Cell. Mol. Biol. 58 (1): 174-181
Published on December 22, 2012
DOI 10.1170/T938

Cellular & Molecular Biology
http://www.cellmolbiol.com
Copyright © 2012. All rights reserved.

PROTECTIVE EFFICACY OF *Solanum xanthocarpum* ROOT EXTRACTS AGAINST FREE RADICAL DAMAGE: PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT EFFECT

S. KUMAR, U. K. SHARMA, A. K. SHARMA AND A. K. PANDEY ✉

Department of Biochemistry, University of Allahabad, Allahabad- 211002, India

Abstract

Free radicals have been implicated in many diseases. They attack biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid peroxidation. Present study reports the phytochemical analysis as well as free radical scavenging and antioxidant activities of *Solanum xanthocarpum* root extracts. Tannins, flavonoids, terpenoids, alkaloids, saponins and steroids were present in different extracts. Total flavonoid content in extracts was quantified and maximum contents were found in ethyl acetate fraction followed by chloroform and ethyl alcohol fractions, respectively. Dose dependent response was observed in metal ion chelating activity of extracts. Comparatively better chelating activity was found in polar extracts. Most of the extracts exhibited significant free radical scavenging activity in DPPH radical scavenging assay. Ethanolic and aqueous extracts accounted for about 40-50% lipid peroxidation inhibition (LPOI) in rat liver homogenate. Antioxidant activity did not show direct correlation with the amount of flavonoid contents in the extracts. However, direct correlation was observed between DPPH free radical scavenging activity and LPOI. Antioxidant activity of the extracts was compared with standard antioxidants. The differential activity observed in extracts could be attributed to the presence of other phytochemicals such as tannins and terpenoids in addition to flavonoids. The study demonstrated appreciable protective efficacy in *S. xanthocarpum* root extracts against free radical damage.

Key words: Antioxidant, DPPH, extracts, free radicals, LPOI, phytochemical analysis, *Solanum xanthocarpum*.

Article information

Received on May 14, 2012
Accepted on July 24, 2012

✉ **Corresponding author**
Tel: +91 9839521138
Fax: +91-532-2461157
E-mail:akpandey23@rediffmail.com

INTRODUCTION

Medicinal plants contain numerous phytochemicals with immense therapeutic value and are considered to be natural and safe in comparison with synthetic drugs (33, 47). Microbial pathogenesis is very much needed to control because many infectious microorganisms are resistant to synthetic drugs. Plants have long been used as an alternative source for medicines and remedy for treating human diseases (31, 37, 40). The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs (22).

Reactive oxygen species (superoxide anion, hydroxyl radicals and hydrogen peroxide, etc) including free radicals are generated by normal physiological processes and various exogenous factors (11). Exogenous sources of reactive oxygen species (ROS) include tobacco smoke, certain pollutants, organic solvents, and pesticides (9, 18, 42). Excessive concentrations of ROS in the human body may be involved in a number of pathological events (2, 26, 29, 49). Lipid peroxidation is a process that produces many pathological events in the cells and organs of man (11, 19, 36, 46). This process causes damage to unsaturated fatty acids, which results in decreased membrane fluidity and leads to many other pathological events.

Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers (30, 50). Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity (22, 51). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging and anti-inflammatory actions (14). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (16).

Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) commonly known as Yellow Berried Nightshade (syn: kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2–3m height found through out India, mostly in dry places as a weed on roadsides and waste lands (1). The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions (6). The whole plant is used for medicinal purpose including fruits and roots (17). Jigrine, a polypharmaceutical herbal formulation, used for treatment of liver ailments contains aqueous extracts of some medicinal plants and *S. xanthocarpum* root is one of them (34).

A literature survey did not reveal any reference to previous work describing the antioxidant activity of *S. xanthocarpum* root. Most of the studies in the literature are focused on *S. xanthocarpum* fruit, because of its well-known and widely documented biochemical properties. Therefore, the present study was aimed at investigating phytochemical constituents, antioxidant effects and the anti-lipid peroxidation activities of *S. xanthocarpum* root extracts.

MATERIALS AND METHODS

Chemicals

2, 2-diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid, ferrozine, propyl gallate , butylated hydroxyl anisole (BHA), tert-butyl-4-hydroxy toluene (BHT), quercetin, Potassium ferricyanide were purchased from Himedia (Mumbai, India). Trichloroacetic acid (TCA), dimethyl

sulfoxide (DMSO) and methanol were procured from Merck (India). The remaining chemicals and solvents used were of standard analytical grade.

Plant Material

S. xanthocarpum roots were collected in the April 2010 from Amelia, Allahabad, Uttar Pradesh, India. The roots were shade-dried at room temperature for 10-15 days. Dried roots were crushed and ground into fine powder with mortar and pestle.

Preparation of Extracts

Fifty gram powdered sample was sequentially extracted with hexane (HX), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC),ethyl alcohol (ET) and water (AQ) in Soxhlet apparatus for 8 h (32, 38). The respective extracts were centrifuged and filtered. Solvent was removed completely under reduced pressure. The dried residues were dissolved in DMSO for determination of antioxidant and anti-lipid peroxidation activities of extracts.

Phytochemical screening

Chemical tests were carried out on different solvent extracts of *S. xanthocarpum* root using standard procedures to identify the constituents (21, 32, 48).

Anthraquinone

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube followed by addition of 1 ml of dilute ammonia. The resulting solution was observed for color changes to violet indicating presence of anthraquinones.

Flavonoids

A few drops of 1% aluminum chloride solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids.

Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. Two ml dilute ammonia was added to 5 ml of the filtrate. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Phlobatannins

The extracts of root sample were boiled with 1% hydrochloric acid. Deposition of a red precipitate was taken as evidence for the presence of phlobatanins.

