International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 2, Issue 4, 2010

Research Article

ANTIOXIDANT ACTIVITY AND ANTIMUTAGENIC EFFECT OF PHENOLIC COMPOUNDS IN *FERONIA LIMONIA* (L) SWINGLE FRUIT

RAMDAS PHAPALE AND SEEMA MISRA -THAKUR *

The Institute of Science, 15, Madame Cama Road Mumbai-400 032 India
Email: seema.prasoon@gmail.com, ramdas.phapale@piramal.com

Received: 09 April 2010, Revised and Accepted: 06 May 2010

ABSTRACT

Feronia limonia (L.) Swingle fruit is much used in India as a stomachic, diuretic, cardiotonic and tonic to the liver and lungs. In this study, free and bound phenolic compound extracts were obtained by successive extractions of *feronia limonia* ripe fruit pulp. Total phenolic content was evaluated following Folin–Ciocalteu method; antioxidant activity by the DPPH assay and antimutagenic potential by the Ames test. The phenolic glycoside extract presented higher (229.0 mg/g, GAE) total phenolic contents followed by phenolic ester (37.5 mg/g) and free phenolics (11.0mg/g). Where as the antioxidant activity was 88.7%, 11.8% and 3.8% respectively. Phenolic glycoside extract showed antioxidant activity higher than that of commercial antioxidant Trolox (64.6%) and Butylated hydroxytoluene (83.2%). At 2500 µl/plate a significant antimutagenic effect was shown by phenolic glycoside extract and the order of antimutagenic activity was found phenolic glycosides > phenolic esters > free phenolics. Results indicated good correlation between total phenol content, antioxidant activity and antimutagenic effect. Phenolic glycosides in *feronia limonia* ripe fruit pulp showed promising antioxidant activity, antimytagenic effect and needs further exploration for their effective use in both modern and traditional system of medicines.

Keywords: *Feronia limonia* (L.) Swingle, Fruits, Total phenolic content, Antioxidant Activity, Antimutagenic Effect

INTRODUCTION

Feronia Limonia (L.) Swingle (wood apple), belonging to the family Rutaceae and is widely distributed in most tropical and subtropical countries. The *feronia limonia* is native and common in India, Sri Lanka, China and Indonesia, where it is cultivated along roads and edges of fields and occasionally in orchards[1]. *Feronia limonia* fruit was known as a medicinal plant already in ancient Greek and Roman times and one of the most important plants of 'Ayurved', the traditional Indian medicine. In India, the fruit is used as a stomachic, diuretic, cardiotonic and tonic to the liver and lungs[2]. Some recent reports identified its use in gastrointestinal disorders[3].

During the last three decades, the antioxidant-based drugs/formulations for the prevention and treatment of some diseases like atherosclerosis, stroke, diabetes, alzheimers disease, and cancer have appeared[4]. This has attracted a great deal of research interest in natural antioxidants, and trend towards the use of natural phytochemicals specially phenolic compounds present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased.

Many research studies have shown that, diets rich in of fruits; vegetables and derived food products have health benefits against cardiovascular disease, chronic disease and certain types of cancer[5]. The principal agents responsible for these protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating compounds and reducing agent[6]. However, more recently the polyphenols have been investigated since they have been found to be beneficial as strong antioxidants and antimutagens[7]. Phenolic compounds are some of the most widespread molecules in nature and widely exist in fruits, nuts, grains and vegetables are reported to have multiple biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties[8]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in body defense systems[9]. Due to their health promoting properties, there has been renewed interest in studying and quantifying phenolic metabolites of fruits, nuts and vegetables.

