CHAPTER IV

Study of Phytochemicals and Antioxidant Properties of Wild Fruits

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated through normal cellular metabolism are responsible for damages to proteins, lipids and nucleic acids in cells which causes many human chronic diseases including atherosclerosis, cancer, diabetes mellitus, cataract, rheumatoid arthritis, and Parkinson's disease and these radicals can also enhance the ageing processes [1–3]. The several natural products from fruits, vegetables and various parts of plants possess high antioxidant properties which directly or indirectly scavenge free radicals inhibiting generated ROS and RNS for further oxidation [4, 5]. Plants rich in antioxidants such as phenolic compounds, vitamin C, tocopherols and carotenoids are attracting to the food industry as replacements for synthetic ones whose use is being restricted because of food safety concerns [6]. The synthetic antioxidants have possibly been used to inhibit lipid oxidative rancidity in foods and to prolong the shelf-life of food products which are the major causes of quality deterioration, nutritional losses, and development of many oxidative stress-related chronic diseases in man [7, 8]. Polyphenols are the most abundant antioxidants and integral part of human diet with total intake of nearly 1 g/day which is much higher compared to vitamin E (12 mg daily), vitamin C (90 mg daily) and vitamin A and its precursors carotenoids (5 mg per day) [9–11]. Phenolic compounds being a large group of bioactive chemicals have diverse biological functions and act as contributors to plant pigmentation, attractants for pollinators, and protective agents against UV light [12–14]. Flavonoids, a small group of phenolic compounds, are powerful antioxidants due to their high redox potential and regular consumption of foods with rich in flavonoids have been found associated with a reduction in the incidence of diseases such as heart disease and cancer [15–18].

In this study, phytochemical constituents, vitamin C content, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties of five wild fruits *viz. G. sapida, O. alismoides, A. dioica, A. bunius* and *E. operculata* from Assam of North East India were investigated and reported.

IV.1 Materials and Methods

IV.1.1 Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2´-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) and quercetin were purchased from Hi-media Laboratories Pvt. Ltd., Mumbai, India**,** Ascorbic acid, H_2O_2 (Hydrogen peroxide) and Folin-Ciocalteu's reagent from Merck, Mumbai, India, Trolox from Sigma Aldrich, Bangalore, India and gallic acid was obtained from Central Drug House Pvt. Ltd., Daryaganj, New Delhi, India. All other reagents used in this study were of analytical grade and used without further purification.

IV.1.2 Sample preparation

The powdered sample prepared as per the procedure mentioned in **Section II.2.3** (**Page No. 67**) was taken for solvent extraction and extracted separately with methanol, hexane, chloroform, acetone and water separately maintaining 1:10 ratio (w/v), stirred, kept for 72 h and filtered using Whatman No. 1. The filtrate was then evaporated until a semi dried powder/sticky mass of plant extract was obtained using Buchi Rotavapor R-215 (Switzerland) and the dried extracts were kept in air-tight containers at 4^oC for further analysis [19].

IV.1.3 Preliminary phytochemical screening

The qualitative phytochemical screening of five different solvent extracts of freeze dried fruits were performed by following the standard methods [19, 20].

Test for alkaloids

Wagner's and Dragendroff's tests

2 mL of each extract was mixed with 2 mL of 1% aqueous HCl, taken into two separate test tubes and 6 drops of Wagner's and Dragendroff's reagents were added. The appearance of a reddish brown precipitate with Wagner's reagent and orange-red precipitate with Dragendroff's reagent indicate the presence of alkaloids.

Test for saponins

1 mL extract mixed with 20 mL of distilled water in a graduated cylinder for 15 min and it was shaken vigorously. Persistence of froth was taken as an indication for the presence of saponins.

Test for cardiac glycosides

Keller-Killiani's test

2 mL of crude extract was treated with 1 mL of glacial acetic acid containing 1-2 drops of $FeCl_3$ solution, followed by addition of 2 mL of concentrated H_2SO_4 along the side of the test tube carefully. The appearance of reddish brown at the junction of two layers indicates the presence of deoxysugar and the appearance of bluish green at upper layer shows the presence of cardiac glycosides.

Tests for steroids

Liebermann Burchard test

1 mL of the extract was taken in a test tube and then treated with 2 mL of acetic anhydride, followed by the addition of few drops of conc. H_2SO_4. The appearance of green colour was taken as an indication for the presence of steroids (terpenoids).

Salkowski's test

2 mL of the extract was taken in a test tube and then treated with 2 mL of chloroform. To this, 2 mL of concentrated sulphuric acid were added to it and shaken gently. The acid layer showing greenish yellow fluorescence and chloroform layer appearing red was considered as an indication for the presence of steroids.

Test for anthraquinones

Modified Borntrager's test

About 0.5 g of the crude extract was taken in a dry test tube and then treated with 5 mL chloroform and shaken well for 5 min. The extract was filtered and an equal volume of 100% ammonia solution was added to the filtrate. Appearance of pink, violet or red colour with the ammonical layer (lower layer) was taken as positive result for the presence of anthraquinones.

Test for coumarins

3 mL of 10% NaOH was mixed with 2 mL of extract and appearance of yellow color indicated the presence of coumarins.

Ferric chloride test for phenols

To 5 mL of extract, 1 mL of 5% ferric chloride solution was added. Greenish black coloration gives an indication of the presence of phenols.

Gelatin test for tannins

To the extract 1% gelatin solution containing 10% sodium chloride solution was added. Formation of white precipitate gives positive indication for the presence of tannins.

Shinoda's test for flavonoids

To the test solution, a small piece of metallic magnesium ribbon was added and concentrated hydrochloric acid was added drop wise. Appearance of pink-tomato red color after few min indicated the presence of flavonoids.

Test for carbohydrates

Molisch's test

2 mL of extract was mixed with few drops of freshly prepared 15% ethanolic α-naphthol solution in a test tube. To this, 2 mL of concentrated sulphuric acid was added carefully along the sides of the test tube to form a layer below the mixture. The appearances of a reddish violet ring at the junction of two layers shows the presence of carbohydrates which disappear on the addition of excess of alkali.

Fehling's test

2 mL of the extract in a test tube was treated with 1 mL of a mixture of equal volumes of Fehling's solutions A and B, and boiled in a water bath for few min. The brick-red precipitate formation gives an indication for the presence of carbohydrates (reducing sugars).

Iodine test for starch

2 mL of iodine solution was mixed with crude extract. Purple or dark blue coloration proved the presence of starch (carbohydrate).

Test for anthocyanins

2 mL of crude extract was mixed with 2 mL of 2 N HCl and ammonia. The formation of pink red turns blue-violet color shows the presence of anthocyanins.

Test for proteins

Ninhydrin test

About 2 drops of Ninhydrin reagent were added to the crude extract and boil for few min. Appearance of blue color gives positive indication for the presence of amino acid.

Millon's test

2 mL of Millon's reagent was mixed with crude extract, formation of white precipitate which turned red upon gentle heating that confirmed the presence of protein.

Test for phlobatannins

About 1 mL of each crude extract was added to a few drops of 2% HCl and the mixture was boiled. The deposition of a red precipitate was taken as an indication for the presence of phlobatannins.

Test for lignin

The formation of red color confirmed the presence of lignin when 2 mL of 2% (w/v) furfuraldehyde was added to the test solution.

IV.1.4 Determination of antioxidant properties

In this study, only methanol extract was taken for antioxidant activity as methanol is the most efficient solvent for extraction of various polar compounds from the plant materials whereas certain groups of non-polar compounds are fairly soluble in methanol. The extracts obtained by using high polar solvents (methanol) exhibited more effective radical scavengers compared to non-polar solvents, indicating that bioactive compounds of different polarity could be present in the plant [21]. Therefore, methanol is commonly used for extraction of bioactive compounds.

IV.1.4.1 DPPH free radical scavenging assay

The free radical scavenging activity of methanol extract of freeze dried fruit was performed by stable DPPH free radical [22]. 1 mL methanolic solution of each extracts (2, 5, 10, 50, 100, 200, 400, 500 µg/mL) were mixed with 3 mL working solution of DPPH (0.1 mM DPPH in methanol) and incubated in the dark at room temperature for 30 min. Absorbance of the preparations of all extracts were measured at 517 nm with an UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) and compared with the corresponding

absorbance of standard ascorbic acid of similar concentrations. 1 mL of methanol was mixed with 3 mL working solution of DPPH which served as blank. The % inhibition was calculated as:

Inhibition (%) = (Absorbance of blank–Absorbance of sample or standard after 30 min)/ (Absorbance of blank after 30 min) ×100.

IC_{50} value of methanol extract of freeze dried sample and standard ascorbic acid were calculated by plotting % inhibition against concentration.

IV.1.4.2 ABTS free radical scavenging assay

Antioxidant activities of methanol extracts of freeze dried fruits were investigated by ABTS assay [23] with some modifications. ABTS radical cation ($ABTS^{\bullet+}$) was generated by the reaction of 7 mM ABTS solution with potassium persulphate (2.45 mM) and allowing the mixture to react for 12 h at room temperature in the dark before use. The $ABTS^{\bullet+}$ solution was diluted with methanol to give an absorbance of 0.706 ± 0.01 at 734 nm. After the addition of 25 – 250 µL of methanolic extracts or ascorbic acid used as standard to 2 mL of diluted $ABTS^{\bullet+}$ solution, the absorbance was measured at 734 nm using UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) exactly after 6 min. The % inhibition was calculated as:

Inhibition (%) = 100 × (Absorbance of blank−Absorbance of sample)/Absorbance of blank.

