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Nutritional and Antioxidant Properties of *Ziziphus oenopolia* (L.) Mill. Fruit.

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Abstract

The preliminary phytochemical investigations of leaf extract of *Ziziphus oenopolia fruit* extract showed the presence of plant secondary metabolites such as alkaloids, saponins, phenolic compounds, tannins, flavonoids, phytosterol and glycosides which plays an important role. The methanolic extract of *Ziziphus oenopolia* fruit showed higher values for quantification assays such as, the total phenolics (198.28 mg GAE/ g extract), tannins (207.36mg TAE/ g extract) and flavonoids (116.66mg RE/ g extract). Similarly, in the *in vitro* antioxidant studies; the methanolic fruit extracts exhibited significant results compared to other extracts, ethyl acetate and hot water extracts were also showed comparable antioxidant activities whereas petroleum ether extracts showed least activity. The antioxidant activities of the Methanolic extract were as follows; DPPH radical scavenging assay (IC_{50} : 16.07µg/ml), ABTS radical scavenging assay (10334.27 µM TE/ g extract), phosphor molybdenum assay (198.17 mg AAE/ g extract), ferric reducing antioxidant power assay (60.30 mM Fe (II)/ mg extract), nitric oxide radical scavenging (65.682%) and metal chelating activity (44.07 mg EDTA Equivalents/ g extract). In conclusion, Better characterization of the antioxidant properties and phytochemical investigation of the *Ziziphus oenopolia fruit* are expected to significantly increase the marketing appeal of these fruit and consequently to increase the interest of food industry in using these fruits, especially in new human health-oriented products.

1. Introduction

A wide range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods and pharmaceutical. A wealth of information and scientific evidences are rapidly accumulating that shows the beneficial effects of a wide variety of fruit components on human health. In this context, fruits and vegetables are immensely valued not only for their nutritional content but also for their potential heath functionality against various degenerative diseases such as cancer, cardiovascular, cataract, diabetes, and neurodegenerative diseases like Alzheimer's and Parkinson's (Amit Kumar *et al*., 2012). They can also play in terms of food provision and food culture of rural poor. Therefore concentration on underutilized fruits research seems to explore importance of it. These fruits are potentially rich

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source of many dietary secondary metabolites like phenolics. There is growing evidence that reactive oxygen species (ROS) are related to many diseases and that foods rich in antioxidants may contribute to overall health and disease prevention. A diet rich in fruit, vegetables, and minimally-refined cereals is associated with a lower incidence of illnesses (Anastasa *et al*., 2007, Arunachalam & Parimelazhagan, 2012). Plant derived or natural compounds like flavonoids, tannins, anthocyanins and other phenolic constituents present in food of plant origin are potential antioxidants (Awika *et al*., 2003). Search for natural antioxidant sources among plants used as food is necessary for health promotion. The compounds which possess antioxidant property also show high biological activity like antimicrobial activity. Hence antimicrobial properties have tremendous potential for extending the shelf life of food products (Bar *et al*., 2009). Phytochemicals are bioactive non-nutrient chemical compounds found in plant foods, such as fruits, vegetables, grains, and other plant foods. They can be categorized into various groups, i.e., Polyphenols, organ sulfur compounds, carotenoids, alkaloids, and nitrogen-containing compounds (Blois, 1958, Cao, 2004).

***Ziziphus oenoplia* is an Important Traditional Medicinal Plant:**

Ziziphus oenoplia Mill. (Family-Rhamnaceae) an important shrub, often found throughout the hot regions of tropical Asia, Which is commonly well known as Jackal jujube in English. It is a folk herbal medicine used as an abdominal pain killer and antidiarrhoeal agent. The present study was an attempt has made to determine the total phenolic, tannin, flavonoid contents and in vitro free radical scavenging properties with antioxidant activity of crude extract from the fruit.

2. Materials and Methods

2.1. Collection of plant material

The fresh leaves of *Ziziphus oenopolia fruit* were collected from Madurai, Tamil Nadu, India during the month of January 2023. The collected Fruits were washed under running tap water to remove the surface pollutants. The Fruits were air dried under shade. The dried fruit material was powdered and used for further studies.

2.2. Chemicals

Sodium nitroprusside, 2, 2 – diphenyl -1 – picrylhydrazyl (DPPH), potassium persulfate, 2,2′ –azinobis (3-ethyl-benzothiozoline)-6-sulponic acid diammonium salt (ABTS), Trolox, silver nitrate were obtained from Himedia, Merck and Sigma. All other chemicals and solvents used were of analytical grade.

