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Study of Anthocyanin Content, Antioxidant Property, UV Absorbance & SPF Analysis of A Few Petals

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ABSTRACT:

Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion as well as for enhancement of the life span and quality of life. It is a well-known fact that Traditional Systems of medicine always played an important role in meeting the global health care needs. Herbal cosmetics use phytochemicals from variety of plants. The botanical ingredients present influence biological functions of skin and provides nutrients necessary for healthy skin. Skin care is major problem faced by many people due to increase in pollution and other factors. The aim of this study was to determine the Anthocyanin content, Antioxidant property, UV Absorbance & Sun Protection Factor (SPF) of few petals namely, Rose, Bougainvillea, Chrysanthemum, Dahlia, Marigold and Ixora. It was found that dry ethanolic and aqueous extracts of Dahlia, Chrysanthemum and Rose has high anthocyanin content and SPF value above 30 compared to other flower petals. Skin cream was prepared using aqueous extract of these three petals. Lavender oil was used for fragrance. Survey was conducted through questionnaire.

Though many plants are known to cure various diseases, knowledge of traditional medicine used by communities in India has been lost due to lack of documentation. Hence efforts were made to visit villages in Karnataka & Mumbai to collect data of plants used for primary health care.

Keywords: Anthocyanin, Antioxidants, UV Absorbance, Sun Protection Factor.

INTRODUCTION:

Plants are universally recognized as a vital part of the world's biological diversity and an essential resource for the planet. Many thousands of wild plants have great economic and cultural importance, providing food, medicine, fuel, clothing and shelter for humans around the world. Many plant species are threatened by habitat transformation, over-exploitation, invasive alien species, pollution and climate change, and are now in danger of extinction.

Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness" (World Health Organization). Ayurveda is a medicinal system primarily practiced in India that has been known for nearly5000years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment. Herbal therapy for skin disorders has been used for thousands of years. Even our biologically close relatives, the great apes, use herbal self-medication (Huffman, 2001).

Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and advanced civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. The benefits of one society were passed on to another, which upgraded the old properties, discovered new ones, till present day. The continuous and perpetual people's interest in medicinal plants has brought about today's modern and sophisticated fashion of their processing and usage.

Petals of brightly coloured flowers have high anthocyanin which is responsible for its bright colour. Anthocyanins helps to remove reactive oxygen species (ROS) hence exhibiting antioxidant property. The primary objective of this work was to identify petals extract with high anthocyanin content, antioxidant property and good SPF value (above 30) which was used to prepare herbal cream.The function of a skin cream is to protect the skin against harshness from the environment and any dry conditions of the skin. A skin cream should aid the skin in carrying out its normal functions. The chemical components sometimes have harmful effects on the skin. Because of that, they more and more choose products with natural components (Anitha, 2012).

MATERIALS AND METHODS:

Petals used for this study includes Rose – *Rosa sps.*, Bougainvillea – *Bougainvillea glabra*, Chrysanthemum – *Chrysanthemum sps.*, Dahlia – *Dahlia pinnata*, Marigold – *Tagetes sps.*, Ixora – *Ixora coccinea.* Drying is one of the oldest methods of food preservation and generally applied to extend the shelf life of the plants used for pharmaceutical purpose. Different storage time of dried wrinkled rose petals had no significant effect on polyphenol and anthocyanin content as well as on antioxidant activity measured in water and methanol extracts prepared from this dry material (Agnieszka

Zawislak *et al.,* 2014). In the ancient classical texts of Ayurveda there are several references of numerous medicinal plants and mode of application of their processed formulations for enhancing complexion, treating acne, treating dark patches, curing boils and carbuncles etc. (Kumar Sarvesh *et al.,* 2012).

Preparation of plant material:

The collected plant materials were thoroughly washed in tap water and distilled water to remove adhered dust particles. Then they were shade dried. Further the dried samples were powdered and used for the extraction of bioactive phytochemicals.