IV.1.4.3 H_2O_2 radical scavenging assay

The ability of the fruit extract to scavenge hydrogen peroxide (H_2O_2) was carried out following the method given by Ruch *et al*. [24]. A solution of hydrogen peroxide (20 mM) was prepared (by adding 22.7 µL of the provided H_2O_2 to 977 µL of Buffer) in Phosphate buffer saline (pH = 7.4). Various concentrations of 2–25 µL of the extracts or standards in methanol were added to 2 mL of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm after 10 min of incubation against a blank solution that contained PBS without hydrogen peroxide. The percentage of H_2O_2 scavenging activity of fruit extract and standard compounds was calculated using the following equation.

Inhibition (%) = 100 × (Absorbance of control − Absorbance of sample)/Absorbance of control.

IV.1.4.4 FRAP antioxidant assay

Antioxidant activity of methanol extracts was determined as per the method of the assay of ferric reducing/antioxidant power (FRAP) of Benzie and Strain [25] with slight modifications. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent), and 20 mM $FeCl_3.6H_2O$ solution in a volume ratio of 10:1:1 respectively. Freshly prepared FRAP reagent (2850 µL) was warmed at 37°C and mixed with 150 µL of the extract and the reaction mixtures were later incubated for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were measured at 593 nm. The standard curve was linear between 25 and 1000 µM trolox. FRAP values were expressed in µM TE (trolox equivalent)/g dry weight.

IV.1.5 Determination of total phenolic content (TPC)

Total phenolic content in freeze dried fruit extract of methanol was determined spectrophotometrically at 765 nm using Folin-Ciocalteu's reagent [22]. 1 mL methanolic solution of extract (1 mg/mL) or standard solutions of gallic acid (10, 20, 40, 60, 80, 100 µg/mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in distilled water and incubated at room temperature for 5 min. After 5 min, 2 mL of 7.5% saturated Na_2CO_3 solution was added to the mixture and the absorbance was taken at 765 nm using UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) after incubation in dark for 30 min at 24°C. Blank reagent was prepared using 1 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2 mL of 7.5% Na_2CO_3 solution. Results were calculated using the calibration curve of gallic acid and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram dried extract (mg GAE/g dried extract).

IV.1.6 Determination of total flavonoid content (TFC)

Total flavonoid content in freeze dried fruit extracts of methanol was also determined spectrophotometrically at 510 nm with slight modifications by Zhishen *et al*. [26]. 1 mL methanolic solution of extract (1 mg/mL) or standard solutions of quercetin (10, 20, 40, 60, 80, 100 µg/mL) was mixed with 0.5 mL of 5% $NaNO_2$ solution and 0.5 mL of 10% $AlCl_3$ solution was added 5 min later. After 5 min of incubation, 2 mL of 4% NaOH solution was added, incubated for 15 min at room temperature and the absorbance level was measured versus the blank at 510 nm using UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA). Blank reagent was prepared by adding the entire reagent without adding sample or standard. A calibration curve was generated using standard quercetin and the total flavonoid

content were expressed as milligrams of quercetin equivalents per gram dried extract (mg QE/g dried extract).

IV.1.7 Determination of vitamin C

The vitamin C content of fresh fruit was determined by the iodine titration method reported by Suntornsuk *et al*. [27]. A standard iodine solution was prepared by dissolving 5 g of potassium iodide (KI) and 0.268 g of potassium iodate (KIO_3) in distilled water in a 500 mL volumetric flask. 30 mL of 3 M H_2SO_4 acid was added to the solution, mixed and the volume of solution was increased to 500 mL with distilled water. A 20 mL of fruit juice extract was titrated against the iodine solution to a light blue colour end point using 1% starch as an indicator. The vitamin C content in sample was calculated as V_1/V_2 and the results were expressed in mg/100 g of fresh fruits.

Where, V_1 was volume of iodine solution consumed to titrate the sample.

V_2 was the volume of the iodine solution consumed to titrate the standard vitamin C solution.

IV.1.8 Statistical analysis

All the experiments were carried out for three independent replicates and the data were represented in terms of mean ± standard deviation. OriginPro 8.5 software (MA 01060, OriginLab Corporation, USA) was used for statistical analysis and executed by the one-way ANOVA and *t*-test at $p < 0.05$. SPSS 13.0 software was utilized for the study of Pearson's correlation.

IV.2 Results and Discussion

IV.2.1 Phytochemical screening

The freeze dried five wild fruits were extracted with five different solvents *viz.* methanol, chloroform, hexane, acetone and aqueous. These extracts were investigated qualitatively for the presence of various bioactive compounds and the results are shown in **Tables IV.1–IV.5**. Interestingly, alkaloids were present in all the five solvent extracts of *G. sapida, A. dioica, E. operculata, A. bunius* and *O. alismoides*. Whereas alkaloids were not detected in hexane and acetone extracts of *O. alismoides* using Dragendroff's test. Alkaloids are known for their numerous pharmacological effects rather than for their toxicity or adverse effects. Alkaloids cause neurological disorders and gastrointestinal trouble when their content in plants is high. Alkaloid contents more than 20 mg/100 g are not within the safe limits for

human consumption [28]. Saponins were detected in all the five extracts of the fruits. Saponins have anti-inflammatory and hemolytic activities, and they have a bitter taste and cholesterol binding properties [29–31]. Cardiac glycoside were not detected in aqueous extracts of all the fruits, while it was found to be present in methanol, chloroform, hexane and acetone extracts except in hexane and acetone extracts of *A. bunius* and *O. alismoides* respectively. Glycosides have been reported to have anti-diarrheal activity [32] and can decrease blood pressure [29]. However, *A. bunius* and *G. sapida* showed the presence of steroids in all the extracts whereas steroids were not detected in acetone extract of *E. operculata* and *O. alismoides* and also not present in methanol and acetone extract of *A. dioica*. Steroids have antibacterial, analgesic and anti-inflammatory properties [33, 34]. In *E. operculata*, methanol, water and acetone extracts showed positive test for anthraquinones and coumarins, while in *O. alismoides* and *G. sapida*, anthraquinones and coumarins were not detected in chloroform and hexane extracts. In *A. dioica*, acetone and water extracts showed positive test for anthraquinones but coumarin was not detected in hexane and acetone extracts. In *A. bunius*, anthraquinone was not detected in chloroform extract and coumarin was not detected in methanol, chloroform and hexane extracts. Coumarins are a potential antioxidants and its antioxidant activity is due to its capability of scavenging free radicals and chelating metal ions [20, 35]. Phenols were detected in all the solvent extracts of all four fruits except in the chloroform extract of *A. bunius* fruit. Positive test for tannins was observed in the methanol and acetone extracts of *G. sapida, A. bunius* and *O. alismoides* and also in the acetone extract of *E. operculata*. Tannins are used for the treatment of dysentery and diarrhea, and have gained immense attention in many fields due to their physiological activities like antioxidant, anti-inflammatory and antimicrobial [23, 36]. The methanol, acetone and water extracts of all the five wild fruits showed positive results for flavonoids. Chloroform and hexane extracts showed negative results for flavonoids in *A. bunius* and *O. alismoides*. Starch was detected in all the five extracts in *A. bunius* whereas methanol and acetone extracts indicated negative results for starch in *E. operculata, G. sapida* and *O. alismoides*. *A. dioica* showed positive results for starch in chloroform and water extracts. Hexane and acetone extracts showed the presence of anthocyanins in *A. bunius* whereas in *E. operculata, O. alismoides* and *A. dioica*, methanol, acetone and hexane extracts were found to contain anthocyanins. *G. sapida* showed negative results for anthocyanin in chloroform extract and found to be present in all other extracts. All the fruits showed positive results for proteins (Millon's test) in methanol and water extracts and phlobatannins were found positive in acetone and aqueous extracts of all the fruits. Phenolic compounds are one of the most abundant groups of plant metabolites [33], comprises about one-third of the dietary phenols, which are widely spread throughout the plant kingdom. They may occur commonly in free

and bound forms linked to cell wall of plant components such as cellulose, lignin, and proteins through ether, ester or acetal bonds [37]. Natural antioxidants are mainly derived from plants in the form of phenolic compounds such as phenolic acids, flavonoids, and tocopherols [38]. Flavonoids, a group of phenolic compounds, act as free radical scavengers as they are potential reducing agents and protect from oxidative cell damage through their water soluble property [20]. Phytochemicals found in the plant materials are known to possess many biologically active compounds and they are responsible for several biological activities such as antioxidant, antimicrobial, anti-inflammatory, antifungal and anticancer activities [39, 40].

Table IV.1: Phytochemical screening of freeze dried fruit extracts of *G. sapida*

Phytochemicals	**Test**	**Me**	**Ch**	**He**	**Ac**	**Wa**
Alkaloids	Wagner's reagent	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	+	-
Steroids (Terpenoids)	Liebermann-Burchard test	+	+	+	+	+
	Salkowski's test	+	+	+	+	-
Anthraquinones	Modified Borntrager's test	+	-	-	+	+
Coumarins		+	-	-	+	+
Phenols	$FeCl_3$ test	+	+	+	+	+
Tannins	Gelatin test	+	-	-	+	-
Flavonoids	Shinoda's test	+	-	+	+	+
Carbohydrates	Molisch's test	+	+	+	+	+
	Fehling's test	+	+	+	+	-
Starch	Iodine test	-	+	+	-	+
Anthocyanins		+	-	+	+	+
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	+	+	+	+

Me, Methanol; Ch, Chloroform; He, Hexane; Ac, Acetone; Wa, Water; (+), Present; (–), Absent.

