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Assessment of *In-vitro* Free Radical Scavenging Activity of *Calendula officinalis* flower Extract using Fenton Reaction

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Research Article

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ABSTRACT

Calendula officinalis is a good source of natural antioxidants, particularly phenolic substances, and other compounds that trap harmful free radicals, It is also known as “marigold” and has been a subject of several chemical and pharmacological studies. It is used in traditional medicine, especially for wound healing, jaundice, blood purification, and as an antispasmodic. There has been growing interest in the health benefits associated with natural compounds and have been demonstrated with the emphasis on antioxidants. The present study focused on the antioxidant activity of *Calendula officinalis* flower Extract in vitro conditions. The total phenolics content of leaf as determined by fenton reaction and was found to be good antioxidant activity as different dose concentrations. The Reducing power of extract was carried out with ascorbic acid as a standard reducing agent. In this plant *Calendula officinalis* flower extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that *Calendula officinalis* flower extract possess free radical and hydroxyl radical scavenging activity as well as antioxidant activity *in vitro*. In conclusion the present study indicates that *Calendula officinalis* flower extract may be a potential source of natural antioxidant.

INTRODUCTION

Calendula officinalis, commonly known as marigold, is used in the Western and Asian countries for its anti-inflammatory properties. They are popular medicinal herbs well known throughout the world because of their vast areas of biological activities such as antimicrobial, anti-oxidant, anti-mutagenic, hepatoprotective, healing and anti-inflammatory. The species *Calendula suffruticosa Vahl*, is a perennial and viscous herbaceous plant, belonging to the family *Asteraceae* and reaching about 40 cm in height; the stem is upright. It is usually wooded at some distance above the base, simple or little branched. The leaves are of a pale green color and lanceolate, slightly wavy and toothed. According to some reports, the extract of this plant possesses some pharmacological activities which include antioxidant action, anti–inflammatory, antibacterial, antifungal, and antiviral properties[1]. Results of one clinical trial showed that *Calendula officinalis* was highly effective in the prevention of acute dermatitis in patients with cancer undergoing postoperative irradiation. It was observed that this plant has cytotoxic effect on tumor cell lines in vitro and anticancer activity *in vivo*. Numerous phytochemical investigations carried out on Calendula species such as *C. officinalis* and *C. arvensis* show that they constitute an enormous reservoir of

potentially active natural molecules, the majority of which are essential oils, Flavonoids, the saponosides, carotenoids organic acids, saccharides, sterols and lipids [2]. These reported pharmacological activities might be related to the antioxidant activity of Calendula extract, although this was not fully substantiated [3]. In fact, there are only very few reports on the antioxidant activity of Calendula extract, and in this report we have done a systematic investigation on the antioxidant potential of this extract in vivo test system. Therefore, we have planned out the antioxidant activity of the *Calendula officinalis* extract using fenton reaction [4].

MATERIALS AND METHODS

Plant material

Calendula officinalis flower was collected from local herbal garden, Raipur (Chhattisgarh), India.

Reagent and authentic samples

The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany). Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by varian.

Preparation of extract

The ability to ferment D-xylose was tested in tubes containing YEPX broth and incubated at 28°C on shaker for 24 h then tubes were kept at stationary condition for 48 h. The yeast cultures were checked for their ability to utilize the pentose sugar (xylose) by plating the isolates on YEPA containing 2% xylose.

Determination of ethanol producing ability of different yeast isolates

Dried powdered of *Calendula officinalis* flower (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After filtration, methanol was evaporated until only water remained through evaporation on water bath at 60-70°C temperature. The dried powder was kept in air tied box.

