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PHARMACOGNOSTICAL AND ANALYTICAL STUDY OF *GUDUCHYADI MEDHYA RASAYANA*: A POTENTIAL HERBAL FORMULA FOR COGNITIVE ENHANCEMENT

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ABSTRACT

Guduchyadi Medhya Rasayana is one of the important Ayurvedic formulation recommended for memory improvement. In Ayurvedic medicine, *Medhya Rasayana* drugs are being used for different types of mental disorders, particularly in promoting memory enhancement and intelligence. Quality of a drug depends on its genuineness of ingredients and the study is focused to demonstrate pharmacognostical and analytical study of *Guduchyadi Medhya Rasayana*. The drug has been prepared in granular form and the result revealed a presence of important microscopic characters of ingredients of *Guduchyadi Medhya Rasayana.* Total Ash value was 3.2967w/w% while, acid insoluble Ash 1.6983w/w. Its Ash value shows very less quantity and revealed authenticity and purity of the drug. In addition, Alcohol soluble extractive 17.6w/w% and water-soluble extractive 37.60w/w%. Bulk density 3 taps 0.547g/ml while, 50 taps 0.570 g/ml. Compressibility was 4.100.Angle of repose 0.4705 and Total sugar value was 53.56w/w% while, Reducing sugar 8.57w/w% which were important for marketing purpose of the drug. Also details of Thin Layer Chromatography (TLC) showed that Rf values for five components. Further, Antioxidant activity of *Guduchyadi Medhya Rasayana*, ABTS assay in Vitro models showed potent Antioxidant activity with percentage inhibition (IC_{50} 101.8 +-7.0 microgram/ml) and it would help to enhance cognitive functions. Hence, it could be concluded that the above parameters could be considered as reference standards in future research studies.

Keywords: *Guduchyadi Medhya Rasayana*, Cognitive decline, Pharmacognosy, Analytical study

INTRODUCTION

Herbal drugs possess various properties called *Rasa, Guna*, *Virya, Vipaka* and *Prabhava* and the importance of a drug was based on its properties and action[1]. *Susruta Samhita* has clearly elaborated on it[2]. The action of a drug intimately related to its chemical structurer in the preponderance of one or two proto elements in them[3]. In Ayurveda that *Rasayana* drugs have a major role in the management of age related disorders and have an antioxidant properties which prevents the free radical injury and delays the ageing

process and rejuvenate both mind and the body[4].Moreover,the *Medhya Rasayana* have beneficial effects more prominently over the mind and it improve the mental functions and intellect. *Guduchyadi Medhya Rasayana* has mentioned in Ayurveda authentic textual in *Chakradatta*[5] and *Yogaratnakara*[6] under the *Rasayanadikara* and it consist of eights herbal drugs. At the present time, it has been increased the demand of plant base pharmaceuticals and some drugs preparations are lack of formal standards. Therefore, the study is an attempt to demonstrate the pharmacognostical and the analytical potential of *Guduchyadi Medhya Rasayana*

Table 1: Ingredients of *Guduchyadi Medhya Rasayana:*

Sr. No	Sanskrit Name	Botanical Name	Family Name	Part Used
1	*Guduchi*	*Tinospora Cordifolia* (Willd)	Menispermaceae	*Kanda*
2	*Apamarga*	*Achyranthes Aspera Linn*	Amranthaceae	*Panchanga*
3	*Vidanga*	*Embelia ribes*Burm.*f.*	Myrsinaceae	*Beeja/Sheeds*
4	*Sankhapuspi*	*Convolvulus Pluricaulis* chois	Convolvulaceae	*Panchanga*
5	*Vacha*	*Acorus Calemus* Linn	Araceae	*Moola*
6	*Haritaki*	*Terminalia Chebula* Zetz	Combretaceae	*Phala Twak*/Pericap
7	*Kushtha*	*Saussurea lappa* c.B Clarke	Compositae	*Moola*
8	*Shatavari*	*Asparagus racemosus* wild	Liliaceae	*Moola*
9	*Go Ghrita* (Cow *Ghee*)			
10	Sugar			

Pharmacognostical Study:

Dried samples of all ingredients have been collected from the Department of the Pharmacy at the Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar and following microscopical characteristic features identified at the Department of Pharmacognosy for confirmation of authenticity.