2.3. Preparation of the fruit extract

About 5 gm of dried fruit powder was weighed and added to 100 mL of Millipore water and boiled for 20 minutes at 90°C in a water bath allowed to cool for some time. Then the extract was filtered by Whatman No 1 filter paper and stored at 4°C for further experiments. The liquid obtained was used for the synthesis of silver nanoparticles.

2.4. Qualitative phytochemical screening of *Ziziphus oenopolia fruit*

The fruit extracts of *F. talboti* were analyzed for the presence of major

phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, flavonol glycosides, cardiac glycosides, phytosterols, fixed oils and fats, and gums and mucilages according to standard methods (Raaman, 2006).

2.4.1. Carbohydrates [Molish's test (Ramakrishnan*et al.*, 1994]

About 100 mg of the extract was dissolved in 5 mL of water and filtered. Two drops of alcoholic solution of α- naphthol was added to 2 mL of the filtrate and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

2.4.2. Proteins [Biuret test (Gahan, 1984]

The extract (100 mg) was dissolved in 10 mL of distilled water and filtered through Whatman No. 1 filter paper. A 2 mL aliquot of the filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of 95% ethanol was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

2.4.3. Amino acids [Ninhydrin test(Yasuma and Ichikawa, 1953]

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) was added to 2 mL of aqueous filtrate. The presence of amino acids was indicated by the presence of characteristic purple colour.

2.4.4. Alkaloids [Hager's test (Wagner *et al.*, 1996]

Solvent free extract, 50 mg was stirred with 5 mL of dilute hydrochloric acid and filtered. To the filtrate, 2 mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

2.4.5. Saponins [Frothing test (Kokate, 1999]

The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicated the presence of saponins.

2.4.6. Phenolic compounds [Ferric chloride test (Mace, 1963]

About 50 mg of the extract was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green colour.

2.4.7. Tannins [Potassium hydroxide test [Williamson *et al.*, 1996]

The extract (0.5 g) was added into 10 mL of freshly prepared 10% potassium hydroxide in a beaker and shaken to dissolve. A dirty precipitate indicated the presence of tannin.

2.4.8. Flavonoids [Alkaline reagent test (Raaman, 2006]

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. A bulky white precipitate indicated the presence of flavonoids.

2.4.9. Glycosides [Borntrager's test (Evans, 1997]

50 mg of extract was hydrolyzed with concentrated hydrochloric acid for two hour on water bath and filtered. To 2 mL of filtered hydrolysate, 3 mL of

chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

2.4.10. Flavonol glycosides [Magnesium and hydrochloric acid reduction (Harborne, 1998]

The extract (50 mg) was dissolved in 5 mL alcohol and few fragments of magnesium ribbon were added. Concentrated hydrochloric acid was added drop wise into the test tube. Development of pink or crimson colour indicated the presence of flavonol glycosides.

2.4.11. Cardiac glycosides [Keller Killiani test (Ngbede *et al*., 2008]

Total 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayered with 1 mL of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of deoxy sugar characteristic of cardinolides.

2.4.12. Phytosterols [Libermann and Burchard's test (Finar, 1986]

About 50 mg of extract was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. An array of colour changes showed the presence of phytosterols.

2.4.13. Fixed oils and fats [Saponification test (Kokate, 1999]

A few drops of 0.5 N alcoholic potassium hydroxide solutions were added to a small quantity of extract along with a drop of phenolphthalein. Then the mixture was heated on boiling water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.4.14. Gums and mucilages

Absolute alcohol test (Whistler and BeMiller, 1993)

The extract (100 mg) was dissolved in 10 mL of distilled water and to this; 25 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

2.5. Quantification Assays

2.5.1Quantification of total phenolics

The total phenolics of the plant extracts were determined according to the method described by Makkar (2003). In this method 50 µl of different plant extracts were taken into a series of test tubes and made up to 1 ml with distilled water. A test tube with 1 ml of distilled water served as the blank. Then, 500 µl of Folin – Ciocalteau Phenol reagent (1 N) was added to all the test tubes including the blank. After 5 minutes, 2.5 ml of sodium carbonate solution (5%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725 nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

2.5.2. Quantification of tannins

The total phenolics contain both tannin and non tannin phenolics. The amount of tannins was calculated by subtracting the non tannin phenolics

from total phenolics. For the determination of non tannin phenolics (Makkar, 2003), 500 µl of each plant samples were incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µl of distilled water taken in a 2 ml eppendorf tube for 4 hours at 4˚ C. After incubation the eppendorf tubes were centrifuged at 4000 rpm for 10 minutes at 4˚ C. The supernatant contains only the non tannin phenolics since the tannins would have been precipitated along with PVPP.

The supernatant was collected and the non tannin phenolics were determined by the same method described for the quantification of total phenolics. The analyses were also performed in triplicates and the results were expressed in Tannic Acid Equivalents (TAE). From these two results, the tannin content of the plant samples were calculated as follows, Tannins = Total phenolics - Non tannin phenolics.

