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# PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT STUDY OF *DOOSHIVISHARI AGADA*

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## ABSTRACT

*Dushivishari Agada* is a compound Herbo-mineral preparation which is explained in context of *Dushivisha*. Present study has been planned to evaluate preliminary phyto-chemical and in-vitro antioxidant activity of *Dushivishari Agada*. Preliminary phytochemical analysis was performed to identify secondary metabolites and in vitro antioxidant activity was measured by means of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Super oxide free radical scavenging assay and total polyphenol content in the aqueous extract of *DVA*. Phytochemical screening of aqueous extract of *DVA* showed the presence of Alkaloids, flavonoids, tannins and phenolic compound. Extract strongly scavenged DPPH radical and superoxide radical with the $IC_{50}$ being 103.36 µg/ml and 137.73 µg/ml respectively. The extract exhibited 86.14 GAE mg/100 gm of total polyphenol content. Our study suggests that presence of these bioactive components in DVA, may serve as a natural source of antioxident for *Ayurveda* practitioners.

**KEYWORDS:** Antioxidant, *Agada, Dushivisha,* Phytochemicals.

**Abbreviation:** *DVA - Dushivishari Agada, DV- Duashivisha, OA- oxidative stress.*

## INTRODUCTION

*Dushivishari Agada* is a compound Herbo-mineral preparation which is explained in context of *Dushivisha*. It is a condition where in a *Visha* (toxins / poisons) settles in body due to

improper elimination from body. This settled poison produces ailment when it congregate with triggering factors like *Dushita Desha* (~Polluted/Contaminated place), *Dushita kala* (~inappropriate time), *Dushita Anna* (~inappropriate food) etc.[1]

In one of previous literary study scholars attempted to correlate concept of *Dushivisha* with Oxidative stress (which occurs due to imbalance between reactive oxygen species (ROS) and antioxidants in the body) and advised that there is a correlation between these two (DV and OA) up to some extent. If we accept this theory then one can conjecture that formulation mentioned for the treatment of DV may possess antioxidant activity because antioxidants are considered as treatment for oxidative stress. In an attempt to examine above said premise present study has been planned to evaluate preliminary phyto-chemical and in-vitro antioxidant activity of *Dushivishari Agada*.

## MATERIAL AND METHODS

**1. Collection of Drug-** The test drug *Dushivishari Agada* was collected from a GMP certified company.

**2**. **Preparation of aqueous extract**. Aqueous extracts of DVA were prepared with cold maceration technique.[3] They were further subjected for qualitative phytochemical screening the aqueous extract was stored in refrigerator for further experimental work.

**3** Preliminary phytochemical analysis and in vitro antioxidant activity were carried out at Columbia Institute of Pharmacy, Raipur (C.G.).

**3.1 Preliminary phytochemical -** It was performed to identify secondary metabolites (phytoconstituent) in aqueous extract of *DVA* .[4]

**3.2 - In vitro antioxidant activity –** It was carried out by following methods.

**3.2.1 Hydrogen-Donating activity[5]** - Hydrogen donating activity was quantified in presence of stable DPPH radical on the basis of Blois method. In various studies for testing antioxidant activity this analysis was used. For titrating the oxidizable groups of natural and synthetic antioxidants DPPH method is exactly suitable and correct. This analysis was based on the diminution of a methanolic solution of the colored free radical DPPH by free radical scavenger.

In brief, the methanolic solution of DPPH (100m M, 2.95 ml), 0.05 ml of tablet solution dissolved in methanol was added at different amount of concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The scavenging efficiency of the extract to specify degree of discoloration was calculated by.

$$\% \text{ AA} = 100 - \{[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100] / \text{Abs}_{\text{DPPH}}\}$$

**3.2.2 Superoxide scavenging activity**[6-7] Superoxide scavenging was carried out by using alkaline DMSO) Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. 2.8ml of an aqueous solution containing nitroblue tetrazolium (56 m M) was added to filtrate (200 ml), potassium phophate buffer (10 mM, pH 7.4) and EDTA (10 mM). Sample of tablets (1 ml) at various concentrations (50-250 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO.

**3.2.3 Determination of total polyphenol content**[8]- Total polyphenol content was determined using colorimetric method. 2.0 ml of the prepared tablet solution was oxidized using Folin- Ciocalteu reagent (400 µl), and sodium carbonate solution (75 g/l) was then added to the reaction mixture to reach a 10.0 ml volume. After 2 h, the suspension was centrifuged for 10 min at 5000 rpm, and absorption was measured at a 760 nm wavelength. The amount was calculated using the gallic acid calibration curve. The results were expressed as Gallic acid equivalent (GAE) mg per 100 ml of the *Dushivishari Agada* extract.

