PHARMACOLOGICAL STUDY OF *ANISOMELES MALABARICA*(L.) R. BR. LEAF FOR ANALGESIC ACTIVITY

Vinay Kumar R. and Kuppast. I. J.

Department of Pharmacology, National College of Pharmacy,Balraj-Urs Road, Shivamogga-577201.

ABSTRACT

The study reveals that use of synthetic medicine will have lot of side effects, toxicity and certain limitations for their manufacturing and maintenances. However it has been claimed that herbal drugs are the best medicines than synthetic medicines. In view of this we have selected some of medicinal plants not evaluated analgesic activity scientifically. Among that *Anisomeles malabarica* (L.)R. Br. is one plant belongs to *Lamiaceae* family, widely distributed throughout the India. It has been used as a folk medicine for treatment of cancer, liver disorder, stomach ailment, fever, anorexia, swelling, rheumatism, cold and cough[1, 2]. The present research has been conducted to investigate the analgesic activity of extracts of leaves of *Anisomeles malabarica* obtained by using analytically standard distilled water, petroleum ether and ethyl acetate.The method "up and down" (OECD Guideline No. 425) of CPCSEA was adopted for acute toxicity studies. The analgesic activity was screened by hot plate model and acetic acid induced writhing models by using rats.From the present study it can be concluded that the plant *Anisomeles malabarica* possess significant analgesic activity. The analgesic activity may probably due to the presence of large amount chemical constituents such as alkaloids, flavonoids, steroids, triterpenoids, and glycosides, etc. However further work is required to screen which phytoconstituents is responsible for analgesic activity.

Keywords:*Anisomeles malabarica,* leaves, analgesic activity, phytoconstituents.

No: of Tables: 2 **No: of Figures: 3** **No: of References:18**

INTRODUCTION

Medicinal herbs have been used for centuries for therapeutic purposes. These medicinal plants consider as a rich resources of active ingredients which can be used in drug development and synthesis. Besides that these plants play a vital role in the development of human cultures around the whole world[3].Many of these herbs with extraordinary therapeuticalefficienciesused without any adverse effects. *Anisomeles malabarica* R. Br. from the family *Lamiaceae* is commonly known as Malabar catmint. Is a traditional medicinal plant, distributed throughout India. It has been used in folk medicine for the treatment of cancer, liver disorder, stomach ailment, fever, cold and cough[2]. It is an aromatic, densely pubescent, perennial herb, 1.2-2m in height[4]. Studies have shown that the main constituent of the *Anisomeles malabarica* contains alkaloids, flavonoids, saponins, tannins, sterols, glycosides, oils and fats, phenolic compound, carbohydrates, protein and amino acid, gums and mucilage were also present[2]. Pharmacologically, it has been documented to possess antioxidant, anti-inflammatory, antiepileptic potential, antifertility, anti-pyretic activity, potential anti-allergic, anti-anaphylactic, anti-bacterial, anticancer, anticarcinogenic, antiperiodic, diaphoretic, emmenagogue and antispasmodic properties[2].According to the International Association for the Study of Pain (IASP), pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage"[5]. Analgesic is a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness[6]. Common drugs for pain relief such as aspirin and morphine have been widely used in recent decades. In most instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), can only relieve 50% of the pain in about 30% of patients[7]. In addition, many of these drugs cause serious side effects. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders[5].As such, research to discover other alternatives to treat pain is crucial. Medicinal herbs have been used for centuries for therapeutic purposes. Many of these herbs with analgesic activity had been used without any adverse effects.The present study aimed to evaluate the analgesic activity of various solvents extract of leaves of *Anisomeles malabarica* in animal models.

Materials and methods

Plant Materials. The leaves of *Anisomeles malabarica* used in this study were collected from a local areas of Shivamogga district, Karnataka state. It was identified and authenticated by Dr. D. Rudrappa, Professor and head of Botany, S.R.N.M. National College of Applied Science, Balraj-Urs Road, Shivamogga, Karnataka state. The leaves of *Anisomeles malabarica* were shade dried and reduced to a coarse powder in a pulvirizer (Sunbeam, Munger, India) using mesh no. 3 and passed through a sieve No. 40 to obtain about 2 kg of powder.

