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Research Article

THE DETERMINATION OF ANTIOXIDANT ACTIVITY OF VATSANABHA (ACONITUM FEROX WALL EX SERINGE.) ROOT EXTRACT PROCESSED IN COW'S URINE AND COW'S MILK USING FRAP METHOD

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ABSTRACT

Ageing is the inevitable truth of the life being a natural process. Effects of free radicals may cause premature ageing which is an unnatural process. To avoid or reduce effects of free radicals body needs antioxidants as a body protector. Synthetic antioxidants like Vitamin A, Selenium are often used by community while antioxidants of natural origin are not so commonly used. Medicinal plants mentioned in Ayurved as a Rasayan may have antioxidant activity. Of which few are studied & many are still awaited to be researched on modern parameters like Vatsanabh. The aim of the study was to obtain antioxidant activity of methanolic extracts of Vatsanabh rootusing FRAP (Ferric Reducing Antioxidant Power) method. Three samples of Vatsanabhroots (Aconitum ferox Wall ex Seringe.) i.e. Crude or Ashuddha Vatsanabh, Vatsanabh processed in Cow's Urine and Vatsanabh processed in Cow's Milk were extracted using soxhlet method by methanol 50%. The absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 700 nm and the total value of antioxidant activity was calculated based on the data absorbance. The results showed that all three extracts showed antioxidant capacity while Shodhit Vatsanabh (in cow urine) have the maximum antioxidant capacity in mmol/100gm equivalent of Ascorbic acid with the value as 739.

INTRODUCTION

Rasayantantra is the name of that branch of Ayurved which describes the methods of withholding ageing, increasing lifespan, intelligence, strength and capacity to get rid of diseases[1].Rasayan[2](Promotive treatment) means the way for attaining excellence of rasa etc. (dhatus). Agadtantra is related with the diseases due to different kinds of poisons and their treatment[3]. Visha is of two types Sthawar & Jangam. Sthawar Visha is of two types Visha & Upavisha.Visha are nine in number &of the nine Vishas; Vatsanabh is considered as best for Rasayan[4]. According to Ayurved; Visha is of tremendous medicinal value but should be used after Shodhan procedures only[5]. Three Shodhan procedures are mentioned regarding Vatsanabh in the texts however which method is superior or best for therapeutic use of Vatsanabh as a Rasayan is nowhere clearly explained[6]. Hence in this study two Shodhan procedures were studied which are having totally different processing. Vatsanabh is one of the most poisonous plants known till today to mankind but still used widely in Ayurved treatment in various diseases as Shwas, Kasa, Pandu, Amwat etc.[7]. Ayurved science considers ageing as natural or physiological phenomenon while premature ageing as unnatural or diseased condition[8,9]. According to modern science also ageing is a natural process and premature ageing is an unnatural process and antioxidants are one of the causative factors for the ageing[10]. Antioxidant action maybe one of the probable modes of action of Rasayan chikitsa or drugs used for Rasayan[11]. Vatsanabh is clearly mentioned as Rasayan in Samhitas[12]; however no study has been conducted for antioxidant action of Vatsanabh as a single drug to the best of our knowledge. It is mentioned as Vatsanabh should be given after mixing with one of these medicines-Yashtimadhu, Mrugshrunga, Murudsheng, Ativisha, Tavakil or Vachain a proportion as 1:7 to avoid its ill effects in of the books about Rasayan Chikitsa[13]; hence individual Rasayan effect of Vatsanabh is yet to be researched. In this research, antioxidant activity was obtained using FRAP (Ferric Reducing Antioxidant Power) method. The advantages of this method are it can determine the total antioxidant content of a material

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based on the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} so that the antioxidant power of a compound is analogous to the ability to reduce thecompound[14].The medicinal plants exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity increases. The antioxidant compound present in the samples forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm by UV-Spectrophotometer[15]. In this study antioxidant activity of methanolic extracts of Crude Vatsanabh (*Aconitum ferox Wall ex Seringe*.) root, Vatsanabh root processed in Cow's Urine and Vatsanabh root processed Cow's Milk were determined and results were analysed.