**(a) Calibration curves of Gallic acid**[9]**-** Accurately weighed 100 mg of Gallic acid was dissolved in 100 ml of distilled water which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with Gallic acid which contains the concentration of 100 µg/ml. Further 10 ml of this solution was taken and made up to 100 ml with Gallic acid which contains the concentration of 10µg/ml. 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10µg/ml. The absorbance of the resulting solutions was then measured at 288 nm using UV spectrophotometer, against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance against concentration in µg/ml and data was subjected to weighed linear regression analysis in Microsoft excel.

## RESULTS

**Table 01: Phytochemical Screening of aqueous extract of DVA.**

| Sl no | Parameter | Test/Reagent | Result |
|---|---|---|---|
| 1. | Carbohydrates | Molisch | + |
| 2. | Monosaccharides | Barfoeds | + |
| 3. | Pentose | Bails | - |
| 4. | Hexose | Selwinoffs | - |
| 5. | Non-reducing sugar | Benedicts | - |
| 6. | Polysaccharide | Iodine test | - |
| 7. | Proteins | Millons test | - |
| 8. | Amino acids | Ninhydrin test | - |
| 9. | Steroids | Liebermann Burchard test | + |
| 10. | Glycosides | Cardiac Glycosides | - |
| 11. | | Coumarin | - |
| 12. | | Anthraquinie | - |
| 13. | Saponins | Foam Test | + |
| 14. | Flavonoids | Lead acetate solution Test | + |
| 15. | Alkaloids | Dragandroff's | + |
| 16. | Tannins and Phenolic | Gelatin Test | + |

**3.2.1** DPPH is stable nitrogen centred free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals respond with appropriate reducing mediator, and then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. As shown in table 2, aqueous extract of *Dushivishari Agada* strongly scavenged DPPH radical with the $IC_{50}$ being 103.36 (Fig 1). The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 95.24.

**Table 2: Free radical scavenging capacity of tablets.**

| Concentration (µg/ml) | DPPH Scavenging % | |
|---|---|---|
| | DVA | Ascorbic Acid |
| 50 | 31.25±0.61 | 95.63±0.24 |
| 100 | 52.36±0.48 | 98.57±0.15 |
| 150 | 64.15±0.57 | 91.24±0.4 |
| 200 | 79.62±0.42 | 92.4±0.11 |
| 250 | 92.18±0.34 | 93.85±0.0.06 |
| $IC_{50}$ | 103.36 | 48.51 |

Values are mean ± SEM of six determinations.

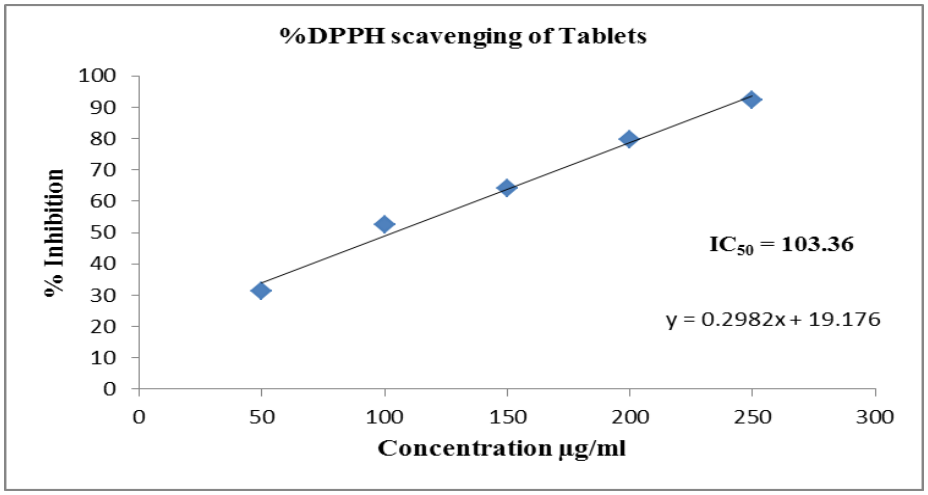


**Fig. 1: $IC_{50}$ values of aqueous extract of *Dooshivishari Agada.***

**3.2.2 Superoxide radical scavenging activity** is measured by Nitro blue tetrazolium (NBT) reduction. The method is based on generation of superoxide radical by auto oxidation of riboflavin in presence of light. Superoxide radical is very harmful to cellular components as an antecedent of more reactive variety. Superoxide is biologically important as it can form singlet oxygen and hydroxyl radical. The overproduction of superoxide anion radical contributes to redox imbalance and is related with dangerous physiological costs. The superoxide radical reduces NBT to a blue colored formazan that can be measured at 560 nm. The decrease of absorbance at 560 nm with extracts and antioxidants indicates the consumption of superoxide anion in the reaction mixture.

