Research Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

EVALUATION OF *IN-VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *CALENDULA OFFICINALIS LEAF* EXTRACT

Priyanka Pandey*[1], Ashis Kumar Sarkar[1], Amit Kumar Dutta[1], Wasim Raja[2]

[1*]School of Studies in Chemical and Biological Sciences, MATS University, Raipur, Chhattisgarh, India.
[2]Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India.

ABSTRACT

The *Calendula officinalis* Linn belong to family Asteraceae is used medicinally in Europe, China and India amongst several places in the world. The local name of this plant is "marigold" and has been a subject of several chemical and pharmacological studies. In traditional medicine system it is used especially for wound healing, jaundice, blood purification, and as an antispasmodic. This plant was also useful in the health benefits associated with natural compounds and have been demonstrated with the emphasis on antioxidants. The phenolic compound in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities. The present study focused on the antioxidant activity of *Calendula officinalis* Leaf Extract in vitro conditions. The dried leaf of *Calendula officinalis* was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer. In this plant *Calendula officinalis* Leaf Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that *Calendula officinalis* Leaf 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* Leaf Extract may be a potential source of natural antioxidant. The results suggested that *Calendula officinalis* Leaf Extract could serve as a potential source of antioxidant and can be used in any preparations for combating free radical mediated damage to the body.

KEYWORDS

Antioxidant activity, Fenton Reaction, Hydroxyl radical, Ascorbic acid, Calendula officinalis and TBARS.

Author for Correspondence:

Priyanka Pandey,
School of Studies in Chemical and Biological Sciences,
MATS University, Raipur, Chhattisgarh, India.

Email: priyankapandey2907@gmail.com

INTRODUCTION

Calendula is a genus of about 12 to 20 species originating from Macaronesia and the Mediterranean (Paolini, *et al*, 2010)[1]. This plant is well known medicinal herbs throughout the world because of their vast areas of biological activities such as antimicrobial, anti-oxidant, anti-mutagenic, hepatoprotective, healing and anti-inflammatory.

Another species of *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)[2]. It is usually wooded at some distance above the base, simple or little branched (Tutin, *et al*, 1976)[3]. *Calendula officinalis* leaves are of a pale green color and lanceolate, slightly wavy and toothed. The flowers of this plant are united in capitules exceeding 2 cm in diameter and of yellow or orange color, the peripheral flowers are tied and united in two rows, while those in the center are tubular and toothed; the fruit is an achene often curved and equipped with peaks (Beniston, 1984)[2]. 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)[4], Flavonoids, (Wilen, *et al*, 2004)[5] the saponosides (Kirmizibekmeza, *et al*, 2006)[6], Carotenoids (Khalid, *et al*, 2012)[7] Organic acids, saccharides, sterols and lipids (Albulescu, *et al*, 2004)[8].

Free radicals generated either exogenously or endogenously inside the body have been implicated in causation of several diseases such as liver cirrhosis (Slater, 1987)[9], inflammation, atherosclerosis (Halliwell and Gutterridge, 1985)[10], diabetes and cancer (Dreher and Junod, 1996)[11], neurodegenerative disease (Knight, 1997)[12], and so forth. The link between free radicals and disease processes has led to considerable research into nontoxic drugs that can scavenge the free radicals. Many plant extract have been shown to possess significant antioxidant potential (Soudhamini and Kuttan, 1989[13], Jose and Kuttan, 1995[14], Sabu and Kuttan, 2003)[15]. *Calendula officinalis* Linn. (Compositae), an herb employed in traditional medicine, has been reported to have several pharmacological activities. The topical application of Calendula officinalis extract was found to possess significant anti-inflammatory activity (Della Logia, *et al*, 1994)[16]. This plant extract also possessed wound-healing activity (Rao *et al*, 1991)[17]. *Calendula officinalis* extract was found to possess preventive activity against acute dermatitis during irradiation during the clinical trials (Pommier *et al*, 2004)[18]. *Calendula officinalis* flower extract was reported to possess an antigenotoxic effect (Perez-Carreon *et al*, 2002)[19]. These finding related to pharmacological activities might be related to the antioxidant activity of Calendula extract, although this was not fully substantiated. According to literature, there are only very few reports on the antioxidant activity of Calendula extract (Cordova *et al*, 2002[20], Herold *et al*, 2003)[21], 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.