Powder Microscopy of the Ingredients of *Guduchyadi Medhya Rasayana:*

Figure 1: *Guduchi*

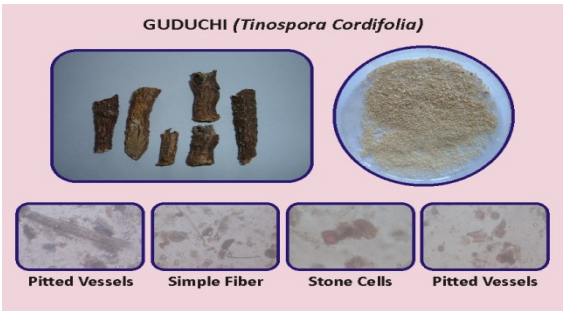


Figure 2: *Apamarga*

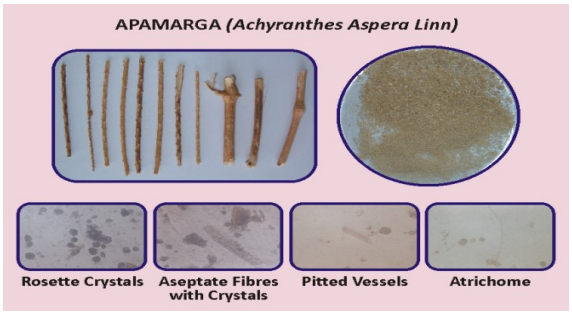


Figure 3: *Vidanga*

Figure 4: *Sankapushpi*

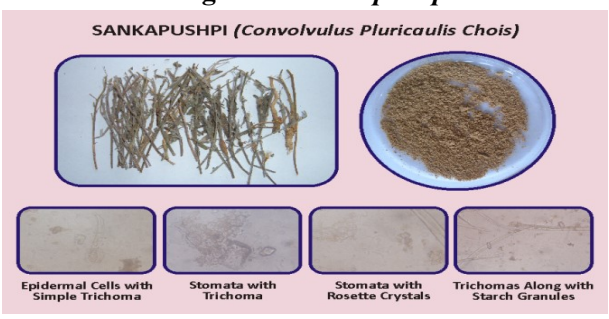


Figure 5: ***Vacha***

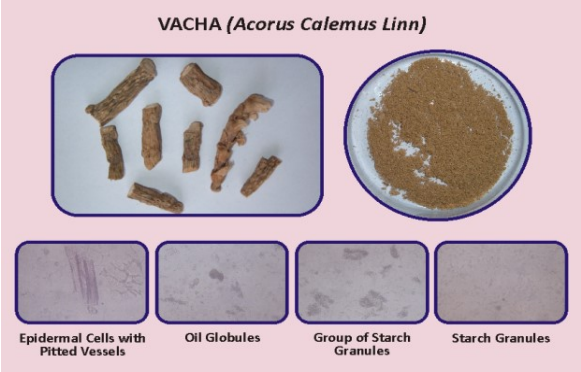


Figure 6: ***Haritaki***

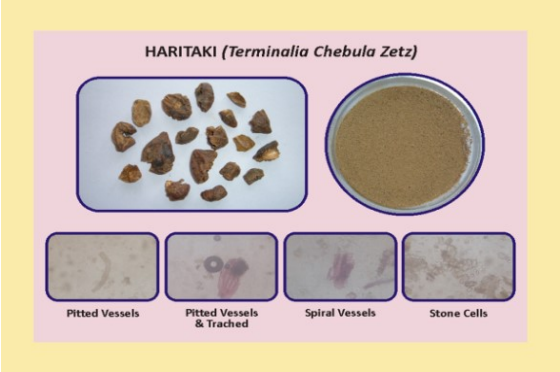
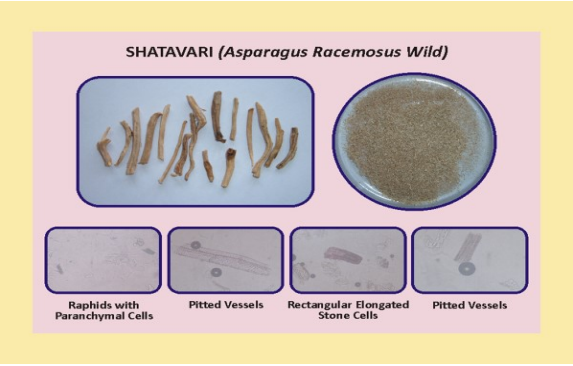


Figure 7*:* ***Kustha***

Figure 8: ***Shatavari***



Method of preparation of *Guduchyadi Medhya Rasayana* in granules form:

Dried ingredients of the *Guduchyadi Medhya Rasayana* powdered properly, at the Department of Pharmacy of I.P.G.T.& R.A, G .A .U according to the powder preparation mentioned in *Sarangadhara Samhita*[7].

