International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.1, pp 155-161, Jan-Mar 2013

In Vitro Phytochemical Screening, Free Radical Scavenging Activity And Anticancer Activity Of Abutilon hirtum (Lam.) Sweet (Malvaceae)

Servin Wesley P[1], Chitra Devi B[2]*[*], Sarmad Moin[1], Sahaya Shibu B[1]

[1]Department of Biotechnology, Karpagam University, Coimbatore, India
[2]Department of Botany, Karpagam University, Coimbatore, India

Corres.author: chitrasaraswathy@gmail.com
Mobile - 00919344112486

Abstract: The aim of the study was to perform *in vitro* phytochemical screening, free radical scavenging and anticancer activity of various extracts of *Abutilon hirtum* (Lam.) Sweet. *Abutilon hirtum* is a perennial herb commonly called as Indian mallow. Traditionally, the plant leaves are used as demulcent, diuretic and used to treat diarrhoea, bladder inflammations, wounds and ulcers. The preliminary phytochemical screening revealed the presence of various phytoconstituents, *in vitro* free radical scavenging activity of various crude extracts were determined using DPPH, FRAP, and reducing power assay. Aqueous extract showed the best result in DPPH assay. FRAP activity was maximum in ethyl acetate extract, which was positively co-related with the total phenol and flavonoid content. The aqueous extract of *A.hirtum* exhibited effective cytotoxic activity towards the human breast cancer cell line (MCF-7) with IC_{50} value 368.7µg/ml. These findings suggest that *Abutilon hirtum* exhibited potential *in vitro* free radical scavenging and anticancer activity.
Keywords: phytochemical screening, antioxidant, anticancer, MCF-7, *Abutilon hirtum.*

Introduction

The genus *Abutilon* comprises about 100–150 species and is distributed in the tropics and subtropics. *Abutilon hirtum* (Lam.) Sweet (*A. hirtum*) [Synonym: *Abutilon graveolens* (Roxb. ex Hornem.) *Wight & Arn.*], a perennial herb belongs to the family Malvaceae. It is commonly called as Belabenda, Indian mallow, Florida Keys etc. In Malaysia, *Abutilon hirtum* is used as a poultice to ease the pain of kidney gravel and often mixed with glutinous rice and applied to ulcers. In Thailand, the roots are used against cough and toothache and as an antipyretic. The leaves or flowers are applied to abscesses[1]. In Kenya the fruits are eaten raw, while the leaves are browsed by goats and camels. Water extract of the bark is given to ease childbirth in Kenya and Uganda. The seeds are an oil source. Nutritional analysis shows that it has crude protein 16-20% and calcium 2.6% [2].

In India, traditionally the leaves are used as demulcent, diuretic and to treat diarrhoea. The decoction of the leaves is used as mouth wash and to cure bladder inflammations, wounds and ulcers[3,4,5] since alkaloids are reported from the roots of the plant[6,7]. The leaf aqueous extract of *A. hirtum* posses hepatoprotective activity[8]. To our knowledge, there are no such scientific reports of phytochemical analysis, free radical scavenging and cytotoxic activity available for this plant species.

So the present investigation was designed to evaluate the *in vitro* phytochemical screening, free radical scavenging activity and anti-cancer activity of *Abutilon hirtum.*

Experimental

Plant materials

The flowering plants of *Abutilon hirtum* (Lam.) Sweet (Malvaceae) were collected from the district of Coimbatore, Tamilnadu situated in the southern region of India during the month of September 2011. It was identified and authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore. The authentication number is BSI/SRC/5/23/2011-12/Tech-1861.

Preparation of the extracts

The plant materials were dried at room temperature, chopped into small pieces, ground into powder and was placed into the extractor of a Soxhlet. The extraction was carried out by using solvents of increasing polarity starting from petroleum ether, chloroform, ethyl acetate, ethanol and water. At the end of the extraction the respective solvents were concentrated by evaporation. The obtained extracts were stored in a refrigerator at 4° C until use.

Preliminary Phytochemical analysis

Qualitative analysis

Qualitative phytochemical analysis was carried out[9,10,11] and the results observed were based on the colour change or precipitate formation after the addition of specific reagents.