MATERIAL AND METHODS

Plant material

Calendula officinalis leaf was collected from Local Herbal Garden, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples

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

Preparation of extract

Dried powdered of *Calendula officinalis* leaf (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 ^{0}C temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Calendula officinalis* bark extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*, 1987[22]. In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final

concentrations) 3.6mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º 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 after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

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

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

RESULTS AND DISCUSSION

The results of the effects of the examined *Calendula officinalis* leaf extract as well as control solutions on OH- radical production. They show that all extract of *Calendula officinalis* leaf *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 No.1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

The body's innate mechanism has many enzymes and nonprotein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. Major compounds include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione, which also play a major role in detoxification and coordinate the body's antioxidant defense processes. The superoxide dismutase is a metalloprotein that scavenges superoxide anions. Catalase is a heme protein, localized in the peroxisome or the microperoxisome, which catalyzes the decomposition of H2O2 to water and oxygen and thus protects the cell from oxidative damage produced by H_2O_2. The glutathione peroxidase catalyzes the reaction of hydroperoxides, which reduces glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide. Glutathione reductase is involved in the regeneration of glutathione that has been converted to GSSG by oxidation and thiol transfer reactions. Glutathione, a major nonprotein thiol, is mainly involved in detoxification (Halliwell and Gutterridge, 1985)[10].

The current study indicates that Calendula officinalis extract effectively scavenged hydroxyl radicals *in vitro*. This types of free 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*.

The *Calendula officinalis* extract 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)[23]. *Calendula officinalis's* 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)[24]. Calendula officinalis's flowers extract are also found to contain lycopene and b-carotene. The active ingredient of Calendula officinalis is Coumarins and this ingredients may contribute to the antioxidant potential of this extract.

Calendula has several ingredients with reported antioxidant activity. The Calendula officinalis extract was found antioxidant potential and having the ability to trigger cellular antioxidants, can be exploited for its use against a number of disorders including cardiovascular diseases, inflammation, and cancer.

Table No.1: Antioxidant activities of *Calendula officinalis* extract using Fenton reaction

S.No	Constrictions (in µl)	% of inhibition	
		Ascorbic Acid	*Calendula officinalis extract*
1	10	22.71	16.28
2	20	38.13	24.23
3	30	46.12	31.25
4	40	52.30	42.50
5	50	61.35	50.10
6	60	73.10	62.63
7	70	79.52	66.41
8	80	82.28	71.21
9	90	86.17	76.28
10	100	90.42	82.85
11	Blank: 0.4320		

