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Evaluation of antioxidant activity of some wild edible fruits collected from Boda and Kolli hills

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ABSTRACT

Free radicals or highly reactive oxygen species are capable of inducing oxidative damage to human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases; hence natural antioxidants have significant importance in human health. The present study is to evaluate the *in-vitro* antioxidant activities of *Catunaregam spinosa, Diospyros ferrea, Murraya koenigii, Tarenna asiatica* and *Zizyphus oenoplia* fruit extracts from different solvents such as aqueous, chloroform and ethanol. The wild edible fruits collected from Boda and Kolli hills, eastern ghats, Tamil Nadu, India. The antioxidant activity of these five edible fruits was evaluated by measuring DPPH, RPA, SO, NO, HO, H_2O_2, FRAP assay. The antioxidant compounds like ascorbic acid were also evaluated. *Catunaregam spinosa, Diospyros ferrea, Murraya koenigii, Tarenna asiatica* and *Zizyphus oenoplia* fruits extract exhibited significant differences in their antioxidant values. The ethanolic extract of *Tarenna asiatica* and *Diospyros ferrea* exhibited significantly higher antioxidant activity than the other extract of *Catunaregam spinosa, Murraya koenigii* and *Zizyphus oenoplia*. It can be concluded that regular use of these edible fruits, help in treating many diseases caused by free radical damage.

Keywords: Kolli and Boda hills, Fruit Extracts, Antioxidant activity and DPPH.

INTRODUCTION

Fruit consumption is not only for taste and personal requirements, but also play a vital role in nutrient supplementation. Macro nutrients such as protein, lipid and carbohydrates and micronutrient such as minerals, fibers, vitamins and secondary phytochemical compounds are the major health supplement to the human body [1, 2]. Diets rich in phytochemicals, such as phenolic compounds have been associated with a reduced risk of diseases such as cancer, inflammation, cardiovascular, cataracts, macular degeneration and neurodegenerative diseases [3, 4, 5]. Tropical fruit consumption is increasing on domestic and international markets due to their nutritional and therapeutic value. India is the second production of fresh and processed fruits worldwide [6]. An antioxidant is a substance that when present at a concentration low compared to that of an oxidisable substrate, significantly delays or prevents oxidation of that substrate. Even though plant phenols are not considered as real antioxidants in the literature, but many *in vitro* studies have demonstrated the antioxidant potential of phenols as direct aqueous phase radical scavengers and as agents capable of enhancing the resistance to oxidation of low density lipoproteins implicated in the pathogenesis of coronary heart disease [7]. Biological systems have evolved with endogenous defense mechanisms to help protect against free radical induced cell damage. Ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E), in their reduced form, which also exert an antioxidant effect by quenching free radicals. A number of other dietary antioxidants exist beyond the traditional vitamins collectively known as phytonutrients or phytochemicals, which are being increasingly appreciated for their antioxidant activity, flavonoids belong to the group of polyphenolic compounds. In plant flavanoids is glucosylated derivatives. They are found in leaves, flowers, fruits, seeds, nuts, grains, spices, and different medicinal plants, beverages such as wine, tea and beer [8, 9]. Wild fruits are receiving increasing interest from researchers because of their medicinal properties, nutritional value, vitamin and mineral contents. Concerning their medicinal properties, the most commonly studied benefit is their antioxidant effects [10-13]. There is an abundance of wild fruits in Boda and Kolli hills, Eastern Ghats, India, because of its vast territory, various landforms and wide-spanning climate. Some of these wild fruits are edible, while some can be used as medicines. However, many wild fruits in Boda and Kolli hills are underutilized, no report about antioxidant activities of wild fruits from Boda and Kolli hills could be found in the literature.

The aim of this study was to systematically evaluate the antioxidant activities of five wild fruits collected from Kolli and Boda hills and to screen out the wild fruits with high antioxidant capacity. From these studies, the full utilization of these wild fruits helpful for the human health benefits.