Deoxyribose assay to assess OH-radical scavenging activity

The OH-radical scavenging activity of *Calendula officinalis* flower extract (10–100 ug/ml) was determined according to the deoxyribose method of in the presence of 100 IM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 IM EDTA, 1 mM H_2O_2, 100 IM L-ascorbic acid, 100 IM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38ºC, 1 hr, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture which was then heated in a boiling water bath for 15 min. Once samples were cooled, the absorbances were read at 532 nm [5]. The IC50 value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent inhibition of hydroxyl radical was calculated as follows: Antioxidant capacity of test compounds was expressed as IC50, the concentration necessary for 50% inhibition concentration of TBARS [6].

$$\% \text{ Inhibition} = \frac{\text{Abs:532 nm Control Abs-532 nm sample Abs} \times 100}{\text{532 nm Control Abs}}$$

Results

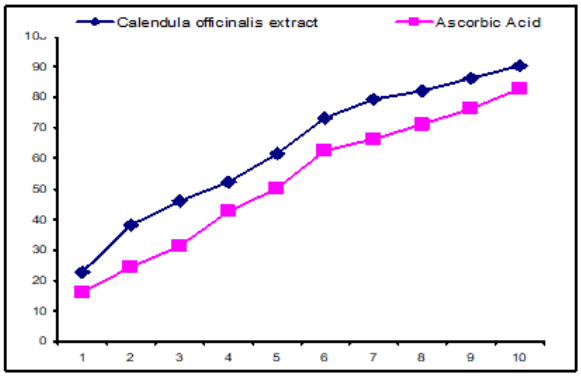
The results of the effects of the examined *Calendula officinalis* flower extract as well as control solutions on OH-radical production. They show that all extract of *Calendula officinalis* flower extract and control solutions as a DMSO inhibited the production of OH-radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Calendula officinalis* presented in Table 1 have reducing power, the free radial OH-scavenging activity of the extract increases with increasing the concentration.

Table 1. Antioxidant activities of *Calendula officinalis* flower extract using fenton reaction

Constrictions (in µl)	% of inhibition	
	Ascorbic Acid	*Calendula officinalis* flower extract
10	22.71	16.40
20	38.13	23.20
30	46.12	29.30
40	50.30	36.75
50	61.35	45.20
60	73.10	54.20
70	79.52	61.85

80	84.28	68.56
90	88.17	73.56
100	96.42	78.56
Blank: 0.4320		

Figure 1. Antioxidant activity of *Calendula officinalis* flower extract



DISCUSSION

Plant kingdom is a huge reservoir of phytochemical ingredients, many of which have been explored for various pharmaceutical applications. Various epidemiological studies have shown a consistent relationship between a diet rich in polyphenolic phytochemical ingredients and a lower risk for many chronic diseases including cancer, heart disease, obesity and type 2 diabetes due to strong free radicals and ROS scavenging properties of these phytochemical ingredients [7-14]. The current study indicates that *Calendula officinalis* flower extract effectively scavenged hydroxyl radicals *in vitro*. These radicals are generated inside the body during the normal metabolism or in presence of xenobiotics. The stable free radicals OH were also scavenged by *Calendula officinalis* extract. These results show that *Calendula officinalis* has a profound effect on the antioxidant defense system *in vitro*. *Calendula officinalis* has been reported to contain flavonoids (including lutein, quercetin, protocatechuic acid, etc.), triterpenoids, and the alkaloid narcissin. Flowers also are rich in carotenoids of which flavoxanthin has been reported to be present at 28.5% of total carotenoids followed by luteoxanthin [15-22]. Flowers are also found to contain lycopene and b-carotene. Coumarins are also an active ingredient in *Calendula officinalis*. These ingredients may contribute to the antioxidant potential of this extract.

