

ANTI-ARTHRITIC STUDY OF AQUEOUS ALCOHOLIC EXTRACTS OF NYCTANTHES ARBOR TRISTIS, VITEX NEGUNDO, BOSWELLIA SERRATA, CYPERUS SCARIOSUS AND COMMIPHORA MUKUL

MAYA KRISHNA GUPTA

Shri Venkateshwara University, Gajraula, Uttar Pradesh

DR. BHUWANENDRA SINGH

Shri Venkateshwara University, Gajraula, Uttar Pradesh

YASH PRATAP SINGH RANA

SD College of Science Atrauli, Aligarh, Uttar Pradesh

ABSTRACT:

The plants which are used in the acute toxicity study were *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* all these plant are known for their Anti-arthritis activity from various traditional literature further the study of this activity with the synergistic effect will have the higher efficacy than the individual extract so for the safety of all the extract and to study the efficacy the primary aim is to consider the acute toxicity study which was done in previous paper. Present study was designed for antiarthritic study of aqueous alcoholic extract of all the five drugs used in the treatment of arthritis which were done *in vivo* where various group of 8 wistar rats. There was no sign of toxicity observed at the dose level of up to 6mg/kg body weight also the anti arthritis activity was significant in method of Freund's adjuvant-induced arthritis.

1. INTRODUCTION

Rheumatoid Arthritis (RA) is a most frequent inflammation disease which affects huge number of the people world widely. Joint disorders are the main symptom if it is not treated early. It is surely fatal disease increases the risk of life or we can say mortality by two times and average life span is decreased by 5 to 7 years. Rheumatoid Arthritis patients suffer from the other diseases such as the cardiac arrest and the disorder of the renal disturbances. Rheumatoid arthritis effects the great percentages of disease that is about 1 percent. Main patient for the disease are females as females are more effected by this disease ^[1]. Various herbal drugs used in the treatment some of them can be used synergistically for the treatment so the aqueous alcoholic extracts of the following drugs were tested for its acute toxicity study:

Commiphora mukul

Guggul (*Commiphora mukul*), a highly valued botanical medicines has been used for centuries in ayurveda to treat several ailments. Various extracts of this drug produces guggulipid in that guggulsterone is the active constituent responsible for its therapeutic activity.

Cyperus scariosus

Traditional medicine as defined by WHO refers to the complementary/alternative/non-conventional/indigenous medicine that is developed based on the theories, beliefs and experiences innate to different cultures, whether interpretable or not, used to maintain health, as well as prevent, attenuate or cure physical and mental illnesses ^[2]. Out of the 7.5 billion world's population, 4.5 billion of them use traditional medicines for primary healthcare. India's population being equivalent to 17.84% of the total world population, around 0.93 billion Indians still use traditional medicines for maintaining primary health ^[3,4].

Boswellia serrata

Boswellia serrata (*Salai/Salai guggul*) (Family: Burseraceae; Genus: *Boswellia*) is a moderate to large sized branching tree that grows in dry mountainous regions of India, Northern Africa and the Middle East^[5,6]. The family of Burseraceae is represented in the plant kingdom with 17 genera and 600 species wide-spread in all tropical regions. There are about 25 known species belonging to Genus *Boswellia*, most of them occur in Arabia, northeastern coast of Africa and India. Since ancient times, three of these species have been considered as ‘true Frankincense’ producing trees^[7,8].

Vitex negundo

Arthritis is one of the most common disease in old geriatric people and occurs in different forms. The most common form is osteoarthritis which results in trauma and infection in the joints. Arthritis is a major problem in oldage people and it needs chronic treatment with analgesics. The chronic treatment may cause adverse effects and that may increase further complications. Hence we need some alternative system of natural medicine of to treat arthritis. *Vitex negundo* Linn. (Verbinaceae) is widely distributed in the region of south Asia and it has anti-inflammatory, antibacterial, antifungal and analgesic properties. This plant is used in the treatment of superficial buises, injuries, sores, and skin infections ^[9,10].

