Evaluation of *In-vitro* Antioxidant and Xanthine oxidase inhibitory activity of selected Indian plants

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ABSTRACT

In the present investigation an attempt was made to find a new herbal derived material with potential Xanthine oxidase inhibitory and free radical scavenging activities. We have screened the Ethanolic extracts of 10 medicinal plants belonging 8 families, regardless of their claimed ethanopharmacological and /or food uses for their XO inhibitory activities using an optimized protocol. The Super oxide, Nitric oxide, hydrogen peroxide radical scavenging activity and Total antioxidant activity of these extracts were investigated employing various established *in vitro* systems. Total phenolic and flavanoid content were also determined. The Xanthine oxidase enzyme inhibitory and the antioxidant activity of Ethanolic plant extract were found to be in the following order. *Piper nigrum* > *Brassica juncea* > *Cuminum cyminum* > *Cinnamomum zeylanicum* > *Coriandrum sativum* > *Cinnamomum tamala* > *Nigella sativa* > *Elettaria cardamomum* > *Syzygium aromaticum* > *Prunus amygdalus*. Among these plant extracts the three plants namely *Piper nigrum ,Cuminum cyminum and Brassica juncea* were found to be most active for the above said activities. The quantitative estimation of revealed the considerable amount of phenols and flavanoids which may be attributed for its antioxidant activity through the inhibition of Xanthine oxidase enzyme. The study showed that many of the tested plant species are potential sources of natural XO inhibitors that can be developed, upon further investigation, into successful herbal drugs for treatment of gout and other XO-related disorders.

KEYWORDS: Free radical scavenging, Total antioxidant, Xanthine oxidase,

INTRODUCTION

In human a given amount of oxygen taken in by the body is always converted to Reactive oxygen species (ROS) such as O_2^-, H_2O_2 and hydroxyl radical (OH) by various enzymatic metabolism system [1]. ROS affects various molecular components of the cell, like fatty acids, proteins and DNA and an excess production of ROS leads to cell degeneration and death [2]. When the amount of ROS exceeds the limit of defense mechanism of the body, many serious diseases may induced such as cancer, arteriosclerosis, gout, alzheimer's disease and various age–related diseases. Among the enzymes concerning oxidation, Xanthine oxidase (XO) serves as an important biological source of oxygen derived free radicals that contribute to the oxidative damage of living tissues [3]. Xanthine oxidase is a key enzyme that catalysis the oxidation of xanthine and hypoxanthine into uric acid and plays a vital role in producing hyperuricemia and gout [4].Gout is a common disease with a worldwide distribution results from the over production or under excretion of uric acid and is greatly influenced by a high dietary intake of food rich in nucleic

acid [5].

Gout is characterized by the deposition of uric acid in the bone joints leading to severe and episodic painful inflammation [6]. Uricosuric drugs which increase the urinary excretion of uric acid, or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout [7]. Allopurinol is a clinically used Xanthine oxidase inhibitor in the treatment of gout, but this drug suffers from many side effects such as hepatitis, nephropathy and allergic reactions [8]. Moreover allopurinol and its active metabolite oxypurinol is catalyzed by Xanthine oxidase itself, resulting in the generation of reactive oxygen species such as Superoxide anion (O_2^-) is involved in various pathological states such as hepatitis, inflammation, ischemia, reperfusion, carcinogenesis and aging [9,10]. Superoxide anion will be subsequently converted to hydrogen peroxide(H_2O_2)by superoxide dismutase in most normal tissues[11].In the presence of transition metals like (fe^{2+}), H_2O_2 will become hydroxyl radical (OH) that are more reactive than super oxide or hydrogen peroxide [12]. Besides the direct toxicity of O^{2-} or it's derivatives,O^{2-} reacts more rapidly with nitric

oxide (NO), generating more toxic species such as peroxynitrite (ONOO-) by which tissue injury will be exacerbated .

With the above scenario thus search for novel Xanthine oxidase inhibitors with a higher therapeutic activity and fewer side effects are desired not only to treat gout, but also to combat various diseases associated with Xanthine oxidase activity. Several plants extracts from China[13], Brazil [14], Vietnam and Australia[15] have shown inhibitory effects on Xanthine oxidase. Natural products provide a vast pool of XO inhibitory potential that can possibly be developed into clinical products. In the present investigation an attempt was made to find a new herbal derived material with potential Xanthine oxidase inhibitory activities using 10 medicinal plants belonging 8 families, regardless of their claimed ethanopharmacological and /or food uses.

