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Original Article
In vitro studies

Chemical analysis and anti-oxidant properties of Hutabhugadi Curna - A polyherbal Ayurveda formulation

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ABSTRACT

Introduction: Hutabhugadi curna (HC) is a polyherbal formulation in Ayurvedic formulary of India (AFI) for treatment of agnimandya (digestive impairment), pandu (anemia), sopha (oedema) and arsa (piles). The current paper is aimed to investigate physico-chemical properties, preliminary phytochemical and proximate composition, including its anti oxidant activity *in-vitro*. ***Methods*:** Estimation of physical parameter, proximate composition, phytochemical screening, total phenol content and antioxidant activity was done using standard methodology. ***Results*:** Physico-chemical and proximate analysis was done to access the composition of the formula. Preliminary phytochemical tests of extracts of HC were performed to find out the different chemical moieties in the formula. Total phenolic content of HC was determined using Folin-Ciocalteau's reagent using tannic acid as a calibration standard. Reducing power assay (RPA) and DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging capacity were carried out to evaluate antioxidant activity *in vitro*. Physical constants and quantity of total protein, carbohydrate, fat and fibre were estimated to obtain calorific value. ***Conclusion*:** Phyto chemical analysis revealed the presence of carbohydrates, saponins, tannins, phenols and coumarins. Total phenolic content of HC was found to be 23.90 mg/g tannic acid equivalents. The classical multidrug formula showed good antioxidant activity.

KEYWORDS

Anti oxidant activity, Ayurvedic formulary of India, phytochemical, proximate analysis, polyherbal formulation.

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Ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing.[1,2] Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues central nervous system injury, gastritis, cancer and AIDS.[3] Due to depletion of immune system natural antioxidants in different maladies consuming antioxidants as free radical scavengers may be necessary.[4] The number of reports on isolations of natural antioxidants, mainly of plant origin, has increased immensely during the last decade.[5] Polyherbal formulations are not being studies extensively for their potential as antioxidants.

Hutabhugadi churna (HC) of Ayurvedic formulary of India (AFI) is a polyherbal drug formulated using one part of ingredients viz. Hutabhuga (*Plumbago zeylanica* Linn. - root), Ajamoda (*Apium leptophyllum* (Pers.) F.V.M.ex Benth - fruit), Saindhava Lavana (Rock salt), Magadha (*Piper longum* Linn. - fruit), Marica (*Piper nigrum* Linn. - fruit), and 5 parts of inredient Pathya (*Terminalia chebula* Retz. – pericarp) (Figure 1). The therapautic uses of HC as per Ayurveda are in treatment of Agnimandya (digestive impairment), pandu (anemia), sopha (oedema) and arsa (piles).[6] In the present study, the formulation was subjected to physical, proximate, and preliminary phytochemical analysis. Methanolic extract of HC was screened for total phenol content, 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging and reducing power assay to evaluate its antioxidant profile *in-vitro*.

MATERIALS AND METHODS

Preparation of Hutabhugadi curna

HC was prepared as per procedure detailed in Ayurvedic Pharmacopoeia of India.[7]

Preparation of extract

Ten grams of HC was exhaustively extracted in Soxhlet apparatus with 150 ml of methanol. The filtrate was evaporated to dryness and the residue was used for phytochemical screening and antioxidant activity profiling.

Physical parameters

Determination of bulk density, tapped density, Carr's index and Hausner's ratio was done using standard procedure.[8]

Proximate analysis

Determination of fat, fibre, protein,[9] and carbohydrate[10] was done using standard procedures. Calorific value was determined by the formula: (Carbohydrate × 4) + (Fat × 9) + (Protein × 4).[9]

Figure 1. ***Hutabhugadi curna***

Phytochemical screening

Phytochemical tests for methanolic extract were performed by standard method.[11]

Estimation of total phenol: The total phenol content of HC was determined by Folin – Ciocalteau method with slight modification, using tannic acid as standard.[12]

Anti oxidant activity of Diphenyl - picryl hydrazyl (DPPH): The antioxidant activity of extract determined by Percent of inhibition as follow: % Inhibition = [(A0 - A1)/A0] ×100.[13]

Reducing power of the HC extract was determined by standard methodology.[14]

RESULTS AND DISCUSSION

Physical parameters like bulk density, tap density, Carr's index and Hausner's ratio were obtained for HC. Protein, fat and carbohydrate are sources of energy in diet and the amount was found to be 4.23 %, 3.35 % and 1.99 % respectively (Table 1). HC revealed the presence of carbohydrates, coumarin, tannin and phenolic compounds (Table 2). The total phenolic content of the extract was determined to be 23.90 mg/g tannic acid equivalents. The concentration 6 µg/ml of HC showed maximum scavenging activity of 67.05% with IC_{50} value of 4.27 µg/ml (Table 3). Reducing power of 6 µg/ml concentration of HC was found to be OD 0.122 at 700 nm (Table 4).

Amount of fibre was found to be 0.10 %. Hence calorific value = (4.23 x 4) + (3.35 x 9) + (1.99 x 4) = 16.92 + 30.15 + 7.96 = 55.03 Kcal.