MATERIALS AND METHODS

Fruits collection and plant identification

Wild edible, mature fruits of *Catunaregam spinosa, Diospyros ferrea, Murraya koenigii, Tarenna asiatica and Zizyphus oenoplia* were collected from the Boda and Kolli hills. The collected fruits specimen was authenticated by Botanical survey of India (BSI), Coimbatore, Tamil Nadu, India and in Department of Botany, National College, Tiruchirappalli.

Preparation of Extract

The fresh fruits were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. 100 gm of powdered material was soxhlet extracted with different solvents, like aqueous, chloroform and ethanol (24 hour each). All the extracts were evaporated in vacuum under reduced pressure. All extracts were stored in sterile glass bottles at room temperature until its screened.

Antioxidant activity methods

The antioxidant activity of the extracts was determined using a DPPH assay [14], Reducing power assay [15], Superoxide anion radical scavenging activity [16], Nitric oxide radical scavenging (NO) assay [17], Hydroxyl radical scavenging (HO) assay [18], Hydrogen peroxide-scavenging activity [19], Ferric reducing antioxidant power (FRAP) assay [20].

DPPH assay

DPPH scavenging activity was carried out by the method of Blois (1958). Different concentrations (1000, 500, 250, 125 and 62.5 µg/ml) of crude extracts were taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2- Picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37° C for 20 minutes. The control was prepared without any extracts. Ethanol was used for base line corrections in absorbance (OD) of samples measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{OD\ Control - OD\ Sample}{\text{OD Control}}$$

Reducing power assay

Different concentrations (1000, 500, 250, 125 and 62.5 µg/ml) of crude extracts were taken in test tubes in triplicates. 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50°C for 20 minutes. After incubation 2.5 ml of 10% TCA was added and were kept for centrifugation at 3000 rpm for 10 minutes. After centrifugation 5 ml of supernatant was taken and to this 5ml of distilled water was added. To this about 1 ml of 1% ferric chloride was added and was incubated at 35°C for 20 minutes. The OD (absorbance) was taken at 700 nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

Superoxide anion radical scavenging activity

The activity was evaluated using nitro blue tetrazolium (NBT) reduction method given by Nishikimi *et al.,* (1972). The reaction mixture consisted of 1ml of NBT solution (156µM) and sample solution at different concentrations (1000, 500, 250, 125 and 62.5 µg/ml). The reaction was started by adding 100µl of phenazine methosulfate solution (60µM, PMS) in phosphate buffer (pH 7.4) to the reaction mixture followed by incubation at 25°C for 5 min and the absorbance at 560nm was measured against blank. Ascorbic acid was used as the standard.

$$Superoxide\ scavenging\ activity\ (\%) = \frac{OD\ Control - OD\ Sample}{\text{OD Control}}$$

Nitric oxide radical scavenging (NO) assay

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentration (1000, 500, 250, 125 and 62.5 µg/ml) of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 hrs, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and the activity was compared with ascorbic acid, which was used as a standard antioxidant. Nitric oxide radical scavenging that is calculated by the Formula:

$$Nitric\ oxide\ radical\ radical\ scavenging(\%) = \frac{OD\ Control - OD\ Sample}{\text{OD Control}} \times 100$$

Hydroxyl radical scavenging (HO) assay

The activity was evaluated by a Fenton reaction ($Fe3^{+}$- ascorbate-EDTA-H_2O_2 system), and the scavenging capacity towards the hydroxyl radicals was measured by using deoxyribose method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1mM, pH 7.4), ferric chloride (20µM), EDTA (100µM), hydrogen peroxide (500 µM), ascorbic acid (100µM) and various concentrations (1000, 500, 250, 125 and 62.5 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 hr at 37°C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90°C) to develop the colour. After cooling, the absorbance was measured at 532nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, According to the following Formula:

$$Hydroxyl\ radical\ scavenging\ (\%) = \frac{OD\ Control - OD\ Sample}{\text{OD Control}} \times 100$$

Hydrogen peroxide-scavenging activity

A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration (1000, 500, 250, 125 and 62.5 μg/ml) of sample was added to a hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as above. All experiments were performed in triplicates. The percentage of inhibition was expressed, According to the following equation:

$$Hydrogen\ peroxide - scavenging\ activity\ (\%) = \frac{OD\ Control - OD\ Sample}{\text{OD Control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

Ferric Reducing Antioxidant Power (FRAP) Assay The ability to reduce ferric ions was measured using the method described by Benzie and Strain, 1996. The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM (tripyridyl triazine) TPTZ solution and 20 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations (1000, 500, 250, 125 and 62.5 μg/ml) was then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated.