MATERIALS AND METHODS

Chemicals

All routine chemicals such as Ascorbic acid, Gallic acid, Catechin, Vitamin E, Sodium nitroprusside, Ferric chloride Potassium thiocynate were obtained from SD Fine Chemicals Ltd., India. Xanthine oxidase from bovine milk was purchased from Sigma (×4500). All other chemicals and reagent used were of analytical grade.

Collection of Plant Material

The Plant materials, of the selected species (*n* = 10), proposed for the present study were collected from Rajiv Gandhi Botanical garden Gaya and were authenticated by Dr.Sankaranarayanan, Assistant Director, Dept of Research and Development, Sairam Siddha Medical College and Research Centre, Chennai. India. The voucher specimen is also available in herbarium file of the same centre.

Preparation of extract

The parts of plants mentioned in Table -1 (100gms each) were washed thoroughly in tap water, shade dried and powdered. The powder was passed through 40-mesh sieve and exhaustively extracted with 90% (v/v) ethanol in Soxhlet apparatus at 60°C.The extract was evaporated under pressure till all the solvent had been removed and further removal of water was carried out by freeze drying. The solid residues were collected, yield was calculated (Table-1) and stored in refrigerator condition until analysis.

Xanthine Oxidase Inhibitory Assay

The Xanthine oxidase activity with xanthine as the substrate was measured spectrophotometrically using the procedure of Owens and Johns [16] with the following modifications. Total volume of the assay mixture is 3.4 ml and consists of the different concentrations of plant extract under study, 0.15 M phosphate buffer (pH7.5) and 100µl of 0.03U/ml Xanthine oxidase enzyme solution. After pre incubation of the test solution at 25°C for 10 min, the reaction was initiated by addition of 1 ml of 0.6 mM substrate solution of xanthine, mixed thoroughly, and monitored through absorption increments read after10 min at 295 nm indicating the formation of uric acid using a Shimadzu UV-1604 series spectrophotometer. Allopurinol was used as a positive control. The Xanthine oxidase inhibitory activity was estimated by comparing the absorbance value of control.The percent xanthine oxidase inhibitory activity of the assayed samples were calculated by using the formula $[(A_0–A_1/A_0) \times 100]$. Where A_0 is the absorbance of the control and A_1 is the absorbance of plant extract or the standard sample.

Xanthine oxidase generated Superoxide anion scavenging assay.

The Superoxide radical scavenging activity generated by the Xanthine/Xanthine oxidase system was determined by the NBT reduction method as described by Valentao,*et al*., [17].The assay mixture consisted of a 50mM sodium carbonate buffer (pH 7.8), 50 mM xanthine, 50 mM nitro blue tetrazolium (NBT), and 0.1mM EDTA in the presence or absence of a tested compounds. The reaction was initiated by adding 5 ml of Xanthine oxidase (20 mM), and the increase in absorbance at 560 nm was measured after 5 min against the corresponding blank solution. L-Ascorbic acid was used as the positive control. The decrease of NBT reduction measured by the absorbance of the reaction mixture correlates with the superoxide radical scavenging activity of the plant extracts. The inhibition percentage of superoxide radical = $[(A_0 –A_1/A_0) \times 100]$, Where A_0 is the absorbance of the control, and A_1 is the absorbance of sample.

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method of Marcocci, *et al*., [18]. Various concentrations of the different extracts were mixed with sodium nitro prusside (5mM) in PBS and a final volume of 3 ml was incubated at 25ºC for 150min. After incubation, samples (0.5ml) were removed and diluted with0.5ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was read at 546 nm. The inhibition of nitric oxide generation was estimated by comparing the

absorbance value of control with that of treatments. Vitamin E was used as standard.

Hydrogen Peroxide Scavenging Activity

The ability of samples to quench H_2O_2 was determined by Ruch, *et al.,* [19]. The different concentrations of the plant extract samples were dissolved in 3.4 ml of phosphate buffered saline (PBS) and mixed with 0.6 ml of 2 mM solution of H_2O_2.The ability of the plant extracts to scavenge the H_2O_2 was calculated by measuring the absorbance of H_2O_2 at 230 nm after10 min in a spectrophotometer. For each concentration, a separate blank sample was used for background subtraction. Ascorbic acid was used as the standard under the same assay conditions. The inhibition of H_2O_2 by the extracts was calculated as follows. Hydrogen peroxide radical scavenging activity (%) = $[(A_0 - A_1/A_0) \times 100]$, where A_0 is the absorbance of the control, and A_1 is the absorbance of Ascorbic acid or the sample.