Table 2: Ingredients of *Guduchyadi Medhya Rasayana granules*

	Ingredients	**Proportion**
1	Powder form of *Guduchyadi Medhya Rasayana*	1 part
2	Sugar	1 part
3	*Go Ghrita* (Cow *Ghee*)	1/4 part
4	Pure water	1/8 part Approximately

The sufficient quantity of water is added to the sugar and then mixed thoroughly and boiled until it appearing form of sugar syrup. Then *Ghee* and fine powder of *Guduchyadi Medhya Rasayana* are added and mixed well. Then mixture is heated gradually until granule formation is appeared Then immediately crushed it in to fine granules form (Fig -9) by using a strainer.

Dosage & Administration of the drug: 5g - 3 times per day after the meal.

Anupana*:* warm water sufficient quantity.

Duration: three months

Analytical Study:

Quantitative Analysis-(Physical Constants)

Before administration of the drug to the patients that purity should be determined. Hence, analytical study has been completed at the Pharmaceutical Chemistry

Laboratory at I.P.G.T. & R.A, G .A.U. at the beginning following physical parameters were carried out.

1. Loss of Drying at 110 ^{0}C
2. Total Ash Value
3. Acid Insoluble Ash Value
4. Alcohol Soluble Extractive
5. Water Soluble Extractive
6. Bulk Density
7. Test for Sugar

- Estimation of Reducing Sugar
- Estimation of Total Sugar

1. Detarmination of loss of Drying at 110^{0}C:

The loss of drying was determined by taking 2g accurately of weighed sample in a dried petri dish. Then dried it in an oven at 110^{0}C till constant weight is obtained. Then noted down the weight after drying. Then percentage was calculated on the basis of air dried sample[8,]

2. Determination of Ash Value:

The ash value of the sample was determined by incinerated about 2g of accurately weighed drug in a dried crucible at a temperature 450^{0}C until free from carbon. Then cooled it at room temperature and weighed. After that the percentage of ash was calculated with the reference to the air dried sample[9].

3. Determination of Acid Insoluble Ash Value:

The ash obtained in (2g) was boiled for 5 minutes with 25 ml of 6 N HCL. Then it filtered with the help of ash less filter paper and washed with hot water till it is free from chloride. Then the filter paper is placed in previously Weighed crucible and incinerated. Finally, the percentage of the acid insoluble ash is calculated with the reference to air dried sample[10].

4. Determination of Alcohol Soluble Extract:

For this study 5g of sample accurately weighed and put in a conical flask. Then added 100ml of 95% ethanol and kept the sample standing for 18 hours. Then after shaking the conical flack continuously for 6 hours, taking precaution against loss of solvent. Then it is filtered with the help of filter paper and measured 20 ml of filtrate into the evaporating disc and kept it for evaporation on the water bath. After proper evaporating the residue, is dried at 105^{0}C in oven to the constant weight and cooled it. Finally, weighed the residue an percentage of alcohol soluble extractive was calculated with reference to air dried sample[11].

5. Determination of Water-Soluble Extract:

Weighed accurately about 5g of sample, then it was macerated with 100ml of chloroform($CHCl_3$) water in a conical flack and continuously shaken for 6 hours and kept for stand for 18 hours. Then filtered rapidly taking precaution against loss of solvent. Then it was filtered and 25ml of the filtrate is taken for evaporation on water bath using in previously weighed evaporating dish. Then extract is dried at 105^{0}C temperature till constant weight is obtained and weight was noted. Then from the weight of residue the percentage of water soluble extractive was calculated with reference to air dried sample[12].

6. Bulk Density

Usefulness of the Bulk Density is to identify the size of container of Bulk chemical and to determine the proper size of container for a given mass of powder so bulk density is important for marketing purposes of drugs.

Bulk density is the weight of powder divided by its bulk volume and it depend upon the

1. Size and Shape of the particle
2. Cohesiveness of the particle

Method:

Powder of trial drug was introduced in to a dry 50ml graduated measuring cylinder up to 50 ml and weighed, then its amount noted. After that cylinder was tapped 3 times and then 50 times with hand on the wooden surface of the table from height of '1'inch at 2 second intervals. Then final volumes was read and noted.

$$\text{Bulk Density} = \frac{\text{Weight of the Sample (g)}}{\text{Volume of the Sample (ml)}}$$

Note-When particles are packed loosely lots of gaps between particles are observed. Hence bulk volume increases making the powder light.

