



PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF HYDROALCOHOLIC EXTRACT OF ANTI-DIABETIC AYURVEDIC FORMULATION.

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ABSTRACT

The study was aimed to evaluate the phytochemical analysis and antioxidant activity of the hydroalcoholic extract of an anti-diabetic Ayurvedic formulation. The qualitative and quantitative phytochemical parameters were studied using standard methods. The antioxidant activity was assessed by using Inhibition of nitric oxide radical, Inhibition of DPPH, ABTS radical cation decolourisation assays and FRAP assays. The phytochemical analysis result showed the presence of carbohydrates, protein, amino acids, steroids, glycoside, saponins, flavonoids, alkaloids, tannins, phenol and starch. The highest phytochemical constituent was found to be flavonoids followed by glycosides, tannins and phenolic content. The antioxidant assay result showed highest percent of inhibition in DPPH assay. It can be concluded that, hydroalcoholic extract of Ayurvedic formulation possess antioxidant property which may be due to the presence of phenolic content and flavonoids.

KEYWORDS: Anti-diabetic, Ayurvedic formulation, antioxidant, phytochemical analysis, Free radicals etc.

INTRODUCTION

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.^[1] India possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. The well known Indian systems of Medicine, namely, the Ayurveda, Siddha and Unani use predominantly plant based raw materials. Most of these traditional preparations and formulations have been found to be a reservoir of pharmaceuticals.^[2]

With the advancement of Ayurvedic tradition and its scientific exploration, several classes of plant species

have been studied in order to evaluate their therapeutic potentials and to isolate the lead compounds. Medicinal plants are of great importance in providing healthcare to a large portion of the population in India.^[3]

The literature of Ayurveda had mentioned use of *Pterocarpus Marsupium*, *Gymnema sylvestre*, *Holarrhena antidysenterica*, *Tinospora cordifolia*, *Rubia cordifolia* and *Terminalia arjuna* Species for the treatment of diabetes (Astanga Hriday Sutrasthan, 15/20^[4] and Chakradutta, 35/8-10,13^[5]). The Ayurvedic formulation was prepared by using all the above mentioned plants for the treatment of diabetes. Medicinal plants are the oldest existing complete medical system in the world and now green-chemistry move toward has demonstrated to be a skillful and early tool for natural drug development. Natural products are important sources for biologically active drugs.^[6] There has been a growing interest in the study of medicinal plants as natural products in diverse parts of the world.^[7] The study of phytochemical constituent and antioxidant property of the Ayurvedic formulation will help to know the possible role of the formulation in the treatment of diabetes. The study was therefore aimed to evaluate the phytochemical parameters (qualitative and quantitative), and antioxidant effect of hydroalcoholic extract of Ayurvedic formulation.

MATERIAL AND METHODS

Collection and Processing of plant materials

The plant materials were collected from Medicinal Plant Garden of National Research Institute of Basic Ayurvedic Sciences (NRIBAS) Kothrud, Pune and Mahabaleshwar region. The plants were identified by the botanist Mrs. Archana Mhase, NRIBAS, Pune and preserved in herbarium for future reference with voucher specimen No. 4398 (*Rubia cordifolia*), 4399 (*Gymnema sylvestre*), 4400 (*Terminalia arjuna*), 4404 (*Pterocarpus Marsupium*), 4405 (*Holarrhena antidysenterica*) and 4406 (*Tinospora cordifolia*). The plant materials were thoroughly washed under running tap water and shade dried.

Preparation of plant extract

Dried plant materials of all the six medicinal plants were powdered with the help of grinder and Ayurvedic formulation was prepared comprising of equal proportion of each plant. This prepared Ayurvedic formulation powder was used for the preparation of hydroalcoholic extract (ethanol:water; 1:1). The extract obtained was then filtered using Whatmann no. 42 (125mm) filter paper and lyophilised using lyophilizer (Labconco Freezone 4.5) at -50°C and 0.020 mBar pressure for 3-4 days. The lyophilised material was stored in air tight container till further use.

Phytochemical analysis

Qualitative parameters

The hydroalcoholic extract of Ayurvedic formulation (HAAF) was subjected to preliminary phytochemical investigations to check the presence of various phytoconstituents such as Carbohydrates, Flavonoids, Sugars, Amino acids, Tannins, Proteins, Glycosides, Steroids, Saponins, Phenol and Starch as per the standard protocols.^[8,9]

Quantitative parameters

The quantitation of the different phytoconstituents like, Carbohydrates^[10], Flavonoids^[11,12,13], Tannins (Lowry *et al.*, 1951), Glycosides^[14,15], Lipids, Total phenolic content^[16,17] and Proteins^[14], were analysed using standard procedures as described.