Superoxide free radical scavenging activity was performed with the aqueous extract of *Dushivishari Agada* and was expressed as $IC_{50}$ value. The $IC_{50}$ was measured for extract and standard compound i.e. ascorbic acid. The $IC_{50}$ value for aqueous extract of *DVA* was 137.73 (table 3 and fig. 2). The superoxide radical scavenging activity was found to dose dependent. The standard drug ascorbic acid scavenged superoxide radical with the $IC_{50}$ being 93.35.

**Table 3: Super oxide scavenging capacity of tablets.**

| Concentration (µg/ml) | Superoxide Scavenging % | |
|---|---|---|
| | DVA | Ascorbic Acid |
| 50 | 23.28±0.52 | 93.35±0.83 |
| 100 | 39.41±0.47 | 93.64±0.31 |
| 150 | 53.72±0.92 | 96.45±0.29 |
| 200 | 69.16±0.34 | 98.82±0.60 |
| 250 | 82.63±0.41 | 95.43±0.75 |
| $IC_{50}$ | 137.73 | 52.38 |

Values are means ± SEM of six determinations

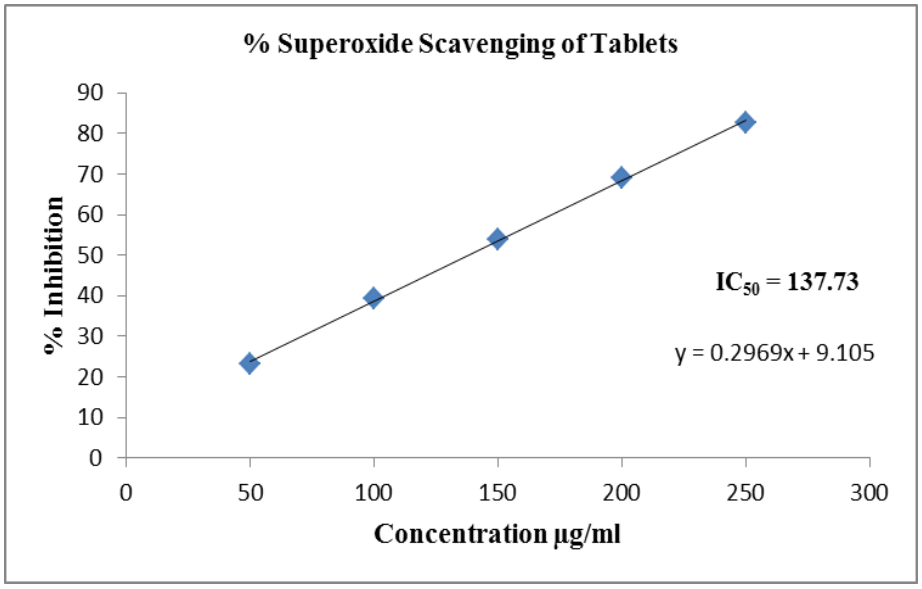


**Fig. 2: $IC_{50}$ values of aqueous extract of *Dushivishari Agada.***

**3.2.3** The aqueous extract of *Dushivishari Agada* was evaluated for investigation of the total phenolic content concentrations in extracts. Measure the standard curve of Gallic acid and set in distilled water for determining absorption data (Table 4). From this Beer's law range and regression coefficient is determined. The linear equation of Gallic acid was found to be y = 0.038 x -0.0019 (Fig 4). The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 4. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of aqueous extract of *DV* was 86.14 GAE mg/gm (Table 5).

