PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 197-204, 2016

Antiproliferative, antioxidant and antimicrobial screening of *Strychnos colubrina Linn* extracts

Preethimol Francis[1]*, SuseemSR[2]

[1]Nirmala College of Pharmacy, Muvattupuzha, Kerala India-686668
[2] SAS, VIT University, Vellore, Tamilnadu India

Abstract : Medicinal plants are the most precious gift to mankind offered by nature. For most of the known health issues there is a natural plant remedy. But most of these plants are not properly exploited. The present study deals with the scientific study of the therapeutic use of a medicinal plant *Strychnos colubrina Linn.* The plant is traditionally used by the tribe for skin cancer and infections. The present study consists of invitro screening of antiproliferative, antioxidant and antimicrobial activity of *Strychnos colubrina Linn* extract The chloroform extract was prepared using ariel plant parts and the antimicrobial activity as assessed by Cup plate method using gram –ve and +ve strains. The antioxidant activity of extract was screened by six different methods that include H_2O_2, superoxide, hydroxyl radical and NO scavenging assays, Reducing power assay and DPPH assay. Also the *invitro* antiproliferative activity was studied by MTT assay on HaCaT skin cell line using the extract. These studies were shown that the extract is having antimicrobial, antioxidant and antiproliferative efficacy.
Key words : *Strychnos colubrina,* chloroform extract antimicrobial, antioxidant, MTT assay.

Introduction

Medicinal plants are the valuable wealth of man-kind. Because of their natural origin, the deleterious effects exert is much less compared to known synthetic compounds. So for a healthy wellbeing, the man should explore and utilize the priceless medicinal wealth offered by nature.

The genus *Strychnos,* is the largest genus of the family Loganiaceae. About 44speciesof Strychnos are endemic to Asia[1]. *Strychnos colubrina Linn (S.colubrina Linn)* is one of the rare species of Strychnos genus which is traditionally used by the tribe for tumors, mania, arthralgia, diarrhoea, pain, chicken pox etc.,[2]. But these beneficial uses are not scientifically studied..

Most of the antimicrobial or anticancer agents available in the market are under the threat of microbial resistance[3]. So it is the need of decade to have new medicinal agents to subside the diseases with minimum side effects. The present study aims to reveal the antioxidant, antimicrobial and antiproliferative activities of *Strychnos colubrina Linn* extract.

Materials and Methods

Plant Material and Extraction

The ariel parts of *Strychnos colubrina Linn* was collected from village of Idukki in the month of October 2014. The plant material was identified and authenticated by the Botany department at Nirmala college of Arts &

Science, Muvattupuzha, Kerala, India. The ariel parts of the plant was shade dried at ambient temperature and the dried materials were crushed into fine powder using an electric blender. About 200g of powdered plant material was weighed and defatted with petroleum ether (1L) using soxhlet apparatus at 40°C for 48hrs. Then the residue was extracted with 750mL of chloroform and methanol successively. The extracts were concentrated in a rotary evaporator to yield a dark brown mass (5 g).

Antimicrobial activity

Micro organisms:Bacteria including *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) were used for the experiment. The clinical strains used in this work are our laboratory collection

Cultivation of Bacteria: The antibacterial activity of extract was determined by agar well diffusion method. The agar plates were seeded with 0.1ml of overnight culture and allowed to incubate for 24hrs. Cups were made in petri plates using sterile cork borer (0.85cm) and 50µLof extract was added to plate well. Then the plates were incubated at 37^0 C for 24Hrs.The extract treated plates were triplicated and the mean zone of inhibition was determined. The zone of inhibition was expreseed in mm[4,5,6,7,8].

Antioxidant Activity

DPPH Assay: The radical scavenging activity of extract was determined by using DPPH assay The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm . 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Different volumes of plant extract were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm.3ml of DPPH was taken as control [9,10,11].

