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In-vitro Antioxidant Activity of methanolic extract of Aerial Parts of *Canna indica* L.

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ABSTRACT

The requirement of compound with antioxidant activity is increasing as there is realization of that formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked in pathogenesis of several human diseases. The herb *Canna indica* L. is commonly known as Devkali, which is widely used in the indigenous system of medicine for the treatment of diuretic, fevers and dropsy. The aerial parts methanolic extract of the plant was studied for its in vitro antioxidant activity in different methods viz DPPH radical scavenging assay, Nitric oxide scavenging assay, Hydrogen peroxide Assay, Hydroxl radical scavenging Assay. Its free radical scavenging activity was estimated by IC_{50} value and the values at various concentrations 10 to 100µg/ml. At 100µg/ml DPPH radical scavenging assay, Hydroxyl radical scavenging assay, Hydrogen peroxide assay and Nitric oxide assay showed maximum inhibition76.70% ,74.36%, 61.37% and 62.84% These results clearly indicate that Aerial Parts of *Canna indica* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Keywords: Aerial Parts, *Canna indica*, Free Radical Scavenging, Antioxidant

INTRODUCTION

Free radicals are reactive species generated in the body during normal metabolic functions or introduced from environment. These species causes cellular damage by reacting with the various biomolecules of body such as membrane lipids, nucleic acid, proteins and enzymes. This damage is the major contributor of many disorders like Cancer, Hepatopathy, Cardiovascular diseases, inflammation, diabetes mellitus, renal failure and brain dysfunction. Body has itself antioxidant system which reacts with reactive species and neutralizes them. This natural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione which protect the body from free radical species and prevent oxidative stress. Synthetic antioxidants like Butylated hydroxyl toluene and Butylated hydroxyl anisole are carcinogenic in nature. So need of natural antioxidant arises. (1) In Ayuvedic system of medicine *Canna indica* L. (Cannanace) commonly known as Devkali has many properties. It is popularly known as Indian Shot. Its flowers are red and attractive. Leaves are fleshy with thin margins. Its root decoction used for treatments of fever, dropsy, dyspepsia. (2) A seed juice used to cure earaches. Leaves show significant analgesic activity. (3) The flower are said to cure eye diseases (4). Flowers contain lutein, ß – carotene, violxanthin. Its leaves haves chemical constituents like lignin, furfural, hemicelluloses.While rhizomes has 5,8- Henicosdine, Tetracosane,Tricosane.Thus, present investigation on based upon above literature carried out , to determine free radical scavenging activity of aerial parts of *Canna indica* L.(5,6)

MATERIALS AND METHODS

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) procures from Himedia Labs, Mumbai. Ascorbic acid, $FeCl_3$, trichloroacetic acid, hydrogen peroxide, sodium nitroprusside, sulphanilamide, H_3PO_4, napthylethylenediamine dihydrochloride. All chemicals used including solvents were of analytical grade.

Plant Material

The whole plant of Canna indica was collected from National Nursery, Konkan Bhuvan, Navi Mumbai. It is authenticated by Prof.D.R.Mahajan, Botanist, Head of Botany Department KTHM College, Nasik, Pune University. A Voucher Specimen (No.03) has been deposited for future reference in the Department of Botany, KTHM College, Nasik, Pune University.

Drug Processing

Aerial parts (Flowers, Leaves, Stems) of plants collected and shade dried. This material then made into powder with the help of grinder. The powder was then used for further study.

Preparation of Methanolic extract

The aerial parts powder of *Canna indica* L. was extracted using methanol as a solvent in Soxhlet extractor. After completion of

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extraction solvents removed using rotary vacuum evaporator. The extracts was then stored in desiccators for further use.

Preliminary Phytochemical Screening

Preliminary phytochemical screening performed to find out nature of chemical constituents that are present in aerial parts of extract. (7, 8)

DPPH Radical Scavenging Activity

DPPH is one of the free radical generally used for testing radical scavenging activity of a plant extract.It is performed by method of Blois.(9) About 10-100 µg/ml of each ml extract or standard was added to 3 ml of DPPH (HiMedia Laboratories Pvt. Ltd., Mumbai) in methanol in a test tube. After incubation at 37°C for 30 minutes the absorbance of each solution was determined at 517 nm using spectrometer Ascorbic acid was used as standard material, with concentrations ranging 10-100 µg/ml.

DPPH radical scavenging activity (%) =[Abs $_{(control)}$ – Abs $_{(stdandard)}$ /Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of DPPH radical + methanol
Abs $_{(stdandard)}$: Absorbance of DPPH radical + extract/standard.