Nyctanthes arbor tristis

Nyctanthes arbor tristis Linn. (Harsingar) is widely used as a decoction in the Ayurvedic system of medicine for treatment of sciatica and arthritis, but it has not yet been screened scientifically. In the present study, the water soluble portion of the alcoholic extract of the leaves of *Nyctanthes arbor tristis* (NAT) was screened for the presence of anti-inflammatory activity.^[11]

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh samples of plants was collected from in the month September, 2018 and was authenticated through Sri Sai Ayurvedic Medical College and Hospital Aligarh having the specimen no. RKT14360 and the herbarium were preserved. Plants and its parts were washed thoroughly to remove impurities and few samples were dried in hot air oven at 55°C. Then grounded to yield fine powder which was further subjected for the extraction processes.

2.2 Preparation of plant extract

The extraction process was carried out by Hot Continuous Extraction (Soxhlet) where the solvent aqueous alcoholic is used for all the drugs which was in the ratio water:alcohol (1:1)^[12].

The yield of aqueous alcoholic extract after the extraction was found to be **2.5%**.

2.3 Experimental animals

For acute toxicity study

30 male Swiss albino mice of body weight from 25-30 g were procured from Mangalaytan University Aligarh. The animals were housed in polypropylene cages in air conditioned room with controlled temperature and alternating 12 hour periods of light and dark were maintained. The animals were acclimatized to standard laboratory conditions prior to experimentation. The guidelines issued by Institutional Animal Ethics Committee of Mangalaytan University Aligarh.

2.4 Freund's adjuvant-induced arthritis

Ankle arthritis was induced by injecting Complete Freund's adjuvant (CFA, 50 µL) into a footpad of the left hind paw of mice. The animals were

separated into six groups (six mice/group):

Group I – Healthy control;

Group II – Arthritic control;

Group III – Mobic 0.125 mg/kg, per os;

Group IV – Aqueous alcoholic extract based on results of acute oral toxicity or human dose, per os;

Group V – Dosage less than twice that of group IV, per os;

Group VI – Dosage less than twice that of group V, per os.

On day 0 and day 28, the anti-arthritic activity of plant extracts was assessed using the following parameters:

paw volume, joint diameter, pain threshold, thermal hyperalgesia, tactile allodynia, and body weight (Kumar et al., 2006).

The diameter of the joints was taken into consideration by using a digital Vernier caliper (Mitutoyo, Japan) on day 0 before CFA injections and than after 28 day it was again taken into consideration. By the subtraction of starting joint diameter and final joint diameter we can calculate difference in joint diameter. On the 28th day, the animals were treated with ether for sedation, than the blood collection is done by retro-orbital puncture for the assessment of various biochemical parameters and histological parameters. By the dissection of ankle area the ankle joints were retrieved with the removal of skin and the. Ankle joints were kept in the formaline solution which was maintained with the concentration of 10%. The sample thus dehydrated and were washed with the help of Xylol and after that dehydrated by the help of alcohol. All the samples were dipped in paraffin and than cutted into 4 to 6 uM slices and after that staining is done by the dye of hematoxyli and eosin. Slices of the ankle joint were examined under a microscope to assess ankle joint damage and the anti-inflammatory efficacy of Aqueous alcoholic extract of drug. The slices were dyed with Safranin O, and the morphology of the cartilage tissue was evaluated following treatment to examine the ability to protect the cartilage tissue. Hematology and blood biochemical assays were performed on

mice blood to assess the amounts of leukocytes, total red blood cells, RF, CRP, AST, ALT, and ALP.

2.5 Composition of diet

The animals were fed on the standard pellet diet, and water was given *ad libitum*. The standard pellet diet comprised 20% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates).

3. RESULT AND DISCUSSION

3.1 For Acute Toxicity Study

Protocol:-

1. Animal species/ strain	Albino Swiss mice.
2. Sex	Male.
3. Body weight	24–30.
4. Animal procured from	Mangalaytan University Aligarh
5. No. of doses groups	12
6. Animals per group	5
7. Route of administration	Oral via gauge.
8. Vehicle of administration	Distilled water
9. Volume of administration	Not more than 1ml as combined volume of plant sample and vehicle.
10. Dosing details	Refer to dosing table (Table 1).
11. Observation period	14 days post dose and 7 days prior to dosing.

1. Clinical Observation

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study as compared to control. Any changes or abnormalities recorded could be an indication of toxicity. The test animals at all

dose levels showed no significant changes in behavior before and after the administration of an oral dose of whole plant powder as slurry. The clinical observation detailed below is in general for the plant material under investigation^[14,15].