Reducing sugars (Fehling's test)

The extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a red coloration/ precipitate indicating presence of reducing sugars.

Terpenoids (Salkowski test)

Two ml chloroform was added to 0.5 g of the extract. Then 3 ml concentrated H_2S0_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Cardiac glycosides (Keller-Killiani test)

0.5 g of extract was dissolved in 5 ml water. Two ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Quantitative determination total flavonoid content

Aluminum chloride colorimetric method (5) as modified by us was used for determination of flavonoids in various extract fractions of root. Small amount (0.2 ml) of extract in pure DMSO (2 mg/ml) was separately mixed with 1.8 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. Tubes were incubated at room temperature for 30 min and then absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared with quercetin solution (1 mg/ml in methanol). Different volumes containing 20-200 µg quercetin were taken in different tubes and volume was raised to 1.8 ml with methanol followed by addition of 0.2 ml DMSO. Rest of the procedure was same as described above. The amount of flavonoids in the test samples was expressed as µg quercetin equivalent/mg sample (µg QE/mg). Experiments were performed in triplicate and the results were expressed as mean ± SEM.

Antioxidant activity measurement

Metal ion chelating activity

The chelation of ferrous ions by the *S. xanthocarpum* root extracts was estimated by the method of Dinis *et al* (10) as modified by us. Modification included dissolution of extracts in DMSO instead of methanol. Briefly, the extract samples (200 µl) of different concentrations were added to a solution of 2 mM/L ferric chloride (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by the formula given below.

Metal ion chelating ability (% Inhibition of ferrozine–Fe^{2+} complex formation) = $[(A_0 - A_1)/A_0] \times 100$

Where A_0 is the absorbance of control and A_1 is absorbance in the presence of the sample/standard *compounds*. The results were expressed as mean ± SD of three replicates.

DPPH radical scavenging activity

The free radical scavenging activity of the extract fractions was measured *in vitro* by DPPH assay (44) as modified by us (30). DMSO was used as solvent for dissolving extracts instead of methanol. Three milliliters of 0.1 mM DPPH solution prepared in methanol was added to 1 ml of the test extracts (500-2000 µg/ml) dissolved in DMSO. The content was mixed and allowed to stand at room temperature for 30 min in dark. The reduction of DPPH free radical was measured by recording the absorbance at 517 nm. The percentage scavenging activities (% Inhibition) at different concentrations of the extracts fractions were calculated using the following formula.

$(\%)\ I = [(A_C - A_S) / A_C] \times 100$

where I is inhibition, A_C and A_S are the absorbance values of the control and the sample respectively. Three replicates were made for each sample and results were expressed as mean ± SD.

Lipid peroxidation Inhibition (LPOI/TBA) assay

The lipid peroxidation inhibition by the *S. xanthocarpum* root extracts was measured by the method of Halliwell and Gutteridge (19) using some modifications (28). Tissue (liver) homogenate was prepared by grinding fresh normal albino rat liver using phosphate buffer saline, pH 7.4 (10% w/v). The homogenate were centrifuged at 3000 rpm for 15 min and clear supernatant were taken for analysis. 100 µl extracts (2 mg/ml) dissolved in respective solvents were taken in test tubes and evaporated to dryness followed by addition of 1 ml of 0.15 M potassium chloride and 0.5 ml of liver homogenate. Peroxidation was initiated by adding 0.2 mM ferric chloride (100 µl). The tubes were incubated at 37^0C for 30 min. The reaction was stopped by adding 2 ml of ice-cold hydrochloric acid (0.25 N) containing 15% TCA, 0.38% TBA and 0.5% BHT. The reaction mixture was incubated at 80^0C for 1 h. The samples were cooled and centrifuged and the absorbance of the pink supernatants was measured at 532 nm. BHA was used as standard for comparison. A similar experiment was performed in the absence of extract samples and standard to determine the amount of lipid peroxidation obtained in the presence of inducing agents, which served as control. All analyses were carried out in triplicate and results were expressed as mean ± SEM. The percentage of lipid peroxidation inhibition (% LPOI) was calculated by using the following formula:

$\text{LPOI}\ (\%) = [(A_0 - A_1) / A_0] \times 100$

Where A_0 is the absorbance of control and A_1 is the absorbance of the standards or samples.

Statistical analysis

All experiments were carried out in triplicate. For determinations of total flavonoid contents and % LPOI activity the data were expressed as mean ± standard error of mean (SEM) while for measurement of antioxidant activity the data were expressed as mean ± standard deviation (SD) and the plots were prepared using Graphpad Prism software.

RESULTS

Phytochemical analysis of S. xanthocarpum root extracts

Extracts were tested for the presence of tannins, flavonoids, terpenoids, cardiac glycosides, anthraquinone, reducing sugars, alkaloids, phlobatanins and saponins. Results are shown in Table 1. Some of the phytochemicals such as flavonoids and alkaloids were present in all the extracts while cardiac glycosides, anthraquinone, reducing sugars and phlobatanins were absent in all the test extracts. Rest of the compounds (tannins, terpenoids and saponins) were mostly found in polar fractions. HX and EA fractions were devoid of most of the test phytochemicals except flavonoids and alkaloids.

Total flavonoid content

Test extracts were quantified for total flavonoid contents (Table 2). EA accounted for maximum flavonoid content (25.17 ± 0.44 µg QE/mg) followed by CH (20.03 ± 0.58 µg QE/mg) and ET (15.83 ± 0.12 µg QE/mg), respectively among all the extracts. Overall order of flavonoid contents was EA> CH> ET> BZ> AC> AQ> HX.

