

## Phytochemical Screening and in-vitro Anti-Inflammatory Activity of Methanolic Extract of *Sterculia foetida* L.

Rubina NS<sup>1</sup>, Mubeen MA<sup>1</sup>, Kiran N<sup>1</sup>, Vijay P<sup>1</sup>, Asma butool S<sup>1</sup>,  
Imad uddin MD<sup>1\*</sup>.

<sup>1</sup>Department of pharmacology, MAK College of Pharmacy, Hyderabad, India.

**Abstract:** *Sterculia foetida* (*S. foetida*) is a tall tree located in India, Malaysia and North East Australia. Decoction of bark in folklore medicine was used for the treatment of dropsy and rheumatism. The main focus of this work was to evaluate in-vitro anti-inflammatory activity of methanolic extract of *sterculia foetida* (MESF) bark using Human Red Blood Cells (HRBCs) membrane stabilization technique. In this study Diclofenac sodium was used as standard drug at a conc. of 200 µg/ml and MESF at different concentrations of 100, 200, 300 and 400 µg/ml. Results of preliminary phytochemical screening revealed the presence of different plant constituents. MESF significantly and dose dependently reduced the rupture of HRBC membrane. Percentage inhibition produced by standard drug was equal to lowest conc. of MESF (100 µg/ml) and further augmented with increase in conc. of MESF up to 400 µg/ml. Based on these results it was divulged for the first time that bark of *S. foetida* has anti-inflammatory activity. This activity was expected due to the presence of alkaloids, flavonoids, steroids and terpenoids in MESF.

**Keywords:** Anti-inflammatory, HRBC membrane, Membrane stabilization, *S. foetida*.

### I. Introduction

Inflammation is a convoluted biotic response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritant [1]. It is a protective response consist of changes in blood flow, increase in permeability of blood vessels, and the migration of fluid, proteins and WBC from the circulation to the site of tissue damage [2]. Rheumatoid Arthritis (RA) is an autoimmune chronic inflammatory disorder which causes painful deformities, [3] affecting more than 1.3 million people. Of these, about 75 percent are women and most often begins between the fourth and sixth decades of life. However, RA can start at any age [4, 5]. Anti-inflammatory drugs such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) was coined to designate such class of drugs having anti-inflammatory, antipyretic and analgesic properties. More than 50 different NSAIDs are introduced in global market and used since 1963. NSAIDs causes gastrointestinal disturbances like intestinal ulcers, damage to mucosa, pain in epi gastric region, nausea, vomiting and hypersensitivity reactions [6]. The precedence of herbal drugs over synthetic drugs is because as they are free from chemicals, fewer incidence of side effects and are commonly used because of widespread availability and low cost. Despite the criticism of herbal drugs in the medical field, most of the drugs which we use today were derived from natural sources [7].

*S. foetida* is a tropical plant belonging to the family Sterculiaceae. It is known as “Janglibadam” in Hindi and Bengali language, and as “Gorapubadam” in Tamil language. Originally plant is from East Africa to north Australia, and grows freely in Myanmar and Sri Lanka [8, 9]. *S. foetida* have anti-inflammatory activity CNS depressant [10], mitogenic [11], anti-feedant [12], anti-obesity [13], anti-fertility [14], anti-oxidant [15], bronchodilatory, antibacterial [16], haemolytic, antifungal [17] and chemosterilant activity. Presence of various constituents such as carbohydrates, steroids, phenols, saponins, proteins and amino acids, alkaloids, flavonoids, cardiac glycosides & tannins were identified by phytochemical screening of *S. foetida* seeds [18]. Leucoanthocyanidine-3-O-alpha-L-rhamnopyranoside and quercetin rhamnoside isolated from root by Chopra RN and Nayer chopra, New Delhi, 1956 [19]. XiaPengFei in 2009 investigated the chemical constituents of *S. foetida* by variety of chromatographic and spectroscopic methods. 95% ethanolic extracts of *S. foetida* was evaluated and reported to contain 46 compounds were isolated from 95% of ethanolic leave extract including 33 flavonoids, 4 coumarins, 6 organic acids and 3 steroids. Compounds named “hypolaetin 3'-methyl ether 8-O-β-D-glucuronide 6"-methyl ester”, hypolaetin 8-O-β-D-glucuronide 6"-ethyl ester, chrysoeriol 7-O-β-D-glucuronide”, “luteolin 7-O-β-D-glucuronide” showed obvious anti-inflammatory activities against croton oil-induced rat ear edema at 20 mg/kg [20].

