

**ASSESSMENT OF IN-VITRO FREE RADICAL SCAVENGING
ACTIVITY OF *CALENDULA OFFICINALIS* FLOWER EXTRACT
USING FENTON REACTION**

***Priyanka Pandey, Wasim Raja and S. Karmakar**

Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur-
492014, Chhattisgarh, India.

Article Received on
12 May 2022,
Revised on 02 June 2022,
Accepted on 23 June 2022
DOI: 10.20959/wjpr20229-24748

***Corresponding Author**

Priyanka Pandey

Central Laboratory Facility,
Chhattisgarh Council of
Science and Technology,
Raipur- 492014,
Chhattisgarh, India.

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.

KEYWORDS: Antioxidant activity, Fenton Reaction, Hydroxyl radical, ascorbic acid, *Calendula officinalis*, Flower Extract, TBARS.

1. INTRODUCTION

Calendula officinalis, commonly known as Marigold, is used in the Western and Asian countries for its anti-inflammatory properties (Akihisa, et. al., 1996). 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 (Beniston, 1984). It is usually wooded at some distance above the base, simple or little branched (Tutin, et. al., 1976). 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 (Chandran and Kuttan, 2008). 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 (Pommier, et. al., 2004). It was observed that this plant has cytotoxic effect on tumor cell lines in vitro and anticancer activity in vivo (Boucaud-Maitre, et. al., 1988). 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 (Couplan, 2012), Flavonoids, (Wilen, et. al., 2004) the saponosides (Kirmizibekmeza, et. al., 2006), Carotenoids (Khalid, et. al., 2012) Organic acids, saccharides, sterols and lipids (Albulescu, et. al., 2004). These reported pharmacological activities might be related to the antioxidant activity of *Calendula* extract, although this was not fully substantiated. In fact, there are only very few reports on the antioxidant activity of *Calendula* extract (Cordova et al., 2002; Herold et al., 2003), 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.

2. 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 - 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 tight box.

Deoxyribose assay to assess OH⁻ radical scavenging activity

The OH⁻ radical scavenging activity of *Calendula officinalis* flower extract (10–100 µg/ml) was determined according to the deoxyribose method of Halliwell, *et. al.*, (1985) in the presence of 100 µM EDTA. FeCl₃, H₂O and ascorbic acid were prepared in degassed H₂O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 µM EDTA, 1 mM H₂O₂, 100 µM L- ascorbic acid, 100 µM FeCl₃, H₂O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38° C, 1 hrs, 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. 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.

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

Antioxidant capacity of test compounds was expressed as IC₅₀, the concentration necessary for 50% inhibition concentration of TBARS.

3. RESULT

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 radical scavenging activity of hydro-methanolic extract of *Calendula officinalis* presented in Table 1 have reducing power, the free radical 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 μ l)	% of inhibition	
	Ascorbic Acid	<i>Calendula officinalis</i> 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		

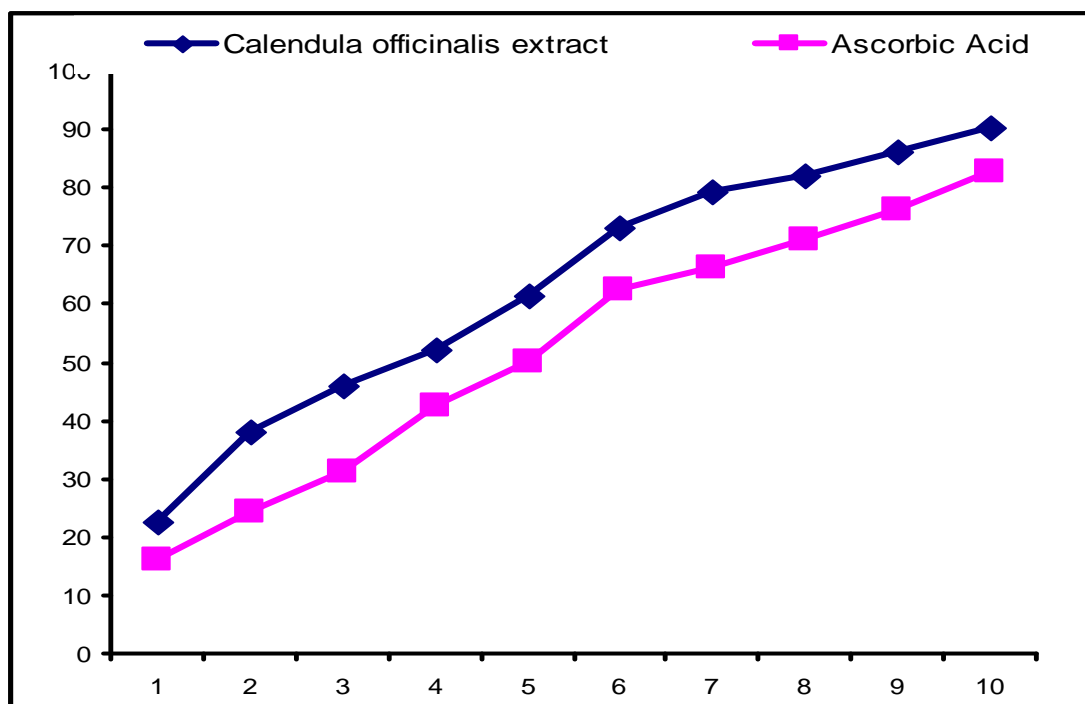


Fig. 1: Antioxidant Activity of *Calendula officinalis* flower extract.