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Test}) \times 100}{\text{Control}}$$

2.3.2. H_2O_2Scavenging activity : The extract was dissolved in 3.4ml of 0.1M phosphate buffer (P_H 7.4)and mixed with 600µl of 43mM solution of hydrogen peroxide .The absorbance value (230nm) of the reaction mixture was recorded after 10 minutes[10,11,12].

$$\% \text{ inhibition} = \left[1 - \frac{\text{Test}}{\text{Control}}\right] \times 100$$

Hydroxyl radial scavenging activity: One milliliter of the final reaction solution consisted of aliquots (500µl) of various concentration of the extract 1µm $FeCl_3$, 1µm EDTA, 20µm H_2O_2, 1µm ascorbic acid and 30µm deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 hour at 37°C and further heated in a boiling water bath for 15 minutes after addition of 1ml of 2-8% (w/v) trichloro acetic acid and 1ml of 1% 2-thio barbituric acid. The colour development was measured of 532nm against a blank containing phosphate buffer[13,14].

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Test}) \times 100}{\text{Control}}$$

Nitric Oxide Scavenging Activity: Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitro prusside (5mmol/L)in phosphate buffered saline (pH-7.4),was mixed with different concentration of the extract prepared in methanol and incubated at 25°C for 30minutes .A control without the test compound, but an equivalent amount of methanol was taken. After 30minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent (1% sulphanilamide,2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with

N-1 naphathyl ethylene diamine dihydrochloride was measured at 546nm and the percentage scavenging activity was measured with reference to the standard[14].

$$\% \text{ inhibition} = \frac{(\text{Control- Test}) \times 100}{\text{Control}}$$

Reducing power activity: Different concentrations of extract were mixed with 2.5ml of phosphate buffer(200mM)(pH 6.6). and 2.5ml of 1% potassium ferric cyanide was added and boiled for 20 minutes at 50°C. After incubation, 2.5 ml of 10% TCA were added to the mixtures followed by centrifugation at 650xg it for 10 minutes. The upper layer (5ml)was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride was added and the absorbance was read at 700nm[15].

$$\% \text{ inhibition} = \frac{(\text{Control- Test}) \times 100}{\text{Control}}$$

Super oxide free radical scavenging activity: Super oxide anion are generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product. 0.02ml of extracts, 0.05ml of Riboflavin solution(0.12mM) , 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5mM] were mixed in test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer [0.067M]. The absorbance of solution was measured at 560nm using DMSO as blank after illumination for 5 min and difference in OD was determined after 30 minutes incubation in fluorescent light. Absorbance was measured after illumination for 30 min. at 560nm on UV visible spectrophotometer. [16]

$$\% \text{ inhibition} = \frac{(\text{Control- Test}) \times 100}{\text{Control}}$$

Invitro anticancer activity by MTT assay:

HaCaT (keratinocyte) were purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 in a humidified atmosphere in a CO2 incubator(NBS, EPPENDORF, GERMANY). The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia)) for 2 minutes and passaged to T flasks in complete aseptic conditions.6.25,12.5,25,50,100µg/ml of sample was added from 1mg/ml stock to grown cells and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT - 5mg/ml dissolved in PBS). It was then incubated at 37^0C for 3 hours. MTT was removed by washing with 1x PBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA)[17,18,19,20,21,22].

% viability = (OD of Test/ OD of Control) X 100

Results:

Antimicrobialactivity:-The extract showed asignificant activity against E.coli and S.aureus.The antimicrobialactivity agaist P.aureuginosa and B.subtilis,is not as significant as the other two. The zone of inhibition is shown in figure-1. The zone of inhibition by the extract is depicted in table.1.From the table it is interpreted that the extract showed maximum activity against E.coli