Preparation of plant extract:

Powdered sample weighing 1g was infused in 20ml of distilled water and ethanol and heated in a water bath maintained at 80^0 C for 20 minutes. The infused solution was then centrifuged at 3000 rpm for 10 minutes. The supernatant was used for further analysis.

Determination of *in vitro* antioxidant property: (Mensor *et al*., 2001)

DPPH (2,2-diphenyl-1-picrylhydrazyl) is dark coloured crystalline powder composed of stable free radical molecule. It is photosensitive chemical, hence prepared in a standard flask totally wrapped in aluminum foil.

The aqueous plant extracts were prepared and plated on ELISA reader plates at different concentrations and 100 microliter of DPPH solution was added to each. The antioxidant activity was measured using an ELISA reader. The results were calculated using the formula:

$$\text{DPPH inhibition (\%)} = \frac{\text{C.V} - \text{T.V}}{\text{C.V}} \times 100$$

Where C.V is Control Value and T.V is Total Value.

Anthocyanin Estimation:

0.5g of sample and 3ml acidified methanol (1% V/V HCl) was incubated in dark at 4^0 C for 24 hrs. 2ml DW and 4.8 ml of Chloroform was added. Centrifuge for 15 mins at 5000 rpm. Supernatant was collected and absorbance was taken at 530 nm & 657 nm (Ruohe Yin, 2010).

Preparation of extract for the study of UV absorption:

The aqueous and ethanolic plant extract was prepared to study the absorbance using UV-VISIBLE spectrophotometer at 290 nm to 320 nm at every 5nm of interval, using distilled water and ethanol as blank (Kaur and Saraf, 2011).

Sun Protection Factor (SPF) determination:

The *in vitro* determination of SPF was done by method described by Mansur *et al*., 1986. The aqueous and ethanolic extract was prepared. Then the absorbance of the extracts was determined from 290 nm to 320 nm at every 5nm interval, using distilled water and ethanol as blank. The results were calculated using the formula:

$$\text{SPF}_{\text{spectrophotometer}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where CF (Correction factor) is 10, EE (λ) is Erythmogenic effect of radiation with wavelength lambda, Abs (λ) is spectrophotometric absorbance values at wavelength lambda. The values of EE(λ)×I (λ) are constant. The obtained absorbance values are multiplied with EE(λ)×I (λ) and then their summation is taken and multiplied with correction factor to obtain the SPF values.

Preparation of Skin cream: (South Gloucestershire Beekeepers' Association, 2010)

The aqueous extract of dried petals which showed high anthocyanin content, antioxidant property and SPF value above 30 were mixed to prepare cream. Mixture of Rose, Dahlia and Chrysanthemum extract was used.

Melt 10g of beeswax wax in 50ml Almond oil. Get the temperature of this mixture to 70^0 C. Dissolve ¼ Teaspoon borax in 50ml extract. Warm this to 70^0 C. When both liquids were at the same temperature mix them together using a whisk and few drops lavender essential oil was added. Continue whisking until cream just starts to firm then pour into containers.

RESULTS AND DISCUSSION:

Antioxidant Property:

Samples	5µl	10µl	15µl
Rose	41.5	24.75	-20.25
Bougainvillea	-45.202	-152.525	-302.02
Dahlia	50	21.31148	-16.6667
Chrysanthemum	11.39241	-37.4684	-116.203
Marigold	60.54591	55.58313	37.22084
Ixora	66.92708	63.02083	-47.9167

Table 1: Aqueous Extract (Dry Petals)

Antioxidant Property:

Samples	5µl	10µl	15µl
Rose	66.24365	63.19797	70.55838
Bougainvillea	78.42566	76.67638	63.8484
Dahlia	68.40796	64.1791	59.95025
Chrysanthemum	66.90647	66.42686	74.82014
Marigold	66.50367	62.34719	58.19071
Ixora	76.05634	76.05634	63.09859

Table 2: Ethanolic Extract (Dry Petals)

UV Absorbance:

Samples	Wavelength(nm)						
	290	295	300	305	310	315	320
Bougainvillea	3.0138	2.8341	3.3113	2.9053	2.97	3.2648	2.985
Rose	3.0643	3.537	3.1355	3.2816	3.1125	3.0381	2.9138
Chrysanthemum	3.5641	3.2775	3.1014	3.3296	3.3737	3.1774	3.9565
Dahlia	3.5338	3.4153	3.5242	3.372	3.0917	3.4618	3.1749
Marigold	1.8784	2.7156	3.1414	3.2148	3.3254	3.0234	3.1351
Ixora	1.9175	2.7946	3.2631	3.2701	3.1749	3.3440	3.6747

Table 3: Aqueous Extract (Dry Petals)

UV Absorbance:

Samples	Wavelength(nm)						
	290	295	300	305	310	315	320
Bougainvillea	2.3763	2.3356	2.2692	2.4856	2.3651	2.3263	2.2253
Rose	2.2877	2.4055	2.4429	2.3875	2.4976	2.4019	2.364
Chrysanthemum	2.2573	2.2562	2.3175	2.2849	2.2367	2.2195	2.467
Dahlia	2.6014	2.6086	2.4658	2.5801	2.4103	2.5396	2.8403
Marigold	3.1727	2.5397	3.3254	3.1654	3.7180	3.5338	3.3737
Ixora	2.0299	2.9949	3.6555	3.4929	3.8060	3.2263	3.4136

Table 4: Ethanolic Extract (Dry Petals)

SPF Value:

SAMPLE	SPF
Rose	32.024
Bougainvillea	30.611
Chrysanthemum	32.706
Dahlia	33.734
Marigold	31.360
Ixora	32.046

Table 5: Aqueous Extract (Dry Petals)

SPF Value:

SAMPLE	SPF
Rose	23.947
Bougainvillea	23.690
Chrysanthemum	22.782
Dahlia	25.195
Marigold	32.979
Ixora	35.116

Table 6: Ethanolic Extract (Dry Petals)

Anthocyanin Content:

SAMPLE	Conc. (mg/0.1g)
Rose	0.088
Bougainvillea	0.065
Dahlia	0.092
Chrysanthemum	0.069
Ixora	0.019
Marigold	0.035

Table 7: For Dry Petals

Natural antioxidants present in herbs and spices are responsible for inhibiting the severe consequences of oxidative stress. Herbs and spices contain certain polyphenols, flavonoids and phenolic which possess radical scavenging activity. Antioxidant properties of plants have been shown due to high content of polyphenols and polyphenolic compounds (Maithri Shekar *et al.,* 2012). From the study conducted it was found that antioxidant property of ethanolic and aqueous extract of dry petals was high in 5µl concentration (Table 1 & 2). The UV Absorbance taken from 290nm – 320nm dry extracts of Dahlia,

Chrysanthemum and Rose showed good absorbance at 295nm (Table 3 & 4). The efficiency of sunscreens is characterized by the sun protection factor (SPF). The SPF is numerical rating system to indicate the degree of protection provided by a sun care products like sunscreen. The higher the SPF number, the greater the protection. The SPF number can range from as low as 2 to 60. These number refers to the product ability to block out the sun's burning rays (C. Malswmtluangi, 2013). SPF value above 30 is consider to be very good. SPF value of Dahlia, Chrysanthemum and Rose in aqueous extract was found to be above 30 (Table 5 & 6). Hence extract of these petals was used in cream preparation. The anthocyanin content of Dahlia petals was found to be 0.092 mg/0.1g (Table 7).

CONCLUSION:

The demand of herbal medicines is increasing rapidly due to their lack of side effects. Research trends in anti-aging skin care products are moving towards developing new plant extracts and botanical ingredients based on their traditional medicinal uses (Neha Singh *et al.*, 2014). Hence it is important to know more about the active constituents present in plants and use them effectively in herbal medicine preparations.

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