Quantitative analysis

The total phenol contents were determined using a protocol[12] with little modification. Briefly, each 0.5 ml extracts were mixed with 2.5 ml Folin-Ciocalteu reagent and 2 ml of 7.5% (w/v) Na_2CO_3. The mixtures were shaken and allowed to react for 30 minutes in the dark for 1 hour. Absorbance was measured at 765 nm, and gallic acid was used as standard. The total phenol content was determined as gallic acid equivalents (GAE) in µg/mg dry weight (DW) of sample. The total flavonoid contents were determined by the $AlCl_3$ method[13]. An aliquot (0.1 ml) of extract was added to 0.3 ml 5% (w/v) $NaNO_2$ and incubated for 5 minutes. 0.3 ml $AlCl_3$ (10% w/v) and 2 ml 1 N NaOH was added, and the total volume was made up to 5 ml with distilled water. After 10 min of incubation at ambient temperature the absorbance was measured at 510 nm by using UV-visible spectrophotometer. Three replicates were made for each test sample. The total flavonoid contents were expressed as Quercetin equivalence (QE) in µg/mg DW of sample.

Determination of free radical scavenging activity by DPPH method[14]

All the five extracts of *A. hirtum* were determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. 100µl of various concentrations were added in 2.9 ml DPPH (60 µM in methanol) solution. The absorbance was measured at 517 nm after 20 minutes of reaction in the dark. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation

% inhibition = $[(A_B - A_S) / A_B] \times 100$.

where A_B is the absorbance of the control reaction (containing all reagents except the test compound), and A_S is the absorbance of the test compound. Ascorbic acid was used as a standard. The % inhibition was plotted against the sample extract concentration and a logarithmic regression curve was established in order to calculate the IC_{50} (inhibitory concentration 50, µg/ml).

FRAP Assay

The FRAP assay was carried out using a protocol[15] with little modifications. The stock solutions contained 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. Extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 minutes in the dark condition. Absorbance of the coloured product [ferrous tripyridyltriazine complex] was then measured at 593 nm. Calculations were made for the conversion of (TPTZ)-Fe (III) complex to TPTZ-Fe (II) compared with those of ascorbic acid. The results were expressed in µM ascorbic acid equivalence (AAE) /g dry weight of extract using standard calibration curve.

Reducing power assay

This assay was carried out using a protocol[16] with little modifications. 3 ml of reaction mixture, containing 250 µl of extracts in 2.5 ml 0.2 M phosphate buffer (pH 6.6) was incubated with 2.5 ml potassium ferricyanide (1% w/v) at 50° C for 20 minutes. The reaction was terminated by adding 2.5 ml of trichloroacetic acid (10% w/v), and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant solution was mixed with 5 ml of distilled water and 1 ml of ferric chloride (0.1%, w/v) solution, and the absorbance was measured at 700 nm against blank contain water and phosphate buffer. Ascorbic acid was used as a standard. Three replicates were made for each sample. Increased absorbance indicated increased reducing power of the sample.

Cytotoxicity assay

The Cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT, Sigma) assay[17] with little modification. The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10 % fetal bovine serum (FBS). 100 µl of cell suspension was seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0 C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hours prior to addition of extracts. The aqueous extract of *A. hirtum* which shows the maximum activity in most antioxidant assays was solubilised in DMSO and diluted in serum free medium. After 24 hrs, 100 µl of the medium containing the extracts at various concentrations (18.75, 37.5, 75, 150, and 300 µg/ml) was added and incubated at 37° C, 5 % CO_2, 95 % air and 100% relative humidity for 48 hours. Triplicate was maintained and the medium containing without extracts were served as control. After 48 h, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37° C for 4 hrs. The medium with MTT was flicked off, and the formed formazan crystals were solubilised in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula. % cell Inhibition = 100- $Abs_{drug}/Abs_{control}$ x100. Nonlinear regression graph was plotted between % Cell inhibition and concentration. IC_{50} was determined using GraphPad Prism software.

Results and Discussions

Preliminary Phytochemical analysis

Qualitative analysis

Phytochemical screening of various extracts of *A.hirtum* aerial parts revealed the presence of alkaloid, flavonoid, terpenoids, tannins and phytosterols. The results has been summarised in Table 1.