Drugs from natural source are of main interest since many decades they are accompanied with several advantages but are still incapacitated because of lack of dose instruction, lack of regulation and medication interactions. The main intent of this study is to evaluate the anti-inflammatory activity of bark of *S. foetida* and to overcome the plight of synthetic drugs and to fabricate the drug with fewer adverse effect.

## II. Material and Methods

**2.1 Chemicals:** Methanol, HCl, Mayer's reagent, Wagner's reagent, Dragendroff's reagent, Hager's reagent, Molisch reagent, Sulphuric acid, Benedict's reagent, Fehling's A & B solutions, Sodium Nitroprusside, Pyridine, NaOH, Glacial Acetic Acid, Acetic acid, Ferric chloride, NaCl, Lead acetate, Millon's reagent, Potassium permanganate, Iodine, HNO<sub>3</sub>, Diclofenac sodium, Potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Normal saline and all the organic chemicals used in the study were of AR grade and purchased from The Scientific Syndicate Hyderabad, India.

**2.2 Plant Collection and Authentication:** Fresh *S. foetida* bark material was collected from local areas of Moinabad India, during the month of January 2015. Taxonomically plant was authenticated by Scientist, In-Charge, Botanical Survey of India, Deccan Regional Centre, Hyderabad Unit, Telangana. Voucher specimen (MAKCP/2015/010) of the plant was deposited for future reference in the laboratory of MAK College of Pharmacy Moinabad, Hyderabad.

**2.3 Plant material extraction:** Fresh plant material was cleaned by washing under running tap water to remove the dust material. Then it was cut in to small pieces and dried under shade for 13 days and fed through mechanical blender (Preethi, India). The dried, coarsely powdered bark (1 kg) was extracted with 500 ml of 99% (v/v) methanol by the hot continuous extraction method using a Soxhlet apparatus at a temperature of 65-70°C. The solvent was evaporated under vacuum yielding a semisolid mass of methanolic extract of *S. foetida*. The efficiency of solvent to extract specific components from the original plant material is called as % yield. Percentage yield of MESF was 6.4% w/w with respect to the dried powder. Extract was stored in air tight container in a refrigerator at 4-2° C until further use.

### 2.4 Phytochemical screening

The preliminary phytochemical assessment was carried out by using standard procedures as described by Khandelwal in 2010 [21], Sofowarain 1993 [22], Trease and Evans 1999 [23].

#### 2.4.1 Test for Alkaloids

**Preparation of stock solution-I:** 200 mg of MESF was dissolved by continuous stirring on a water bath for 5 min in 8ml of 1% HCl and then it is filtered. Filtrate obtained was divided in to 4 equal parts to use in different identification tests.

##### 2.4.1.1 Mayer's Test

2ml of the Filtrate from stock -I was treated with few drops of Mayer's reagent (Potassium Mercuric Iodide solution). Formation of a yellow colored precipitate indicates the presence of alkaloids.

##### 2.4.1.2 Wagner's Test

2ml of Filtrate from stock-I was treated with few drops of Wagner's reagent (Iodine in Potassium Iodide). Presence of alkaloids was indicated by the formation of brown/reddish precipitate.

##### 2.4.1.3 Dragendroff's Test

Next 2ml fraction from stock-I was treated with 1ml of Dragendroff's reagent (solution of Potassium Bismuth Iodide). Red precipitate formation indicates the presence of alkaloids.

##### 2.4.1.4 Hager's Test

2ml of MESF Filtrate from stock-I was treated with 1ml of Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

**Preparation of Stock solution-II:** 200 mg MESF was dissolved in 15 ml of distilled water (DW) and filtered. The MESF filtrate were used for the detection of following test.