Total Antioxidant Activity

The Total antioxidant activity of the extracts was measured by use of a linoleic acid system by the method of Mitsuda *et al*., [20].The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid in Tween 20 and 50 ml of phosphate buffer (pH 7.0).The mixture was then homogenized. A 0.5 ml of different concentration of the extracts and standard sample (in ethanol) was mixed with 2.5 ml of linoleic acid emulsion and 2 ml phosphate buffer. The reaction mixture was incubated at 37°C in the dark to accelerate the peroxidation process. The levels of peroxide formation were determined according to the thiocyanate method by sequentially adding 5ml of 75% ethanol 0.1 ml of ammonium thiocyanate, 0.1 ml sample solution and 0.1 ml ferrous chloride. Vitamin E was used as positive control which was treated similarly. After mixing for 3 min, the peroxide values were determined by reading the absorbance at 500 nm.

Determination of total phenolic content

Total phenolic content in the lyophilized extracts were determined with the Folin–Ciocalteu's reagent (FCR) according to a published method of Slinkard and Singleton [21]. 100 mg of the sample dissolved in 0.5 ml of water was mixed with 2.5 ml FCR (diluted1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°Cfor 90 min. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extracts).

Determination of Total Flavonoid Content

The total flavonoid content of the extracts were determined by a colorimetric method as described in the literature of Zhishen *et al*., [22]. Aliquots of sample containing 100mg of test drugs were mixed with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite solution were added. After 6 min, 0.15 ml of aluminium chloride solution was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Statistical Analysis

All data was analyzed statistically with Statistica/Macsoftware (Prism, USA).The experimental results were mean ± SD of three parallel measurements. IC_{50} values were obtained through linear regression analysis the plot of concentration (200,400, 800 and1000μg/ml) against percent inhibition.

RESULTS

Xanthine Oxidase Inhibitory Assay

In this study, the ethanolic extracts of 10 plants belonging to 8 different families were investigated as potential XO inhibitors. Their free radical scavenging activities were also studied. The selected plants and their yield in ethanolic extract are summarized in (Table 1).Among the different plant extracts the ethanolic extracts of *Nigella sativa* and *Brassica juncea* showed the maximum yield of 18.00% and 16.42 % w/w respectively. The Xanthine oxidase inhibitory activity of different extract was shown in the Table II.The Xanthine oxidase enzyme inhibitory activity of the plant extracts were found to be in the following order. *Piper nigrum > Brassica juncea > Cuminum cyminum > Cinnamomum zeylanicum > Coriandrum sativum > Cinnamomum tamala > Nigella sativa > Elettaria cardamomum > Syzygium aromaticum > Prunus amygdalus*. Among these plant extracts the three extracts such as *Piper nigrum, Brassica juncea* and *Cuminum cyminum* had greater than 80% inhibition of Xanthine oxidase at 1000μl concentration. So the IC_{50} values were determined only for these three extracts and it was found to be 156.45, 172.67 and 212.34 μg/ml respectively.

Xanthine oxidase generated Superoxide anion scavenging assay.

Table –III depicts the Xanthine oxidase generated super oxide scavenging activity of the different plant extracts along with the positive control Ascorbic acid. In the present investigation all the plant extracts scavenged the super oxide radical considerably. But in correlation with the Xanthine oxidase inhibitory activity here also the above said three extracts namely *Piper nigrum Brassica juncea* and *Cuminum cyminum* scavenged the super oxide radicals to a maximum extent with the IC_{50} values corresponds to162.63, 145.36 and143.93 µg/ml respectively.