RESULT AND DISCUSSION

Antioxidant

Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. Keeping the adverse effects of synthetic antioxidants, researchers moved their interest in isolating natural antioxidant compound [21] which are very effective to control the oxidative stress and hence prevent the initiation of diseases propagate by free radical scavengers. The antioxidant activity of a drug may depend on the free radical scavenging activity [22]. Few studies on fruits extract such as *Phoenix dactylifera* [23], *Zizyphus mauritiana* [24] fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* [25] have been done earlier. However, the present studies of five wild edible fruits from Boda and Kolli hills have the sympathetic antioxidant activity.

DPPH Free Radical scavenging activity

The antioxidant activity of the extracts was determined by using a DPPH method. The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are major factor in biological damages caused by oxidative stress. The effects of ethanol, chloroform and aqueous extracts of five edible fruits was evaluated for its antioxidant activity on different *in vitro* models like DPPH radical scavenging activity in a concentration dependent manner (Tables 1). All the tested extracts possessed antioxidant properties, but to varying degrees, ranging from 9.6 to 84.8**%.** Generally ethanol extract showed the better DPPH scavenging activity than aqueous and chloroform extract. A maximum scavenging activity was offered by ethanol of *Tarenna asiatica* (84.8 %) and *Diospyros ferrea* (83.6%) were the selected samples found to interact with DPPH radicals and thereby stabilize their hyperactivity. The measurements showed that antioxidant activities of fruits extracts increased with increasing amount of sample concentration. *Catunaregam spinosa, Murraya koenigii* and *Zizyphus oenoplia o*f the ethanol, chloroform and aqueous extract of edible fruits show lower in reduction rate. The lower activity of aqueous extract might be due to the fact that active principles of plant material are generally located in the conduit structures called the apoplast (cytoplasm) and symplast (cell wall) the aqueous maceration alone is not sufficient to extract these compounds from the structures whereas ethanol may partially solubilize the membrane of the plant cells and storage organs, helping leach the chemical away [26].

Table 1: DPPH assay

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E
1000	89.4	42.1±3.3	64.3±1.7	68.5±2.1	73.2±2.9	75.4±3.0	83.6±2.4	52.9±1.5	53.7±1.0	54.2±2.5	75.4±3.4	78.1±3.3	84.8±2.1	58.4±3.4	61.3±1.0	69.1±1.1
500	78.7	33.6±3.7	52.8±2.3	55.4±2.4	65.1±1.3	66.9±2.3	69.6±3.5	42.2±1.6	45.6±1.8	46.3±2.1	66.6±2.0	67.2±2.5	69.0±2.6	44.9±0.3	47.8±0.9	59.4±3.2
250	63.9	24.2±2.1	35.9±2.3	39.8±2.6	53.3±2.1	51.1±2.3	54.3±3.7	31.5±1.7	34.2±1.3	36.1±2.6	55.8±0.3	57.5±1.7	59.3±1.5	37.3±0.1	39.5±0.9	49.8±2.3
125	55.5	12.4±1.7	25.4±1.1	29.8±1.5	42.7±0.2	45.8±1.1	47.6±1.8	26.0±1.3	21.1±1.9	27.5±3.0	42.1±0.4	45.7±3.4	48.5±3.9	25.2±1.6	27.0±0.3	30.1±0.4
62.5	41.3	9.6±3.8	13.7±1.7	17.8±1.1	31.8±1.8	34.0±1.7	37.7±1.9	16.9±1.5	17.9±3.7	19.1±2.2	34.3±2.3	35.2±0.8	38.2±4.9	15.6±0.3	17.5±0.3	19.4±0.9

