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Preliminary Phytochemical Screening and Comparison study of *In Vitro* Antioxidant activity of selected Medicinal Plants

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Abstract

The current evaluation was to assess the preliminary phytochemical screening and *in vitro* antioxidant activity of four selected medicinal plants *Croton bonplandianum* (Bon Tulsi), *Origanum majorana* (Sweet marjoram), *Vitex negundo* **(**Nirgundi) *and Indigofera linnaeia*, (Birdsville Indigo) were evaluated for their free radical scavenging effects using ascorbic acid as standard antioxidant. The phytochemical investigation of the ethanol leaf extract of four individual medicinal plants was appeared for flavanoids using quality method. The leaves ethanol extracts of four plants were also investigated for their antioxidant and free radical scavenging potential by using distinct *in vitro* tests such as super oxide radical scavenging assay, hydroxyl radical scavenging assay, lipid peroxidation scavenging assay. The total phenolic and flavonoid contents were assessed by Folin-Ciocalteaus and aluminium chloride reagents. The preliminary phytochemical screening for leaves of four different medicinal plants exhibited the presence of flavonoids. The consequence of above study indicated that the *Origanum majorana* ethanolic leaf extract have maximum free radical scavenging activities at dose dependent manner compare to other plant extracts and also this plant possessed huge amount of phenolic and flavonoid contents respectively when compared to other Indian medicinal plants. The results procured in the current investigation illustrated that the leaves of *Origanum majorana* are a hidden cause of essential antioxidants which may be due to its sufficient phenolic and flavonoid contents.

Key words: Medicinal plants, Antioxidant activity, Flavanoid

Introduction

Traditional systems of medicine continue to be widely practiced on many accounts, population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human aliments.[1,2] Among ancient civilization, India has been known to be rich repository of medicinal plants which contains many secondary metabolities like flavanoids, phenols, terpinoids, alkaloids, glycosides, tannins, steroids, saponins and many other phytoconstituents which are the source of medicinal activity that have been found to be an excellent antioxidant property [3].

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Croton bonplandianum Baill., Family Euphorbiaceae Due to the resemblance of the leaves and flower cymes to that of Tulsi, this plant is often called Ban tulsi locally [4].The contains secondary metabolites are alkaloids, saponins, steroids, flavonoids, tannins, terpenoids and phenolic compounds.[5]. It is a medicinal herb used in many health related problems like cholera, boils, bowel complaints, diarrhoea, dysentery, insanity, acute constipation, abdominal dropsy, internal abscesses, cold and cough, lungs infection, bronchitis, asthma, jaundice, liver complaints, reduce pain, sprains, headache. It is also used for the treatment of scurvy, malaria, chicken pox, eye diseases, skin diseases, rheumatism, epilepsy and many other diseases [6, 7]. This study was aimed at investigating the effects of antioxidant activity of ethanol leaf extract of the of *Croton bonplandianum* Baill.

Origanum majorana (family-Lamiaceae), Commonly known as Sweet marjoram, Majorana hortensis or Marwa in India [8]. Marjoram has many uses with numerous health benefits. Digestive benefits **(**Increasing the efficiency of digestion by increasing digestive enzymes and saliva, improving appetite, relieving nausea, eliminating flatulence, preventing intestinal infections, relieving diarrhea and constipation) [9,10]. Marjoram is a great antiseptic, antibacterial, antifungal, and antiviral agent and used in a variety of common illnesses (Food poisoning, Staph infection, Tetanous infection in wounds, Typhoid, Malaria, Influenza, Common cold, Mumps, Measles). Another benefit of marjoram is the enhancement of the cardiovascular and circulatory system (Lowering the blood pressure, greatly reducing the risk of hypertension, preventing the buildup of cholesterol).Anti-inflammatory effects like (Asthma, Muscle spasms, Sinus headaches, Migraines, Fever, Body aches) [11]. Topical application for (Painful joints, Sore muscles, Sprains, Back aches, Toothaches [12,13], Emotional and neurological benefits like (Relieving insomnia, Reducing stress, Calming anxiety, Minimizing emotional reactions, Increasing control of sexual desire). The herb contains important phyto constituents like tannins, glycosides, terpenes, flavonoids, linalool and cavacrol [14, 15].

