www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 3(11): 82-86

www.thepharmajournal.com
Received: 10-12-2014
Accepted: 24-12-2014

Nidhi Singh
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamilnadu, India.

Meenu G
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamilnadu, India.

Anamika Sekhar
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamilnadu, India.

Jayanthi Abraham
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

Correspondence:
Jayanthi Abraham
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

Evaluation of antimicrobial and anticancer properties of finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) extracts

Nidhi Singh, Meenu G, Anamika Sekhar and Jayanthi Abraham

Abstract
Phenolic acids from finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum)* were isolated as fractions in an organic solvent and their anticancer and antimicrobial properties were evaluated. Antimicrobial properties were studied using Kirby Bauer well diffusion technique and the polyphenols of millet showed antimicrobial activity on *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Shigella dysenteriae, Enterococcus* sp. *and Salmonella* sp. GC-MS analysis was done to confirm the compounds present in the extract. The cytotoxicity assay was done to screen for the anticancer activity against HepG2 hepatic cancer cell lines. Further, *in silico* studies were performed using AutoDock 4.0 to validate the antimicrobial results obtained. From the observation, it could be inferred that millet polyphenols of millets could be used as a natural source of antimicrobials and antioxidants, especially for minimizing the risk of diseases arising from oxidative deterioration and also cytotoxic effects.

Keywords: polyphenols, pathogens, GC-MS, HepG2, *in silico*.

1. Introduction
Millets are a group of cereal crops grown worldwide for food and staples, in harsh environments environments such as those at risk of drought [1]. The millets include species in several genera, mostly in the sub-family Panicoideae, of the grass family Poaceae. Pearl millet, commonly known as 'bajra' is a food for poor people. It is one of the four most important cereals (rice, maize, sorghum and millets) grown in the tropics and is rich in iron and zinc, contains high amount of antioxidants and these nutrients may be beneficial for the overall health and wellbeing. The grains are known to contain about 12 % proteins, 5 % ether extractives (including fats) and 67 % carbohydrates. It is known to be a rich source of minerals and vitamins of the B group[2]. It has been already studied extensively for its carbohydrates, proteins, vitamins and minerals, thus now more attention is given to studies related to minor compounds like flavonoids, phenolics, phospholipids and fatty acids. Daniel *et al*. [3] revealed the presence of 0.9 % dry wt. of flavones, 0.75 % phospholipids and 5.4 % fatty acids with phenols being 4.08 µg/g. Finger millet is also called as 'ragi' which is consumed without dehulling is also a principle food grain of people belonging to low income groups. It is rich in polyphenols and tannins and also reported as a good source of antioxidants. Phenolics are the most abundant secondary metabolic products of plants. Plant polyphenols are an extensively used in cancer research due to their potent antioxidant properties and have been implicated for disease resistance in various researches. The presence of phenolic acids in cereal grains has been confirmed in several studies [4, 5]. The polyphenolic content in cereals is usually less than 1% of dry matter and the main polyphenols in cereals are phenolic acids and tannins [6, 7] whereas flavonoids are present in small quantities. Studies are reported with respect to the contents of phenolic acids and tannins in different varieties of ragi [8, 9]. Pearl millet (*Pennisetum glaucum*) is another rich variant of polyphenols and one of the most widely cultivated millet in India. Polyphenols are the most important phytochemicals of the millet because of their nutraceutical potentials such as antioxidant activity, anti-inflammatory, anti-carcinogenic, antimicrobial, antidiarrhoeal, antiulcer, and anti-cardiovascular properties. Besides, polyphenols are also useful in the management of several physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, prevention of oxidation of low-density lipoproteins (LDLs) and maintaining health of the gastrointestinal tract.

The present study has focused on the antibacterial and anticancer effects of polyphenols extracted from the milled fractions of finger and pearl millets.

2. Materials and methods

2.1 Sample Collection and processing

Pearl millet (*Pennisetum glaucum*) and Ragi (*Eleusine coracana*) seeds were procured from the local market, washed to remove foreign particles and dust before further treatments. These were further allowed to germinate and then dried under the sun for 3 days, powdered and stored in air tight containers for further use.