Extraction. Powdered leaves of the *Anisomeles malabarica* were subjected to extraction using different solvents such as distilled water, petroleum ether and ethyl acetate. Aqueous leaf extract was prepared by using cold maceration technique, about 125g of the dried leaves powder was immersed in 500ml distilled water for 2 weeks at 20-25^{0}C. The ethyl acetate extract and petroleum ether extract was prepared by soxhlet extraction method in soxhlet extractor for 48 hours in 8 batches of 35g each. The extract was concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally desiccator dried. The extract so obtained was labelled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered leaves of the plant. These extracts are then used for the activities.

Phytochemical Screening. All the three extracts were screened for their various phytoconstituents using standard procedures[8, 9].

Animals. Healthy young adult male and non-pregnant female Swiss albino mice (20 - 30 g) of either sex and young adult Swiss albino rats (150-300g) were used for the acute toxicity study and pharmacological studies (Analgesic activity) using aqueous, ethyl acetate and petroleum ether extracts of the leaves of *Anisomeles malabarica*. The animals were procured from Central Animal House, National College of Pharmacy, Shivamogga, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions at room temperature 27^0 ± 2^{0}C with relative humidity 65 ± 10% and 12 hours Light/dark cycle. All the animals were fed with rodent pellet diet (Krishvet feeds, Bengaluru,) and water was allowed ad-libitum under strict hygienic condition. Prior to the experiment, the animals were fasted for 12hours with water given adlibitum and weighed. Ethical clearance (Clearance number: NCP/IAEC/CL//02/2016-2017) for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies. Acute toxicity study for the *Anisomeles malabarica* extracts was carried out using OECD guideline-425 (modified, adopted March 23, 2006), the sequential test that uses a maximum of five animals. A test dose of 2000mg/kg or exceptionally 5000mg/kg may be used in situation where experiment has information indicating that the test material is likely to be nontoxic. The acute toxicity studies of aqueous leaf extract, ethyl acetate leaf extract and petroleum ether leaf extract was carried out according to above prescribed methods. At 2000mg/kg sample did not produce any observable toxic effects during entire duration of study. So, there was no mortality of the albino mice was found at 2000mg/kg body weight hence this dose is considered as lethal. To study analgesic activity, the test samples were administered in the dose of 200mg/kg body weight which is equal to 1/10th of 2000mg/kg body weight.

Study Design. The animals were randomly assigned to five groups of six animals each for the two different experimental models.

The first group served as control group receiving distilled water (0.5ml) orally. The second groups served as standard group and were given standard drugs, Diclofenac sodium (4mg/kg each) administered orally. The third, fourth and fifth groups were administered by petroleum ether leaf extract, ethyl acetate leaf extract, and aqueous leaf extract of *Anisomeles malabarica*respectively at a dose of 200mg/kg orally.

Hot Plate Model. Evaluation of analgesic activity of the extract was carried out using hot plate method[10]. The rats were placed on a hot plate maintained at 55^0C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before and at 30, 60, 95, and 120min after the administration of the treatments.

Acetic acid induced writhing test. Writhing was induced in rats by intraperitoneal administration of 1ml/100g of body weight of 0.3% acetic acid. The number of writhing movements was counted for 10min, the test was performed 30min after administration of vehicle or drug. Acetic acid (0.1ml/10gm) was used as irritant. Test animals were administered with the plant extract prior to acetic acid treatment. After a period of 10min, the animals were observed for writhes (indicated by stretching of abdomen with simultaneous stretching of atleast one hind limb) [11].Percentage of inhibition was calculated using the formula.

$$\% \text{ inhibition} = \frac{\text{average writhes in control groups - writhes in drug group}}{\text{Writhes in control groups}} \times 100$$

Statistical analysis. All the values were expressed as mean ± S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by pair wise comparisons of Turkey's HSD (honestly significant difference) test. A probability level of $P<0.05$ was considered moderately significant, $P<0.01$ is considered as significant and $P<0.001$ is considered as highly significant.

