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Research Article

AN ASSESSMENT OF ANTIOXIDANT POTENTIAL AND *IN-VITRO* SPF ACTIVITY OF *EUGINIA JAMBOLANA AND* BETA *VULGARIS*

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ABSTRACT

Euginia jambolana and Beta *vulgaris* are the most common herbal dyes and its having strong antioxidant properties and Sun protection factor properties. These types of herbs contain various organic and inorganic molecules (pigments) and their mixture, it had nature of absorption light in the visible region of 400-800nm. The aim of present research work is to focus on analysis of the antioxidant activity and *in-vitro* Sun protection factor value of *Euginia jambolana and* Beta *vulgaris*. In the phytochemical analysis of *Euginia jambolana and Beta vulgaris* it was found that they contain glycosides, alkaloids, tannin, saponin and flavonoids as well as herbal dyes. The antioxidant properties of ethanolic extract of both herbs were analysed by 1,1-Diphenyl- 2-picrylhydrazyl percentage free radical inhibition activity. The antioxidant activity was found to be 91.59% ± 0.25% for *Euginia jambolana* and 23.94 ±32.29 % was found for *Beta vulgaris*. The Sun protection factor value was found to be 2.278 ± 0.127 *Eugenia jambolana*, and 2.195 ± 0.219 was found for *Beta vulgaris*. The future prospects of the study lie in developing novel suitable formulations of these herbal dyes.

Keywords: Antioxidants, SPF, DPPH, free radical, phytochemical

INTRODUCTION

Natural dyes are obtained from various herbs which have their own properties such as turmeric, saffron, pomegranate, tomato, indigo, beet root and black plums without any side effect[1]. In India, about 450 plants are known for yield natural dyes. Among this 50 plant are found to be more efficient natural dyes from plants.[2] These types of herbs contain various organic and inorganic molecules (pigments) and their mixture because of absorption of light in the visible region of 400-800nm. This absorption of light which is depend on the structure of chemical constituent of the colour pigment, which contains such kind of chromophores in the dye yielding herbs to display the plethora of colours. The current preference for the use of herbal colouring agent or dye is due to their healthfulness and excellent performance. And other hand synthetic colouring agent have been banned because they cause various side effect and harmful or are carcinogenic [3-4].

Herbal dyes are classified on the basis of their chemical structure are following as: Flavones (yellow and brown) mostly 90% of flavonoids are yellow and also having photosensitivity of the chromophore, Iso-quinolones (yellow colour), Chromene (orange yellow), Napthoquines (brown to purple grey), Anthraquinones (Red), Benzophenone (purple and black), Indigoids (blue) and Vegetable tannins (neutral) [5]. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins. The main characteristic of antioxidant is ability to trap the free radical. And various antioxidant activity methods are used to monitor and compare the antioxidant activity of herbs such as (e.g. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, the Superoxide anion radical (O2), the hydroxyl radical (OH), or the proxy radical (ROO) [6]. Such herbal dyes are used to protect the human body from harmful effect of UV radiation of sun rays. The Sun protection factor of herbs by comparing the amount of time needed to produce sunburn on sunscreen protected skin to the amount of time needed to cause sunburn on unprotected skin [7].

Now the herbal dyes are commonly used in cosmetic industries due to without any side effect by its use are safe for human skin, and also it protects from UV radiation and also having anti-ageing properties. In these article we studied about herbal dyes and comparison of antioxidant activity and *in-vitro* SPF value of *Euginia jambolana and Beta vulgaris* for development and formulation of herbal dyes product in future work.

MATERIAL AND METHOD

The fruit of *Euginia jambolana,* and Root of *Beta vulgaris,* leaves of were collected from different sources and its botanical identity was authenticated & confirmed by Taxonomist Dr. Smt. Ranjana Shrivastava Head of Dept, Botany Govt. V. Y. T. (P.G) Autonomous College, Durg (C.G.) & dried under shed. A voucher specimen has been deposited in Department of Botany, Govt. V. Y. T. (P.G) Autonomous College, Durg (C.G.) & Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari (C.G.).

Preparation of extract

Extraction of fruits of *Euginia jambolana* and root of *Beta vulgaris* can be done by decoction process. In this process, the drug is boiled with water for a stated period usually 10 minutes. After boiling, the liquid is strained and water is passed through the content of the strainer to make the required volume. This process is mainly used for vegetable drugs of hard and woody nature having thermos table water soluble constituents [8, 11, 15].

Evaluation of Physico-chemical properties

Evaluation of physicochemical properties can be determine by various physicochemical parameters such as total ash (acid insoluble ash, water soluble ash) , extractive value (water soluble extractive value, alcohol soluble extractive value) , loss on drying , were calculated as per Indian Pharmacopoeia in table no.2 and 3 [15,16,20] .

