Phytochemical screening and evaluation of antioxidant potential of *Feronia limonia* leaves and fruit extracts

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ABSTRACT

Traditionally various parts of *Feronia limonia* (wood apple) are used to treat human ailments. The objective of the present study was to investigate the phytochemicals content, antioxidant activity from the sequential extracts (petroleum ether, chloroform and aqueous) of leaves, pulp and rind from *Feronia limonia*. Estimation of total phenolics showed that petroleum ether extract showed higher phenolics reported as GAE mg/100mg extract (leaf extract- 56.37±4.06; Pulp extract- 55.87±0.17; rind extract-56.37±6.54) content than other extracts and total tannin content was high in aqueous extract expressed as TAEmg/100mg extract (leaf extract- 912.5±23.33; Pulp extract-520.5±7.07; rind extract- 747.5±28.28) and flavonoids was found to be high in chloroform extract of leaf and rind (leaf-113.8 RE mg/100mg ; rind- 86.2 mg/100mg). *In vitro* antioxidant activity was evaluated by employing different assays, including DPPH, ABTS radical scavenging assays, FRAP, phosphomolybdenum reduction assay, hydroxyl radical scavenging activities and metal chelating ability. Total antioxidant acivity (TAA) was found to be high in aqueous extract of all the three parts. Metal chelating activity was found to be high in aqueous extract of rind and petroleum extract of rind.

KEY WORDS: *Feronia limonia*, Phytochemicals, Antioxidant assay.

1. INTRODUCTION

Plants are not only primary source for our food but also they have immense therapeutic potential. Plant metabolites (phytochemicals) are used to cure many of the human ailments. Wood apple is a rare plant tree species with enormous medicinal values. Wood apple is scientifically named as *Feronia limonia* is a well known woody plant grown in temples of Tamilnadu belongs to Rutaceae family. Other Indian names of the plants include; vilamaram (tamil), kaitha (Hindi), Bekalu (kanada). Traditionally, extracts of this plants parts such as leaves, fruit has be used to cure liver and lung malfunction, and constipation etc. Ethanopharmacological properties of *F.limonia* have been documented in various studies. Unripened fruits are useful in treating diarrhorea and dysentery (Panda, 1999; Asima and Pakrashi 1994). The ripened fruit pulp with jiggery is used to cure diabetics (Kangralkar et al., 2010). Extract of leaves of *F.limonia* is prescribed for indigestion, stomachache and leaf extract is known to possess wound healing activity (Rajasab et al., 2003; Ranjan Priya, 2000, Ilango et al., 2012). Bark is useful in treating liver disease (Rastogi and Mehrotra, 1995) and gummy substance from the stem is useful in treating haemorroids (Joshi, 2004). Various plant secondary metabolites are responsible for these medicinal properties of *F.limonia* plant extracts.

Free radicals generated in human body due to external and internal sources leads to irreversible damage to the biological molecules, subsequently impair many vital functions of the body. Recent studies have shown that free radicals damage is responsible for many of the disease conditions such as alzhimers, cancer, diabetics etc (Farrukh, 2006). Natural phytochemicals such as phenolics, flavonoids and tannins are shown to be good source for free radicals scavengers. Therefore, fruits and vegetables are exploited for antioxidants. In the present study, extracts from leaves, pulp and rind has been subjected to quantitative determination of phenolics, flavonoids and tannins and subsequently antioxidant capacity of the extracts.

2. MATERIALS AND METHODS

2.1. Plant material: Leaves and fruits were collected from the local places, Coimbatore and botanical authenticity of the plant was confirmed at Botanical Survey of India-Southern Regional centre, , TNAU campus, Coimbatore (BSI/SRC/5/23/2013-14/tech dated 26.8.2013). Leaves were shade dried and powdered. Unripened fruit was collected and the pulp and rind was separated. The dried in oven at 40˚C for 48 hours and powdered. Uniformly sieved leaf, pulp and rind powder was used for extraction.

2.2. Preparation of extract: The powdered sample was sequentially extracted using petroleum ether, chloroform and aqueous solvents in Soxhlet apparatus for 6 hours. The extract was concentrated using rotary evaporator (Heidolph, Laborota 4000) and then completely dried to get paste. The yield of the extract was calculated and expressed as dried weight of plant material. The dried extract was dissolved at a concentration of 1mg/ml and used for phytochemical analysis and antioxidant assays.