MATERIALS AND METHODS

Collection and selection of drug

One kilogram of fully matured Vatsanabh (*Aconitum ferox Wall ex Seringe*.) roots were collected from the local market of Uttarakhand in India and were botanically authenticated by Pharmacognosists and sample specimens were kept for future reference. Cow's urine (Gomutra) and Cow's milk (Godugdha) were collected from the local cow shed in the morning and were used for Shodhan procedures of the root.

Equipment for Shodhan

Stainless steel plates circular shapes 2 in number; stainless steel vessel having capacity of 1.5 lit used as Dolayantra, stainless steel rod (35 cm.), cotton thread 30 cm. in length, measuring mug (capacity of 1 L), muslin cloth (45 cm × 45 cm), stainless steel spatula (length 30 cm), digital weighing machine, pyrometer, LPG stove, LPG cylinder, Clay pot of 1 litre capacity.

Shodhan Procedures

Each Shodhan Procedure was carried out in different batches by using two different media (cow's milk and cow's urine) individually.

Shodhan Procedure of Vatsanabh using cow's urine as a media[16]

100 gm. of clean and dried roots of Vatsanabh were made into pea sized pieces and were kept in a clay pot having one litre of cow's urine in it for three consecutive days. The pot was kept on the terrace having plenty of sunlight in the month of April. Each day the cow's urine was replaced with the fresh one. On the fourth day, the roots were washed with water; the outer cortical layers were peeled off and the product was again washed with warm water. The pieces were dried in sunlight and kept in an air tight glass container and the final product was labelled as Shodhit Vatsanabh (in cow urine).

Shodhan Procedure of Vatsanabh using cow's milk as a media[17]

100 gm. of clean and dried roots of Vatsanabh were made into pea sized pieces. These pieces were tied in a muslin cloth into a poultice which was suspended in the centre of a pot with the help of a steel rod. Cow's milk was poured in the vessel to completely immerse the poultice. It was then heated on a LPG stove for three hours at 100°C. Level of milk was maintained above poultice level by repeatedly adding boiled hot milk whenever required. Later, the pieces of Vatsanabh were taken out and washed with hot water. The outer cortical layers of the roots were peeled off. After proper drying in sunlight the Vatsanabh pieces were then kept in an air tight glass container as Shodhit Vatsanabh (in cow milk).

Anti-oxidant Assay: Ferric Reducing Antioxidant Power assay (FRAP Method)

Materials and equipment for FRAP assay[18]

- Deionized water
- Potassium ferricyanide
- Sodium chloride
- Potassium chloride
- Disodium hydrogen phosphate
- Potassium dihydrogen phosphate
- Hydrochloric acid
- Trichloroacetic acid
- Ferric chloride
- Centrifuge tubes
- Pipette
- Water bath
- Vortex shaker
- Centrifuge
- UV-Spectrophotometer

Preparation of Reagents

0.2 M phosphate buffer (pH 6.6): 8 gm. of sodium chloride, 0.2 gm. of potassium chloride, 1.44 gm. of disodium hydrogen phosphate, 0.24 gm. of potassium dihydrogen phosphate was taken in a 1,000 mL standard flask and 800 mL of distilled water was added and the pH was adjusted at 6.6 using hydrochloric acid and adjusted the volume with deionised water.

Potassium ferricyanide (1%): 1 gm. of potassium ferricyanide was dissolved in 100 mL of deionised water.

Trichloroacetic acid (10%): 10 gm. of trichloroacetic acid was dissolved in 100 mL of deionised water.

Ferric chloride (0.1%): 100 mg of ferric chloride was dissolved in 100 mL of deionised water.