Table IV.2: Phytochemical screening of freeze dried fruit extracts of *E. operculata*

Phytochemicals	**Test**	**Me**	**Ch**	**He**	**Ac**	**Wa**
Alkaloids	Wagner's reagent	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	+	-
Steroids	Liebermann-Burchard	+	+	+	-	+
(Terpenoids)	test					
	Salkowski's test	+	+	+	+	-
Anthraquinones	Modified Borntrager's	+	-	-	+	+
	test					
Coumarins		+	-	-	+	+
Phenols	$FeCl_3$ test	+	+	+	+	+
Tannins	Gelatin test	-	-	-	+	-
Flavonoids	Shinoda's test	+	-	+	+	+
Carbohydrates	Molisch's test	+	+	-	+	-
	Fehling's test	+	+	+	+	+
Starch	Iodine test	-	+	+	-	+
Anthocyanins		+	-	+	+	-
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	+	+	+	+

Me, Methanol; Ch, Chloroform; He, Hexane; Ac, Acetone; Wa, Water; (+), Present; (–), Absent.

Table IV.3: Phytochemical screening of freeze dried fruit extracts of *A. dioica*

Phytochemicals	**Test**	**Me**	**Ch**	**He**	**Ac**	**Wa**
Alkaloids	Wagner's	+	+	+	+	+
	Dragendroff's	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	+	-
Steroids (Terpenoids)	Liebermann-Burchard test	-	+	+	-	+
	Salkowski's test	+	-	+	+	-
Anthraquinones	Modified Borntrager's test	-	-	-	+	+
Coumarins		+	+	-	-	+
Phenols	$FeCl_3$ test	+	+	+	+	+
Tannins	Gelatin test	+	-	-	+	-
Flavonoids	Shinoda's test	+	-	+	+	+
Carbohydrates	Molisch's test	+	+	+	+	+
	Fehling's test	+	+	+	+	-
Starch	Iodine test	-	+	-	-	+
Anthocyanins		+	-	+	+	-
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	-	-	+	+

Me, Methanol; Ch, Chloroform; He, Hexane; Ac, Acetone; Wa, Water; (+), Present; (–), Absent.

Table IV.4: Phytochemical screening of freeze dried fruit extracts of *A. bunius*

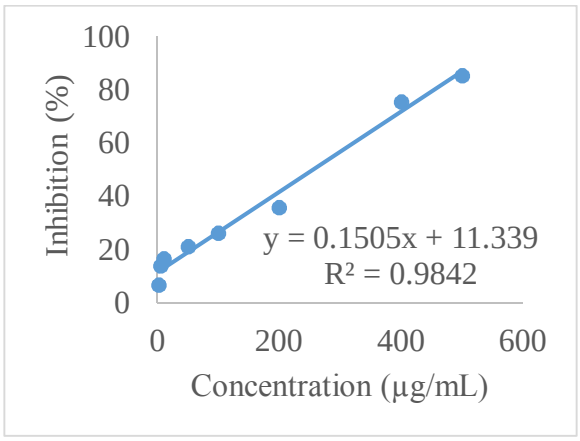
Phytochemicals	**Test**	**Me**	**Ch**	**He**	**Ac**	**Wa**
Alkaloids	Wagner's reagent	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	-	+	-
Steroids (Terpenoids)	Liebermann-Burchard test	+	+	+	+	+
	Salkowski's test	+	+	+	+	-
Anthraquinones	Modified Borntrager's test	+	-	+	+	+
Coumarins		-	-	-	+	+
Phenols	$FeCl_3$ test	+	-	+	+	+
Tannins	Gelatin test	+	-	-	+	-
Flavonoids	Shinoda's test	+	-	-	+	+
Carbohydrates	Molisch's test	+	+	-	+	-
	Fehling's test	+	+	+	+	+
Starch	Iodine test	+	+	+	+	+
Anthocyanins		-	-	+	+	-
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	+	-	+	+

Me, Methanol; Ch, Chloroform; He, Hexane; Ac, Acetone; Wa, Water; (+), Present; (–), Absent.

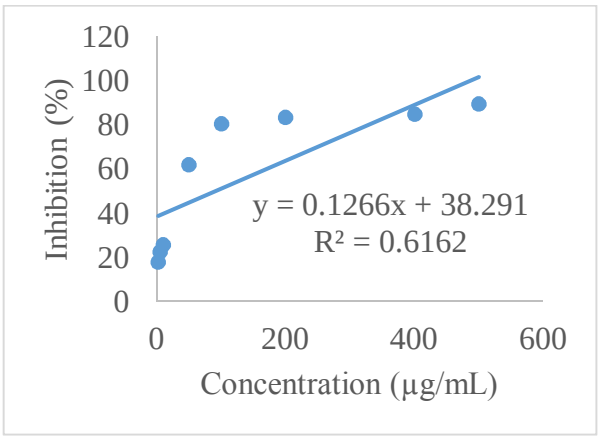
Table IV.5: Phytochemical screening of freeze dried fruit extracts of *O. alismoides*

Phytochemicals	**Test**	**Me**	**Ch**	**He**	**Ac**	**Wa**
Alkaloids	Wagner's	+	+	+	+	+
	Dragendroff's	+	+	-	-	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	-	-
Steroids (Terpenoids)	Liebermann-Burchard test	+	+	+	-	+
	Salkowski's test	+	+	+	+	-
Anthraquinones	Modified Borntrager's test	+	-	-	+	+
Coumarins		+	-	-	+	-
Phenols	$FeCl_3$ test	+	+	+	+	+
Tannins	Gelatin test	+	-	-	+	-
Flavonoids	Shinoda's test	+	-	-	+	+
Carbohydrates	Molisch's test	+	+	+	+	+
	Fehling's test	-	+	+	+	-
Starch	Iodine test	-	+	+	-	+
Anthocyanins		+	-	+	+	-
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	-	+	+	+

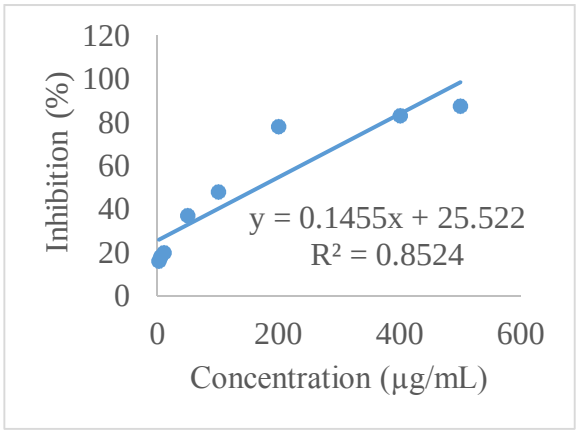
Me, Methanol; Ch, Chloroform; He, Hexane; Ac, Acetone; Wa, Water; (+), Present; (–), Absent.



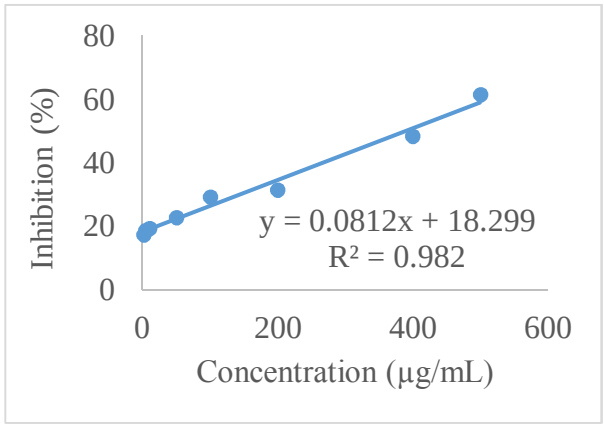
A: *Grewia sapida*



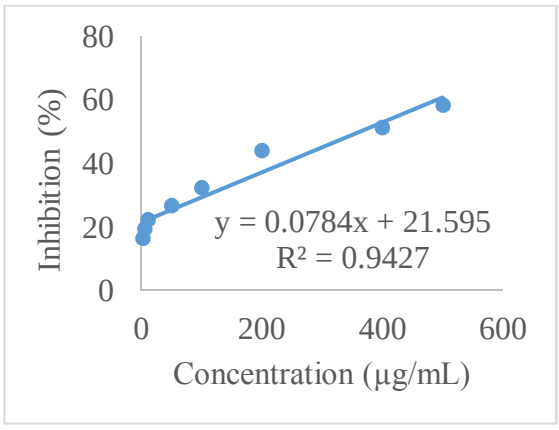
B: *Eugenia operculata*



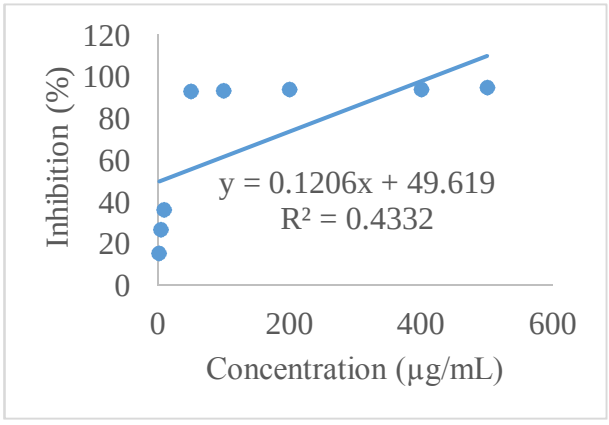
C: *Aporosa dioica*



D: *Antidesma bunius*



E: *Ottelia alismoides*



F: Ascorbic acid

Fig. IV.1: Plot of inhibition (%) against extract concentration for calculation (µg/mL) of IC_{50} value of DPPH.