2.5.3.Quantification of flavonoids

The flavonoid contents of the extracts were quantified according to the method described by Zhishen*et al*. (1999). About 500 µl of all the plant extracts were taken in different test tubes and 2 ml of distilled water was added to each test tube. A test tube containing 2.5 ml of distilled water served as blank. Then, 150 µl of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150 µl of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 ml of 4% NaOH was added to all the test tubes which were then made up to 5 ml using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in Rutin equivalents (RE).

2.6. In vitro Antioxidant Assays

DPPH ˙scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method of Braca*et al*. (2001). Sample extracts at various concentrations were taken and the volume was adjusted to 100 µl with methanol. About 3 ml of a 0.004 % methanolic solution of DPPH was added to the aliquots of samples and standards (BHA, BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 µl of methanol in 3 ml of methanolic DPPH solution. The tubes were allowed to stand for 30 minutes at 27°C. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH˙ concentration.

2.6.1. ABTS˙+ scavenging activity

The total antioxidant activity of the samples was measured by ABTS

radical cationdecolourization assay according to the method of Re *et al*. (1999). $ABTS^{\bullet+}$was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulphate in the dark for 12–16 hours at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 25°C to give an absorbance of 0.700 ± 0.02 at 734 nm. About 1 ml of diluted ABTS solution was added to various concentrations sample solution and 10 µl of Trolox (final concentration 0–15 µM) in ethanol. A test tube containing 1 ml of diluted ABTS solution and 30 µl of ethanol served as the negative control. All the test tubes were vortexed well and incubated exactly for 30 minutes at room temperature. After incubation the absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol. The results were expressed as the concentration of Trolox having equivalent antioxidant activity expressed as µM/ g extracts.

2.6.3 Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto*et al*. (1999). A triplicate of 300 µl of samples and standards (BHT and Rutin) were taken into test tubes. About 300 µl methanol taken in a test tube was considered as the blank. All the test tubes were added with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and vortexed well to mix the contents. The mouth of the test tubes were covered with foil and incubated in a water bath at 95°C for 90 minutes. After the samples were cooled to room temperature. The absorbance of the mixture was measured at 695 nm against the reagent blank. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents /g extract.

2.6.4 **Ferric reducing antioxidant power (FRAP) assay**

The antioxidant capacities of different extracts of samples were estimated according to the procedure described by Pulido*et al.* (2000). FRAP reagent (900 µl), prepared freshly and incubated at 37°C, was mixed with 90 µl of distilled water and 30 µl of test sample or methanol (blank). Vitamin E, Trolox and Rutin were used as the standards. All the test tubes were incubated at 37°C for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5 ml of 20 mM TPTZ in 40 mMHCl, 2.5 ml of 20 mM $FeCl_3.\ 6H_2O$ and 25 ml of 0.3 M acetate buffer (pH-3.6). At the end of incubation, the absorbance of the blue colour developed was read immediately at 593 nm against the reagent blank. Methanolic solutions of known $FeSO_4.7H_2O$ concentration ranging from 500 to 4000 µM were used for the preparation of the calibration curve. The parameter Equivalent Concentration was expressed as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4\ 7H_2O$.

2.6.5 Nitric oxide scavenging activity

The procedure is based on the method of Sreejayan and Rao (1997), where 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 Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, 2 ml of sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH-7.4) was mixed with 100 µl sample solution of various extracts and standards (BHT and Rutin) and incubated at room temperature for 150 minutes. After the incubation period, 2 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N- (1-naphthyl) ethylene diaminedihydrochloride) was added to all the test tubes. The same reaction mixture without the sample was used as the negative control. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer). The scavenging activity (%) was calculated as: Scavenging activity (%) = [(Control OD – Sample OD)/Control OD] X 100 the absorbance of the mixture was measured at 695 nm against the reagent blank. Ascorbic acid was used as the reference standard and the results were expressed as milligrams of ascorbic acid equivalents /g extract.

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reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N- (1-naphthyl) ethylene diaminedihydrochloride) was added to all the test tubes. The same reaction mixture without the sample was used as the negative control. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer). The scavenging activity (%) was calculated as: Scavenging activity (%) = [(Control OD – Sample OD)/Control OD

3. Results and Discussion

3.1. Preliminary phytochemical screening study of *Ziziphus oenopolia fruit*

Primary metabolites such as carbohydrates, proteins and amino acids were evaluated in leaf of the *Ficustalboti*. The secondary metabolites such as alkaloids, saponins, phenolic compounds, tannins, flavonoids, phytosterol and glycosides were found to be variously distributed in leaf (Table 1). Saponins, phytosterol, alkaloids, flavonoids and phenolics were present in higher level in leaf aqueous extract. The glycosides and flavonol glycosides are present in appreciable amount in the leaf part of the plant. From these results, *Ziziphus oenopolia fruit* plant extracts ensure the presence of secondary metabolites such as phenolics, sterols, triterpenes and alkaloids. Thus it can acts possibly as good natural antioxidant.

