



CODEN [USA]: IAJPBB

ISSN : 2349-7750

## INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

Available online at: <http://www.iajps.com>

Research Article

### EVALUATION OF *IN- VITRO* RHEUMATOID ARTHRITIC ACTIVITY OF POLYHERBAL ETHANOLIC EXTRACT CONTAINING FORMULATIONS FOR SELECTED POTENTIAL INDIAN HERBS

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**Article Received:** February 2022**Accepted:** February 2022**Published:** March 2022**Abstract:**

*Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response.<sup>[3]</sup> One fifth of the world's elderly suffer with arthritis. In the anti-arthritic activity the production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. The mechanism of denaturation probably involves electrostatic hydrogen, hydrophobic and disulphide bonding. Anti denaturation study which includes the albumin denaturation is performed by using Bovine serum albumin (BSA). When BSA is heated it undergoes denaturation and express antigens associated with type 3 hypersensitivity reactions and that is related to diseases such as serum sickness, glomerulonephritis, rheumatic arthritis, and lupus erythematosus.*

**Key words:** *Inflammatory, Arthritis, Medical response, Lysis*

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Please cite this article in press R.Margret Chandira et al, *Evaluation Of In- Vitro Rheumatoid Arthritis Activity Of Polyherbal Ethanolic Extract Containing Formulations For Selected Potential Indian Herbs*, Indo Am. J. P. Sci, 2022; 09(3)

## INTRODUCTION:

Inflammation is a normal protective response to tissue injury which involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair.[1] It is characterized by redness, swelling, pain, stiffness of joint and loss of joint function. Inflammation is associated with membrane alterations, increase in vascular permeability and protein denaturation. [2] Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response.[3] One fifth of the world's elderly suffer with arthritis.[4] The current treatment of arthritis includes minimization of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs.[5,6] Due to adverse reactions of the NSAIDs and disease modifying anti-rheumatic drugs, the arthritic patients tend to search for other treatments that are effective and less toxic. Therefore, complementary and alternative medicines are commonly preferred by such patients.[ 7]

*Polygonumglabrum*: the tribes of Chhattisgarh use the root paste as a medicine for snake bite [20]. In some areas the root stock is used for the treatment of jaundice and piles. [8] The leaves are used as an antimalarial agent in Sudan. [9] In south India *Polygonumglabrum* leaves are used for the treatment of dysentery. [10] A decoction of the leaves and seeds are used as cardio tonic, astringent and anthelmintic.[11]The whole plant decoction is used as a remedy for colic pain, pneumonia and the boiled paste is applied in cuts and wounds.[12]Apart from medicinal use, the whole plant is powdered and used as bait for fishing. Peels from stem are used for treating rheumatism.[13]

*Ochnaobtusata* DC. (FamilyOchnaceae).is a small tree up to 8 m tall. The family is characterized by the presence of secondary metabolites like flavonoids, terpenoids. [14]And it is extensively used in Indian traditional medicine for the treatment of epilepsy, menstrual complaints, lumbago, asthma, ulcers, and as an antidote to snake bites. [15] Several studies conducted on *Ochnaspecies* revealed the presence of glycosides, saponins, steroids, flavones and fatty acids. [16] The leaves and roots of *O. obtusata*are used for ulcer, asthma and bronchitis and also possess anti-ulcer genic activity. [17]

*Canthiumdiococcum* Ethanolic extract of whole plant of *Canthiumdiococcum* for anti-inflammatory activity in Wister albino rats in various models of anti-inflammatory activity viz. Carrageenan induced paw edema, Formalin induced paw edema, fresh egg white induced paw edema and cotton pellet induced granuloma model. Results showed the extract with anti-inflammatory activity and suggests a potential alternative to NSAIDS like diclofenac.[18]ethanolic extract of *Canthiumdiococcum* for anti-diabetic in an alloxan induced diabetic rat model. Results showed a significant drop in fasting blood sugar in a dose-dependent manner, with an effect on the beta-cell population in the pancreas. The extract showed almost equipotent anti-diabetic activity compared to standard drug Glibenclamide. [19] Ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against Egg-albumin induced arthritis model.[20]

In the recent studiesof the author ethanolic extract of the above plants and the polyherbal formulations with different fractions of ethanolic extract showed good anti-oxidant activity.The present study is designed to evaluate the ant arthritic activity for the different polyherbal formulations

## MATERIALS AND METHODS:

### Plant source and authentication:

*Polygonumglabrum*, *Ochnaobtusata* DC,and *Canthiumdiococcum* were collected from Tirumala Hills, Tirupati, and Chittoor district of Andhra Pradesh, near Seshachalam and Tirumala Hills. The plant specimen was verified to be of the correct species by Dr. MadhavaSetty, a botanist from the Department of Botany, S. V. University, Tirupati .