7. Test for Sugar Estimation:

- **Estimation of Reducing Sugar**

25 ml of prepared stock solution is taken along with 10 ml each Fehling-A and Fehling –B solution and taken in to a beaker. This mixture is heated on hot plate, and boiled for 3 minutes. The precipitate of Cu_2O is filtered through Whatman filter paper No 1 and washed the precipitate with the hot water till free from Fehling solution. Then precipitate of Cu_2O is dissolve in acidic ferric ammonium sulphate solution and titrated against 0.1N $KMnO_4$ solution by using Orthophenanthroline using as an indicater. When the reaction is completed that orange colour solution has changed in to greenish blue in colour. Then reading of $KMNO_4$ is noted and calculated the copper obtained. The weight of reducing sugar is equalent to the weight of copper and then compared with Hammont table and percentage of Reducing Sugar has calculated.

- **Estimation of Total Sugar**

25ml of prepared stock solution is taken and 5ml of 6N HCL is added and heated over the water bath for 3 minutes between 67-71^0 C temperature. Then the hydrolyzed solution is kept for getting cool and neutralized with Conc 20% NaOH using Phenolphthalein as an indicator. (pink colour is obtained at the end reaction) Then it is diluted with distil water up to 100ml in volumetric flask. This became the stock solution for estimation of total sugar. So from this solution 25ml is taken in to a beaker and added 25ml each of Fehling A and Fehling B .Then further procedure is similar which has mentioned under the estimation of reducing sugar. After calculation of the total sugar then Non reducing sugar of the sample is calculated.

Non-Reducing Sugar= Total Sugar-Reducing Sugar

Results of the Analytical Study:

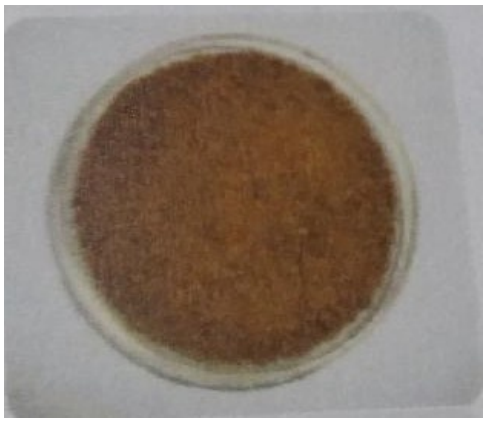


Figure - 9: *Guduchayadi Medhya Rasayana* Granules

Organoleptic Properties:

Colour : Dark Brown
Taste : Sweet
Odour : Aromatic

Table 3: Physico-chemical Properties of *Guduchayadi Medhya Rasayana*

Sr.No	Parameters	Result
1	Total Ash Value	3.2967 w/w%
2	Acid Insoluble Ash Value	1.6983w/w%
3	Alcohol Soluble Extractive	17.6 w/w%
4	Water Soluble Extractive	37.60w/w
5	Bulk Density 3 taps	0.547 g/ml
	50 taps	0.570 g/ml
6	Compressibility	4.100
7	Angle of repose	0.4705
8	Total Sugar Value	53.56w/w%
9	Reducing Sugar Value	8.57w/w%
10	None Reducing Sugar Value	44.99 w/w%

Phytochemical Investigation:

For Determination of phytoconstituents of the *Guduchyadi Medhya Rasayana* following test were carried out,

1. Thin Layer Chromatography
2. Anti-oxidant Activity

1. T.L.C (Thin Layer Chromatography):

Thin Layer Chromatography is a very commonly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction[13].

The following procedures have been carried out during the study.

Stationary Phase - Silica Gel
Mobile Phase - Toluene: Ethyl Acetate
9 : 1
Principle - Adsorption

Preparation of Stationary Phase:

Taken a glass plate and slurry spreaded that silica gel as a thin layer. Then plate is allowed to dry, on air and activated by heating on an oven at 110^0C for 30 minutes. Then it is called as stationary phase.

Preparation of The Solution:

Weighed 2 g of sample and put in to 100ml of beaker then added 20ml of distil water. After that added 4 ml of Con .HCL and heated sometimes and filtered it. Then taken the filtrate and added $CHCl_3$ for acid hydrolysis. After that there were 2 layers appeared called organic and aqueous layers. Organic layer used as extract solution.

Preparation of The Mobile Phase:

It has been prepared by mixing of Toluene and Ethyl Acetate in the ratio of 9:1 and transferred in to a clean dried 100 ml beaker. The beaker has covered by glass lid and kept a side for saturation.

Method:

Activated T.L.C plate is taken and kept two drops separately from extract solution using a capillary tube at least 1cm above from the base of the plate. Then the T.L.C plate has kept inside the saturated beaker until that solvent reaching to the upper end of the T.L.C plate. Then it has taken and dried and put inside the UV chamber.