Reducing power assay

The reducing power of the tested extracts range from 0.03 to 2.13% for 62.5 to 1000 μg/ml, respectively (Tables 2). A maximum scavenging activity was offered by ethanolic extract of *Tarenna asiatica* (2.13%) and *Diospyros ferrea* (1.89**%**). The antioxidant activity is due to their redox properties which can act an important role in absorbing and neutralizing the free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [27] (Osawa, 1994). Tanaka and coworker [28] have observed a direct correlation between antioxidant activities and reducing power of certain fruits extracts. The reducing properties are due to the presence of reductones [29] which helps to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[30]. In this assay, depending on the reducing power of antioxidant compounds, the yellow colour of the test solution changes into various shades of green and blue. Therefore, by measuring at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Table 2: Reducing power assay

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**
1000	2.222	0.79±0.01	0.80±0.04	0.86±0.01	1.48±0.1	1.59±0.02	1.89±0.01	1.31±0.04	1.57±0.02	1.63±0.04	2. 01±0.02	2.09±0.01	2.13±0.04	0.51±0.06	0.62±0.01	0.95±0.06
500	2.145	0.67±0.03	0.64±0.03	0.75±0.02	1.35±0.08	1.47±0.02	1.76±0.03	1.29±0.03	1.44±0.03	1.53±0.03	1.92±0.003	1.88±0.03	1.96±0.03	0.46±0.02	0.55±0.02	0.83±0.07
250	2.015	0.48±0.08	0.43±0.02	0.66±0.03	1.23±0.2	1.33±0.01	1.51±0.01	1.22±0.01	1.32±0.02	1.45±0.03	1.75±0.02	1.75±0.02	1.82±0.01	0.34±0.01	0.46±0.02	0.77±0.01
125	1.912	0.28±0.12	0.34±0.06	0.49±0.01	1.10±0.03	1.24±0.005	1.37±0.01	1.12±0.03	1.24±0.08	1.34±0.05	1.61±1.2	1.64±0.06	1.76±0.03	0.21±0.05	0.34±0.03	0.62±0.08
62.5	1.715	0.03±0.2	0.14±0.02	0.23±0.02	1.06±0.04	1.13±0.01	1.15±0.02	1.05±0.02	1.10±0.06	1.22±0.01	1.42±0.003	1.54±0.03	1.63±0.03	0.11±0.002	0.26±0.02	0.56±0.01

Superoxide scavenging assay

Superoxide has been observed to directly initiate lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical [31]. Superoxide anions derived from dissolved oxygen by the phenazine methosulfate (PMS)-Nicotinamide adenine dinucleotide (NADH) system will reduce NBT in this system [32]. From the results, All the tested extracts possessed antioxidant properties ranging from 21.3 to 82.9%. Generally ethanol extract showed better Superoxide scavenging activity than aqueous and chloroform extract. A maximum scavenging activity was offered by ethanol of *Zizyphus oenoplia* (81.4%) and *Diospyros ferrea* (82.9%) were the selected samples found to interact for the antioxidant. *Catunaregam spinosa, Murraya koenigii* and *Tarenna asiatica o*f the ethanol, chloroform and aqueous extract of edible fruits show lower in reduction rate (Tables 3). Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [33]. Numerous biological reactions generate superoxide anions which are highly toxic species [32].