Nitric oxide scavenging activity

Table-IV explains the Nitric oxide scavenging activity of plant extracts which is compared with standard Vitamin E. In contrast with the above activities all the selected plants extracts scavenged nitric oxide radicals considerably. The scavenging activity of all extracts ranges between 70 to 80 % when comparable to the standard vitamin-E, which showed the inhibition of nitric oxide radicals in range of 80%.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity was shown in the Table –V. Among all the extracts the extracts of *Piper nigrum, Brassica juncea* and *Coriandrum sativum* inhibited the hydrogen peroxide radicals to a maximum extent. At the concentration of 1mg/ml the hydrogen peroxide scavenging ability of these plant extracts were found to be 88.37%, 83.31% and 82.67% respectively. In concurrence with the scavenging activity of these extracts the IC_{50} values were also found to minimum. For *Piper nigrum* it was 151.22 µg/ml for *Brassica juncea* it was 160.87 µg/ml and for *Coriandrum sativum* it was 170.65 µg/ml respectively.

Total Antioxidant Activity

Since we had made an attempt to study the individual free radical scavenging property of our selected plants extracts we wanted to know the total antioxidant activity also. The total antioxidant activity of plant extracts was measured using ferric thiocyanate test, which determines the amount of peroxide produced at the initial stage of lipid peroxidation. Antioxidant scavenging activity of different plant extracts and standard Vitamin E were shown in the Figure-I. Similar to the Xanthine oxidase inhibitory activity the total antioxidant activity was found to be high for the three plant extracts namely *Piper nigrum, Brassica juncea* and *Cuminum cyminum.* Among the three, *Piper nigrum* showed 89.26 % antioxidant activity which is little less than that of standard vitamin E with the value of 90.72%.

Total phenol and Flavanoid Content

The total phenol and flavonoid content of the different plants extract was depicted in Table-VI. All the plant extracts possessed considerable quantity of phenols and flavonoids The highest free radical scavenging potential of *Piper nigrum* and *Brassica juncea* may due to its presence of high phenol and flavonoids contents, with a inhibitory potential of Xanthine oxidase enzyme which is responsible for the production of above said oxidants.

DISCUSSION

Reactive oxygen species (ROS) such as singlet oxygen (•O_2), hydrogen peroxide (H_2O_2) and hydroxyl (•OH) radical are often generated as byproducts of biological reactions or from exogenous factors. Accumulating evidence suggests that even under normal physiological conditions, reactive oxygen species (ROS) are generated in aerobic cells, and therefore, exposure to ROS is inevitable [23].Among the enzymes concerning oxidation, Xanthine oxidase has been recognized as a key enzyme in pathology such as ischemia reperfusion and hypertension. Xanthine oxidase (XOD), an enzyme widely distributed in mammal tissues, catalyzes metabolism of hypoxanthine to Xanthine (XA), and then Xanthine to uric acid in the presence of molecular oxygen, this being accompanied with super oxide (O_2^-) generation and plays a vital role in producing hyperuricemia and gout.

The uric acid is one of the major contributors of plasma antioxidant capacity; however under condition of increased oxidative stress there occurs depletion of local antioxidants like superoxide dismutase, glutathione peroxidase and catalase. This pathophysiology results in increased production of ROS especially super oxide ions as the endothelial nitric oxide synthase uncouples to produce superoxide ions instead of nitric oxide, increasing subsequently favoring antioxidant – pro oxidant urate redox shuttle[24].When serum uric acid levels are elevated the uric acid which was previously physiologically antioxidant paradoxically becomes pro- oxidant. In this study, we investigated the Xanthine oxidase inhibitory potential as well as scavenging effect of our plant extracts on Xanthine oxidase generated super oxide radicals.

As a result, we found that three extracts namely *Piper nigrum*, *Brassica juncea* and *Cuminum cyminum* could effectively inhibit Xanthine oxidase and scavenge the super oxide radicals generated by the hypo-xanthine/xanthine oxidase reaction system. The phytoconstituent piperdine in *piper nigrum* and a compound named BjCHI1 and kaempferol glycosides present in *Brassica juncea* may be responsible for the inhibitory activity of Xanthine oxidase and there by the

hyperuricemia effect. Reshmi,*et al*.,has isolated several piperdine compounds from the methonolic extract of *piper nigrum* and showed its antiproliferative activity [25].According to Hyun Ah Jung, *et,al.,*the leaves from *Brassica juncea* containing kaempferol glycosides, such as kaempferol-3-O-(2-O-sinapoyl)-β-d-glucopyranosyl-(1→2)-β-d-glucopyranoside-7-O-β-d-glucopyranoside,kaempferol-3-O-β-d-glucopyranosyl-(1→2)-β-d-glucopyranoside-7-O-β-d-glucopyranosyl-(1→6)-β-d-glucopyranoside and kaempferol-3-O-(2-O-sinapoyl)-β-d-glucopyranosyl-(1→2)-β-d-glucopyranoside-7-O-β-d-glucopyranosyl-(1→6)-β-d-glucopyranoside possessed the antioxidant activity[26].The presence of these compounds in the seeds were accounted for the XO inhibitory activity observed in our present investigation.

