ANTIOXIDANTS, TOTAL PHENOLIC, FLAVONOID AND PHYTOCHEMICAL SCREENING OF TRADITIONAL HERBAL PRODUCT

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Abstract: The objectives of the present study is to determine Antioxidant activities, total phenolic and flavonoid contents as well as the phytochemical screening of traditionally used Herbal Medicine 'Arjunarishta'. Total phenolic and flavonoids contents were determined spectrophotometrically five times at the interval of four months over the period of two years using gallic acid and quercitin as standards. Antioxidants activity was evaluated in vitro by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The antioxidant activity was also determined five times at the interval of four months over the period of two years, to know is there any changes in the antioxidant activity as well as total phenolic and flavonoid contents as the time passes. For antioxidant activity the samples were prepared in distilled water of five different concentrations 5%, 2.5%, 1.25%, 0.625%, 0.3125%, and 0.15625%. It shows highest antioxidant property at higher concentration and least antioxidant property at lowest concentration. Also the antioxidant activity changes by the time intervals.

Key Words: Antioxidant activity, Phenolic, flavonoid, DPPH

Introduction

Free radicals are continuously generated in the human body as an effect of oxidative metabolism and thus results the incidence of severe illness such as coronary heart disease, cancer, neurodegenerative ailments, diabetes mellitus, autoimmune disease and aging[1,2,3]. Antioxidants are substance which can protect human body from free radicals and reactive oxygen species (ROS) induced cronic diseases [4]. The interrelationship between society and nature, and the importance of herbal medicine to human health has recently become widely acknowledged, which has drawn attention to the fact that loss of biodiversity, destruction or unscientific use of medicinal plants can have direct and indirect effect on the well being of humans. Human health cannot consider alone, as it is extremely dependent on the quality of the environment in which we live; for people to be healthy, they needs healthy environment and a proper medical care system that provides eco-friendly, bio-friendly, cost effective and relatively safe treatments [5,6,7].

In 21st century herbal medicines are gaining importance in mainstream healthcare as greater number of people seeks relatively safe remedies and approaches to healthcare. The demand of herbal medicines, herbal health products, herbal pharmaceuticals, nutraceutical, food supplements and herbal cosmetics etc. is increasing globally due to the growing recognition of these products as mainly non toxic, having in the main less side effects, better compatibility with physiological flora, and availability at affordable prices [8,9].

Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. Standardization of herbal medicines is essential in order to assess of quality drugs, based on the concentration of their active principles, antioxidants, total phenolic and total flavonoids, phytochemicals. The quality assessment of herbal medicines is of paramount importance in order to justify their acceptability in modern system of medicine [10].

For the study we have selected *Arjunarishta,* a herbal medicine. Each 10 ml of this medicine contains *Arjuna Tvak*- 3.066 mg, *Draksa*- 1.533 mg, *Madhuka, Dhataki*- each 613.888 mg, *Guda*- 3.066 mg. And it is prescribed for the treatment of increased palpitation, excessive perspiration, dryness of mouth, increased pulse rate etc.

This herbal medicine have been analyzed for the Antioxidant activity by DPPH assay [11], total phenolic [12] and total flavonoids [13] and phytochemical screening have been done.

Materials and Methods:

The sample were collected from the local herbal medicine store.

DPPH Assay:

Scavenging of DPPH free radicals is the basis of a common antioxidant assay. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is a free radical which has an unpaired valence electron at one atom of its nitrogen bridge.DPPH is a stable free radical in methanolic solution. In its oxidized form, DPPH radical has an absorbance maximum centered on 517nm. The antioxidant activity is calculated by measuring the reduced intensity of purple DPPH in the presence of test compound. The DPPH method as simple, rapid and convenient for screening many samples for free radical scavenging activity.

The investigational samples were prepared in distilled water. DPPH reagent were prepared at a concentration of 0.1mM in 100% Methanol. After Repeated pilot experiments the mentioned concentration range was finalized for the study.

Total Phenolic content:

The extracts were prepared 1mg/ml in Methanol. 0.2 ml of this was added to 0.8 mL of Folin-Ciocalteu reagent. 2.0mL of 7.5 % Na_2CO_3 was added along with 7 ml of distilled water. Gallic acid (0.05-0.3 mg/ml) was used as standard for deriving a calibration curve. All the tubes were incubated in dark for 2 hours. Absorbance was read at 765 nm on UV-Vis spectrophotometer. The calibration curve was plotted with absorbance values of Gallic acid dilutions on y- axis and concentration on X- axis. Regression line equation was obtained as (y=2.768x + 0.7138). The absorbance of sample was substituted in the equation to give concentration equal to Gallic acid from graph (C).