Table. IV.6: DPPH free radical scavenging activity of methanolic extract of wild fruits

Fruit extract/ Standard	Concentration (µg/mL) and its inhibition (%)								IC_{50}
	2	5	10	50	100	200	400	500	
G. sapida	6.73± 0.27[a]	13.97± 0.75[a]	16.57± 0.38[a]	21.25± 0.43[a]	26.17± 0.25[a]	35.83± 0.41[a]	75.47± 0.45[a]	85.43± 0.25[a]	257.67± 2.52[a]
E. operculata	17.97± 0.64[b]	22.49± 0.96[b]	25.64± 0.83[b]	61.96± 0.19[b]	80.55± 1.01[b]	83.42± 0.37[b]	85.01± 0.64[b]	89.65± 0.55[b]	92.33± 4.16[b]
A. dioica	16.11± 0.46[c]	18.34± 0.37[c]	20.05± 0.73[c]	37.05± 0.41[c]	48.07± 0.46[c]	78.23± 0.46[c]	83.24± 0.37[c]	87.48± 0.46[c]	168.01± 2.65[c]
A. bunius	17.48± 0.32[b]	18.91± 0.48[d]	19.38± 0.16[d]	22.94± 0.56[d]	29.37± 0.64[d]	31.41± 0.48[d]	48.44± 0.36[d]	61.41± 0.39[d]	395.02± 3.61[d]
O. alismoides	16.64± 0.52[c]	19.64± 0.28[e]	22.38± 0.16[e]	26.79± 0.52[e]	32.43± 0.28[e]	44.22± 0.47[e]	51.49± 0.36[e]	58.51± 0.72[e]	364.33± 5.51[e]
Ascorbic acid	15.80± 0.56[d]	27.10± 0.75[f]	36.43± 0.71[f]	93.23± 0.41[f]	93.60± 0.50[f]	94.17± 0.55[f]	94.33± 0.65[f]	95.07± 0.45[f]	16.67± 2.52[f]

IC_{50} value in µg/mL; Values were expressed as mean of 3 replicates ± standard deviation; The data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.2 Antioxidant property

IV.2.2.1 DPPH free radical scavenging activity

The evaluation of antioxidant properties of five wild edible fruits was performed by DPPH radical scavenging assay. DPPH stable free radical method is an easy, rapid and sensitive way to examine the antioxidant activity of a specific compound or plant extracts. **Fig. IV.1** shows the plot of inhibition (%) against concentration of methanol extracts of fruits and standard ascorbic acid for DPPH assay. The results of DPPH assay and its IC_{50} are presented in **Table IV.6** and it was observed that the radical scavenging capacity increased with increasing concentration of sample. IC_{50} value is the required concentration to scavenge 50% of the free radical and lower IC_{50} value of sample indicates higher antioxidant power. At 500 µg/mL concentration, *E. operculata* fruit extract (89.65 ± 0.55%) showed highest percentage of inhibition and *O. alismoides* showed the lowest (58.51 ± 0.71%). While the standard ascorbic acid showed 95.07 ± 0.45% inhibition at the same concentration. The study showed that the highest radical scavenging activity was exhibited by the methanol extract of *E. operculata* (IC_{50} = 92.33 ± 4.16 µg/mL) whereas the methanol extract of *A. bunius* showed lowest activity (IC_{50} = 395.02 ± 3.61 µg/mL). The IC_{50} value of *Punica granatum* fruit reported by Khomdram *et al.* [41] is (398.54 ± 47.6 µg/mL) which is close to that of *A. bunius* and higher than the other four fruits of this study. Preethi *et al.* [42] was also reported the IC_{50} value of *Muntingia calabura* fruit as 90 ± 0.04 µg/mL which is comparable to that of *E. operculata* fruit (92.33 ± 4.16 µg/mL). Banerjee *et al.* [43] also reported the IC_{50} value of *Syzygium cumini* fruit skin extract as 168 µg/mL which is same with the result of *A. dioica* (168.01 ± 2.65 µg/mL). The IC_{50} values of *Lannea microcarpa* fruit extracts reported by Bationo *et al.* [44] ranges from 29.55 ± 1.80 µg/mL to 46.67 ± 2.05 µg/mL which are comparatively lower than the five wild fruits of this study. Wang *et al.* [45] reported IC_{50} value of *Morus alba* fruit extract as 14.38 ± 2.83 mg/mL which is also lower than the value obtained in the present study. The methanol extract of *Leucas indica* exhibited IC_{50} value of 365.9 µg/mL similar to the IC_{50} value of *O. alismoides* (364.33 ± 5.51 µg/mL) [46]. The IC_{50} value of seed extracts of *Abelmoschus moschatus* (38.1 µg/mL) reported by Gul *et al.* [47] indicated good antioxidant potential. Junejo *et al.* [48] reported the IC_{50} value of the *Diplazium esculentumas* as 138.8 µg/mL which is higher than *E. operculata* fruit (92.33 ± 4.16 µg/mL) but lower than other fruits studied. The methanol extract of *Mespilus germanica* reported by Nabavi *et al.* [49] exhibited IC_{50} value of 419 ± 3.2 µg/mL which is also higher than the values obtained in the present study.

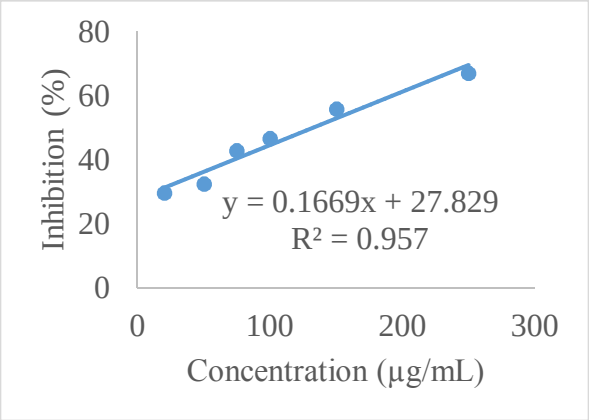
Table IV.7: ABTS radical scavenging activity of methanolic extract of wild fruits

Fruit extract/ Standard	Concentration (µg/mL) and its inhibition (%)						IC_{50}
	20	50	75	100	150	250	
G. sapida	29.69±0.58^{a}	32.50±0.65^{a}	42.87±0.51^{a}	46.72±1.39^{a}	55.89±1.05^{a}	66.95±0.29^{a}	134.33±4.05^{a}
E. operculata	28.93±0.35^{b}	41.01±0.46^{b}	66.03±0.51^{b}	78.69±0.73^{b}	81.61±0.45^{b}	90.17±0.65^{b}	52.66±1.15^{b}
A. dioica	32.22±0.29^{c}	52.11±0.51^{c}	71.38±0.72^{c}	80.19±0.86^{c}	85.88±0.73^{c}	91.91±1.02^{c}	27.33±1.52^{c}
A. bunius	24.77±0.75^{d}	36.91±1.19^{d}	46.36±0.52^{d}	52.48±1.06^{d}	63.73±0.29^{d}	80.74±0.89^{d}	105.33±3.06^{d}
O. alismoides	13.07±1.23^{e}	23.43±1.38^{e}	31.59±1.08^{e}	33.69±0.86^{e}	41.69±0.46^{e}	57.38±0.62^{e}	201.00±6.55^{e}
Ascorbic acid	36.09±0.87^{f}	38.52±1.17^{f}	55.55±1.03^{f}	66.86±0.66^{f}	73.51±0.81^{f}	79.43±1.16^{f}	73.67±3.21^{f}

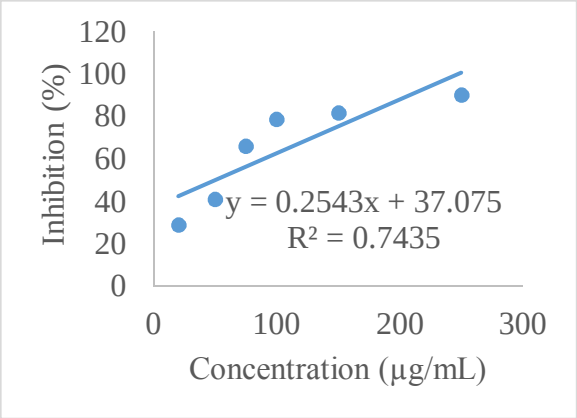
IC_{50} value in µg/mL; Values were expressed as mean of 3 replicates ± standard deviation; The data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.2.2 ABTS free radical scavenging assay