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. According to Hemnani and Parihar, [27] the potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids, degradation of DNA and oligonucleosomal fragments.Nitric oxide or reactive nitrogen species, formed during its reaction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds alter the structure and function of many cellular components. In our present study all the selected plants extracts scavenged nitric oxide radicals considerably. This is attributed to the fact that all the Indian spicy plants contain many important phyto-chemical constituents which can protect the body from the ill effects of oxidants.

Hydrogen peroxide is formed by two-electron reduction of O_3 which is not a free radical, but an oxidizing agent. In the presence of O_3 and transition metal ions, the H_2O_2 can generate OH radical via Fenton reaction. Mahakunakorn *et al.*, [28] reported, that in addition, H_2O_2 can easily cross the cell membrane and exerts an injurious effect on tissues through a number of different mechanisms such as, perturbing intracellular Ca^{2+} homeostasis, increasing intracellular ATP, inducing DNA damage, and cell apoptosis.So the removal of H_2O_2 is important for the antioxidant defence mechanism. In the present investigation the plant extracts effectively scavenged the H_2O_2 radicals. The maximum scavenging activity was exhibited by the three extracts namely *Piper nigrum, Brassica juncea and Coriandrum sativum*. The presence aromatic acids containing compounds such as 2-decenoic acid, E-11-tetradecenoic acid, capric acid, undecyl alcohol tridecanoic acid and undecanoic acid in *Coriandrum sativum* were reported by Md. Nazrul Islam Bhuiyan, *et al*., [29]may be responsible for the Hydrogen peroxide scavenging activity observed in the present study.

In correlation with the above investigations the *Piper nigrum* shows 89.26 % antioxidant scavenging activity which is little less than that of standard vitamin E with the value of 90.72%. According to Jiang *et al.,*[30] flavanoid generally occur as O-glycosides in which one or more of the hydroxyl groups are bound to sugars. As stated by Robards and Antolovvich [31] this glycosylation renders flavanoids more water soluble, making them store readily in the cell vacuole where they are commonly found. The above-mentioned effects might be expected for our plant extracts in reducing the lipid peroxidation. The highest Xanthine oxidase inhibitory activity and free radical scavenging potential of *Piper nigrum* and *Brassica juncea* observed in our present investigation may due to the presence of high phenol and flavanoid contents. The presence of significant quantities of flavanoid in these two plant extracts are considered as the major contributors towards the antioxidant activity in our present study. According to Pietta, [32] flavanoids are a class of secondary plant phenolics with powerful antioxidant properties. The obtained results suggest that the studied plants can form a good source of effective crude inhibitors for Xanthine Oxidase enzyme which can be used in the treatment of gout and other XO-related disorders. However, further biological investigations are needed, particularly using animal models, to verify the reported inhibitory activities under *in vivo* conditions.

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Table -I Selected parts of the plants for the proposed study and their percentage yield

Botanical name of plant	Family	Parts used	Common name	Ethanolic extract Yield expressed in % (w/w)
Brassica juncea	Brassicaceae	seed	Brown mustard	16.42
Elettaria cardamomum	zingiberaceae	seed	Cardamom	8.69
Cinnamomum tamala	Lauraceae	leaf	Cinnamon tamala	11.68
Cinnamomum zeylanicum	Lauraceae	Inner bark	Cinnamom	11.02
Syzygium aromaticum	Myrtaceae	Flower bud	Clove	15.12
Coriandrum sativum	Apiaceae	Seed	Coriander	14.08
Cuminum cyminum	Apiaceae	Seed	Cumin	15.44
Nigella sativa	Ranunculaceae	Seed	Nigella	18.00
Piper nigrum	Piperaceae	Fruit	Pepper	15.44
Prunus amygdalus	Rosaceae	Fruit	Almond	8.10