However, as far as we know, no literatures on antioxidant activity and antimutagenic effect of *feronia limonia* fruit ripe pulp have been published. Thus, the objective of the present work was to extract the phenolic compounds present in *feronia limonia* fruit pulp and determine their total phenolic content, antioxidant activity and antimutagenic effect to evaluate their probable contribution as antioxidants and antimutagens, which could be eventually used as potential agents in phytomedicine.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent were obtained from Merck, Germany, DPPH [1,1-diphenyl-2-picrylhydrazyl], Butylated hydroxytoluene (BHT), Trolox were obtained from Sigma-Aldrich (Germany). Gallic acid monohydrate, Ascorbic acid and Sodium azide were obtained from S.D.Fine-chem (Mumbai, India). All solvents and other chemicals used for this study were of analytical reagent grade and obtained from Ranbaxy Fine Chemicals Ltd. (Mumbai, India). Molten soft agar was obtained from HiMedia (Mumbai, India) The Salmonella typhimurium strain TA100 were obtained from Trinova Biochem (Germany). Presently, it is being maintained in laboratory at the Primal Life Sciences, Mumbai, India.

Fruit samples, pre-treatment and storage

The fruits of *Feronia limonia* (L) Swingle were harvested from different trees of same origin during January 2008 in a rural area of Pune Distric Maharashtra, India. After harvesting, the fruits were identified and authenticated in the Department of Botany, The Institute of Science, Mumbai, India. All the healthy fruits of uniform size and appearance were tested for maturity. The mature fruits were kept in the sun for two weeks to fully ripen[10]. The outer wooden rind was broken with hammer and scooped out pulp were sieved through mesh to remove seeds and fibrous particles. Pulp homogenisded under nitrogen for 20 min and kept in an airtight container in a freezer (at -18°C) until further use.

Extraction

Phenolic compounds in ripe fruit pulp were extracted as methanol soluble free phenolics and bound phenolic compounds i.e. phenolic glycosides released after acid hydrolysis, and phenolic esters released after alkaline hydrolysis, as described by Matteo et al., b 2004, and Shela et al., 2004[11,12]with some modifications.

Extraction of free phenolics

In order to collect the free phenolic compounds, four gram of pulp was sonicated in 40 mL with a mixture of methanol and water (4:1, v/v), for 30 min. After centrifugation at 1000 rpm for 10 min, supernatant removed in separating funnel and extraction was repeated two more time. Combined supernatant was extracted twice with 50 mL hexane. Upper organic layer were discarded and lower aqueous layer was divided in to two equal parts and evaporated under reduced pressure at 40°C. One part of residue was reconstituted with 10 mL mixture of water and formic acid (99.7:0.3, v/v) and other was reconstituted with 10 mL DMSO for antimutagenic activity. The residual pulp, obtained after sonication and filtration, was divided into two equal parts and used for phenolic glycosides and phenolic esters phenolic compound extractions.

Extraction of phenolic glycosides

One part of residual pulp was digested with 100mL of 2 N Hydrochloric acid in methanol at 95°C for 60 min, After cooling the solution was filtered and the filtrate extracted twice with 50 mL diethyl ether: light petroleum ether (1:1, v/v). Upper organic layer discarded and the lower aqueous layer was extracted five times with 50 mL mixture of of diethyl ether and ethyl acetate (1:1, v/v). The combined organic layer was divided in to two equal parts and evaporated under reduced pressure at 40°C. One part of residue was reconstituted with 100 mL of 0.1% HCl in methanol and other was reconstituted with 5 mL DMSO for antimutagenic activity.

Extraction of phenolic esters

Second part of residual was digested with 100mL of 2M NaOH at room temperature and shaken under nitrogen gas for 20 h .The mixture was then acidified at pH 2–3 in 10M of hydrochloric acid in a cooling-ice bath and extracted with 100mL of hexane to remove lipids by a seperating funnel. The final aqueous layer was extracted five times with 50mL of diethyl ether–ethyl acetate (1:1, v/v) using seperating funnel. The combined organic layer was divided in to two equal parts and evaporated under reduced pressure at 40°C. One part of residue was reconstituted with 20 mL of water–formic acid (99.7:0.3, v/v) and other was reconstituted with 5 mL DMSO for antimutagenic activity.