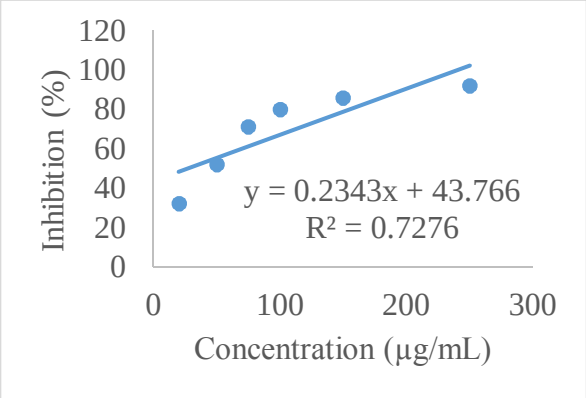
The ABTS method is known as a rapid method for determining the antioxidant activity and employed to screen samples in order to obtain high content of natural antioxidants in foods [50]. ABTS free radical scavenging activities in methanol extracts of five wild edible fruits and standard ascorbic acid are presented in **Table IV.7** and this method also showed antioxidant activities in a concentration-dependent manner. **Fig. IV.2** shows the plot of inhibition (%) against the extract concentration and standard and the ABTS IC_{50} values were calculated from the equations of the respective graphs. *A. dioica* fruit extract (91.91 ± 1.02%) exhibited the highest percent of inhibition at concentration of 250 µg/mL with an IC_{50} value of 27.33 ± 1.52 µg/mL whereas *O. alismoides* (57.38 ± 0.62%) showed the lowest percent of inhibition at the same concentration with IC_{50} value of 201.00 ± 6.55 µg/mL which indicated that the fruit extract of *A. dioica* had better antioxidant capacity among the fruits studied. While the standard ascorbic acid showed 79.43 ± 1.16% inhibition at the same concentration with an IC_{50} value of 73.67 ± 3.21 µg/mL. Al-Rimawi *et al.* [51] reported IC_{50} value of *Tragopogon porrifolius* plant as 60.8 µg/mL which is lower than most of the fruits studied herein. IC_{50} value of methanolic extract of *Hodgsonia heteroclita* reported by Basumatary *et al.* [52] was 28.58 ± 0.42 µg/mL which is found similar to the value of *A. dioica* (27.33 ± 1.52 µg/mL) and is much lower than the other fruits studied. The IC_{50} value of the methanolic extract of *Amoora rohituka* (73 µg/mL) was found similar to the value of standard ascorbic acid (73.67 ± 3.21 µg/mL) reported by Umesh *et al.* [53]. Ethanolic fruit extracts of *Solanum americanum* and *Solanum torvum* reported by Fidrianny *et al.* [54] were found with an IC_{50} of 4.73 µg/mL and 2.36 µg/mL respectively which are much lower than the results of the present study. The ethanolic extract of *Lagenaria siceraria* (95.98%) exhibited highest percent of inhibition at concentration of 50 mg/mL with an IC_{50} value of 19 mg/mL, which is much higher compare to the present study [55]. Sharma *et al.* [56] reported IC_{50} value of methanolic extract of *Docynia indica* as 49.83 ± 0.24 µg/mL, comparable to the value of *E. operculata* (52.66 ± 1.15 µg/mL) and *Garcinia pedunculata* as 535.70 ± 4.04 µg/mL, which is much higher in comparison to the values of the five wild fruits studied herein. The ABTS assay of methanolic fruit extract of *Phoebe cooperiana* was evaluated to 268.114 µM/g with an IC_{50} value of 94.6 ± 1.35 µg/mL, which is much higher compare to the IC_{50} of *E. operculata* (52.66 ± 1.15 µg/mL), *A. dioica* (27.33 ± 1.52 µg/mL) and standard ascorbic acid ((73.67 ± 3.21 µg/mL) [57].



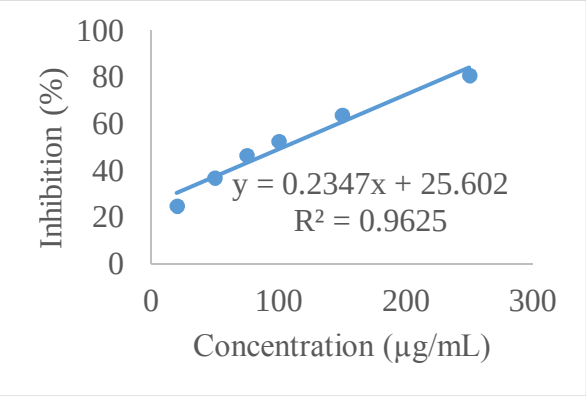
A: *Grewia sapida*



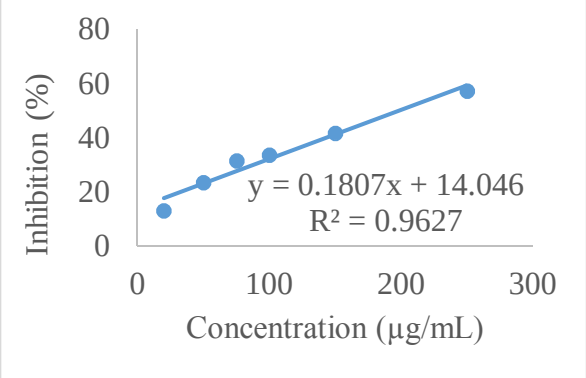
B: *Eugenia operculata*



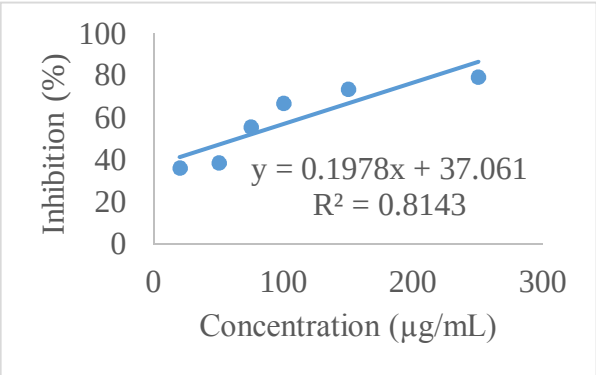
C: *Aporosa dioica*



D: *Antidesma bunius*



E: *Ottelia alismoides*



F: Ascorbic acid

Fig. IV.2: Plot of inhibition (%) against extract concentration and standard for ABTS assay.

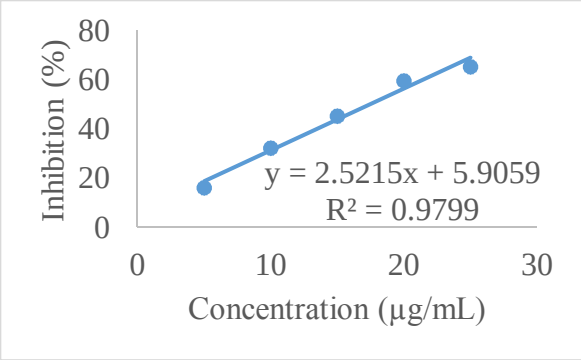
Table IV.8: Hydrogen peroxide scavenging activity of methanolic extract of wild fruits

Fruit extract/ Standard	Concentration (µg/mL) and its inhibition (%)					IC_{50}
	5	10	15	20	25	
G. sapida	16.03±0.18^{a}	32.37±0.26^{a}	45.21±0.38^{a}	59.58±0.29^{a}	65.46±0.36^{a}	17.66±0.25^{a}
E. operculata	13.24±0.09^{b}	27.63±0.65^{b}	36.07±0.31^{b}	47.57±0.12^{b}	62.19±0.25^{b}	20.57±0.21^{b}
A. dioica	19.29±0.15^{c}	37.65±0.32^{c}	40.13±0.11^{c}	61.05±0.14^{c}	68.26±0.28^{c}	16.57±0.25^{c}
A. bunius	5.94±0.15^{d}	18.01±0.23^{d}	27.20±0.16^{d}	32.47±0.11^{d}	55.01±0.07^{d}	24.37±0.06^{d}
O. alismoides	5.26±0.31^{e}	13.50±0.24^{e}	24.04±0.18^{e}	37.45±0.19^{e}	52.41±0.14^{e}	24.47±0.12^{d}
Ascorbic acid	10.41±0.31^{f}	27.89±0.16^{b}	41.94±0.24^{f}	51.45±0.12^{f}	60.52±0.28^{f}	19.77±0.15^{e}

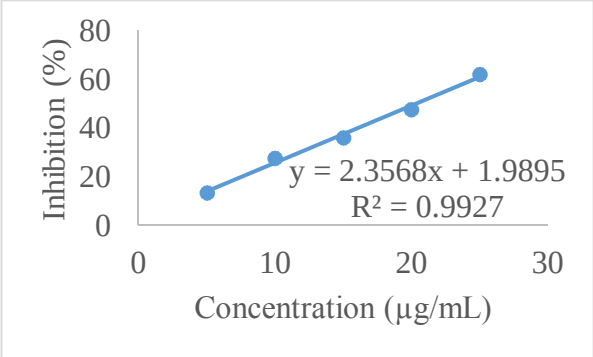
IC_{50} value in µg/mL; Values were expressed as mean of 3 replicates ± standard deviation; The data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.2.3 H_2O_2 scavenging assay

