ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2020.SP1.184

Research Article

In vitro antioxidant activity, total phenolic and flavonoid contents of aqueous and alcoholic extract of bilwadi agad

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Received: 15.04.20, Revised: 12.05.20, Accepted: 10.06.20

ABSTRACT

Introduction: The present study was undertaken to explore the antioxidant properties of Bilwadi agad. Bilwadi agad is one such formulation mentioned in classics which is the first drug of choice in acute toxicity conditions. The ingredients present in bilwadi agad are also known for their rasayana karma. The primary objective of this study was to evaluate the antioxidant effect of Bilwadi agad.

Methods: To fulfil the aim of the study two extract i.e aqueous and alcoholic extract of bilwadi agad were prepared and analysed in laboratory using different concentrations (5%, 10%, 15%) of extract. Aqueous and Alcoholic Extract of Bilwadi Agad had been prepared by reflex extraction method. Total phenolic content, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and total flavonoid content of bilwadi agad were determined.

Results: The results obtained during course of study were found significant, which showed antioxidant property of bilwadi agad. The obtained values clearly indicates that Bilwadi agad with both the extracts shows antioxidant activity or percentage inhibition of free radicals. The alcoholic extract showed more significant result in comparison to aqueous extract, it can be stated that more the concentration of extract more will be antioxidant property.

Conclusion: Present study has confirmed the antioxidant potentials of our preparation supporting its application as preventive remedy for various autoimmune and degenerative disorders. Finally it may be concluded that further study is required to isolate and identify the pure moiety responsible for antioxidant action of drug.

Keywords: antioxidant. alcoholic and aqueous extract, bilwadi agad, DPPH, phenolic content, flavonoid content, Rasayana.

INTRODUCTION

Ayurveda is an ancient holistic medical system that originated in India more than 5000 years ago. It is considered as Upveda of Atharvveda. The Atharva veda is measured to be the mother of curing systems in India.

स्वस्थस्य स्वास्थ्य रक्षणम् आतुरस्य विकार प्रशमनम् च I (च.सू.30/26)

Prevention is better than cure, this was long ago understood by our acharyas. The specialty of Ayurveda is that before the treatment +of disease, it describes preservation of health i.e the primary motive of Ayurveda is to conserve the health and to cure the disease is its secondary aim (Shukla , 2012).

Ayurveda was divided in eight different branches called as Ashtang Ayurveda, Agadtantra is one of the part of this division. Since ancient time Agadtantra was considered one of the important branch in Ashtang Ayurveda by all eminent acharyas.

In ayurvedic samhitas and various reference granthas, acharyas used agadas as medicine in the treatment of diseases. Agadas are used in rasyanachikitsha .e.g. bhallataka rasayana. Acharya Charak have told that visha in tilmatra (minute dose) can be used as rasayana while, visha when used by appropriate yukti can be converted to bheshaja (medicine). Rasayana yog contains drugs which show promising effect as antioxidant substances. Rasayana drug are the drugs which reduces or diminishes the oxidative stress produced in body (Kuchewar et al., 2014). By the use of Rasayana, our body automatically

develops a defence mechanism against the foreign pathogens which further boost up the function of different systems of our body, it increases our immunity that helps to fight in different diseased condition (Chulet and Pradhan, 2009).

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भुजंगलूतोन्दुरुवृश्चिकाद्यै विषूचिकाजीर्णगरज्वरैश्च |
आर्त्तान् नरान् भूतविधअर्षितांश्च स्वस्थीकरोत्यन्जनपाननस्यै: || (अ.स.42/87-88) (अ.ह्र.36/84-85)

Bilwadi agad is one of the formulation which shows rasayana properties due to its ingredients which has incredible properties, they are – Bilwa moola, sursa pushp, karanj phal, tagar moola, suraha twak(devdaru), vyosh (sunthi, maricha, pippli), nisha dwya (haridra, daruharidra), phal triya (bibhitaki, haritaki, amalki) and aja mutra as bhavana dravya. This yog has been indicated in poisonous conditions like sarpa, luta, vrischik, mushak dansa, jwar, visuchika, ajeerana and gar vish (Gupta, 2011; Shastri ,1988) Ingredients of bilwadi agad like haritaki, bibhitaki, amalaki, haridra, sursa, karanj etc shows rasayana properties.

Antioxidants are the compounds which prevent free radicals, produced through cell metabolism. These free radicals are very reactive and damage the healthy cells, tissues and other parts of our body. These radicals, oxidize macromolecule in the body, such as proteins, lipids, nucleic acid and are responsible for various diseases. Now a day's plant based natural antioxidants are used because of their strong ability of capturing the free radicals (Mohan et al. 2019; Rahman and Parvin, 2014). Bilwadi agad has such ingredients which are antioxidant in nature. Due to presence of rasayana guna in ingredients of bilwadi agad present study was carried out, to evaluate its antioxidant property using different concentration of aqueous and alcoholic extracts.

