in shade for about 3 to 4 weeks. Dried materials were cleaned manually and kept over dry plastic sheet to investigate different organoleptic features. The magnifying glass and scale were used to measure the parameters like shape, size, colour, odour, taste, texture, weight. After determination of organoleptic features, dried plant materials were grinded by using electronic grinder and filled in an air tight container forfurther experimental work.

Physicochemical Studies

Different physicochemical parameters of powdered drug like ash values (total ash, water soluble ash andacid insoluble ash), extractive values (water soluble extractives and alcohol soluble extractives) and fluorescence pattern were determined by official methods as prescribed in Indian Pharmacopoeia., 1996 and WHO guidelines., 1992 on quality control methods for medicinal plants.

Determination of Ash Values

Determination of Total Ash

The clean and dry crucible (silica) was taken and its weight was noted. 10 g of powdered plant materials of *Grewia asiatica* and *Crataegus oxyacantha* were weighed in crucible and powder plant material

washeated up to 300˚C for 3-4 hours until the whole powder turns into ash. The crucible was cooled and weighed again. The difference in the weight was noted and percentage of total ash was calculated.

$$\text{Total ash (\%w/w)} = \frac{\text{Weight of ash (g) Weight of sample used (g)}}{} \times 100$$

Determination of Water Soluble Ash

One gm of ash obtained as above was weighed and 10 ml of distilledwater was added. The mixture was kept on a rotary shaker at 140 rpm for 8 hours and filtered through ashless filter paper. The ash remained on the paper was kept in acrucible (Silica) and burnt to ash again in a muffle furnace for 15 min at 450ºC. The weight of ash obtained was noted and percentage of water soluble ash was determined by subtracting weight of insoluble matter from weight of total ash and calculated with reference to air dried plant material using following formula:

$$\text{Water soluble ash (\%w/w)} = \frac{\text{Total ash - Water insoluble residue in total ash}}{\text{Weight of sample}} \times 100$$

Determination of Acid Insoluble Ash

One gram of total ash was weighed and 10 ml of concentrated H_2SO4 was added. The mixture was kept on a shaker at 140 rpm for 8 hours and filtered through ashlessfilter paper. The ash remained on the paper was kept in a crucible (Silica) and burnt to ash again in a muffle furnace for 3-4 hours. The weight of ash obtained was notedand percentage of acid insoluble ash was determined.

$$\text{Acid insoluble ash (\%w/w)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of Extractive Value

Determination of Water Soluble Extractive Value

Five grams of powder of both plants were weighed and added into a conical flask. 100 ml of distilledwater was added into it and kept on a rotator shaker (140 rpm) for 24 h. After 24 h it was filtered and 25ml of filtrate was dried in hot air oven set at 80˚C for 24 h and weighed again. The difference in the weight was determined and percentage of water soluble extractive was calculated with respect tothe weight of air dried material.

Determination of Alcohol Soluble Extractive Value

Five grams of powder was weighed and added into a conical flask. 100 mlof ethanol was added into it and kept on a rotator shaker (140 rpm) for 24 h. After 24 h it was filtered and 25 ml of filtrate was dried in hot air oven set at 80˚C for 24 hand weighed again. The difference in the weight was determined and percent of alcohol soluble extractive was calculated with respect to weight of air dried material.

Fluorescence Analysis

Fluorescence analysis was carried out in accordance with the procedure reported by Chase and Pratt., 1949 and Kokoshi *et al*., 1958. One mg of powdered drug of *Grewia asiatica* fruit and *Crataegus oxyacantha* leaves were placed on a petriplate and treated with different solvents like 1 ml of 1 N HCl, 1ml of 1N NaOH,1 ml of Iodine, 1 ml of Ferric chloride, 1 ml of ammonia, 1 ml of methanol, 1 ml of

chloroform one by one and observed for characteristic fluorescence colour when exposed under UV light at 365 and 254nm.

RESULT & DISCUSSION

Morphological and Organoleptic Characters

Morphological studies revealed that *Grewia asiatica* berries are round in shape, purple or cherry red in colour with sweet acidic taste. Leaves of *Crataegus oxyacantha* are green in colour, with glabrous broad ovate or obovate shape and serrate margin with characteristic faint taste (Table 4).

Table-4: Morphological characteristics of *Grewia asiatica* berries and *Crataegusoxyacantha* leaves

S. No	Morphological feature	*Grewia asiatica*(Berries)	*Crataegus oxyacantha* (Leaves)
1.	Shape	Almost round	Glabrous, broad ovate or obovate
2.	Colour	Purple or cherry red	Green
3.	Taste	Sweet acidic	Characteristic faint
4.	Size/Margin	1.0 to 1.9 cm in diameter, 0.8 to 1.6 cmin vertical height	Serrate margins with three to five lobes
5.	Weight/Length	0.5 to 2.2g	15 mm-5 cm long

Physicochemical Parameters

The different physicochemical parameters like Total ash, acid insoluble ash, water soluble ash, water soluble extractives and alcohol soluble extractives values of powder of *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves were determined. The values obtained are presented in Table 5.