Table 3: Superoxide anion radical scavenging activity

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**
1000	81.4	64.7±0.3	65.1±0.9	68.3±0.1	66.5±0.2	77.1±1.6	82.9±0.4	63.7±0.3	67.1±0.9	71.2±1.1	64.7±2.6	70.8±0.5	76.4±0.6	66.5±0.2	77.1±1.6	81.4±0.4
500	69.4	52.7±0.7	58.6±0.4	61.0±0.7	59.4±0.4	64.9±1.5	70.3±0.9	58.7±0.7	52.6±0.4	68.3±1.2	58.7±0.4	61.9±0.1	69.3±1.1	59.4±0.4	64.9±1.5	70.3±0.9
250	57.7	44.9±2.6	48.2±1.0	56.6±2.1	43.5±0.1	53.2±0.05	61.9±1.5	42.5±1.8	48.2±1.5	52.9±0.3	42.2±1.5	46.6±1.2	52.5±1.3	43.5±0.1	53.2±0.05	61.9±1.5
125	49.5	38.4±2.3	40.6±0.4	53.7±2.7	37.3±1.5	45.4±2.0	49.4±1.6	37.1±0.9	40.06±0.4	57.7±1.8	38.3±1.8	33.7±1.0	47.7±0.1	37.3±1.5	45.4±2.0	49.4±1.6
62.5	36.7	21.3±2.1	25.9±0.5	29.9±2.1	29.4±0.3	32.9±0.5	34.2±0.05	21.4±1.7	29.9±0.5	35.9±2.1	21.8±1.4	28.9±1.0	33.6±1.4	29.4±0.3	32.9±0.5	34.2±0.05

Hydroxyl radical scavenging assay

All the tested extracts possessed antioxidant properties, but to varying degrees, ranging from 12.9 to 84.1%. Generally ethanol extract showed better Hydroxyl radical scavenging activity than aqueous and chloroform extract. A maximum scavenging activity was offered by ethanol of *Tarenna asiatica* (84.1%) and *Diospyros ferrea* (81.4%) were the selected samples. *Catunaregam spinosa, Murraya koenigii and Zizyphus oenoplia* of the ethanol, chloroform and aqueous extract of edible fruits show lower in reduction rate (Tables 4). The hydroxyl radical can induce oxidative damage to DNA, lipids and proteins [34]. The hydroxyl radical scavenging ability of the extracts was determined by its ability to compete with deoxyribose for hydroxyl radical. In this assay, 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acid conditions to develop a pink chromogen (thiobarbituric acid reactive species) with a maximum absorbance at 532 nm [35].

Table 4: Hydroxyl radical scavenging (HO) assay

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**
1000	88.3	63.3±0.02	71.4±0.02	79.1±0.06	62.9±0.1	72.1±0.1	81.4±1.9	67.1±0.02	77.2±0.01	80.9±0.007	70.5±0.04	76.2±0.01	84.1±0.01	66.5±0.04	76.2±0.01	77.5±0.06
500	78.9	59.5±0.04	67.5±0.01	68.7±0.03	46.4±0.4	66.5±1.0	72.1±0.8	51.7±0.03	65.1±0.02	73.8±0.01	65.3±0.03	64.5±0.01	77.5±0.02	51.4±0.03	63.5±0.01	68.5±0.01
250	64.8	33.1±0.08	44.4±0.01	53.6±0.03	35.3±0.3	58.7±1.1	56.4±2.1	46.4±0.03	55.9±0.03	62.4±0.02	45.9±0.04	55.6±0.02	62.4±0.09	45.4±0.04	54.6±0.02	57.4±0.04
125	53.4	24.4±0.02	36.3±0.02	48.5±0.01	24.4±0.2	37.4±1.2	48.1±0.7	35.8±0.01	47.5±0.02	56.9±0.1	38.1±0.01	49.5±0.01	51.2±0.02	38.1±0.01	42.5±0.01	49.2±0.08
62.5	41.2	16.4±0.02	22.4±0.009	36.1±0.01	12.9±0.1	24.1±1.5	35.5±1.5	24.5±0.011	34.8±0.009	44.1±0.03	23.3±0.03	34.5±0.01	39.1±0.02	26.8±0.01	32.5±0.003	48.8±0.01

Nitric oxide Scavenging assay

All the tested extracts possessed antioxidant properties, but to varying degrees, ranging from 12.1 to 76.1%. Generally ethanol extract showed better Nitric oxide Scavenging activity than aqueous and chloroform extract. A maximum scavenging activity was offered by ethanol of *Tarenna asiatica* (76.1%) and *Diospyros ferrea* (73.9%) were the selected samples found to interact with DPPH radicals and thereby stabilize their hyperactivity. The measurements showed that antioxidant activities of fruits extracts increased with increasing amount of sample concentration. *Catunaregam spinosa, Murraya koenigii and Zizyphus oenoplia o*f the chloroform and aqueous extract of edible fruits show lower in reduction rate (Tables 5). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [36, 37]. Nitric oxide is an essential bio regulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodialation and control of blood pressure [38-41]. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems, including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [42]. NOS is synthesized in a variety of cell types from multiple mammalian species and can produce consistent, high concentrations of NO upon induction with cytokines and or bacterial lipopolysaccharide (LPS) [43].