2.3. QUANTIFICATION OF PHYTOCHEMICALS

2.3.1. Determination of Phenolics: The total phenolic content in various extracts of leaf, pulp and rind of *F.limonia* was estimated using Folin-Ciocalteu reagent according to the standardized procedure. Twenty µl of the plant extracts (dissolved in the respective solvents) were taken in a test tube and made up to the volume of 1.0 ml using distilled water. 0.5 mL of freshly prepared Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL of 20% sodium carbonate solution were added successively in each tube. Then the mixtures were stirred and left in the dark room for 40 min for colour development. The absorbance was recorded at 725 nm against the reagent blank using spectrophotometer (Systronics-106). A calibration curve of gallic acid was prepared in the range of 20-100 µg/ml. The total phenol content of the extract was calculated using the standard curve and expressed as gallic acid equivalent, (GAE) mg/100mg extract.

2.3.2. Determination of Tannins: About 0.1 ml of extract was added to all the test tubes and distilled water was added to make up to 7 ml. To this the reaction mixture, 1ml of (0.008M) potassium ferric cyanide and 1ml of (0.02M) ferric chloride in 0.1M HCL were added. Optical density (OD) was measured at 700 nm using Spectrophotometer. Tannic acid was used as standard. A calibration curve of tannic acid was prepared in the range of 20-100 µg/ ml. The total tannin content of the extract was calculated using the standard curve, and expressed as tannic acid equivalent, (TAE) mg/100mg extract.

2.3.3. Determination of Flavonoids: An aliquot (1ml) of extracts or standard solutions of rutin (20, 40, 60, 80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. About 0.3 ml of $NaNO_2$ (5% w/v) was added to the above mixture and and the tubes were incubated at room temperature for 5 min. Then, 0.3 ml $AlCl_3$ (10 % w/v) was added then 2 ml of 1M NaOH was added. The final reaction volume was made up to 10 ml with distilled water and the content was stirred thoroughly. Absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg rutin equivalents (RE).

2.4. *IN VITRO* ANTIOXIDANT ASSAYS

2.4.1. Free radical scavenging activity on DPPH: Diphenly picrylhydrazyl (DPPH) was used as free radical source to assess the radical scavenging capacity of the plant extracts. Various concentrations of plant extract (20, 40, 60, 80 and 100 µL) of the sample was taken in a test tube and made up to 3.0ml with 0.1mM methanolic DPPH. 100 µL methanolic solutions with DPPH were used as negative control. The tubes were incubated at 37˚C for 20 min and the absorbance was recorded at 517 nm using methanol as blank. Free radical scavenging activity of the samples was calculated using the following formula; DPPH radical scavenging activity (%) = $[(A_o - A_t)/A_o]*100$; Where A_o is absorbance of the control, A_t is absorbance of the sample extract (Ali and Raheleh, 2013).

2.4.2. ABTS cation scavenging capacity: ABTS cation radicals were produced by reacting 7 mM ABTS solution with 2.4 mM ammonium persulphate in dark for 12-16 h at room temperature. Prior to assay, the above solution was diluted with ethanol. Plant extract (100 µl) was added to test tube containing 900 µl of ABTS solution and vortexed for 10sec. The tubes were incubated at room temp for 6 min and OD at 734 nm was measured. The ABTS scavenging activity was calculated using the following formula; ABTS scavenging activity = [1- (Absorbance of test sample / Absorbance of control] *100. (Re et al., 1999).

2.4.3. Modified FRAP: Plant extract (0.1 ml) was added to 0.9 ml of ethanol (96% v/v) and made up to 5 ml distilled water and then 1.5 ml of HCl(1M), 1.5 ml of 1% potassium ferric cyanide,0.5 ml of 1% SDS, 0.5 ml of 0.2% ferric chloride were added in sequence. The tubes were incubated in water bath at 50˚C for 20 min. The tubes were rapidly cooled and OD was measured at 750nm. FRAP was calculated using ascorbic acid standard curve and expressed as AEAC mg /100mg extract.

2.4.4. Total Antioxident Assay -Phosphomolybdenum assay method: 100µL of extract was added to test tube containing 1ml of reagent solution (0.6mM sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Ascorbic acid Was prepared in 1mM dimethylsulphoxide was used as standard. The test tubes were was incubated in water bath at 95˚C for 90 min. and then OD was measured at 695nm. Instead of extract distilled water in reagent solution was used as blank. The result reported as milligram of ascorbic acid equivalent per 100 milligram of the extract obtained (Shirwaikar et al., 2006).

