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Original Article

Determination of Phytoconstituents & antioxidant Potential of extracts of fruit of Fragaria Vesca

Jeevan Menaria[1*], Dr Jai Singh Vaghela[1], Ashok Choudhary[2]

[1]Bhupal Nobles College of Pharmaceutical Sciences, Bhupal Nobles University, Udaipur, Rajasthan, 313001
[2]Department of Pharmacology, Dr S.N. Medical College, Jodhpur
** jeevanmenaria@gmail.com*

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ABSTRACT

F*ruits of Fragaria vesca it is belonging to vitaceae family, commonly known as strawberry available in every fruit seller. In these study try to findout antioxidant activity of these fruit. Prepare two different extract of fruit aqueous and ethanolic extract & find out Phytoconstituents and antioxidant activity of both extract. Fragaria vesca Phytoconstituents possess wide range of Phenolic, flavonoids compound possess. In vitro antioxidant was detected by DPPH assay, reducing power assay.The ethanolic, aqueous of fragaria vesca concentrates of & ascorbic acid exhibited antioxidant potential possessing IC50 48.14, 66.0 µg/ml & 14.40 µg/ml (Diphenylpicrylhydrazyl radical). The Reducing power assay possess 10.10, 11.51 µg/ml. Free radical scavenging difference due to solvent difference, it have better antioxidant activity which is helpful for controlling excess free radical in body.*

Keywords: *Fragaria vesca, Phytoconstituents, antioxidants.*

1. INTRODUCTION

Free radicals in body causes oxidative stress which is produced by reactive oxygen genus. It grow in cellular metabolism which may be in physiological cell at low to high concentration. It affects all over body systemic components i.e. Lipids, Proteins, DNA etc. (Valko et al., 2006, Halliwel et al 2006, Marnett et al 1999). In our body possess wide number of free radicals present, it interfere with various electrons i.e. oxidation, reduction and produced oxidative stress, which is responsible for many pathological conditions, including cancer, neurological disorders (Siems et al 1995, Stadtman et al 2004, Wang et al 1996), atherosclerosis, hypertension, ischemia/perfusion (Jenner et al 2003,Lyras et Ial 1997), diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease,and asthma (Sayre et 2001, Tosniwal et al 1997, Kukreja et al 1992)

Free radicals has been possess electrically molecules, radicals responsible for oxidation, reduction, neutralization themselves. It possess unpaired electron, antioxidant are responsible for its activity or deactivity.

Human body developed defensive mechanism to protect organ and cells against reactive oxygen species. Antioxidant i.e. ascorbic acid, Tocopherols, Carotenoids, Lipoic acid (Jacob et al 1995)

Fragaria vescas leaf active constituents which are mainly present are cumene, α-pinene, thuja-2,4 - diene, β-pinene (Afzalzadeh el al., 2015), 3-p-menthene,6 hepten-2-ol,7 mentha-1(7), 8-dien, 8 hexenyl acetate,9 α-terpinene, p-cymene, limonene, β-ocimene (Z),β-ocimene, γ-terpinene, trans linalool oxide, terpinolene(Fontana et al., 2013), cis-linalool oxide, linalool, nonanal, menth-2-en-1-ol,trans-pinocarveol, cis verbenol,trans-verbenol, citronellal, menthone, pinocarvone, borneol, nonanol, mentha-1,5-dien-8-ol, menthol,terpinen-4-ol, cymen-8-ol, myrtenol(Yadav et al., 2009).

2. METHODOLOGY

Gathering& Identification of Plant

The Fruits of vitis has been collected from local market. Vitis were desiccated under shadowy, segregate, crushed through the grinder.

***Corresponding author**:

Jeevan Menaria,
Research Scholar, Bhupal Nobles College of Pharmaceutical Sciences, Bhupal Nobles University, Udaipur, Rajasthan, 313001
Email: jeevanmenaria@gmail.com
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Preparation of extract

Maceration: 20 gm powdered was placed in a air tight container filled with 1000 ml distilled water. Leaved it for 7 days and gently shaked it. After 7 days extract was filtered out by using whattmann filter paper and dry it (Pourmorad F et al 2006).

Phytochemical Screening: Phytochemical screen of the extracts was conceded out according to the standard procedures, The Aqueous and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannin (Kokate et al 2002).

a. Carbohydrate

- **Molish test** -2ml of extract with Molish reagent. Observed that violet ring was formed.
- **Fehling test**-1ml of extract, it was treated with Fehling A and Fehling B. This mixture was heated at water bath for 5 minutes observed that red precipitate was formed.
- **Benedict's test**-1ml of extract and 1ml of Benedict reagent was added in a test tube. This mixture was heated at water bath for 7 minutes observed that red colour was formed.
- **Barfoed test**-2ml of extract was taken in test tube. This mixture added 1ml of barfoed's reagent red precipitate was observed.