1. Condition of fur	Normal
2. Damage area of skin	Normal
3. Subcutaneous swelling or lumps	Normal
4. Abdominal detention	Normal
5. Eye dullness	Normal
6. Eye opacity	Normal
7. Pupil diameter	Normal
8. Ptosis (drooping of upper eyelid).	Normal
9. Colour and condition of faeces	Normal
10. Wetness or soiling of perineum	Nil
11. Condition of teeth	Normal
12. Breathing abnormalities	Normal

2. ANTI INFLAMMATORY ACTIVITY

The findings of mice paw swelling in Figure 1 indicated that the values in the healthy control group, after 1 h, 3 h, 5 h, 24 h, 48 h, 96 h, 120 h, and 144 h differed significantly from the other groups ($p < 0.05$). At a 400 mg/kg of dosage, there was a reduction in edema of the mice's feet compared to the inflammatory control group during post-dosing period, except for the time of 5 h ($p < 0.05$). At 3 h and 5 h after causing inflammation, the swelling in using *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* increased from 41.28% to 58.11% and 62.45%, respectively. This might be explained that the time period from 3 h to 5 h was the time when carrageenan exerted the highest inflammatory effect. At all the time points of the survey, the *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* sample at a dose of

400 mg/kg could reduce mice paw edema, although the decrease is not comparable to that of diclofenac 5 mg/kg. Thus, at an oral dosage of 400 mg/kg, *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* displayed anti-inflammatory activity, with a later onset than diclofenac at a dosage of 5 mg/kg. This finding was consistent with prior research on *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* anti-inflammatory properties (Kosala et al., 2018, 2019). *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* has been revealed to contain a wide range of polyphenols, saponins, anthraquinones, and terpenoids and has been proven to promote better anti-inflammatory properties than Indomethacin (Huyut et al., 2017). Food polyphenols have been shown to have anti-inflammatory activity by inhibiting Cyclooxygenase 2 induced by 12-O-tetradecanoylphorbol- 13-acetate, stimulant-induced tumorigenesis in mice skin, and COX activity in macrophages based on the induction of lipopolysaccharides (Yoon and Baek, 2005; Hounkong et al., 2014; Su et al., 2016).

5.3 Effect of the *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* on ankle joint edema

Complete Freund's Adjuvant-induced arthritis is a widely used chronic test model because the clinical and pathological changes of the trial can be compared with those observed in rheumatoid arthritis in human (Singh et al., 2003). In the Complete Freund's Adjuvant model, chronic inflammation is expressed through an increase in the volume of the injected foot (Jalalpure et al., 2011). In the arthritic control group, the results increased the mean joint diameter from 2.72 -0.09 mm to 5.85 _ 0.41 mm after 28 days of Complete Freund's Adjuvant injection. This increase is a common phenomenon in rheumatoid arthritis (Garg and Azim, 2021). Secondary lesions occur in arthritic mice due to cell-mediated immunity, and their increase suggests immunostimulant action (Pandey et al., 2012). After 28 days, the ankle joint diameter gradually decreased in all *Nyctanthes Arbor Tritis*, *Vitex Negundo*, *Boswellia*

Serrata, Cyprus Scariosus And Commiphora Mukul Extract- treated groups and the Mobic groups. The difference in joint diameter between all Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract- treated groups and Mobic control group on day 28 was statistically significant ($p < 0.05$) compared to the arthritic control group. However, the difference between the Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract-treated group at a dosage of 400 mg/kg and the Mobic control groups was not statistically significant ($p < 0.05$). The findings also revealed that, in reducing joint swelling, the treatment of Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract at two dosages of 200 and 400 mg/kg showed the same efficacy with the Mobic treatment (Table 2). The secondary response is the expression of cell-mediated immunity in adjuvant arthritis, and 'Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract¹⁰ demonstrated an increase of this response, implying immunomodulatory function of Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract¹. Complete Freund's Adjuvant-induced polyarthritis has been shown to be associated with an immune-mediated inflammatory response, and the only subject documented to develop polyarthritis following Complete Freund's Adjuvant treatment is mice (Nielen, 2006)

After 28 days, all Complete Freund's adjuvant -treated mice had a substantial ($p < 0.05$) increase in paw volume compared to the healthy control group. Mice paw volume after 28 days of using Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract¹ at the dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg was significantly alleviated. Simultaneously, at the significance level, it was discovered that the Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract¹ treatment at 400 mg/kg efficiently decreased the mice leg volume and could be comparable to the Mobic control group at 0.125 mg/kg dosage. The

ability of "Cao Khai" to inhibit mice paw edema can be understood due to its inherent immunosuppressive activity. Furthermore, the inhibitory effect of "Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract100 on leg edema was found to be comparable to that of Mobic drugs. This result suggested a potent inhibitory effect by "Nyctanthes Arbor Tritis, Vitex Negundo, Boswellia Serrata, Cyprus Scariosus And Commiphora Mukul Extract100 of cellmediated immunity in rheumatic diseases.