2.4.5. Metal chelating assay: Metal chelating capacity of the extract was estimated using ferrous as metal source. 100µL of the extract was added to 50 µL of the solution of 2mM $FeCl_2$. The contents were mixed thoroughly and incubated at room temperature for 10 min. OD was measured at 562nm against distilled water as blank. The metal chelating capacity of the extract was calculated using following formula: Metal chelating capacity (%) = [(Ao –At)/Ao]*100, Where Ao is absorbance of the control, A_t is absorbance of the sample extract. (Ali and Raheleh, 2013)

2.4.6. Hydroxyl Radical Scavenging Activity: The scavenging activity of Feronia *limonia* extracts on hydroxyl radical was measured according to thefollowing procedure Resat Apak et al. Initially, about 100microlitres of the extract sample was added to the reagent solution containing 1.5ml of phosphate buffer (pH-7.0), 0.5 ml of 10mM 2-Deoxy D-ribose, 0.25 ml of 20 mM EDTA disodium salt, 0.25 ml of 20 mM Ferrous Chloride solution.About 1.9ml of distilled water and 0.5ml of 10mM hydrogen Peroxide was rapidly added. The reaction was initiated by incubating the mixture for 4hr at 37°C in a water bath. The reaction was arrested by the addition of 2.5ml of 2.8% TCA. The reaction mixture was incubated at 100 ˚C in boiling water bath for 10 minutes after the addition of 2.5ml of 1% TBA. The absorbance was measured at 520nm.The reaction mixture without the sample was used as control and the blank was distilled water. The % hydroxyl Radical scavenging activity (HRSA) is calculated by the following formula: % HRSA = [(Ao – At)/Ao]*100, Where Ao is absorbance of the control, A_t is absorbance of the sample extract.

3. RESULTS AND DISCUSSION

In the present study, traditionally used medicinal plant *F.limonia* (wood apple) plant extracts were prepared from leaf, unripened fruit pulp and rind. Petarolium ether, chloroform and aqueous solvents were used in order to extract the plant metabolites that can be solubilized in these three different solvents. Soxhlet extract method is efficient technique to get all that metabolites because of repeated extract of the material with the solvent system. The yield percentage of the each extract is tabulated as dry weight of the plant parts (Table 1). Highest recovery of the extract was recorded in chloroform extract of pulp (10.19 %) followed by approximately equal recovery of aqueous leaf (8.71%) and rind (8.42%) extracts. Total phenolics content was high in petroleum ether extract of all three plant parts (petroleum ether-leaf: 56.2; petroleum ether-pulp 55.7;

petroleum ether-rind 56.2 (GAE mg/100mg extract)). Whereas, tannin content was found to be high in all of three parts aqueous extracts. Total flavonoid was more in chloroform extract of all three parts used (Table 1).

Table.1.Yield percentage of extract recovered from various solvents, total phenolics, tannins and Flavonoid content in *F.limonia* parts

Sample	Yield of crude Extract (%)	Total Phenolics(GAE mg/100mg extract) Mean±SD	Tannins (TAE mg/100mg extract) Mean±SD	Flavonoid(RE mg/100mg extract) Mean±SD
FPL	4.59	56.38±4.07	25±1.4	7.95±3.6
FPP	5.74	55.88±0.2	24.75±1.1	49.25±15.5
FPR	2.25	56.38±6.5	29.25±13.8	54.25±19.9
FCL	3.66	57.25±5.3	29.25±3.9	113.8±57.6
FCP	10.19	15±1.4	184.25±37.1	20.8±2.1
FCR	2.2	11.5±3.2	40±6.4	86.2±26.9
FWL	8.71	29.75±8.5	912.5±28.3	29.8±15.1
FWP	7.74	30.38±13.3	520.5±7.1	6.85±0.4
FWR	8.42	34±4.9	747.5±28.3	39.9±53.9

(FPL- petroleum ether leaf; FPP-Feronia petroleum ether pulp; FPR-Feronia petroleum ether rind, FCL-Feronia chloroform leaf, FCP- Feronia chloroform pulp, FCR-Feronia chloroform rind, FWL-Feronia water leaf, FWP-feronia water pulp, FWR-Feronia water rind)

3.1. Free radical scavenging activity on DPPH: DPPH is stable free radical which gives intense color to the solution which is reduced by the scavenging activity of the extract. The free radical scavenging activity assay showed noticeable scavenging power of various extracts (table 2). Aqueous extract of the leaf showed maximum DPPH scavenging activity followed by pulp and rind extracts. Petroleum and chloroform extracts of all three parts showed almost similar amount of scavenging activity. Free radical scavenging capacity of plant extract is attributed to hydrogen donating power of the phytochemicals present it (Philips, 2010).