4. 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, (Green, 1982) heart

disease (Hung, 2004), obesity and type 2 diabetes (Schroeder, 2007) due to strong free radicals and ROS scavenging properties of these phytochemical ingredients.

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 (including faradiol, oleanolic acid, beta-amyrin, calenduladiol, etc.), and the alkaloid narcissin (Matysik et al., 2005). Flowers also are rich in carotenoids of which flavoxanthin has been reported to be present at 28.5% of total carotenoids followed by luteoxanthin (Kishimoto et al., 2005). Flowers are also found to contain lycopene and β -carotene. Coumarins are also an active ingredient in *Calendula officinalis*. These ingredients may contribute to the antioxidant potential of this extract.

5. 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.

ACKNOWLEDGMENT

The authors are thankful to Director General, Chhattisgarh Council of Science and Technology, Raipur (Chhattisgarh) India, for providing facility and technical support to carry out the above work.

6. BIBLIOGRAPHY

1. Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M, Kumaki K. Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. *Phytochemistry*, 1996; 43: 1255–1260.
2. Albulescu M, Alexa N, Cojan C. *Calendula Officinalis* flowers, source of extracts with antioxidant activity. *Annals of West University of Timisoara. Series Chemistry*, 2004; 13(2): 169-76.
3. Beniston N, Beniston W. *Fleurs d'Algérie*. Enterprise National du Livre, p.359; 1984.
4. Boucaud-Maitre Y, Algernon O, Raynaud J. Cytotoxic and antitumoral activity of *calendula officinalis* extracts. *Pharmazie*, 1988; 43: 220–221.
5. Chandran PK, Kuttan R. 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: 58–64.
6. Cordova CA, Siqueria IR, Netto CA, Yunes RA, Volpato AM, Cechinel Filho V, Curi-Pedrosa R, Crecynski-Pasa TB (2002): Protective properties of butanolic extract of the *Calendula officinalis* L. (marigold) against lipid peroxidation of the rat liver microsomes and as free radical scavengers. *Redox Rep*, 7(2): 95–102.
7. Couplan F, *Les plantes et leurs noms: Histoires insolites*. Editions Quae, 2012; 223.
8. Green LC, Wagner DA, Glogowski J. Analysis of nitrate, nitrite and (15N) nitrate in biological fluids. *Anal Biochem*, 1982; 126: 131-138.

9. Halliwell B, Gutteridge JMC (1985): Free Radicals in Biology and Medicine. Oxford, Clarendon Press, pp. 107–170, 627–630.
10. Herold A, Cremer L, Calugaru A, Tamas V, Ionescu F, Manea S, Szegli G (2003). Antioxidant properties of some hydroalcoholic plant extracts with anti inflammatory activity. Roum Arch Microbiol Immunol, 62(3–4): 217–227.
11. Hung H, Joshipura KJ, Jiang R, et al. Fruit and vegetable intake and risk of major chronic disease. J Nat Cancer Inst, 2004; 96: 1577- 1584.
12. Khalid KHA, Teixeira JA. Biology of *Calendula Officinalis* Linn: focus on pharmacologic, biological activities and agronomic practices. Medicinal and Aromatic Plant Science and Biotechnology. Medicinal and Aromatic Plant Science an Biotechnology, 2012; 6(1): 12-27.
13. Kirmizibekmeza H, Bassarello C, Piacente S, Pizza C. Triterpene Saponins from *Calendula arvensis*. Verlag der Zeitschrift f'ur Naturforschung, 2006; 61(9): 1170-3.
14. Kishimoto S, Maoka T, Sumitomo K, Ohmiya A (2005): Analysis of carotenoid composition in petals of *Calendula* (*Calendula officinalis* L.). Biosci Biotechnol Biochem, 69(11): 2122–2128.
15. Matysik G, Wojciak-Kosior M, Paduch R (2005): The influence of *Calendula officinalis* flos extract on cell cultures and the chromatographic analysis of extracts. J Pharm Biomed Anal, 38(2): 285–292.
16. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon X. Phase III randomized trial of *calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. J Clin Oncol, 2004; 22: 1447–1453.
17. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon X (2004): Phase III randomized trial of *Calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Clin Oncol, 22(8): 1447–1453.
18. Schroeder H. Protective mechanisms of the mediterranean diet in obesity and type 2 diabetes. J Nutr Biochem, 2007; 18: 149- 160.
19. Tutin T, Heywood H. Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae). Cambridge University Press, 1976; 534.
20. Wilen RW, Barl B, Slinkard AE. Feasibility of Cultivation *Calendula* as a Dual Purpose Industrial Oil seed and Medicinal Crop. Future for Medicinal and Aromatic Plants, 2004; 199-206.