Extraction and Clinical Application of *Calendula officinalis* L. Flowers Cream

Khulood M. Alsaraf, Ibrahim S. Abbas, Eman F. Hassan

1 Department of Pharmacy / University of Al-Esraa / Iraq
2 Collage of Pharmacy / University of Mustansiriyah / Iraq
3 Collage of Agriculture / University of Kerbala / Iraq

ABSTRACT

The results of the HPLC analysis indicated that marigold flowers extract contain nine different active compounds, including Vitexin 11.40%, Rutin 12.29%, Quercetin-3-3galactosid 12.64%, Luteolin-7-glucose 9.27%, Quercetin-3-glucoside 7.38%, Quercitrin 9.83%, Myricetin 10%, Luteolin 10.72%, Apigenin 7.08% and kampferol 9.37%. The results of a clinical study showed the effect of marigold flowers extract cream as an antioxidant which protected the skin in particular from oxidative damage after sunburn and reduced the symptoms of skin aging. This effect was evident in both concentrations 10% and 15% when compared with control. Control response rate, 10% of marigold flowers cream and 15% of flowers extract cream were reached to 76%, 85% and 92% within two weeks of treatment respectively. The Conclusion of this study showed the importance of marigold flowers extract as a source of bioactive compounds such as rutin and quercetin derivatives, vitexin, luteoline, apigenin and kampferol which act as an antioxidant to restore skin health and aging resistance. This study approved that marigold flowers extract characteristics can make it the main ingredients in the preparation of topical agents for the treatment of various skin desases.

Keywords: Marigold, Apigenin, kampferol, vitexin.

Introduction

It's widely known that the largest organ in the body is the skin; it's about twenty square feet of total area. The main functions of the skin are the protection of the body from invasive elements and microbes in particular, regulating the temperature of the body and allows the sense of touch, cold and heat [1]. There are different factors that affect the skin such as ultraviolet (UV) radiation, dehydration, invasion of microorganisms like bacteria, mechanical trauma and physical injuries [2].
The chemical products used for cosmetics or skin care are gaining attention because of the negative effect on skin and health. Also the chemical additives of skin care products may lead to skin irritation and allergic reactions [3], such as the harmful effect of some preparation containing steroids for skin bleaching. Recently the herbal or medicinal plants were used as source for skin care products. The extracts of herbs showed more powerful antioxidant activity and keep the skin looking not only healthy but also more youthful [4]. The most benefit of herbal extracts in skin care is belongs to their natural agents such as omega-3, vitamins, flavonoids compounds such as apigenin and quercetin. Also the plant parts extracts contain natural nutrients like vitamin E that keeps skin healthy [5]. The marigold (*Calendula officinalis*) plant (fig.1)

![Plate of *C. officinalis* L.](image)

was considered as one of the most important medicinal plants. The activity of marigold is belonging to active compounds such as flavonoids that considers as free radicals scavenging and antioxidant [6].

The aim of this study was included the colaction of plant flowers, extraction and detection of active compound with evaluation the clinical activity as skin nutritive agent.

**Materials and Methods**

**Plant material**
The flowers of marigold plant were collected during April season from medicinal plants garden of pharmacognosy department of college of pharmacy/ Almustansiryah University. The flowers were cleaned and dried at room temperature in the shade for about 10 days.

**Extraction of flavonoids**

100 gram of flower powder was extracted by soxhlet apparatus with ethanol (90,500 ml) till exhaustion. The extract was concentrated by using rotary evaporated then mixed with distilled water (50 ml) and extracted with ethyl acetate (ethyl acetate 30 ml ) the upper layer (ethyl acetate layer ) was separated by a separator funnel, then dried by using anhydrous sodium sulphate, and labeled as Ethyl acetate extract ( as shown in scheme 1 )[7]

![Scheme 1: Extraction of flavonoid](image)

**Test for flavonoids**

Ethanolic KOH (2ml) was added to 1 ml of ethanolic extract of marigold flowers, yellow color indicates the presence of flavonoids [8].

**HPLC analysis Method**

Column phenomenex C-18, 3 micrometer particle size (50X 2.0 mm I.D Column ) Shimadzul C- 6A.Japan .
**Mobile phase:**

Linear gradient of solvent A 0.1 % formic acid: Solvent B was (6.3: 1, V/V) of acetonitrile: methanol: 0.1 % formic acid, gradient program from 0 % B to 100% for 10 minutes. Flow rate 1.2 ml / min. [9].