Table 1: Phytochemical screening of *Ziziphus oenopolia fruit* extract

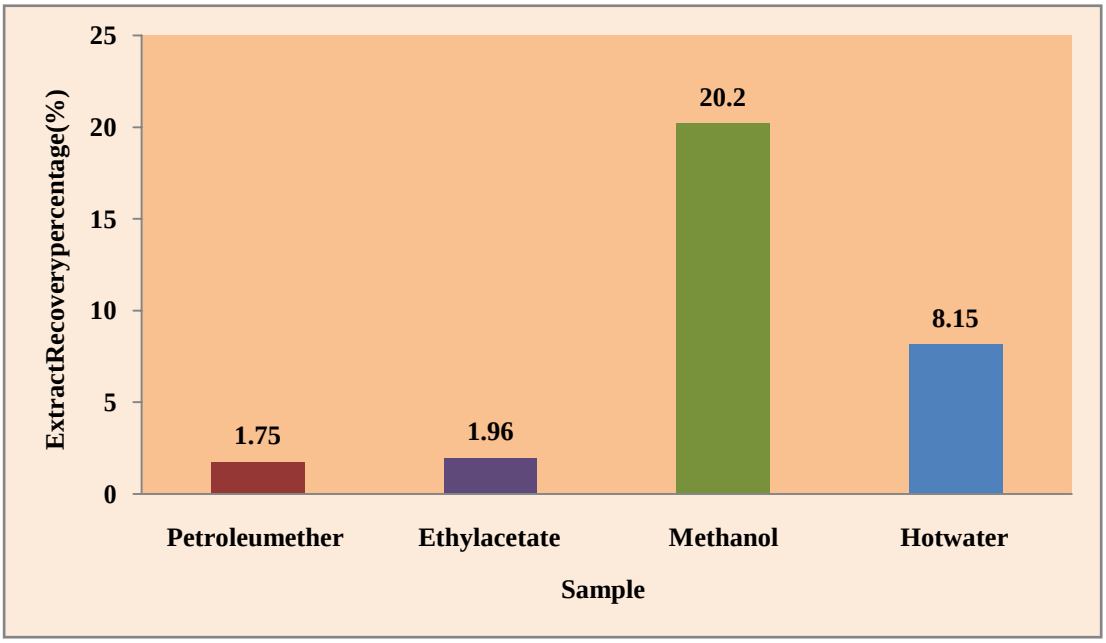
Phytochemicals	Leaf extract
Carbohydrates	++
Proteins	++
Amino acids	+
Alkaloids	+
Saponins	++
Phenolic compounds	+++
Tannins	++
Flavonoids	+++
Glycosides	+
Flavonol glycosides	++
Cardiac glycosides	-
Phytosterols	++
Fixed oils and fats	++
Gums and mucilages	-

(+): Presence of chemical compound, (-): Absence of chemical compound
(+) < (++) < (+++): Based on the intensity of characteristic colour.

3.2 Extract recovery percentage:

The percentage yield of *Ziziphus oenopolia fruit* fruit in different solvent extracts are presented in the Figure 1. The maximum yield was obtained from methanol extract of fruits, where the yield percentage was 20.2 and second highest yield was recovered from hot water extraction. The yield percentage of petroleum ether and ethyl acetate was found to be very low compared to polar solvents. This result presumed that *Ziziphus oenopolia fruit* fruits contain more amounts of high polar phytocompounds.

Fig. 1: **Extract Recovery Percentage of *Ziziphus oenopolia fruit* fruit**



3.3 Quantification assays

3.3.1 Total phenolics

The content of extractable phenolic compounds in the *Ziziphus oenopolia fruit* fruit extracts were determined through a linear gallic acid standard curve ($y = 0.031x + 0.162$; $R^2 = 0.981$). The amount of total phenolics present in various extracts were statistically analysed and tabulated in Table 3. Among the four different extracts the methanolic extract contains highest amount of phenolics (198.28 ± 3.05mg GAE/ g extract) whereas the petroleum ether extract contains lowest amount (1.61 ± 0.56mg GAE/ g extract) of phenolics. The decreasing order of total phenolics present in different extracts of *Ziziphus oenopolia fruit* fruit are methanol> hot water >ethyl acetate > petroleum ether. The lowest content of total phenolics in petroleum ether extract may be probably due to the low solubility of major polar phenolic compounds in petroleum ether. Plant phenolics present in the fruit have received considerable attention because of their potential antioxidant activity. The phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Chang *et al.*, 2001). These high levels of total phenolics present in methanolic extract of *Ziziphus oenopolia fruit* fruit confirms that, it may possess high potentiality of free radical scavenging activity.