Table-5: Ash Values and Extractive Values of *Grewia asiatica* Fruits and *Crataegus oxyacantha* Leaves Whole Powder

Standardizationparameters	*Grewia asiatica(%w/w)*	*Crataegus oxyacantha (%w/w)*
Total ash	5.83±0.20	4.5±0.1
Acid insoluble ash	0.23±0.03	0.62±0.370
Water soluble ash	2.3±0.15	2±0.1
Water soluble extractive	12.66±3.05	25.3±1.154
Alcohol soluble extractive	20±2	33.33±3.055

Determination of Fluoroscence Pattern

Fluoroscence pattern of *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves powder when treated

with different solvents and exposed to UV radiation of short and long wavelength were determined as given in Table 6.When the powdered drugs were treated with different reagents and observed under UV light, they emit radiations of various colour. The colour change for crude powder was distinctiveand reproducible revealing solvent properties of phyto-constituents.

Table 6: Fluorescence Characteristics of *Grewia asiatica* Fruits and *Crataegusoxyacantha* Leaves with Different Solvents

Solvents	*G. asiatica*		*Crataegus oxyacantha*	
	Short	**Visible**	**Short**	**Visible**
NaOH	Yellowish green	Colourless	Colourless	Colourless
HCl	Blue	Yellow	Colourless	Transparent
Iodine	Dark brown	Wine red	Brown	Dark red
Ferric chloride	Bottle green	Light green	Dark green	Pale yellow
Ammonia	Dark green	Yellow	No colour	Transparent
Methanol	Light green	Light green	Grey	Transparent
Chloroform	Colourless	Colourless	Blue	Green

Phytochemical Studies

Successive Extraction

500 gm of coarsely powdered fruits of *Grewia asiatica* and *Crataegus oxyacantha* leaves were subjected to successive extraction technique usingpetroleum ether, chloroform and methanol as solvent. Percentage yield of all extracts and colour were noted (Table7).

Table 7: Percentage Yield Of Different Extracts of *Grewia asiatica* Berries and *Crataegus oxyacantha* Leaves

S. No.	Types of Extract	Colour	Percentage yield (w/w)
1	Petroleum ether extract of *Grewia asiatica*	Grey blue	18.3
2	Chloroform extract of *Grewia asiatica*	Bottle Green	12.5
3	Methanol extract of *Grewia asiatica*	Brownish red	15.42
4	Petroleum ether extract of *Crataegus oxyacantha*	Dark brown	13.5
5	Chloroform extract of *Crataegus oxyacantha*	Reddishorange	18
6	Methanol extract of *Crataegus oxyacantha*	Yellowish	21.2

Solubility Studies

Solubility pattern of petroleum ether, chloroform and methanol extracts of *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves were determined in different solvents like hexane, diethyl ether, ethyl acetate, acetone, ethanol, acetic acid and water. Results are shown in Table 8.

Table 8: Solubility pattern of *Grewia asiatica* and *Crataegus oxyacantha* extracts in Different Solvents. Where PEGA, CEGA, MEGA, PECO, CHCO and MECO Represents Petroleum, Chloroform and Methanol Extract of *Grewia asiatica* and *Crataegus oxyacantha* Respectively.

Solvents	PEGA	CEGA	MEGA	PECO	CHCO	MECO
Hexane	Soluble	Insoluble	Insoluble	Soluble	Soluble	Insoluble
Diethyl ether	Soluble	Soluble	Insoluble	Soluble	Soluble	Insoluble
Ethyl acetate	Insoluble	Soluble	Soluble	Soluble	Insoluble	Soluble
Acetone	Insoluble	Soluble	Soluble	Insoluble	Soluble	Soluble
Ethanol	Insoluble	Sparingly Soluble	Soluble	Insoluble	Insoluble	Soluble
Acetic acid	Insoluble	Insoluble	Soluble	Insoluble	Insoluble	Soluble
Water	Insoluble	Insoluble	Soluble	Insoluble	Insoluble	Soluble

Preliminary Phytochemical Screening

Preliminary phytochemical characterization was done to determine the presence of various phytoconstituents in *Grewia asiatica* fruits and *Crataegus oxyacantha* leaves. Results are shown in Table 9:

Table 9: Phytochemical Screening of Extracts of *Grewia asiatica* Fruits and *Crataegus oxyacantha* Leaves

Phyto Constituents	Chemical Test	PEGA	CEGA	MEGA	PECO	CECO	MECO
Alkaloids	Mayer Test	_	_	_	_	+	+
	Dragendroff Test	_	+	_	_	_	_
	Hager Test	_	_	_	_	+	_
	Wagner Test	_	_	_	_	_	+
Flavanoids	Lead acetate Test	_	+	+	_	+	+
	Shinoda Test	_	_	+	_	_	+
	Alkaline Reagent test	_	+	+	_	+	+
Triterpenoids	Salkowski Test	+	+	_	_	+	_
	Libermann Burchard Test	_	_	_	+	+	+
Tannins	Lead acetate	_	-	_	_	_	+
	Ferric chloride Test	_	_	+	_	_	_
Saponins	Froth Test	_	_	+	+	_	_
Glycosides	Legal Test	_	+	+	_	+	_
	Keller Killiani Test	_	_	_	_	+	+
Carbohydrates	Molisch Test	_	_	_	_	_	+
	Benedict test	_	_	+	_	+	_
Protein Test	Biuret test	_	_	+	_	_	_
Fats	Solubility test	+	_	_	+	_	_

Conclusion

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. Extracts thus obtained were subjected to preliminary phytochemical screening like solubility studies and phytochemical characterization to determine the phytoconstituents present. Based on phytochemical tests and percentage yield the chloroform and methanol extract were subjected to estimation of phytochemicalslike flavonoids and phenolic content determination.

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