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NOTE

Antioxidant Activity of The Successive Extracts of *Calendula officinalis* Leaves

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Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like analgesic, antiinflammatory, antioxidant, hypoglycemic agents and many more. The successive extracts of *Calendula officinalis* leaves were screened for *in vitro* antioxidant properties using the standard procedures. The successive extracts such as petroleum ether, ethyl acetate, methanol and water and 50 % crude methanol extracts exhibited IC_{50} values of respectively in DPPH and respectively in nitric oxide radical inhibition assays. The values are comparable with the standards such as ascorbic acid and quercetin. The *Calendula officinalis* leaves are showing antioxidant activity.

Key Words: *Calendula officinalis*, Antioxidant, DPPH, Nitric oxide, Peroxidation, Free radical scavenging.

Calendula officinalis belong to the family compositae, commonly called as pot marigold is an important herb used in our Indian system of medicine. It is a small herb, which grows to 2 feet in height with yellow to gold flowers and found in Mediterranean region. It is a native to Central Europe. Folklore uses of *Calendula officinalis* are for the treatment of chronic colitis, ulcers, in cases of neuropsychiatry disorders[1], antimicrobial and in reproductive diseases. From the literature it was revealed that no systematic work has been carried out in the leaf part of the plant. The major active constituents which were isolated from the plant are sesquiterpene and flavonol glycosides[2,3]. The amount of active constituent obtained from the plant varies depending upon its age and maturity. Thus, studies were carried out on the leaf part of the plant, for its potential antioxidant activity.

Lipid peroxidation considered as the outmost important biochemical assay which is involved in pathogenesis of many diseases like diabetes mellitus, atherosclerosis, tumor, myocardial infarction and also in the process of ageing. Free radicals generally called as reactive oxygen species (ROS) are synthesized *in vivo* from a various biochemical reactions and tends to form a chain in the system. These free radicals are the major points in lipid peroxidation. Plants containing flavonoids[2] have been reported to possess strong oxidant properties. Thus in the present investigation the successive extraction of *Calendula officinalis* leaves was screened for *in vitro* antioxidant properties using standard operating procedures.

The plant was collected from the Nursery Garden of Shirpur, Maharashtra, India in the month of May 2007. The plant was authenticated from the sources and a voucher specimen has been procured in the Department of Pharmacognosy, SVKM'S, NMIMS University, SPTM, Maharashtra India.

Preparation of extracts and standards: The successive extracts of the shade dried powdered leaves of *Calendula officinalis* was prepared with different solvents as per the order of their polarity in Soxhlet apparatus. The solvents were evaporated with the help of rotary evaporated to get a solid residue. The solid residue was placed in a vacuum desicator and was further used for the experiments. The *in vitro* experiments, a weighed quantity of the extract was dissolved in DMSO or methanol and used. Solution of ascorbic acid and quercetin were used as standards for *in vitro* studies were prepared in distilled DMSO.

DPPH Method: The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical[4]. A total of 100 µL of the methanolic extract (from 20 to 40 µg mL in DMSO solution). After the incubation period at 37 ºC for 50 mim. The absorbance of each solution was determined at 490 nm the corresponding blank readings were also noted and the remaining DPPH was calculated. IC_{50} values is the concentration of sample required to scavenging 50 % DPPH free radical.

Nitric oxide radical inhibition assay: Aqueous solution of sodium nitroprusside at physiological pH spontaneously released nitric oxide, which can be estimated by the use of Griess IIIosvoy reaction[5]. The scavengers of nitric oxide reduce the production of nitric oxide. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5) and the extract or the standard solution (0.5 mL) was incubated at 25 ºC for 2.5 h. After incubation, 0.5 mL of the reaction mixture containing nitric oxide was pipette out and were mixed with 1 mL of sulphanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completion diazotization. 1 mL of 1-naphthylamine (5 %) was added, mixed and allowed standing for 0.5 h a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. IC_{50} values is defined as the concentration of sample required to inhibit 50 % of the nitric oxide radical.

***In vitro* assay:** The successive extracts of *Calendula officinalis* exhibited antioxidant activity in DPPH and nitric oxide radical inhibition assay as an evidence by the lowering of IC_{50} values (Tables 1 and 2). The successive extracts such as petroleum ether, ethyl acetate, methanol, water and 50 % crude methanol extract exhibited IC_{50} values 250.16 ± 1.57, 16.00 ± 0.57, 27.66 ± 1.20, 175.83 ± 1.35 and 27.33 ± 1.86 µg/mL, respectively in DPPH and 23.00 ± 0.85, 47.00 ± 0.57, 55.00 ± 1.23, 152.33 ± 0.84 and 73.66 ± 1.05 µg/mL, respectively in nitric oxide radical inhibition assay. These values were observed to be more than those which were obtained from the ascorbic acid and quercetin used as standards.

TABLE -1
ANTIOXIDANT ACTIVITY OF *Calendula officinalis*
LEAVES EXTRACTS USING DPPH METHOD

Test compound	IC_{50} values ± SE* (µg/mL)
Petroleum ether extract	250.16 ± 1.57
Ethyl acetate extract	16.00 ± 0.57
Methanol extract	27.66 ± 1.20
50 % Methanol crude extract	27.33 ± 1.86
Aqueous crude extract	175.83 ± 1.35
Ascorbic acid	75.66 ± 1.52
Quercetin	55.00 ± 0.77

*Average of 10 determination.

TABLE-2
ANTIOXIDANT PROPERTY OF *Calendula officinalis* LEAVES EXTRACTS
USING NITRIC OXIDE RADICLE INHIBITION ASSAY

Test compound	IC_{50} values ± SE* (µg/mL)
Petroleum ether extract	23.16 ± 0.85
Ethyl acetate extract	47.00 ± 0.57
Methanol extract	55.00 ± 1.23
50 % Methanol crude extract	73.66 ± 1.05
Aqueous crude extract	152.33 ± 0.84
Ascorbic acid	22.66 ± 0.98
Quercetin	18.50 ± 0.88

*Average of 10 determination.

Thus it can be stated that free radical oxidative stress has a major role in the pathogenesis of a wide range of clinical disorders resulting from different natural antioxidant defences. Among the 5 extracts of *Calendula officinalis* leaves and 2 standards tested for antioxidant activity using DPPH method, the ethyl acetate successive extract showed the maximum antioxidant activity with IC_{50} values of 16.00 ± 0.57 µg/mL, respectively. The methanol extract showed antioxidant activity with IC_{50} values 27.66 ± 1.20 µg/mL. The 50 % crude methanolic extract showed IC_{50} values 27.33 ± 1.05.98 µg/mL, respectively. However petroleum ether extract exhibited the lowest antioxidant activity with an IC_{50} value of 250.16 ± 1.57 µg/mL. The standards exhibited IC_{50} values 75.66 ± 1.52 and 55.00 ± 0.77 µg/mL, respectively.

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