AIMS AND OBJECTIVES

To evaluate the antioxidant effect of Bilwadi agad

MATERIAL AND METHOD

Collection of drug

All the ingredients of bilwadi agad (100gm) were collected each in pure form from their natural habitat. The main ingredient of bilwadi agad like bilwamoola, sursapusphmanjari were collected from Rishikul campus Haridwar in winter season. Karanj phal, triphala, sunthi, maricha, pippli and tagar were bought from pannalal store Haridwar. Suraha and Daru haridra were collected from Dhanaulty in Uttarakand. Haridra was collected from Himachal Pradesh, 7 litres of goat urine was collected from farmer house in Haridwar. All the ingredients of bilwadi agad were identified and verified by eminent experts of Dravyaguna Dept. at Rishikul Campus-Haridwar Uttarakand Ayurved University .

Preparation of drug

All these ingredients were washed thoroughly in running water to remove soil and other foreign particles. These were cut into pieces and dried in shade. Powder of ingredients of bilwadi agad was prepared at Hans Pharmacy, Sidcul, Haridwar. The powder of all these ingredients were mixed thoroughly, the process of bhavana with ajamutra were carried out in Rasashastra Department at Rishikul campus Haridwar. Total 7 bhavana were given to prepare bilwadi agad. The obtained bilwadi agad was blackish- brown in colour with smell of ajamutra.

ANALYTICAL STUDY

Prepration of extract:

Test sample: Aqueous and Alcoholic Extract of Bilwadi Agad had been prepared by reflex extraction method.

For aqueous extraction - 20 gram of bilwadi agad was added to 200 ml of solvent (distilled water) and kept in reflex extractor for 1 hour. Filtered with filter paper and residue was kept in water bath until it reaches to dry form. From this process 3.6 gram dry extract was obtained.

Now from the dry extract, we took 500 mg, 1 gram and 1.5 gram of dry extract and 10 ml of water is added in each, from which 5%, 10% and 15% concentration of aqueous extract of bilwadi agad was obtained.

For alcoholic extraction -20 gram of bilwadi agad was added to 200 ml of solvent (methanol) and kept in reflex extractor for 1 hour. Filtered with filter paper and residue was kept in water bath until it reaches to dry form. from this process 3 gram extract was obtained.

10 ml of methanol was added to 500 mg, 1 gram and 1.5 gram of dry extract respectively from which 5%, 10% and 15% concentration of alcoholic extract of bilwadi agad was obtained.

Antioxidant Study:

DPPH free radical scavenging assay (Brand Williams et al., 1995)

Test Sample: Aqueous and alcoholic extract of Bilwadi Agad (5%, 10% and 15%).

Standard Sample: **Ascorbic acid (15 %)**

Standard sample i.e. Ascorbic acid along with blank was observed by bleaching the purple colour of 2.2 Diphenyl -1-picryl hydrazyl. after bleaching, 0.1 ml solution was added to 1.4 ml

of DPPH, the solution was kept in dark for 30 min, then the absorbance of the solution was measured using spectrophotometer at 517 nm, finally the percentage inhibition was calculated using following formula.

$$\text{Percentage Inhibition (\%)} = \frac{(A_0 - A_1) \times A_0}{100}$$

where:
A_0 is the Absorbance of Blank
A_1 Absorbance of Test sample and Ascorbic Acid

Determination of total phenolic content
Folin Ciocalteau method was used (Waterhouse, 2002)
Test Sample: 1 ml
1 ml of sample solution was added in 2.5 ml of 10 % Folin ciocalteau reagent and 2.5 ml Na_2CO_3 (7.5 %) was added sequentially then, samples were incubated at 45°C for 45min. After incubation the sample was warmed for 1 minute and cooled subsequently after cooling the absorbance was measured at 760 nm using spectrophotometer. The average absorbance values obtained at different concentrations were used to plot the calibration curve. Using this calibration plot, the concentration of phenol in test samples was calculated, similarly using garlic acid calibration curve, phenolic content was measured using garlic acid equivalent per gram of dried extract (μg GAE/g sample).

Determination of flavonoid content
To determine total flavonoid content in the sample aluminium chloride colorimetric method (Chang et al., 2002) was used.
1 ml sample, 1 ml standard quercetin solution (50, 100, 150, 200 μg /ml) and 4 ml of water were taken in volumetric flask after 5 min 0.3 ml of 5 % sodium nitrite solution, 0.3 ml of 10% aluminium chloride was added to it. The solution was then incubated for 6 min at room temperature; finally 2 ml of Sodium hydroxide was added to the above prepared solution and the final volume of the solution was kept 10 ml using distilled water. The absorbance of the solution was taken at 510 nm using spectrophotometer; the results were expressed as Quercetin equivalents (mg Quercetin/g dried extract).