2.2 Preparation of extracts

Soxhlet apparatus was used for the purpose of solvent extraction and care was taken not to exceed the boiling point of the solvent. Pearl millet and finger millet were extracted with hexane followed by ethyl acetate. The solvent extracts were vacuum dried and stored at 4 ^{0}C for further analysis. The extracts were filtered through Whatman Filter Paper No.1 and then concentrated under reduced atmospheric pressure. The extracts were isolated and then dissolved in hexane and ethyl acetate preparing 3 samples, that is, ragi in hexane, ragi in ethyl acetate and pearl millet in hexane and were stored in -20 ^{0}C for further use.

2.3 Test organisms

The bacteria used as test organisms in this study were *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Shigella dysenteriae, Enterococcus* sp. and *Salmonella* sp. All the clinical isolates were acquired from Microbial Biotechnology Laboratory, VIT University, Vellore, Tamil Nadu, India. Bacterial clinical isolates were maintained on Nutrient agar.

2.4 Antimicrobial study

Antibacterial activity was determined by the well diffusion method of Kirby Bauer. The Muller-Hinton agar media were prepared and the sterilized media were poured into the sterile Petri plates. The spread plate method was followed using cotton swabs with 9 different isolates of bacteria. After the media were cooled and solidified 9 mm diameter wells were punctured in the agar and 25 µl, 50 µl, 75 µl and 100 µl of 25 mg/ml of each sample were loaded in the wells. The plates were incubated for 24 h at 37 ^{0}C. After incubation, the zone of inhibition was observed and the diameter was measured.

2.5 Antioxidant assay

DPPH assay was performed to calculate the total antioxidants present in the extracts. To perform this assay, ascorbic acid (10 mg/ml) was used as the standard and 10 mg/ml of extracts were used for the assay. 100 µl of 0.1 mM DPPH (bought from Sigma Aldrich), 100 µl of extract and 1800 µl of methanol was added to the reaction mixture. This mixture was incubated in dark for 30 min and absorbance was read at 517 nm which is standard for DPPH [10],

Percent inhibition or percent activity was calculated based on the following formula:

$$\% \text{ Scavenging activity} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

2.6 Gas Chromatography Mass Spectrometric Assay

The obtained extracts were analyzed for gas-chromatography mass spectrometry to identify the number of compounds and molecular weight of the compounds. GC-MS analysis was carried out on a GC Clarus 600 model and an Elite-5MS (30.0 m, 0.25 mm ID, 250 µm df column). The total run time was 30 min and helium was used as the carrier gas with a gradient temperature schedule of 60 ^{0}C for first 2 min followed by an increase of 10 ^{0}C to 300 ^{0}C with a hold time of 4 min. The injector temperature was kept at 300 ^{0}C and the mode of operation of the ion trap was the electron impact mode with a scan range of m/z from 50 to 600. A further sample was loaded using the injector and the separation was obtained in the form of a chromatogram. Each peak was searched in the Nist library data for similar results for identification of the compounds obtained.

2.7 *In vitro* cytotoxicity assay

The human hepatic cancer cell lines (HepG2) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37 ^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. To check the anticancer activity against hepatic cancer cell lines MTT assay was performed [11, 12]. The cells were placed into 96 well microtitre plated and incubated for a period of 48 h at 37 ^{0}C. After incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ^{0}C for 4h. The medium with MTT was then removed and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ Cell inhibition} = 100 - \frac{\text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

Nonlinear regression graph was plotted between percentage of cell inhibition and Log concentration and IC_{50} was determined using GraphPad Prism software.

2.8 Molecular Docking studies

Docking studies are computational techniques for the exploration of the possible binding modes of a ligand to a given receptor, enzyme or other binding site[13]. In this study, we used AutoDock 4.0 to evaluate the binding energy of ligands inside the known 3D structure of the target enzyme. AutoDock 4.0 software consists of two main programs, autogrid that pre-calculates grid maps of interaction energies for various atom types of ligand with a macromolecule and autodock, which performs the docking of the ligand to specified grids [14]. Considering the well obtained *in vitro* results, it was worthwhile to go for docking to study the binding inside the active site of topoisomerases, potential target for antibacterial agents and support the *in vitro* results.