Preliminary phytochemical screening

Plant is a biosynthetically prepared in laboratory not only for the primary metabolites such as carbohydrates, proteins & lipid that are utilized as food by man but also for secondary metabolites like alkaloids, glycosides, tannins, volatile oil etc. that exerts a physiological & therapeutic effects. The mother extract obtained by successive decoction method were then subjected to various presence of qualitative chemical constituents such as glycoside , alkaloid , tannin , volatile oil , saponin, gum and mucilage etc are observed on table no.5 [21].

Antioxidant activity determination

The hydrogen atoms or electrons donation ability of polyphenol-rich extract was measured from the bleaching of purple colored methanol or ethanol solution of DPPH. This spectrophotometric method uses stable radical 1,1-Diphenyl- 2-picrylhydrazyl (DPPH) as a reagent [21, 22]. Four ml of the aqueous extracts dissolved in methanol or ethanol were added to 2.5 ml of a 0.1 mM solution of DPPH. After a 30 min incubation period at room temperature the absorbance was recorded against a blank at 515 nm. Percentage inhibitions of both extract were observed on table no. 6 [23-31].

Formula

I% = (Ao – As/ Ao) ×100

Where, I = Percentage inhibition DPPH activity,
Ao = The absorbance of the standard solution,
As = The absorbance of the test compound**.**

Determination of *In vitro* SPF (Sun Protecting Factor)

For sample preparation 100mg of extract was weighed and made up the volume up to 10 ml with aqueous (water) which gave 10,000 micro g ml of extract. Then 1 ml was taken out of it and made up the volume up to 10 ml which gave 1000 µg ml. Further took 2 ml of the above dilution and made up the volume up to 10ml which produced 200 µg ml of the extract. Then absorbance values of each aliquot prepared were determined from 290-320nm, at 5 nm intervals, taking water as blank using Shimadzu UV- Visible spectrophotometer (Shimadzu 1700, Japan) Value are shown in table no. 7 and 8 [54-57]. The efficacy of sunscreen is usually expressed by the Sun Protection Factor **(**SPF**) .**SPF is defined as the UV energy required to produce a Minimal Erythematic Dose **(**MED**)**on protected skin, divided by the UV energy required to produce a MED on unprotected skin **.**

SPF= Minimal erythematic dose in sunscreen – Protected skin / Minimal erythematic dose in non sunscreen – Protected skin

Where, MED is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythematic on unprotected skin**.** The higher the SPF, the more effective is the product in preventing sunburn**.** [31, 34-36]

The observed absorbance values at 5 nm intervals (290-320nm) were calculated by using formula:

Formula

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE(\lambda) \times 1(\lambda) \times Abs(\lambda)$$

Where, **CF**= correction factor [10]
EE (λ) = erythmogenic effect of radiation with wavelength λ., Abs (λ) = Spectrophotometric absorbance values at wavelength λ.
I (λ) = Intensity with wavelength λ.

RESULT AND DISCUSSION

Physicochemical parameter

The determination of physicochemical parameter is very important parameter to determination of adulterants and improper handling of drugs. Table no.1 and 2 shows the result of various physico-chemical parameters of herbs by using standard parameter. Loss on drying method used to determine the moisture content in herbs as per standard. Ash value used to determine the quality and purity of herbs. Ash values are indicative to some extent of care taken in collection and preparation of drug for market and of foreign matter content of natural drug. The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic constituents. Adhering dirt, sand as well as variation caused by calcium oxalate may be determined by acid-insoluble ash content. Total ash value usually consists of carbonates, phosphates, silicates & silica. The acid insoluble ash consists of mainly silica and indicates contamination with earthy matter. The water soluble ash is used to determination of the amount of inorganic elements present in drugs. The extractive values are useful to determination of the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent.

Table 1: Physico-chemical parameters of *Eugenia jambolana*

S.No.	Physical Constants	Yield	Standard.
1.	Loss of drying.	2.0%	N.M.T=3.0%
2.	Total ash value.	7.5%	**N.**M.T=11%
3.	Acid insoluble ash value.	0.05%	N.M.T=1%
4.	Water soluble ash value.	3.5%	N.M.T=5.0%
5.	Water soluble extractive value.	9.5%	N.L.T=11%

*Each result is the average of three measurements ±SD.