Metal ion chelating ability of root extracts

S. xanthocarpum root extracts accounted for differential degree of metal ion chelation ability which is indicated

Table 1. Phytochemical analysis of *S. xanthocarpum* root extracts.

	Extracts						
Phytochemicals	**HX**	**BZ**	**CH**	**EA**	**AC**	**ET**	**AQ**
Tannins	-	-	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+	+
Cardiac Glycosides	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-
Reducing sugars	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-
Saponins	-	-	-	-	+	-	+

Phytochemical analysis of different extracts of *S. xanthocarpum* root was done as shown in Methods section.
HX - hexane, BZ - benzene; CH - chloroform; EA - ethyl acetate; AC - acetone; ET - ethanol; AQ - water; + present/detected; - not detected.

by reduction in formation of red coloured complex. ET extracts demonstrated appreciable chelation potential at all the test concentrations. Maximum % inhibition observed with ET extract was about 65%. Percent inhibition of colour production as a function of activity of different extracts has been graphically represented in Fig. 1. The figure shows that formation of the Fe^{2+}-ferrozine complex is not complete in the presence of test extracts, indicating that some of the extracts chelate the iron. The percentage of metal chelating capacity of root extracts linearly increased in dose dependent manner (from 400 to 1000 µg/ ml). The metal ion chelating capacity (% inhibition of Fe^{2+}-ferrozine complex formation) at 1000 µg/ml concentration of ET, HX and AQ extracts were found to be about 64, 45 and 41% respectively. The over all order of chelating activity of extracts was found to be ET, HX, AQ, CH, BZ, AC and EA, respectively at all test concentrations. Standard pure compounds BHA, BHT, PG, QU and AA demonstrated higher chelating capacity (about 65, 86, 90, 30 and 63%, respectively) at a concentration of 400µg/ml (Fig. 2). BHA, BHT and PG produced concentration dependent incremental response in chelating activity while inverse activity response was observed with increasing concentration of QU and AA.

Table 2. Contents of total flavonoid in *S. xanthocarpum* root extracts.

Extract	Flavonoids (µg QE/mg)
HX	4.80 ± 0.21
BZ	11.17 ± 0.60
CH	20.03 ± 0.58
EA	25.17 ± 0.44
AC	7.33 ± 0.39
ET	15.83 ± 0.12
AQ	6.20 ± 0.47

The values are represented as µg quercetin equivalent per milligram of sample (µg QE/mg). The results are expressed as mean ± SEM (n = 3). Abbreviations: HX- hexane, BZ - benzene, CH - chloroform, EA-ethyl acetate, AC - acetone, ET - ethyl alcohol, AQ -water.

Free radical scavenging activity of S. xanthocarpum root extracts by DPPH assay

Free radical scavenging activity of *S. xanthocarpum* root extracts at different concentrations (250, 500 and 1000 µg/ml) was evaluated by the DPPH method (Fig. 3). The degree of discoloration indicates the scavenging potentials of the extracts. Most of the polar extracts demonstrated appreciable free radical quenching potential (above 85% inhibition) at all the concentrations tested. At 250µg/ml concentration EA, ET, AQ extracts exhibited same scavenging activities (about 85 %) while at the same concentration AC and HX fractions showed about 94 and 20 % free radical scavenging activities, respectively. Marginal increase in activity was observed at higher concentrations. EA, AC, ET and AQ extracts exhibited about 88-95% free radical scavenging activity at a concentration 500 µg/ml while HX extract showed minimal activity. Further increase in activity was observed at 1000 µg/ml concentration for all the root extracts. In general, with increasing concentration of root extracts there was gradual increase in free radical scavenging activity. The order of activity of extracts may be represented as AC, ET, EA, AQ, BZ, CH and HX, respectively. However activity pattern of first four extracts was more or less similar. Standard antioxidants namely BHA, BHT, QU and AA have already been reported to produce 95-99% scavenging activities even at 500 µg/ml concentration by our research group (30).

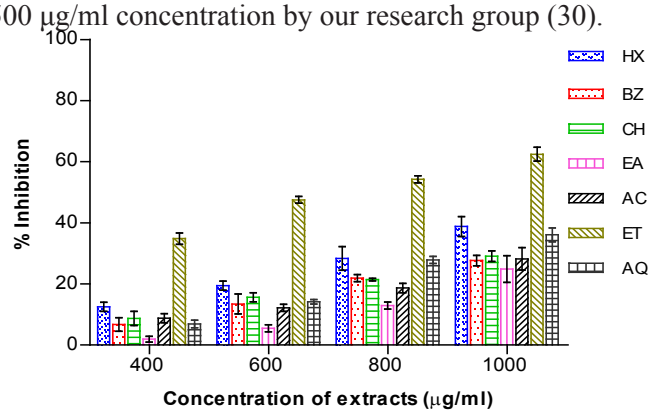

Figure 1. Metal ion chelating ability of *S. xanthocarpum* root extracts (Phytochemicals present in root sample were extracted with hexane (HX), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethyl alcohol (ET) and water (AQ) as described in methods section. Metal ion chelating activity of extracts was measured at different concentrations and absorbance was recorded at 562nm. The results are expressed as mean ± SD of three replicates).