Vitex negundo, (family- Verbenaceae) commonly known as the five-leaved chaste tree, is a large aromatic shrub, widely used in folk medicine.[16] *In vitro* and animal studies have shown that chemicals isolated from the plant have potential anti-inflammatory, antibacterial, antifungal and analgesic activities. *Vitex negundo* is used for treating stored garlic against pests and as a cough remedy in the Philippines [17]. Roots and leaves used in eczema, ringworm and other skin diseases, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, backache; seeds used as vermicide. It is also used to control population of mosquitoes. Traditionally the leaves of *Vitex negundo Linn.* are documented to possess antibacterial, antitumor, astringent, febrifuge, sedative, tonic and vermifuge. It has been reported to posses potent pharmacological properties like anti-inflammatory, anti-rheumatic, antibiotic, Hepatoprotective, antioxidant, anticonvulsant, oxidative stress, anti-androgen, snake venom neutralization and anti-allergic activities. The various chemical constituents like flavonoids, flavones glycosides, volatile oil, triterpenes, tannins and many others were identified in this plant [18 19].

The plant *Indigofera linnaei Ali* (family-Fabaceae), commonly known as Birdsville indigo is a Trailing, branched, slender annual or perennial herbs with woody rootstock, 15–50 cm high with a long taproot. Leaves imparipinnate. *Indigofera* is a large genus of over 750 species of flowering plants belonging to the family Fabaceae [20-23]. They are widely distributed throughout the tropical and subtropical regions of the world. *Indigofera linnaei* is one of important species used to alleviate pain. The herbs are generally used for toothache, insect stings, snake bites and swellings, relieve ulcer pain. anthelmintic, tonic, skin disorders, for toothache, ulcer, solid tumors, epilepsy, anti-nociceptive, analgesic and anti-inflammatory, antioxidant, rheumatism, arthritis, antimicrobial, antidyslipidemic, anti-fertility, antiscorbutic, diuretic, jaundice and to treat burns, liver disease and psychiatric illness, promoting growth of hair, chronic bronchitis, asthma, hydrophobia, in gastropathy and also used as thermogenic, laxative, expectorant etc [23-25].

Material and Methods

Determination of superoxide radical scavenging activity:

Riboflavin photoreduction method:

By McCord JM and Fridovich I method, 1969, Superoxide scavenging activity of the selected plant ethanol extract was determined, which depends on light induced superoxide generation by riboflavin and corresponding reduction of nitroblue tetrazolium, 0.1ml of different concentration of plant extract, and 0.1 ml of 6µM ethylenediamine tetraacetic acid containing NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube , and final volume was made upto 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and there after the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes

The percentage inhibition of superoxide scavenging activity = $[(A_0-A_1)/A_0] \times 100$

Where, A_0 is the absorbance of control

A_1 is the absorbance with addition of plant extract / ascorbic acid.

Determination of Hydroxyl radical scavenging activity:

Deoxyribose degradation method:

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (Elizabeth K and Rao MNA, 1990). Fenton reaction mixture consists of 200 µl of 10mM ferrous sulphate ($FeSO_4$, $7H_2O$), 200µl of 10 mM EDTA and 200 µl of 10mM 2-deoxyribose and was mixed with 1.2 ml of 0.1M phosphate buffer (pH 7.4)and 200 µl of plant extract. Therefore, 200 µl of 10mM H_2O_2 was added before the incubation at 30^0C for 4 hours. Then, 1ml of this Fenton reaction mixture was treated with 0.2ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% of thiobarbituric acid and 1.5 ml of 20% acetic acid.the total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100^0C for 1 hour. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added.following vigorous shaking, the tubes were centrifuged at 4000 rmp for 10 minutes and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extracts/compounds was determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay.

