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Research Article

Study of *invitro* anti-Oxidant Activity of *Ipomea Pes-Caprae*

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ABSTRACT

The traditional medicinal plant ipomea pes- caprae belongs to convolvuceae family. The present study has been undertaken to find out the antioxidant activity of the whole plant extract of *Ipomea pes-caprae*. Plant was subjected to extraction by cold maceration by using ethanol as a solvent. Antioxidant activity such as 1,1-Diphenyl,2-Picryl,Hydrazyl (DPPH) Radical Scavenging Activity, Hydroxyl Radical Scavenging Activity, Reducing Power, Metal chelating activity were determined. Physicochemical analysis was carried out to identify the chemical constituent of the plant and showed the presence of alkaloid, sugar, steroid, glycoside, saponins, Terpenoids and phenol compounds. The result of free radical scavenging activity of EEIP by DPPH reduction revealed that the test compound is electron donor and could react with free radicals to convert them to more stable product and terminate radical chain reaction. For the measurement of reducing ability we investigated the Fe^{3+} to Fe^{2+} transformation. The metal chelating capacity of the EEIP and standard anti oxidants are determined by accessing the ability to complete with bipyridil and thiocyanate for $Fe3^{+}$ and $Fe2^{+}$ respectively. The formation of ferrous bipyridil, ferric – thiocyanate is not complete in the presence of EEIP. The ability of chelating is increased with increased concentration. So, it can be assumed that the plant extract chelate the iron. The experiment demonstrates that action of plant extract as per oxidation protector may be related to its iron binding ability.

Keywords: Ipomea pes-caprae, DPPH Radical Scavenging Activity metal Chelating Activity

Introduction

Antioxidants are Phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. Anti oxidants are reduce the rate of particular oxidation reaction in a specific context, where oxidation reactions are chemical reaction that

involve the transfer of electrons from a substance to an oxidizing agent. In vitro and in vivo studies have shown that antioxidants help prevent the free radical damage that is associated with cancer and heart disease. Antioxidants can be found in most fruits and vegetables but also culinary herbs and medicinal herbs can contain high levels of antioxidants. The antioxidant level of herbs can be as high as 465mmolper100g. Antioxidants Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by hemolytic cleavage of covalent bond of a molecule by the loss of a single electron from a normal molecule or by addition of a single electron to a normal molecule. Free radicals are formed as part of our natural metabolism but also by environmental factors, including smoking, pesticides, pollution and radiation. Free radicals are unstable molecules[1] which react easily with essential molecules of our body, including DNA, fat and proteins. However when oxygen is partially reduced it becomes "activated" and reacts with a variety of biomolecules. This partial reduction occurs in one, two and four electrons to O2, which leads to successive formation of reactive oxygen metabolites (ROMs). There are 5 possible species Superoxide anion (O_2)**,** Hydroperoxyl radical (HO_2)**,** Peroxide ion (HO_2) **,** Hydrogen peroxide (H_2O_2) **,** Hydroxyl radical (OH). The first line of defense against O_2 and H_2O_2 mediated injury are anti oxidant enzymes such as SOD, GPx, and CAT. Anti oxidant enzymes, together with the substance that are capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cells to counter act the cation of oxygen metabolites. All the reducing agents there by form the protective mechanism, which maintain the lowest possible levels of ROMs inside the cell[2]. Many studies show that antioxidants may reduce the risk of cancer. A large randomized trial on antioxidants and cancer risk was the Chinese Cancer Prevention Study (1993). This study showed that a combination of the antioxidants beta-carotene, vitamin E and selenium significantly reduced incidence of cancer. However, the Alpha-Tocopherol / Beta-Carotene Cancer Prevention Study (1994) showed that intake of beta-carotene increased lung cancer rates of male smokers. Everyone knows that cholesterol causes heart diseases and tries to limit cholesterol intake. But a more important cause of fatty buildups in the arteries is the oxidation of low-density lipoprotein cholesterol. The use of dietary supplements of antioxidants could reduce the risk of cardiovascular disease, but there is no hard evidence. At this stage, studies only show that the intake of foods, naturally rich in antioxidants reduces this risk.

MATERIALS AND METHODS

Collection plant material

The *Ipomea pes –caprae* was collected from the sea beaches area of Nellore, Andhra Pradesh, India. It was identified and authenticated by prof.P. Jayaraman, Ph.D. National institute of herbal science, plant anatomy research centre (PARC) Chennai, India .where voucher specimen has been deposited. The acquisition number of *Ipomea pes-caprae* was **PARC/2009/396.**

Methods of extraction

The root of *Ipomea pes- caprae* was cut into small pieces, dried and pulverized to coarse powder. About 500gms of powder was extracted by cold maceration method. The solvent was filtered and distilled off. The extracts were then concentrated in vacuum under reduced pressure using rotary vacuum evaporator and dried in desiccators.