IC_{50} value is the concentration of the sample required to scavenge 50% free radical of sample.

Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell.(10) Stock solutions of EDTA (1mM), $FeCl_3$ (10 mM), ascorbic acid (1mM), H_2O_2 (10mM) and deoxyribose (10mM) were expressed in distilled deionized water. The assay was performed by adding 0.1 ml of EDTA , 0.01 ml of $FeCl_3$, 0.01 ml of $FeCl_3$, 0.1 ml of H_20_2, 0.36 ml of deoxyribose,1.0 ml of MECI (Concentrations used were 10,20,40,60,80 and 100 µg/ml) in distilled water,0.33 ml of Phosphate buffer (50 mM,pH 7.4) and 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37 ^{0}C for 1hr. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA (S. D. Fine Chem. Ltd., Mumbai.) And 1.0 ml of 0.5% TBA to develop the pink chromogen measured at 532nm.

OH^- scavenged (%) = [Abs $_{(control)}$ – Abs $_{(standard)}$ / Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of the control reaction and
Abs $_{(standard)}$: Absorbance of the extract/standard.

Nitric Oxide Radical Scavenging Activity

Sodium nitroprusside (5mM) in phosphate–buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10-100 µg / ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance at standard solutions of potassium nitrite, treated in the same way with Greiss reagent. (11)

NO scavenged (%) = [Abs $_{(control)}$ – Abs $_{(standard)}$ / Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of the control reaction and
Abs $_{(standard)}$: Absorbance of the extract/standard.

Scavenging Of Hydrogen Peroxide

The ability of the Canna indica to scavenge hydrogen peroxide was determined according to the method of Ruch.(12) A solution of hydrogen peroxide (2 mmol/l) (Fine Chem Industries, Mumbai) was prepared in phosphate buffer (pH 7.4). *Canna indica* (10–100 µg /ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. (13)

% scavenging activity [H_2O_2] =[Abs $_{(control)}$ – Abs $_{(standard)}$ / Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of the control and
Abs $_{(standard)}$: Absorbance of the extract/standard.

IC_{50} value is the concentration of the sample required to scavenge 50% free radical of sample. All the assay similarly performed using Ascorbic acid Standard compound.

Statistical Analysis

Data reported are means of three assays. All measurements were performed in triplicate. The data are given as means ±standard error of mean (S.E.M.). $P < 0.05$ value considered significant. Version 5.0 (Graph Pad Software, San Diego, CA, USA) used.

RESULTS

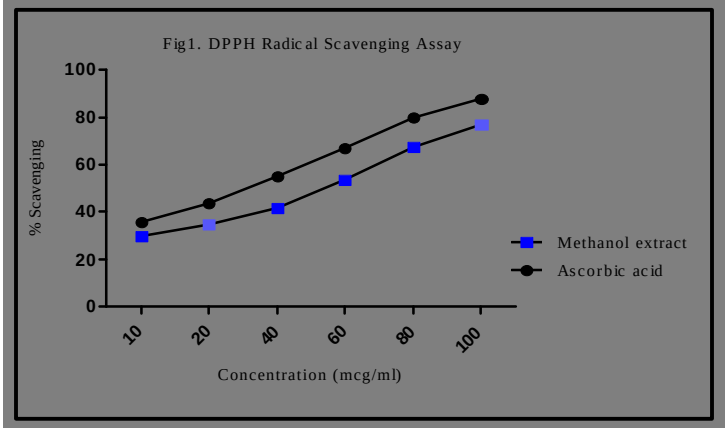
The methanol extract yield of *Canna indica* L. was found to be 9.68 % w/w. Phytochemical screening revealed the presence of Phenols, Steroids, Flavanoids, and Saponins.

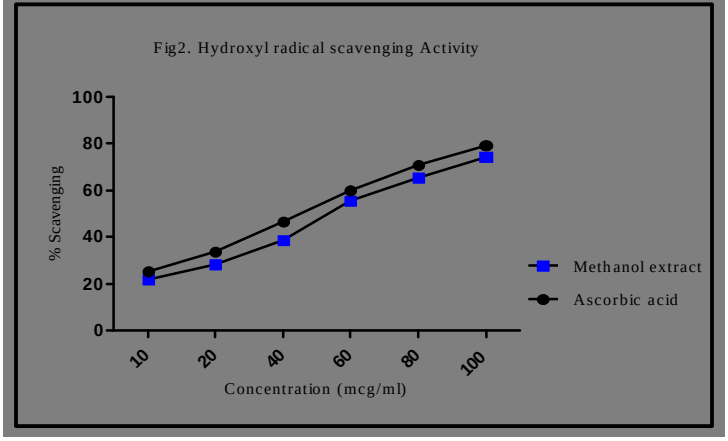
Table1. Antioxidant IC_{50} values in different in-vitro models.