Results:

$$\text{Rf Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

T.L.C Report on *Guduchyadi Medhya Rasayana* Granules:

Table 4.1: Under 254 Nanometer

Number of Spots	Travelled Distance of Solute	Travelled Distance of Solvent	Rf Value
Spot-1	0.9	6 cm	0.15
Spot-2	4.8	6cm	0.8

Table 4.2: Under 336 Nanometer

Number of Spots	Travelled Distance of Solute	Travelled Distance of Solvent	Rf Value
Spot-1	0.9cm	6cm	0.15
Spot-2	1.4cm	6cm	0.23
Spot-3	2.5cm	6cm	0.41
Spot-4	4.8cm	6cm	0.8
Spot-5	5.4cm	6cm	0.9

Table 4.3: After Spray Reagent: Venilin + H_2SO_4

Number of Spots	Travelled Distance of Solute	Travelled Distance of Solvent	Rf Value
Spot-1	0.9cm	6cm	0.15
Spot-2	1.4cm	6cm	0.23
Spot-3	4.8cm	6cm	0.8

2. Anti-Oxidant Activity:

Role of Anti-oxidants on the Human Body:

Human have evolved a highly sophisticated and complex antioxidant protection system. That functions interactively and synergistically to neutralize free radicals. Thus, anti-oxidant are capable of stabilizing or deactivating free radical before they attack cells and they are absolutely critical for maintaining optimal cellular and systemic health and well–being. Naturally there is a dynamic balance between the amount of free radical produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects.

The amount of anti oxidant principles present under normal physiological condition may be insufficient to neutralize free radical generated. Therefore, it is obvious to enrich the diet as well as therapeutic supplements with antioxidant to protect against harmful diseases[14].

Considering the above important of this area it has been evaluated that **Antioxidant activity of *Guduchayadi Medhya Rasayana* using in *Vitro* Models.**

For this study ABTS (2,2Azinobis (3-Ehyl Benzothiazoline - 6 Sulfonicacid) Diamonium Salt) method has been adopted and it has been carried out at Herbal Technology Section of Industrial Technology Institute in Sri Lanka.

- **ABTS (2,2Azinobis (3-Ehyl Benzothiazoline - 6 Sulfonicacid) Diamonium Salt) Method[15] :**

This is a measure of antioxidant activity as opposed to antioxidant concentration which might induce a proportion of biologically inactive antioxidants. It also permits the measurement of antioxidant activity of mixtures of substances and assist to distinguish between additive and synergistic effects. The assay is based on interaction between antioxidant and ABTS+ radical cation which has a characteristic color showing maxima at 645,734 and 815nm.

Observation and Results:

The result from **ABTS** assay showed that the water extract *Guduchyadi Medhya Rasayana* showed potent Antioxidant activity with percentage inhibition **(IC_{50} 101.8 +_ 7.0 microgram/ ml)** .

DISCUSSION

Pharmacognostical study proved that presence of important microscopic characters of ingredients of *Guduchyadi Medhya Rasayana* (Fig 1 to Fig 8) and the physicochemical parameters (Table -3) that loss of drying revealed less value of moisture content could prevent Bacterial, Fungal, or Yeast growth on present Sample. In addition, Ash value of a drug gives on idea of the earthy matters such as Silica or the Inorganic composition and other impurities present along with the drug. The present samples the total Ash and Acid insoluble Ash values showed in very less quantity. So, it gives positive contribution to Authenticity and Purity of the drug. Regarding Extractive values which are primarily useful for the determination of exhausted or adulterated drugs on the basis of solubility constituents in narrated solvents. The total sugar value of the drug is 53.56w/w%, because during the preparation of *Guduchyadi Medhya Rasayana* that sugar added equal part to the ingredients. Moreover, Bulk density, tap density and compressibility physical parameters used to determine particle property and derived property of Bulk Chemical and to determine the proper size of container which is the important for marketing purpose of the drug (www.faqs.org) ,(www.sphinxsai.com). Thin Layer Chromatography showed (Table 4.1) the spots and having same Rf values indicates the presence of identical components in the sample (Tables 4.2, 4.3)

CONCLUSION

In view of the above, the powder microscopic features and organoleptic characters revealed the genuine identification of plant materials of *Guduchyadi Medhya Rasayana.* Physico-chemical parameters proved the quality and purity of its ingredients. Thin Layer Chromatography (TLC) results showed that identical chemical constituents are presented in the drug which could be useful for future research. In addition, ABTS assay revealed that the water extract of *Guduchayadi Medhya Rasayana* having potent anti-oxidant activities with percentage inhibitions of IC_{50}101.8±7.0 µ/ml. Hence, the antioxidant property of *Guduchayadi Medhya Rasayana* would help to minimize the cognitive decline.

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