#### 2.4.2 Test for Carbohydrates

##### 2.4.2.1 Molisch's Test

1ml of filtrate from stock-II was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. Then add 2ml of conc. H<sub>2</sub>SO<sub>4</sub> carefully by the sides of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

#### **2.4.2.2 Benedict's test**

1ml of filtrate from stock-II when mixed with 2ml of Benedict's reagent and boiled. Presence of the carbohydrates is indicated by the formation of reddish brown precipitate.

#### **2.4.2.3 Fehling's Test**

1ml of Fehlings A is mixed with 1 ml of Fehlings B solutions. Boil the mixture for 1 min and add 2ml of filtrate from stock-II and heat in boiling water bath for 5-10min, first yellow and then brick red precipitate was observed.

#### **2.4.3 Detection of cardiac glycosides:**

##### **2.4.3.1 Legal's Test:**

1ml of filtrate from stock-II was treated with 1ml of each sodium nitroprusside and pyridine. Presence of cardiac glycosides is indicated by the formation of pink to blood red colour.

##### **2.4.3.2 Keller-kilani test:**

2ml of filtrate from stock-II was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. Above mixture is added to 2ml of concentrated H<sub>2</sub>SO<sub>4</sub> taken in another test tube. Presence of cardiac glycosides is indicated by the presence of brown ring at the interface. Just below the brown ring violet ring was also appeared.

#### **2.4.4 Detection of saponins:**

##### **2.4.4.1 Foam Test:**

30mg of MESF was shaken with 2 ml of water. Test is positive if foam produced persists for ten minutes.

#### **2.4.5 Detection of phytosterols (steroids and terpenoids):**

##### **2.4.5.1 Salkowski's Test:**

100 mg of MESF was added to a test tube containing 2ml of chloroform. To the above solution 2ml of conc. H<sub>2</sub>SO<sub>4</sub> was added carefully along the sides of the test tube and shaken gently. Appearance of reddish brown color at the interface indicates the presence of steroidal ring, i.e. Glycone portion of the glycoside (terpenoid).

##### **2.4.5.2 Libermann Burchard's test:**

MESF (100 mg) was mixed with 2ml of chloroform and acetic acid each. The solution obtained is boiled first and then rapidly cooled in ice. After cooling add 2ml of conc. H<sub>2</sub>SO<sub>4</sub> along the sides of test tube. At the junction if brown ring appears and upper layer turns to green indicates the presence of glycoside. Formation of deep red color indicates the presence of triterpenoids.

#### **2.4.6 Detection of phenols:**

##### **2.4.6.1 Ferric Chloride Test:**

0.5 ml of filtrate from stock-II was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

#### **2.4.7 Detection of tannins:**

##### **2.4.7.1 Gelatin Test**

0.5ml of filtrate from stock-II was treated with 1% gelatin solution containing small amount of sodium chloride. Formation of white precipitate indicates the presence of tannins.

##### **2.4.7.2 Lead acetate test**

0.5 ml of filtrate from stock-II is mixed with 2-3 drops of lead acetate solution, presence of white precipitate indicates tannins.

##### **2.4.7.3 Acetic acid test**

0.5ml of filtrate from stock-II is mixed with 2-3 drops of acetic acid, presence of red color solution indicates tannins.

#### **2.4.7.4 Dilute potassium permanganate solution test**

0.5ml of filtrate from stock-II was mixed with 2-3 drops of KMNO<sub>4</sub> solution, discoloration of solution indicates presence of tannins.

#### **2.4.7.5 Dilute iodine solution test**

0.5ml of filtrate was treated with 2-3 drops of iodine solution transient red colour is produced indicating the presence of tannins.

### **2.4.8 Detection of flavonoids**

#### **2.4.8.1 Alkaline Reagent Test**

1ml of filtrate from stock-II was dissolved in 1 ml of DW of which 0.5ml was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed. Formed yellow color was disappeared after the addition of few drops of dilute sulphuric acid which indicated the presence of flavonoids.

