IJPSR (2013), Vol. 4, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 April, 2013; received in revised form, 12 June, 2013; accepted, 18 August, 2013; published 01 September, 2013

EVALUATION OF ANTI-OXIDANT, ANTI-INFLAMMATORY AND CYTOTOXICITY POTENTIAL OF *HEMIGRAPHIS COLORATA*

T.T. Akhil* and Punieethaa Prabhu

Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode- 637 215, Nammakkal, Tamil Nadu, India

Keywords:

DPPH, cytotoxicity, Anti-inflammatory, RBC

Correspondence to Author:

T.T. Akhil

B. Tech. Biotechnology, Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode- 637 215, Nammakkal, Tamil Nadu, India

E-mail: akhiltthilak@gmail.com

ABSTRACT: Plants have been used as a source of natural products for various therapeutic processes from a long time. The proposed work illustrates the study of the importance of natural products in terms in-*vitro* approaches. On account of this study the phytochemical constituents of various extracts of *Hemigraphis colorata* (Blume) are evaluated. The plant was traditionally known for its wound healing activities. Crude plant extract was prepared by percolation method using a Soxhlet apparatus. The solvents used were Hexane, Chloroform, Acetone and Ethanol .The *in-vitro* antioxidant capacity was analysed through DPPH and reducing power assays. Anti-inflammatory properties of various extracts from the whole plant are analyzed through human RBC membrane stabilisation method. The anti-oxidant and anti-inflammatory activities were more for the ethanolic extracts of *Hemigraphis colorata* when compared to acetone, chloroform and hexane extracts.The in-*vitro* cytotoxicity analysis of ethanolic extract was also carried out in the present study using trypan blue method

INTRODUCTION: Natural products especially which are derived from plants have been used as a source for various therapeutic processes for long times from the human civilisation. The most common strategy of drug development from plants is careful observation of use of natural resources in flock medicine in different cultures by ethanopharmacology [1]. Free radicals have been implicated in numerous diseases, cancer, inflammation and neurodegenerative disorders [6]. The current drugs which are used as anti-inflammatory drugs can cause severe side effects.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(9).3477-83

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(9).3477-83

Ethanopharmacological approach of analysing the phytochemical properties of various extracts of *Hemigraphis colorata* (Family: Acanthaceae) is used in the present study. The anti-oxidant, anti-inflammatory potential of this plant is exploited in the present study. *Hemigraphis colorata* plant is claimed in flock medicine for its very good wound healing activity [2].

Hemigraphis colorata (Blume) H.G. Hallier leaf paste on the wound promotes wound healing in mice but the oral administration was in effective [2]. The phytochemical constituents on *Hemigraphis colorat*a were identified by examining the crude extracts of its leaves and stem using various solvents, screened the antibacterial activity against selected pathogens [7]. The crude aqueous, acetone, benzene, chloroform, ethanol, and petroleum ether extracts of the plant had phenols, carbohydrates, steroids, saponins, coumarins, tannins, proteins,

flavonoids, alkaloids. The hypoglycaemic and anti-diabetic properties of *H. colorata* was identified for the first time using wistar rats and Swiss albino mice by [3].

Glucose lowering effect and antidiabetes activity studied using glucose tolerance test with different extracts .n-Hexane and ,to some extent ethanolic extracts were found to lover the levels of blood glucose in glucose feed rats. The pharmacological actions of ethanolic extracts of leaves of *Hemigraphis colorata* and Clerodendron *phlomoides* were studied by [4] which reviewed the anti-inflammatory effects, antioxidant activities; wound healing properties, effects on arthritis by analysing Phytochemical profiles.

The preliminary photochemical studies of methanolic extract of *H. colorata* to evaluate wound healing activity of the methanolic extract ointment of dried leaves of *H. colorata* in albino rats and found that increase in tensile strength of treated wounds may be due to the increase in collagen concentration per unit area and stabilisation of fibers[5].An attempt to screen various compounds responsible for the anti-oxidant and anti-inflammatory properties by *in vitro* assays is carried out in the present study.

MATERIALS AND METHODS:

Collection of Plant: *Hemigraphis colorata* plant species were collected from Mannuthy, Thrissur district of Kerala during the month of July to September 2012 was dried at room temperature and powdered in a mixer grinder for further analysis.