Total phenolic contents

Total phenolic content of all three extracts were determined using Folin-Ciocalteu reagent method as described earlier by Miroslaw T et al., 2006 [13]. To 100 µl of each extract and gallic acid standard solutions (0.2,0.4,0.5,0.6 and 0.7 mg/mL) 2.5 mL of Folin-Ciocalteu reagent (1N) added and followed by 7.5 mL of 7.5% sodium carbonate solution was added and mixed well. After 2h incubation at room temperature, the absorbance was measured at 765 nm. The results were expressed as milligram of gallic acid equivalent (GAE) per gram of dry weight (DW).

DPPH radical scavenging activity

The scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) free radicals by three extracts and known antioxidant was performed as described by Farrukh A et al., 2006 with some minor modifications [4]. An aliquot of 200 µl of free phenolic (0.25-2.5 mg/ml), phenolic esters (0.07-0.7 mg/ml), phenolic glycosides (0.015-0.15 mg/ml), Trolox (0.02-0.20 mg/ml) and BHT (0.015-0.15 mg/ml) were mixed with 800 µl of 50 mM Tris-HCl buffer (pH 7.4), the mixture was then added to 1 mL of 500 µM DPPH in ethanol. Methanol (200 µl) only was used as the experimental control. The reaction mixtures were incubated for 30 min at at room temperature and the decrease in absorbance was measured at 517 nm. The percent inhibition was calculated from the following equation and results were expressed as "percentage inhibition" (%) of the DPPH on of dry weight.

% Inhibition = [(Abs. of control – Abs. of test sample)/ Abs. of control] x 100

EC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [14].

Antimutagenic effects

The Antimutagenic Effect of all extracts was studied using the tester strains of *Salmonella typhimurium* (TA100) through the standard plate incorporation Ames test as described by Negi PS et al., 2003 [15]. Each extract (625, 1250 and 2500 µg /plate) were assayed by plating with 2 ml molten soft agar containing 0.1 ml of 10 h old culture of *Salmonella typhimurium* (TA100) strains.

Sodium azide was used as a diagnostic mutagen (1.5 µg per plate) in the positive control and plates without test samples and without sodium azide was considered as negative controls. His+ revertants were counted after incubation of the plates at 37 °C for 48 h. The mutagenicity of sodium azide in the absence of test samples was defined as 100% inhibition. Each extract was assayed using triplicate plates. The % inhibition was calculated according the formula given below.

$$[\% \text{ Inhibition} =(1-T)/(M) * 100]$$

Where *T* is the number of revertants per plate in the presence of mutagen and the test sample and *M* is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and the denominator. The antimutagenic effect (% inhibation) between 25-40% was considered moderate and strong when more than 40%. The antimutagenic effect (% inhibation) less than 25% was considered as weak and was not recognised as positive result[16].

Statistical method

All experiments were performed in three replicates and results are reported as Mean ± SD.

RESULTS AND DISCUSSION

Phytochemicals, especially phenolics, in fruits, nuts and vegetables are suggested to be the major bioactive compounds for health benefits [7, 8]. This study was designed to screen the total phenolic content antioxidant activity and antimutagenic effect of free and bound forms phenolic made by applying solvent extraction and hydrolysis from *feronia limonia* ripe fruit pulp of Indian origin.

The total phenolic content was determined using *Folin-Ciocalteau* reagent to evaluate their correlation with antioxidant activity and antimutagenic effect. In present study, we observed that the residue obtained after free phenolic compound extract produced red colour upon treatment with heat and mineral acids. A red solution was insoluble in water, but soluble in amyl alcohol, is a characteristic of anthocyanidins. Anthocyanidins are produced from the acid hydrolysis of procyanidins and this would tend to confirm that glycoside bound phenolic compound show presence of anthocyanidins [17].