Phytochemical studies on *Ipomea pes- caprae*

Phytochemical studies was carried out to identify the chemical constituent of the plant. Preliminary photochemical tests showed the presence of alkaloid, sugar, steroid, glycoside, saponins, Terpenoids and phenol compounds.

Phytochemical analysis

The different extract was subjected to different chemical test for the detection of different phytoconstituents using standard procedure[3].

In vitro anti oxidant study:

- 1,1-diphenyl, 2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity
- Hydroxyl Radical Scavenging Activity
- Determination of Reducing Power
- Metal Chelating Activity

INVITRO ANTI OXIDANT EXPERIMENTS[4]

1,1-Diphenyl,2-Picryl,Hydrazyl (DPPH) Radical Scavenging Activity

DPPH scavenging activity was measured by spectrophotometric method. To a ethanolic solution of DPPH (100µM, 2.95ml),0.05 ml of test compounds were dissolved in propylene glycol was added at different concentration 100-1000µg/ml. Equal amount of propylene glycol was added to the control absorbance was recorded at 517nm after 5min.

Hydroxyl Radical Scavenging Activity

Hydroxyl scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the FE^{3+}/ascorbate/EDTA/H_2O system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS formation. The reaction mixture contained deoxyribose (2.8mM), $FeCl_3$ (0.1mM), EDTA (0.1mM), H_2O_2 (0.1mM), ascorbate (0.1mM), KH_2PO_4-KOH buffer (20mM,Ph 7.4) and various

concentrations (EEIP 100-3-00 µg/ml) and standard mannitol (100 µg/ml) of the drug in a final volume of 1 ml. the reaction mixture was incubated for 1 hr at 37°c deoxyribose degradation was measured at 532nm.

Determination of Reducing Power[5]

The reducing power of ethanolic root extract of *Ipomea pes-caprae* was determined according to the following method. 10 mg of ethanolic root extract of *Ipomea pes-caprae* in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml,0.2M,pH6.6) and potassium ferricyanide [$K_3Fe(CN)_6$](2.5 ml 1%). The mixture was incubated at 50°c for 20min. A portion (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Metal Chelating Activity[6]

Metal chelation property for ferric ion (FE^{3+}) was estimated using thiocyanate method. Here different ratios of the extract (1:2.5 to 1:10 ratio) were mixed with a fixed concentration of ferric chloride (10 µg). The mixture was incubated for 30min. At the end of the incubation, 1ml of potassium thiocyanate (25%) was added and absorbance of ferric – thiocyanate complex (reddish brown complex was measured at 459nm. The results were compared with EDTA (1:10) metal chelation property for ferrous ion was estimated by using 2.2-bipyridil method. Here different concentrations of the extract were mixed with a fixed concentration of ferrous sulphate (10 µg). The mixture was incubated for 30min. at the end of the incubation 2ml of 2, 2-bipyridil (1mM) was added and absorbance of ferrous-bipyridil complex (pink-coloured complex) was measured at 525nm. The results were compared with EDTA.

Statistical Analysis

All the results were expressed as Mean ± SEM and *** $P < 0.001$, ** $P < 0.005$ and * $P < 0.05$ considered as a significant (n.s-not significant). Statistical comparisons between control and treated groups were carried out using TWO-way analysis of variances (ANOVA) and differences between groups assessed using Dunnet's t test. All statistical analysis can be done by using Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

This dissertation covered the analgesic, anti oxidant and anti arthritic activity of whole plant extract of I*pomea pes caprae.* As a part of the preliminary Phytochemicals test was performed to identify the presence of chemical constituents on support of literature review are shown in Table 1. Reduction of DPPH radicals can be observed by the decrease in the absorbance at 517nm. The scavenging capacity of EEIP was found to be $p<0.01$ results are shown in Table 2. The reducing power of EEIP increased with increasing concentration of EEIP. 500 µg, 375 µg concentration showed significant $p<0.01$. When compared to control. Results are shown in Table 3. EEIP chelated Fe^{2+} (42.13%) and Fe^{3+} (43.63%) significantly $p<0.01$ at 1:10 ratio of iron: EEIP and chelating ability for metal transition ion(Fe^{2+}, Fe^{3+}) increased in a dose dependent manner respectively. EEIP at all tested concentration exhibited significantly $p<0.01$ chelation when compared against control. In similar condition, EDTA exhibited $p<0.01$ chelation for $Fe^{2+}$and $p<0.01$ chelation for Fe^{3+}respectively, which is significant $p<0.01$ when compared with control. Results were shown in table 4. The traditional medicinal plant *Ipomea pes- caprae* belongs to convolvuceae family. Earlier folklore claims reports that the plant is used in analgesic gout inflammatory and rheumatism conditions. So present

