ISSN 2229-3566
Research Article

IJRAP
www.ijrap.net

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF AN AYURVEDIC FORMULATION: BALARISHTAM

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Received on: 10/10/11 Revised on: 06/11/11 Accepted on: 20/11/11

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ABSTRACT
Balarishtam is a popularly and effectively used traditional ayurvedic formulation for disorders of connective tissues like joints, bones and cartilage .Clinically it is observed that it has profound activity in inflammatory and degenerative disorders of connective tissues like rheumatoid arthritis, other types of infective and inflammatory arthritis, osteoarthritis, spondylosis ,and in many painful syndromes of soft connective tissues .The present study on physicochemical parameters, evaluates the ph, specific gravity, total solids and alcohol % of balaristam .The preliminary qualitative evaluation of phytochemicals show the presence of Carbohydrates, Phenols, Flavonoids, Tannins, Terpenoids, Anthraquinone and Fixed Oil The quantitative estimation of total reducing sugar, total non-reducing sugar, total tannins and phenols are also performed .The antioxidant property of the product is also evaluated by DPPH method, and the product shows antioxidant property also.
KEY WORDS: Balarishtam, antioxidant, physicochemical, phytochemical, connective tissue disorders

INTRODUCTION

Arishtas are aqua-alcoholic preparations, described in Ayurveda. Balarishtam is described in the classical text Bhaishajjyaratnavali in the context of vathavyadhi (connective tissue disorders). It is used to alleviate, neuromuscular and the connective tissue disorders. It bestows strength, nourishment and digestive power to the body. It is a combination of *Withania somnifera, Sida rhombifolia, Woodfordia fruticosa, Holostemma ada-kodein, Ricinus communis, Alpinia calcarata, Syzygium aromaticum, Vetiveria zizanioides, Tribulus terrestris* and *Merremia tridentata*. The processing involves fermentation of the combination with jaggery for a period of one month[1,2]. The study aims a preliminary phytochemical screening and an evaluation of the antioxidant property of Balarishtam.

MATERIALS AND METHODS

Samples of Balarishtam were collected from production line at The Arya Vaidya Pharmacy (Coimbatore) Factory, Kanjikode. Chemicals used are AR-grade sourced from Merck and Nice Chemicals.

Physico And Phytochemical Analysis

The physicochemical parameters such as specific gravity pH, total solids, acidity and alcohol content are determined as per method described in Indian Pharmacopoeia[3]. The procedure followed for preliminary phytochemical analysis[4, 5, 6] is described here.

Test For Carbohydrates

Fehling's Test**:** To 1.0ml of sample, equal quantities of Fehling's solution A and B are added and heated. The formation of brick red precipitate indicates presence of carbohydrate.

Molisch's Test**:** To 2.0ml of extract, 1.0ml of Molisch's reagent is added. Concentrated H_2SO_4 is added through the sides of the test-tube. Purple / reddish-violet colour at the junction of the two liquids indicates presence of carbohydrates.

Test For Phenols

Phosphomolybdic acid Test: Few drops of extract are mixed with Phosphomolybdic acid. Formation of blue colour indicates presence of phenols.

Test For Flavonoids

Lead acetate Test: To 5.0ml of sample, 1.0ml of lead acetate solution is added. Flocculent white precipitate shows presence of flavonoids.

The sample is treated with Sodium hydroxide. Formation of yellow colour indicates presence of flavonoids.

Test For Tannins

Braemer's Test: To 2-3 ml of sample, alcoholic Ferric Chloride solution is added. Dark blue or greenish black colouration shows presence of tannins.

Potassium Dichromate Test: To a few drops of sample, strong Potassium dichromate solution is added. Yellow colour precipitate indicates presence of tannins and phenolic compounds.

Test For Steroids / Terpenoids

Liebermann-Burchard Test: To 1.0ml of sample, 1.0ml of chloroform, 2-3ml acetic anhydride and 1-2drops of conc. HCl are added. Dark green colouration indicates presence of steroids while pink/red colouration indicates presence of terpenoids.

Test For Alkaloids

Dragendorff's Test: To 1.0ml of solution, 1.0ml of Dragendorff's reagent is added. An orange-red precipitate indicates presence of alkaloids.

Mayer's Test: To 1.0ml of sample, 1.0ml of Meyer's reagent is added. Whitish –yellow / cream precipitate indicates presence of alkaloids.

Test For Anthraquinone

Borntrager's Test: To a few drops of sample, a few ml of 10% Ferric Chloride solution and 1.0ml HCl are added. It is cooled, filtered and then the filtrate is shaken with diethyl ether. The ether extract is further treated with strong ammonia. Pink/deep-red colouration of aqueous layer indicates presence of Anthraquinone glycosides.

Quantitative Determination Of Total Phenolic Content

The total phenolic content is determined using Folin-ciocalteu reagent (Singleton et al, 1965). Appropriately diluted standard and samples are made up to 3.5ml with distilled water in a series of test tubes. These solutions are then treated with 0.5ml 2N Folin-ciocalteus reagent and incubated for 3 minutes at room temperature. The reaction is neutralized by addition of 1ml 20% sodium carbonate. The reaction mixture is further incubated at room temperature for 90 minutes and the absorbance read at 760nm using Shimadzu 1800 UV-Vis spectrophotometer. The results are expressed as Gallic acid equivalent in percentage of sample, using a standard curve generated with Gallic acid[7].

Quantitative Determination Of Carbohydrates

Carbohydrate is estimated by titration method using Fehling's solutions. Equal amount of Fehling's solution A & Fehling's solution B was titrated against prepared standard Arishtam solution

using methyline blue indicator. Total reducing sugar before and after inversion are estimated and total non- reducing sugar determined[3].