Determination of lipid peroxidation inhibition activity

Induction of lipid peroxidation by Fe^{2+}/ascorbate system:

Inhibition of lipid peroxidation was determined by the method developed by Ohkawa H et al, 1979. Rat liver tissue weighing 10 gm was homogenized with a polytron homogenizer in ice-cold tris-Hcl buffer to produce a 25% w/v homogenate. The homogenate was centrifuged a 4000 rpm for 10 minutes. An aliquot of supernatant 0.1ml was mixed with 0.1 ml of plant extract of different concentration, followed by addition of 0.1 ml of potassium chloride (30 ml), 0.1 ml of ascorbic acid (0.06mM) and 0.1 ml of ammonium ferrous sulphate (0.16mM) and were incubated for 1 hour at 37^0C. the reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20% acetic acid (pH 3.5). the total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 100^0C for 1 hour. After the mixture had been cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture were added. Following vigorous shaking, the tubes were centrifuged at 400 rpm for 10 minutes and the absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay.

Results and Discussion

The extract of *Croton bonplandianum*, *Origanum majorana*, *Vitex negundo* and *Indigofera linnaei* were found to scavenge the superoxides generated by photo reduction of riboflavin. *Croton bonplandianum* and *Origanum majorana* at concentration of 30, 60, 90 and 120 µg produced dose dependent inhibition of superoxide radicals as compare to other plant extract and the mean values are represented in table-1.

Degradation of deoxyribose mediated by hydroxyl radicals generated by Fe^{3+}/ascorbate /EDTA/ H_2O_2 system was found to be inhibited by these extracts. All the extracts at 100, 150 and 200µg/mL levels scavenged the hydroxyl radicals in a dose dependent inhibition of hydroxyl radicals and the mean values were found to be shown in table-2.

Lipid peroxides generated by induction of Fe^{3+} /ascorbate on rat liver homogenate was found to be inhibited by the addition of these extracts. *Croton* bonplandianum at a concentration of 50, 75 and 100 µg/mL inhibited the lipid peroxides in a dose dependent manner and the mean values were found to be shown in table -3. The concentration for 50% inhibition was calculated by plotting a graph between concentrations vs. optical densities.

Considerable secondary bioactive constituents containing polyphenolic structures supported as nutraceuticals to supplement food for maintain health care now a day. All most of them are asserted to shows antioxidant activity. Currently, much attention has been focused on the use of natural antioxidants to protect the human from different harmful disorders. The antioxidant potential of the ethanol extract of *Croton bonplandianum*, *Origanum majorana*, *Vitex negundo* and *Indigofera linnaei* of Indian traditional plants was investigated in comparison with the known antioxidant ascorbic acid (AA) by *In vitro* studies. The polyphenolic compounds are well known as free radical scavengers and protect human beings from harmful disorders like diabetes, cancer etc. the presence of terpenoids, flavonoids phytoconstituents might be responsible for the comparative antioxidant

activity with that of known antioxidant ascorbic acid. Since reactive oxygen species are involved in stress and stress related disorders. The extract of these plants may be beneficial in preventing the initiation or progression different disorders. The diabetes being stress related, these phytoconstituents presence might prevent diabetes.

Conclusion

It is noticable from the above study that *Croton bonplandianum, Origanum majorana, Vitex negundo* and *Indigofera linnaei* have significant antioxidant contents and activity. *Origanum majorana and Croton bonplandianum* are better in this regard than other two plants. The regular use of *Origanum majorana* leaf as a natural health supplement can be beneficial in the treatment of neurological disorders associated with free radical damage. Keeping in view these all four plants having antioxidant property in dose dependent manner, these plants can also used alone or in combination form for better consequences of antioxidant activity to protect the body from harmful effects of free radicals.

