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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *CALENDULA OFFICINALIS* LEAVES

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ABSTRACT

Antioxidant activity of different extracts (water, alcohol and hydroalcohol) of *Calendula officinalis* leaves was studied for its free radical scavenging property on in vitro models as 1,1-diphenyl-2-picryl hydrazyl (DPPH). The extract showed good dose-dependent free radical scavenging property. Hydro-alcoholic extract of *Calendula officinalis* leaves exhibited 82.58% inhibition by scavenging free radicals. *In-vitro* anti-oxidant studies of *Calendula officinalis* demonstrated the suppression of both inflammation and arthritis. The results indicate that the antioxidant property of the extract may be due to the high content of phenolic compounds. These results clearly indicate that *Calendula officinalis* is effective against free radical mediated disease.

Keywords: Antioxidant, Calendula officinalis, Free radicals, Phenolic compound, Reductive ability.

INTRODUCTION

Free radicals [reactive oxygen species (ROS)] are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, these radicals can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer. [1,2] Although many anti-oxidant defense systems consisting of enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and nonenzymatic (ascorbic acid, glutathione and α-tocopherol) compounds can maintain the balance between free radical generation and protection from damage by these radicals but these anti-oxidant systems do not provide complete protection from against ROS under conditions of severe oxidative stress.[3,4]

Calendula officinalis is traditionally used for the treatment so many diseases. Its stem is used for the treatment of fever. Plant is reported to have hepatoprotective and hypoglycemic activity. Some plants are known to possess hepatoprotective activity due to their antioxidant property. It is also established that diabetes is associated with low level of antioxidants and many plants show hypoglycemicproperty due to their antioxidant potential. As the plant under consideration possesses both hepatoprotective and hypoglycemic activity it may have good antioxidant property.[5,6] Thus, the present study has been directed to investigate the antioxidant activity of *Calendula officinalis* leaves by in vitro model.

MATERIAL & METHODS

Preparation of extracts

Calendula officinalis leaves was collected from Forest Research Institute (FRI), Dehradun and authenticated by Mr. S. K. Srivastava, Department of Botanical Survey of India, Dehradun, U.K. The leaves of Calendula dried, crushed in motor pestle and passed through sieve no. 44 and then extracted by water, ethanol and hydroalcohol by cold maceration process. Then extract filter by using filter paper. The filtrate is placed in china disc and evaporates the filtrate. Finally collect the crude extract. Calculate its % yield.

Qualitative phytochemical analysis

The preliminary chemical tests were carried out for the extract of Calendula to identify the presence of various phytoconstituents.[7]

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Determination of antioxidant activity of different extracts of the Calendula officinalis.

DPPH radical scavenging assay

To the ethanolic solution of DPPH (100µM) an equal volume of test drug was added, dissolved in distilled water at various concentrations (10µg to 50µg/ml). After incubation for 20 minutes at room temperature absorbance was recorded at 517nm. Blank was carried out in the same manner without the drug. For rate kinetic study absorbance was taken immediate after addition of DPPH up to 500 seconds. The experiment was conducted in triplicate.

$$\text{DPPH Scavenged (\%)} = \frac{A_{CONTROL} - A_{TEST}}{A_{CONTROL}} \times 100$$

Where Acontrol is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the *Calendula officinalis* extracts were expressed comparing with standard.[8]

RESULTS AND DISCUSSION

Several concentrations ranging from 5–250 µg/ml of all three extract of *Calendula officinalis* leaves were tested for their antioxidant activity by DPPH free radical scavenging model. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration. Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing[9]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm[10] and tabulated in table no. 2.

Table 1: % yield of the different extract of *Calendula officinalis* leaves.

Solvent	**% yield**
Water	15.3%
Ethanol	9.6%
Hydro-alcohol	6.5%

Table 2: % inhibition of different extracts of the Calendula officinalis leaves.

Conc.	**Ascorbic acid**		**Water Extract**		**Ethanolic Extract**		**Hydro-alcoholic Extract**	
(µg /ml)	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
5	0.017	95.99%	0.351	17.21%	0.401	5.424%	0.359	15.33%
25	0.014	96.69%	0.301	29.09%	0.392	7.547%	0.319	24.76%
50	0.012	97.17%	0.217	48.82%	0.377	11.08%	0.241	43.16%
100	0.011	97.40%	0.151	64.38%	0.288	32.07%	0.191	54.95%
150	0.004	99.05%	0.119	71.93%	0.116	72.64%	0.145	65.80%
200	0.002	99.52%	0.094	77.83%	0.094	77.38%	0.078	81.32%
250	0.001	99.76%	0.085	79.82%	0.090	77.75%	0.074	82.58%

CONCLUSION

Hydro-alcoholic extract of *Calendula officinalis* leaves exhibited antioxidant effect by inhibiting free radical. *In-vitro* anti-oxidant studies of *Calendula officinalis* demonstrated the suppression of both inflammation and arthritis. One of the causes of rheumatoid arthritis was denaturation of proteins and inhibition denaturation was one of the in vitro tests to screen anti-oxidant drugs. From the preliminary screening study, it showed the presence of coumarins and alkaloids. Anti-oxidant activity of alkaloid and coumarin has been recognized long back in rodents and reviewed exhaustively. Some examples include tocopherol, vitamin C etc. Some examples include tocopherol, vitamin etc. Hence proper isolation of the active constituent/s might help in the findings of new lead compounds in the fields of antioxidant drug research. Studies related to active constituent which reduced free radicals are necessary to understand the mechanism of action in relation to the observed antioxidant activity[11,12].

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REFERENCES

1. B Ames, Micronutrients prevent cancer and delay aging, Toxicol. Lett, 1998, 102, 5-18.
2. DA Cox and Cohen ML, Effects of oxidized low density lipoprotein on vascular contraction and relaxation, Pharmacol, 1996, 48, 3-9.
3. L Cesaratto, C, Vascotto S Calligaris and G Tell, The importance of redox state in liver damage, Ann. Hepatol, 2004, 3, 86-92.
4. C Evans, NJ Miller, Factors affecting the antioxidant activity determined by the ABTS radical cation assay, Free Radic. Res, 1997, 195, 26-27.
5. T Finkel and NJ Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature, 2000, 408, 239-247.
6. S Ganapaty, VM Chandrashekhar, HR Chitme, NM Lakshmi, Free radical scavengingactivity of gossypin and nevadensin: An in vitro relation, Indian Journal of Pharmacology, 2007, 39, 281-283.
7. J Liu, K Kurashiki, K Shimizu, R Kondo, Structure-activity relationship for inhibition of 5alpha-reductase by triterpenoids isolated from Ganoderma lucidum, Bioorg Med Chem, 2006, 14 (24), 8654-60.
8. W Liu, H Wang, X Pang, W Yao, X Gao, Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of Ganoderma lucidum, Int J Biol Macromol, 2010, 46(4), 451-457.
9. A Shirwaiar,. Shirwaikar, I.S.R. Punitha, Antioxidant studies on the methanol stem extract of Coscinium fenestratum, Natural Product Sciences, 2007,13, 40-45.
10. G Spiteller. Peroxidation of linoleic acid and its relation to aging and age dependent diseases, Mech Ageing Dev, 2001, 122, 617–657.
11. A Temraz and H Walid, Characterization of antioxidant activity of extract from Artemisia vulgaris, Pak. J. Pharm. Sci., 2008, 21, 321-326.
12. YS Velioglu, G Mazza, L Gao, BD Oomah, Antioxidant activity and total phenolics inselected fruits, vegetables, and grain products. Journal of Agricultural Food & Chemistry, 1998, 46, 4113–4117.

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