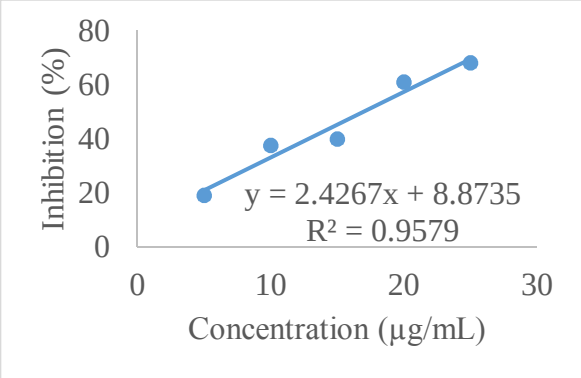
Hydrogen peroxide is a weak oxidizing agent that can inactivate a few enzymes directly by oxidation of essential thiol (-SH) groups. It can penetrate biological membranes and once within the cell, H_2O_2 can possibly react with Fe^{+2} and possibly Cu^{+2} to form hydroxyl radical which may be the origin of many of its toxic effects [58]. Scavenging of H_2O_2 by the extracts may be attributed to their phenolic compounds which can donate electrons to H_2O_2 thereby neutralizing it to water [59, 60]. H_2O_2 scavenging activities of methanol extracts of the fruits are presented in **Table IV.8**. The **Fig. IV.3** shows the plot of inhibition (%) against extract concentration or standard and the H_2O_2 IC_{50} value calculated from the equations of the respective graphs. The H_2O_2 IC_{50} value for methanolic extract of *O. alismoides* (24.47 ± 0.12 µg/mL) was found to be the highest and that of *A. dioica* fruit (16.57 ± 0.25 µg/mL) was found to be the lowest indicating stronger antioxidant capacity of *A. dioica* fruit, while the standard ascorbic acid showed an IC_{50} value of 19.77 ± 0.15 µg/mL. Gul *et al*. [47] reported the IC_{50} values of seed extracts of *Abelmoschus moschatus* that ranged from 22.6 ± 5.0 to 26.3 ± 4.0 µg/mL. These values are similar in comparison to the results of our study. The IC_{50} value of the methanol extract of *Mespilus germanica* reported by Nabavi *et al.* [49] was 1138 ± 77.1 µg/mL which is much higher compared to that of the fruits studied herein. Subramanian *et al*. [61] reported the H_2O_2 IC_{50} values of *Shorea roxburghii* stem bark for methanol and acetone extracts as 63.67 and 87.18 µg/mL, respectively. These values are higher than the IC_{50} values of the five fruits studied (**Table IV.8**). Similarly, Pawar *et al*. [62] reported that the IC_{50} values of ethanol extract of *Asteracantha longifolia* as 60.77 ± 1.34 µg/mL which was found higher than that of the present five wild fruits studied. Rahman *et al*. [63] also studied the H_2O_2 scavenging activity and the IC_{50} value of *Citrus macroptera* fruit peels for ethanol extract was reported as 216.49 µg/mL which is much higher than the results of the present investigation. The IC_{50} value of methanolic extract of *Syzygium cumini* for H_2O_2 scavenging activity was 42.03% at 1.2 mg/mL which is much higher in comparison to the values of the present study [64]. The IC_{50} values of peel and pulp extracts of citrus fruits were in the range between 0.11 ± 0.002 mg/mL and 0.20 ± 0.003 mg/mL and these values are much higher compared to the present study [65]. Mudoi *et al.* [66] also reported IC_{50} value of methanolic extract of *Garcinia pedunculata* as 2 mg/mL which was found higher than that of the present wild fruits studied. The IC_{50} value of the investigated fruits are much lower in comparison to the value of hydro-methanol extract of the seeds of *Swietenia mahagoni* (66.10 µg/mL) [67].



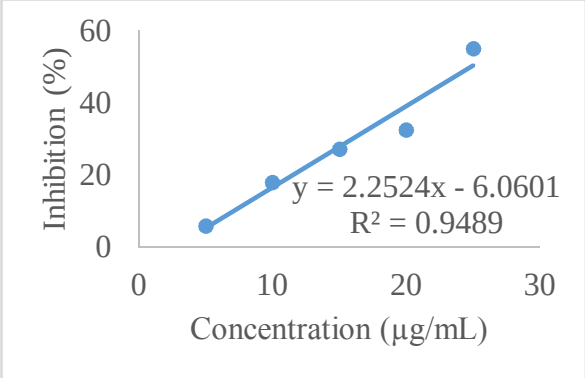
A: *Grewia sapida*



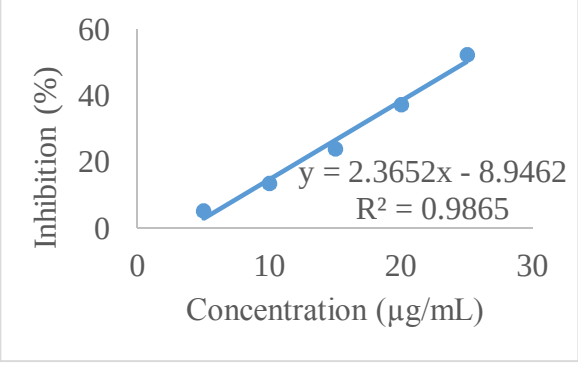
B: *Eugenia operculata*



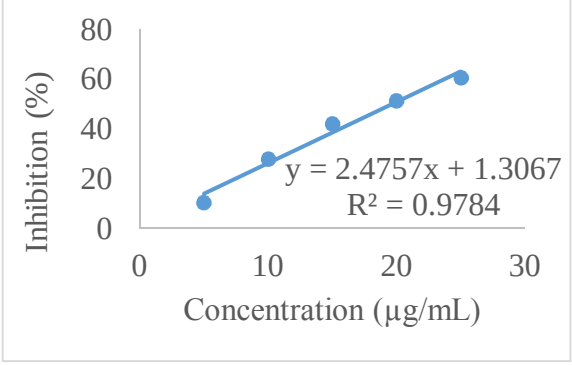
C: *Aporosa dioica*



D: *Antidesma bunius*



E: *Ottelia alismoides*



F: Ascorbic acid

Fig. IV.3: Plot of inhibition (%) against extract concentration and standard for H_2O_2 assay.

IV.2.2.4 Ferric reducing antioxidant power (FRAP) assay

FRAP assay is a simple, inexpensive and may offer putative index of antioxidant activity. This method measures the ferric reducing potential of sample at low pH, forming an intense blue color as the ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex which is reduced to the ferrous (Fe^{2+}) form that can be detected at an absorbance of 593 nm [25]. The results of FRAP assay of the fruits studied are presented in **Table IV.9** and the values were calculated from the linear regression equation of standard trolox (y = 0.0004x + 0.1687; r^2 = 0.9664) shown in **Fig. IV.4**. *E. operculata* fruit exhibited higher FRAP value of 281.58 ± 8.79 µM TE/g dried extract (DE) indicating stronger antioxidant capacity and *O. alismoides* fruit showed the lowest FRAP value of 44.08 ± 7.64 µM TE/g DE. Wong *et al.* [68] studied the antioxidant activities of the twenty five tropical edible plants and the FRAP value reported were found ranging from 25 to 300 mol trolox/g. The FRAP value of *Melastoma malabathricum* (5878.35 ± 0 .05 µM AEAC/g DE) reported by Nayak *et al.* [69] was found higher than the results of our study. In one study reported by Namiesnik *et al.* [70], the aqueous extract of *Vaccinium corymbosum* exhibited FRAP value of 94.10 ± 9.3 µM TE/g DE which is higher than *A. bunius* (61.58 ± 3.82 µM TE/g DE) and *O. alismoides* (44.08 ± 7.64 µM TE/g DE) of this study. The FRAP results reported by Alothman *et al.* [71] were smaller (0.59 ± 0.15 – 31.9 ± 0.95 µmol Fe (II)/g) than present studied fruits (44.08 ± 7.64 to 281.58 ± 8.79 µM TE/g DE). Similarly, the FRAP results of this study are also in accordance with the FRAP values of wild edible plants reported by Wong *et al.* [72] which are in the range of 12.84 ± 2.95 to 45.01 ± 6.69 µmol TE/g DE.

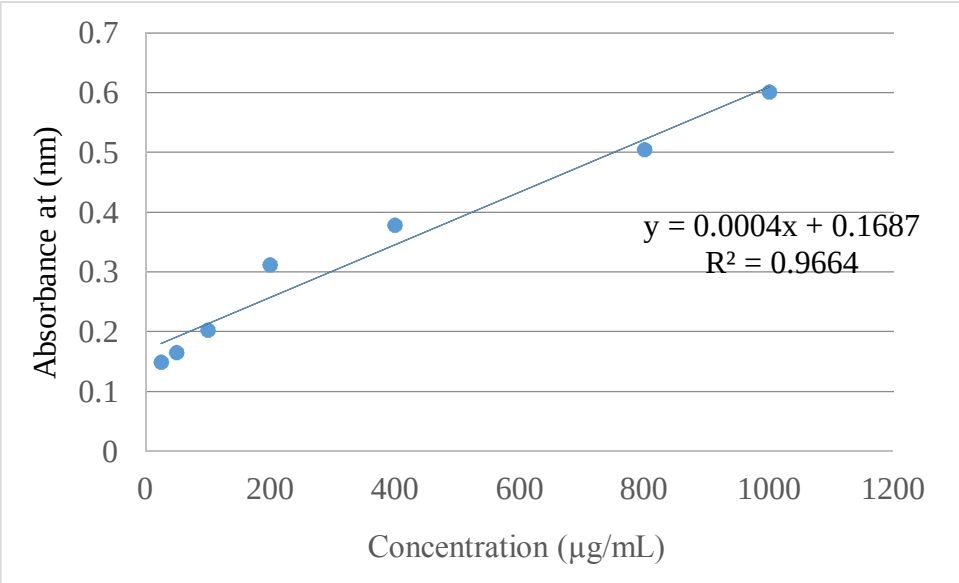


Fig. IV.4: Trolox standard curve for FRAP assay.

Table IV.9: FRAP value, TPC, TFC and vitamin C content of wild fruits

Plants	FRAP value (µM TE/g DE)	Total phenolic content (mg GAE/g DE)	Total flavonoid content (mg QE/g DE)	Vitamin C (mg/100 g fresh fruit)
G. sapida	62.40±10.40^{a}	294.35±4.69^{a}	116.95±10.71^{a}	8.60±0.30^{a}
E. operculata	281.58±8.79^{b}	226.74±2.10^{b}	108.76±7.02^{b}	6.60±1.12^{b}
A. dioica	106.58±5.20^{c}	146.71±2.81^{c}	72.51±8.83^{c}	6.12±0.61^{b}
A. bunius	61.58±3.82^{d}	119.36±1.39^{d}	64.32±8.82^{d}	7.30±1.45^{c}
O. alismoides	44.08±7.64^{e}	93.86±1.17^{e}	43.27±5.36^{e}	3.68±0.84^{d}

DE, Dried extract; Values were expressed as mean of 3 replicates ± standard deviation; The data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.3 Total phenolic and flavonoid contents

The phenolic contents in the methanol extracts of fruits were determined using the equation obtained from the curve of standard gallic acid (**Fig. IV.5;** $y = 0.0212x + 0.3098$; $r^2 = 0.9971$) and flavonoid contents were also determined through an equation obtained from the curve of standard quercetin (**Fig. IV.6;** $y = 0.0013x + 0.0802$; $r^2 = 0.9828$). Total phenolic and total flavonoid contents of the five edible wild fruits were displayed in **Table IV.9**. A graphical comparison of the total phenolic contents (TPC) and total flavonoid contents (TFC) of five wild edible fruits are also shown in **Fig. IV.7**.

