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Research Article

FORMULATION & *in-vitro* ANTIOXIDANT ANALYSIS OF ANTI-AGEING CREAM OF *CARICA PAPAYA* FRUIT EXTRACT

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ABSTRACT

Skin ageing is the outcome of constant deterioration process because of damage of cellular DNA and proteins. It is the phase of gradual decline of efficiency and metabolic activities. Free radical cause oxidative alterations in collagen, elastin materials and changes in membrane characteristics and encourage polymerization reactions. *Carica papaya* Linn. is one such herbal drug commonly known as papaya belongs to the family *Caricaceace*. The fruit is not just delicious and healthy, but whole plant parts, fruits, roots, bark, peel, seeds and pulp are also known to have excellent medicinal properties for the treatment of different diseases. Papaya is a rich source of three powerful antioxidants vitamin C, vitamin A and vitamin E; the minerals, manganese and potassium; the B vitamin pantothenic acid and folate and fibers. The present study is aimed to formulate topical anti-aging cream based on the antioxidant potential of herbal extract of papaya namely CPEAF, CPEE, CPXE, CPHAE, CPCE and CPPEE, thus obtained were stored in desiccator and suitable topical cream base for effective containing of fruit extract was developed namely FOEAF, FOEE, FOXE and FOHAE. The dry extracts and formulations were tested for in-vitro antioxidant activity. Results showed that dried extracts CPEAF and CPXE and their corresponding formulations FOEAF and FOXE are high in their reducing power and free radical scavenging (CPEAF > CPXE > CPEE > CPHAE) and (CPEAF > CPXE > CPEE > CPHAE). The study could be established that herbal creams without side effects can be used as a barrier to protect and avoid aging of skin.

KEY WORDS: Herbal cream, Carica, Anti-ageing, Antioxidant, Free radical Scavenging, Reducing Power, Cosmetics.

INTRODUCTION

Use of plants and plant products as medicines has been documented in the history since centuries. Volumes of literatures have been written describing the use of various herbs, shrubs and plants.[1] Natural compounds extracted and isolated from different parts of the plants such as leaves, fruits, stem, roots, and seeds have been shown to have admirable medicinal value[2]. Skin aging is the result of continual deterioration process because of damage of cellular DNA and protein. Aging process is classified into two different types, i.e. sequential skin aging and photo-aging. Both types have different medical and historical features. Sequential skin aging is widespread and probable process characterized by physiological alteration in skin function. In the aging process keratinocytes are unable to form an efficient stratum corneum and rate of formation from neutral lipids slows down resulting dry pale skin with wrinkle. In contrast, photo aging is instigated by over exposure to ultra violet rays from sunlight. It is characterized by dry, pale and thin skin, exhibiting fine wrinkles as well as deep furrows caused by the ineffectiveness of epidermal and dermal components linked with elastosis and heliodermatitis. Medicnal plants have already proved useful in complementary medicine.[3,4] The main objective of this paper is to evaluate the anti-ageing potential of *Carica papaya* fruit extract to evaluate its utility as skin

rejuvenator and as an antioxidant. Ageing is the stage of gradual wearying of body efficiency and metabolic activities after reaching a stage of maturity. Free radicals cause oxidative changes in collagen, elastin material and membrane characteristics that induce polymerization reactions. *Carica papaya* holds significant antioxidant and free radical scavenging potential. The basic objective of this thesis is to formulate & evaluate of anti-ageing cream of *Carica papaya* fruit extract in order to assess its usefulness in relieving of Oxidative stress and related conditions. This assessment is presented from both pharmacognostic and pharmaceutical perspective. Herbal products are very popular among people from the times immemorial due to their minimum risk of side-effects with maximum efficacy. The data generated from the study showed that the cream prepared from Papaya fruit extract showed significant better anti –ageing efficacy. Thus, the cream prepared from Papaya fruit extract may prove to be an anti-ageing preparation and can be used for retarding the symptoms of ageing. However, the results found are only directional and further study can be made on the basis to get additional data and evidence about the Papaya fruit, and combined effects of various botanical extracts can also be utilized on skin renewal. An antioxidant is a molecule capable of constraining the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals; these radicals could start chain reactions that cause damage to cells. Antioxidants cut off these chain reactions by removing free radical, and inhibit oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as tocopherol, ascorbic acid, thiols or polyphenols.[5]