**Table 4: Absorbance by Gallic acid in different concentration.**

| Concentration (µg/ml) | Absorbance at 760 nm |
|---|---|
| **0** | **0** |
| **1** | 0.038 |
| **2** | 0.069 |
| **3** | 0.108 |
| **4** | 0.145 |
| **5** | 0.205 |
| **6** | 0.228 |
| **7** | 0.265 |
| **8** | 0.298 |
| **9** | 0.336 |
| **10** | 0.379 |

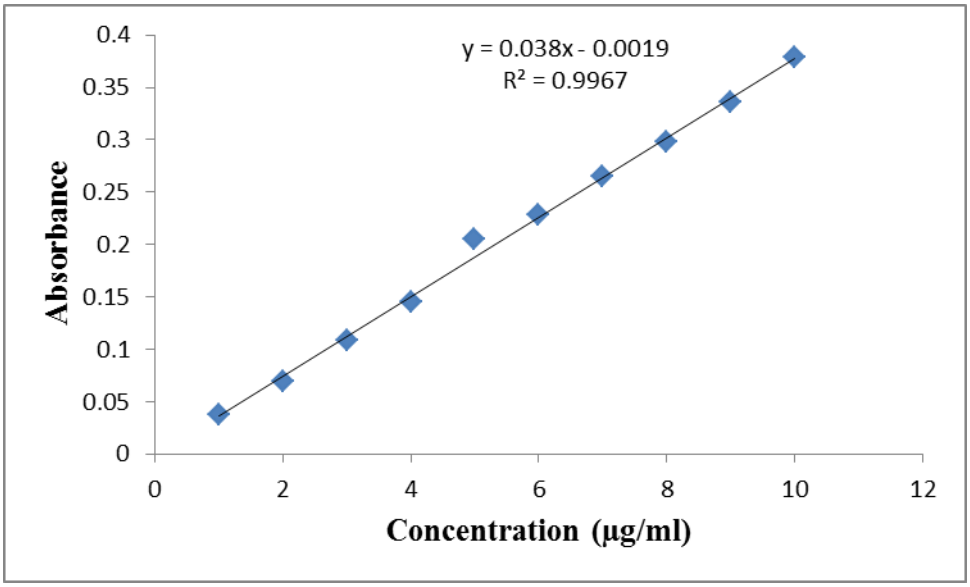


**Fig 3: Calibration curve of Gallic acid in distilled water.**

**Table 5: Determination of total polyphenol content of tablets.**

| Extract | Total polyphenol content (GAE mg/gm) |
|---|---|
| Tablets | 86.14±0.73 |

Data expressed as Gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations.

## DISCUSSION

The phytochemical investigation of Aq. extract of DVA revealed the presence of Alkaloids, flavonoids, saponins, steroids tannins and phenolic compound. The flavonoids, tannins and phenolic compound are known to be useful in the treatment of various diseases such as cancer, hepatotoxicity, ulcerated tissue, cardiovascular diseases, diabetes etc. Hence, the presence of these bioactive components in Aq. Extract of DVA, may serve as a potential source of drug for Ayurveda practitioners.

The important mechanisms for measuring antioxidant activity are known to be the proton radical scavenging activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the oil. The rates of DPPH scavenging activity of oil are most likely due to the occurrence phenolic complexes. Our study clearly indicated that the Aq. Extract of DVA exhibited containing of phenolic compounds which was significantly correlated with the DPPH radical scavenging activity.

From results, it was found that the Aq. Extract of DVA showed free radical scavenging activity. They take out, donated their electrons to the superoxide and scavenge them to avoid

their additional interaction with NBT are followed by inhibition of formation of blue colored formazan product.[10-11] The findings of results revealed that Extract displayed flavonoids content, which was significantly correlated with the superoxide radical scavenging activity.

From the result of DPPH and superoxide radical scavenging activity it was observed that the Extract showed highest DPPH radical scavenging activity and maximum superoxide radical scavenging activity. It indicates the presence of different character of antioxidant components in crude extract of DVA.

From these results it can be concluded that antioxidant activity of extract depends on the presence of quality of active constituents, because each in vitro antioxidant model has different mechanism to reduce free radicals. Earlier many researchers have reported that the antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents.

In general plant flavonoids and phenols, are very much effective free radical scavenging and antioxidants.[12] Polyphenol and flavonoids are used for the prevention and cure of various diseases and skin disorders, which are mainly associated with free radicals. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions.[13-14]

The compounds such as the flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants.[15] The findings of total polyphenol content of DVA support the study of DPPH and superoxide scavenging capacity.

## CONCLUSION

Our study suggests that presence of these bioactive components in DVA, may serve as a natural source of antioxident for *Ayurveda* practitioners in their clinical practice.

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