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Table 1: Qualitative Analysis –Phytochemical constituents from ethanol leaf extracts of following medicinal plants

S/No.	Phytoconstituents	*Croton bonplandianums*	*Origanum majorana*	*Vitex negundo*	*Indigofera linnaei Ali*
1	Alkaloids	+++	+	++	+
2	Flavonoids	+++	+++	+++	+++
3	Saponins	++	++	++	+
4	Tannins	++	+	+	++
5	Phlobatannins	-	-	-	-
6	Glycosides	+++	+++	++	++
7	Sterols	++	+	+++	++
8	Resins	+	++	+	+
9	Phenols	++	+	++	+
10	Anthraquinones	-	-	+	-
11	Terpinoids	++	-	++	++
12	Cardiac glycosides	++	++	++	++

+=Present, -=Absent

Figure-1

Table2: Percentage Inhibition of superoxide radicals using photo reduction method

Plant extracts	Quantity (µg/mL)					
	15	**30**	**60**	**120**	**240**	**480**
CB	5.7±1.24	12.4±2.63	20.81±6.21	50.31±8.22	61.52±8.61	76.05±7.61
OM	14.21±2.32	20.32±6.39	26.29±5.39	51.63±7.65	70.38±8.32	77.43±6.55
VN	16.012±0.23	28.015±1.21	37.081±3.02	46.64±4.61	60.21±7.25	69.15±7.19
IL	13.21±0.85	26.40±2.20	32.61±6.24	48.05±6.86	69.26±7.25	74.34±7.68
AA	23.05±0.35	38.9±1.84	52.05±5.86	61.74±6.24	73.08±6.37	89.19±7.05

Values are mean±SEM of five samples, CB-*Croton bonplandianum*, OM-*Origanum majorana*, VN-*Vitex negundo*, IL-*Indigofera linnaei*, AA- Ascorbic acid.

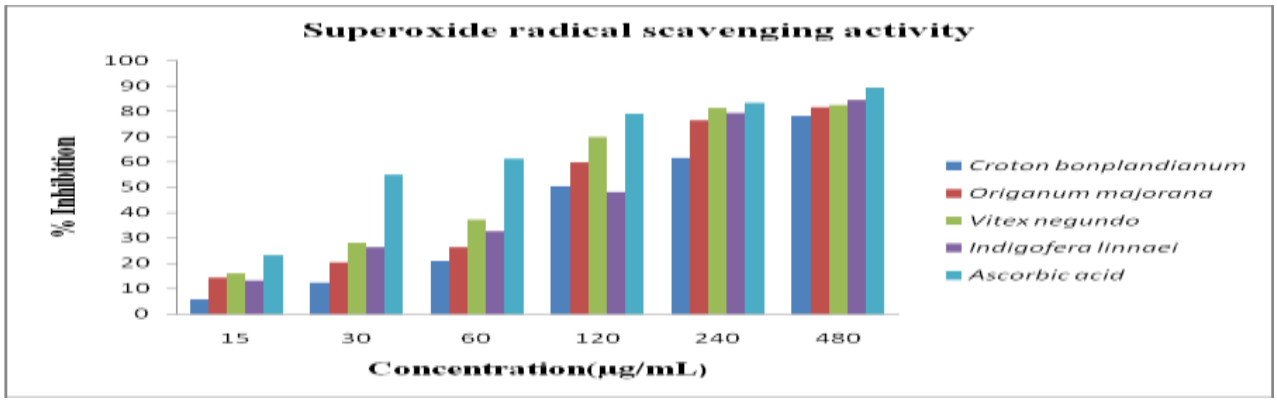

Figure-2

Table 3: Percentage Inhibition of Hydroxyl radical using deoxyribose method

Plant extracts	Quantity(µg/mL)					
	10	**20**	**40**	**80**	**160**	**320**
CB	9.81±0.05	19.06±1.21	32.24±3.21	55.012±3.35	63.25±4.51	81.09±5.21
OM	12.34±0.10	27.05±0.83	39.61±2.31	56.12±3.65	71.08±3.75	81.24±4.98
VN	6.8±1.11	14.32±2.31	38.41±5.42	51.05±3.44	72.15±4.21	79.08±6.08
IL	8.75±1.08	15.58±2.42	36.41±3.48	53.08±3.25	68.23±4.52	71.09±3.44
AA	16.03±2.14	32.14±2.45	51.46±5.24	67.105±2.45	84.52±4.58	91.05±6.45