During docking the grid parameters were 60×60×60 Å with points separated by 0.375 Å. Also the X, Y and Z coordinated were specified as -32.137, 5.574, 4.657 for *Pseudomonas aeruginosa* topoisomerases. While, *Klebsiella pneumoniae* topoisomerase was found at coordinates -25.767, 9.145, -4.632 being X, Y and Z following the grid parameters 60×60×60 Å separated by 0.375 Å.

3. Results and discussion

The ragi and pearl millet seeds when allowed to germinate produces sprouts, which contain large quantity of proteins and

among them some are biologically active for e.g., lectins, ribosome inactivating proteins, enzyme inhibitors, arcelins, chitinases and canatoxin 11. They are majorly known as enzyme inhibitors compromising both amylase and proteinase inhibitors. The present work employs the extraction of phytochemical compounds of sprouted ragi and pearl millet seeds through various solvents and the biological activities of obtaining extracts is studied. The ethyl acetate and hexane extracts obtained after Soxhlet extraction were stored at 4 °C for further biological activities. The extract of ragi in ethyl acetate inhibited all the pathogenic microorganisms except *E. coli*. The maximum zone of inhibition was manifested against *Pseudomonas aeruginosa* (22 mm) and *Klebsiella pneumoniae* (17 mm) as shown in table 1.

Table 1: Antimicrobial activity of the extracts.

Pathogen	Ethyl acetate (ragi)				Hexane (ragi)				Hexane (pearl millet)			
	25	50	75	100	25	50	75	100	25	50	75	100
***Enterococcus* sp.**	-	-	-	17	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	1	14	14	22	-	-	-	-	-	-	-	-
S. aureus	-	-	11	14	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	13	-	-	-	-
Proteus mirabilis	-	-	14	15	-	-	-	-	-	-	-	-
Shigella dysenteriae	-	-	-	14	-	-	-	-	-	-	-	-
***Salmonella* sp.**	-	-	1.2	16	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	14	17	-	-	-	-	-	-	-	-
Serratia marcescens	-	8	11	13	-	-	-	-	-	-	-	-

*diameter of the zones measured in mm.

3.1 GC-MS analysis

The chromatogram obtained after the mass spectral analysis depicted the presence of peaks relative to compounds present in the extracts. Since the ethyl acetate extract of ragi grains showed promising antimicrobial results, GC-MS analysis of the extract was performed to obtain chromatogram as depicted in figure 1. Among the compounds present 1,2 benzenedicarboxylic acid (BDC) was most prominent and was tested for its antibacterial activity *in silico* using molecular docking techniques.

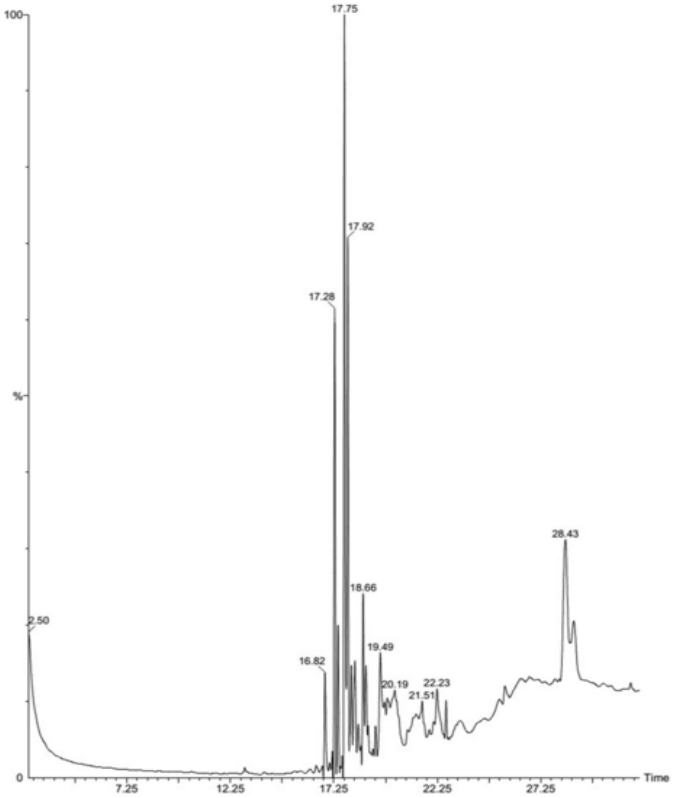

Fig 1: GC-MS chromatogram of ragi grain ethyl acetate extract.