RESULTS

Hot Plate Test. The results of the analgesic effect of the various solvent extract of the leaves of the *Anisomeles malabarica* in rats of either sex weighing 150-300g at 200mg/kg body weight using hot plate method are summarized in Table1. The results showed that there was no significant difference on the thermal stimulus in rats treated with distilled water (negative control) throughout the 120min observation. In comparison to the control treated animals, the significant increase in there action time to thermal pain was detectable in both standard drug treated group and groups received solvent extracts of leaves of *Anisomeles malabarica*. Fig. 1 illustrates the analgesic effect of control group, standard drug treated group and groups received various solvent leaf extracts of *Anisomeles malabarica* using hot plate model. Diclofenac sodium elicited significant analgesic activity within 15 min following administration as evidenced by the

gradual increase throughout the observation period.

Writhing test. The results of the analgesic effect of the various solvent extract of the leaves of the *Anisomeles malabarica* on acetic acid induced writhing model are summarized in Table 2. The results showed that all the three leaf extract of *Anisomeles malabarica* at a treatment of 200mg/kg body weight will exhibitedsignificant analgesic activity when compared to control treated animals. Fig. 2and 3 illustrates the analgesic effect of control group, standard drug treated group and groups received leaf extracts of *Anisomeles malabarica* on acetic acid induced writhing model. Finally it has been showed that the leaf extract of *Anisomeles malabarica* has significant effects on analgesic activity.

Table 1: Table showing the effect of various leaf extracts of *Anisomeles malabarica* on analgesic activity by hot plate model.

SL NO	GROUPS	DOSE MG/KG	REACTION TIME (IN SECONDS)			
			30min	60mim	90min	120min
1	Control	0.5ml	3.33±0.21	3.0±0.25	3.5±0.22	3.0±0.25
2	Diclofenac Sodium	10	10.5±0.50***	12.16±0.70***	14.33±0.61***	16.33±0.76***
3	Petroleum ether extract	200	5.66±0.21***	7.0±0.25***	8.16±0.65***	10.16±0.65***
4	Ethyl acetate extract	200	8.33±0.33***	10.33±0.66***	12.5±0.56***	13.66±0.55***
5	Aqueous extract	200	6.5±0.42***	7.5±0.42***	8.16±0.40***	10.66±0.49***

Note: Data was analyzed using one way ANOVA followed by pairwise comparision. Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

Figure 1: Histogram showing the effect of various leaf extract of *Anisomeles malabarica* on analgesic activity by hot plate model.

