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DETERMINATION OF TOTAL FLAVONOID CONTENT AND HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *ARJUNARISHTA*

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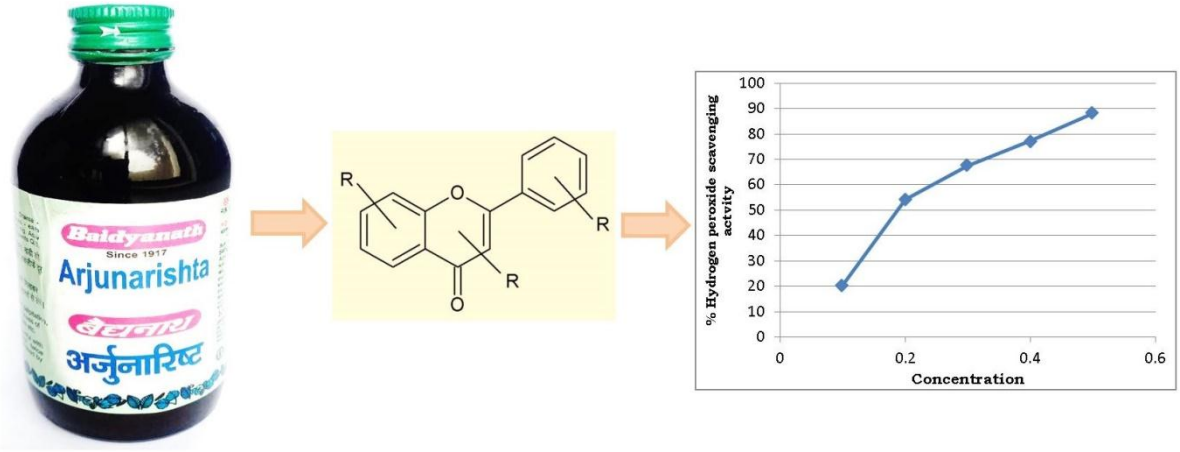
ABSTRACT

Flavonoids are secondary metabolites with C6-C3-C6 carbon skeleton exhibiting in phenyl benzopyran structure. Due to involvement of ethanol, *Arjunarishta* contains different flavonoids. These flavonoids were measured by colorimetric method at 510 nm and then antioxidant activity was determined by hydrogen peroxide scavenging spectroscopic method at 230 nm. Total flavonoid content of *Arjunarishta* was found to be 44.54 Rutin equivalents. It was found that, *Arjunarishta* was found antioxidant by hydrogen peroxide scavenging in dose-dependent manner.

Graphical abstract



INTRODUCTION

Arjuna is medicinal plant indigenous to India, botanical source is *Terminalia arjuna*, belonging to family Combretaceae. Its bark is used as a cardiotonic and cardioprotective in anginaand poor coronary circulation; asa diuretic in cirrhosis of liver and for symptomatic relief in hypertension;externally in skin diseases, herpesand leukoderma (Khare CP). It was reported to contain phytochemicals of various classes like tannins, polyphenols, flavonoids, triterpenoids, sterols and saponins. Flavonoids so far reported to be present in *Arjuna* bark are Arjunone, Luteolin, Baicalein, Kempferol, Oligomeric proanthocyanidins, Pelargonidin and Quercetin (Amalraj and Gopi, 2017).

The present aim was aimed towards quantification of total flavonoid contents and determination of hydrogen peroxide scavenging activity of *Arjunarishta.*

MATERIALS AND METHODS

Procurement of chemicals

Entire research work was undertaken on marketed formulation *Arjunarishta* manufactured by Baidyanath Ltd, Patna, India. Rutin was purchased from Yugo Labs, Mumbai while hydrogen peroxide was brought from Arihant Traders.

Detection of flavonoids

Presence of flavonoids in marketed formulation *Arjunarishta* was detected by Shinoda test, wherein sample was treated with magnesium turnings and con. hydrochloric acid.