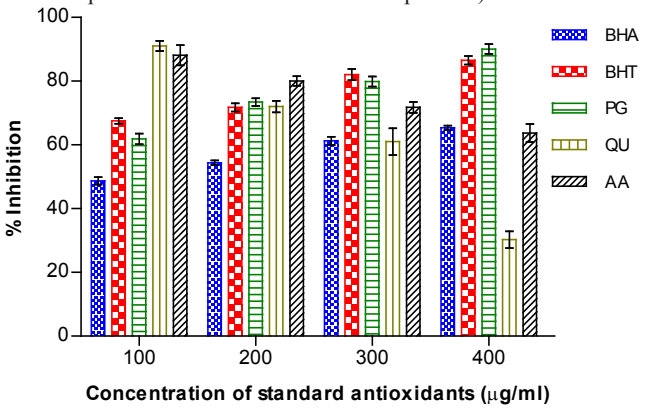

Figure 2. Metal ion chelating ability of standard antioxidant compounds. (The chelating activity of BHA, BHT, PG (propyl gallate), QU (Quercetin) and AA (ascorbic acid) was measured at different concentrations and absorbance was recorded at 562nm as described in Methods section. The results are expressed as mean ± SD of three replicates).

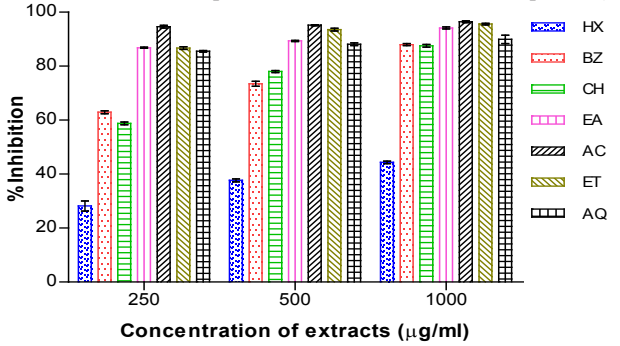

Figure 3. Free radical scavenging activity of *S. xanthocarpum* root extracts by DPPH assay (Phytochemicals present in root were extracted with HX, BZ, CH, EA, AC, ET, AQ and radical scavenging activity of the extracts was measured at three different concentrations as described in methods section. The results are expressed as mean ± SD of three replicates).

Lipid-peroxidation inhibition by S. xanthocarpum root extracts

The liver homogenate of albino Wistar rats undergo rapid peroxidation when incubated separately with ferric chloride. The iron induced production of peroxide in turn attacks the biological material. This leads to the formation of MDA (malonodialdehyde) and other aldehydes which form a pink chromogen with TBA showing maximum absorbance at 532 nm (27). It was observed that some of the root extracts of *S. xanthocarpum* showed anti lipid peroxidation activity *in vitro* (Fig. 4) and have capability of protecting liver from peroxidative damage. Two polar extracts namely ET and AQ exhibited significant protective efficacy against lipid peroxidation activity in rat liver tissue. The % LPOI activity in ET and AQ extracts was found to be about 45% and 51%, respectively. Rest of the extracts accounted for least activity with % LPOI ranging between 3-14%.

Comparative assessment of relationship between DPPH radical scavenging activity and anti lipid peroxidation activity of extracts was done by plotting curve between % inhibition in DPPH assay and % LPOI (Figure 5). A significant positive correlation was observed between the DPPH radical scavenging assay and the % LPOI for various root extracts at $P < 0.05$ indicating appreciable antioxidant potential in the extracts.

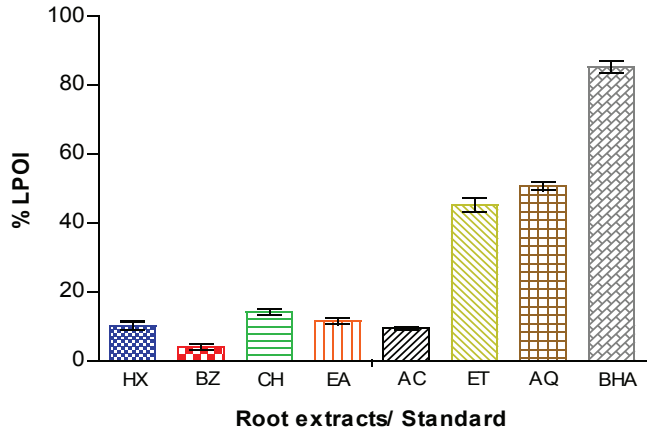

Figure 4. Inhibition of iron induced lipid peroxidation (% LPOI) in rat liver tissue by *S. xanthocarpum* root extracts (Phytochemicals present in root were extracted with HX, BZ, CH, EA, AC, ET, AQ and % LPOI was measured as described in methods section. BHA was used as standard for comparison. The results are expressed as mean ± SEM of three replicates).

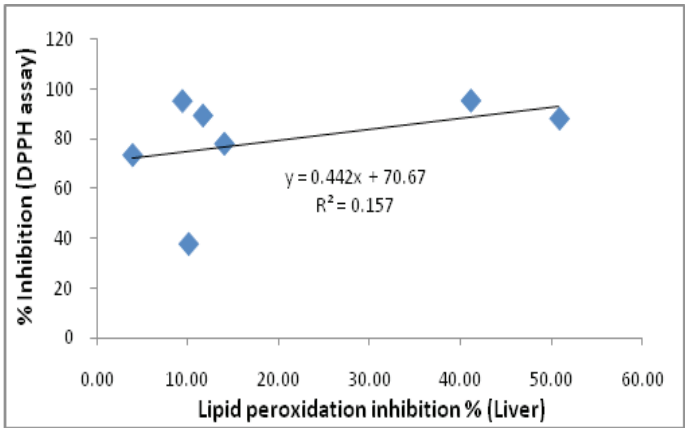

Figure 5. Relationship between DPPH free radical scavenging activity and %LPOI in liver of albino Wistar rats ($P < 0.05$)

DISCUSSION

Phytochemical analysis indicates that all the extract fractions derived from *S. xanthocarpum* root are rich in flavonoids and alkaloids. Tanins, terpenoids and saponins showed distributory effect among the various polar and non polar extracts of the root i.e., they were concentrated among the polar fractions (Table 1). However terpenoids were equally distributed in non-polar and polar extracts. It is apparent that non-polar and polar fractions accounted for low flavonoid content while extracts derived in medium polar solvents showed higher content of flavonoids.