Table 2: Physico-chemical parameters of *Beta vulgaris*

S.No.	Physical Constants	Yield	Standard.
1.	Loss of drying.	55%	N.M.T= 79%
2.	Total ash value.	1.0%	N.M.T= 1.0%
3.	Acid insoluble ash value.	0.3%	N.M.T= 0.5%
4.	Water soluble ash value.	0.004%	N.M.T= 0.005%
5.	Water soluble extractive value.	17.0%	N.L.T= 20%

*Each result is the average of three measurements ±SD.

Preliminary phytochemical screening

The herbs or powdered drugs are showed different colour with different chemical reagents when seen on naked eye. The different colour are observed shows the presence with different phytoconstituents. The phytochemical screening is used to determine the presence of primary and secondary metabolites. Primary metabolites such as carbohydrates, lipid and proteins and other than secondary metabolites such as glycoside, alkaloids, saponin etc.

Table 3: Determination of phytochemical screening of *Euginia jambolana* and *Beta vulgaris*

S. No.	Chemical Tests for	*Eugenia jambolana*	*Beta vulgaris*
1.	Carbohydrates	+ve	+ve
2.	Proteins	-ve	-ve
3.	Amino Acids	-ve	-ve
4.	Alkaloids	-ve	-ve
5.	Glycosides Anthraquinones	-ve	-ve
	Cardiac.	-ve	-ve
	Cyanogenetic.	-ve	-ve
6.	Flavonoids.	-ve	-ve
7.	Steroids & Triterpenoids.	-ve	-ve
8.	Tannins & Phenolics	+ve	-ve
9.	Saponins	-ve	-ve
10.	Resins	-ve	-ve
11.	Mucilage	-ve	-ve
12.	Gum	-ve	-ve
13.	Fixed Oils	-ve	-ve
14.	Volatile Oils	-ve	-ve

* here +ve for presences, -ve for absent, and each result is the average of three measurements ±SD.

Antioxidant activity determination

The determination of antioxidant activity from natural product mostly DPPH method has been widely used. This method has such advantages of being an easy, stable and rapid way to study the antioxidant activity of herbal dyes or any other herbs, which acts as free radical scavengers. In this method, when DPPH reacts with an antioxidant compound, the colour changes from deep violet to yellow colour and get the absorbance at 517nm by using UV spectrophotometer. In this study, the DPPH absorption inhibition ranged from 91.59 ± 0.25% for *Euginia jambolana*, and 23.94±32.39 for *Beta vulgaris.* So it has been concluding that the *Euginia jambolana* extract have good antioxidant activity as compared with *Beta vulgaris.*

Table 4: Percentage inhibition of extract of *Euginia jambolana* and *Beta vulgaris*

Concentration(µg/ml)	% Inhibition of ascorbic acid	% Inhibition of *Euginia jambolana*	% Inhibition of *Beta vulgaris*
25	93.53%	91.59%	14.11%
50	93.81%	91.62%	23.94%
75	94.79%	91.65%	32.88%
100	94.87%	91.66%	45.30%
125	99.16%	91.70	56.53%

*Each result is the average of three measurements ±SD.

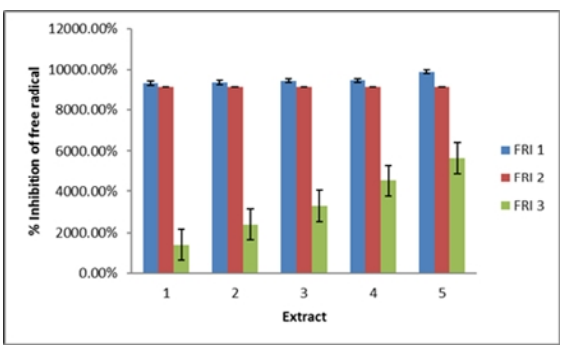


Figure 1. A graph represent the percentage of free radical inhibition activity by DPPH reagent, in which FRI 1 for L-ascorbic acid , FRI 2 for *Eugenia jambolana* extract , FRI 3 for *Beta vulgaris* extract.

The results are expressed as mean ± S.E.M. The statistical analysis values Statistical tests as well as mean and S.E.M calculations and graphical representation of result were performed.

***In-vitro* SPF value**

The *in-vitro* SPF value method has been widely used to study the sun protection factor of any photo protective herbs. It help us to study the prevention from sunburn and reduce the harmful effects of the sun such as premature skin aging and skin cancer by using *in-vitro* SPF value method. In this method ,SPF value of extracts were found to be for *Eugenia jambolana* 2.278 ± 0.127,for *Beta vulgaris* 2.195 ± 0.219.The herbs are used in the formulation and produced a stable photo protective herbal. *Eugenia jambolana* gives higher SPF value which is used as the combination with extract.