3.3.2 Tannins

Tannins are water soluble polyphenols present in many fruits and have been

also recognized as antioxidants. The amount of tannins present in different extracts of *Ziziphus oenopolia fruit* fruit are represented in Table 3. The maximum amounts of tannins are present in methanolic extract of fruit (207.36 ± 11.39mg TAE/ g extract). Whereas considerable result was obtained from hot water extract (115.78 ± 12.41mg TAE/ g extract). Petroleum ether and ethyl acetate extracts revealed minimum amount of tannin content which may be due to the lower solubility of these compounds in low polar solvents. Many tannin components were suggested to be anti-carcinogenic and have been shown to reduce the mutagenic activity of a number of mutagens. Numbers of carcinogens and/or mutagens produce oxygen free radicals for interaction with cellular macromolecules. The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their anti-oxidative properties, which are important in protecting against cellular oxidative damage. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds (Chung *et al.*, 1998). The significant amount of tannins present in this wild edible fruit can be a good indication for its higher antioxidant activity.

Table 3: Total phenolic, Tannin and Flavonoid contents of *Ziziphus oenopolia fruit* fruit

Solvents	**Total phenolics** (mg GAE/ g extract)	**Tannins** (mg TAE/ g extract)	**Flavonoids** (mg RE/ g extract)
Petroleum ether	1.61 ± 0.56	2.10 ± 3.80	38.66 ± 3.79
Ethyl acetate	29.46 ± 5.01	9.82 ± 9.78	102.33 ± 5.03^{b}
Methanol	**198.28 ± 3.05^{a}**	**207.36 ± 11.39^{a}**	**116.66 ± 6.66^{a}**
Hot water	85.53 ± 2.61^{b}	115.78 ± 12.41^{b}	90.66 ± 5.77

Values are mean of triplicate determination (n=3)±standard deviation,
GAE-Gallic Acid Equivalents, TAE- Tannic Acid Equivalents, RE- Rutin Equivalents
Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}$ in each column.

3.3.3.Flavonoids

The Table 3 represents the results of flavonoids present in the wild edible fruit of *Ziziphus oenopolia fruit* (The result were expressed through the rutin standard equivalents ($y = 0.031x + 0.162$; $R^2 = 0.981$) because rutin is an important and one of the major flavonoids found in varity of plants). The results revealed that higher amount of flavonoids is present in methanolic extract of fruit (116.66 ± 6.66 mg RE/ g extract), followed by ethyl acetate (102.33 ± 5.03 mg RE/ g extract). The increasing order of flavonoid content presented in the fruit follows the order of petroleum ether < hot water < ethyl acetate < methanol. Flavonoids are the most

common and widely distributed group of plant phenolic compound, which usually are very effective antioxidant because of the scavenging ability conferred by their hydroxyl group. Flavonoid compounds from plants are known to be good natural antioxidant (Darmanyan, *et al*., 1998). The beneficial effect of flavonoids in human beings is the antioxidant activity attached to structural diversity which makes them to exhibit anti-neoplastic, anti-inflammatory, anti-hepatitis, anti-allergic, anti- bacterial, anti-mutagenic, anti-thrombosis and antiviral activities (Dahl *et al*., 2007). These higher amounts of flavonoids in the *Ziziphus oenopolia fruit* fruits suggest that it may possess great antioxidant potential with significant biological activities.

***In vitro* Antioxidant Assays**

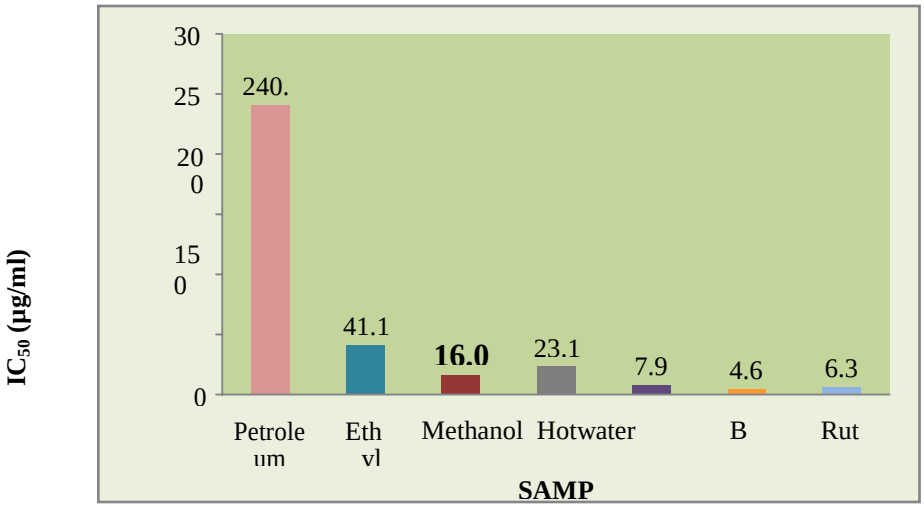
3.3.4 DPPH$^{\bullet}$ scavenging activity