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (7, 25). Polyphenols, particularly flavonoids, which are widely distributed in the plant kingdom, and are present in considerable amount in fruits, vegetables, spices and medicinal herbs have been used to treat many human diseases, such as diabetes, cancer and coronary heart disease (4). Flavonoids have also been shown to exhibit the antioxidative, antiviral, antimicrobial and anti-platelet activities (20). Our experiments demonstrated presence of differential amount of flavonoids in different extracts (Table 2).

The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions (43). Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (18, 19). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of samples possessing chelating activity the formation of red coloured complexes is decreased. Therefore measurement of the rate of color reduction helps to estimate the chelating activity of the coexisting chelator present in the samples. Our results have shown that the absorbance of coloured complex decreased linearly which indicated that the formation of Fe^{2+}-ferrozine complex was not completed in the presence of *S. xanthocarpum* root extracts suggesting chelation of iron by phytochemicals present in these plants. Several reports (18, 19, 43) on chelation of iron by other plant extracts also substantiate these findings. In this study extracts of *S. xanthocarpum* root and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine.

It has been reported that chelating agents, which form sigma bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (15). The data presented in Fig. 1 revealed that ET, HX and AQ extracts demonstrated marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to iron binding capacity.

DPPH assay is one of the most commonly used methods for screening antioxidant activity of plant extracts. It has been largely used as a quick, reliable and reproducible pa-

rameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts. DPPH produces violet colour in methanol solution. It is reduced to a yellow coloured product, diphenylpicryl hydrazine. Antioxidants react with DPPH, a stable nitrogen-centered free radical, and convert it to α, α-diphenyl-*β*-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extracts (Fig. 3). The decrease in absorbance by the DPPH radical with increasing concentration of the extracts in dose dependent manner results in the rapid discolouration of the purple DPPH, suggesting that extracts of *S. xanthocarpum* have radical scavenging antioxidant activity due to its proton donating ability (3). The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. Several extract fractions exhibited significantly higher inhibition percentage (stronger hydrogen –donating ability) which can be positively correlated with total phenolic content (44). In our study the flavonoid contents were estimated in all extracts. Their amount ranged between 4.80 ±0.21 to 25.17 ±0.44 µg QE/mg extract (Table 2). Therefore marked radical scavenging activity observed in most of the extracts could be attributed to the presence of significant quantity of flavanoids in addition to other phytochemicals.

Lipid peroxidation causes damage to unsaturated fatty acids, which results in decreased membrane fluidity and leads to many other pathological events (11, 19, 46). Redox chemistry of iron plays an important role in both the occurrence and the rate of lipid peroxidation. Fe^{3+} reacts with lipid hydroperoxides to form peroxyl radicals that initiate a chain reaction by reacting with other molecules. A probable alternative fate of peroxyl radicals is to form cyclic peroxides or lipid peroxides. These cyclic peroxides, lipid peroxides, and cyclic endoperoxides undergo fragmentation to aldehydes such as MDA and polymerization products. MDA is usually taken as a marker of lipid peroxidation (LPO) and oxidative stress, and its reaction with two molecules of TBA yields a pinkish red chromogen (TBARS, i.e., thiobarbituric acid reactive substances) with an absorbance maximum at 532 nm (24, 27). Among *S. xanthocarpum* root extracts ET and AQ extracts accounted for substantial anti lipid peroxidation activities (Fig. 4). It may be inferred that major phytoconstituents present in some of the active extracts such as flavonoids along with tannins, terpenoids and alkaloids are responsible for quenching Fe^{3+} and thereby preventing oxidative damage to lipids leading to protection of liver and other tissues (19, 39, 43). The study also established significant correlation between anti lipid peroxidation activity and free radical scavenging activity of the *S. xanthocarpum* root extracts.

Many reports tend to show that secondary metabolites such as alkaloids, flavonoids, tannins and other compounds of phenolic nature are the responsible compounds for the antioxidant and antimicrobial activities in higher plants (3, 8). Phytochemical screening has revealed that extracts from root of *S. xanthocarpum* possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins . Therefore, the presence of these phytochemicals could to some extent justify the observed antioxidant activities in the current study (13). These results are in agreement with many studies realized on other plant species (3, 30, 31, 33, 39, 40) attributing antimicrobial and antioxidant activities to the presence of secondary metabolites.

Present study has established that phytochemicals present in *S. xanthocarpum* root extracts have capability to quench free radicals generated in the system and neutralize metal ion mediated oxidative damage. Therefore *S. xanthocarpum* root could be regarded as a source of future medicinal compounds.

ACKNOWLEDGEMENTS

SK acknowledges financial support from UGC, India in the form of Rajiv Gandhi National fellowship. UKS and AKS also acknowledge the financial support from University of Allahabad, Allahabad, India in the form of UGC-CRET research fellowships.

Other articles in this theme issue include references (52-79).

REFERENCES

1. Anonymous, TheWealth of India—Raw materials vol. IX: Rh–So. CSIR, New Delhi, 1989, pp. 393–394.

2. Auroma, O.I., Nutrition and health aspects of free radicals and antioxidants. *Food Chem. Toxicol.* 1994, **32:** 671–683.

3. Boussaada, O., Chriaa, J., Nabli, R., Ammar, S., Saidana, D., Mahjoub, M.A., Chraeif, I., Helal, A.N. and Mighri, Z., Antimicrobial and antioxidant activities of methanol extracts of *Evax pygmaea* (Asteraceae) growing wild in Tunisia. *World J. Microbiol. Biotechnol.* 2008, **24:** 1289-1296.