Total phenolic contents

Total phenolic content of all three extracts were determined using Folin-Ciocalteu reagent method, which is based on the complex formation of molybdenum-tungsten blue in alkaline solution[18].

The phenolic contents, expressed as mg gallic acid equivalents per gram of dry weight (mg/g GAE dw), were found to be the highest, i.e.229.0±0.6 mg/g in phenolic glycosides, after that 37.5 ± 0.5 mg/gm in phenolic esters and lowest 11.0 ± 0.6mg/gm in free phenolic extract. There was a remarkable difference between total phenolic content in free, phenolic esters and phenolic glycosides are showed in Fig.1.

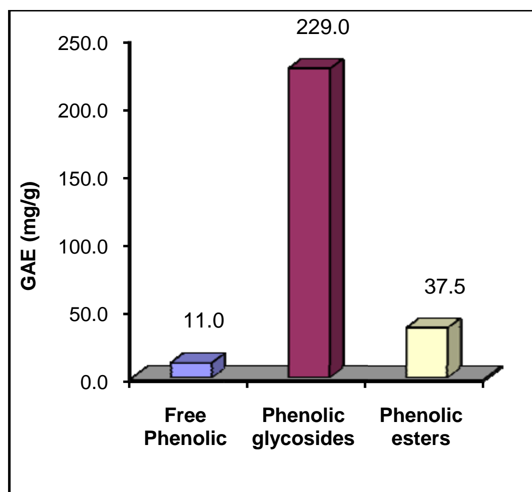

Fig. 1: Total phenolic content of fruit extracts

It was found that free and bound phenolic compound were present in fruit in the following fractions: free phenolics, methanol-soluble phenolic esters and, methanol-soluble phenolic glycosides[19]. The phenolic glycosides released after acid hydrolysis showed significantly higher total phenolic contents than the extractable phenolic contents in other free and ester form, indicating that the major phenolic compound in *feronnia limolnia* fruit pulp were not extractable by aqueous methanol but released upon acid hydrolysis. Our finding are in agreement with Shela G et al. (2004) [12] who have reported that phenolic content of Jaffa sweeties and white grapefruits occurred mostly in the bound form, and the bound form showed higher phenolic content than the free form. These studies indicate that ripe fruit pulp of *feronia limonia* have varying total phenolic contents in based on their free or bound from and the bound form phenolic glycosides released after acid hydrolysis showed higher total phenolic content than other ester and free form.

DPPH radical scavenging activity

DPPH free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid peroxidation. The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruit and vegetable juices or extract[20]. The percentage inhibition of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) solution was recorded maximum for phenolic glycosides (88.7±0.8%) followed by BHT (83.2±0.5%), Trolox (64.6 ± 1.2%), phenolic esters (11.8 ±0.7%) and free phenolics (3.8±0.09%) extracts at a concentration of 15 µg/ml. Fig.2 shows the comparison of DPPH % inhibition between all three extract and known antioxidant. Anthocyanidins in phenolic glycosides extract are responsible for their strong antioxidant activity and our findings are in agreement with other workers[20,21], who have reported that the fruits rich in anthocyanins have strong antioxidant activity. The dose-response curve for the free radical scavenging activity of studied extracts of phenolic glycosides, phenolic esters, free phenolics and standards at different concentrations are presented in Fig 3. The % inhibition of all extract and standards on the DPPH radical was found to be strongly dependent on concentration. Our results are accordance with Amin I, Tan SH (2002) [14]who have reported that, scavenging effects of seaweed water extract, vitamin E and BHT on the DPPH radical increased sharply with increasing concentration to a certain extent and then slowly increased. Fig.3.shows the comparison of the mean concentration for 50% free radical scavenging activity (EC_{50}) of phenolic glycosides, phenolic esters, free phenolics, BHT and Trolox against 250 µM DPPH radical. The EC_{50} of phenolic glycosides 0.03 ± 0.009 mg/ml, which is less than 0.06 ± 0.005 mg/ml of BHT and 0.11 ± 0.007 mg/ml of Trolox. A lower EC_{50} indicates a higher antioxidant activity [23]. The DPPH % inhibition in phenolic esters and free phenolics found to be very less so EC_{50} value of both was not calculated.