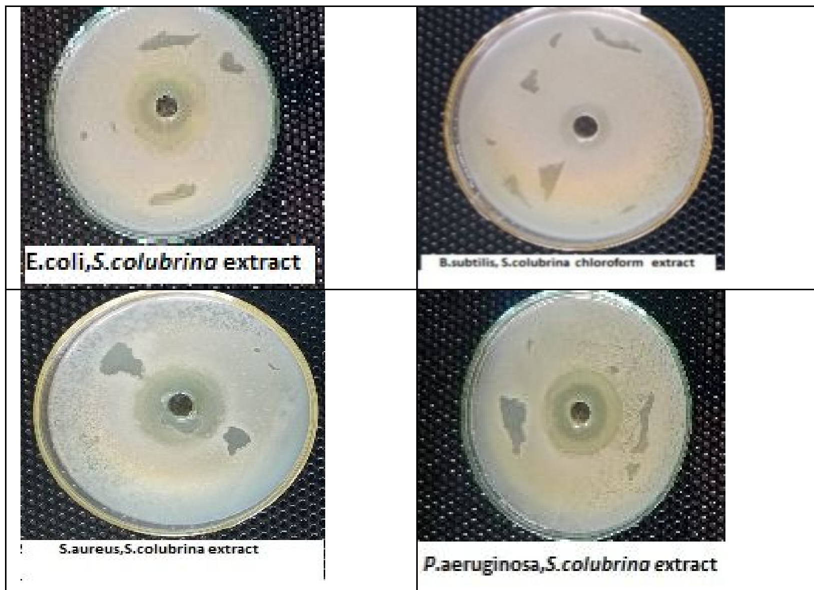

Figure.1:-The zone of inhibition shown by extract against different bacterial strains

Table1:The zone of inhibition for Antimicrobial effect of *Strychnos colubrina Linn* extract by cup-plate method

Sl.No.	Bacterial Strain	Standard	Extract
1.	B.subtilis	27.2±1.32	10±1.10
2.	P.aeureginosa	30.35±2.13	16.12±1.45
3.	S.aureus	30±2.33	18±0.23
4.	E.coli	29.15±1.11	19.0±2.53

Values are expressed as Mean±SEM.

Antioxidantactivity: The invitro antioxidant screening of *Strychnos colubrina Linn* extract showed significant protection against oxidizing agents/free radicals. The Table 2 and 3 shows the percentage inhibition by the extract by different *invitro* antioxidant screening methods. The maximum potency was shown at 2000 µg/ml concentration. In No scavenging assay the percentage inhibition of extract at 2000 µg/ml concentration is comparable to the standard

Table:2 The percentage inhibition in different methods of antioxidant activity by *Strychnos colubrina Linn* extract

Sl. No	Concentration (µg/ml)	Percentage Inhibition							
		DPPH Assay		NO scavenging assay		Hydroxyl radical scavenging assay		Superoxide scavenging assay	
		Standard	Extract	Standard	Extract	Standard	Extract	Standard	Extract
1.	125	10.24±3.0	9.5±1.3	57.44±4.2	11.73±3.0	31.15±3.5	71.73±2.15	33.33±4.25	34.97±3.0
2.	250	32.23±0.5	28.64±2.1	64.53±1.9	42.82±1.1	49.27±0.9	55.62±1.18	41.66±2.05	43.69±1.85
3.	500	38.48±1.3	36.03±1.6	68.08±3.0	80.44±2.5	71.25±5.1	40.93±2.47	66.66±1.8	50.91±4.11
4.	1000	66.12±2.1	57.75±0.4	71.63±2.6	85.7±0.75	75.36±3.0	44.53±0.54	75±1.55	60.78±5.13
5.	2000	69.14±0.7	60.05±3.2	92.90±2.0	86.91±1.5	80.67±1.0	50.45±1.8	91.66±0.88	79.45±4.3

Values are expressed as Mean±SEM

Table:3 The percentage inhibition in H2O2 scavenging assay and Reducing power assay by *Strychnos colubrina Linn* extract

Sl.No	Concentration (µg/ml)	H2O2 scavenging assay	Reducing power assay
		Percentage Inhibition by Extract	Percentage Inhibition by Extract
1.	125	6.03±1.12	20.23±1.5
2.	250	7.75±0.57	39.38±3.0
3.	500	8.64±4.1	42.03±2.8
4.	1000	9.5±1.22	43.44±1.56
5.	2000	10.05±4.23	45.22±0.50

Values are expressed as Mean±SEM

But the antioxidant activity by H2O2 scavenging assay showed a less effective response compared to other screening methods

***In vitro* anticancer activity by MTT Assay:-**The result of MTT assay is shown in table.4 and figure.2. The extract is having a potent antiproliferative effect on HaCaT cell line.