Table.2.DPPH scavenging activities of petroleum ether, chloroform and aqueous extract leaf, Pulp and rind of *F.limonia*

Sample	DPPH radical scavenging activity (%)				
	Sample concentration µL				
	20	40	60	80	100
FPL	42.05	43.74	44.81	48.25	47.06
FPP	45.55	46.27	47.06	47.29	48.36
FPR	46.61	45.26	45.09	47.7	44.08
FCL	45.43	47.01	48.81	51.52	51.57
FCP	49.49	51.74	62.23	66.23	67.53
FCR	49.04	51.74	53.6	56.03	54.45
FWL	66.12	89.51	94.41	95.43	94.08
FWP	57.44	69.44	77.45	85.45	91.09
FWR	59.86	69.61	77.73	84.66	87.03

3.2. ABTS radical cation scavenging activity: The efficacy of various extracts to scavenge stable cation free radical ABTS is expressed in table 3. Petroleum ether extract of leaf showed maximum ABTS scavenging capacity (98.44%) chloroform extract of pulp and rind also showed same amount of radical scavenging capacity (98.44%).

Table 3 ABTS scavenging activities of petroleum ether, chloroform and aqueous extract leaf, Pulp and rind of *F.limonia*

Sample	ABTS scavenging activity (%)	Sample	ABTS scavenging activity (%)
FPL	98.39±1.2	**FCR**	98.39±0.7
FPP	58.31±2.3	**FWL**	90.93±1.0
FPR	64.68±2.8	**FWP**	96.79±0.2
FCL	98.39±0.5	**FWR**	76.77±4.6
FCP	99±0.2		

3.3. Metal Chelating Activity: Ferrous ion chelating capacity of various extracts is shown in Figure 1. Metal chelating capacity of the extracts increased with increasing concentration of the extracts. Aqueous extract of rind showed maximum metal chelating capacity (38.62%) followed by petroleum ether extract of rind (31.32%).

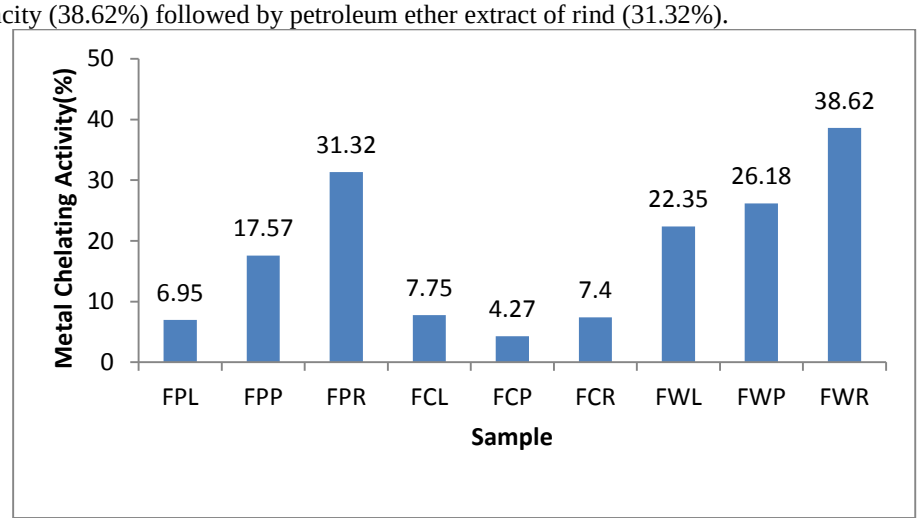

Figure.1.Metal chelating capacity of petroleum ether, chloroform and aqueous extract leaf, pulp And rind of *F.limonia*

3.4. Total antioxidant Activity: Total antioxidant activity (TAA) was estimated and expressed as ascorbic acid equivalent per mg of extract obtained in the respective solvents (Table 4). Aqueous extract of the rind showed highest AEAC value of 201.5 (mg AA/100mg extract) followed by chloroform extract of the leaf extract 102.75 (mg AA/100mg extract).