Table 5: *In vitro* SPF of *Eugenia jambolana*

Wavelength (nm)	EE(λ)×I(λ) Employed	Absorbance (A)	EE(λ) × I(λ) × Absorbance (A)
290	0.0150	0.1934 ± 0.001	0.0029 ± 0.000015
295	0.0187	0.1834 ± 0.001	0.0149 ± 0.000081
300	0.2874	0.1740 ± 0.002	0.05007 ± 0.000057
305	0.3278	0.1653 ± 0.01	0.00541 ± 0.0032
310	0.1864	0.157 ± 0.002	0.02932 ± 0.00037
315	0.0837	0.1466 ± 0.01	0.12270 ± 0.00837
320	0.0180	0.1393 ± 0.002	0.00256 ± 0.00003
			Σ= 0.2278 ± 0.0127

*Each result is the average of three measurements ±SD

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE(\lambda) \times 1(\lambda) \times Abs(\lambda)$$

=10 (0.2278 ± 0.0127)

SPF= 2.278 ± 0.127

Table 6: *In vitro* SPF OF *Beta vulgaris*

Wavelength (nm)	EE(λ) × I(λ) Employed	Absorbance (A)	EE (λ) × I (λ) × Absorbance.
290	0.0150	0.064 ± 0.02	0.0009 ± 0.03
295	0.0817	0.064 ± 0.02	0.0052 ± 0.002
300	0.2874	0.062 ± 0.02	0.0179 ± 0.008
305	0.3278	0.058 ± 0.01	0.0191 ± 0.005
310	0.1864	0.052 ± 0.01	0.0097 ± 0.004
315	0.0837	0.056 ± 0.05	0.0047 ± 0.004
320	0.0180	0.039 ± 0.001	0.0007 ± 0.00001
			Σ= 0.2195±0.0219

*Each result is the average of three measurements ±SD

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE(\lambda) \times 1(\lambda) \times Abs(\lambda)$$

= 10 (0.2195 ± 0.0219)

SPF = 2.195 ± 0.219.

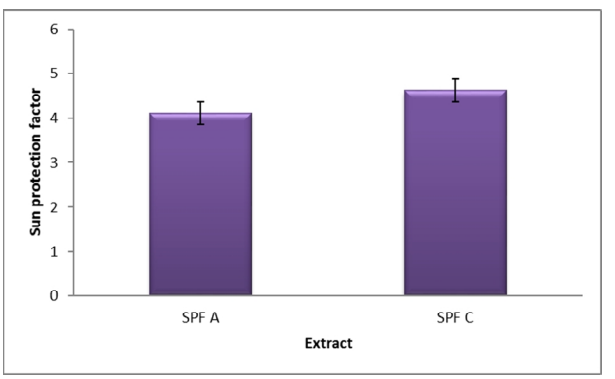


Figure 2. A graph represents the in-vitro SPF value of *Eugenia jambolana* extract and *Beta vulgaris* extract, where SPF A for *Eugenia jambolana* extract, SPF C for *Beta vulgaris* extract.

The results are expressed as mean ± S.E.M. The statistical analysis values Statistical tests as well as mean and S.E.M calculations and graphical representation of result were performed.

CONCLUSION

Herbal dyes are not only having colouring property but also having the wide range of medicinal properties like antioxidant, UV protection and antiageing properties. Nowadays, there is increasing awareness among people about herbal dyes. Due to their non-toxic properties, less side effects, more medicinal values, herbal dyes are used in food products and in pharmaceutical industry. Because of that we selected two herbs to study antioxidant activity and SPF value of *Eugenia jambolana* and *Beta vulgaris*. Phytochemical screening was carried out according to standard methods. Extracts shows the presence of carbohydrates, glycosides, flavonoids, tannins. It has been reported that topical application of *Eugenia jambolana* and *Beta vulgaris* extract prior to UV radiation result in significant protection against UV induced cutaneous edema and erythema. Hence selected plant extract could form an important constituent of photo protective formulation. By adding various constituent's product synergistic effects with herbal extract and its photo protective activity high range herbal photo protective formulation could be designed. .The herbs are used in the formulation and produced a stable photo protective herbal. *Eugenia jambolana* gives higher SPF value which is used as the combination with extract. The antioxidant activity was performed by the DPPH method. The antioxidant activity of the extracts was compared with the standard ascorbic acid .Antioxidant activity of ascorbic acid was considered 100% and activity for the other extracts was determined with respect to it. The *Euginia jambolana* extract have good antioxidant properties as well as SPF value so , this extract are safe because of that for further development of pharmaceutical industry to formulate the natural plant pigments into therapeutically beneficial pharmaceutical formulations/dosage forms for safe use.

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