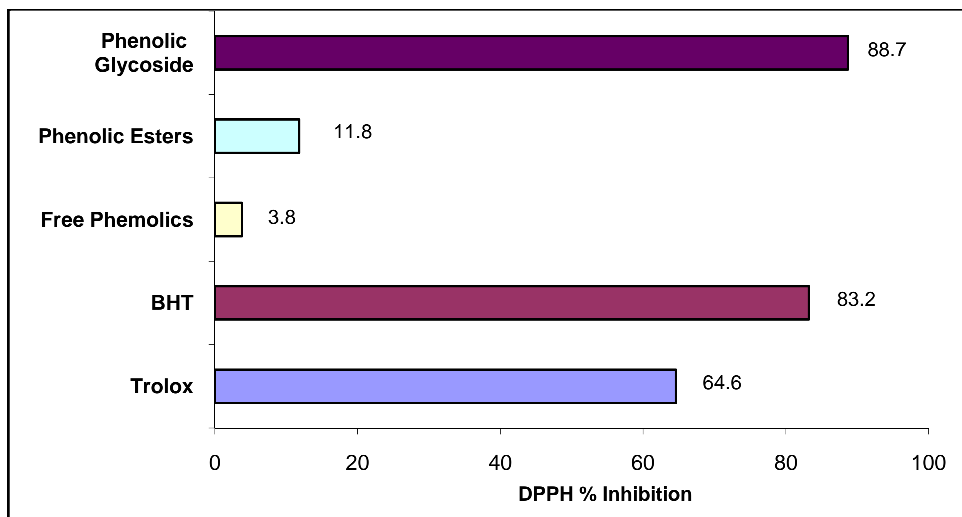

Fig. 2: Comparison of DPPH % inhibition between all three extract and known antioxidant

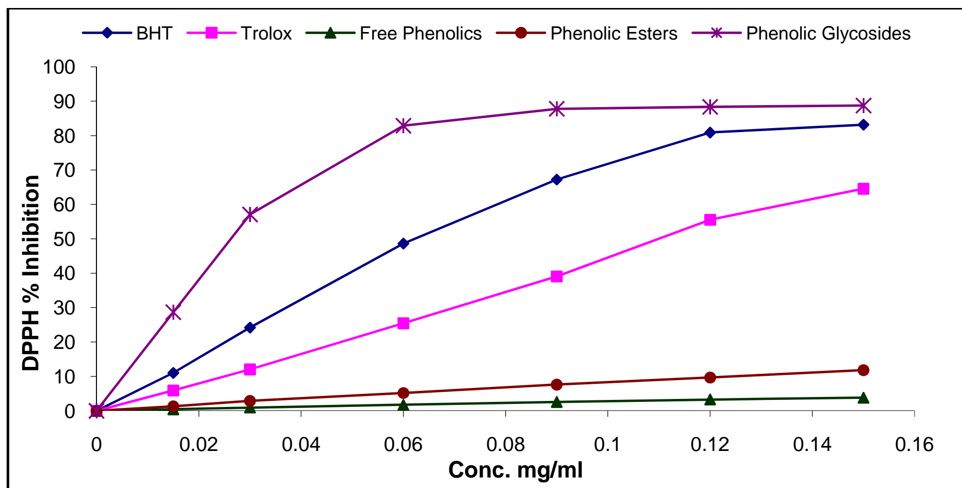

Fig. 3: Dose-response curve for the free radical scavenging activity of BHT, Trolox, free phenolics, phenolic esters and phenolic glycosides in *Feronia limonia* fruit pulp

Antimutagenic Effects

The protective action of free phenolic, phenolic esters and phenolic glycosides against the mutagenicity of sodium azide was evaluated by the Ames test using *S. typhimurium* TA100, as presented in Fig. 4. Free phenolic, phenolic esters and phenolic glycosides showed an antimutagenicity ranging from weak to strong, depending on concentration of the test samples.