### Chemicals and reagents:

All the chemical are used analytical grade and the Egg Albumin ,Bovine Serum And Diclofenac sodium obtained from sigma Aldrich

### Preparation of poly herbal extract:

Aerial parts of *Polygonumglabrum*, *Canthiumdiococcum*,*Ochnaobtusata* were collected and dried. Then the material was blended to form a fine powderand extracted Ethanol using Soxhlet apparatus for 6 hrs at 50°C and water by maceration The solvent was completely removed by rotary evaporator (Rotavapor® R-210, BUCHI Corporation) and respective extracts preserved for various investigations.

### Preparation of ethanol extract of four selected polyherbal formulations using different portions

The above extract used for the preparation of five different poly herbal formulations with varying proportions and working formula given in the table.1

	FORMULATIONS	PGEE	CDEE	OOEE
1	FORMULATION 1	1	1	1
2	FORMULATION 2	2	1	1
3	FORMULATION 3	1	2	1
4	FORMULATION 4	1	1	2

#### Preliminary phytochemical studies: [28-30]:

Previously various preliminary phytochemical tests were performed using standard procedures and the above formulations showed the presence of mainly carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids and saponins which majorly responsible for the desired activity.

#### Evaluation of in-vitro anti-arthritis of polyherbal formulations:

##### Inhibition of protein denaturation method using bovine serum [31 -34]

Preparation of the standard solution: The standard solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5 % w/v aqueous solution) and 0.05 ml of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test solution: The test solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test control solution: This solution (0.5 ml) was prepared using 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.

#### Experimental procedure:

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

**Percentage inhibition =  $\frac{[100-(\text{optical density of test solution} - \text{optical density of product control})}{(\text{optical density of test control})} \times 100$ .**

#### Inhibition of protein denaturation method using egg albumin [35-38]

Preparation of the standard solution: The standard solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test solution: The test solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of different concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml).

Preparation of the test control solution: This solution prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of distilled water.

#### Experimental procedure:

All above solutions were incubated at  $37 \pm 2^\circ\text{C}$  in incubator for 15 min and then heated at  $70^\circ\text{C}$  for 5 min. After cooling their absorbance were measured at 660 nm by using vehicle as a blank. The percentage inhibition of protein denaturation was calculated by using the following formula

**Percentage inhibition =  $\frac{[100-(\text{optical density of test solution} - \text{optical density of product control})}{(\text{optical density of test control})} \times 100$ .**

#### HRBC membrane stabilization method [39-45]:

The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and was mixed with equal volume of sterilized alsevers solution. The blood solution was centrifuged in a centrifugation

machine at 3,000 rpm for 15 min and the upper layer was carefully removed with a syringe or sterile pipette. The packed cells remained at the bottom were separated and washed with isosaline solution and a 10% v/v suspension was made with isosaline. Human red blood cells suspension was used for the study.

**Preparation of the standard solution:** The standard solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of distilled water. **Preparation of the test solution:** The test solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of extract solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of

distilled water. **Preparation of test control:** The test control solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline, 2.5 mL of distilled water.

#### Experimental procedure:

All the assay mixtures were incubated at 37°C for 30 min and centrifuged at the rate of 3,000 rpm. The supernatant liquid was poured out and the hemoglobin content was estimated by UV spectrophotometer at 560 nm. The percentage of human red blood cell membrane stabilization or protection against hypotonicity induced hemolysis was calculated by using the following formula.

$$\text{Percentage protection} = 100 - \left[ \frac{\text{optical density sample}}{\text{optical density control}} \times 100 \right]$$