Papaya, a juicy and tasty fruit, belonging to family *Caricaceae* is scientifically known as *Carica papaya L.* It is grown in various parts of the world, including India, tropical America and Europe. Papaya tree is basically a short lived Indian tree. Carica papaya contains many biologically active compounds. Two important compounds are Papain and Chymopapain, which aid in digestion. Papain is also used to treat arthritis.[6]

MATERIALS AND METHODS

The matured fruits of *Carica papaya* were collected from the local market of Roorkee city in the month of June and were authenticated by the Botanist of department of biology, S. S. D. P. C. Girls College, Roorkee. A voucher specimen (STIBAS/Corr/2014-15/1313) was deposited in the Department of Botany. Cetostearyl alcohol, cetomacrogol 1000, white petroleum jelly and propylene glycol was gift sample from Salud Care (I) Pvt. Ltd., Roorkee. All other chemicals and solvents were of analytical reagent grade.

Preparation of Extracts:

The Successive Extraction: Firstly the outer layer and seeds of the fruits were removed and the pulp was collected. The pulp was dried at temperature not exceeding 60ºC using hot air ovens (Universal Hot Air Oven). About 200g of dried fruit pulp was extracted for 8 hours with petroleum ether (40-60ºC) with soxhlet apparatus. The petroleum ether extract was filtered and air dried. The air dried extract was repacked in the soxhlet apparatus and exhaustively extracted with chloroform for 8 hours. The chloroform extract was filtered and again air-dried. Then extracted plant material was repacked in the soxhlet apparatus and exhaustively extracted with ethanol and water for 8 hours respectively. Extracts filtered and evaporated and their yield, color and consistency were recorded.

Extraction Method for Biflavones: Powdered drug (100 g) of *Carica papaya* was extracted separately in the soxhlet extraction apparatus using ethanol (95%) for 12 Hours. The resultant alcoholic extract was then air-dried and stored in vacuum desiccators. The dried alcoholic extract

was suspended in water. The alcoholic extract was mixed with *n*-hexane and the *n*-hexane portion was discarded after separation. To the aqueous portion, dichloromethane was added and the dichloromethane portion was collected and extracted with ethyl acetate. The ethyl acetate portion was collected and solvent was completely removed. The yield of the ethyl acetate fraction was noted. The ethyl acetate fraction was subjected to qualitative chemical test and thin layer chromatography studies for flavonoids.[7]

Extraction Method for Xanthones: Powdered drug (70 g) of *Carica papaya* was cold macerated (at 30-40ºC) using methanol (95%) for 24 hours. The alcoholic extract was then mixed with n-hexane (for the removal of the fatty material may be present in the extract) and the *n*-hexane portion was discarded after separation. Methanolic portion was collected and solvent was completely removed. The yield of the ethanol was noted. The ethanolic extract was subjected to qualitative chemical test and thin layer chromatography studies for Xanthone.[8]

Preparation of Topical Anti-Ageing Cream: The ingredients mentioned in the table were weighed accurately and prepared, by melting or heating, both the phases separately to same temperature i.e. 70ºC and mixed with continuous stirring till cream was formed. The different extract obtained from *Carica papaya* fruit were added in concentration of 5% at the end. Perfume was added after cooling of the product.[9]

Table 1: Formulation of Topical Anti-ageing Creams

S. No.	Name of Ingredients	QTY for 100 gm.
1	Cetostearyl Alcohol	8
2	Glyceryl Monostearate	2
3	Cetomacrogol 1000	2
4	Liquid Paraffin	5
5	White Petroleum Jelly	10
6	Propylene Glycol	20
7	Glycerine	5
8	Disodium EDTA	1
9	Ascorbic Acid	25
10	Purified Water	q. s.
11	Perfumes	q. s.