A Significant antimutagenic effect (52.0 ± 1.3 %) was shown by phenolic glycosides at the dose of 2500 µg /plate followed by ester bound (12.6 ± 0.9 %) and free phenolic (3.1 ± 1.4 %)at same dose per plate against the mutagenicity of sodium azide. The order of antimutagenic effect is phenolic glycosides > Ester bound > free phenolic extract showed in Fig.2.

In this study, significant antimutagenic effect was shown by anthocyanidin released after acid hydrolysis of fruit pulp. Our work is accordance with Kazimierz Ggsiorowski et al., (1997) [23], who have reported that anthocyanins isolated from Aronia melanocarpa fruits exert a marked antioxidative influence and exhibit a strong antimutagenic activity against the standard mutagens action. The antimutagenic effect depends on the number and position of phenolic hydroxyl groups as can be seen in flavones. Blocking of hydroxyl groups by alkylation or acetylation decreases antimutagenic activities [24].

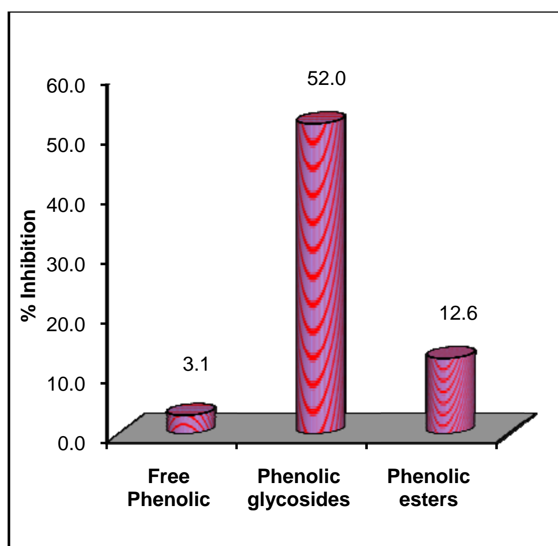

Fig. 4: Antimutagenic effect of fruit extracts

Several studies showed a correlation between phenolic content with antioxidant activity and antimutagenic effect[25]. Antioxidant activity of free phenolics, phenolic esters and phenolic glycoside extracts increased with an increasing amount of the extracts. The correlation coefficients between antioxidant activities, antimutagenic effect with total phenolic content of the free phenolic, phenolic ester, and phenolic glycosides are showed in Fig. 5a and 5b. The best correlation (R^2=0.998) observed between total phenolic content and antioxidant activities (DPPH % Inhibition) and a good correlation (R^2=0.994) observed between total phenolic content and antimutagenic effect. These data are in accordance with others, who have shown that high total polyphenol contents increases

antioxidant activity [12,26, 27] and there is a linear correlation between phenolic content and antioxidant activity. The correlations indicate that phenolic compounds were responsible for the antioxidant and antimutagenic effect exhibited in this study. High total phenolic content is generally regarded as an indication of high total antioxidant capacity and antimutagenic effect[28]. It has also been suggested that compounds, which possess antioxidant activity can inhibit mutation and cancer because they can scavenge free radical or induce antioxidative enzyme[29]. The results suggested that the anthocyanidins from phenolic glycosides contributed significantly to the antioxidant activity and antimutagenic effect of the *Feronia limonia* ripe fruit pulp. *Feronia limonia* ripe fruit can be considered an important source of phenolic compounds, which exhibit their antioxidant activity and antimutagenic effect. A bound form of phenolic glycosides in fruit pulp is supporting its potential use as an antioxidant and antimutagenic phytochemicals. However, further work is required to determine which anthocyanidins are responsible for antioxidant and antimutagenic effects.