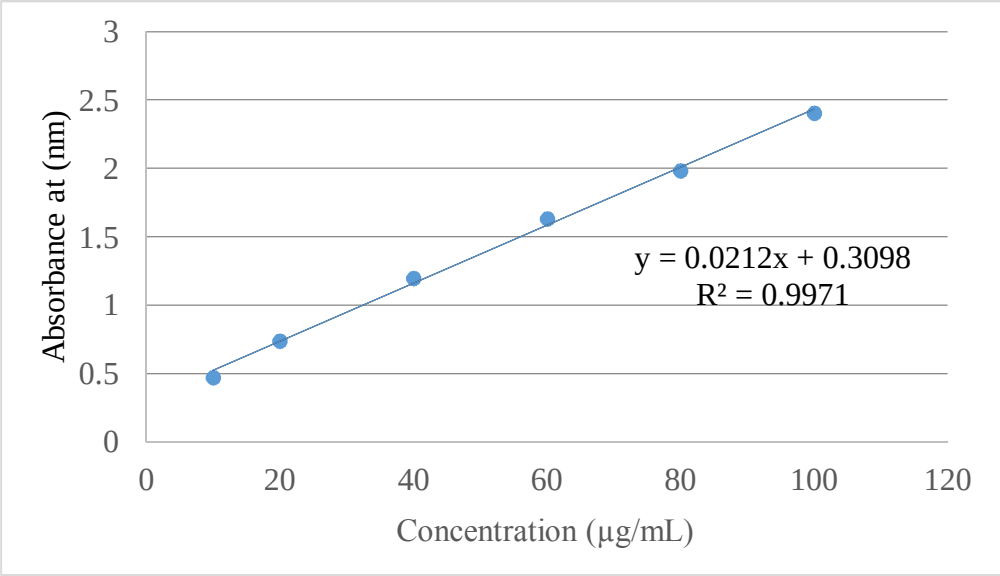


Fig. IV.5: Gallic acid standard curve for determination of total phenolic content.

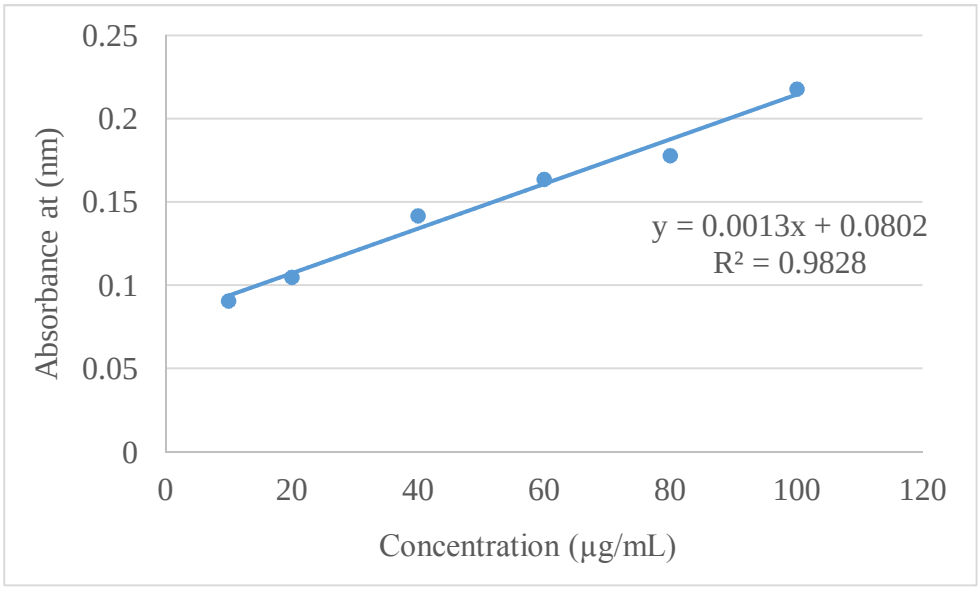


Fig. IV.6: Quercetin standard curve for determination of total flavonoid content.

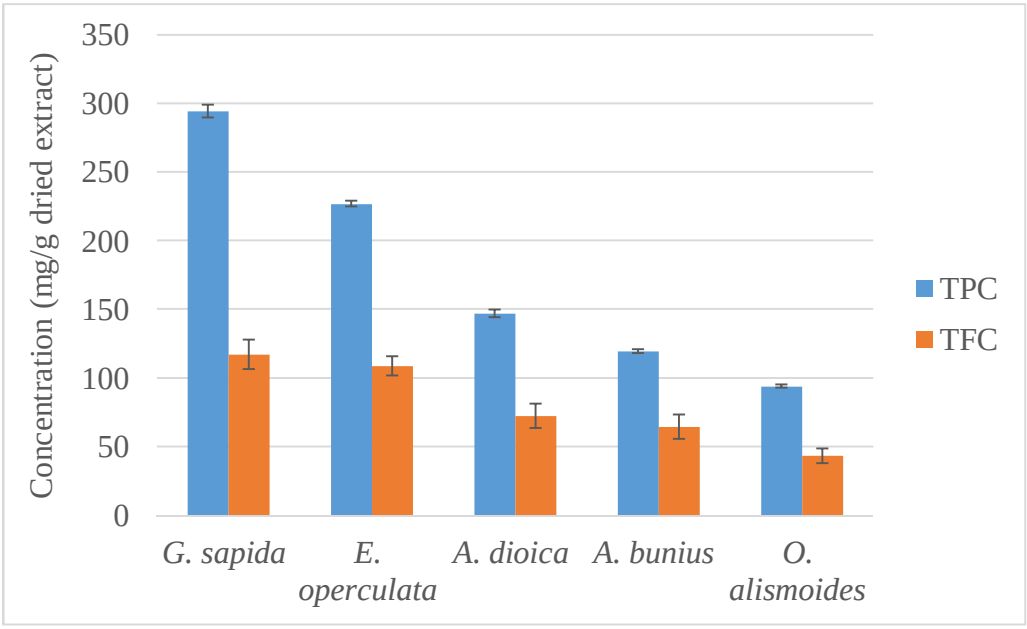


Fig. IV.7: Bar diagram showing the total phenolic and flavonoid contents in the fruits.

The levels of total phenolic contents in the methanolic extracts of fruits varied significantly from 93.86 ± 1.17 to 294.35 ± 4.69 mg gallic acid equivalent (GAE)/g DE. The total phenolic content was found highest in *G. sapida* (294.35 ± 4.69 mg GAE/g DE) followed by *E. operculata* (226.74 ± 2.10 mg GAE/g DE), *A. dioica* (146.71 ± 2.81 mg GAE/g DE), *A. bunius* (119.36 ± 1.39 mg GAE/g DE) and *O. alismoides* (93.86 ± 1.17 mg GAE/g DE). Devi and Mazumder *et al*. [73] reported antioxidant activity of aqueous and ethanol fruit extracts of *Eugenia operculata* and reported that the total phenolic content for aqueous and ethanol extracts were 88.1 and 58.6 mg/g GAE respectively. Samappito and Butkhup [74] also examined methanolic extracts of *Antidesma bunius* for flavonoid, anthocyanin, phenolic acids constituents and antioxidant activity. Results indicated that the fruit extracts contain high amounts of flavonoids (397.90 mg/100 g FW), anthocyanin (141.94 mg/100 g DE) and phenolic acid compounds (13.56 mg GAE/g DE). Butkhup and Samappito [75] also investigated *Antidesma bunius* fruits for their changes in physico-chemical properties, antiradical activity and polyphenolic compounds. Results showed that the total phenolic compounds were in the range from 19.60 to 8.66 mg GAE/g fresh weight and the fruit possess the highest antioxidant activity with an average IC_{50} value of 100.08 mg/mL. In this study, the total flavonoid content was found highest in *G. sapida* (116.95 ± 10.71 mg QE/g DE) followed by *E. operculata* (108.76 ± 7.02 mg QE/g DE), *A. dioica* (72.51 ± 8.83 mg QE/g DE), *A. bunius* (64.32 ± 8.82 mg QE/g DE) and *O. alismoides* (43.27 ± 5.36 mg QE/g DE). *G. sapida* fruit extract exhibited higher contents of both total phenolics and total flavonoids than the other fruits which attributed to better antioxidant capacity in *G. sapida* fruit. Jorjong *et al*. [76] reported fourteen Mao-Luang (*Antidesma bunius*) cultivars from Northeastern Thailand for their phytochemicals and antioxidant activities and the reported phenolic content was 345.68 ± 9.12 mg GAE/100 g dry weight, flavonoid contents was 289.60 ± 19.52 mg catechin equivalent/100 g dry weight and total anthocyanin was 131.30 mg/100 g dry weight. Prakash *et al*. [77] worked on some wild fruits from Sikkim Himalayan region of India, the total phenolic content reported by them varied from 7.3 to 119.2 mg GAE/g. However, the total phenolic contents of 56 wild fruits from South China reported by Fu *et al*. [78] ranged from 0.49 ± 0.04 to 54.8 ± 3.05 mg GAE/g wet weight. Saikia *et al*. [79] reported phenolic content (4.62–14.74 mg GAE/g dry weight) and flavonoid content (0.65– 7.72 mg QE/g dry weight) in some non-conventional green leafy vegetables which are lower in comparison to the present study. The phenolic content of *Pyrus pashia* (98 ± 5 mg GAE/ g DE) was found comparable to the value of *O. alismoides* (93.86 ± 1.17 mg GAE/g DE) [80]. The levels of TPC in the evaluated wild fruits were found higher compared