4. Broadhurst, C.L., Polansky, M.M. and Anderson, R.A., Insulin-like activity of culinary and medicinal plant aqueous extracts in vitro. *J. Agric. Food Chem.* 2000, **48:** 849–852.

5. Chang, C., Yang, M., Wen, H. and Chern, J., Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analaysis.*2002, **10:** 178-182.

6. Chopra, R.N., Nayar, S.L., Chopra, I.C., Glossary of Indian Medicinal Plants. CSIR, New Delhi, 1956, pp. 230.

7. Cook, N.C. and Samman, S., Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutri. Biochem.*1996, **7:** 66-76.

8. Cordell, G.A., Quinn-Beattie, M.L. and Farnsworth, N.R., The potential of alkaloids in drug discovery. *Phytother. Res.* 2001, **15:** 183-205.

9. Davies, K. J. A., Oxidative stress: The paradox of aerobic life. *Biochem. Soc. Symp*.1994, **61:** 1–34.

10. Dinis, T. C. P., Madeira, V.M. C. and Almeida, L. M., Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.*1994, **315:**161–169.

11. Drueke, T.B., Khoa, T.N., Massay, Z.A., Witko-Sarsat, V., Lacour, B., and Descamps-Latscha, B., Role of oxidized low-density lipoprotein in the atherosclerosis of uremia. *Kidney Int. Suppl*.2001, **78:** S114–S119.

12. Duh, P. D., Antioxidant activity of burdock (Arctium lappa Linne): Its scavenging effect on free radical and active oxygen. *J. Am. Oil Chemist's Soc*. 1998, **75:** 455–465.

13. Fabri, R.L., Nogueira, M.S., Braga, F.G., Coimbra, E.S. and Scio, E., *Mitracarpus frigidus* aerial parts exhibited potent antimicrobial, antileishmanial, and antioxidant effects. *Biores. Technol.* 2009, **100:** 428–433.

14. Frankel, E., Nutritional benefits of flavonoids. International conference on food factors: Chemistry and Cancer Prevention. Hamamatsu, Japan, Abstracts, 1995, C6- 2.

15. Gordon, M. H., The mechanism of the antioxidant action in vitro. In: *Food antioxidants,* Hudson, B. J. F. (Ed.), Elsevier Applied Science, London, 1990, pp. 1–18.