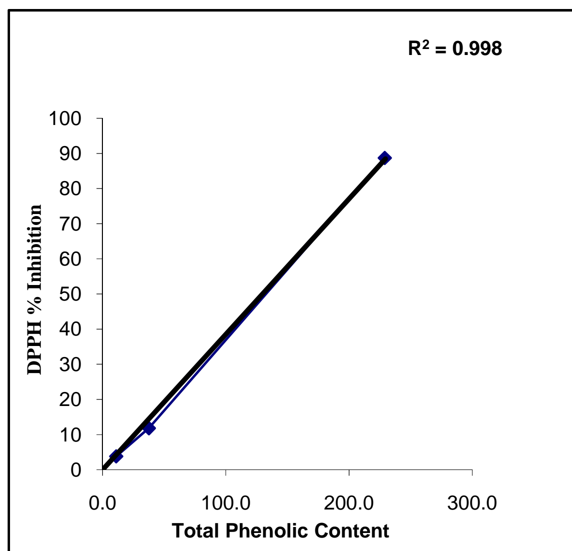

Fig. 5a: The correlation coefficients between total phenolic content and DPPH % Inhibition of free phenolic, phenolic ester, and phenolic glycosides.

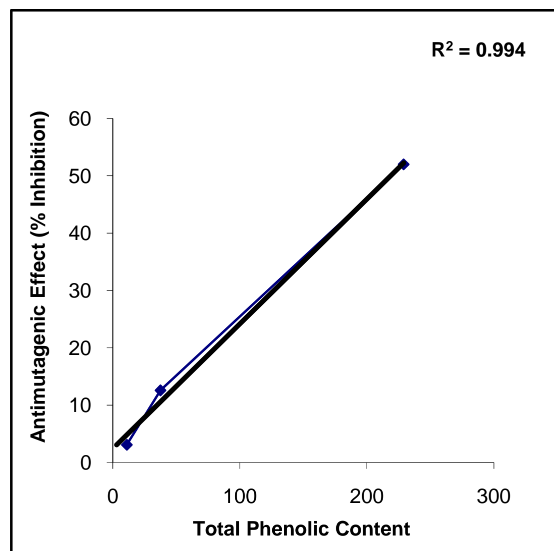

Fig. 5b: The correlation coefficients between total phenolic content and antimutagenic effect of free phenolic, phenolic ester, and phenolic glycosides.

ACKNOWLEDGEMENT

The authors are thankful to the Piramal Life Sciences, Mumbai for providing the necessary facilities to carry out Ames test of this research work. We are also thankful to 'The Institute of Science' Mumbai for its financial support.