to Thai fruits that ranged from 1.3 to 214 mg GAE/g DE [81]. Ikram *et al*. [82] reported the TPC of selected Malaysian under-utilized fruits that varied from 0.65-32 mg GAE/g. These values are much lower than the values obtained in the present study. Singh *et al*. [83] also reported the TPC of wild edible fruits of North-West Himalaya, India that varied from (58.83 ± 14.50) to (4496.39 ± 318.00) mg GAE/100 g fresh weight and these values are lower than the fruits of this study. Similarly, Gul *et al.* [47] investigated the TPC in the different seed extracts of *Abelmoschus moschatus* which were reported in the range of 1.56 to 3.74 mg GAE/g DE which are much lower compared to the values of the fruits of this study. Mudoi *et al*. [66] reported TFC of *Garcinia pedunculata* (71.4 ± 0.84 mg QE/100 g DE) which is much lower than the values obtained in the study. Seal [84] also reported the TFC of the acetone extract of *Fagopyrum cymosum* (52.17 ± 0.01 mg QE/g) higher than the values of *O. alismoides* (43.27 ± 5.36 mg QE/g DE). The flavonoid content in the extract of *Mellilotus officinalis* (57 ± 5.4 mg QE/g DE) reported by Pourmorad *et al.* [85] was found higher than *O. alismoides* (43.27 ± 5.36 mg QE/g DE) but lower than the results of other fruits studied herein. The extraction yield of phenolic and flavonoid contents from plant materials depend on the polarity of solvent used for extract preparation [21]. Fruits and vegetables are very good sources of polyphenols, flavonoids, anthocyanins and several other compounds, and possess favorable effects on human health as antioxidant and antibacterial agents [86, 76]. Phenolic compounds such as phenolic acids and flavonoids are major ingredients of fruits which play a significant role in the nutritional properties of the fruits [87]. The higher contents of polyphenols and flavonoids correspond to stronger antioxidant capacity and they have various indispensable roles in reducing the risk of many human diseases especially oxidative stress related diseases [86]. Several studies have revealed that the phenolic compounds in the plants have antioxidant activities which may be due to their redox properties, hydrogen donors, and singlet oxygen quenchers [22, 88].

IV.2.4 Evaluation of vitamin C contents

Ascorbic acid, also known as vitamin C is a water-soluble vitamin that is naturally present in a variety of fresh fruits and vegetables. The fresh fruit of *G. sapida* (8.6 ± 0.30 mg/100 g) showed higher vitamin C content (**Table IV.9**) followed by *A. bunius* (7.30 ± 1.45 mg/100 g), *E. operculata* (6.60 ± 1.12 mg/100 g), *A. dioica* (6.12 ± 0.61 mg/100 g) and *O. alismoides* (3.68 ± 0.84 mg/100 g). The variation of vitamin C content per 100 g fresh sample of five wild edible fruits are shown in **Fig. IV.8**. Khomdram *et al*. [41] reported the vitamin C content in wild endemic fruits of the Manipur region of India that ranged from 6.91 mg/100 g

in *Prunus armeniaca* to 375.68 mg/100 g of fresh weight in *Phyllanthus emblica*. Bhatt *et al.* [89] reported higher vitamin C contents in wild edible fruits of Indian Himalayan region that varied from 2.02 to 33.15 mg/g. The vitamin C content of *A. bunius* (7.30 ± 1.45 mg/100 g) was found similar to the *Prunus spinosa* (7.73 mg/100 g) reported by Morales *et al.* [90]. When compared to the vitamin C content of *Sclerocarya birrea* (128.3 mg/100 g) and *Adansonia digitata* (141.3 mg/100 g), the vitamin C content in the present study is significantly lower [91]. Arunachalam *et al.* [92] also reported vitamin C content of *Ficus amplissima* (10.83 + 0.25 mg/100 g) comparable to the values of *G. sapida* (8.60 ± 0.30 mg/100 g) and *A. bunius* (7.30 ± 1.45 mg/100 g). Fruits and vegetables normally can provide about 90% of a person's dietary vitamin C requirement. The average dietary intake of vitamin C for an adult human being is about 50 mg per day [41, 93]. Vitamin C is required during various growth stages of human life and being a powerful reducing agent, it plays a significant role in absorbing and neutralizing free radicals and thereby protects the body from harmful effects [41, 77].

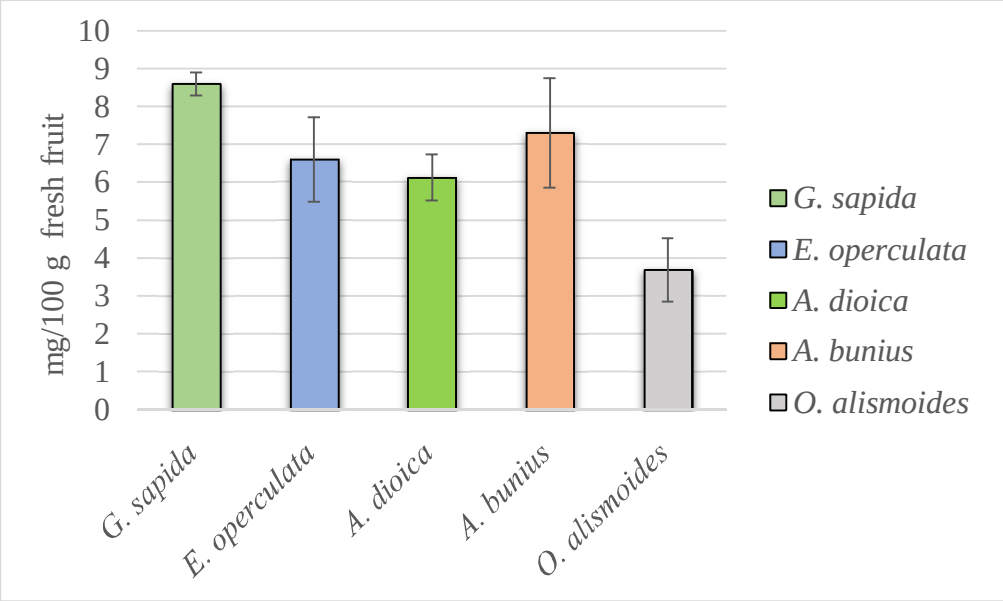


Fig. IV.8: Variation of vitamin C contents of fresh sample of five wild edible fruits.

IV.3 Pearson's correlation study

The Pearson's correlation among the results of antioxidant activities (DPPH, ABTS, H_2O_2, FRAP), TPC, TFC and vitamin C of five wild edible fruits were investigated (**Table IV.10**). In the study, DPPH assay was found to be positively strong correlated with ABTS (r = 0.737) and H_2O_2 (r = 0.690) assays. The DPPH assay was negatively correlated with FRAP (r = −0.831), TPC (r = −0.526) and TFC (r = −0.639). ABTS assay showed moderate positive correlation with H_2O_2 (r = 0.597) and exhibited negative correlation with other parameters. On the other hand, FRAP had an intermediate positive correlation with TFC (r = 0.525) and a mild positive correlation with TPC (r = 0.349) and vitamin C (r = 0.093). Pearson's correlation study showed that TPC was positively strong correlated with TFC (r = 0.971) significantly at $p<0.01$ which was found in accordance with the results reported by Narzary *et al.* [86]. The vitamin C was also found positively correlated with TPC and TFC.

Table IV.10: Pearson's correlation coefficients of antioxidant activities (DPPH, ABTS, H_2O_2, FRAP), TPC, TFC and vitamin C content in the wild fruits

	DPPH	ABTS	H_2O_2	FRAP	TPC	TFC	Vitamin C
DPPH	1						
ABTS	0.737	1					
H_2O_2	0.690	0.597	1				
FRAP	-0.831	-0.612	-0.202	1			
TPC	-0.526	-0.212	-0.627	0.349	1		
TFC	-0.639	-0.396	-0.618	0.525	0.971[a]	1	
Vitamin C	-0.187	-0.371	-0.469	0.093	0.752	0.777	1

a, Correlation is significant at p<0.01 (2-tailed).

IV.4 Conclusion

In this study, the phytochemical screening of different solvent extracts of the fruits exhibited the presence of various bioactive compounds such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids which have potentials for pharmacological uses. The results demonstrated that the methanol extracts of *E. operculata* fruits (DPPH and FRAP assays) and *A. dioica* fruits (ABTS and H_2O_2 assays) showed potent antioxidant capacity. The methanol extract of *G. sapida* fruit showed the highest total

phenolic content (294.35 ± 4.69 mg GAE/g DE) and highest total flavonoid content (116.95 ± 10.71 mg QE/g DE). The highest vitamin C content (8.60 ± 0.30 mg/100 g fresh fruit) was also observed in *G. sapida* fruit. Pearson's correlation study indicated a strong positive correlation of DPPH assay with ABTS and H_2O_2 assays. ABTS assay showed a moderate positive correlation with H_2O_2 assay and a strong positive correlation of TPC with TFC was observed. This study suggests that the fruits could play a positive role against the diseases caused by oxidative stress and could inhibit the development of various diseases such as cancer, cardiovascular diseases, neurological diseases, aging, etc. Therefore, consumption of these edible wild fruits may not only fulfil the nutritional requirement, but will have a great role for nutraceutical development. Further, isolation and identification of bioactive compounds responsible for antioxidant activity is encouraged.

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