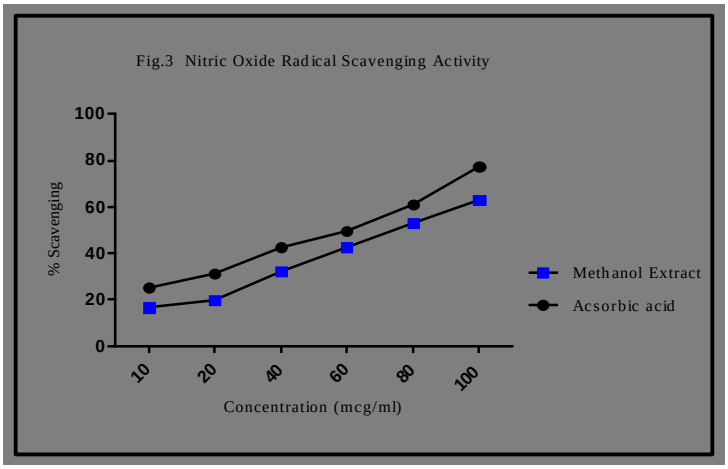
Sr. No	Assay	Methanol Extract (µg/ml)	Ascorbic Acid (µg/ml)
1	DPPH scavenging assay	55.21	32.04
2	Hydroxyl radical scavenging assay	53.91	46.17
3	Nitric oxide assay	75.33	62.15
4	Hydrogen Peroxide Assay	82.70	58.23

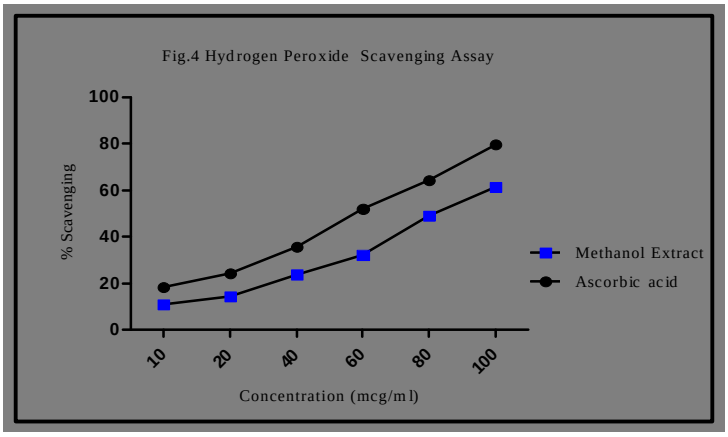
DISCUSSION

Various concentrations ranging from 10-100 µg/ml of methanol extract were tested for their free radical scavenging activity. It was observed that free radical scavenged by Canna indica in dose dependent manner. DPPH is one of the free radicals generally used for test-









ing preliminary radical scavenging activity of a compound or a plant extract. DPPH radical is a stable free radical in an aqueous or methanol solution. It accepts an electron or hydrogen radical to become stable diamagnetic molecule. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy. The antioxidant activity measured by the capacity of odd electron of the radical becomes paired off in the presence of extract (hydrogen donor). When it becomes paired off, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. Substances which are able to perform this reaction can be considered as antioxidants and hence radical scavengers. *Canna indica* L. showed concentration dependent decrease in absorbance and increase in scavenging activity. (14) Hydroxyl radical is highly reactive species formed in biological systems and have been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells. In addition, this species is one of the quick initiators of lipid peroxidation process; abstracting hydrogen atoms from unsaturated fatty acids It will further disrupt biomembarane causes cell damage. It's very important to scavenge hydroxyl radical. *Canna indica* resist hydroxyl radical generation. (15) The Nitric oxide radical scavenging assay procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. *Canna indica* L. compete with oxygen which results in decreased production of nitric oxide. Similar activity shown by Ascorbic acid. (16) Hydrogen peroxide itself is not very reactive; it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_20_2 is very important throughout systems. Scavenging of H_20_2 by Canna indica extracts may be attributed to their phenolics contents, which can donate electrons to H_20_2 and neutralizing it to water. (17)

CONCLUSION

Canna indica aerial parts showed different levels of antioxidant activity in all models studied. It possess good activity in scavenging DPPH radical, Hydroxyl radical, Nitric oxide radical and scavenging of H_2O_2 . Further investigation of individual phytocompounds, their isolation, identification, their role as free radical scavenger is needed.

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