REFERENCES

1. Gupta VC, Hussain SJ, Imam S. Important folk-medicinal plants and traditional knowledge of tribals of Aurangabad and Nasik forest divisions of Maharashtra, India. Hamdard Medicus.1997; 40:59-61.
2. Mukhlesur R. Alexander I. Gray. Antimicrobial constituents from the stem bark of Feronia limonia. Phytochemistry.2002; 51(1):73-77.
3. Saima Y, Das AK, Sarkar KK, SenSr AK, Sur P. An antitumor pectic polysaccharide from *Feronia limonia*. International Journal of Biological Macromolecules.2000; 27(5):333-335.
4. Farrukh A, Iqbal A, Zafar M. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants.Turk J Biol.2006; 30: 177-183.
5. Maron DM, Ames B N. Revised methods for Salmonella mutagenicity test. Mutat. Res. 1983; 113:173–215.
6. Ramazan M, Olcay D, Derya U, Elif K. Antioxidant and antimicrobial activities of extracts from tubers and leaves of Colchicum balansae Planchon. Journal of Medicinal Plants Research.2009; 3(10): 767-770.
7. Rocha-Guzman NE, Herzog A, Gonzalez-Laredo RF, Ibarra-Perez FJ, Zambrano-Galvan G, Gallegos-Infante JA. Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*). Food Chemistry.2007; 103:521-527.s
8. Guanghou S, Lai PL. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. Journal of Chromatography.2002; 977(A): 89-96.
9. Sahaa MR, Hasana SM, Aktera R, Hossaina MM, Alamb MS, Alam MA, et al., In vitro free radical scavenging activity of methanol extract of the leaves of mimusops elengi linn. Bangl. J. Vet. Med.2008; 6 (2): 197–202.
10. Julia F. Morton, Miami, FL: Wood-Apple. P. 190-191. In: Fruits of warm climates. 1987.
11. Matteo B, Emanuele M, Maria FC. Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flours.Journal of Chromatography.2004;1057(A):1-12.
12. Shela G, Milena C, Ivana M, Ratiporn H, Yong-Seo P, Soon-Teck J, et al., Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits Food Chemistry .2004; 84:503-510.
13. Miroslaw T, Krzysztof D, Danuta G,Bogdan S. HPLC determination of phenolic acids and antioxidant activity in concentrated peat extract-a natural immunomodulator.Journal of Pharmaceutical and Biomedical Analysis.2006; 41: 182-188.
14. Amin I, Tan SH. Antioxidant Activity of Selected Commercial Seaweeds. Mal. J. Nutr.2002; 8(2): 167-177.
15. Negi PS, Jayaprakasha GK, Jena BS. Antioxidant and antimutagenic activities of pomegranate peel extracts. Food Chemistry.2003; 80:393-397.
16. Ikken Y, Morales P, Martinez A, Marin ML, Haza AI, Cambero MI. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. Journal of Agricultural and Food Chemistry.1999; 47; 3257–3264.
17. Mohd F, Abu B, Maryati M, Asmah, R, Jeffrey F. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). Food Chemistry.2009; 113:479-483.
18. Chandrika M, Liyana-Pathirana, Fereidoon S. Importance of Insoluble-Bound Phenolics to Antioxidant Properties of Wheat. J Agric Food Chem.2006; 54:1256-1264.

19. Yanli Y, Xuewu D, Xiaoyi W, Xinguo S, Mouming Z, Jian S. et al., Identification of Major Phenolic Compounds of Chinese water chestnut and their Antioxidant Activity. Molecules. 2007;12:842-852.
20. Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ. Anthocyanin antioxidants from edible fruits. Food Chemistry.2004; 84:23–28.
21. Gracia-Alonso M, de Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo JC.Evaluation of antioxidant properties of fruits.Food Chemistry.2004;84:13-18.
22. Ljiljana S, Mihajlo S, Vesna N, Ljubisa N, Dusica R, Jasna C, et al., Antioxidant Activity and Total Phenolic and Flavonoid Contents of Hieracium pilosella L. Extracts. Sensors. 2009; 9:5702-5714.
23. Kazimierz G, Katarzyna S, Barbara B, Beata K, Magda J, Jan O.Antimutagenic activity of anthocyanins isolated from Aronia melanocarpa fruits.Cancer Letters. 1997;119:37-46.
24. Philip MS, Richard RM.Presence of proanthocyanidins in mutant green *Sarracenia* indicate blockage in late anthocyanin biosynthesis between leucocyanidin and pseudobase. Plant Science. 1998; 135:16-16.
25. Elvira G, de Mejia, Eduardo C, Guadalupe LP. Antimutagenic effects of natural phenolic compounds in beans.Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999; 441(1): 1-9.
26. Maryam z, Farrukh a, iqbal a. The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants.International journal of pharmacy and pharmaceutical sciences. 2009; 1:88-95.
27. Proteggente AR, Pannala AS, Paganga G, Van Buren L , Wagner E, Wiseman S, et al., The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radical Research 2002; 36:217-233.
28. Yean-Yean S, Philip JB. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry. 2007; 88: 411-417.
29. Hochstein, Atallah AS.The nature of oxidant and antioxidant system in the inhibition of mutation and cancer. Mut. Res. 1988; 202:363-375.