Total flavonoids Content:

Total flavonoid content was estimated by Aluminium Chloride colorimetric method. To 1.5 mL of $AlCL_3$ 500 µL of extract was added (1mg/mL). Blank was prepared by adding 500 µL of distilled water instead of extract. Quercitin was used as a standard (20-100 µg/mL). All the tubes were incubated at RT for 60 min and absorbance was read at 420 nm. The total flavonoid content expressed as quercetin equivalent (QE) was calculated based on the calibration curve, using y = 0.018x -0.094 b, where x is the absorbance and y is the concentration (mg QE) of the methanolic quercetin solutions.

Phytochemicals:

The given herbal medicine were tested for the following phytochemicals [14]. All the extracts were used directly (as such) for each of the following tests.

Test for flavonoids: To 2 mg of the extracts few drops of NaOH was added to give intense yellow colour, which further decolorizes on addition of few drops of concentrated HCl, confirms the presence of flavonoids.

Test for saponins (Foam Test): To 2mg of the extracts, 5mL of distilled water was added and shaken for the formation of froth which confirms the presence of saponins.

Test for cardiac glycosides: 2mg of extracts was treated with 2 mL of glacial acetic acid containing a drop of FeCl3 solution. This was under layered with 1 mL of concentrated H2SO4. A brown ring obtained at the interface indicates the presence of de-oxy sugar characteristics of cardenolide.

Test for terpenoids: 2 mg of extracts was treated with 2 mL of chloroform and concentrated H2SO4 was carefully added to form a layer. A reddish brown colour formation at the interface confirms the presence of terpenoids.

Test for tannins: 2mg of extracts was boiled in 2mL of water for 5-10 minutes and filtered. Ferric chloride (0.1%) was added to this and a brownish green or blue black coloration formed confirms the presence of tannins.

Test for phenols (Ferric Chloride Test): To 10mg of the extracts add 3-4 drops of FeCl3 was added and was checked for the appearance of bluish black precipitate.

Results and Discussion:

Antioxidant activity by DPPH:

The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. The method is widely used due to relatively short time required for the analysis. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical is very stable, reacts with compounds that can donate hydrogen atoms, and has a UV–Vis's absorption maximum at 517 nm. The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical.The mean antioxidant activity of extracts of Arjunarishta is presented in Table-1.

Table 1: DPPH assay showing mean antioxidant activity of extracts of *Arjunarishta*, the herbal medicine.

Concentration %	Initial analysis	4 month analysis	8 month Analysis	12 month analysis	16 month analysis
5	**100.268**	**94.23268**	**93.175**	**89.411**	**80.583**
2.5	**98.465**	**90.68611**	**80.94**	**81.243**	**78.376**
1.25	**83.163**	**75.10772**	**71.13**	**70.041**	**64.784**
0.625	**53.216**	**56.57938**	**58.19**	**55.863**	**50.441**
0.3125	**31.067**	**39.14485**	**37.73**	**36.581**	**34.289**

As the percentage concentration decreases the antioxidant activity also goes on decreasing. The herbal medicine *Arjunarishta* shows consistent DPPH activity over the period of two years.

Total phenolic and total flavonoids content:

Table- 2: Total Phenolic and Total Flavonoids content in herbal medicine *Arjunarishta*.

	Initial analysis	4 month analysis	8 month analysis	12-month analysis	16 month Analysis
Total phenolic content %	**35.22**	**34.26**	**34.09**	**33.69**	**31.05**
Total flavonoids content %	**25.52**	**24.56**	**24.02**	**23.21**	**21.23**

The percentage of total phenolic content and total flavonoids content shows that an antioxidant activity is mainly due to presence of phenolic and flavonoids compounds.

Phytochemical: Sample was screen for phytochemical content qualitatively as per the procedure prescribed in materials and methods and results are presented in Table-3

Table-3: Phytochemical present in the herbal medicine *Arjunarishta.*

Name of the phytochemicals	+ = present / - = absent
Saponins	+
Flavonoids	+
Phenols	+
Terpenoids	+
Cardiac Glycosides	+
Tanins	+

The phytochemical screening showed the presence of above phytochemicals.

Conclusion:

From this study it is observed that the herbal medicine Arjunarishta is a source of natural antioxidants with free radical scavenging potential. The phenolic content and the flavonoids content present in the compound may play a significant role in this free radical scavenging capacity. The systematic study carried out for over the period of time shows that the composition of phytochemical does not change which helps to maintain the original property of herbal medicine *Arjunarishta*.

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