#### **2.4.8.2 Sulphuric acid test**

To 0.5ml of filtrate from stock-II add few drops of sulphuric acid (66% or 80%) flavones and flavonoids get dissolved in it and give deep yellow solution. Chalcones give red to red-bluish solutions and flavanes give orange to red colours.

#### **2.4.8.3 Lead acetate Test**

0.5ml of filtrate from stock-II was treated with few drops of lead acetate solution. Presence of flavonoids is indicated by the appearance of yellow color precipitate.

### **2.4.9 Detection of proteins and amino acids**

#### **2.4.9.1 Millon's test**

1 ml of filtrate from stock-II when mixed with 2ml of Millon's reagent, white precipitate appeared. Presence of protein was confirmed by the appearance of red color upon gentle heating.

#### **2.4.9.2 Ninhydrin Test:**

1ml of filtrate from stock-II was added to 2ml of 0.2% Ninhydrin reagent. Solution obtained is boiled for few minutes. Amino acid are confirmed by the formation of blue colour.

## **2.5 In-vitro Anti-Inflammatory activity by Membrane stabilization test**

### **2.5.1 Preparation of Human Red Blood cells suspension**

*In vitro* Anti-inflammatory activity of MESF was evaluated by using membrane stabilization method delineate by shinde *et al* [24] and olajide *et al* [25]. Fresh human blood was collected from a healthy volunteer (3ml) who was not administered by any anti-inflammatory drug two weeks forgoing to the experiment. Collected blood is then transferred to the centrifuge tubes containing anti-coagulant 0.5M EDTA. Blood was then centrifuged at 3000 rpm for 10min. The serum was separated and washed three times with isosaline (0.85% and pH 7.2). The volume of the blood was measured and 10% v/v suspension was prepared with isosaline.

### **2.5.2 Heat induced hemolysis**

MESF of different concentrations were prepared from 100-400µg/ml with isotonic buffer saline and labelled as test. The control sample was prepared by taking only isotonic buffer solution. And the standard solution was prepared by taking Diclofenac Sodium (200 µg/ml) as standard drug in isotonic saline solution. Than the test, control and standard solutions were mixed with 0.1 ml of 10% v/v RBC suspension and heated at 51°C for 20 min followed by cooling and centrifugation at 2500 rpm for 3 min and the supernatants was collected and absorbance was measured at 560nm. By using absorbance percentage inhibition of Hemolysis was calculated as follows:

Percentage inhibition = (Abs control–Abs test sample or Abs of standard sample) X 100 /Abs control .

## **III. Results and Discussion**

The percentage yield of the MESF was found to be 6.4%. It showed the presence of different constituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, phenols, tannins, flavonoids, proteins and amino acids as presented in Table 1. Among these, based on literature review the constituents which are reported to possess anti-inflammatory activity are alkaloids [26], flavonoids [27], tannins [28], steroids [29] and terpenoids [30]. These constituents were found in *S. foetida* leaves and their alcoholic extract

publicized to possess anti-inflammatory activity against rat paw edema and chronic granuloma pouch model, reported by Mujumdar AM in 2000 [10], and later in 2004 Naik DG, isolated active constituent taxer-14-en-3-beta-ol which was proved to be having anti-inflammatory activity [31]. MESF bark was also shown to possess above said constituents which was justified by positive results of different types of specific screening tests. Based on these results the present study was conducted to evaluate in-vitro anti-inflammatory activity by Membrane Stabilization Test.