b. Protein and Amino Acids

- **Biuret's test**- 1ml of extract treated with 1ml of 10% sodium hydroxide solution in a test tube and heated. In this solution added 0.7% copper sulphate solution. Observed that violet colour was formed indicate that protein in present
- **Milion's test-**3ml of extract was treated with 5ml of Milion's reagent. A white precipitate was formed. This mixture was heated turned to brick red colour it is show protein is present.
- **Ninhydrin test**-2ml of extract was treated with 4-5 drops of 5% ninhydrin solution. These was heated in water bath blue colour was observed. Concluded that amino acid is present.

c. Glycoside test

- **Brontrager's test**- Take 3ml of extract, diluted sulphuric acid was added. This mixture was boiled 5-6 minutes and filtered. Leave it for chilling, after these 3ml of benzene was added and shake it. A layer as separated mixture ammonia was added. Observed that pink to red ammonical layer was formed resulted that antharaquinones glycosides is present.
- **Keller**-killani test-2ml of extract takes. In this sample added 3ml of glacial acetic acid and a drop of 5% ferric chloride were added in test tube 2-5 drops of concentrated sulphuric acid was added the side of test tube, observed that blue colour was formed in acetic layers. Result that cardiac glycosides is present.

d. Saponin test

- **Froth test**- leaf extract diluted with distilled water and shake regularly in graduated cylinder for 20 minutes. No change observed in this extract. Result that forth tests is negative.

e. Alkaloid test

Extract was diluted with hydrochloric shake it well and filtered.

- **Mayer test**- 2ml extract, added few drop of Mayer reagent. A white creamy precipitate formed.
- **Dragendroffs test**- 2ml of extract added few drop of Dragendroffs reagent in a test tube. Red precipitate formed.
- **Hager's test** 3ml of leaf, added few drop of Hager reagent in a test tube. Yellow precipitate was formed. Result that alkaloid is present.
- **Wagner's test**- 2ml of extract added few drop of Wagner's reagent in a test tube. A reddish brown precipitate was formed. Result that alkaloid is present.

f. Flavonoids test

- **Lead acetate test**- Extract was treated with few drop of lead acetate solution. Yellow precipitate was formed.
- **Alkaline reagent test**- Extract was treated with few drop of sodium hydroxide (NaOH) in test tube yellow colour is formed. In this mixture added few drop of concentrated sulphuric acid (conc.H2SO4) mixture become colourless.

g. Triterpenoid and terpenoid test.

- **Salkowski's test**- Extract was treated with chloroform and filtered. This filtrate was added few drop of concentrated sulphuric acid (conc.H2SO4), shake it allowed to stand. Two layers are turn red, result that steroid are present.
- **Liebermann**- bur chard's test –fresh was treated with chloroform. This solution added few drops of acetic anhydride boiled it and cooled. Few drops of concentrated sulphuric acid were added through side of test tube. Observed that brown ring at junction of two layers was turned green indicating that a steroid is present.

h. Tannins and phenolic compounds

- **Ferric chloride test**- Extract dissolved in water. This solution added 2ml of 5% ferric chloride. Violet colour was observed.
- **Lead acetate test**- Extract was dissolved in distilled water. These mixture added few drop of lead acetate solution. White precipitate was observed.
- **Dilute iodine test**- Extract was, added dilute iodine solution were added. Red colour was observed.

Invitro Antioxidant Assay:

Determination of antioxidant activity of the leaves in Fragaria Vesca

1. DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control (Alam et al 2014).

The % radical scavenging activity of the plant extracts was calculated using the following formula,

Percentage inhibition = Ac – At*100/Ac

Ac = Absorbance of control, **At** = Absorbance of test

2. Reducing power assay

The Fe3+ reducing power of successive extracts of Fragaria Vesca. was determined by the method. The extract 0.75 ml at various concentrations (20-100 µg/ml) were mixed with 0.75 ml of phosphate buffer (0.2 M, pH.7.0) and 0.75 ml of potassium hexacyanoferrate [K3Fe(CN)6] (w/v 1 %), followed by incubating at 500 C in a water bath for 20min. The reaction was stopped by adding 0.75ml of trichloroacetic acid solution (TCA) (10 %) and then centrifuged at 3000rpm for 10 min. Then 1.5 ml of supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride solution (0.1 %w/v). After 10 min. the absorbance at 700 nm was measured as the reducing power. Higher the absorbance of the reaction mixture indicated greater the reducing power (Hemlatha et al 2013).

3. RESULT

Preliminary Phytochemical Analysis:

The phytochemical analysis of Fragaria Vesca were revealed the presence of various chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids, carbohydrate etc.