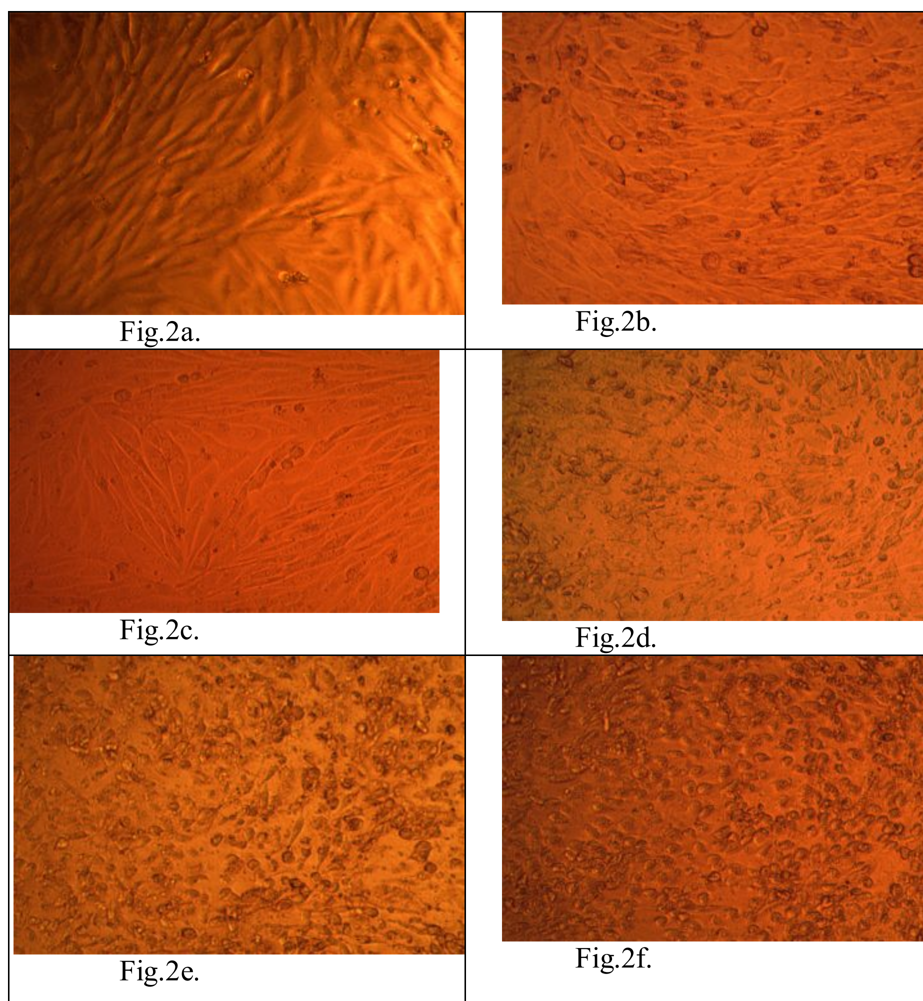

Figure.2: Viability of cell line in antiproliferative activity of *S.colubrina Linn* by MTT Assay:2a-Control;2b-. 6.25µg/mL sample treated;2c-12.5µg/mL sample treated; 2d-. 25µg/mL sample treated;2e-50 µg /mL sample treated;2f-100 µg /mL sample treated

Table:4 Percentage viability of cells in MTT Assay

Sample Concentration (µg/ml)	Percentage Viability
6.25	95.69±1.30
12.5	88.29 ±0.04
25	78.32±2.30
50	56.38±1.01
100	39.17±0.77

Values are expressed as Mean±SEM

Discussion:

The antimicrobial study revealed that the extract is having broad spectrum of activity against both gram positive and gram negative microbes. Because of this wide antimicrobial activity the extract can be used in the treatment of various infections or inflammations. The mechanismof action should be identified by further works.

Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. DPPH is a stable free radical at room temperature. As the electron became paired in the presence of free radical scavenging the absorption vanishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. The bleaching of DPPH molecules can be correlated with the number of available hydroxyl groups. We can infer that, the activity of the extract may be probably due to the presence of substance with an available hydroxyl group. The extracts are able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine [23,24,25] The discoloration increases as the radical scavenging activity increased. The results showed that as the increased concentration of extract of *Strychnos colubrina Linn* showed a proportional increase in activity. The saponins or flavonoids or other phenolic compounds in the extract may reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen.[26]

In H_2O_2Scavenging activity, the Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [27]. The extract had shown only a mild H_2O_2 scavenging activity.