DPPH radical scavenging activities of *Ziziphus oenopolia fruit* fruit extracts are presented in the Figure 2. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equations prepared from the concentrations of the extracts versus percentage inhibition of free radical formation. A lower IC_{50} value indicates greater antioxidant activity. From the results, it is clear that methanolic fruit extract of *Ziziphus oenopolia fruit* possess lowest IC_{50} value of about 16.07µg/ml. This study confirms that the fruit extract of *Ziziphus oenopolia fruit* contains more antioxidant phytochemicals. The petroleum ether extract found to have a minimal antioxidant activity (IC_{50} value 240.73 µg/ml) because most of antioxidant phytochemicals found to dissolve only in high polar solvents. Moreover the results are compared with that natural (Rutin) and synthetic antioxidants (BHA and BHT). The IC_{50} values of standards and extracts are in the order of BHA <Rutin< BHT < methanol extract < hot water extract < ethyl acetate extract < petroleum ether extract.

The model of scavenging the stable free radical is widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Collera-Zuniga *et al*., 2005). It has also been found that ascorbic acid, α-tocopherol, flavonoids, tannins, and aromatic amines reduce and decolorize DPPH by their hydrogen donating ability (Chittaranjan *et al*., 2012). It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to their phenolics contents, suggesting a causative relationship between total phenolic content and antioxidant activity (Rice-Evans *et al*., 2004). In the present study also there exists a significant correlation between DPPH scavenging activity and total phenolics. Previous studies in the genus Syzygium, have indicated that the phyto constituents belong to the group of phenolic compounds (Neergheen, 2006). Consistent with these reports, we have also found that the extracts of *Ziziphus oenopolia fruit* possess a very potent

antioxidant property which may be contributed by the phenolics and flavonoids in the extracts.

Fig. 2: DPPH radical scavenging activities of *Ziziphus oenopolia fruit* fruit



3.3.5 ABTS$^{\bullet+}$ scavenging activity

The ABTS$^{\bullet+}$ scavenging activity of different extracts of *Ziziphus oenopolia fruit* fruit and positive control are depicted in Table 4. In the present study, methanolic extract was found to have a higher scavenging activity (10334.27 ± 80.73 µM TE/ g extract) compared to positive controls (Rutin and BHT). These commercially available antioxidants possess Trolox equivalents values such as 8423.95 ± 96.32 µM TE/ g and 9942.69 ± 109.84 µM TE/ g respectively. The descending order of TEAC value for fruit extracts and standards is methanol extract >Rutin> BHT > hot water extract > ethyl acetate extract > petroleum ether extract. ABTS$^{\bullet+}$ assay is an excellent tool to determine the antioxidant activity of hydrogen donating antioxidants (scavenging aqueous phase radicals) and of chain breaking antioxidants (scavenging lipid peroxyl radicals). Reduction of ABTS$^{\bullet+}$ can be even more efficient than that of DPPH. The total antioxidant ability of all the studied. samples seems to be sufficient for functioning as potential nutraceuticals when they are ingested along with nutrients. Most fruits tested with high antioxidant capacity in the DPPH assay, also showed a high antioxidant capacity in ABTS assay (Changwei *et al*., 2008). The high correlation may partly result from a similar mechanism and also both antioxidants are soluble in methanol. The high level of antioxidant activity can be taken as a good evidence for nutraceutical property of *Ziziphus oenopolia fruit* fruit.

Table 4: ABTS cation radical scavenging activities and Phosphomolybdenum assay of *Ziziphus oenopolia fruit* fruit

Sample	**$ABTS^{\bullet+}$ scavenging activity** (µM TE/ g extract)	**Total antioxidant capacity** (mg AAE/ g extract)
Petroleum ether	605.94 ± 147.24	91.28 ± 3.15
Ethyl acetate	7031.56 ± 510.07	159.28 ± 10.15^{c}
Methanol	**10334.27 ± 80.73^{a}**	**198.17 ± 7.08^{b}**
Hot water	7937.14 ± 487.81^{c}	136.06 ± 0.63
BHT	8423.95 ± 96.32^{b}	437.19 ± 6.85^{a}
Rutin	9942.69 ± 109.84^{a}	443.51 ± 6.43^{a}

Values are mean of triplicate determination (n=3) ± standard deviation**,** TE- Trolox Equivalents. AAE- Ascorbic Acid Equivalents.

Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}$ in each column.

3.3.6 **Phosphomolybdenum assay**

The phosphomolybdenum assay was used to determine the total antioxidant capacity of wild edible *Ziziphus oenopolia fruit* fruits and the results are presented in Table 4. However, the results are estimated from linear Ascorbic Acid standard curve (y = 0.020x - 0.048; R^2= 0.991). Among the different extracts, the methanolic extract of *Ziziphus oenopolia fruit* fruit showed higher reducing ability compared to other extracts (198.17 ± 7.08 mg AAE/ g extract**)**. Next significant values were obtained for ethyl acetate and hot water extracts, which were 159.28 ± 10.15 and 136.06 ± 0.63 mg AAE/ g extract respectively. The decreasing reducing ability of *S.calophyllifolium*extracts and commercially available antioxidants follows the order of Rutin> BHT > Vitamin E > Methanolic extract > Ethyl acetate extract > Hot water extract > Petroleum ether extract.

Total antioxidant activities reflect the capacity of non-enzymatic antioxidant defence system. In the phosphomolybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/Mo^{5+} complex at acidic pH. The high reducing ability of methanolic extract confirms that more amount of Mo^{6+} is reduced to Mo^{5+} by the ability of high amount of bioactive compounds (Chang *et al*., 2001). Such compounds are the reason for electron transfer or hydrogen ion transfer in this assay. The results revealed that all four extracts show significant total antioxidant capacity and this is a better indication for antioxidant property of this wild edible fruit.

3.3.7 Ferric reducing antioxidant power assay (FRAP)

The ferric reducing power assay relies on the production of a blue colour which is directly proportional to the antioxidant activity of the extracts (Huang *et al.*, 2005). The intensity of the blue colour produced by the extract was measured spectrophotometrically and the results are illustrated in Figure 3. The higher ferric reducing power was observed for methanolic extract of *Ziziphus oenopolia fruit* fruit and was 60.30 ± 0.05 mM Fe (II)/ mg extract. The ethyl acetate and hot water extracts also showed significant reducing power (33.33 ± 0.99 34.27 ± 1.30 mM Fe (II)/ mg extract respectively). From the results it was also clear that methanol, ethyl acetate, hot water extracts have more reducing power compared to that of Vitamin E.

FRAP is a simple inexpensive assay and may offer presumed index of antioxidant activity. The FRAP assay measures the ability of antioxidants to reduce the ferric 2, 4, 6-tripyridyl-S-triazine complex [Fe (III)-(TPTZ)2]$^{2+}$ to intensely blue colored ferrous complex [Fe(II)- (TPTZ)2]$^{2+}$ in acidic medium (Chandran *et al*., 2006) Many reports have demonstrated that the reducing power of natural plant extracts and essential oils might be strongly correlated with their antioxidant activities (Stratil*et al.*, 2006). The obtained results are in agreement with the findings from the ABTS assay and confirm that the antioxidant power of the *Ziziphus oenopolia fruit* which can be due to electron transfer (Chanda *et al*., 2011), reported that the ferric reducing antioxidant power of fruit juice correlated well with the polyphenol concentrations and they suggested that higher antioxidant activity of fruit juice might be due to the presence of phenolics. The preceding results clearly indicates that the fruit extracts studied can act as electron donors and react with free radicals, and convert them to more stable products, thus terminating the radical chain reaction.