Table 1: Phytochemical Analysis of Fragaria Vesca

Phyto constituents	Chemical tests	Ethanol	Aqueous
Alkaloids	Dragendroffs	_	-
	Hager's test	_	-
	Mayer' test	_	-
	Wagner's test	+	-
Carbohydrate	Molish test	-	-
	Fehling's test	+	-
	Benedict's test	-	-
	Barfoed's test	-	-
Proteins	Biuret's test	+	+
	Milion's test	-	-
	Precipitation test	+	-
Amino tests	Ninhydrin test	-	-
	Xanthorproteic test	-	-
Steroids	Salkowski's test	+	_
Flavonoids	Shinoda test	+	+
Glycoside	Brontrager's test	-	-
	Legal's		
Tannins and Phenolic	Zinc HCl test	-	+
	With 5% ferric chloride	-	+
	With KMnO4	+	-
	With lead acetate	-	+

4. INVITRO STUDY

Determination of antioxidant activity of the Fragaria Vesca

a. DPPH radical scavenging activity

The DPPH radical scavenging activity increases with increasing concentration. Therefore in this study, the antioxidant properties of ethanol and aqueous extract of leaves of Fragaria vesca were examined for DPPH radical scavenging activity.

Scavenging of DPPH free radical by various extract

Table 2: scavenging % of DPPH free radical by various extract

Concentration (µg/ml)	Standard (Ascorbic acid) % scavenging	Ethanolic extract % scavenging	Water extract % scavenging
5	39.10	18.53	16.55
10	56.75	26.17	23.43
25	63.91	37.85	28.25
50	71.09	48.30	38.62
100	74.16	57.96	45.54
200	88.74	76.50	57.77

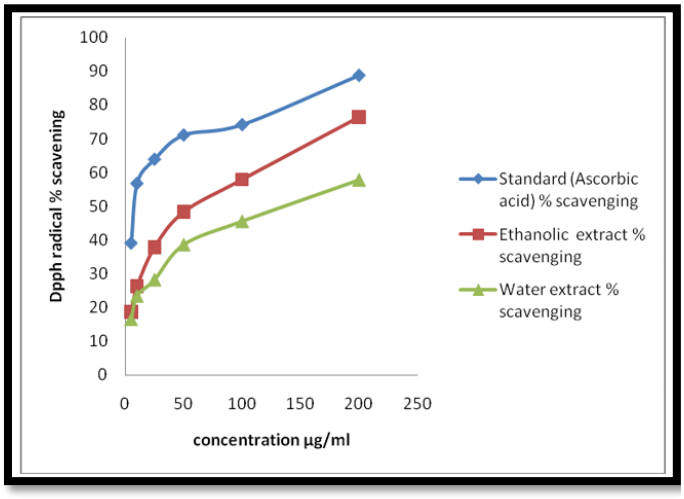

Fig 1: DPPH radical % scavenging

Table 3: Determination IC50 Value DPPH radical % scavenging

S.NO	SAMPLE	IC_{50} VALUE µg/ml
1.	Ascorbic acid	14.40
2.	Ethanolic extract	48.18
3.	Water extract	66.20

b. Reducing power

The reducing power of ethanol and aqueous extracts of Fragaria vesca increases with increase in the concentration. Ethanol and aqueous extract of Fragaria Vesca exhibited good reducing power. The antioxidant properties of ethanol and aqueous extract of leaves of Fragaria Vesca were examined for reducing power scavenging activity.

Scavenging of reducing power by various extract:

Table 4: Scavenging % of reducing power by various extract

Concentration (µg/ml)	Standard (Ascorbic acid) % scavenging	Ethanolic extract % scavenging	Water extract % scavenging
20	26.24	24.5	11.5
40	35.38	14.34	16.6
60	39.56	21.36	21.73
80	49.29	29.27	32.02
100	57.20	46.72	42.29
200	74.39	55.98	47.16

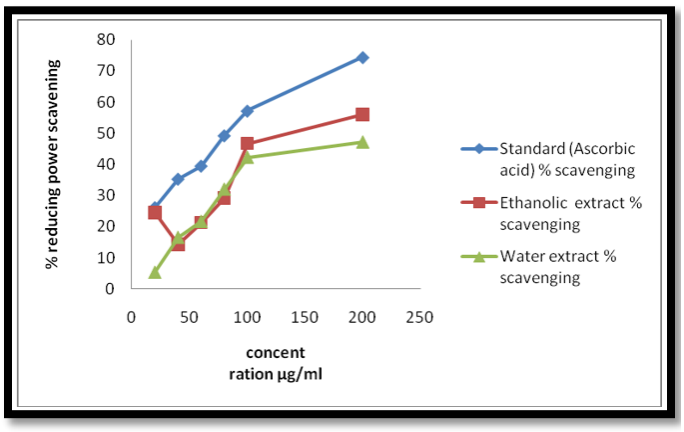

Fig 2: Reducing Power % scavenging

Determination IC50 value

Table 5: Determination IC50 Value Reducing power % scavenging

S.NO	SAMPLE	IC_{50} VALUE µg/ml
1.	Ascorbic acid	45.35
2.	Ethanolic extract	10.10
3.	Water extract	11.51

5. CONCLUSION

Resent herbal medicine research is widely used for treating any noxious disease, as per these evidence hints that fruits of Fragaria Vesca possess wide range of antioxidant potential. Antioxidant potentiality results indicate that it is much more useful in minimizing free radical disease.

6. FUNDING SUPPORT

None

7. CONFLICT OF INTEREST

There is no conflict of interest.

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