16. Gryglewski, R.J., Korbut, R. and Robak, J., On the mechanism of antithrombotic action of flavonoids. *Biochemical Pharmacol*. 1987, **36:** 317- 321.
17. Gunaselvi, G., Kulasekaren. and Gopal, V., Anthelmintic activity of the extracts of *Solanum xanthocarpum* Schrad and Wendl fruits (Solanaceae). *Int. J. PharmTech. Res*. 2010, **2(3):** 1772-1774.
18. Halliwell, B. and Gutteridge, J. M. C., Free radicals in biology and medicine. Clarendon Press, Oxford, UK, 1989, pp. 23–30.
19. Halliwell, B., and Gutteridge, J.N.C., Mechanism of damage of cellular targets by oxidative stress: lipid peroxidation. In: *Free Radicals in Biology and Medicine*, Halliwell, B., and Gsutteridge, J.M.C. (Eds.), Oxford University Press, Oxford, UK, 1999, pp. 284–313.
20. Halliwell, B., Gutteridge, J.M.C. and Cross, C.E., Free radicals, antioxidants and human disease: where are we now? *J. Lab. Clin. Med*. 1992, **119**: 598-620.
21. Harborne, J.B., Phytochemical Methods. Chapman and Hall Ltd., London, 1973, 49-188.
22. **I**namdar, N., Edalat, S., Kotwal, VB. and Pawar, S., Herbal drugs in milieu of modern drugs. *Int. J. Green Pharmacy.* 2008, **2:** 2-8.
23. Ito, N., Fukushima, S., Hasegawa, A., Shibata, M., and Ogiso, T., Carcinogenecity of buthylated hydroxyanisole in F344 rats. *J. Natl. Cancer Inst.* 1983, **70:** 343–347.
24. Janero, D.R., Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* 1990, **9:** 515–540.
25. Kessler, M., Ubeaud, G. and Jung, L., Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol*. 2003, **55:** 131-142.
26. Kirkinezos, I.G. and Moraes, C.T., Reactive oxygen species and mitochondrial diseases. *Seminars on Cell Development Biology.* 2001, **2:** 257–449.
27. Kosugi, H. and Kato, T. et al., Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem*. 1987, **165:** 456-464.
28. Kumar, S. and Pandey, A.K., Antioxidant, lipo-protective and antibacterial activities of phytoconstituents present in *Solanum xanthocarpum* root. *Int. Rev. Biophysical Chem. (IREBIC).* 2012, **3:** 42-47.
29. Lee, H.C., and Wei, Y.H., Mitochondrial alterations, cellular response to oxidative stress and defective degradation of proteins in ageing. *Biogerontology*. 2001, **2:** 231–244.
30. Mishra, A., Kumar, S., Bhargava, A., Sharma, B. and Pandey, A. K., Studies on *in vitro* antioxidant and antistaphylococcal activites of some important medicinal plants. *Cell. Mol. Biol.* 2011, **57 (1):** 16-25.
31. Mishra, A. K., Mishra A., Bhargava A. and Pandey, A. K., Antimicrobial activity of essential oils from the leaves of *Cinnamomum spp. Natl. Acad. Sci. Lett.* 2008, **31**: 341-345.
32. Mishra, A.K., Misra, A., Kehri, H.K., Sharma, B. and Pandey, A.K., Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvuluria lunata*, the pathogenic dematiaceous moulds. *Ann. Clin. Microbiol. Antimicro.* 2009, **8**: 9.
33. Mishra, A.K., Singh, B.K. and Pandey, A.K., *In vitro* antibacterial activity and phytochemical profiles of *Cinnamomum tamala* (Tejpat) leaf extracts and oil. *Reviews in Infection*. 2010, **1:** 134-139.
34. Najmi, A. K., Pillai, K.K., Pal, S.N. and Aqil, M., Free radical scavenging and hepatoprotective activity of jigrine against galactosamine induced hepatopathy in rats. *J. Ethnopharmacol.*2005, **97:** 521–525.
35. Nikaido, H. and Varra, M., Molecular basis of bacterial outer membrane permeability, *Microbiol. Rev.* 1985, **1:**1-32.
36. Noguchi, N., and Niki, E., Lipid peroxidation. In: *Antioxidant Status, Diet, Nutrition, and Health,* Papas, A.M. (Ed.), CRC Press, London, 1999, 8–13.
37. Nostro, A., Germano, M.P., D'Angelo, V., Marino, A. and Cannatelli, M.A., Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol***.** 2000, **30**: 379 - 384.
38. Pandey, A.K., Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parthenium histerophorus*: an in vitro study. *Natl. Acad. Sci. Lett.* 2007, **30**: 383-386.
39. Pandey, A.K., Mishra, A.K., Mishra, A., Kumar, S. and Chandra, A., Therapeutic potential of *C. zeylanicum* extracts: an antifungal and antioxidant perspective. *Int. J. Biol. Med. Res.* 2010, **1:** 228-233.
40. Pandey, A.K., Pandey, P., Sharma, S. and Maheshwari, D.K., Antibacterial potential of extracts of *Lantana camara* – A prominent weed of northern India. *Univ. J. Phytochem. Ayur. Heights*. 2005, **1:** 18-23.
41. Perez, C., Pauli, M. *and* Bazerque, P., An antibiotic assay by the well agar method. *Acta. Biol. Med. Exp.* 1990, **15:** 113–115.
42. Robinson, E. E., Maxwell, S. R. J., and Thorpe, G. H. G., Aninvestigation of antioxidant activity of black tea using enhanced chemiluminescence. *Free Radical Res.* 1997, **26:** 291–302.
43. Singh, R. and Arora, S., Attenuation of free radicals by acetone extract/fractions of *Acacia nilotica* (l) Willd. Ex del. *J. Chinese Clin. Med*. 2007, **2:** 196-204.
44. Singh, R.P., Chidambara Murthy, K.N. and Jayapakash, G.K., Studies on the antioxidant activity of Pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J. Agric. Food Chem*. 2002, **50:** 81-86.
45. Sofowora, A., Medicinal plants and traditional medicine in Africa. Spectrum Books, Ibadan, 1993**:** 150.
46. Spiteller, G., Peroxidation of linoleic acid and its relation to ageing and age dependent diseases. *Mechanisms of Ageing and Development.*2001, **122:** 617–657.
47. Szelenyi, I . and Brune, K., Herbal remedies for asthma treatment: Between myth and reality. *Drugs Today.* 2002, **38**: 265.
48. Trease, G.E. and Evans, W.C., In: *Pharmacognosy,* Bailliere Tindall, London,1989, **11:** 45-50.
49. Werns, S.W. and Lucchesi, B.R., Myocardial ischemia and reperfusion: the role of oxygen radicals in tissue injury. *Cardiovascular Drugs Ther.* 1989, **2:** 269–761
50. Yen, G. C., and Duh, P. D., Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. *J. Agric. Food Chem.* 1994, **42:** 629.
51. Zheng, W., and Wang, S. Y., Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 2001, **49:** 5165–5170.
52. Singh, M. P., and Kumar, V., Biodegradation of vegetable and agrowastes by *Pleurotus sapidus*: A noble strategy to produce mushroom with enhanced yield and nutrition. *Cell. Mol. Biol.* 2012, **58** (1): 1-7.
53. Pandey, V. K., Singh, M.P., Srivastava, A. K., Vishwakarma S. K., and Takshak, S., Biodegradation of sugarcane bagasse by white rot fungus *Pleurotus citrinopileatus. Cell. Mol. Biol.* 2012, **58** (1): 8-14.
54. Ruhal, A., Rana, J. S., Kumar S., and Kumar, A., Immobilization of malate dehydrogenase on carbon nanotubes for development of malate biosensor. *Cell. Mol. Biol.* 2012, **58** (1): 15-20.
55. Vishwakarma, S. K., Singh, M. P., Srivastava A.K. and Pandey, V. K., Azo dye (direct blue) decolorization by immobilized extracellular enzymes of *Pleurotus* species. *Cell. Mol. Biol.* 2012, **58** (1): 21-25.
56. Dash, S. K., Sharma, M., Khare, S. and Kumar, A., *rmpM* gene as a genetic marker for human bacterial meningitis. *Cell. Mol. Biol.* 2012, **58** (1): 26-30.
57. Bertoletti, F., Crespan, E. and Maga, G., Tyrosine kinases as essential cellular cofactors and potential therapeutic targets for human immunodeficiency virus infection. *Cell. Mol. Biol.* 2012, **58** (1): 31-43.
58. Sandalli, C., Singh, K., and Modak, M. J., Characterization of catalytic carboxylate triad in non-replicative DNA polymerase III (pol E) of *Geobacillus kaustophilus* HTA. *Cell. Mol. Biol.* 2012, **58** (1): 44-49.
59. Kaushal, A., Kumar, D., Khare, S. and Kumar, A., *speB* gene as a specific genetic marker for early detection of rheumatic heart disease in human. *Cell. Mol. Biol.* 2012, **58** (1): 50-54.