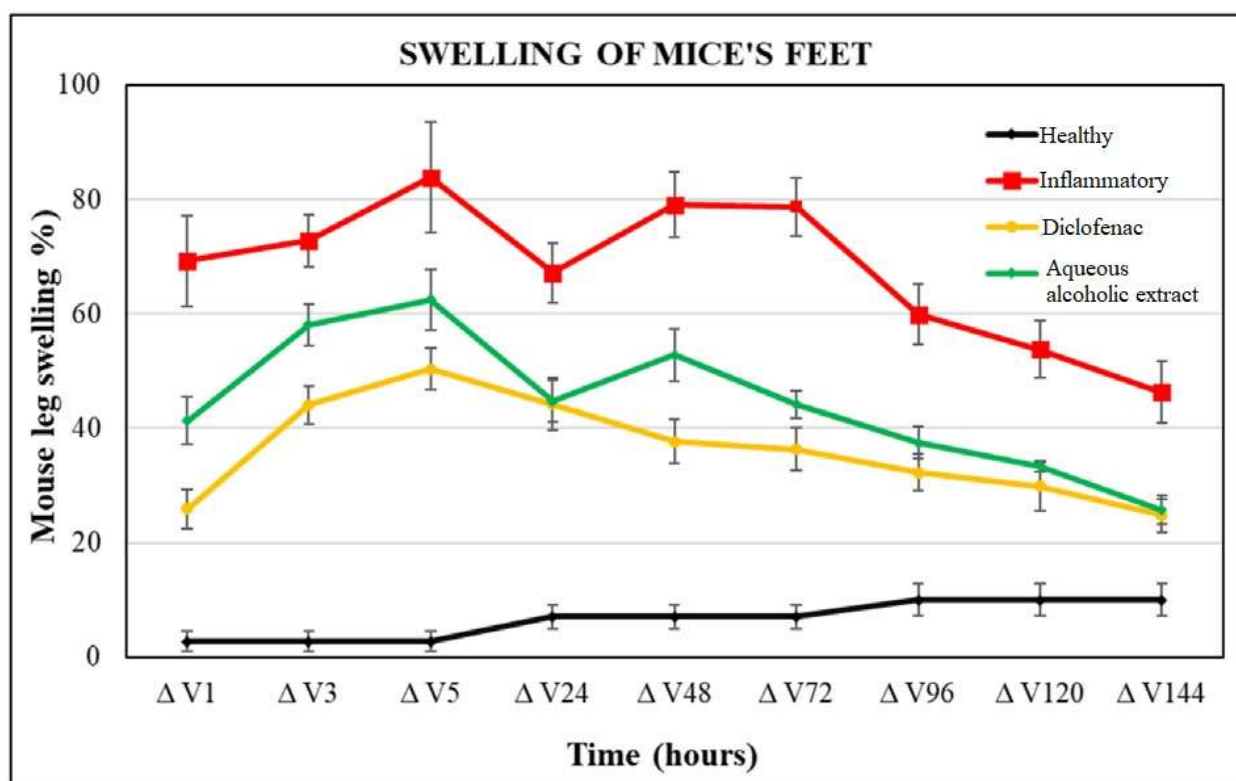


Figure1 Swelling degree of the mice's paw of the experimental batches over time.