## RESULTS:

**Table no .2 Inhibition of protein denaturation method using bovine serum**

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	51.92±0.4	37.12±0.4	44.82±0.24	34.22±0.12	39.36±0.34
50	57.81±0.5	43.24±0.6	51.91±0.3	42.91±0.24	46.18±0.4
100	63.14±0.9	49.42±0.6	54.64±0.6	45.82±0.19	69.26±0.8
200	79.12±1.5	57.12±2.5	71.24±1.3	53.64±0.9	61.92±1.4
400	85.46±0.8	65.46±0.4	78.36±0.6	67.74±0.25	64.82±0.6
800	92.16±0.9	74.24±0.2	84.29±0.9	72.92±0.16	76.19±0.8
1000	94.28±1.0	81.26±0.5	89.16±0.6	79.29±0.24	82.28±0.28

**Table no .3 Inhibition of protein denaturation method using egg albumin**

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	52.32±0.8	46.12±0.25	49.42±0.24	42.12±0.5	46.89±0.5
50	58.24±0.6	48.24±0.62	53.91±0.3	44.24±0.2	49.12±0.12
100	65.04±1.2	51.42±0.24	57.64±0.6	48.42±0.4	52.22±0.4
200	73.24±1.4	56.12±2.5	69.24±1.3	51.12±1.5	58.25±2.15
400	76.08±1.18	63.46±0.4	74.36±0.6	59.46±1.4	64.14±0.41
800	79.16±1.5	72.24±0.2	76.29±0.9	68.24±1.2	73.43±0.12
1000	86.14±1.2	79.26±0.5	83.16±0.6	74.26±0.5	81.12±0.15

Table no .4 HRBC membrane stabilization method

Conc µg/ml	Standarad solution	F1	F2	F3	F4
10	56.92±0.4	41.56±0.46	44.56±1.2	39.56±0.4	39.96±0.14
50	62.81±0.5	49.21±0.42	51.21±1.5	45.21±0.6	50.21±1.3
100	89.14±0.9	82.13±1.3	84.13±0.9	82.13±0.25	81.13±1.6
200	91.59±1.5	85.14±0.2	89.14±1.4	78.14±1.2	84.14±1.2
400	94.26±0.8	89.46±1.4	92.46±0.4	84.46±0.6	86.46±0.9
800	96.59±0.9	90.49±1.2	94.49±0.5	89.49±1.3	92.49±0.5
1000	98.98±1.0	92.98±0.25	96.98±0.26	93.98±0.4	93.98±0.6

**DISCUSSION:**

The Ethanolic extracts of *Polygonumglabrum*, *Canthiumdicoccum*, *Ochnaobtusata*, are formulated into four formulation with different portion as given in the above formula and evaluated for *in-vitro* anti-arthritis activity with concentrations varying from 10 µg/ml to 800 µg/ml by the *in-vitro* Inhibition of protein denaturation method using bovine serum, Inhibition of protein denaturation method using egg albumin and HRBC membrane stabilization method comparing with standard drug as Diclofenac sodium. In the five formulation F2 and F4 having the significantly more inhibition of protein denature and membrane stabilization which are concentration dependent.

**CONCLUSION:**

The above result gives a conclusion that the polyherbal formulations with the different concentration have the anti-arthritis activity and the F2 and F4 having significantly more potential. Further investigation is required to use the two formulations in the treatment of Rheumatoid Arthritis

**Acknowledgement:**

We the authors are thankful to the management and staff of VMRF, Salem, Chennai, Department of Pharmacognosy for providing facilities and encouragement.

**Conflict of interest:**

No conflict of interest

**REFERENCES:**

1. Vane JR. Inhibition of prostaglandins synthesis as a mechanism of action for aspirin like drugs. *Nature*, 1971; 231(2): 232-235.
2. Umapathy E, Ndebua EJ, Meeme A, Adam B, Menziura P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albucasetosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell

migration during acute inflammation. *J Med Plant Res*, 2010; 4(5): 789-795.