### 3.1 In-vitro anti-inflammatory activity

#### Membrane stabilization test

MESF (100-400µg/ml) inhibited the heat induced hemolysis of RBCs in a dose dependent manner and was compared with diclofenac sodium as shown in Table 2 and Fig 1. Percentage inhibition of MESF (100, 200, 300 and 400 µg/ml) and diclofenac sodium (200 µg/ml) was found to be 88.33%, 93.62%, 94.76%, 95.47% and 88.94% respectively. During inflammation, as there is an increase in the vascular permeability and migration of neutrophils followed by the destruction of lysosomes resulting in the release of bactericidal enzymes and proteases that are responsible for tissue damage and different types of inflammatory disorders. Lysosomal membranes are similar to HRBC membrane components [32]. Exposure of RBCs to injurious substances such as hypotonic medium, phenylhydrazine, heat, methyl salicylate results in the lysis of the membranes, accompanied by haemolysis and oxidation of hemoglobin [33]. Inhibition of heat induced RBC membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of *S. foetida*. MESF analogous to Diclofenac sodium, stabilized RBC membrane and thus it can be extrapolated to the stabilization of neutrophils lysosomal membranes. Therefore it can be stated that MESF can block the release of lysosomal contents and protects the tissue from damage and inflammation.

**Table 1: Phytochemical screening of MESF**

S No.	Phytochemical tests	MESF
1.	<b>Alkaloids:</b>	
	a) Mayers test:	+ve
	b) Wagners test:	+ve
	c) Dragondroffs test:	+ve
	d) Hagers test:	+ve
2.	<b>Carbohydrates:</b>	
	a) Molisch test:	+ve
	b) Benedicts test:	+ve
	c) Fehlings test:	+ve
3.	<b>Cardiac glycosides:</b>	
	a) Legals test:	+ve
	b) Killer kilani test:	+ve
4.	<b>Saponins:</b>	
	a) Foam test:	+ve
5.	<b>Phytosterols:</b>	
	a) Salkowskis test:	+ve
	b) Libermann Buchard test:	-ve
6	<b>Phenol:</b>	
	a) Ferric chloride test:	-ve
7	<b>Tannins:</b>	
	a) Gelatin test:	-ve
	b) Lead acetate solution test:	+ve
	c) Acetic acid solution:	-ve
	d) Dil.Kmno4:	+ve
	e) Dil.I2 solution test:	+ve
8	<b>Flavonoids:</b>	
	a) Alkaline reagent test	+ve
	b) Lead acetate test	-ve
	c) H2SO4 test	-ve
9	<b>Proteins and aminoacids:</b>	
	a) Ninhydrin test	-ve
	b) Millons test	+ve

**+ve = present, -ve = absent**

**Table 2: Membrane stabilization Assay**

Type of drug	Concentration of drug(µg/ml)	Mean ± SEM	Percentage inhibition
Control	-	1.265±0.0051	-
Diclofenac sodium	200	0.1399±0.0050	<b>88.94<sup>***</sup></b>
MESF	100	0.1475±0.0049	<b>88.33<sup>***</sup></b>
MESF	200	0.0807±0.0042	<b>93.62<sup>***</sup></b>
MESF	300	0.0662±0.0051	<b>94.76<sup>***</sup></b>
MESF	400	0.05725±0.0055	<b>95.47<sup>***</sup></b>

All the values are expressed as Mean ± SEM and analyzed by one –way ANOVA followed by Dunnetts multiple comparison test. n=3, \*\*\*=p<0.001.

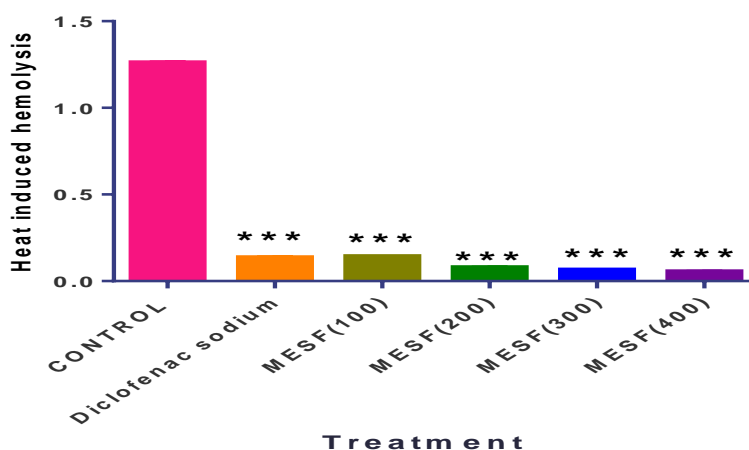


Figure 1. All the value are expressed as Mean ± SEM and analyzed by one –way ANOVA followed by Dunnetts multiple comparison test. n=3, \*\*\*=p<0.001.