Table 5: Nitric oxide radical scavenging (NO) assay

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E
1000	77.8	54.3±2.0	57.5±1.1	68.4±0.1	62.4±1.2	67.4±1.1	73.9±1.0	57.8±1.1	60.3±0.2	64.4±1.2	63.52±1.1	70.2±0.5	76.1±1.1	55.8±0.5	54.7±1.1	61.8±0.2
500	71.2	42.7±1.9	45.3±0.8	56.3±1.2	56.1±0.9	59.3±1.5	66.3±1.0	49.3±0.1	53.7±0.9	56.3±1.3	55.49±1.2	52.4±0.3	68.7±1.3	41.3±0.7	43.6±0.1	49.5±1.1
250	64.8	34.8±1.1	35.7±0.9	41.8±1.9	42.9±1.1	46.8±2.1	53.2±1.0	40.7±1.2	43.8±1.1	48.1±1.1	43.71±1.3	47.7±1.1	59.4±0.9	32.1±1.1	33.2±0.5	35.7±0.1
125	52.5	23.2±1.2	25.2±1.1	35.9±1.6	34.8±1.8	37.6±1.2	41.5±1.0	31.8±0.5	26.2±0.1	35.3±0.9	33.62±0.1	37.5±0.9	49.9±0.6	24.4±0.1	27.9±1.5	29.2±1.1
62.5	34.5	14.1±0.5	18.8±1.9	21.2±1.2	25.6±1.4	28.3±3.5	32.8±1.0	19.1±0.4	20.2±0.3	21.4±1.1	21.49±1.2	25.4±0.1	32.2±2.1	12.1±0.9	15.3±0.1	16.4±1.9

Hydrogen peroxide scavenging assay

The ability of tested extracts of wild edible to scavenge hydrogen peroxide is fruits range from 1.003 to 2.133% for 62.5 to 1000 µg/ml, respectively shown in Table 6 and compared with that of ascorbic acid as standard. Biological systems can produce hydrogen peroxide. Hydrogen peroxide can be formed *in vivo* by several oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. It is used in the respiratory burst of activated phagocytes [44]. *Tarenna asiatica* (2.133%) and *Zizyphus oenoplia* (1.968%) has effective hydrogen peroxide scavenging activity. It is known that H_2O_2 is toxic and induces cell death *in vitro* [45]. Hydrogen peroxide can attack many cellular energy-producing systems. For instance, it deactivates the glycolytic enzyme glyceraldehyde-3- phosphate dehydrogenase [46].

Table 6: Hydrogen peroxide-scavenging activity

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E	AQ	CH	E
1000	2.222	1.049±0.01	1.051±0.04	1.059±0.01	1.146±0.01	1.165±1.2	1.461±0.01	1.133±0.04	1.571±0.02	1.833±0.04	1.878±0.02	1.941±0.01	2.133±0.04	1.816±0.06	1.955±0.001	1.968±0.06
500	2.145	1.037±0.03	1.045±0.03	1.055±0.02	1.137±0.02	1.157±0.008	1.356±0.03	1.121±0.003	1.444±0.03	1.743±0.03	1.622±0.003	1.768±0.03	2.086±0.03	1.671±0.02	1.858±0.02	1.830±0.007
250	2.015	1.025±0.008	1.037±0.02	1.045±0.03	1.130±0.04	1.144±0.001	1.246±0.01	1.116±0.01	1.332±0.02	1.635±0.03	1.465±0.02	1.553±0.02	1.925±0.01	1.478±0.01	1.636±0.002	1.775±0.001
125	1.912	1.010±0.02	1.021±0.06	1.039±0.01	1.103±0.01	1.124±0.005	1.137±0.01	1.112±0.03	1.242±0.08	1.504±0.05	1.222±1.2	1.311±0.06	1.876±0.03	1.219±0.05	1.44±0.03	1.527±0.008
62.5	1.715	1.003±0.02	1.014±0.006	1.025±0.02	1.062±0.04	1.073±0.01	1.121±0.02	1.105±0.02	1.148±0.006	1.321±0.01	1.112±0.003	1.168±0.03	1.660±0.03	1.001±0.002	1.231±0.002	1.365±0.011