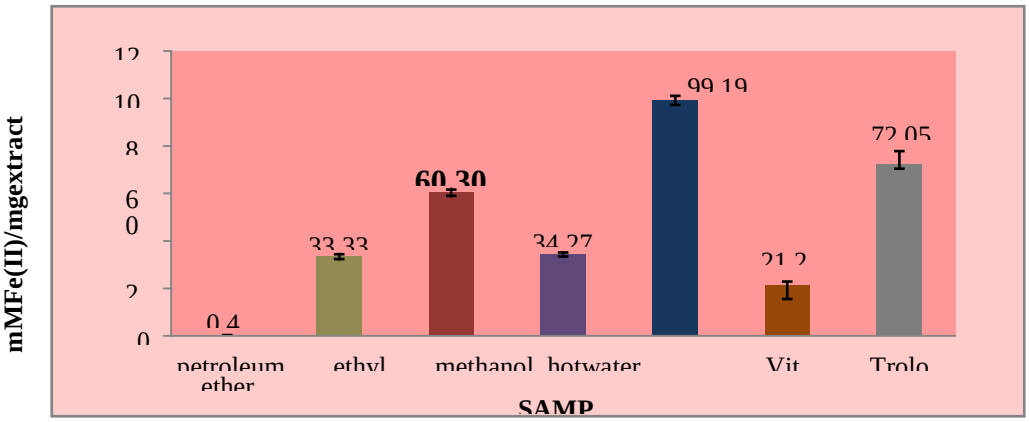
3.3.8 Nitric oxide scavenging activity

The relative nitric oxide scavenging potential percentage of *Ziziphus oenopolia fruit* fruit extracts were studied and are illustrated in Figure 4. At the concentration of 100µg/ml, methanol and ethyl acetate extracts showed greater percentage of nitric oxide scavenging activity (65.68 ± 3.32% and 52.73 ± 2.91% respectively). On the other hand hot water extract showed significant nitric oxide scavenging activity (68.51 ± 1.50 %) at a concentration200 µg/ ml. However, at 200 µg/ ml concentration, petroleum ether extract expressed low scavenging percentage compared to other extracts of *Ziziphus oenopolia fruit* fruit.

Nitric oxide (NO) or reactive nitrogen species such as NO_2, N_2O_4, N_3O_4, NO_3, and NO_2 formed during the reactions of nitrogen with oxygen or with superoxides are very reactive. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Large amounts of NO, per oxynitrite and other reactive nitrogen oxide species are considered to be potentially cytotoxic and capable of injuring the surrounding cells (Chanda & Rakholiya, 2011). Plant/plant products may have the

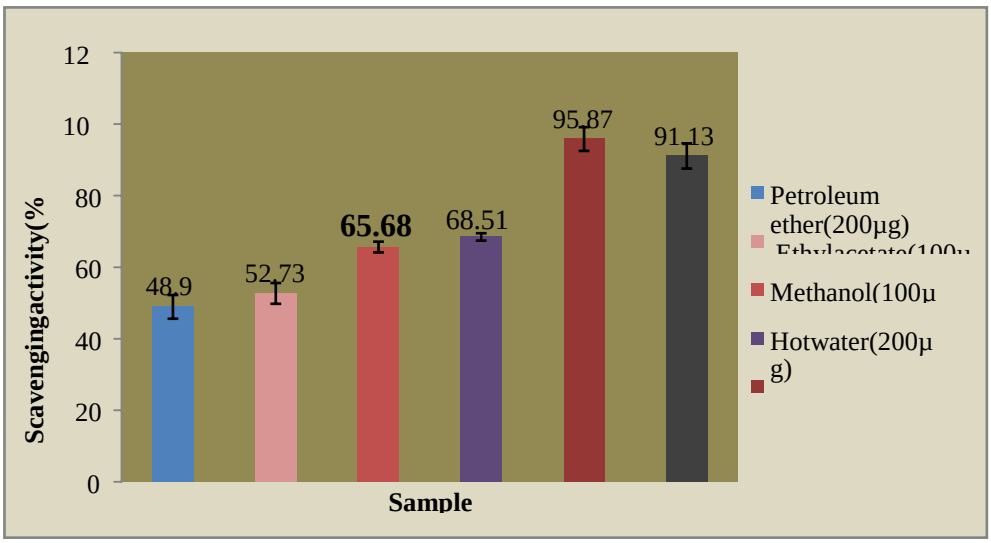
property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO present in the human body (Moncada*et al.*, 1993).

Fig. 3: FRAP assay of *Ziziphus oenopolia fruit* fruit



Values are mean of triplicate determination (n=3) ± standard deviation Statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}$.

Fig. 4: Nitric oxide scavenging activity of *Ziziphus oenopolia fruit* fruit



Values are mean of triplicate determination (n=3) ± standard deviation statistically significant at $p<0.05$ where $^{a}>^{b}>^{c}>^{d}$. In this assay the nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the *Ziziphus oenopolia fruit* fruit extract also possessed potent nitric oxide scavenging activity. The phenolic compounds of these fruits such as

flavonoids, tannins, and pro-anthocyanidins may be responsible for the radical scavenging activity of the extracts.

4. Conclusion

Indigenous knowledge based traditional medicinal practices are commonly used to treat various ailments. Now a day's such practices are reduced due to modern culture and unawareness. Majority of people in developing countries are not having a sufficient knowledge regarding such plants. In this context the antioxidant properties and phytochemical investigation of the *Ziziphus oenopolia fruit* are expected to significantly increase the marketing appeal of these fruit and consequently to increase the interest of food industry in using these fruits, especially in new human health-oriented products. Further, detailed exploration, chemical studies and screening for medicinal properties will provide cost effective and reliable source of medicine for the welfare of humanity.

5. References

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