Quantitative Determination Of Tannin

The tannin content in arishtam is estimated by Folin-Denis Method. Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins present. The intensity is measured using UV-Vis spectrophotometer at700nm. The standard tannic acid is prepared by dissolving 100 mg of tannic acid in 100 ml of distilled water. To100µl of appropriately diluted arishtam 0.5ml Folin-Dennis reagent 1ml sodium carbonate solution are added and diluted to 1ml. It was incubated for 30 minutes. The absorbance is read using Shimadzu UV-Vis spectrophotometer (1800). The concentration of tannin is calculated from the standard graph and is expressed in mg/g of Tannic acid equivalents[7].

Determination Of Antioxidant Activity Using DPPH

Anti-oxidant activity of Balarishtam is estimated by DPPH (2'2-diphenyl-1-picryl hydrazine) radical scavenging activity (Blois; 1985). To various concentrations of the sample, methanolic solution containing DPPH radicals (0.1mM) is added and shaken vigorously. The reaction mixture is then left to stand for 30 minutes in dark. After the incubation period, absorbance is measured at 517nm against corresponding test blanks. The percentage inhibition of DPPH free radical is calculated using the formula,

% Inhibition = (Control – Sample) x 100 / Control

The sample concentration providing 50% inhibition (EC_{50}) was calculated from the graph of RSA percentage against sample concentration. Gallic acid is used as standard[8].

RESULTS AND DISCUSSION

Balarishtam commercially prepared in Arya Vaidya Pharmacy (Coimbatore) Ltd is taken for the study. The physicochemical parameters like alcohol%, acidity, total solids, specific gravity and ph were tested. The results are as shown in the table 2. Since the alcohol content in the aristam acts as a preservative the product need not be preserved.

The result of preliminary phytochemical screening is detailed in table 3. The result shows the presence of carbohydrates, phenols, flavonoids, tannins, terpenoids, anthraquinone.

The flavanoids[9], Terpenoids[10] and tannins[11] are potential anti inflammatory agents that help in most of the inflammatory connective tissue disorders; probably the analgesic anti inflammatory activity of balaristam is addressed, by these activities of the secondary metabolites in the aristam[11].

The antioxidant properties of phenols especially flavanoids and tannins[12], helps to relieve the oxidative stress induced by free radical scavenging and provides nourishment and wellness to the body.

The quantification of carbohydrates, total phenols and tannins are also done, the results are as shown in the table 4.

The antioxidant defense system is compromised in inflammatory conditions like rheumatoid arthritis. There is a shift in the oxidant/antioxidant balance in favor of lipid peroxidation[13] which could lead to the tissue damage observed in such diseases. Therefore the activity of antioxidants in such inflammatory conditions is very much helpful to prevent bone and tissue damage and there by reduce the pain and inflammation associated with such conditions.

The antioxidant property of balaristam is also studied using the DPPH method and is detailed in table 5 and fig.1. The assay yielded EC_{50} at 56.86 µg/ml of the sample.The analgesic and anti-inflammatory properties shown by balaristam in the treatment of vatavyadhis can also be attributed to its antioxidant property. The strong and effective secondary metabolites along with the antioxidant property of balaristam help in effective management of pain in vatavyadhi and also improve the general health and wellness.

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TABLE.1 SHOWING INGREDIENTS OF BALARISHTAM

S/N	Ingredients
1	*Sida rhombifolia*
2	*Withania somnifera*
3	*Woodfordia fruticosa*
4	Jaggery
5	*Holostemma ada-kodien*
6	*Ricinus communis*
7	*Alpinia calcarata*
8	*Syzygium aromaticum*
9	*Vetiveria zizanioides*
10	*Tribulus terrestris*
11	*Merremia tridentata*

TABLE.2 SHOWING PHYSICO-CHEMICAL PARAMETERS

S/N	Parameter	Observed value
1	Specific gravity	1.0650
2	pH	3.26
3	Total Solid	23.23%
4	Acidity	0.65
5	Alcohol	5.91%

TABLE-3 SHOWING RESULT FOR PRELIMINARY PHYTO-CHEMICAL ANALYSIS

S/N	Phyto constituent	Observation
1	Carbohydrate	Present
2	Phenols	Present
3	Flavonoids	Present
4	Tannins	Present
5	Steroids	Absent
6	Terpenoids	Present
7	Alkaloids	Absent
8	Anthraquinone	Present
9	Fixed Oil	Present

TABLE-4 QUANTITATIVE ESTIMATION OF MAJOR PHYTO-CHEMICALS

S/N	Phyto-chemical	Observed value
1	Tannin	3.96mg/g
2	Total reducing sugar	17.41%
3	Total non-reducing sugar	0.22%
4	Total phenolic content	9.46%

TABLE-5 SHOWING ANTI-OXIDANT ACTIVITY BY DPPH ASSAY

S/N	Concentration (µg/ml)	Inhibition (%)
1	21.14	22.857
2	42.28	41.428
3	63.42	56.426
4	84.56	72.142
5	105.7	80

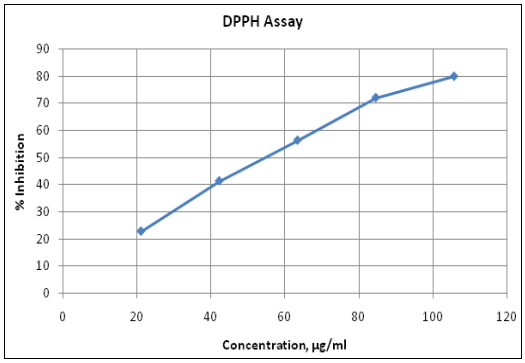

FIG.1 SHOWING DPPH ASSAY ON BALARISHTAM

Source of support: Nil, Conflict of interest: None Declared