**Preparation of marigold Cream:**

Oil in water (O/W) emulsion – based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (olive oil; cetyl alcohol) then added the flower extract. The components were heated to 75 °C. The preservatives (methyl paraben, propyl paraben) and other water soluble components (triethanolamine, propylene glycol) were dissolved in the aqueous phase and heated to 75 °C, after heating the aqueous phase was added in portions to the oil phase with continuous stirring until cream is formed then left at room temperature until cooled.

**Characterization of marigold cream formulas:**

1- Physical properties: The cream was observed for color, odor and appearance [6].
2- Determination of PH: About 0.05g of the cream weighed and dissolved in 50 ml distilled water and its pH was measured [7].
3- Viscosity: Rheological measurement regarded as sensitive tools for detecting structural changes in pharmaceutical cream and should be regarded as an integral part of the quality evaluation of pharmaceutical creams.

The Viscosities of formulated sufflawer creams were measured by Brook Field Viscometer (DV –II) at temperature .

**Clinical study**

The clinical study was carried out at AL-Razi center for alternative medicine, Bagdad / Iraq. Ninety patients were included in this study and divided to three groups randomly, group treated with conventional treatment (nutritive cream vitamins A, C and E lipolic complex) as control, group B treated with 10 % marigold cream and Group C treated with 15 % marigold cream .All symptoms such as skin aging, skin damaged by the sun light, oxidative
damage after sun burn or after chemicals treatment were followed for 6 months. All clinical studies were conducted in accordance with the ethical committee of college of pharmacy / Almustansiriya University.

Statistical analysis

The analysis of the statistical data was conducted by the Spss statistical program and all the recorded data was applied for analysis of variance (ANOVA) and least significant difference (LSD) at level 0.01 was used to compare the studied parameters [10].

Results

HPLC analysis of marigold flowers extract

The results were referred to the extract, had different flavonoids glycosides including Vitexin 11.40%, Rutin 12.29%, Quercetin-3-3galactosid 12.64%, Luteolin-7-glucose9.27%, Quercetin-3-glucoside 7.38%, Quercitrin 9.83%, Myricetin 10%, Luteolin 10.72%, Apigenin 7.08% and kampferol 9.37 % as shown in Fig2 and 3.

Fig. 2: Active fractions of *Calendula officinalis* oil
Properties of marigold cream

The prepared cream formula as shown in table (1) was white in color with good odor and elegant and homogenized appearance. The produced cream ph was recorded to be 6 which are perfect for the intended topical application hence the skins pH is from 4.5 to 6. The cream's viscosity was found to be different at different revolution per minute (rpm).

Viscosity was decrease at 0.5 rpm to 20rpm from 4897 to 442 centipoise . If the rate was decreased, the viscosity of the cream will increase. Therefore the cream viscosity is inversely proportional to the rate of the shear.

Table [1]: Composition of marigold cream

| Compositions   | F1    | F2    |
|----------------|-------|-------|
| Flowers extract| 10 %  | 15 %  |
| Stearic acid   | 6 %   | 6 %   |
| Cetyl alcohol  | 3 %   | 3 %   |
| Propylene glycol| 4 %  | 4 %   |
| Triethanolamine| 2 %   | 2 %   |
| Methyl paraben | 0.05 %| 0.05 %|
| Propyl paraben | 0.05 %| 0.05 %|
| Distilled water| QS    | QS    |

F1: Formula one  
F2: Formula two  
QS: quantity for saturation

Clinical study

Age and sex distribution in this study is presented in table (2). The age groups (19 -24), (24 – 30), (30-40) and (40 -55) were about 67.7%, 15.55%, 8.88 % and 7.77 respectively. The percentage of males about 23.33% and female percentage about 76.66% .

The results in table (3) showed the effect of marigold flowers extract cream as antioxidant agent which protect the skin specifically in face from oxidative damage after sun burn and decrease the symptoms of skin aging, this effect was obvious in both concentrations 10 % and 15% when compared with control.