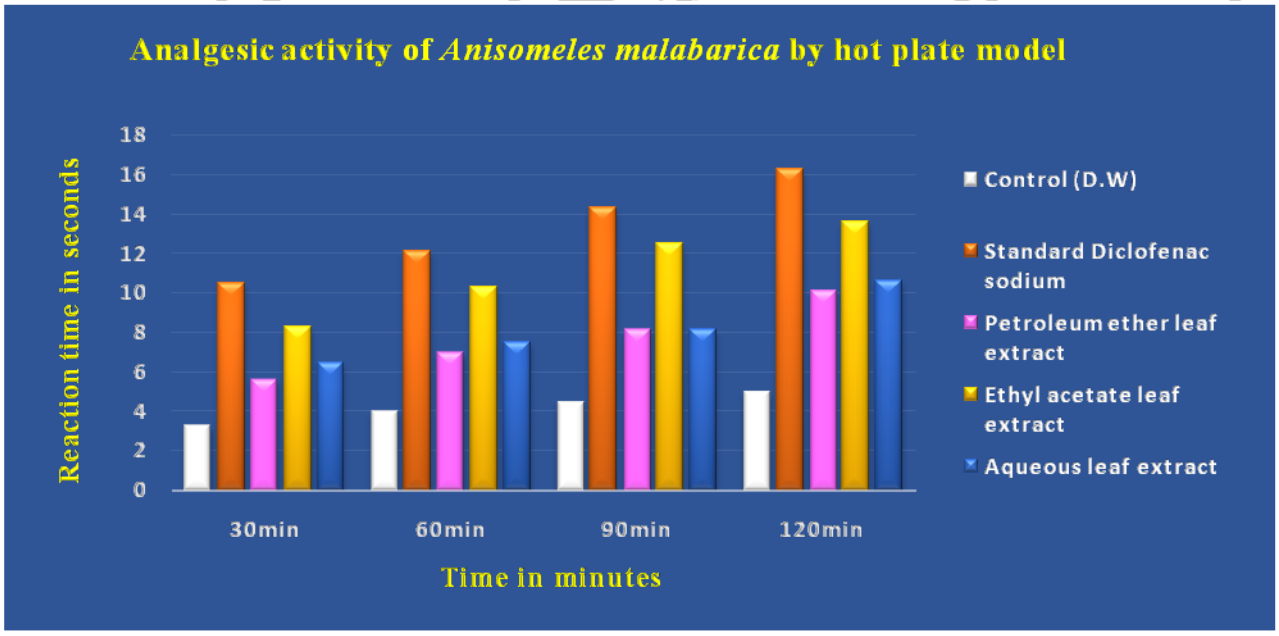


Table 2: Table showing the effect of various leaf extracts of *Anisomeles malabarica* on analgesic activity by acetic acid induced writhing model.

SI NO	GROUPS	DOSE(MG/KG)	NO OF WRITHING (PER 30 MIN)	% OF INHIBITION
1	Control (D.W)	0.5ml	24.16±1.014	--
2	Diclofenac sodium	10	5.66±0.66***	76.58%
3	Petroleum ether extract	200	11.33±0.49***	53.11%
4	Ethyl acetate Extract	200	9.83±0.73***	59.31%
5	Aqueous extract	200	11.33±0.49***	53.11%

Note: Data was analysed using one way ANOVA followed by pairwise comparision. Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

Figure 2: Histogram showing the effect various leaf extract of *Anisomeles malabarica* on analgesic activity by acetic acid induced writhing model.

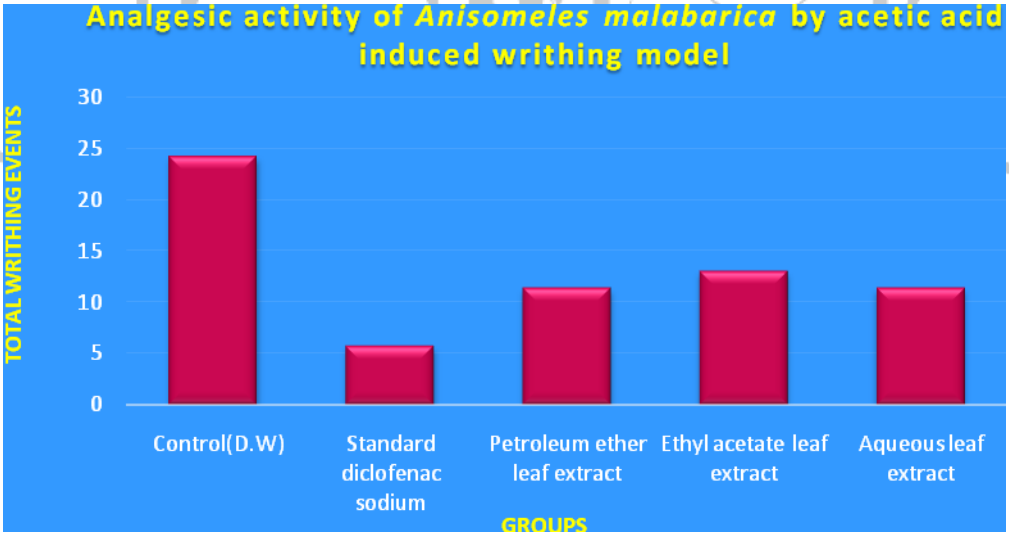
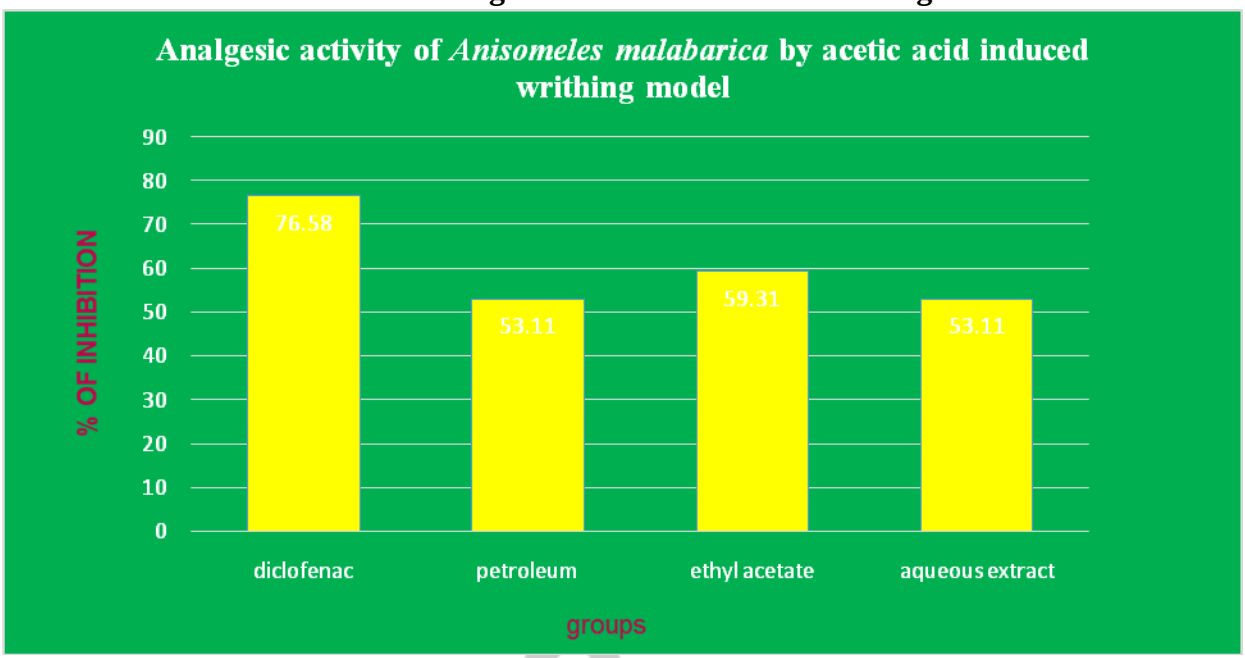