RESULTS

Table 1 shows the DPPH free radical scavenging assay at different concentrations, Table 2 shows total Phenolic and Flavonoid content at different concentrations, while table 3 shows Comparative values of Phenolic and Flavonoid content in different extracts. The obtained values clearly indicates that Bilwadi agad with both the extracts shows antioxidant activity or percentage inhibition of free radicals. At 5% aqueous concentration the percentage inhibition was found 10.48%, at 10% aqueous concentration the percentage inhibition was found 20.79% and at 15% aqueous concentration the percentage inhibition was found 25.60%. On the other hand percentage inhibition of alcoholic extract of bilwadi agad at 5% concentration was found 12.37%, at 10% concentration was found 22.68% and at 15% concentration it was observed as 28.69%. The alcoholic extract showed more significant result in comparison to aqueous extract, it can be stated that more the concentration of extract more will be antioxidant property. Similarly total phenolic content of bilwadi agad with 15% concentration of aqueous and alcoholic extract showed absorbance of 0.132 and 0.146 . This absorbance seems to be much equal to gallic acid . Also flavonoid content found in aqueous extract was 67 mg of quercetin equivalent / gm oil and 87 mg of quercetin equivalent / gm oil in alcoholic extract. That means, bilwadi agad has satisfactory results of antioxidant property. because more the total phenolic and flavonoid content more will be antioxidant property.

DISCUSSION

Till date, several workers reported the biological activities of phenolics in which they considered phenols as potent antioxidants and radical scavenger's (Kahkonen et al., 1999; Rice Evans et al., 1995; Sugihara et al. 1999). Bilwadi agad showed potent antioxidant activity, each ingredients of bilwadi agad have antioxidant properties. The ingredients of bilwadi agad like Aegle marmelos displayed marked antioxidant property (Rahman and Parvin, 2014). Emblica officinalis works as a Immunmodulator, reduces stress and ageing process (Sharma et al., 2017) .Terminalia belerica showed antioxidant property (Deb et al., 2016) , Terminalia chebula exhibited antioxidant activity (Upadhayay et al. , 2014), Karanjin has antioxidant properties (Yadav et al. 2011), Turmeric has maximum absorbance so it has a great antioxidant property(Gupta and Ghosh, 1999), ginger has an equal antioxidant effect to that of ascorbic acid (Mashhadi et al. 2013). Yao et al. (2010) in their study reported significant positive correlation between the antioxidant activity and the contents of total flavonoids and total phenolics in celery. This is because of chemical constituents present in each plant. According to Adesegan et al. 2009 phenolics and flavonoids, constitutes a major group of compounds, these compounds acts as primary antioxidant and are also known to protect DNA from oxidative damage, they also help to

Table 1: Showing DPPH free radical scavenging assay of *Bilwadi agad* at different concentrations

S No.	Sample	Percentage Inhibition
1	Aqueous Extract (5 %)	10.48
2	Aqueous Extract (10 %)	20.79
3	Aqueous Extract (15 %)	25.60
4	Alcoholic Extract (5 %)	12.37
5	Alcoholic Extract (10%)	22.68
6	Alcoholic Extract (15 %)	28.69
7	Ascorbic Acid	83.16

Table 2: Showing determination of total Phenolic and Flavonoid content at different concentrations

S. No	Sample	Concentration	Absorbance
1	Gallic Acid	10 µg/ml	0.251
2		20 µg/ml	0.430
3		40 µg/ml	0.6561
4		60 µg/ml	0.971
5	Aqueous Extract	15 % solution	0.132
6	Alcoholic Extract	15 % solution	0.146

Table 3: Comparative values of Phenolic and Flavonoid content in different extracts

S. No.	Sample Name	Phenolic Content (mg of gallic acid equivalent /gm oil)	Flavonoid content (mg of quercetin equivalent / gm oil
1	Aqueous Extract	0.642	67
2	Alcoholic Extract	1.642	87

inhibit the growth of tumours cells and possess antimicrobial and anti-inflammatory properties. As per Hussain et al., (1987) these are known to react with hydroxyl radicals, Afanasev et al., (1989) reported reaction with superoxide anion radicals while Torel et al. (1986) observed its reaction with lipid peroxy radicals.

CONCLUSION

The present study affirms the in vitro antioxidant potential of aqueous and alcoholic extract of the bilwadi agad with results comparable to those of the standard compounds such as gallic acid or ascorbic acid. In this study we adopted three methods for determination of antioxidant property. In DPPH free radical scavenging assay method, our drug showed that it has good percentage inhibition of free radicals at all concentration of drug. The other method was determination of total phenolic content and determination of total flavonoid contents. Bilwadi agad showed sufficient phenolic and flavonoid content, which shows that it has good antioxidant property. This study has helped us to understand the importance of herbal drugs or medicines as well as this formulation. Present study has confirmed the antioxidant potentials of our preparation supporting its application as

preventive remedy for various autoimmune and degenerative disorders. Finally it may be concluded that further study is required to isolate and identify the pure moiety responsible for antioxidant action of drug.

Conflict of interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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