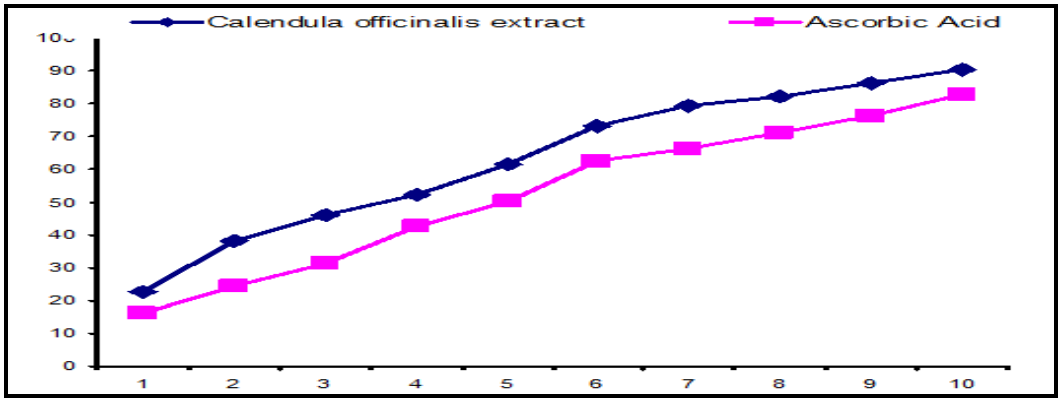

Figure No.1: Antioxidant Activity of *Calendula officinalis* leaf extract

CONCLUSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. 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. Some literature on Calendula officinalis are 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 bark 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 leaf in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude School of Studies in Chemical and Biological Sciences, MATS University, Raipur, Chhattisgarh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Paolini J, Barboni T, Desjobert J M, Djabou N, Muselli A, Costa J. Chemical composition, interspecies variation and seasonal variation in essential oils of Calendula arvensis L, *Biochemical Systematics and Ecology,* 38(5), 2010, 865-874.
2. Beniston N, Beniston W. Fleurs d'Algerie, *Enterprise National du Livre,* 1984, 359.
3. Tutin T, Heywood H. Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae), *Cambridge University Press,* 1st Edition, 1976, 505
4. Couplan F. Les plantes et leurs noms: Histoires insolites, *Editions Quae,* 1st Edition, 2012, 223.
5. Wilen R W, Barl B, Slinkard A E. Feasibility of Cultivation Calendula as a Dual Purpose Industrial Oil seed and Medicinal Crop, *Future for Medicinal and Aromatic Plants,* 626(629), 2004, 199-206.
6. Kirmizibekmeza H, Bassarello C, Piacente S, Pizza C. Triterpene Saponins from Calendula arvensis, *Verlag der Zeitschrift for Naturforschung,* 61(9), 2006, 1170-1173.
7. Khalid K H A, Teixeira J A. Biology of Calendula Officinalis Linn: focus on pharmacologic, biological activities and agronomic practices, *Medicinal and Aromatic Plant Science and Biotechnology,* 6(1), 2012, 12-27.
8. Albulescu M, Alexa N, Cojan C. Calendula Officinalis flowers, source of extracts with antioxidant activity, Annals of West University of Timisoara, *Series Chemistry,* 13(2), 2004, 169-176.
9. Slater T F. Free radicals and tissue injury: Fact and fiction, *Br J Cancer,* 55(8), 1987, 5-10.
10. Halliwell B, Gutterridge J M C. Free Radicals in Biology and Medicine, *Oxford, Clarendon Press,* 3rd Edition, 1985, 107-170, 627-630.
11. Dreher D, Junod A F. Role of oxygen free radicals in cancer development, *Eur J Cancer,* 32A(1), 1996, 30-38.
12. Knight J A. Reactive oxygen species and the neurodegenerative disorders, *Ann Clin Lab Sci,* 27(1), 1997, 11-25.
13. Soudhamini K K, Kuttan R. Inhibition of chemical carcinogenesis by curcumin, *J Ethnopharmacol,* 27(1-2), 1989, 227-233.
14. Jose J K, Kuttan R. Antioxidant activity of Emblica officinalis Gaertn, *J Clin Biochem Nutr,* 19(2), 1995, 63-70.
15. Sabu M C, Kuttan R. Antioxidant activity of Indian herbal drugs in rats with alloxan induced diabetes, *Pharm Biol,* 41(7), 2003, 500-505.
16. Della Loggia R, Tubaro A, Sosa S, Becker H, Saar S, Isaac O. The role of triterpenoids in the topical anti-inflammatory activity of Calendula officinalis flowers, *Planta Med,* 60(6), 1994, 516-520.
17. Rao S G, Udupa A L, Rao G, Rao P G M, Kulkarni D R. Wound healing activity of Calendula officinalis, *Fitoterapia,* 62(6), 1991, 508-510.
18. Pommier P, Gomez F, Sunyach M P, 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, *Clin Oncol,* 22(8), 2004, 1447-1453.
19. Perez-Carreon J I, Cruz-Jimenez G, Licea-Vega J A, Arce Popoca E, Fattel Fazenda S, Villa-Trevino S. Genotoxic and anti genotoxic properties of Calendula officinalis

extracts in rat liver cell cultures treated with diethylnitrosamine, *Toxicol in Vitro*, 16(3), 2002, 253-258.

20. Cordova C A, Siqueria I R, Netto C A, Yunes R A, Volpato A M, Cechinel Filho V, Curi-Pedrosa R, Crecynski-Pasa T B. 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), 2002, 95-102.
21. Herold A, Cremer L, Calugaru A, Tamas V, Ionescu F, Manea S, Szegli G. Antioxidant properties of some hydroalcoholic plant extracts with anti inflammatory activity, *Roum Arch Microbiol Immunol,* 62(3-4), 2003, 217-227.
22. Halliwell B, Gutteridge J M, Aruoma O I. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals, *Anal Biochem,* 165(1), 1987, 215-219.
23. Matysik G, Wojciak-Kosior M, Paduch R. The influence of Calendula officinalis flos extract on cell cultures and the chromatographic analysis of extracts, *J Pharm Biomed Anal,* 38(2), 2005, 285-292.
24. Kishimoto S, Maoka T, Sumitomo K, Ohmiya A. Analysis of carotenoid composition in petals of Calendula (Calendula officinalis L), *Biosci Biotechnol Biochem,* 69(11), 2005, 2122–2128.
25. Gazim Z C, Rezende C M, Fraga S R, Filho B P, Nakamura C V, Cortez D A. Analysis of the essential oils from Calendula Officinalis growing in Brazil using three different extraction procedures, *Brazilian Journal of Pharmaceutical Sciences,* 44(3), 2008, 391-395.
26. Rubiaceae. *Cambridge University Press,* 1976, 534.

Please cite this article in press as: Priyanka Pandey *et al.* Evaluation of *in-vitro* free radical scavenging activity of *calendula officinalis leaf* extract, *International Journal of Research in Pharmaceutical and Nano Sciences,* 7(6), 2018, 230-235.