Preparation of Extracts: Crude plant extract was prepared by percolation method using a Soxhlet apparatus [8]. About 10 grams of plant material was packed uniformly into a thimble and extracted with 150 ml of different solvents separately based on the increasing order of polarity n-Hexane, chloroform, acetone, and ethanol were the solvents used. The extraction process was continued till the solvent in siphon tube of the apparatus became colourless. The crude extract was filtered and concentrated under vacuum and controlled temperature. The extract was stored in refrigerator at 4 °c until further use.

Qualitative Phytochemical Analysis: The preliminary phytochemical analyses were carried out using the whole plant extracts of Hemigraphis colorata followed by [9] Phenols and tannins were identified by ferric chloride test, test for flavonoids were performed using lead acetate solution, saponins were identified by froth test, glycosides were identified by adding concentrated H_2SO_4, steroids by Salkowski's test, alkaloids by adding Dragendroff's reagent, and reducing sugars with Fehling's test.

Determination of Anti-Oxidant Activity

1. **DPPH Free Radical Scavenging Assay:** The free radical scavenging capacity of the *Hemigraphis colorta* whole plant extract was determined using DPPH according to the method of Blios with slight modifications[12]. DPPH solution (0.004% w/v) was prepared freshly in 80% methanol and was added to 1ml sample solutions containing various extracts ranging from concentration 100µg/ml - 500µg/ml .The mixture was allowed to stand at room temperature in dark for 30 minutes and vortexed. The absorbance was read at 517nm using spectrophotometer. Reference standard used was Ascorbic acid while DPPH solution without any extract served as control.80% methanol solution was used as blank solution. All tests were performed in triplicates. Percentage scavenging of the DPPH free radical was measured using the following equation,

Radical scavenging activity (%) =

$$(A_{control} - A_{Test})/A_{control} \text{ X } 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{Test} is the absorbance in the presence of the extracts.

2. **Reducing power (FRAP) Assay:** The Fe^{3+} reducing power of the whole plant extract of *Hemigraphis colorata* was determined by the method of Oyaizhu with slight modifications [12]. Different concentrations (100 - 500µg/ml) of the various extracts of were mixed with phosphate buffer (0.2 M, pH6.6) and made up to 1ml , 0.5 ml potassium ferrocyanide (1%), followed by incubation at 50°C in a water bath for 20 min. After incubation, 0.5 ml of Trichloroacetic acid (10%) was added to terminate the reaction. The upper portion of the

solution (1ml) was mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution in UV spectrophotometer. All tests were performed in duplicates. A higher absorbance of the reaction mixture indicated greater reducing power. Standard: Ascorbic acid

3. **Anti-inflammatory properties:** The anti-inflammatory properties of *H. colorata whole plant* extracts were analysed by Human red blood cell membrane stabilization method [10]. Fresh blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride) and were centrifuged at 3000 rpm and the packed cells obtained were washed with isosaline (0.85% NaCl, pH 7.2) thrice and 10% (v/v) suspension was made. Solutions of different concentrations ranging from 50 -300 µg/ml of the solvent extracts of *H. colorata* were prepared.

   Assay mixture contained the active drug, 1 ml of phosphate buffer (0.15 m pH 7.4) 2 ml of hyposaline (0.25% Nacl) and 0.5 ml of 10% RBC suspension. Control tube contained 2 ml of distilled water instead of hyposaline. All the tubes were incubated at 37°C for 30 min. They were centrifuged at 3000 rpm for 20 minutes and the haemoglobin content in the supernatant was estimated using UV spectrophotometer at 560 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated.

% inhibition of haemolysis =

$$[OD_1\text{-}OD_2/OD_1] \text{ X } 100$$

Where, OD_1 = Optical density of hypotonic-buffered saline solution, OD_2 = Optical density of test sample in hypotonic solution.

4. **Cytotoxicity screening:** The *in vitro* short term cytotoxicity of ethanolic extract of *H. colorata* was assessed using DLA (Daltons Lymphoma Ascites) tumor cell lines by Trypan blue exclusion method. The cell lines were obtained from Amala Cancer Research Institute, Thrissur, Kerala, India

   Cell viability was determined by trypan blue exclusion method [13] .Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the ethanolic extracts of *H. colorata* was dissolved in DMSO and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension .These assay mixture were incubated for 3 hours at 37°C. Further cell suspension was mixed with 0.1 ml of % trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells was counted separately.