3.2 Antioxidant assay

The effective ragi ethyl acetate extract was tested for antioxidant property based on DPPH radical scavenging assay resulted in an antioxidant potential of 54%. Ascorbic acid was taken as the standard and compared with the extract, which confirmed the effective antioxidant potential. The high antioxidant content could be a contribution to polyphenols present in the extract [15, 16]. There have been some reports on free radical quenching action of finger millet [17] and other plant sources like sage [18], rice hulls [19], china seeds [20], etc. This suggests that the extract may also have potent anticancer properties, thus further cytotoxicity assay was done.

3.3 Cytotoxicity assay

HepG2 liver cancer cell lines were chosen for the study and the extract was administered in different concentrations. The results suggested a dose dependent growth of anticancer, with an increase in the concentration of the extracts there was a mild decrease. Zhang *et al* [21] has reported antiproliferative activity of proso millet against human breast cancer and HepG2 liver cancer cell lines. Furthermore, with better purification and concentration the extracts could be used in cancer therapy. Figure 2 shows the graph plot of cell viability of cancer cells against the increasing concentration of the extracts.

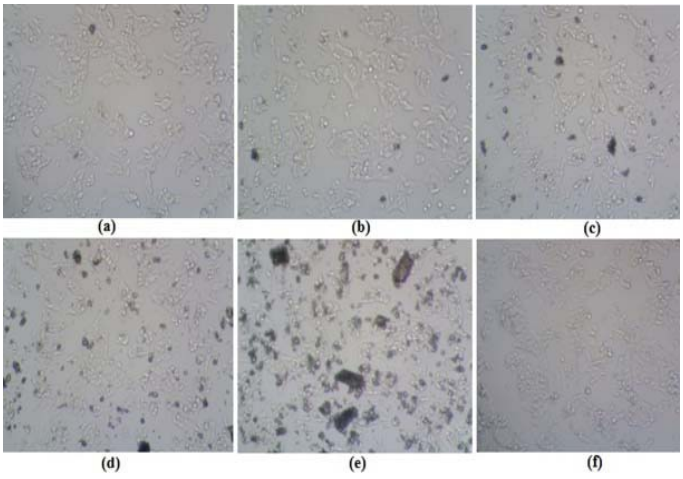

Fig 2: Cytotoxicity of ragi (ethyl acetate) extract against HepG2 cell lines.

3.4 Molecular docking studies

To validate the *in vitro* results of antimicrobial analysis, molecular docking studies were done, which were in accordance with the zone of inhibition results. The binding energies stated the binding efficiency of the ligand with the target topoisomerases to be strong and efficient.

In silico results revealed that the ligand showed the minimum binding energy of -6.32 and -6.94 Kcal/mol, due to dipole-dipole and hydrogen bond interaction, with amino acids of topoisomerases of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively. The docked result of ligand with secondary structure of topoisomerase of *P. aeruginosa* is given in figure 3a and the hydrogen bond interactions are found with LEU at position 264. Furthermore, 2D plot of hydrogen bond forming amino acids with target ligand is depicted in figure 3b. *K. pneumonia* topoisomerase docking (Figure 4a) with ligand showed interactions with HIS 259. Also 2D plot is shown in figure 4b representing the hydrogen bond formation with specific bond distances.

