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NOTE

Antioxidant Activity of The Successive Extracts of *Tagetes erectus* Flowers

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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 *Tagetes erectus* flowers 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. *Tagetes erectus* flowers are showing antioxidant activity.

Key Words: *Tagetes erectus*, Antioxidant, DPPH, Nitric oxide, Peroxidation, Free radical scavenging.

Tagetes erectus flowers belong to the family compositae. It is a small shrub, which grows to 1-2 m and is used widely in our traditional system of medicine for curing various diseases like ulcers, laxation and in the treatment of eye diseases. The leaves are used in kidney troubles and in muscular pains and are applied on boils and carbuncles. Infusion of plant is used against rheumatism, cold and bronchitis[1]. In Unani medicine, a confection of tender leaves and purified sugar is prescribed in anuria, retention of urine and kidney troubles. The flowers contain pigments as quercetagetin and quercetagetrin[2]. From the literature cited very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for its therapeutic activity.

Lipid peroxidation is the outmost important biochemical assay which is involved in pathogenesis of many diseases like diabetes mellitus, atherosclerosis, tumor, myocardial infraction 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[3] have been reported to possess strong oxidant properties. Thus in the present investigation the successive extraction of *Tagetes erectus* flowers was screened for *in vitro* antioxidant properties using standard operating procedures.

The plant was collected from the wild sources of Shirpur forest, Maharashtra, India in the month of May 2008. The plant was identified and authenticated from standard resources.

Preparation of extracts and standards: The successive extracts of the shade dried powdered flowers of *Tagetes erectus* 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 dimethyl sulphoxide (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 was prepared (from 20 to 40 µg mL in DMSO solution). After the incubation period at 37 °C for 50 min, 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 with oxygen to produce nitrite ions. The nitrite ions 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 *Tagetes erectus* exhibited antioxidant activity in DPPH and nitric oxide radical inhibition assay as 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 251.16 ± 1.57, 16.00 ± 0.57, 26.67 ± 1.20, 174.82 ± 1.35 and 27.34 ± 1.86 µg/mL, respectively in DPPH and 23.00 ± 0.85, 46.02 ± 0.57, 55.09 ± 1.23, 151.33 ± 0.84 and 72.68 ± 1.05.98 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 *Tagetes erectus* FLOWERS EXTRACTS USING DPPH METHOD

Test compound	IC_{50} values ± SE* (μg/mL)
Petroleum ether extract	251.16 ± 1.57
Ethyl acetate extract	16.00 ± 0.57
Methanol extract	26.67 ± 1.20
50 % Methanol crude extract	27.34 ± 1.86
Aqueous crude extract	174.82 ± 1.35
Ascorbic acid	74.66 ± 1.52
Quercetin	55.00 ± 0.77

*Average of 8 determination.

TABLE-2
ANTIOXIDANT PROPERTY OF *Tagetes erectus* FLOWERS EXTRACTS USING NITRIC OXIDE RADICLE INHIBITION ASSAY

Test compound	IC_{50} values ± SE* (μg/mL)
Petroleum ether extract	23.00 ± 0.85
Ethyl acetate extract	46.02 ± 0.57
Methanol extract	55.09 ± 1.23
50% Methanol crude extract	72.68 ± 1.05
Aqueous crude extract	151.33 ± 0.84
Ascorbic acid	22.66 ± 0.98
Quercetin	18.50 ± 0.88

*Average of 8 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 five extracts of *Tagetes erectus* flowers and two standards tested for antioxidant activity using DPPH method, the ethyl acetate successive extract showed the maximum antioxidant activity with IC_{50} values of 15.00 ± 0.57 μg/mL, respectively. The methanol extract showed antioxidant activity with IC_{50} values 26.67 ± 1.20 μg/mL. The 50 % crude methanolic extract showed IC_{50} values 27.34 ± 1.05.98 μg/mL, respectively. However petroleum ether extract exhibited the lowest antioxidant activity with an IC_{50} value of 251.16 ± 1.57 μg/mL. The standards exhibited IC_{50} values 74.66 ± 1.52 and 55.00 ± 0.77 μg/mL, respectively. Thus from the above investigation it can be stated that antioxidant are essential as they play an important role in the defensive and ageing process of mankind.

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