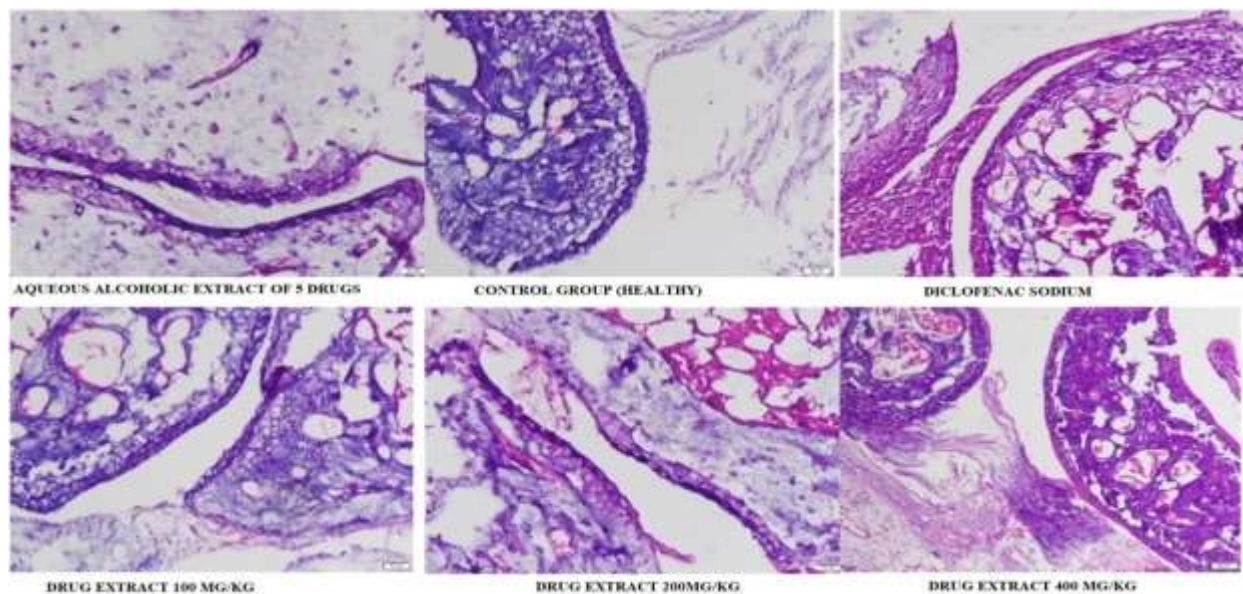


Figure 2. Hematoxylin and Eosin staining results.