The percentage of response for control, 10% marigold cream and 15% marigold cream were reached to 76%, 85% and 92% on 2 weeks of treatment respectively, while these percentages were reached to 81% and
92% for 10% marigold cream and 15% marigold cream respectively during 4 weeks of treatment the percentage of control reached to 71 % and 75 % and respectively at 2 and 4 weeks of treatment. The percentage of control, 10 % marigold cream and 15% marigold cream after 2 weeks from the end of treatment were reached to 73 %, 80 % and 91 % respectively.

**Table 2: Age and sex distribution in the study population**

| Age ( year) | control | 10% marigold cream | 15% marigold cream | Total |
|-------------|---------|--------------------|--------------------|-------|
| 19 - 24     | 20      | 66.66              | 18                 | 60    | 23 | 76.66 | 61 | 67.7 |
| 24 - 30     | 3       | 10                 | 8                  | 26.66 | 3  | 10    | 14 | 15.55 |
| 30 - 40     | 4       | 13.33              | 2                  | 6.66  | 2  | 6.66  | 8  | 8.88 |
| 40 - 55     | 3       | 10                 | 2                  | 6.66  | 2  | 6.66  | 7  | 7.77 |
| Total       | 30      | 100                | 30                 | 100   | 30 | 100   | 90 | 100 |

**NU = Number**

**SD: stander deviation value**

**LSD: least significant difference**

**Discussion**

Hydrophilic creams were chosen for incorporation of Marigold extract because of several positive properties. As the emulsions o/w with a hydrophilic external phase, they are miscible with water and skin secretions and thus are easily removed from skin or clothing [11]. They are not occlusive because they leave little residue on the skin. Properly designed o/w creams are elegant systems, pleasing in both appearance and fell post application [12].

The activity of marigold cream as antioxidant and nutritive for skin may be belonging to its compounds such as rutine, quercetine, apigenin, luteolin, kampferol and vitexin. The reported pharmacological actions of plant extract as well as pure compounds isolated from it have been demonstrated to possess multiple pharmacological activities such as cytotoxic, anti-inflammatory activity, antibacterial and antiviral activity, that effective in treatment of acne and wound healing [13].

Marigold was highly efficacious in the prevention of acute dermatitis in cancer patients undergoing postoperative irradiation [14].
Medicinally the Marigold flower extract was used to decrease inflammation and stimulate the growth of skin and mucus membranes [15] [16]. The hydro alcoholic extract of Marigold can suppress the activities of 5-lipoxygenase (5LO) and cyclooxygenase-2 (COX-2) (key enzymes) in the formation of pro inflammatory eicosanoids from arachidonic acid. Marigold extract was found to scavenge superoxide radicals generated by photo reduction of riboflavin and hydroxyl radicals generated by Fenton reaction and inhibited in vitro lipid peroxidation [17]. Pharmacological studies have confirmed that Marigold exhibit a broad range of biological effects, such as antimicrobial and immunomodulatory effect [18]. Anti-inflammatory [19]. Antioxidant [20]. Wound healing [21]. And anti-tumoral [22]. Consequently, preparations with plant extracts rich in the above active constituents, such as Marigold extract, may provide the wide range of therapeutic, protective or preventive effects [23] [24].

Conclusion
Depend on the results of this study the marigold flowers extract can be used in burns, cuts, rashes, acne, eczema, psoriasis and any other skin conditions. Also the marigold flowers extract has a high content of flavonoids that act as antioxidants. Also depend on antioxidant activity, marigold cream can used as natural skin nutritive agent.

References
1- Jolene H. Eat prity.2014. Nutrition for beauty, inside and out. China: Edition 1.
2- Geneva, 2005; Available from: http// www.who. Int/ iris / handle / 10665 / 69229.
3- Bucca B. pino A. Almonti A. Forte G. 2014. Toxic metals report Requ. Toxicol pharmacology. 68 (3): 447 – 67.
4- Ibrahim S. Abbas . 2013. Effect of biological competition of weeds on Growth and volatile Oil yield of marigold (Calendula officinalis L.) As medicinal plant used in herbal medicine of Iraq. Int.J. of pharm. And pharmaceutical Research. 6(1). 217 – 219.
5- Bashir S, Giliani AH .2008. Studies on the antioxidant and analgesic activities of Aztec marigold (Tagetes erecta) flowers. phytother Res. 22: 1692 -1694 . doi: 10.1002 / ptr .2550 .[PubMed ] [cross Ref].
6- Khare Cp.2004.Encyclopedia of India medicinal Plants. Germany, Springer – verlag Publisher, 2004; 116 -117.