Quantitative analysis

The maximum percentage yield was obtained in ethanol extract (5.34 %) followed by water (4.16 %). The total phenol (55 µg) and flavonoid (40 µg) content of the *A.hirtum* was present maximum on ethyl acetate extract followed by ethanol and aqueous extracts (Table 2).

Determination of free radical scavenging activity by DPPH method

Free radicals are involved in many disorders like neurodegenerative diseases and cancer. Antioxidants through their scavenging power are useful in control of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[18]. Therefore each extract of *A.hirtum* is tested with DPPH to determine their free radical scavenging ability. The result of the DPPH scavenging assay is shown in Figure 1. It is found that antioxidant compounds in aqueous and ethyl acetate extracts have exhibited effective free radical scavenging activity. The IC_{50} values were calculated from the logarithmic regression curve. The highest free radical (DPPH) scavenging activity was observed in aqueous extract with IC_{50} value 120 µg/ml followed by ethyl acetate and ethanol extracts (202 µg/ml and 270 µg/ml respectively), may due to the presence of flavonoids, tannins and phenolic compounds that act as primary antioxidants or free radical scavengers[19].

FRAP (ferric reducing antioxidant power)

The antioxidant potential of five different extracts were estimated for their ability to reduce 2, 4, 6-tripyridyl-*s*-triazine (TPTZ)-Fe (III) complex to TPTZ-Fe (II). The reducing ability of five different extracts of *A.hirtum* was in the range of 227.11 - 454.23 µM AAE/g dry weight (**Table 3).** The high FRAP value was observed in ethyl acetate followed by aqueous and ethanol extracts which corresponds to the presence of total phenol and flavonoid content. There are couple of reports stated that different solvent extracts showed different potentiality in different assay. It is reported that the methanol extract of *Asparagus officinalis* has a higher antioxidant capacity than acetone extract of the same plant based on the DPPH scavenging assay, while antioxidant potential of acetone extract is higher than methanol extract in ABTS assay[20]. Similarly the methanol extract of *Nelumbo nuficera* Gaertn rhizome obtained the highest antioxidant activity based on DPPH assay, while dichloromethane extract showed the strongest antioxidant capacity in β-carotene bleaching experiment[21].

Table 1: Phytochemical screening of various extracts of *Abutilon hirtum*

Phytochemicals	Petroleum ether	Chloroform	Ethyl acetate	thanol	Water
Alkaloids	+	+	+	+	J
Carbohydrates	J	J	+	+	+
Glycosides	-	-	+	+	+
Saponins	-	-	-	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	-	-
Flavonoids	-	-	+	+	+
Phenolic compounds	-	-	+	+	+
Protein	-	+	-	-	-
Amino acids	-	-	-	-	-
Fats and oils	-	-	+	+	-
Gum and Mucilages	+	+	-	-	-
Phytosterols	+	+	+	+	+
Tannins	-	-	+	+	+

(+) present, (-) absent

Table 2: Total Phenol and Flavonoid contents of crude extracts of *Abutilon hirtum* (Lam.) Sweet

Extracts	µg GAE/g DW	µg QE/g DW
Petroleum ether	15 µg	38 µg
Chloroform	20 µg	31 µg
Ethyl acetate	55 µg	40 µg
Ethanol	45 µg	31 µg
Water	40 µg	32 µg

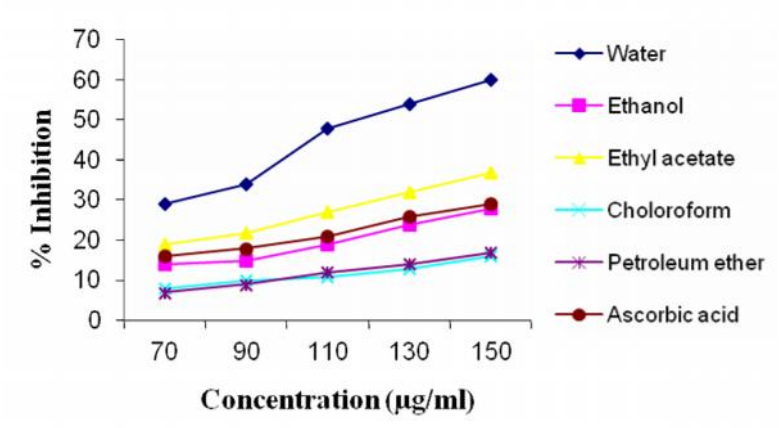

Figure 1: DPPH scavenging activity of various solvent extracts.