In vitro analysis of antioxidant activity

Ferric reducing antioxidant potential (FRAP) assay

The reducing power of plant extracts was analysed according to the method of Oyaizu.^[18] About 200 mg of plant extract in 1ml of distilled water was mixed with phosphate buffer and potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction mixture was incubated and a portion of trichloroacetic acid was added. The upper layer of the solution was mixed with distilled water and $FeCl_3$. Absorbance was measured at 700 nm. Butylated Hydroxy Toluene (BHT) was used as the reference standard.

Inhibition of Nitric oxide radical

This test was carried out by the Griess reaction.^[19,20] The reaction mixture containing sodium nitroprusside in phosphate buffered saline and plant extracts and the reference compound in different concentrations was incubated. After each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent was added. The absorbance was measured at 546 nm. Ascorbic acid served as a positive control for the test.

Inhibition of DPPH radical

The free radical scavenging activity of extract was measured by 1; 1-diphenyl-2-picrylhydrazil (DPPH) with the help of the method of Blois^[21] and Gomez-Alonso *et al.*^[22] 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentration of plant extracts. After 30 min incubation, absorbance was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as the reference standard. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

ABTS radical cation decolourisation assay

In this assay, the oxidant is generated by persulfate oxidation of 2, 2'-azino-bis (3-ethylbenzoline-6-sulfonic acid)-(ABTS²⁻) as described by Re *et al.*^[23] ABTS radical cation (ABTS⁺) are produced by reacting ABTS. To determine the antioxidant activity, different concentration of plant extract was added to 0.3ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was read at 745nm and the percentage inhibition was calculated.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The results of the qualitative phytochemical analysis of the hydroalcoholic extract of Ayurvedic formulation is depicted in Table 1. The results of qualitative analysis shows the presence of carbohydrates, protein, amino acids, steroids, glycoside, saponins, flavonoids, alkaloids, tannins, phenol and starch on the hydroalcoholic extract of Ayurvedic formulation.

Quantitative phytochemical analysis

The quantitative phytochemical analysis of hydroalcoholic extract of Ayurvedic formulation (HAAF) was carried out to know the quantity of the phytochemical constituents in the extract. The results obtained in the study are presented in Table 2 and diagrammatic representation of quantitative phytochemical constituents of hydroalcoholic extract of Ayurvedic formulation (HAAF) in Figure 1.

The results indicate that the extract contains the highest concentration of flavonoids followed by glycosides, carbohydrates, tannins, proteins, total phenolic content and lipids.

In vitro antioxidant potential assessment using biochemical assays

The antioxidant potential was assessed using biochemical assays to evaluate the free radical scavenging activity of hydroalcoholic extract of Ayurvedic formulation. The standard plots were generated for Ferric reducing antioxidant potential (FRAP), Inhibition of nitric oxide radical, Inhibition of DPPH and ABTS radical cation decolourisation assays using suggested standard compounds and were used for calculating the IC₅₀ values of the extract. The IC₅₀ values

of the extract for Inhibition of nitric oxide radical, Inhibition of DPPH and ABTS radical cation decolourisation assays are illustrated in the Table 3 and FRAP assay values in the Table 4.

The highest percent inhibition of free radical for the extract was observed in DPPH assay followed by ABTS radical cation decolourisation assay and Inhibition of nitric oxide radical assay. For FRAP assay 200 µg of BHT equivalent per 1000 µg of the hydroalcoholic extract of Ayurvedic formulation value was obtained.

Table 1: Qualitative tests of hydroalcoholic extract of Ayurvedic formulation (HAAF).

Sr. No.	Test	Test Method	Presence/ Absent
1	Carbohydrates	Molish's Test	+
2	Reducing sugar	Fehling's Test	-
3	Pentose sugar	Phloroglucinol test	-
4	Hexose sugar	Cobalt Chloride Test	+
5	Protein	Biuret Test	+
6	Amino acid	Ninhydrin Test	+
		Test for cysteine	+
7	Steroids	Libermann-Burchard Test	+
8	Glycoside	General test	+
9	Cardiac Glycosides	Legal test	+
		Keller-Killiani test	+
10	Anthroquinone glycosides	Borntrager's test	-
11	Coumarin glycosides	No aromatic order	+
		No green fluorescence	-
12	Saponins	Foam test	+
		Lead acetate test	+
13	Flavonoids	Shinoda test	+
		Lead acetate test	+
		Alkaline reagent test	+
14	Alkaloids	Mayer's test	+
		Wagner's test	+
15	Tannins	Lead acetate test	+
16	Phenol	FeCl ₃ test	+
		Folin-ciocaltauereagent	+
17	Starch	Iodine test	+

(+): Present; (-): Not present.