#### IV. Conclusion

In conclusion, the evaluation of *S. foetida* bark for anti-inflammatory activity indicated that it possess potential activity against inflammation which was confirmed by the stabilization of the HRBC membrane possibly due to the presence of Alkaloids, tannins, flavonoids, steroids and terpenoids. In terms of stabilizing membrane it was clearly seen in the results that percentage inhibition of MESF (100 µg/ml) was equal to that of standard drug (200 µg/ml) and it was increased with increase in concentration from 100-400 µg/ml. These results rationalize the use of bark decoction in the treatment of Rheumatism. Further *S. foetida* bark extract can be used for the isolation and development of suitable safe profile compounds for the treatment of inflammatory disorders like RA. We are currently underway to evaluate in-vivo biological activities of *S. Foetida* bark.

#### References

- [1]. L. Ferrero-Miliani, O.H. Nielsen, P.S. Andersen, S.E. Girardin, Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation, Clin. Exp. Immunol, 147 (2), 2007, 227-235.
- [2]. A.B. Abbas, A.H. Lichtman, "Ch.2 Innate Immunity". In Saunders. Basic Immunology. Functions and disorders of the immune system (Elsevier 3<sup>rd</sup>ed, 2009) ISBN 978-1-4160-4688-2.
- [3]. "Handout on Health: Rheumatoid Arthritis". National Institute of Arthritis and Musculoskeletal and Skin Diseases. August 2014. Retrieved July 2, 2015.
- [4]. R. Lozano, M. Naghavi, K. Foreman, S. Lim, K. Shibuya, V. Aboyans, J. Abraham, T. Adair, "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010", Lancet, 380 (9859), 2012, 2095–128.
- [5]. Y. Alamanos, P.V. Voulgari, A.A. Drosos, Voulgari, Drosos, "Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review", Semin. Arthritis Rheum, 36 (3), 2006, 182–188.
- [6]. K.D. Tripathi, *Essentials of medical pharmacology seventh edition* (New Delhi, India, JaypeeBrothers Medical Publishers, 2013)
- [7]. Herbal advantages available at- <http://herbal-medicine-benefits.tumblr.com/rss>
- [8]. C.A. Orwa, K.R. Mutua, R. Jamnadass, S. Anthony. Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>) 2009.
- [9]. G.W. Staples, D.R. Herbst. A Tropical Garden Flora: Plants Cultivated in the Hawaiian Islands and other Tropical Places. Honolulu, Hawaii: Bishop Museum Press.
- [10]. A.M. Mujumdar, Naik, R.J. Waghole, D.K. Kulkarni, M.S. Kumbhojkar, Pharmacological studies on sterculia foetida leaves, Pharm Biol. 38(1), 2000, 13-17.
- [11]. D.G. Scarpelli. Mitogenic activity of Sterculic acid, a Cyclopropanoid fatty acid. *Science*, 185(4155), 1974, 958-960.