work was focused to evaluate analgesic, antioxidant and antiarthritic activity by formaldehyde induced arthritis. Physicochemical analysis was carried out to identify the chemical constituent of the plant. Preliminary photochemical tests showed the presence of alkaloid, sugar, steroid, glycoside, saponins, Terpenoids and phenol compounds. The result of free radical scavenging activity of EEIP by DPPH reduction revealed that the test compound is electron donor and could react with free radicals to convert them to more stable product and terminate radical chain reaction. Many anti oxidant have reducing ability as one of the possible mechanism to exhibit anti oxidant property. For the measurement of reducing ability we investigated the Fe^{3+} to Fe^{2+} transformation. The reducing capacity of a compound serves as the significant indicator for its potential anti oxidant activity. Many anti oxidants exerts the metal chelating ability, which may be responsible for their anti oxidant property. The metal chelating capacity of the EEIP and standard antioxidant are determined by accessing the ability to complete with bipyridil and thiocyanate for $Fe3^{+}$ and $Fe2^{+}$ respectively. The formation of ferrous bipyridil, ferric – thiocyanate is not complete in the presence of EEIP. The ability of chelating is increased with increased concentration. So, it can be assumed that the plant extract chelate the iron. The experiment demonstrates that action of plant extract as per oxidation protector may be related to its iron binding ability.

Table-1

Preliminary phytochemical screening of *ipomea pes-caprae*

Chemical test	Ethanol Extract
Alkaloids	+
Carbohydrate	+
Sugar	+
Steroids	+
Tannins	+
Proteins	-
Terpenoids	+
Flavonoids	+
Anthocyanins	-
Quinines	-

+ Present

- Absent

Table -2

Free radical scavenging activity of EEIP by DPPH Reduction

S.NO	Concentration of EEIP (µg/ml)	% Inhibition
1	Control	-
2	100	30.20±0.087**
3	200	57.84±0.215**
4	400	69.44±0.062**
5	600	76.57±0.088**
6	800	80.56±0.0736**
7	1000	85.58±0.0112**
8	Standard BHT(1000)	88.74±0.054**

- EEIP: Ethanolic Extract of *Ipomea Pes-caprae.*
- Values are mean±SEM of 6 parallel measurements.
- Statically significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)
- All values are significant **p<0.01 when compared against control.

Table -3

Hydroxyl radical scavenging activity EEIP and Mannitol

S.NO	Concentration (µg/ml)	% inhibition of hydroxyl Radical
1	Control	_
2	EEIP(50)	67.08±2.2473**
3	EEIP(100)	75.20±0.9121**
4	EEIP(200)	77.60±0.687**
5	Standard(mannitol 100)	81.03±1.0112**

- EEIP: Ethanolic Extract of *Ipomea Pes-caprae.*
- Values are mean ± SEM of 6 parallel measurements.
- Statically significant test for comparison was done by ANOVA, followed by Dunnet's't' test (n=6)
- All values are significant **p<0.01 when compared against control.

Table – 4

Effect of EEIP and EDTA on Fe^{2+}/Fe^{3+} metal chelation

Iron: drug	OD at 525nm	% inhibition of Fe^{2+}	OD at 460nm	% inhibition of Fe^{3+}
1:00 control	0.308	0	1.021	0
1:0.25EEIP	0.254	17.46±0.602**	0.9470	7.24±0.163**
1:0.5 EEIP	0.225	26.67±0.445**	0.8560	16.11±0.171**
1:1 EEIP	0.212	30.90±0.395**	0.7770	23.89±0.168**
1:2.5 EEIP	0.206	32.85±0.536**	0.710	30.41±0.319**
1:5 EEIP	0.196	36.35±0.417**	0.690	32.39±0.173**
1:10 EEIP	0.178	42.13±0.336 **	0.554	46.63±0.470**
1:10 Standard (EDTA)	0.0667	78.18±0.575**	0.149	85.39±0.186**

- EEIP: Ethanolic Extract of *Ipomea Pes-caprae.*
- EDTA: Ethylene Diamine Tetra Acetic acid
- Values are mean ± SEM of 6replecates.
- Statically significant test for comparison was done by ANOVA, followed by Dunnet's' test (n=6)
- Fe^{2+} and Fe^{3+} were quantified by Fe^{2+} - dipyridyl complex (525nm) and Fe^{3+}-thiocyanate complex (460nm), respectively.
- All values are significant **p<0.01 when compared against control.

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