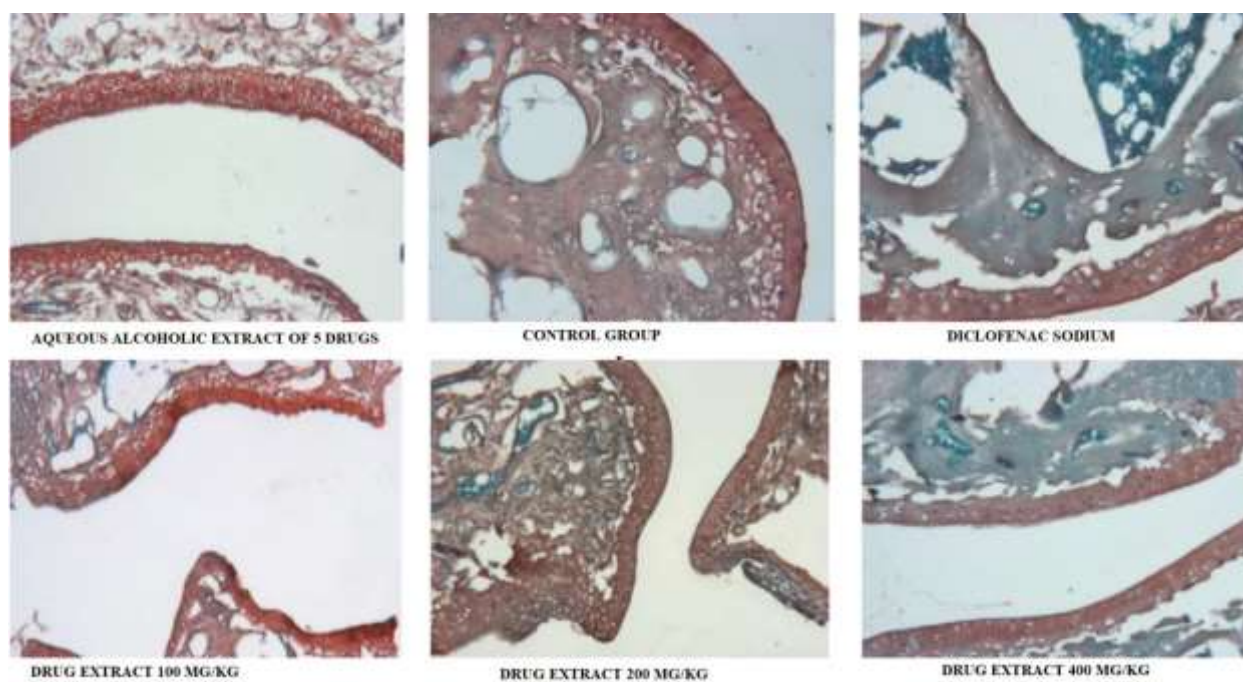


Figure 3. Safranin O staining results.

3. CONCLUSION

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the Wistar rats from all the dose groups. There was no mortality recorded even at the highest dose level i.e.5g/kg body weight, which proves that aqueous alcoholic extracts of *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* have no toxic effect in Wistar rats. The results have indicated that the aqueous alcoholic extract of *Nyctanthes arbor tritis*, *Vitex negundo*, *Boswellia serrata*, *Cyprus scariosus* and *Commiphora mukul* plant is safe and can be used for efficacy studies for different activities at the effective dose of 600 mg/kg.

Where when we consider the anti-arthritis activity of the extract than there is significant efficacy of the extract as compared to the standard drug. This may be due to the synergistic effect of all the drugs which were used traditionally as the anti-arthritis drugs. Further there is more things to study for the activity portion of the different extracts of the plants.

4. REFERENCE

1. Kirtikar KR & Basu, *Indian Medicinal Plants*, International Book Distributors, Volume 2, 2005; 85.
2. WHO/EDM/TRM/2000.1 (2000) General guidelines for methodologies on research and evaluation of traditional medicine, Geneva.
3. Cordell GA. Natural products in drug discovery-creating a new vision. *Phytochem Rev.* 2002;1:261–273. doi: 10.1023/A:1026094701495.
4. Usha T, Middha SK, Bhattacharya M, Lokesh P, Goyal AK (2014) Rosmarinic acid, a new polyphenol from *Baccaurea ramiflora* Lour. leaf: a probable compound for its anti-inflammatory activity antioxidants 3(4):830–842
5. Maupetit P. New constituents in olibanum resinoid and essential oils. *Perfumer Flavorist.* 1984;9:19–37.

6. Leung AY, Foster S. 2nd ed. New York: John Wiley and Sons; 1996. Encyclopedia of common natural ingredients used in food, drugs and cosmetics; pp. 389–91.
7. Wallis TE. 5th ed. London: J and A Churchill Limited; 1967. Textbook of Pharmacognosy; pp. 500–1.
8. Evans WC. 14th ed. London: WB Saunders Company Ltd; 1996. Trease and Evans Pharmacognosy; p. 289.
9. Wallis TE. 5th ed. London: J and A Churchill Limited; 1967. Textbook of Pharmacognosy; pp. 500–1.
10. Evans WC. 14th ed. London: WB Saunders Company Ltd; 1996. Trease and Evans Pharmacognosy; p. 289.
11. R.S. Saxena, R. B. Gupta Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor tristis* Linn. an Indian medicinal plant, Journal of Ethnopharmacology Volume 11, Issue 3, August 1984, Pages 319-330
12. Mukherjee P. K., Quality Control of Herbal Drugs, Business Horizons Publishers; 2002; page-398.
13. Angel Sánchez-Lamar, Gladys Fonseca, Jorge Luis Fuentes, Renata Cozzi, Enrico Cundari, Mario Fiore, Ruggero Ricordy, Paolo Perticone, Francesca Degrassi and Rosella De Salvia, Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts, Journal of Ethnopharmacology; 2003; 89, pp.295–300.
14. Kar, D. M., B. K. Nanda, D. Pradhan, S. K. Sahu and G. K. Dash, Analgesic and antipyretic activity of fruits of *Martynia annua* Linn. Hamdard Medicus, 2004, 47(1): 32-35.
15. Butler, L.G., Rogler, J.C., Biochemical mechanisms of the antinutritional effects of tannins. In: Ho, C.-T., Lee, C.Y., Huang, M.T. (Eds.), Phenolic Compounds in Food and their Effects on Health I. American Chemical Society, Washington, DC; 1992; pp. 298–304.