Reducing power assay

Figure 2 show the reducing power of five different extracts as a function of their concentration. The presence of reductants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, measuring the colour change at 700 nm can monitor the Fe^{2+}concentration. The reducing power of the various extracts of *A.hirtum* increased with increase in the concentration. The reducing power of aqueous and ethanol extracts at 100 µg/ml was 0.454 and 0.428 (Abs at 700 nm) respectively, which remained slightly lower than that of ascorbic acid (0.532) at the same concentration, used as positive control due to the lower amount of reduction in the extracts, which could react with radicals to stabilize and terminate radical chain reactions.

Table 3: FRAP activity of *Abutilon hirtum* extracts.

Extracts	µM AAE/ g DW
Petroleum ether	227.11 µM
Chloroform	283.89 µM
Ethyl acetate	454.23 µM
Ethanol	255.50 µM
Water	369.04 µM

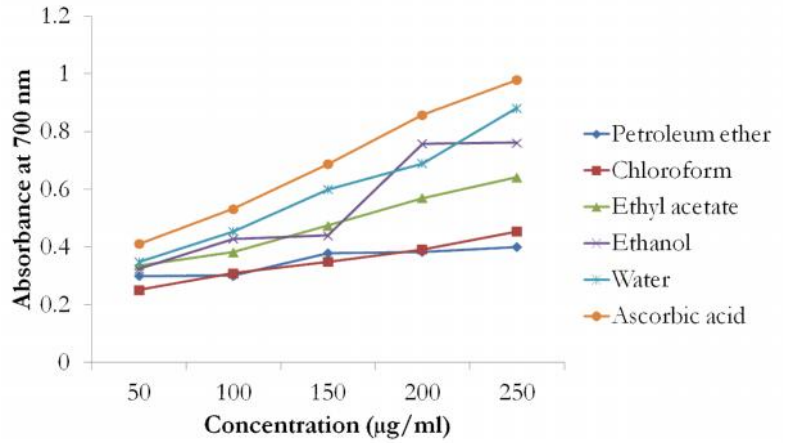

Figure 2: Reducing power ability of various solvent extracts.

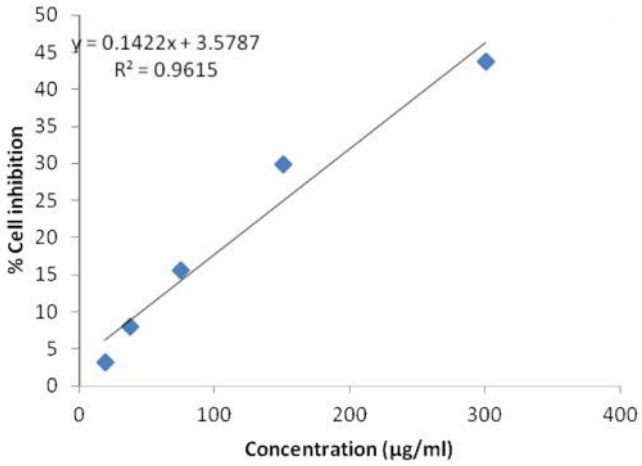

Figure 3. Graph showing the relationship between concentration and % cell inhibition of MCF-7.