Figure 3: Histogram showing effect of various leaf extract of *Anisomeles malabarica* on percentage inhibition of writhing in acetic acid induced writhing model.



DISCUSSION

Analgesics are drugs that act on peripheral or central nervous system consciousness[12]. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain[13]. The animal models employed for screening of analgesic activity in this study are pain-state model which include hot plate method and acetic acid induced writhing test. Both methods are useful in illustrating both centrally and peripherally mediated antinociceptive responses. While the hot plate method involves higher brain functions and is regarded a supraspinally organized response[14]. In hot plate model, in comparison with control, the diclofenac sodium produce more significant antinociception effect during all observation times, followed by the various leaf extract of *Anisomeles malabarica* are shows highly significant analgesic effect. In acetic acid induced writhing model, analgesic activity was determined by comparing the percent writhing inhibition by the crude solvent extracts of leaves of *Anisomeles malabarica* in comparison to the control and the standard groups. The more the writhing inhibition by the test groups, the more the positive activity. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase PGE_1 and PGE_2 peripherally[15]. NSAIDs can inhibit COX in peripheral tissue and therefore with the mechanism of transduction of primary afferent nociceptors[16]. The mechanism of analgesic activity of leaf extract of *Anisomeles malabarica* could be probably due to the blockade of the effect or release of endogenous substance that excite pain nerve endings similar to that of Diclofenac sodium and NSAIDs. Thus, the reduction in the number of writhing

indicates that leaf extract of *Anisomeles malabarica* might exert analgesic activity by inhibition of prostaglandin synthesis or action of prostaglandin.*Anisomeles malabarica* at the dose of 200mg/kg showed the analgesic activity in the tested model. Analgesic activity may due to the large number of chemical constituents like flavonoids, alkaloids, glycosides, triterpines and other active constituents present in *Anisomeles malabarica*. Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Flavonoids affect arachidonic acid metabolism in different ways. Some flavonoids specifically block cyclooxygenase or lipooxygenase, whereas others block both enzymes. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase[17, 18]. More investigations are necessary to prove the analgesic activity of *Anisomeles malabarica* by other models and further studies are necessary to identify the exact mechanism of analgesic activity of *Anisomeles malabarica* and to isolate the active compound(s) responsible for this pharmacological activity.