RESULTS AND DISSCUSSION:

Plant collection and extraction: The *H. colorata* plant collected and the dried whole plant parts was powdered and extracted successively using with hexane, acetone, chloroform and ethanol in the increasing order of polarity and their colour and consistency were studied. The ethanolic extract showed maximum average extractive yield of 2.4% (w/w) and least value for acetone extract of yield 0.5% (w/w). The results were tabulated in **table 1**.

TABLE 1: COMPARISON OF VARIOUS SOLVENT EXTRACTS OF *H. COLORATA* BY CONTINUOUS HOT PERCOLATION METHOD BY SOXHLET APPARATUS

Solvent used (150 ml)	Color and consistency	Polarity	Boiling point of solvent	Average yield of extraction (% w/w)
Hexane	Yellowish ,sticky	1.88	69	1.3
Chloroform	Dark green	5.6	35	1.4
Acetone	Green	21	56	0.5
Ethanol	Yellowish green	24.3	79	2.4

Qualitative Phytochemical Analysis: The phytochemical characteristics of *H. colorata* revealed the presence of active compounds. The results were summarized in **table 2**. Analysis of the plant extracts revealed the presence of phyto-constituents which are known to exhibit medicinal as well as physiological activities [9]. Phytochemicals such as phenols, tannins, alkaloids, glycosides, steroids, saponins etc. were found in the extracted compounds. Ethanolic extract showed the presence of flavonoids, steroids, phenols and tannins, saponins, glycosides and reducing sugars. The observations were in accordance with [7].

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *H. COLORATA*

Compounds	Hexane extract	Acetone extract	Chloroform extract	Ethanolic extract
Alkaloids	-	-	+	-
Flavonoids	-	-	-	+
Steroids	+	-	-	+
Phenols and tannins	-	+	+	+
Saponins	-	+	+	+
Glycosides	-	-	-	+
Reducing sugars	+	+	+	+

+ indicates presence, - indicates absence

DPPH Radical Scavenging Assay: Antioxidants react with DPPH, a stable free radical which is reduced to DPPH-H and as a consequence the absorbance is decreased from the DPPH radical to the DPPH-H. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [12]. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were performed in triplicates. Percentage scavenging of the DPPH free radical was measured and expressed in graph along with error value at 5% significance as in **fig. 1**.