CONCLUSION

Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc. Experimental data revealed that there might be correlation between total phenolic and antioxidant capacity of different extracts of lemon grass. However, some literature demonstrated that antioxidant was not solely dependent on phenolic content but it may be due to other phytoconstituents as tannins, triterpenoid or combine effect of them. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as in vivo and in vitro assays will be necessary in discovering new biological antioxidants. In the present study, the phenolic content of *Calendula officinalis* flower extract was found to be high which might have responsible for its antioxidant and free radical scavenging activity in the in vitro study models. Thus our results were congruent with the findings of others. Further studies can be designed to prove the antioxidant activity of *Calendula officinalis* flower in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it. Observations of the study suggested that ethanolic floral extract has high phenolic, flavonoids, β-carotene, lycopene, tannin, chlorophylls contents as compared to aqueous extract of *C. officinalis*. Further phytochemical constituents in ethanolic floral extract endowed with high total antioxidant activity, free, superoxide anion, nitric oxide, hydroxyl radicals scavenging activity and reducing power, though the activity was low as compared to ascorbic acid. Thus, the dietary supplementation ethanolic extract of *C. officinalis* may provide protection against degenerative changes associated with free radicals induced damage (aging, cancer, alzheimer disease, etc) besides improve the food quality by retarding oxidative degeneration of food lipids.

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REFERENCES

1. Akihisa T, et al. Triterpene alcohols from the flowers of *compositae* and their anti-inflammatory effects. Phytochemi. 1996;43:1255-1260.
2. Albulescu M, et al. *Calendula officinalis* flowers, source of extracts with antioxidant activity. Series Chemistry. 2004;13:169-70.
3. Beniston N, et al. Fleurs d'Algérie. Enterprise National du Livre. 359;1984.
4. Boucaud-Maitre Y, et al. Cytotoxic and antitumoral activity of *Calendula officinalis* extracts. Pharmazie. 1988;43:220-221.
5. Chandran PK, et al. Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. J Clin Biochem Nutr. 2008;43(2):58-64.
6. Cordova CA, et al. Protective properties of butanolic extract of the *Calendula officinalis L.* (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. Redox Rep. 2002;7:95-102.
7. Couplan F. Plants and their names: Unusual stories. Plants and their names. 2012:1-224.
8. Green LC, et al. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical biochemistry. 1982;126:131-138.
9. Halliwell B, Gutterridge JMC (1985): Free Radicals in Biology and Medicine. Oxford, Clarendon Press,627–630
10. Couplan F, et al. Plants and their names: Unusual stories. Plants and their names. 2012:1-224.
11. Green LC, et al. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical biochemistry. 1982;126:131-138.
12. Halliwell B, Gutterridge JMC. Free Radicals in Biology and Medicine. Oxford, Clarendon Press. 1985;627–630.
13. Herold A, et al. Antioxidant properties of some hydroalcoholic plant extracts with antiinflammatory activity. Roum Arch Microbiol Immunol. 2003;62:217-227.
14. Hung HC, et al. Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst. 2004;96:1577-1584.
15. Khalid KA, et al. Biology of *Calendula officinalis* Linn: focus on pharmacology, biological activities and agronomic practices. Medicinal and Aromatic Plant Science and Biotechnology. 2012;6:12-27.
16. Kırmızıbekmez H, et al. Triterpene saponins from *Calendula arvensis*. Zeitschrift für Naturforschung B. 2006;61:1170-1173.
17. Kishimoto S, et al. Analysis of carotenoid composition in petals of calendula *(Calendula officinalis L.)*. Bioscience, biotechnology, and biochemistry. 2005;69:2122-2128.
18. Matysik G, et al. The influence of *Calendulae officinalis* flos extracts on cell cultures, and the chromatographic analysis of extracts. J Pharma Biomedical Anal. 2005;38:285-292.
19. Pommier P, et al. Phase III randomized trial of *Calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. J Clinic Oncol. 2004;22:1447-1553.
20. Schröder H, et al. Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. J Nutr Biochem. 2007;18:149-160.
21. Tutin T, et al. Flora Europaea: *Plantaginaceae* to *Compositae* (and *Rubiaceae*). Cambridge University Press.534;1976.
22. Wilen RW, et al. Feasibility of cultivation calendula as a dual purpose industrial oilseed and medicinal crop. Acta Horticulturae. 2004:199-206.