Determination of total flavonoid content

Total flavonoids content of *Arjunarishta* was determined as per method explained by Chang et al. 2002 with slight modifications. Dilutions of rutin (1 -5 mg/ml) were prepared and mixed with 0.3 ml of 0.5 Msodium nitrite,

0.3 ml of aluminium chloride and 2 ml of 1 M sodiumhydroxide. Total volume was made up to 10 ml with distilled water andabsorbance of each dilution was noted on digital colorimeter (Elico) at wavelength 510 nm.A graph of concentration of rutin (mg/ml) was plotted against their absorbances noted at 510 nm; and best fit equation was determined on MS Excel.

About 1 ml of marketed formulation was diluted to 10 ml with water. Fromthis solution, 1 ml was added with 0.3 ml of 0.5 M sodium nitrite, 0.3 ml of aluminium chloride and 2 ml of 1 M sodium hydroxide. Total volume wasmade up to 10 ml with distilled water and absorbance of sample was noted on digital colorimeter (Elico) at wavelength 510 nm.

Determination of hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of *Arjunarishta* was determined as permethod explained by Kim. 2013 with slight modifications. To get 40 mM, about 0.1 ml of hydrogen peroxide was added to prepare 100 ml with distilledwater.Absorbance of blank solution i.e. 40 mM hydrogen peroxide was noted againstphosphate buffer.
Each of different dilutions of *Arjunarishta* was added with 0.6 ml of 40 mM hydrogen peroxide and total volume was made to 10 ml with phosphate buffer(pH 7.4). Absorbance of each dilution was noted after 10 mins on Jasco V-630spectrophotometer at 230 nm. Then, percent hydrogen peroxide scavengingactivity was determined using formula,

$$Scavenging\ activity\ (\%) = \frac{A230\ of\ control - A230\ of\ sample}{A230\ of\ control} \times 100$$

where, A230 are the absorbances of control and sample at 230 nm.

RESULTS AND DISCUSSION

After procurement of all required chemicals, Shinoda test was performed on marketed formulation, *Arjunarishta*. Dark pink colour of sample treated with magnesium turning and hydrochloric acid indicated the presence of flavonoids in marketed formulation.

Considering the significance of flavonoids, it was rational to determine flavonoid contents of given marketed formulation of *Arjunarishta*. Total flavonoid contents of *Arjunarishta* were found to be –Rutin equivalents. The results were presented in Fig.2.

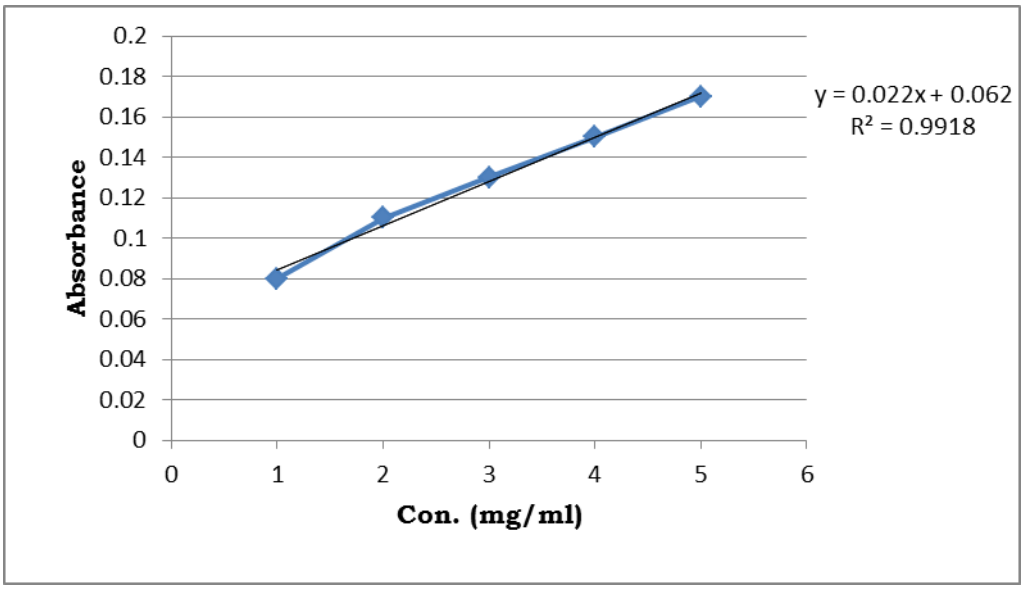


Fig. 2: Plot of concentration of rutin absorbance at 510 nm. It was used as calibration curve as it followed Beer-Lambert's law.