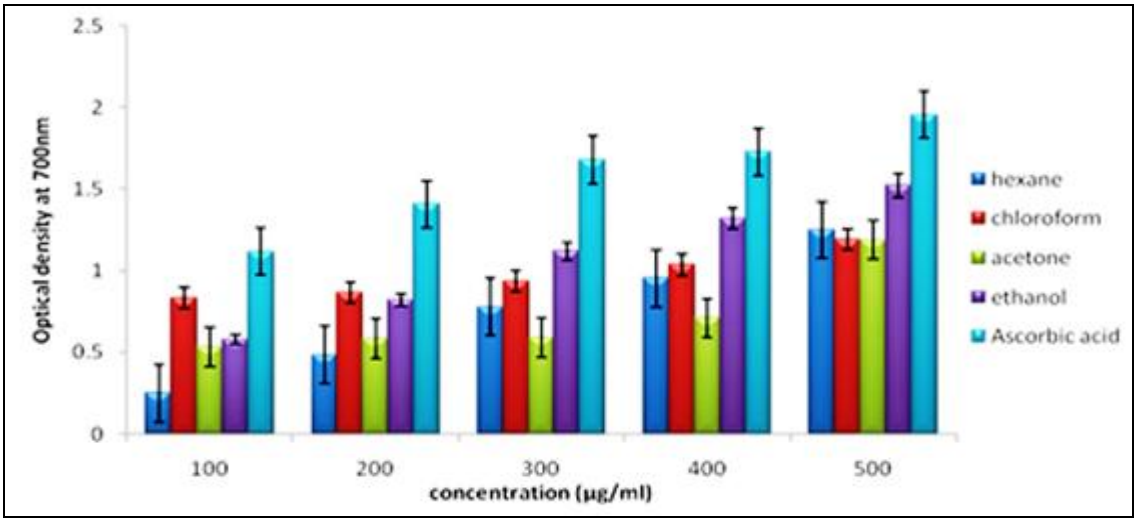

FIGURE 1: DPPH RADICAL SCAVENGING ASSAY OF VARIOUS EXTRACTS WITH STANDARD ERROR AT 5% OF SIGNIFICANCE

The percentage of radical scavenging property was more for ethanolic extract per 100µg of extract was 91.10 ± 1.71% and that of the control was 97.76 ± 0.70 %. The radical scavenging activities of extracts of leaf was expressed as the IC_{50} value (µg/ml), i.e., the concentration necessary to decrease the DPPH concentration by 50%. The results revealed that ethanolic extract of *H. colorata* has maximum activity when compared with the standard ascorbic acid. The concentration necessary to decrease the DPPH concentration by 50% of ethanolic extract was found to be 54.88mg/ml. The present study revealed that ethanolic extracts of *H. colorata* exhibits proton donating ability and could serve as good antioxidants by scavenging free radicals [13].

Reducing Power (FRAP) Assay: Reducing power is the measure to test the reductive ability of anti-oxidant and is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of various plant extracts [15].

The formation of Prussian blue colour was measured at 700 nm against an appropriate blank solution in UV spectrophotometer. All tests were performed in triplicates and the result is expressed in **fig. 2** along with error value at 5% significance. A higher absorbance of the reaction mixture indicated greater reducing power. The results obtained shows ethanolic extract of *H. colorata* have more reducing power when comparing with other extracts of the plant. Hexane extract showed lower absorbance while ethanolic extract had good increasing value for increasing concentration [14].

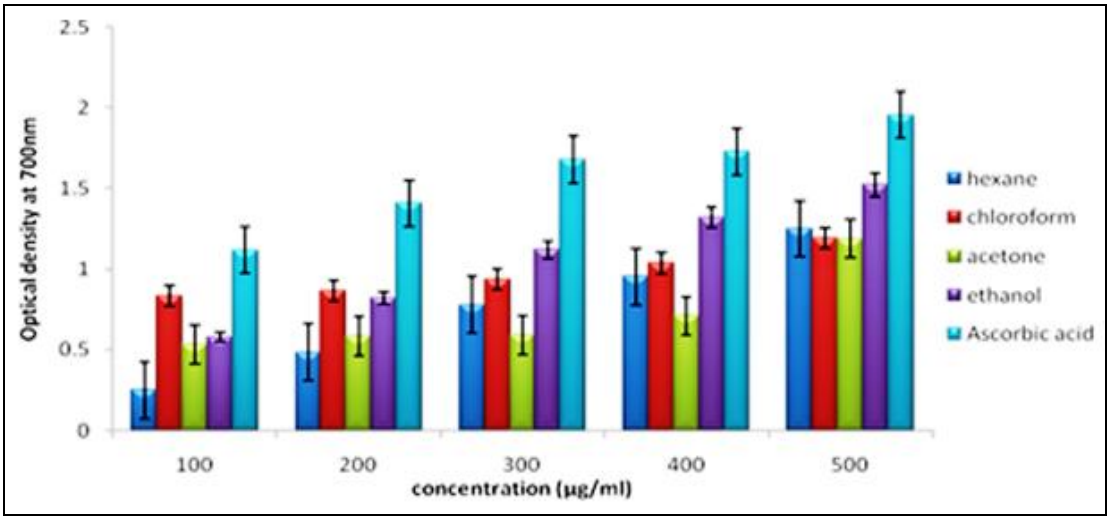

FIGURE 2: RESULTS OF FRAP ASSAY WITH STANDARD ERROR AT 5% OF SIGNIFICANCE

Anti-inflammatory analysis: The *in vitro* anti-inflammatory assay was carried out by HRBC membrane stabilization method. The ethanolic extract showed good percentage of membrane stabilisation when compare to hexane, acetone, and chloroform extracts. Diclofenac sodium was used as the reference standard. The absorbance of human blood treated with the assay mixture at 560 nm is tabulated in **Table 3**. The percentage inhibition of haemolysis or membrane stabilization was calculated and plotted in **fig. 3** by mentioning 5% significance of error value. Ethanolic extract of *H. colorata* showed a good percentage of inhibition of haemolysis when compared to the other extracts. The results were comparable with [12].