Cytotoxicity assay

The anticancer activity of the aqueous extract of *A. hirtum* was investigated using MTT assay on human breast cancer cell lines (MCF-7). A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. In this study, viability of the MCF-7 cancer cells decreased with increasing concentration (18.75-300 µg/ml) of the extract, depicted in the

plotted graph showing the relationship between concentration and % inhibition of cultured cells (figure 3). Aqueous extract of *A.hirtum* has exhibited a high cell inhibition rate of 43.71 % at 300 µg concentration with IC_{50} value of 368.7 µg/ml on MCF-7 cell line (figure 4), which is quite comparable to the potent antiproliferative activity of *Phyllanthus amarus* in the human adenocarcinoma cell line MCF-7[22]. Similarly the *Wrightia tomentosa* also has been reported to inhibit the proliferation of human breast cancer cell lines, MCF-7 and MDA-MB-231[23]. The inhibitory property of *Abutilon indicum* on human melanoma (SK-MEL28 cell lines) and human non-small cell lung cancer (NCI-H23 cell lines) has been studied[24], since the rate of increase of cancer incidence and the lack of anticancer drugs has forced scientists to pharmacological and chemical investigations in the area of medicinal plants to search for anticancer agents[25], this study also made an effort to report the anticancer activity of *A.hirtum* for the first time.

Conclusion

The phenolic and flavonoid compounds, commonly found in the plants have been reported to posses multiple biological effects including antioxidant activity. The plant species as identified for their high levels of *in vitro* free radical scavenging activity may be valuable in the future studies to discover new healing drugs.

Acknowledgement

The authors thank Karpagam University, Coimbatore, Tamil Nadu, India for providing necessary facilities. The authors are grateful to Dr. R. Gopalan, (Fmr. Scientist, BSI) Professor and Head, Department of Botany, Karpagam University for his valuable support.

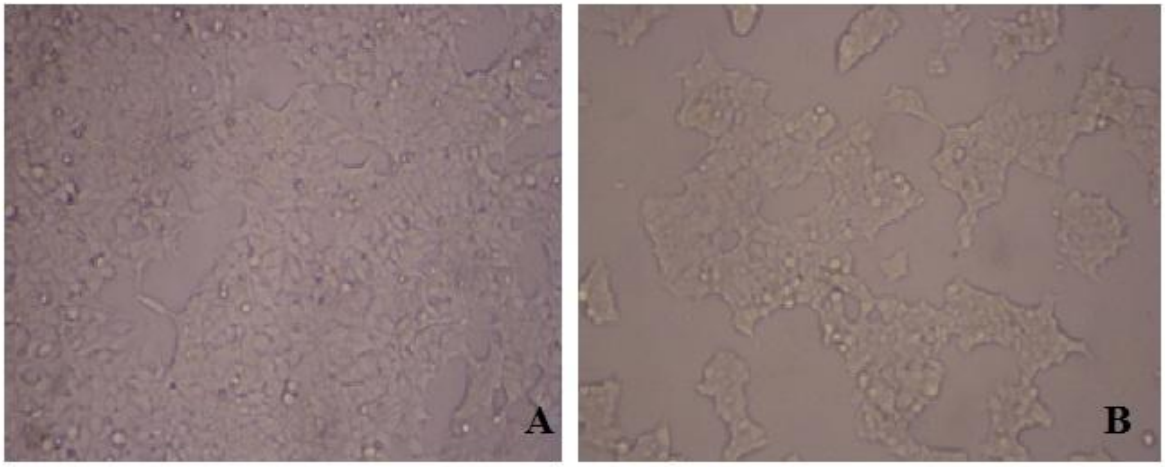

Figure 4. Cytotoxic activity of aqueous extract of *Abutilon hirtum* on MCF-7 cells.
Cells were photographed in culture conditions. (A) Control (B) treated with 300 µg/ml of aqueous extract.

References

1. Perumal B., *Abutilon hirtum* (Lamk) Sweet In: van Valkenburg, J.L.C.H. and Bunyapraphatsara, N. (Eds.), Plant Resources of South-East Asia: Medicinal and poisonous plants 2, Backhuys Publisher, Leiden, the Netherlands, 2001, 12(2), 30-31.
2. SEPASAL. [Survey of Economic Plants for Arid and Semi-Arid Lands] database. Royal Botanic Gardens, Kew, Richmond, United Kingdom, http://www.kew.org/ceb/sepasal/, Accessed November 2009.
3. Kapoor S.L. and Kapoor L.D., Medicinal plant wealth of the Karimnagar district of Andhra Pradesh, Bull. Med. Ethnobotanic. Res., 1980, 1,120-144.
4. Pullaiah T., Medicinal plants of India, Regency publications, India, 2002, 1, 9.
5. Chandana B.K., Saxena A.K., Sangeeta S., Sharma N., Gupta D.K., Singh K., Jyotsna S., Bhadauria M., and Qazia G.N., Hepatoprotective activity of *Woodfordia fruticosa* flowers against carbon tetrachloride induced hepatotoxicity, J. Ethnopharmacol., 2008, 1(19), 218–224.
6. Mauersberger H.R., Textile fibers: their physical, microscopic and chemical properties, 6th Edition, John Wiley and Sons, New York, United States, 1954, 1283.
7. Avijeet J., Manish S., Lokesh D., Anurekha J., Rout S.P., Gupta V.B., and Krishna K.L., Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica*