Rutin solutions of different concentrations obeyed the Beer and Lambert's Law and showed increase in absorbance as increase in concentration at 510 nm. Absorbance of diluted marketed formulation was 0.16. Hence, using best fit equation obtained for rutin, total flavonoid content of marketed formulation *Arjunarishta* was found to be 44.54 Rutin Equivalents per g dry weight.

The protective effects of flavonoids in biological systems are attributed to theirability to transfer free radical electrons, chelate metal catalysts (Ferrali et al.,1997), activate antioxidant enzymes (Elliott et al., 1992), reduce alpha-tocopherolradicals (Hirano et al., 2001), and inhibit oxidases (Cos et al., 1998).

Antioxidant activity of *Arjunarishta* was determined as its hydrogen peroxide scavenging ability. It was observed that, at 230 nm, absorbance of hydrogen peroxide treated with *Arjunarishta* decreases in dose-dependent manner. Higher the antioxidant content in

sample, lesser was the absorbance of hydrogen peroxide at 230 nm (Fig. 3).

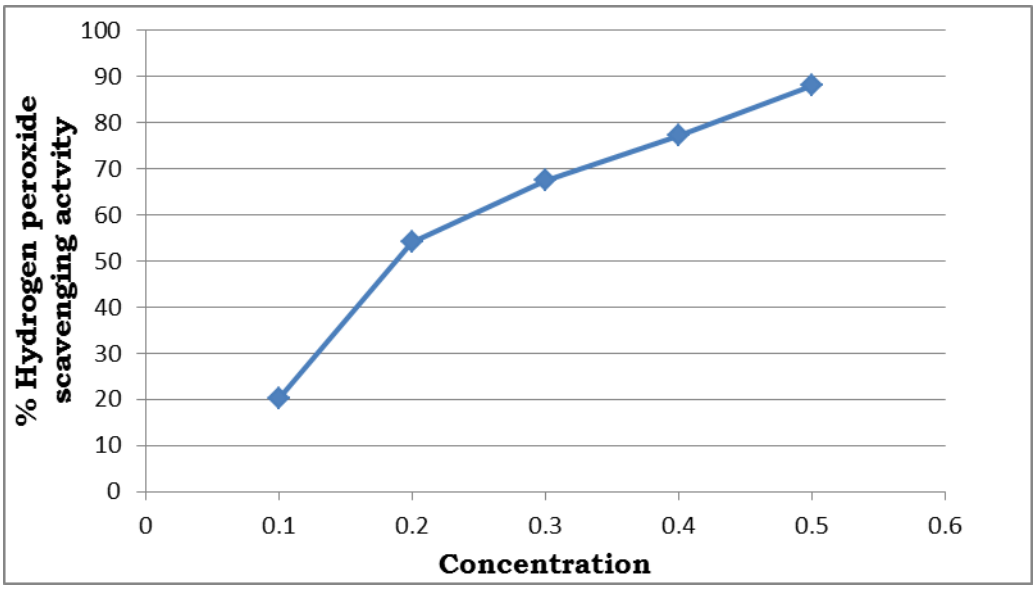


Fig. 3: Plot of concentration of formulation versus % hydrogen peroxide scavenging activity. It was found dose-dependent with amount of *Arjunarishta* added to fixed quantity of hydrogen peroxide.

As *Arishta* formulation contains ethanol, it solubilises almost all flavonoids.

Ciumarnean et al. 2020 concluded that flavonoids have an important involvement in preventing cardiovascular disease, mainly due to their antiatherogenic, antithrombotic, and antioxidant properties.

CONCLUSION

Flavonoids are very important secondary metabolites and exhibits numerous pharmacological activities. In Arjunarishta, there are different flavonoids which due to antioxidant ability, are cardioprotective in action.

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