Table 2: Quantitative analysis of Ayurvedic formulation (HAAF).

Sr.No.	Phytochemical	Concentration (µg/ml)
1	Carbohydrates	179.70
2	Flavonoids	262.20
3	Tannins	071.24
4	Glycosides	200.00
5	Proteins	051.14
6	Lipids	013.65
7	Total Phenolic Content	039.28

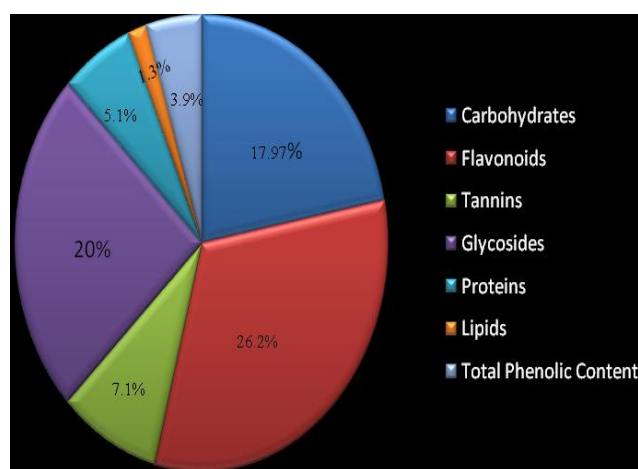


Figure 1: Diagrammatic representation of quantitative phytochemical constituents of hydroalcoholic extract of Ayurvedic formulation (HAAF).

Table 3: Antioxidant analysis of Ayurvedic formulation (HAAF) using DPPH, NO Radical scavenging, ABTS assay.

S.No.	Assay/ Method	IC ₅₀ (µg/ml)	% inhibition
1.	DPPH	543	92.17
2.	NO Radical scavenging	793	63.69
3.	ABTS	561	89.46

Table 4: Antioxidant analysis of Ayurvedic formulation (HAAF) using FRAP assay.

S.No.	Assay/ Method	Concentration of extract (µg/ml)	µg of BHT/ 1000µg of extract
1.	FRAP	1000	200

The study results indicate the presence of steroids, glycoside, saponins, flavonoids, alkaloids, tannins and phenol in the extract of anti-diabetic Ayurvedic formulation.

The Plant extracts have been used for a wide variety of purposes for many thousands of years. The therapeutic properties of plants are attributed due to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins. These natural compounds physiologically affect the body of human beings, interacting with the pathogens and interrupting their growth at different stages of development thus making the body disease free.^[24]

Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases^[25], and have possible benefits to the humanity. Phenolics are commonly found in medicinal plants and have been reported to have multiple biological effects, including antioxidant activity. The Indian system of Medicine owes its origin.^[26]

Free radicals are chemical entities that can exist separately with one or more unpaired electron. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation etc.^[27]

A substance may act as antioxidant due to its ability to reduce reactive oxygen species by donating hydrogenion.^[28,29] The reducing property of hydroalcoholic extract of Ayurvedic formulation implies that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity as the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor.^[30,31]

The results of *in vitro* antioxidant assays indicate that the hydroalcoholic extract of Ayurvedic formulation (HAAF) possesses good antioxidant potential or activity.

CONCLUSION

It may be concluded that the hydroalcoholic extract of Ayurvedic formulation used for the treatment of diabetes possesses antioxidant property which may be attributed to the antioxidant principles that are present in the plant. Further, studies are needed not only to confirm but also to isolate and characterize the active principle responsible for the activity.