dioica Roxb. Leaves, J. Ethnopharmacol., 2000, 115, 61–66.
8. Srinivas Reddy K., Sanjeeva kumar A., Gnananath K., and Ganapathy S., Hepatoprotective potential of *Abutilon hirtum* Sweet leaves in carbon tetrachloride induced hepatotoxicity, Asian J. Biomed. Pharm. Sci., 2011, 1(3), 26-31.
9. Harborne J.B., Methods of plants analysis: In phytochemical methods, 2nd Eds, UK: Chapman and hall, 1984.
10. Trease G.E. and Evans W.C., Introduction and general methods in pharmacognosy, 13th Eds, K: Cambridge University press, 1989.
11. Raaman N., Phytochemical techniques, New India publishing agency, New Delhi, 2006.
12. Hendra R., Ahmad S., Oskoueian E., Sukari A., and Shukor M.Y., Antioxidant, anti-inflammatory and Cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff Fruit, BMC Complement, Altern. Med., 2011, 11, 110.
13. Ismail H.I., Chan K.W., Mariod A.A., and Ismail M., Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts, Food Chem., 2010, 119, 643-647.
14. Shimada K., Fujikawa K., Yahara K., and Nakamura, T., Antioxidative properties of xanthan on the anti-oxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem., 1992, 40, 945–948.
15. Benzie I.F.F. and Strain J.J., The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay, Anal. Biochem., 1996, 239, 70-76.
16. Oyaizu M., Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr., 1986, 44, 307–315.
17. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. immunol. Methods., 1983, 65, 55-63.
18. Koleva I. I., Van Beek T.A., Linssen J.P.H., Groot A. De, and Evstatieva L.N., Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, Phytochem. Anal., 2002, 13, 8-17.
19. Polterai O., Antioxidants and free-radical scavengers of Natural Origin, Curr. Org. Chem., 1997, 1, 415–440.
20. Sun T., Powers J.R., and Tang J., Evaluation of the antioxidant activity of *Asparagus*, broccoli and their juices, Food Chem., 2007, 105(1), 101-106.
21. Yang D., Wang Q., Ke L., Jiang J., and Ying T., Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn.) rhizome, Asia Pac. J. Clin. Nutr., 2007, 16(1), 158-163.
22. Gauri Abhyankar P., Suprasanna B.N., Pandey K.P., Mishra K.V., Rao and Reddy V.D., Hairy root extract of *Phyllanthus amarus* induces apoptotic cell death in human breast cancer cells, Innovat. Food. Sci. Emerg. Tech., 2010, 11, 526–532.
23. Bandana Chakravarti, Ranjani Maurya, Jawed Akhtar Siddiqui, Hemant Kumar Bid, Rajendran S.M., Prem Yadav P., and Rituraj Konwar., *In vitro* anti-breast cancer activity of ethanolic extract of *Wrightia tomentosa*: Role of pro-apoptotic effects of oleanolic acid and urosolic acid, J. Ethnopharmacol., 2012, 142, 72–79.
24. Srikanth P., Karthik P.S., Sirisha M., and Sashikanth Chitti., Evaluation of Antioxidant and Anticancer Properties of Methanolic Extracts of *Abutilon indicum* and *Blumea mollis*, J. Pharm. Res., 2012, 5(4), 2373-2376.
25. 0Koduru S., Grierson D.S., Van de Venter M., and Afolayan A.J., Antitumor activity of *Solanum aculeastrum* berries on three carcinoma cells. Int. J. Cancer Res., 2006, 2(4), 397–406.

*****