In Hydroxyl radial scavenging activity**,** the highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of *Strychnos colubrina* on the inhibition of free radical mediated deoxyribose damage was assessed by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore was determined at 532 nm[28]. The extract showed a competent hydroxyl radical scavenging activity with that of the standard**.**

The NO scavenging method is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions[29]. The extract had shown potent NO scavenging activity at concentrations of 100, 200and 400µg/mL. The activity of extract at these concentrations is comparable with the standard.

As per the reducing power assay results, the extract showed a moderate antioxidant efficacy efficacy. The reducing power of extract might be due to their hydrogen donating ability. The reducing power of a compound is related to its electron transferability and may serve as a significant indicator of its potential antioxidant activity possibly, the extracts could react with radicals to stabilize and terminate radical chain reactions[30]

Superoxide has been observed to directly initiate lipid Peroxidation. Ferrous iron can initiate lipid peroxidation by the fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides

into peroxyl and alkoxyl radicals. Superoxide anion radical plays an important role in the formation of other reactive oxygen-species such as hydroxyl radical, hydrogen peroxide, or singlet oxygen in living systems [31], so evaluating the scavenging effects of *S. colubrina* on superoxide radicals is one of the most important ways to clarify the mechanism of any antioxidant activity. At 200µg/mLconcentration, it showed a maximum antioxidant activity

Invitro anticancer activity by MTT assay:

HaCaT is a spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin[32]. The agents that decrease the proliferation on these cells are an indication of their use in skin proliferation. The results are represented in the graph. It shows that the viability decreases with increase in concentration of extract. At concentrations of 100µg/mL it showed a better cytotoxic effect

Acknowledgement:

The authors wouldlike to thank the VIT University for providing the support for carrying out this work

References

1. Ohiri F.C, Verpoorte R and Baerheim S.A., The African *Strychnos* species and their alkaloids: a review, Journal of Ethnopharmacology, 1983, 9, 167-223.
2. Nambiar V.P.K, Sasidharan N, Renuka C and Balagopalan M., KFRI Research Report, 1985, 42, 94.
3. Kumar A and Schweizer H.P., Bacterial resistance to antibiotics: active efflux and reduced uptake, Advanced Drug Delivery Reviews, 2005, 57(10), 1486–1513.
4. Pesaramelli K, Vellanki J, Keerthi D.V and Chaitanya S., Evaluation of antibacterialactivity of herbs, International Research Journal Of Pharmacy, 2012, 3(8), 230-32.
5. Kavitha R, Damodharan N, Uwadokakelechi D and Olabiyi B.B., Ultrasonic-Assisted Extraction and Antibacterial Activites of Protein Recovred from White Button Mushroom (Agaricus bisporus), Int.J. PharmTech Res, 2015,8(5), 991-95
6. Helmina B.S, Tonel B, Lamek M and Partomuan S., Antioxidant and Antibacterial Activity of Some Leaves Extracts (Methanol, Ethyl Acetate and N-Hexane) of Scurrula fusca G.Don. Int. J. PharmTech Res, 2015,8(9), 24-30.
7. Ajmer S.G and Sonika R. Synthesis., Antibacterial and Antifungal Activity of 2,5- Disubstituted-1,3,4-oxadiazole Derivatives. Int.J. PharmTech Res,2014,6(7), 2015-2021.
8. Anjali T, Pusp R.S.G, Prateek P and Ankit K., Design, Synthesis, SAR, Docking and antibacterial evaluation: Aliphatic amide bridged 4-aminoquinoline clubbed 1,2,4- triazole derivatives. International Journal of ChemTech Research, 2016,9(3),575-588.
9. Chang S.T, Wu J.H, Wang S.Y, Kang P.L, Yang N.S and Shayur L.F., Antioxidant activity of extracts from Acasia confuse bark and heartwood, Journal of Agricultural and food chemistry, 2001, 49, 3420-3424.
10. Das P and Himaja M., Antioxidant, anti-arthritic and hypoglycemic activity of Oxalis corniculata Linn. leaf extracts, Int. J. PharmTech Res. 2015, 8(7), 51-57.
11. Faten A.S, Francois K , Manal D and Chadi S., Composition, in Vitro Antioxidant and Antileishmanial activities of Vitex agnus-castus L. and Thymus syriacus Boiss. Essential Oils, Int. J. Chem Tech Res. 2015,8(8), 53-60
12. Ruch R.J, Cheng S.J and Klaunig J.E., Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen*,* 1989, 10, 1003-1008.
13. Ruch R.T, Cheng S.J and Klaunig J.E., Spin trapping of superoxide and hydroxyl radicals, methods in enzymology, 1984, 105, 198-209.
14. Halliwell B and Gutteridge J.M.C., Free Radicals in Biology and Medicine,Oxford University Press, UK, 1989, 231-250.
15. Halliwell B, Gutteridge J.M.C and Aruoma O.I., The deoxyribose method: a simple 'test tube' assay for determination of rate constant for reaction of hydroxyl radicals, Analytical biochemistry, 1987, 165, 215-219.