Table– II Xanthine oxidase inhibitory activity of ethanolic extracts of different plants and Standard Allopurinol

Botanical name of plant	Xanthine oxidase inhibitory activity (%)			
	200µgms/ ml	400µgms / ml	800µgms/ ml	1000µgms/ ml
Brassica juncea	13.56± 0.47	26.40±0.52	44.59±0.52	83.31±0.64
Elettaria cardamomum	05.76±0.78	12.55±0.52	55.53±0.63	65.63±0.76
Cinnamomum tamala	07.34±1.34	12.45±0.47	43.58±0.70	69.21±0.61
Cinnamomum zeylanicum	11.52±1.45	22. 45±0.39	65.12±0.45	79.46±0.72
Syzygium aromaticum	7.56±1.29	12.55±0.52	45.53±0.63	65.63±0.76
Coriandrum sativum	9.23±0.52	15.74±0.70	52.49±0.55	71.37±0.75
Cuminum cyminum	12.78±0.56	30.12±0.45	68.10±0.29	83.06±0.89
Nigella sativa	10.67±0.53	17.73±0.52	32.45±0.44	65.63±0.76
Piper nigrum	15.55±3.67	28.24±0.59	68.47±0.64	88.17±0.74
Prunus amygdalus	02.50±0.23	06.30±0.53	18.31±0.30	37.33±0.54
Allopurinol	16.78±0.92	31.56±0.82	67.34±0.46	88.24±0.35

Each value represents the mean ±SEM (n=3)

Table –III Super oxide radical scavenging activity of ethanolic extracts of different plants and Standard Ascorbic acid

Botanical name of plant	Super oxide radical inhibitory activity (%)			
	200 µgms/ml	400µgms/ml	800 µgms/ml	1000µgms/ ml
Brassica juncea	15.16±1.00	32.59±0.52	59.48±0.71	79.40±0.61
Elettaria cardamomum	14.56±0.45	23.17±1.02	49.48±0.60	78.44±0.54
Cinnamomum tamala	10.08±1.6	21.66±0.64	36.63±0.73	75.33±0.52
Cinnamomum zeylanicum	07.35±0.68	15.50±0.50	32.34±0.61	58.55±0.71
Syzygium aromaticum	08.33±0.57	15.53±0.63	28.74±0.70	58.46±0.64
Coriandrum sativum	13.34±0.76	27.17±0.64	53.45±0.79	78.06±1.2
Cuminum cyminum	07.41±0.53	13.58±0.70	29.67±0.66	69.24±0.65
Nigella sativa	09.42±0.63	17.58±0.67	49.73±0.52	73.46±0.58
Piper nigrum	18.45±0.59	35.47±0.64	72.41±0.68	84.38±0.62
Prunus amygdalus	07.63±0.63	16.31±0.30	28.23±0.58	56.42±0.69
Ascorbic acid	16.67±0.78	34.62±0.80	73.87±2.54	83.39±0.54

Each value represents the mean ±SEM (n=3)

Table –IV Nitric oxide radical scavenging activity of ethanolic extracts of different plants and Standard Vitamin E

Botanical name of plant	Nitric oxide radical inhibitory activity (%)			
	200µgms / ml	400µgms / ml	800µgms/ml	1000µgms/ ml
Brassica juncea	18.42±0.64	33.48±0.71	60.21±0.11	80.49±0.71
Elettaria cardamomum	16.43±0.55	29.48±0.60	59.43±0.39	79.55±0.41
Cinnamomum tamala	12.45±0.60	26.63±0.73	56.63±0.36	77.54±0.47
Cinnamomum zeylanicum	11.34±0.36	25.34±0.61	57.24±0.13	77.29±0.54
Syzygium aromaticum	09.49±0.55	15.74±0.70	41.76±0.54	71.37±0.75
Coriandrum sativum	18.37±0.61	33.45±0.79	62.14±0.71	79.77±0.54
Cuminum cyminum	10.54±0.61	19.67±0.66	53.32±0.43	71.31±0.61
Nigella sativa	08.70±0.51	19.73±0.52	50.51±0.25	75.41±0.56
Piper nigrum	11.30±0.66	24.41±0.68	54.34±0.46	80.31±0.51
Prunus amygdalus	13.40±0.62	28.23±0.58	59.65±0.32	78.49±0.71
Vitamin E	19.56±0.83	38.45±0.67	64.18±0.39	84.76±2.67