Values are mean±SEM of five samples

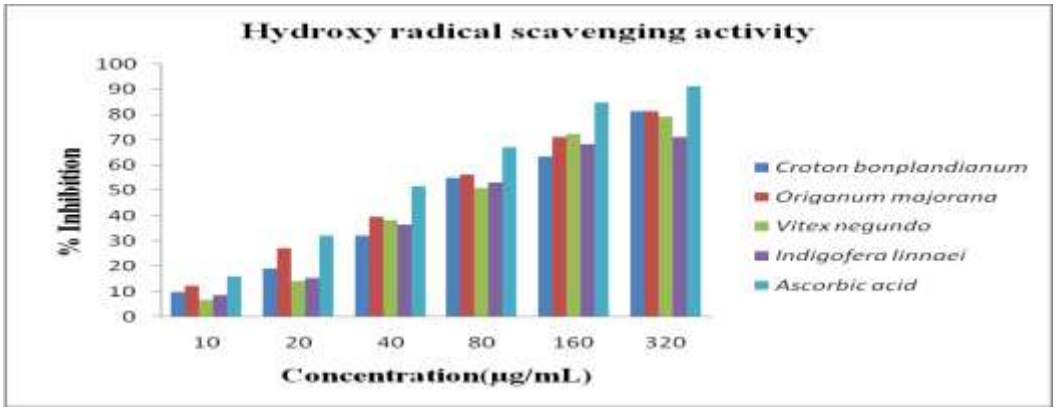

Figure-3

Table 4: Percentage Inhibition of Lipid peroxidation using thiobarbituric acid method

Plant extracts	Quantity(µg/mL)				
	25	50	100	200	400
CB	13.62±0.42	29.06±0.08	36.21±1.55	54.36±3.32	68.28±8.40
OM	18.05±1.09	36.12±1.57	43.81±1.19	62.24±6.41	78.35±8.86
VN	15.21±0.87	33.07±2.29	49.72±2.61	66.52±3.82	81.16±5.83
IL	14.48±0.27	32.17±2.24	57.03±3.24	59.08±7.28	77.02±8.56
AA	25.22±0.98	41.47±1.24	64.37±3.58	82.25±5.68	94.55±4.86

Values are mean±SEM of five samples

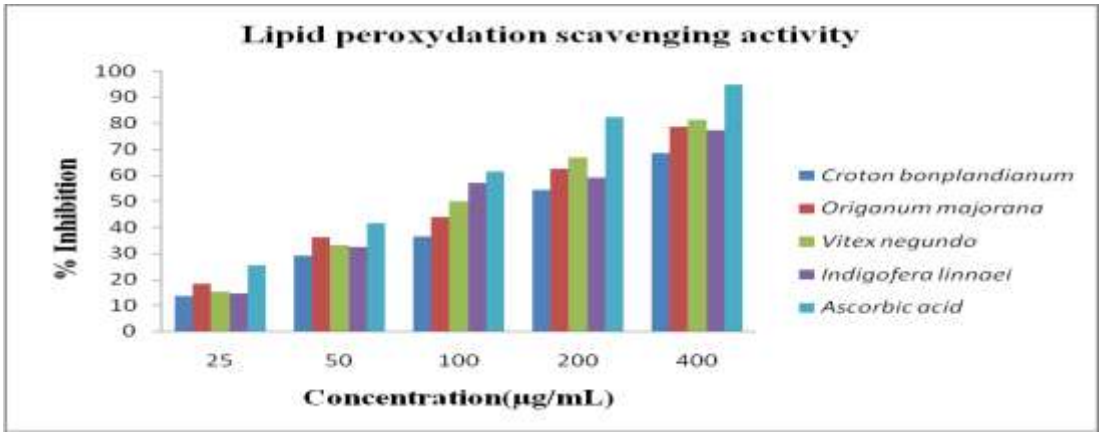

Figure-4

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