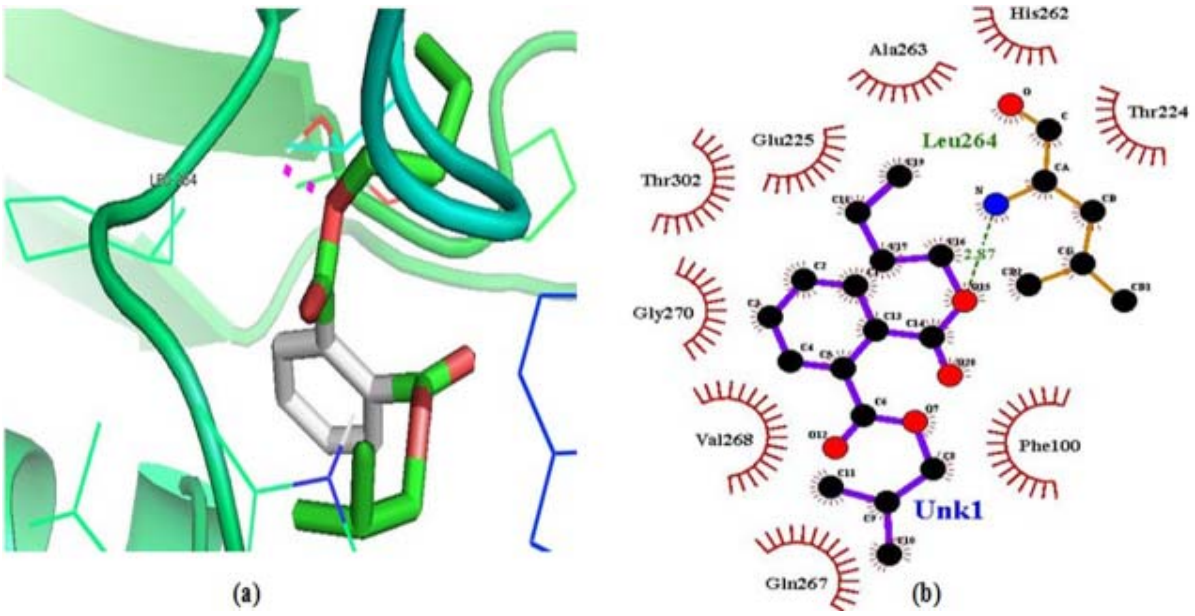

Fig 3: (a) 3D complex of *Pseudomonas aeruginosa* topoisomerase and ligand interactions. (b) 2D Ligplot showing the bond length and hydrogen bonds.

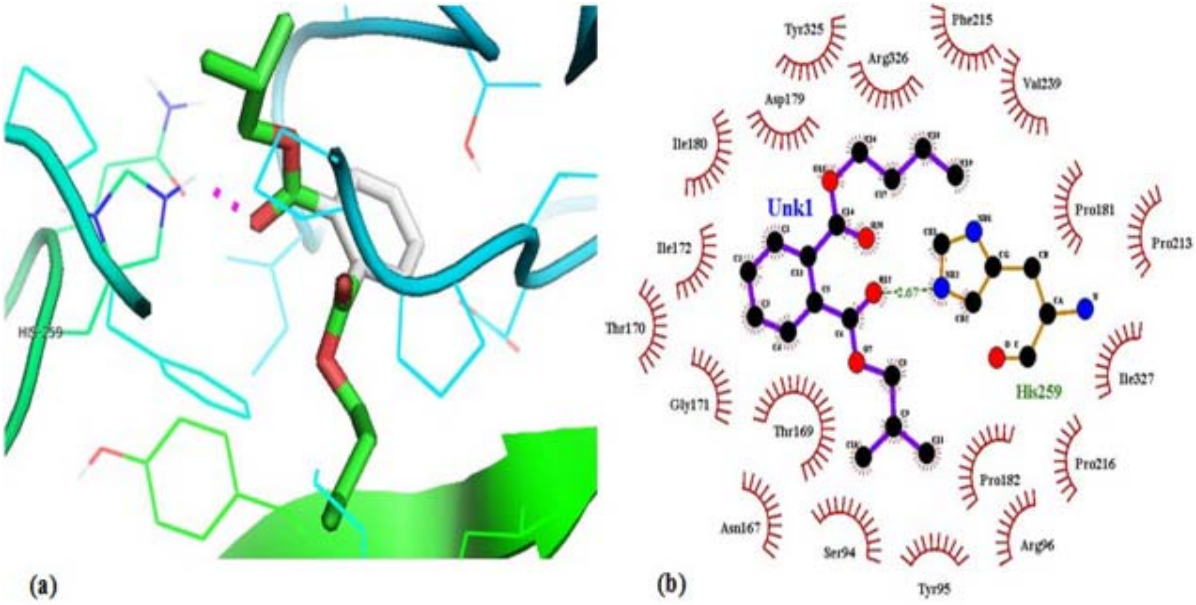

Fig 4: (a) 3D complex of *Klebsiella pneumoniae* topoisomerase and ligand interactions. (b) 2D Ligplot showing the bond length and hydrogen bonds.