Table 1. Physico-chemical parameters of *Hutabhugadi curna*

Parameter	Results
Bulk Density (gm/ml)	0.455
Tap Density (gm/ml)	0.556
Carr's Index	26.2
Hausner's Ratio	0.818
Protein (%w/w)	4.23
Carbohydrate (%w/w)	1.99
Fat (%w/w)	3.35

Table 2. Phytochemical screening of methanolic extract of *Hutabhugadi curna*

Tests	Color if positive	Methanolic extract	Inference
Alkaloid			
Dragendroff's test	Orange red precipitate	No precipitate	Absent
Wagner's test	Reddish brown precipitate	No precipitate	
Mayer's test	Dull white precipitate	No precipitate	
Hager's test	Green yellowish turbid	No precipitate	
Carbohydrate			
Molisch's test	Violet ring	Violet ring at the bottom	Present
Fehling's test	Brick red precipitate	Brick red precipitate	
Benedict's test	Red precipitate	Reddish brown ppt	
Sterols			
Liebermann-Buchard test	Dark green solution	Greenish yellow colour in the upper layer	Absent
Salkowski test	Bluish red to cherry red	Brown colour in the lower layer	

Saponins			
On shaking with water	Stable froth	Stable froth formed	Present
Tannins			
With $FeCl_3$	Dark blue or green color	Dark blue colour	Present
Flavonoids			
Shinoda's test	Red to pink	Yellow colour	Absent
Phenol			
with $FeCl_3$	Blue to blue black, green	Bluish black colour	Present
Coumarins			
With 2N NaOH	Dark yellow	Yellow colour	Present
Triterpenoids			
Liebermann-Buchard test	Pink	Brown colour	Absent
Tin and thionyl chloride test	Pink	Brown colour	
Resins/ Wax			
With distilled water, acetone	Turbidity	No turbidity	Absent
Quinone			
Dark pink, purple, red	Dark pink, purple, red	Brown	Absent
Amino acids			
Violet colour	Violet colour	Brown	Absent
Carboxylic acid			
Brisk effervescence	Brisk effervescence	Brown	Absent

Table 3. DPPH assay of *Hutabhugadi curna*

Concentration	OD at 517 nm	% Inhibition
Control	0.343 ± 0.004	0
1 µg/ml	0.299 ± 0.001***	12.83
2 µg/ml	0.262 ± 0.005***	23.62
3 µg/ml	0.213 ± 0.002***	37.9
4 µg/ml	0.186 ± 0.0008***	45.77
5 µg/ml	0.134 ± 0.003***	60.93
6 µg/ml	0.113 ± 0.004***	67.05

Value = Mean ± SEM; IC_{50} 4.27 µg/ml ***P<0.001 in comparison to control

Table 4. Reducing power of assay of *Hutabhugadi curna*

Concentration	OD at 700 nm
Control	0.05 ± 0
1 µg/ml	0.060 ± 0.002728^{ns}
2 µg/ml	0.078 ± 0.001667**
3 µg/ml	0.084 ± 0.001453***
4 µg/ml	0.093 ± 0.001667***
5 µg/ml	0.107 ± 0.01048***
6 µg/ml	0.122 ± 0.001202***

Value = Mean ± SEM; OD – optical density ^{ns} *P>0.05* in comparison to control **P<0.01*in comparison to control ***P<0.001* in comparison to control

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.[15] The test formulation HC contains 5 herbal and a mineral ingredient of different chemical nature. Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of energy, and as structural components. Ribose is an important component of coenzymes and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development.[16] Coumarin has clinical medical value as an edema modifier. Coumarin and other benzopyrones, such as 5,6-benzopyrone, 1,2-benzopyrone, diosmin, and others, are known to stimulate macrophages to degrade extracellular albumin, allowing faster resorption of

edematous fluids.[17] Tannins are anticarcinogenic and antimutagenic. Tannins and related compounds inhibit superoxide radicals. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins thus serve as a natural defense mechanism against microbial infections. Tannins can also be used in food processing to increase the shelf-life of certain foods. Tannins have also been reported to exert physiological effects such as acceleration of blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses.[18] Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers.[19]

Antioxidants block the process of oxidation by neutralizing free radicals. It has been suggested that the antioxidant activity of plants might be due to their phenolic compounds.[20] In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effect.[21] In present study antioxidant activity of HC was performed using two *in-vitro* models; DPPH scavenging and reducing power assay. DPPH is a stable nitrogen centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation which is measured at 517 nm. From the result it was found that radical scavenging activity of HC extract increased with increasing concentration.

The presence of antioxidants in the sample would result in the reducing Fe^{3+} to Fe^{2+} by donating an electron resulting in light green colour, which was measured at 700 nm. HC showed increasing reductive power on increase of concentration. Higher the absorbance of the sample, better will be reducing activity.

CONCLUSION

The physical properties, phytochemical composition and antioxidant properties of *Hutabhugadi curna* AFI was tested methodically. Further research in this regard would throw more light on multi-herb Ayurvedic therapeutics.

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CONFLICTS OF INTEREST

Nil

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ABOUT AUTHORS

Mrs. Priyadarshini MSc has worked as Junior Research Fellow in UGC Major Research Project on standardisation of Ayurveda formulations for a period of 3 years. She has authored 5 research papers. Along with drug standardisation she has expertise in evaluation of *in vitro* antioxidant, assay for thyroid hormones by ELISA, biochemical analysis and microbial load analysis.

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GRAPHICAL ABSTRACT

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