7- Dania F, Alsaffar, Ibrahim S. Investigation of the main Alkaloid of London Rocket (Sisymbrium irol) as a wild medicinal plant grown in Iraq. International Journal of Pharmaceutical Sciences Review and Research. 2016; 39(1):279-281

8- Thamer M., Suhad F., Ibrahaim S.Aljubory, Qabas N.letef. 2016. Phytochemical Study and Antibacterial Activity of Crude Alkaloids and Mucilage of Cordia myxa in Iraq. Int.J.pharm Sci-Rev.Res. 39(1). No.45. 232: 238.

9- Noor S.Jaafar, Maha N. Hamad, Ibrahim S. Abass, Iman S. Jaafer.2016. Qualitative phytochemical comparison between flavonoids and phenolic acids contents of leaves and fruits of melia azedarach (family: meliaceae) cultivated in Iraq by HPLC and HPTLC. Int J. of Pharmacy and Pharmaceutical Sciences. Vol.8 (10) .242-250.

10- Steel G .D.R, J .H.1960. Terrie. Principles and procedures of statistics. Mc. Graw – Hill press New York.

11- Betageri G, Prabhu S. 2002. Semisolid preparations. In: Swarbrick J, Boylan JC (eds) Encyclopedia of Pharmaceutical Technology. 2nd ed., vol. 3. New York, Basel: Marcel Dekker, Inc., pp. 2436-2457.

12- Flynn GL. 2002. Cutaneous and transdermal delivery – Processes and systems of delivery. In: Banker GS, Rhodes CT (eds) Modern Pharmaceutics. 4th ed. New York, Basel: Marcel Dekker, Inc., pp. 187-235.

13- BP. Muley, SS. Rhadabadi, NB. Banarase. 2009. Phytochemical Constituents and Pharmacological Activities of Calendula officinalis Linn (Asteraceae): A Review, Tropical Journal of Pharmaceutical Research, 8(5): 455.

14- Jimenez –Media, E. Garacia – Lora, A. Paco, L .Algarral, L. Collado, A, Garride F. 2006. A new extract of the plant calendula officinalis produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation, BMC cancer. 6.119.

15- Renata Dawid. 2013. Medicinal plants used in treatment of inflammatory skin diseases. J. Postepy Dermatol Alergol. Jun; 30(3): 170–177.

16- Jura Bernatoniene, Ruta Masteikova. 2011. Topical application of Calendula officinalis (L.): Formulation and evaluation of hydrophilic cream with antioxidant activity. J. of medicinal plant research. April; 5(6):868-877.
17- Herold A, Cremer L, Calugaru A, et al. 2003. Hydroalcoholic plant extracts with anti-inflammatory activity. Roum Arch Microbiol Immunol. 62(12):117–129.

18- Attard A, Cuschieri A. 2009. In vitro immunomodulatory activity of various extracts of Maltese plants from the Asteraceae family. J. Med. Plant. Res., 3(6): 457-461.

19- Amoian B, Moghadamnia AA, Mayandarani M, Amoian MM, Mehrmanesh S. 2010. The effect of Calendula extract on the plaque index and bleeding in gingivitis. Res. J. Med. Plant. 4(3):132-140.

20- Fonseca YM, Catini CD, Vicentini FTMC, Nomizo A, Gerlach RF, Fonseca MJV. 2010. Protective effect of C. officinalis extract against UVB-induced oxidative stress in skin: Evaluation of reduced glutathione levels and matrix metalloproteinase secretion. J. Ethnopharmacol. 127: 596-601.

21- Preethi KC, Kuttan G, Kuttan R. 2009. Anti-inflammatory activity of flower extract of C. officinalis Linn. and its possible mechanism of action. Ind. J. Exp. Biol., 47: 113-120.

22- Ukiya M, Akihisa T, Yasukawa K, Tokuda H, Suzuki T, Kimura Y. 2006. Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (C. officinalis) flowers. J. Nat. Prod. 69: 1692-1696.

23- Freedman BM. 2009. Topical polyphenolic antioxidants reduce the adverse effects of intense pulsed light therapy. J. Cosmet. Laser Ther. 11(3): 142-145.

24- Hadfield RA, Vlahovic TC, Khan T. 2008. The use of marigold therapy for podiatric skin conditions. Foot Ankle J. 1(7): 1-8.