TABLE 3: RESULTS OF ABSORBANCE VALVES HRBC MEMBRANE STABILIZATION TEST AT 560 NM

Solvents used	Diclofenac sodium	Acetone	Chloroform	Hexane	Ethanol
Concentration (µg/ml)	**Optical Density at 560 nm**				
50	0.66±0.07	0.66±0.2	0.722±0.25	0.66±0.18	0.68±0.21
100	0.392±0.9	0.52±0.38	0.624±0.43	0.52±0.23	0.56±0.38
150	0.181±1.2	0.44±0.56	0.578±0.7	0.44±1.3	0.47±0.56

All readings are represented as mean± standard error value at 5% level of significance

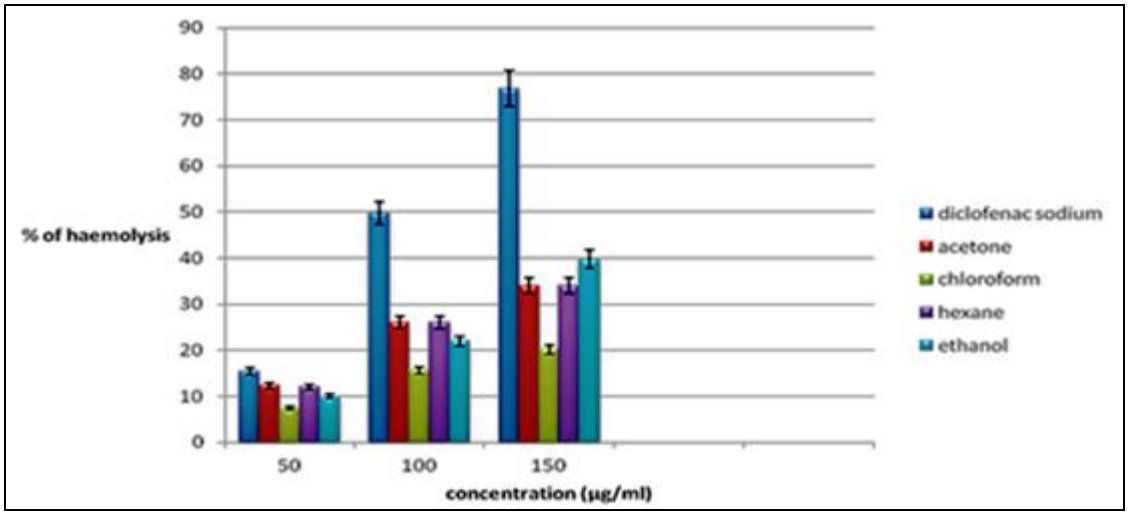

FIGURE 3: RESULTS OF HAEMOLYSIS DISPLAYED WITH ERROR VALUE OF 5% SIGNIFICANCE

The reference drug Diclofenac sodium showed 76.79 % of haemolysis while it was 39.34%, 34%, 20.4%, 34% for ethanolic, acetone, chloroform, and hexane extracts respectively, in the concentration of 150µg/ml. The anti-inflammatory effects of the *H. colorata* extracts may be due to the presence of flavonoids, tannins, phenols etc. The enzyme phospholipase A2 is responsible for the formation of mediators of inflammation such as prostaglandins. By attracting polymorphonuclear leucocytes to the site of inflammation, they can lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins. Prostaglandins are main compounds responsible for inducing pain during inflammation.

Cytotoxicity assay: The trypan blue exclusion method was used to study the cytotoxicity when the DLA cells obtained from the carrier mice were treated with different concentrations of the ethanolic extract of *H. colorata* for 3 hours the relative cell survival progressively decreased in a dose dependent manner .The cytotoxic effect of ethanolic extract of *H. colorata* against the activity against DLA cell lines is relatively poor which is in accordance with the report on *H. colorata* which exhibit low cytotoxicity against DLA cell lines up to a concentration of 200 µg/mL in a short-term bioassay. The results were tabulated in **table 4**.

TABLE 4: *IN VITRO* CYTOTOXICITY STUDY

Sl no.	Concentration (µg/ml)	Percentage of cell death
1	10	0
2	20	0
3	50	2
4	100	10
5	200	20

CONCLUSION: The results obtained suggests that the ethanolic extracts of *Hemigraphis colorata* possess good antioxidant capacity to scavenge various free radicals which may be due to the presence of phytochemicals like flavonoids, tannins, saponins, and phenolic compounds .The anti-inflammatory properties of the various extracts of *Hemigraphis colorata* comparatively less in the human RBC membrane stabilization test.