***In-vitro* Antioxidant activities**

Reducing Power:

Reducing power is linked with antioxidant activity and serves as a significant reflection of the antioxidant activity.[10] Compounds with reducing power are electron donors and can decrease the oxidized intermediates of lipid peroxidation processes; so that they can act as primary and secondary antioxidants.[11] The reducing power was determined by the method[12]of 1.0 ml sample was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of tri chloro acetic acid (600mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6mM) and absorbance was measured at 700 nm. Butylated hydroxy toluene (BHT) was used as positive control.[13]

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity:

Hydrogen Peroxide scavenging activity of sample was determined using a modification of the method by Gow Chin Yen and Hui-Yin Chen.[14] 4mM solution of H_2O_2 was prepared in phosphate–buffered

saline (PBS, pH 7.4). H_2O_2 concentration was determined using UV-VIS Spectrophotometer from absorbance at 230nm. 20-400 µg sample corresponding to 0.10, 0.15, 0.20, 0.25ml of 1mg/ml plant extract stock solution in 4ml distilled water were added to 0.6ml hydrogen peroxide-PBS solution. Absorbance of H_2O_2 at 230nm was determined 10 minutes later against a blank solution containing plant extract in PBS without H_2O_2. Different concentrations of Butylated hydroxy toluene (BHT) was added in place of plant extract, in 4ml distilled water and the solution was added to 0.6ml H_2O_2 solution in PBS. Absorbance was determined 10 minutes later against a blank solution similar to that above.[15] Percentage free radical scavenging activity was calculated using the formula:

$$\% \text{ SA} = \frac{A_C - A_E}{A_C} X100$$

Where A_C=Absorbance of control, A_E=Absorbance of Extract, % SA=Percentage scavenging activity

Free radical scavenging assay:

Effect of the sample extracts on DPPH radical was measured using this method. Amount of 200 µl of the sample extract (0.62 – 4.96 mg/ml) or ascorbic acid (0.04 – 1.28 mg/ml) were added to 1 ml of 0.1 mM DPPH in 80% methanol. The mixture was shaken vigorously and left to stand in dark room for 30 min at room temperature. Absorbance of the solution was measured using UV-VIS Spectrophotometer at 517 nm with deionized water as blank.[13, 16] the capability of sample to scavenge the DPPH radical was calculated according to the equation as follows:

$$\% \text{ SA} = \frac{A_C - A_E}{A_C} X100$$

Where A_C=Absorbance of control, A_E =Absorbance of Extract, % SA=Percentage scavenging activity

RESULT AND DISCUSSION

Reducing Power

The results of this study shows that the reducing power of the FOHAE was less active than all extracts and FOEAF, as defined by the intensity of UV absorption even its highest concentration was less active than the lowest concentration (25µg/ml) of BHT. **(Figure 1, Table 2)**, the ethyl acetate fraction shows intense absorptions as compared to other extracts. FOEE showed significant absorption only at higher concentration (400µg/ml), FOXE shows higher activity than FOEE and FOHAE but lesser than the BHT and ethyl acetate fraction. In an overall reducing power analysis the tests can be arranged as.

BHT > FOEAF > FOXE >FOEE >FOHAE

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity:

The results of this study shows that the reducing power of the FOHAE was less active than all extracts and FOEAF, as defined by the intensity of UV absorption even its highest concentration was less active than the lowest concentration (25µg/ml) of BHT. **(Figure 2, Table 3)**, the ethyl acetate fraction shows intense absorptions as compared to other extracts. FOEE showed significant absorption only at higher concentration (400µg/ml), FOXE shows higher activity than FOEE and FOHAE but lesser than the BHT and ethyl acetate fraction. In an overall reducing power analysis the tests can be arranged as.