4. Conclusion

The present work elaborates the antimicrobial, antioxidant and anticancer potential of two varieties of millets. Also the molecular docking studies give supporting information with regard to the antimicrobial effect obtained in the process suggesting that there could be a good scope of future perspectives to be worked upon.

5. References

1. Lu H, Zhang J, Liu KB, Wu N, Li Y, Zhou K, *et al*. Earliest Domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. Proceedings of the National Academy of the United States of America. 2009; 106:7367-7372.
2. Léder I. Sorghum and millets, Cultivated plants, Primarily as Food Sources, (Ed. G. Fuleky)

Encylopedia of Life Support System. EOLSS Publishers, Oxford. 2004.
3. Daniel M, Denni M, Chauhan D. Polyphenols, phospholipids and fixed oil composition of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Int J Pharma Life Sci 2012; 3:2098-2102.
4. McKeehen JD, Busch RH, Fulcher RG. Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to fusarium resistance. J Agr Food Chem 1999; 47:1476-1482.
5. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Vitic 1977; 28:49-55.
6. Deshpande SS, Cheryan M, Salunke DK. Tannin analysis of food products. Crit Rev Food Sci Nutr 1984; 24:401-449.
7. Towo EE, Svanberg U, Ndossi GD. Effect of grain pre-treatment on different extractable phenolic groups in cereals and legumes commonly consumed in Tanzia. J Sci Food Agr 2003; 83:980-986.
8. Mbithi-Mwikya S, Van Camp J, Yiru Y. Huyghebaert Nutrient and antinutrient changes in finger millet (*E. coracana*). LWT-Food Sci Technol 2000; 33:9-14.
9. Subba Rao MVSST, Muralikrishna G. Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (ragi, *E. coracana*, Indaf-15). Food Chem 2001; 72:187-192.
10. Sreeramulu D, Reddy CV, Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. Ind J Biochem Biophys 2009; 46:112-5.
11. Liu M, Li XQ, Weber C, Lee CY, Brown J, Liu RH. Antioxidant and antiproliferative activities of raspberries. J Agric Food Chem 2002; 50:2926-2930.
12. Sun J, Liu RH. Apple Phytochemical Extracts Inhibit Proliferation of Estrogen-Dependent and Estrogen-Independent Human Breast Cancer Cells through Cell Cycle Modulation. J Agric Food Chem 2008; 56:11661-11667.
13. Tomi IHR, Al-daraji AHR, Abdula AM, Al-Marjani MF. Synthesis, antimicrobial and docking study of three novel 2,4,5-triarylimidazole derivatives. J Saudi Chem Soc; In Press, 2013.
14. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, *et al*. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998; 19:1639-1662.
15. Subba Rao MVSST, Muralikrishna G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (ragi, *E. coracana* Indaf-15). J Agr Food Chem 2002; 50:889-892.
16. Barros L, Joao Ferreira M, Queiros B, Ferreira ICFR, Baptista P. Total phenol, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushroom and their antioxidant activities. Food Chem 2007; 103:413-419.
17. Sripriya G, Chandrashekharan K, Murty VS, Chandra TS. ESR spectroscopic studies on free radical quenching action of finger millet (*E. coracana*). Food Chem 1996; 57:537-540.
18. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desfex DC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. J. Agr. Food Chem. 2000; 48(10):5030-5034.
19. Ramarathnam N, Osawa T, Namikii M, Kawakishi S. Chemical studies on novel rice hulls antioxidants. J. Agr. Food Chem. 1989; 37:316-319.
20. Taga MS, Miller EE, Pratt DE. China seed as source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 1984; 61:928-931.
21. Zhang L, Liu R, Nio W. Phytochemical and antiproliferative activity of Porso millet. PLoS ONE 2014; 9(8):e104058. doi:10.1371/journal.pone.0104058.