The present study has been found useful in the identification of several phytoconstituents like phenols, tannins, steroids, flavanoids, saponins, reducing sugars; glycosides were present in the ethanolic extract of whole plant of *Hemigraphis colorata.* , which may be responsible for the anti-oxidant and anti-inflammatory actions. The active fraction obtained from the ethanolic extract of whole plant can be used for further studies leading to possible drug development process involving the *in silico* process.

The cytotoxicity studies on the ethanolic extracts had poor cytotoxic effects which revealed that the extracts were not too toxic to the cells, which adds advantage to the drug development process. Development of phytomedicines is relatively inexpensive and less time consuming. Various environmental factors may lead to variations in the phytochemical content of the plant materials.

REFERENCES:

1. Rates SMK: Plant sources as drugs, Toxicon, Elsevier 2001; 39: 603-613
2. Subramonian A , Evans DA, and Sreekandan Nair G: Effect of *Hemigraphis colorata* (Blume) H.G. Haller leaf on wound healing and inflammation in mice , Int.Journal of Pharmacology 2001; 33:283-285
3. Gayathri V, Lekshmi P, Padmanabhan RN: Antidiabetes and hypoglycaemic properties of *Hemigraphis colorata* in rats, International J.Pharam Science 2011; 4(2): 224-328
4. Joen RS, Thanasekaran J, Chao-Chien C, Yueh-Chung C and Shanmuga P: Pharmacological actions of an ethanolic extracts of the leaves *Hemigraphis colorata* and *Clerodendron phlomoides*, Clinical molecular medicine 2011; 3(2):1-3.
5. Saravanan J, Shariff WR, Joshi Narasimhachar H, Varatharajan R, Joshi VG, Karigar Asif A : Preliminary Pharmacognostical and Phytochemical Studies of Leaves of *Hemigraphis colorata*, Research J. Pharmacognosy and Phytochemistry, 2011; 2(1): 15-17
6. Alviano DS and Alviano CS : Plant extracts-search for new alternatives to treat microbial diseases current pharm. Biotechnology 2011; 10: 106-121
7. Vimala Thankappan Anitha, Johnson Marimuthu @ Antonisamy and Solomon Jeeva: Anti-bacterial studies on *Hemigraphis colorata* (Blume) H.G. Hallier and *Elephantopus scaber* L, Asian Pacific Journal of Tropical Medicine 2011; 20:52-57
8. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur: Phytochemical screening and extraction: a review, Internationale Phamaceutica Sciencia 2011; 1(1): 98-106.
9. RNS Yadav and MuninAgarwala: Phytochemical analysis of some medicinal plants Journal of Phytology 2011; 3(12): 10-14.
10. Vijender K, Zulfiqar AB, Dinesh K, Khan NA, and Chashoo IA: Evaluation of anti-inflammatory potential of leaf extracts of *Skimmia anquetilia*, Asian Pacific Journal of Tropical Biomedicine, Elsevier 2012; 627-630.

11. Talwar GP: Handbook of Practical Immunology. National Book Trust, New Delhi, 1974: 329.
12. Arunachalam Kumar, Suchetha Kumari N and Divya Bhargavan: Evaluation of in vitro antioxidant potential of ethanolic extract from the leaves of *Achyranthes aspera* AJPCR 2012; 5(3): 146-148
13. Pracheta Veena Sharma, Ritu Paliwal, Sadhana Sharma: Preliminary phytochemical screening and *in vitro* antioxidant potential of *Euphorbia neriifolia* Linn. Int.J.PharmaTech Res 2011; 3(1):24-132.
14. Nazeema TH and Girija: Comparative phytochemical investigations and in-vitro pharmacological studies of two medicinal species of *Jatropha* from South India. European Journal of Experimental Biology 2012; 2(24): 21-426.

How to cite this article:
Akhil TT and Prabhu P: Evaluation of anti-oxidant, anti-inflammatory and cytotoxicity potential of *Hemigraphis colorata*. *Int J Pharm Sci Res* 2013: 4(9); 3477-3483. doi: 10.13040/IJPSR. 0975-8232.4(9).3477-83