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REFERENCE

1. Farnsworth NR, Soejarto DD. Global importance of medicinal plants. In: Akerele, O., Heywood, V., Syngé, H. (Eds.) "The Conservation of Medicinal Plants", Cambridge University Press, Cambridge, 1991; 25-51.
2. Arora S, Kaur K, Kaur S. Indian medicinal plants as a reservoir of protective phytochemicals. *Teratogenesis Carcinogenesis Mutagenesis*, 2003; 1: 295-300.
3. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. Review: The Ayurvedic Medicine *Clitoria ternatea*-From traditional use to scientific assessment. *J Ethnopharmacol.*, 2008; 120: 291-301.
4. Ashtanga Hridaya of Vagbhat Translated by Prof. K.R.Srikantha Murthy, Fourth Edition, 2000, Krishnadas Academy, Varanasi, India.
5. Chakradatta. A Treatise on Principles and practices of Ayurvedic Medicine, Text in English by P.V.Sharma, 1st Edition, 1994, Chaukhambha Vishwabharti, Oriental Publisher and Distributors, Varanashi, India.
6. Saleh AI, Al-Dosari M, Abdul MS, Alsheikh M, Abdel-kader MS. Evaluation of the hepatoprotective effect of *Fumaria parviflora* and *Momordica alsamina* from Saudi folk medicine against experimentally induced liver injury in rats. *Res. J. Med. Plants*, 2009; 3(1): 9-15.
7. Gazzaneo Ir, De lucena PRF, Paulino de Albu QU. *In vitro* antioxidant activities of *Ocimum* species: *Ocimum basilicum* and *Ocimum sanctum*. *J. Ethnobiol. Ethnomed.*, 2005; 1: 9.
8. Sadasivam S, Manickam A. Biochemical methods. New age International (P) Limited, Publishers, New Delhi, 1996.
9. Kokate CK. Practical Pharmacognosy. 4th ed. Reprint. Delhi: Vallabh Prakashan, 1996; 10-321.
10. Khandelwal KR. Practical Pharmacognosy Technique and Experimental. Pune: Nirali Prakashan, 2001; 149-156.

11. Peach K, Tracey MV. Modern methods of plant analysis. 1956; 3: Springer Verlag, Berlin.
12. Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. International Journal of ChemTech Research, 2010; 2(3): 1698-1701.
13. Trease GE, Evans WC. Pharmacognosy. 17th edn; Bahive Tinal, London, 1985; 149.
14. Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. Protein measurement with the Folin Phenol Reagent, J. Biol. Chem, 1951; 193: 265-275.
15. Trease GE, Evans WC. Pharmacognosy. 11th edn; Brailliar Tiridel Can. Macmillian Publishers, 1989.
16. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1, 1974, McGill Queen's University Press Montreal and London.
17. Mallick CP, Singh MB. Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi, 1980; 286.
18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 1986; 44: 307-15.
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR. Analysis of nitrate, nitrite and 15N in biological fluids. Anal Biochem, 1982; 126: 131-136.
20. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging property of *Ginkgo biloba* extract EGb 761. Biochem Biophys Res Commun, 1994; 201: 748-755.
21. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, 1958; 181: 1199-1200.
22. Gomez-Alonso S, Fregapane G, Salvador MD, Gordon MH. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem, 2003; 51: 667-72.
23. Re R, Pellegrini N, Protoggenete A, Pannala , Yang M, Rice-Evans C. Antioxidnat activity applying an improved ABTS radical cation decoloration assay. Free Radic Biol Med, 1999; 26: 1231-7.
24. Mittal J, Sharma MM, Batra A. *Tinospora cordifolia*: a multipurpose medicinal plant- A review. Journal of Medicinal Plants studies, 2014; 2(2): 32-47.
25. Lukmanul HF, Girija A, Boopathy R. Antioxidant property of selected Ocimum species and their secondary metabolite content. J. Med. Plants Res. 2(9): 2008; 250-257.
26. Mondal SK, Goutham Chakraborty, Gupta M, Mazumber UK. *In vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. Ind J Exp Bio, 2006; 44: 39-40.
27. Shatvalekar S. AtharvaVeda Samhita. 1st ed. Balsad, Maharashtra, India: Suyadhyay Mandal Kila Pardee publication; 1943; 25.
28. Khanam S, Shivprasad HN, Kshama D. *In vitro* antioxidant screening models: a review. Indian J Pharm Educ, 2004; 38: 180.
29. Jayaprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extract on peroxidation models *in vitro*. J Agric Food Chem, 2001; 55: 1018.
30. Duh PD, Tu YY, Yen GC. Antioxidant activity of aqueous extract of harn jyr (*Chrysanthemum morifolium* Ramat). Lebensmittel- Wissenschaft and Technologie, 1999; 32: 269.
31. Dreosti IE. Antioxidant polyphenols in tea, coca and wine. Nutrition, 2000; 16: 692.