BHT > FOEAF > FOXE >FOEE >FOHAE

Free radical scavenging assay

Effect of the standard (Ascorbic acid) and sample extracts on DPPH radical is shown in Table 4 and corroborated by Figure 3 at concentrations as low as 0.62 mg/ml, where STD had 34.33 % efficiency, FOEAF mopped up to 28.33 % as compared to 19.67 %, 15.22 % and 7.49 % for FOEE, FOXE and FOHAE respectively. Similarly, STD, FOEAF, FOEE, FOXE and FOHAE scavenged 78.89 %, 72.31 %, 53.34 %, 45.36 % and 27.42 % respectively at a maximum concentration of 4.96 mg/ml.

Conc. (µg/ml)	BHT	FOEAF	FOXE	FOEE	FOHAE

400	1.62±0.01	1.33±0.015	0.66±0.02	0.96±0.04	0.18±0.015
200	0.72±0.011	0.62±0.02	0.53±0.026	0.54±0.015	0.11±0.015
100	0.69±0.015	0.29±0.02	0.24±0.015	0.35±0.57	0.08±0.011
50	0.51±0.13	0.24±0.015	0.19±0.02	0.21±0.01	0.02±0.005

n=3±SD

Table 2: Reducing Power of BHT (Standard) and different formulation of Topical Anti-ageing Cream

Conc. (mg/ml.)	**Scavenging activity (in percentage)**				
	BHT	**FOEAF**	**FOEE**	**FOXE**	**FOHAE**
0.0250	41.73±0.20	38.72±0.15	31.48±0.10	29.57±0.12	10.23±0.14
0.0375	64.73±0.15	58.14±0.17	50.14±0.17	47.29±0.12	23.67±0.12
0.0500	82.35±0.15	73.49±0.19	66.84±0.15	67.03±0.10	31.82±0.18
0.0625	92.40±0.20	79.83±0.10	75.44±0.17	69.55±0.10	39.58±0.15

n=3±SD

Table 3: H_2O_2 Percentage scavenging of BHT (Standard) and various formulation of Topical Anti-ageing Cream

Conc. (mg/ml.)	**Scavenging activity (in percentage)**				
	STD	**FOEAF**	**FOXE**	**FOEE**	**FOHAE**
0.62	34.33±0.17	28.13±0.17	19.67±00.15	15.22±0.20	7.49±0.05
1.24	43.26±0.15	34.69±0.10	30.23±0.15	23.668±0.21	11.56±0.02
2.48	66.31±0.10	61.11±0.20	46.21±0.20	32.16±0.15	18.59±0.10
4.96	78.89±0.18	72.31±0.20	53.34±0.10	45.36±0.17	27.42±0.11

n=3±SD

Table 4: Free radical scavenging of Standard (Ascorbic acid) and various formulation of Topical Anti-ageing Cream