Ascorbic acid (0.1%): 1 mg of ascorbic acid was dissolved in 1 mL of water.

Standard Solution

Working solutions of Ascorbic acid were prepared with different concentrations viz. 100 µmol/ L, 200 µmol/ L, 400 µmol/ L, 600 µmol/ L, 800 µmol/ L and 1000 µmol/ L respectively. These working solutions were used for calibration.

Sample Preparation

Ashuddha Vatsanabh: 1.250 gm. sample was refluxed with 20 mL of 50 % methanol for 3 hrs. and then volume made up to 50 mL with methanol.

Shodhit Vatsanabh (in cow urine): 1.088 gm. sample was refluxed with 20 mL of 50 % methanol for 3 hrs. and then volume made up to 50 mL with methanol.

Shodhit Vatsanabh (in cow milk): 1.047 g sample was refluxed with 20 mL of 50 % methanol for 3 hrs. and then volume made up to 50 mL with methanol.

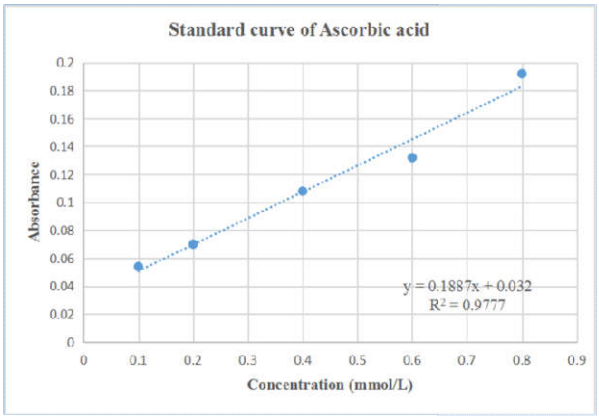
The working solutions thus obtained were used for Antioxidant study

Procedure[19]

1ml of the above said working solutions of the samples were taken in test tubes and labelled as sample. 2.5. ml of 0.2 M Phosphate buffer (6.6pH) and 2.5ml of 1% Potassium ferricyanide were added. This solution was mixed well with vortex mixer and incubated at 50^0C on water bath for 20 minutes. 2.5 ml of 10% Trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. Then 2.5ml of the supernatant solution was taken in the test tube and mixed with 2.5 ml of water. 0.5 ml of 0.1% of Ferric chloride was added to this solution and mixed well with vortex mixer. Immediately absorbance was measured of the sample solution in UV spectrophotometer at 700 nm using control solution. From the linear equation of standard the antioxidant capacity of the sample equivalent to standard Ascorbic acid was determined.

Standard Curve of Ascorbic acid

The curve of regression was obtained using the value of absorbance (y) and concentration of Ascorbic acid (x). The regression equation was y = 0.1887x + 0.032 and coefficient of determination value (R^2) was 0.9777 as shown in the figure.



RESULTS

Absorbance

Table 1

1.	Ashuddha Vatsanabh	0.285
2.	Shodhit Vatsanabh (in cow's urine)	0.902
3.	Shodhit Vatsanabh (in cow's milk)	0.311

Antioxidant capacity in mmol/100gm equivalent of Ascorbic acid

Table 2

1.	**Ashuddha Vatsanabh**	**223.78**
2.	Shodhit Vatsanabh (in cow's urine)	739
3.	Shodhit Vatsanabh (in cow's milk)	283.07