- [12]. P. Usharani and P. Rajasekharreddy. Toxic and antifeedant activity of *Sterculia foetida* L. seed crude extract against *Spodoptera litura* F and *Achaea janata* L. *Journal of Biopesticide*, 2(2), 2009, 161-164.
- [13]. E.T. Sheehan and M.G. Vavich, Delay of sexual maturity of the female rat by *Sterculia foetida* oil. *J.Nutrition*, 1965, 8-12.
- [14]. M. Rupesh and D. Nitin. Studies on the medicinal uses of wild trees of Nagpur district. *International Journal of Life Sciences and Pharma Research*, 2(1), 2012, 21-24.
- [15]. E. Manivannan, R. Kothai, B. Arul, S Rajaram. In vitro antioxidant properties of *Sterculia foetida* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(3), 2011, 43-51
- [16]. K.R. Kudle, M.R. Donda, MR Kudle, R. Merugu, Y. Prashanthi, M.P.P. Rudra. Fruit (epicarp and endocarp) extract mediated synthesis of silver nanoparticles from *sterculia foetida* plant and evaluation of their antimicrobial activity. *Nanoscience and Nanotechnology: An International Journal*, 3(3), 2013, 56-59.
- [17]. K.M. Schmid, G.W. Patterson. Effects of cyclopropanoid fatty acids on fungal growth and lipid composition, *Lipids*, 23(3), 1988, 248-52.
- [18]. G. Shahabadkar. Shamsundar and Swamy Paramjyothi, Preliminary pharmacognostical and phytochemical investigation on *Sterculia foetida* Linn. seeds *African Journal of Biotechnology*, 9 (13), 2010, 1987-1989
- [19]. R.N. Chopra, S.L. Nayer, I.C. Chopra, Glossary of Indian medicinal plants, CSIR: New Delhi, 1956, p. 24
- [20]. X.I.A. Pengfei, S. Shuang, F. Ziming, Z. Peicheng, Chemical constituents from leaves of *Sterculia foetida*. *China Journal of Chinese Materia Medica*, 34(20), 2009, 2604-2606.
- [21]. K.R. Khandelwal, D.R. vrunda sethi, Practical pharmacognosy techniques and experiments twentieth edition, 2009. (Pune; India Nirali publications)
- [22]. E.A. Sofowara, Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., 1993 (Ibadan, Nigeria) pp. 191-289.
- [23]. G.E. Trease, W.C. Evans. Pharmacognosy, 11th ed. 1989 (Bailliere Tindall, London) pp. 45-50
- [24]. R.M. Shinde, A.S. Phadke, A.M. Nari, A.A. Mungantiwar, V.J. Dikshit, M.N. Saraf, Membrane stabilization activity, a possible mechanism of action for the anti-inflammatory Activity of *Cedrus Deodora* wood oil, *Fitoterapia*, 70, 1999, 251-257.
- [25]. O.A. Olajide, J.M. Makinde, D.T. Okpako, S.O. Awe, Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of *Bridelia ferruginea* stem bark, *J. Ethnopharmacol.* 71, 2000, 153-160.
- [26]. A.L. Souto, J.F. Tavares, M.S. da Silva, M.F.F.M. Diniz, P.F. Athayde-Filho, J.M.B. Filho, Anti-Inflammatory Activity of Alkaloids: An Update from 2000 to 2010 *Molecules*, 16, 2011, 8515-8534.
- [27]. P. Rathee, H. Chaudhary, S. Rathee, D. Rathee, V. Kumar, K. Kholi, Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflamm Allergy Drug Targets*, 8(3), 2009, 229-235
- [28]. R. Amarowicz, Tannins: the new natural antioxidants? *Eur. J. Lipid Sci. Technol.* 109, 2007, 549-551.
- [29]. S. Snehal. Patel\*, K. Jignasha. Savjani, Systematic review of plant steroids as potential antiinflammatory agents: Current Status and Future perspectives *The Journal of Phytopharmacology*, 4(2), 2015, 121-125.
- [30]. R.D.C. Da Silveira e Sá, L.N. Andrade, D. Pergentino de Sousa. A Review on Anti-Inflammatory Activity of Monoterpenes. *Molecules*, 18, 2013, 1227-1254.
- [31]. D.G. Naik, A.M. Mujumdar, R.J. Waghole, A.V. Misar, S.W. Bligh, A. Bashall, J. Crowder. Taraxer-14-en-3beta-ol, an anti-inflammatory compound from *Sterculia foetida* L. *Planta Medica*. 70(1), 2004, 68-9.
- [32]. V.M. Mounnissamy, S. Kavimani, V. Balu, Q.S. Drlin: Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Canjera rehedii*. *Iranian J Pharmacol Therapeut*, 6, 2008, 235-237.
- [33]. M. Ferrali, C. Signorini, L. Ciccoli, M. Comporti, Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochem J*, 285 (1), 1992, 295-301.