Each value represents the mean ±SEM (n=3)

Table –V Hydrogen peroxide radical scavenging activity of ethanolic extracts of different plants and Standard Vitamin E

Botanical name of plant	Hydrogen peroxide radical inhibitory activity (%)			
	200 µgms / ml	400µgms / ml	800 µgms / ml	1000µgms / ml
Brassica juncea	14.59±0.52	28.45±2.34	61.16±1.00	83.31±0.64
Elettaria cardamomum	06.17±1.02	17.55±0.52	51.56±0.45	70.68±0.67
Cinnamomum tamala	09.66±0.64	22.45±0.44	60.08±1.6	72.59±0.58
Cinnamomum zeylanicum	09.80±0.50	30. 45±0.44	64.35±0.68	73.51±0.44
Syzygium aromaticum	05.53±0.63	14.55±0.52	39.33±0.57	65.63±0.76
Coriandrum sativum	13.17±0.64	32.74±0.70	69.34±0.76	82.67±0.80
Cuminum cyminum	06.58±0.70	18.12±0.45	47.41±0.53	69.21±0.61
Nigella sativa	06.58±0.67	19.73±0.52	43.42±0.63	63.48±0.57
Piper nigrum	18.47±0.64	38.74±0.59	68.45±0.59	88.37±0.74
Prunus amygdalus	04.31±0.30	16.30±0.53	22.63±0.63	37.33±0.54
Vitamin E	20.45±1.23	34.67±0.78	69.56±0.89	90.12±0.45

Each value represents the mean ±SEM (n=3)

Table –V Total Phenol and Flavanoid Content of the different plant extracts

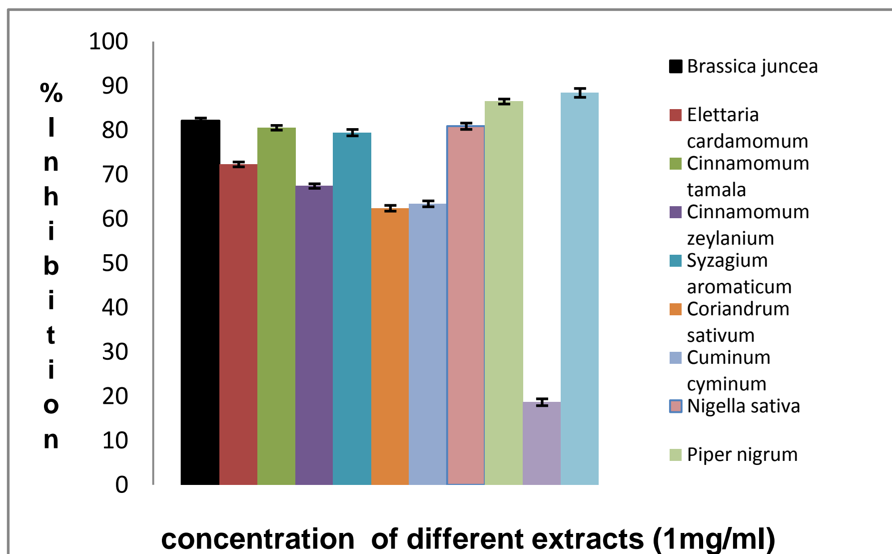
Botanical name of plant	Total phenol content (mg/g)	Total flavonoid content (mg/g)
Brassica juncea	43.48±0.45	272.024±0.16
Elettaria cardamomum	5.44±0.36	50.84±0.32
Cinnamomum tamala	28.02±0.76	45.58±0.23
Cinnamomum zeylanicum	2.04±0.64	35.26±0.49
Syzygium aromaticum	18.56±0.09	63.10±0.49
Coriandrum sativum	39.42±0.57	105.60±0.32
Cuminum cyminum	07.76±85	73.44±0.51
Nigella sativa	14.00±0.75	54.00±0.37
Piper nigrum	90.72±0.11	282.24±0.16
Prunus amygdalus	9.56±0.76	31.58±0.39

Each value represents the mean ± SEM (n = 3).

Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.

Total flavanoid content was expressed as mg catechin equivalent/g dried extract.

Fig-1 Total Antioxidant scavenging activity of 1mg/ml different plant extract and standard Vitamin E



Each value represents the mean ±SEM (n=3)