60. Datta, J. and Lal, N., Application of molecular markers for genetic discrimination of *fusarium* wilt pathogen races affecting chickpea and pigeonpea in major regions of India. *Cell. Mol. Biol.* 2012, **58** (1): 55-65.

61. Siddiqi, N. J., Alhomida, A. S., Khan, A. H. and Onga, W.Y., Study on the distribution of different carnitine fractions in various tissues of bovine eye. *Cell. Mol. Biol.* 2012, **58** (1): 66-70.

62. Ong, Y. T., Kirby, K. A., Hachiya, A., Chiang, L. A., Marchand, B., Yoshimura, K., Murakami, T., Singh, K., Matsushita, S. and Sarafianos, S. G., Preparation of biologically active single-chain variable antibody fragments that target the HIV-1 GP120 v3 loop. *Cell. Mol. Biol.* 2012, **58** (1): 71-79.

63. Singh, J., Gautam, S. and Bhushan Pant, A., Effect of UV-B radiation on UV absorbing compounds and pigments of moss and lichen of Schirmacher Oasis region, East Antarctica. *Cell. Mol. Biol.* 2012, **58** (1): 80-84.

64. Singh, V. P., Srivastava, P. K., and Prasad, S. M., Impact of low and high UV-B radiation on the rates of growth and nitrogen metabolism in two cyanobacterial strains under copper toxicity. *Cell. Mol. Biol.* 2012, **58** (1): 85-95.

65. Datta, J. and Lal, N., Temporal and spatial changes in phenolic compounds in response *Fusarium* wilt in chickpea and pigeonpea. *Cell. Mol. Biol.* 2012, **58** (1): 96-102.

66. Sharma, R. K., JAISWAL, S. K., Siddiqi, N. J., and Sharma, B., Effect of carbofuran on some biochemical indices of human erythrocytes *in vitro*. *Cell. Mol. Biol.* 2012, **58** (1): 103-109.

67. Singh, A. K., Singh, S. and Singh, M. P., Bioethics A new frontier of biological Science. *Cell. Mol. Biol.* 2012, **58** (1): 110-114.

68. Adedeji, A. O., Singh, K. and Sarafianos, S. G., Structural and biochemical basis for the difference in the helicase activity of two different constructs of SARS-CoV helicase. *Cell. Mol. Biol.* 2012, **58** (1): 115-121.

69. Singh, S., Choudhuri, G., Kumar, R. and Agarwal, S., Association of 5, 10-methylenetetrahydrofolate reductase C677T polymorphism in susceptibility to tropical chronic pancreatitis in North Indian population. *Cell. Mol. Biol.* 2012, **58** (1): 122-127.

70. Sharma, R. K., Rai, K. D. and Sharma, B., *In* vitro carbofuran induced micronucleus formation in human blood lymphocytes. *Cell. Mol. Biol.* 2012, **58** (1): 128-133.

71. Naraian, R., Ram, S., Kaistha S. D. and Srivastava J., Occurrence of plasmid linked multiple drug resistance in bacterial isolates of tannery effluent. *Cell. Mol. Biol.* 2012, **58** (1): 134-141.

72. Pandey, A. K., Mishra, A. K., And Mishra, A., Antifungal and antioxidative potential of oil and extracts, respectively derived from leaves of Indian spice plant *Cinnamomum tamala. Cell. Mol. Biol.* 2012, **58** (1): 142-147.

73. Mishra, N., and Rizvi, S. I., Quercetin modulates na/k atpase and sodium hydrogen exchanger in type 2 diabetic erythrocytes. *Cell. Mol. Biol.* 2012, **58** (1): 148-152.

74. Kumar, A., Sharma, B. and Pandey, R. S., Assessment of stress in effect to pyrethroid insecticides, λ-cyhalothrin and cypermethrin in a freshwater fish, *Channa punctatus* (Bloch). *Cell. Mol. Biol.* 2012, **58** (1): 153-159.

75. Srivastava N., Sharma, R. K., Singh, N. and Sharma, B., Acetylcholinesterase from human erythrocytes membrane: a screen for evaluating the activity of some traditional plant extracts. *Cell. Mol. Biol.* 2012, **58** (1): 160-169.

76. Singh, M.P., Pandey, A. K., Vishwakarma S. K., Srivastava, A. K. and Pandey, V. K., Extracellular Xylanase Production by *Pleurotus* species on Lignocellulosic Wastes under *in vivo* Condition using Novel Pretreatment. *Cell. Mol. Biol.* 2012, **58** (1): 170-173.

77. Shukla, A., Singh, A., Singh, A., Pathak, L.P., Shrivastava, N., Tripathi, P. K., Singh, K. and Singh, M.P., Inhibition of *P. falciparum* pfATP6 by curcumin and its derivatives: A bioinformatic Study. *Cell. Mol. Biol.* 2012, **58** (1): 182-186.

78. Michailidis, E., Singh, K., Ryan, E. M., Hachiya, A., Ong, Y. T., Kirby, K. A., Marchand, B., Kodama, E. N., Mitsuya, H., Parniak, M.A. and Sarafianos, S.G., Effect of translocation defective reverse transcriptase inhibitors on the activity of n348i, a connection subdomain drug resistant HIV-1 reverse transcriptase mutant. *Cell. Mol. Biol.* 2012, **58** (1): 187-195.

79. Parveen, A., Rizvi, S. H. M., Gupta, A., Singh, R., Ahmad, I., Mahdi, F., and Mahdi, A. A., NMR-based metabonomics study of sub-acute hepatotoxicity induced by silica nanoparticles in rats after intranasal exposure. *Cell. Mol. Biol.* 2012, **58** (1): 196-203.