16. Panda B.N, Raj A.B, Shrivastava N.R and Prathani A.R., The evaluation of nitric oxide scavenging activity of Acalypha indica Linn Root, Asian Journal Research Chemistry, 2009, 2(2), 148–150.
17. Yen G.C and Duh P.D., Antioxidative properties of methanolic extracts from peanut hulls, journal of the American oil chemistry society, 1993, 70, 383-386.
18. Elizabeth K and Rao M.N.A., Oxygen radical scavenging activity of curcumin, International Journal of Pharmaceutics, 1990, 58, 237–240.
19. Liu F, Ooi V.E.C and Chang S.T., Free radical scavenging activity of mushroom polysaccharide extracts, Life Science, 1997, 60, 763-771.
20. Gayathri S, Divyalakshmi D, Mithula S, and Rupachandra S., Invitro anticancer effects of methanolic seed coat extract of Momordica dioica against human carcinoma cell lines. Int.J. ChemTech Res, 2016,9(2), 284-289.
21. Osman M.E, Eid M.M, Khattab O.H, El-Hallouty S.M, El-Manawaty M and Mahmoud D.A., In vitro cytotoxicity of biosynthesized Ag/CS NP against MCF7, PC3 and A549 Cancer cell lines. Int.J. PharmTech Res. 2015,8(5)1011-1017.
22. Ghaisas M.M, Navghare V.V, Takawale A.R, Zope V.S and Deshpande A.D., Hepatoprotective activity of aqueous and ethanolic extract of *Trichosanthes dioica* roxb. in ferrous sulphate-induced liver injury**,** Pharmacologyonline, 2008, 3, 296 -305
23. Kiranmayi G, Ramakrishnan G, Kothai R and Jaykar B., in-vitro Anti-Cancer activity of Methanolic extract of leaves of Argemone mexicana Linn. Int. J. Pharm Tech Res.2011,3(3).1329-1333
24. Blois M.S., Antioxidant determination by the use of a stable free radical, Nature, 1958, 26, 1199-1200.
25. Blois M.S., Antioxidant activity of grape seed extracts on peroxidation models *in-vitro*, J Agri Food Chem, 2001, 55, 1018.
26. Nadia S.A, Azman A. A., Review of Flavonoid Quercetin: Metabolism, Bioactivity and Antioxidant Properties. Int.J. PharmTech Res.2014,6(3), 933-941.
27. Kumaravel R.S, Maleeka Begum S.F, Parvathib H and Senthil K., Phytochemical screening and *in vitro* antioxidant activity of ethyl acetate leaf extracts of *Pterocarpus marsupium* Roxb (Fabaceae), Int J Curr Sci, 2013, 9, 46-55.
28. Peitta P.G., Flavonoids as antioxidants, Journal of Natural Products, 2000, 63, 1035-42.
29. Nabavi S.M et al., Determination of antioxidant activity, phenol and flavonoids content of *Parrotiapersica Mey*, Pharmacologyonline, 2008, 2, 560-567.
30. Sreejayan N and Rao M.A., Nitric oxide scavenging by curcuminoids, J Pharmacol,1997,49(1),105-7.
31. Patel D.S, Shah P.B and Managoli N.B., Evaluation of *In-vitro* Anti-oxidant and Free Radical Scavenging activities of *Withania somnifera* and *Aloe vera,* Asian J. Pharm. Tech, 2012, 2,143-147.
32. Stief T.W., The physiology and pharmacology of single oxygen. Med. Hypotheses*,* 2003,60, 567-572.

*****