1. Dixit KP, Mittal S. Herbal sources of anti-arthritis potential: A comprehensive review. *Int J Pharm Biomed Res*, 2013; 4(2): 88-92.
2. 4. Murugananthan G, Sudheer KG, Sathya CP, Mohan S. Anti-arthritis and anti-inflammatory constituents from medicinal plants. *J App Pharm Sci*, 2013; 3(4): 161-164.
3. Patil KR, Patil CR, Jadhav RB, Mahajan VK, Patil PR, Gaikwad PS. Anti-arthritis activity of bartogenic acid isolated from fruits of *Barringtonia racemosa* Roxb. (Lecythidaceae). *Evid. Based Complement. Alternat. Med*, 2011; 1-7.
4. Jadhav RB, Patil CR, Surana SJ, Bhatnagar SP, Patil MJ. Rheumatoid arthritis and herbal drugs: current status and future prospects, in *Phytopharmacology and Therapeutic Values II*. J. N. Govil and V. K. Singh Studium Press, Houston, Tex, USA: 2007; 277-300.
5. 7. Rao JK, Mihaliak K, Kroenke K, Bradley J, Tierney WM, Weinberger M. Use of complementary therapies for arthritis among patients of rheumatologists. *Ann Intern Med*, 1999; 131(6): 409-416.
6. Shiddamallayya N, Azra Yasmeen, Gopakumar K. Medico-botanical survey of kumarparvathakukkesubramanya, Mangalore, Karnataka. *Indian Journal of Traditional Knowledge*. 2010; 9(1):96-99.
7. ElTahir A, Satti GM, Khalid S A. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on maytenusenegalemsis. *Journal of Ethnopharmacology*. 1999; 64(3): 227-233.
8. Soudahmini E, Ganesh M, Senthil PL, Madhu C, Divakar. Herbal remedies of Madugga tribes of Siruvani forest, South India. *Natural Product Radiance*. 2005; 4(6): 492-499.
9. Shankar LH, Mishra PK. Study of aquatic medicinal plants of Hazaribagh district of



- Jharkhand, India. International research journal of pharmacy. 2012; 3(4): 405-409.
10. Koche DK, Shirsat RP, Syed Imran, Mohd. Nafees, Zingare AK, Donode KA. Ethnomedicinal Survey of nagzira wild life sanctuary, District Gondia (M.S.) India-Part II. Ethnomedicinal Leaflets. 2008; 1(8): 532-537.
  11. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary, Springer Science & Business Media, LLC, NY, USA. 2007: 509.
  12. Okigawa M, Kawano N, Aqil M, Rahman WJ. Total synthesis of Ochna flavones. Chem. Soc. Perkin Trans , 1, 1976, 580- 583.
  13. 15.Kamil M, Khan NA, Ilyas M, Rahman W. Biavones from Ochnaceae-a new biavone from Ochnapumila. Indian Journal of Chemistry, 22B, 1983, 608.
  14. Oliveira MCC, Carvalho MG, Werle AA. New biflavonoid and other constitutions from Luxemburgianobilis EICHL. Journal of Brazilian Chemistry Society, 13, 2002, 119-123.
  15. Estevam CS, Oliveira FM, Conserva LM, Lima LF, Barros SCP, Rocha EMM, Andrade EHA. Preliminary screening of constituents of Ourateanitida Av. (Ochnaceae) for In vivo antimalarial activity/. Brazilian Journal Pharmacognosy, 2005, 195-198.
  16. Rajarajeswari N, Ramalakshmi.S, et al “GC-MS Analysis of bioactive components from the ethanolic leaf extract of Canthiumdicoccum (Gaertn.) Teijsm&Binn” J.Chem.Pharm.Res., 2011, 3(3): 792-798.
  17. 19.Patel PD, Patel NJ, et al “In-Vivo evaluation of Pleurotussajorcaju mycelium extract for Anti-inflammatory activity” Pharmacologyonline, 2011, 2: 784-789.
  18. Asim KG, Manasi B, et al “Anti-inflammatory activity of root of Alpinialangala Wild” Chron Young Sci, 2011, 2(3): 139- 143.
  19. 21.Petra Mann, Britta Tofern, MackiKaloga and EckartEich. Flavonoid sulfates from the Convolvulaceae. Phytochemistry, 1999; January: Volume 50: Issue 2:26: 267-271.
  20. 22. YNShukla, Anil Srivastav, Sunil Kumar and Sushil Kumar. Phytotoxic activity and antimicrobial constituents of Argyreiaspeciosa and Oentherabiennis. Journal ofEthnopharmacolgy 1999; 67 (2): 41-245.
  21. Rahman A, Ali M, Khan NZ. Argyroside from Argyeia nervosa seeds.Pharmazie 2003 Jan; 58 (1): 60-62.
  22. Mishra SH and Chaturvedi.SC.Antibacterial and antifungal of the oil and unsaponifiable matter of Argyreia nervosa. Indian drugs Pharmaceutical Industry, 1978; 13 (5): 29-31
  23. 25. Gokhlae AB, Damre AS and Saraf HN. Investigations into the immunomodulatory activity of *Argyreia nervosa*. Journal of Ethnopharmacolgy , 2003; Jan 84 1: 109-114
  24. Shaw Cross WE. Recreational use of ergoline alkaloids from *Argyreia nervosa*. Journal Psychoactive drugs. 1983; Oct-Dec 15(4): 251-259.
  25. 27.C. Kaur, H.C. Kapoor Anti-oxidant activity and total phenolic content of some Asian vegetablesInt. J. Food Sci. Technol., 37 (2002), pp. 153–161.
  26. Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy. 26th ed. Pune: NiraliPrakashan; 2006. p. 593-7.
  27. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Academic Press; 1998. p. 193-204.
  28. V. Satyanarayana, S. Jaya KumariEvaluation of Ethanolic polyherbal formulation for anti-oxidant activity j. Global trends pharm sci, 2017; 8(3): 4217 – 4225
  29. 31.M. Sivakumar\*, D. Chamundeeswari, E. SusithraComparative in-vitro anti-arthritis studies on the various extracts of Glycosmispentaphylla dc rootsJournal of Pharmacy Research 2014,8(7),986-989
  30. Gellias and M. N. A. Rao, Indian J. Expt. Biology, 26, 540-542 (1988).
  31. Sakat SS, Juvekar AR, Gambhire MN. In-vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci. 2010;2:146–155.
  32. GodhandaramanSangeetha, RamalingamVidhya In vitro anti-inflammatory activity of different parts of *Petalium murex* (L.) International Journal of Herbal Medicine 2016; 4(3): 31-36
  33. 35.Alamgeer, Hasan UH, Uttra AM, Rasool S. Evaluation of in vitro and in vivo anti-arthritis potential of *Berberiscalliotrys*. Bangladesh J Pharmacol.2015; 10: 807-19.
  34. 36.Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziura P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albucasetosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plant Res, 2010; 4(5): 789-795.
  35. Dixit KP, Mittal S. Herbal sources of anti-arthritis potential: A comprehensive review. Int J Pharm Biomed Res, 2013; 4(2): 88-92.
  36. Volluri SS, Bammidi SR, Chippada SC, Meena V. In-vitro anti-arthritis activity of methanolic extract of *Bacopamonniera*, IJCEPR, 2011; 2: 156-159.

37. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Sarsf MN: Membrane stabilization activity- a possible mechanism of action for the anti-inflammatory activity of Cedrusdeodara wood oil. *Fitoterapia*.1989, 70: 251-257.
38. 40.Chioma A Anosike1\*, Onyechi Obidoa2 and Lawrence US Ezeanyika1 Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanumaethiopicum) DARU Journal of Pharmaceutical Sciences 2012, 20:76.
39. Puspall De, SubhradeepSarkar and Madhumita J Mukhophadhyay Study the antioxidant and In vitro Anti-inflammatory activity by membrane stabilization method of Amaranthusgangeticus leaf extract *Journal of Pharmacognosy and Phytochemistry* 2017; 6(4): 103-105
40. \*G. PrakashYoganandam,K.Ilango,Sucharita De. Evaluation of Anti-inflammatory and Membrane Stabilizing Properties of various extracts of Punicagranatum L.(Lythraceae) /Int.J. PharmTech Res.2010,2(2).
41. Mahendra V. Kardile , Umesh B. Mahajan# , Haidarali M. Shaikh, Sameer N. Goyal and Chandragouda R. Patil\* Membrane Stabilization Assay For Anti-Inflammatory Activity Yields False Positive Results For Samples Containing Traces Of Ethanol And Methanol *World Journal of Pharmacy and Pharmaceutical Sciences* Vol 5, Issue 3, 2016.
42. ShaziaTantary , Akbar Masood , AashiqHussainBhat , Khalid Bashir Dar ,Mohammad AfzalZargar, Showkat Ahmad Gani1\* In vitro Antioxidant and RBC membrane Stabilization Activity of Euphorbia wallichii Free Radicals and Antioxidants, 2017; 7(1): 13-22.
43. R. Manivannana and D. Sukumar\* The RBC membrane stablisation in an in vitro method by the drug isolated from Leucasaspera *International Journal of Applied Science and Engineering* 2007. 5, 2: 133-138