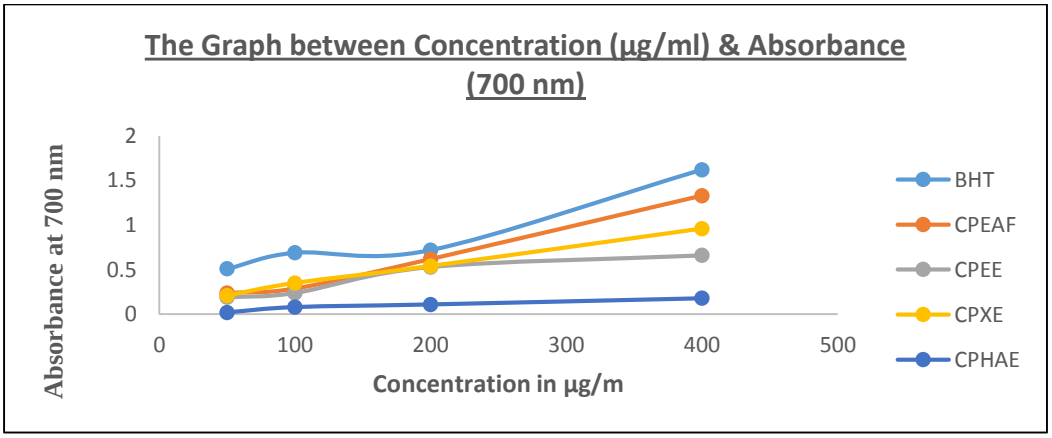

Figure 1: The graph between Concentration (µg/ml) & Absorbance at 700 nm

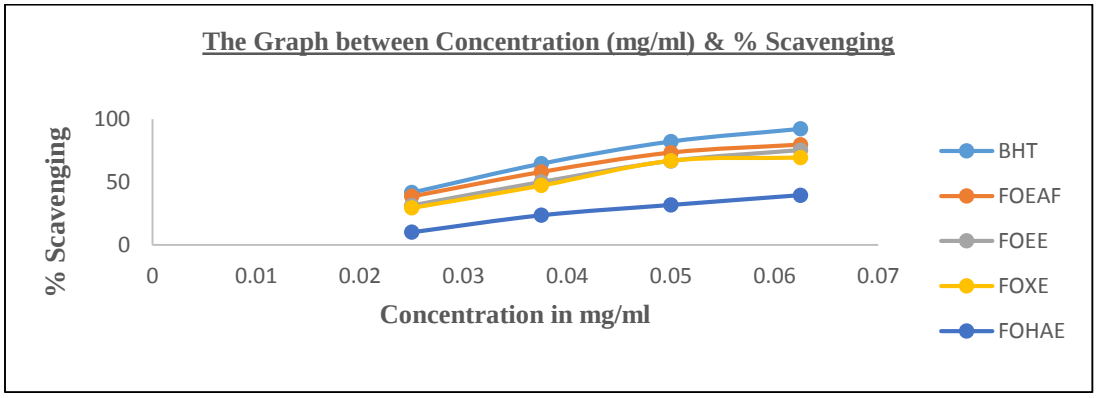

Figure 2: The Graph between Concentration (mg/ml) & % Scavenging

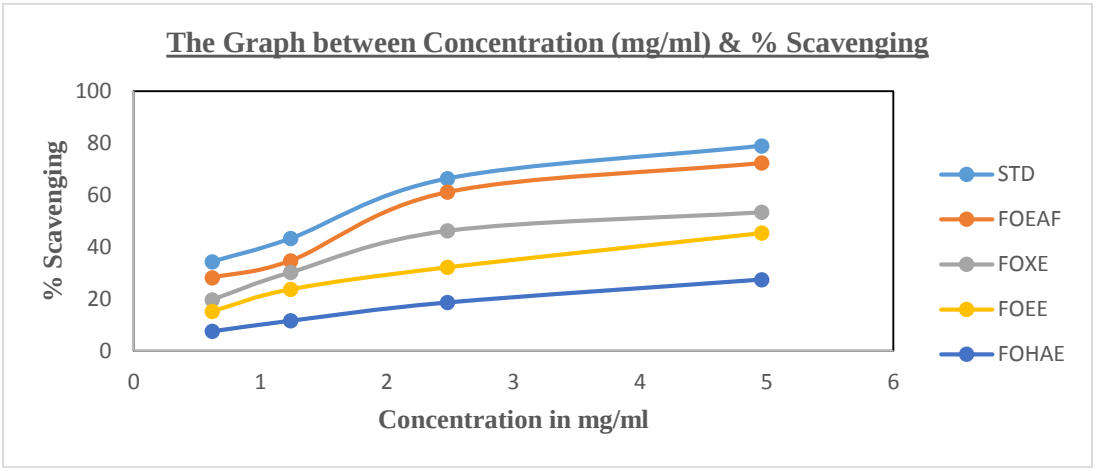

Figure 3: The Graph between Concentration (mg/ml) & % Scavenging

CONCLUSION

From all results and considerations it can be determined that the cream prepared from *Carica papaya* fruit extract showed significantly better anti-ageing efficacy as compared to the control. The cream prepared from *Carica papaya* fruit extract is an ideal oil-in-water cream with good consistency, smooth & shining texture, good stability and may prove to be an anti-ageing preparation and can be used for retarding the symptoms of ageing and showing skin-renewal activity. The outcomes obtained in this study are only directional and further investigations can be made on this ground, to get additional data and information about *Carica papaya* fruit, and combined effects of various botanical extracts can also be studied on skin renewal.

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