DISCUSSION

Vatsanabh root (*Aconitum ferox Wall ex Seringe root*); is toxic due to its chief active principle an alkaloid named as Aconitine. According to Ayurveda and modern science; it is one of the most poisonous plants [20, 21]. Despite it is used widely in Ayurved treatment in various diseases in many medicinal preparations after proper processing termed as Shodhan procedures. These processings are done in specific media[22]. Shodhan procedures enhance therapeutic properties of Vatsanabh, reduce its toxicity & convert it into medicine. In Ayurvedic literature, media like Gomutra (Cow's urine), Godugdha (Cow's milk) and Ajadugdha (Goat,s milk) has been mentioned for Shodhan procedures of Vatsanabha[23]. This study focuses on antioxidant effect of Vatsanabh rootafter processed by using Gomutra (cow's urine) as a media and Godugdha (cow's milk) as a media while antioxidant action of crude roots was also determined. In the previously conducted study, in vitro Vatsanabh root extract showed good antioxidant activity. Previous study also indicates that other than phenols and flavonoids there are some compounds present in the roots which are responsible for antioxidant activity in *Aconitum ferox*[24]. However in that study crude Vatsanabh roots were used without processed by any Shodhan procedure with any media. However in the Ayurved literature use of Vatsanabh roots is advised only after Shodhan procedure[25]. Hence the Determination of antioxidant activity of crude Vatsanabh (*Aconitum ferox Wall ex Seringe*.) roots was done along with roots processed in cow's urine and cow's milk using FRAP Method.

This research used FRAP (Ferric reducing antioxidant power) method to obtain total antioxidant activity. Ascorbic acid was used as a standard solution because ascorbic acid functions as secondary antioxidant that captures free radicals and prevents chain reactions. That's because Vitamin C has a free hydroxyl group that acts as a catcher of basal radicals and it has a polyhydroxy group which will increase antioxidant activity. Measurement of antioxidant activity used this FRAP method with Ascorbic acid solution as reference standard. The addition of Trichloroacetic acid was expected to precipitate the potassium ferricyanide$K_3Fe(CN)_6$complex deposited. The addition of $FeCl_3$ is to form a complex of green to blue colour (blue berlin). Reduction power was a potential indicator of an antioxidant compound. Reduction power in this case was expected from the ability of an antioxidant to convert Fe^{3+} to Fe^{2+}. Compounds that have the reduction power may act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms to form radicals more stable. The reaction was:

$K_3Fe(CN)_6 K_4Fe(CN)_6$

$Fe^{3+} + e^- \longrightarrow Fe^{2+}$

The analysis of antioxidant activity using FRAP assay depends on capacity of extract to reduce Fe^{3+} to Fe^{2+}. The Fe^{2+} solution has specific pale green colour which is equivalent with antioxidant present in samples[26]. The colour change was developed for all three samples but there was difference in the intensity of the colour which develops. The difference may be linked to the difference in the antioxidant constituents of the three samples or extracts. Table 1 shows the values of the absorbance of the root extracts of all three samples. The absorbance of each sample is different due to the difference in antioxidant constituent of the root extract. The reducing power was given graphically in terms of concentration versus absorbance. In this assay, increased absorbance indicated increased reducing power. Shodhit Vatsanabh (in cow's urine) extract showed maximum absorbance and in turn maximum antioxidant capacity in mmol/100gm equivalent of Ascorbic acid. A previous study confirms that Shodhan procedure by using cow's urine as a media is better than Shodhan procedure by using cow's milk as a media as far as physicochemical parameters are concerned[27]. Same observation is mentioned in one of renowned books of Dravyaguna Vigyan[28].This study confirms that Vatsanabh root should be processed in Cow's urine as a media for used as a purpose of Rasayan or Shodhan

procedure in Cow's urine enhances antioxidant activity of Vatsanabh.

CONCLUSION

From this study, it is concluded that all three samples of Vatsanabh roots shows significant antioxidant activity. Both the Shodhan procedures increases antioxidant activity of the Vatsanabh roots. After comparing antioxidant activity of all three samples it can be concluded that Shodhan procedure of Vatsanabh (*Aconiumferox Wall ex seringe.)* in cow's urine significantly increases Rasayan or antioxidant effect of Vatsanabh root than cow's milk. Shodhit Vatsanabh in cow's urine is far more better than Shodhit Vatsanabh in cow's milk other two samples as far as antioxidant activity is concerned.

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