Table.4.Total Antioxidant activity (TAA) of petroleum ether, chloroform and aqueous extract Leaf, pulp and rind of *F.limonia*

Sample	AEAC(mg AA/100mg extract)	Sample	AEAC(mg AA/100mg extract)
FPL	16.25±13.0	**FCR**	70±9.6
FPP	4.75±5.3	**FWL**	304±82.7
FPR	25.25±4.1	**FWP**	223±10.2
FCL	102.75±19.5	**FWR**	201.5±13.0
FCP	89±4.6		

3.5. Hydroxyl radical Scavenging Assay: All the extracts showed significant level of hydroxyl radical scavenging capacity (figure 2). The percentage of hydroxyl radical scavenging activity increased in dose dependent manner. Petroleum ether extract of leaf part showed maximum activity (75.5%) followed by chloroform extract of pulp (71.14 %). Hydroxyl radicals are very active and they react with vital biological molecules such as DNA, protein which results in inactivation these molecules. Plant extract are shown to posess hydroxyl radical scavenging capacity (Rajamanikandan, 2011).

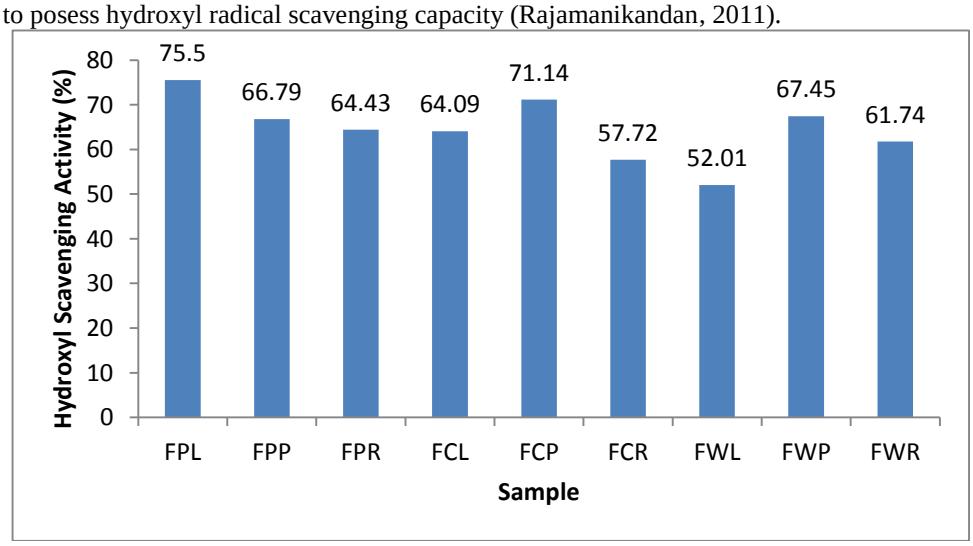

Figure.2.Hydroxyl radical scavenging activities of petroleum ether, chloroform and aqueous Extract leaf, pulp and rind of *F.limonia*

3.6. Modified FRAP: Ferric reducing/antioxidant power (FRAP) was estimated and expressed as ascorbic acid equivalent per mg of extract obtained in the respective solvents (Table 5).Aqueous extract of leaf showed highest AEAC value of 297.5(mg AA/100mg extract) followed by aqueous extract of rind extract 247.5 (mg AA/100mg extract).

Table.5.Ferric reducing/antioxidant power (FRAP) of petroleum ether, chloroform and aqueous Extract Leaf, pulp and rind of *F.limonia*

Sample	AEAC(mg AA/100mg extract)	Sample	AEAC(mg AA/100mg extract)
FPL	127.87±31.64	**FCR**	72.38±12.6
FPP	160±16.9	**FWL**	297.5±2.8
FPR	134.63±43.3	**FWP**	169.12±10.1
FCL	114.5±0.0	**FWR**	247.5±20.5
FCP	208.13±48.3		

CONCLUSION

Extracts of *F.limonia* plant parts are traditionally used to treat various diseases. Phytochemical screening revealed that aqueous extract of leaf, pulp and rind showed high amount of tannins. Chloroform extract of leaf and rind found to containg high quantity of flavonoids. Total antioxidant activity was more in aqueous extract of leaf compared to pulp and rind.

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