CONCLUSION

Analgesic activity of various leaf extracts of *Anisomeles malabarica*(L.) R. Br. was carried out by using two models namely hot plate model and acetic acid induced writhing model. In the present study all the test samples (petroleum ether leaf extract, ethyl acetate leaf extract and aqueous leaf extracts) exhibited significant ($P < 0.001$) analgesic activity. Among these test samples ethyl acetate leaf extract exhibited more analgesic activity when compare to control. It can be concluded that active constituents responsible for analgesic activity might be present in the leaf extracts. However, further studies are necessary to find the exact mechanism of analgesic effect and to isolate the active compound(s) responsible for this pharmacological activity.

REFERENCES

Ramaraj, R., & Unpaprom, Y. (2013). Medicinally Potential Plant of *Anisomeles malabarica* (L.) R. Br. *Journal of Agr. Research & Extension*, *30*(3), 29-39.

Vijayalakshmi, R., & Ranganathan, R. (2012). Chemopreventive effect of *Anisomeles malabarica* whole plant extracts during DMBA induced hamster Buccal pouch carcinogenesis. *Asian J Pharm Clin Res*, *5*, 185-188.

Rasool Hassan, B. A. (2012). Medicinal plants (importance and uses). *Pharmaceut Anal Acta*, *3*, e139.

http://indiabiodiversity.org/biodiv/species/show/228742

Fan, S. H., Ali, N. A., & Basri, D. F. (2014). Evaluation of analgesic activity of the methanol extract from the galls of Quercus infectoria (Olivier) in rats. *Evidence-Based Complementary and Alternative Medicine*, *2014*.

Tripathi, K. D. (2013). *Essentials of medical pharmacology*. JP Medical Ltd.

Hewitt, D. J., Hargreaves, R. J., Curtis, S. P., & Michelson, D. (2009). Challenges in analgesic drug development. *Clinical Pharmacology & Therapeutics*, *86*(4), 447-450.

Khandalwal, K. R., & Sethi, V. K. (1999). Practical Pharmacognosy Techniques and Experiments. *Nirali Prakashan*, 146-148.

Gokhale, S. B., Kokate, C. K., & Purohit, A. P. (2015). A text book of Pharmacognosy. *Nirali Prakshan, Pune, India*, 108-111.

Eddy, N. B., & Leimbach, D. (1953). Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics*, *107*(3), 385-393.

Ghosh, M. (2005). Fundamentals of Experimental Pharmacology Hilton and Company. *Kolkata, India*.

Tripathi, K. D. (2004). Textbook of Essentials of Medical Pharmacology. *published by Jaypee Brothers, Medical Publishers Pvt. Ltd*.

Shreedhara, C. S., Vaidya, V. P., Vagdevi, H. M., Latha, K. P., Muralikrishna, K. S., & Krupanidhi, A. M. (2009). Screening of Bauhinia purpurea Linn. for analgesic and anti-inflammatory activities. *Indian journal of pharmacology*, *41*(2), 75.

Chapman, C. R., Casey, K. L., Dubner, R., Foley, K. M., Gracely, R. H., & Reading, A. E. (1985). Pain measurement: an overview. *Pain*, *22*(1), 1-31.

Kumar, V., Singh, P. N., & Bhattacharya, S. K. (2001). Anti-inflammatory and analgesic activity of Indian Hypericum perforatum L.

Adzu, B., Amos, S., Kapu, S. D., & Gamaniel, K. S. (2003). Anti-inflammatory and anti-nociceptive effects of Sphaeranthus senegalensis. *Journal of Ethnopharmacology*, *84*(2-3), 169-173.

Middleton, E. (1998). Effect of plant flavonoids on immune and inflammatory cell function. In *Flavonoids in the living system* (pp. 175-182). Springer, Boston, MA.

http://dx.doi.org/10.1016/bs.apha.2014.10.007