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is simple and broadly employed method for the evaluation of antioxidant capacity [47], is based on the capacity of antioxidants to reduce ferric (III) ions to ferrous (II) ions [20]. From the results, all the tested extracts possessed antioxidant properties range from 1.11 to 2.18 %. A maximum scavenging activity was offered by ethanol of *Tarenna asiatica* (2.18%) and *Diospyros ferrea* (1.98%) were the selected samples found to interact as an antioxidant actvity. *Catunaregam spinosa, Murraya koenigii and Zizyphus oenoplia o*f the chloroform and aqueous extract of edible fruits show lower in reduction rate (Tables 7). FRAP assay treats the antioxidants as reductant in a redox linked colorimetric reaction. FRAP assay measures the reducing potential of antioxidant to react on ferric tripyridyltriazine (Fe3+-TPTZ) complex and produce blue colour of ferrous form which can be detected at absorbance 593 nm. Antioxidant compounds which act as reducing agent exert their effect by donating hydrogen atom to ferric complex and thus break the radical chain reaction [20].

Table 7: Ferric reducing antioxidant power (FRAP) assay

Conc	AC	*Catunaregam spinosa*			*Diospyros ferrea*			*Murraya koenigii*			*Tarenna asiatica*			*Zizyphus oenoplia*		
		AQ	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**	**AQ**	**CH**	**E**
1000	2.222	1.57±0.02	1.58±1.0	1.68±0.11	1.75±0.03	1.83±1.2	1.98±0.1	1.57±0.01	1.61±0.2	1.69±1.1	1.83±0.1	2.01±0.4	2.18±0.02	1.56±0.4	1.59±1.2	1.64±1.2
500	2.145	1.44±0.1	1.47±1.1	1.55±0.04	1.59±0.09	1.67±0.1	1.73±0.01	1.44±0.01	1.48±0.02	1.53±0.9	1.78 ±0.4	1.8±0.02	2.11±0.01	1.46±0.2	1.46±0.1	1.56±1.1
250	2.015	1.33±0.6	1.41±0.3	1.43±0.01	1.44±0.06	1.58±0.1	1.68±0.2	1.31±0.04	1.35±0.6	1.38±1.2	1.64±0.1	1.67±0.05	1.84±0.04	1.62±1.1	1.43±1.0	1.49±0.9
125	1.912	1.25±1.5	1.39±0.02	1.36±0.07	1.34±0.055	1.49±1.2	1.52±0.3	1.25±0.02	1.27±1.4	1.29±0.4	1.56 ±0.04	1.58±0.03	1.63±0.03	1.21±0.01	1.32±0.1	1.42±0.2
62.5	1.715	1.11±0.5	1.23±0.1	1.29±0.5	1.34±0.09	1.37±0.1	1.4±0.7	1.16±0.01	1.19±1.3	1.20±1.1	1.33±1.5	1.38±0.1	1.41±0.05	1.19±0.2	1.28±1.1	1.36±0.1

CONCLUSION

The ethanol, chloroform and aqueous extract of *Catunaregam spinosa, Diospyros ferrea, Murraya koenigii, Tarenna asiatica* and *Zizyphus oenoplia* edible fruits has an effective source of natural antioxidants. It is used as a possible food supplement as well as in pharmaceutical industry also. It could contribute to prevent damage by reactive oxygen species. It can be used in stabilizing food against oxidative deterioration. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components in all five edible fruits. The dietary intake of these edible fruits can be useful in the management of oxidative stress and could be used in medicines to treat the various ailments.

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