J. Appl. Environ. Biol. Sci., 6(9)72-77, 2016
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ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Central Analgesic Property of Extracts and Essential Oils from *Inula viscosa* And *Anacyclus valentinus* (*Asteraceae*) In Wistar Rats

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Received: June 2, 2016
Accepted: August 4, 2016

ABSTRACT

The methanolic extractsand essential oils of *Inula viscosa* and *Anacyclus valentinus* were screened for their central analgesic activity by the tail immersion method *in vivo* using rats. Methanolic extracts at doses of 300 and 500 mg/Kg, essential oils at doses of 0.06 and 0.1ml/Kg and standard drugs (paracetamol and tramadol) were orally administered to animals. A significant ($p<0.05$) analgesic effect comparable to the standard drugswas shown and the most important effect was recorded to the methanolic extract of *I. viscosa* (500mg/Kg) with a latency time of 15.76 ± 0.03 Sec and a percentage analgesia of 83%.

KEYWORDS: central analgesic, *Inula viscosa, Anacyclus valentinus,* tail immersion, polyphénols, essential oils.

1. INTRODUCTION

Herbal medicine has known for a few years back into favor. Today, natural products play an important rolein the discoveryof new drugs. Algeria is indeed recognized by its varietal diversity in medicinal and aromatic plants, the *Asteraceae*. Also known as *Compositae*, this family is one of the most important and largest plant familieswith over 1000 genera and 23000 species [1, 2].

Our choice fell on two annual medicinal plants in this family: The sticky fleabane (*Inula viscosa* or *Dittrichia viscosa*) and valence anacycle (*Anacyclus valentinus*). Traditional data report that these plants are known for their antifungal [3, 4], anti-diabetic [5, 6] and anti-inflammatory activities [7]. In some parts of the country, *A. valentinus* is often used in culinary preparations [8].

Pain is defined as an undesirable sensory and emotional experience associated with potential or actual tissue damage [9]. In many cases, it represents the only symptom for the diagnosis of several diseases. It is associated with necrosis, inflammation, spasm, asthma, surgical interventions, trauma and rheumatoid arthritis. In pain, motivational, affective, and cognitive aspects are involved [10, 11].

Drugs which are in use for the management of this disorder are peripheral analgesic (salicylic acid) and central analgesic (opioids) [12]. Opiate analgesic drugs, which are important for the treatment of chronic pain, are generally considered to act on specific receptors in different regions of the central nervous system [13].

However, the prolonged use these modern drugs may cause side and toxic effects, such as hepatotoxicity and gastric disorders. In addition, opiates can develop dependence [13]. Consequently, there is a need to develop new analgesic agents with minimum side effects.

This study therefore aims at examining the central analgesic action of the methanolic extracts and essential oils of *Inula viscosa* and *Anacyclus valentinus* on male Wistar rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Paracetamol (Doliprane ®) and Tramadol (Supramadol ®) which is a centrally acting opioid analgesic were obtained from a local pharmacy and diluted in sterile normal saline.

2.2. Animals

The *invivo* tests were carried out in accordance with ethical guidelines and were performed on male Wistar rats"*Rattus norvegecus*"(weighing 150 – 200 g).These animals were provided by the Experimental Station of the University of Mascara/Algeria. They were kept at a temperature of 24 ± 2 °C with, a humidity of 50 - 60 % and a 12/24 hour photoperiod and were allowed for food and water *ad libitum*. The rats were fasted for 12 hours before administration of products.

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2.3. Plant material

The plant material consists of leaves and flowers of *I.viscosa* from Chorfa in the region of Mascara and *A.valentinus* from El Bayadh/Algeria. Both plants were harvested in October- December under the control of botanists from the University of Mascara.

2.4. Preparation of methanolic extracts

The collected plants were dried under shade at room temperature (27- 30°C) for 15-30 days, and then pulverized to fine powder. The methanolic extract of both plants was obtained by maceration of 30g of powder in 300 ml of methanol (80%) for 24h at room temperature with constant stirring. The same powder was extracted two times. To remove the solvent, combined filtrates obtained were concentrated under reduced pressure and dried at 45°C [14, 15].

2.5. Isolation of essential oils

100g of collected parts mixed with 1000 ml were hydrodistillated in a Clevenger-type apparatus for 3h [16]. Cyclohexane was used as a collecting solvent, which was removed under reduced pressure in a rotary evaporator [17].

The yields of methanolic extracts and essential oils of *I. viscosa* and *A. valentinus* were calculated according to [18] and both extracts were kept at (4°C) for bioassay.

2.6. Acute toxicity

The acute toxicity test for methanolic extracts and essential oils obtained from *I. viscosa* and *A. valentinus* were performed as per OECD guideline 423. Groups of ten rats (5 males and 5 females) received doses of 300, 600, 1000, 1500, 2000, 2500 mg/kg of methanolic extract and doses of 0.3, 0.5, 1, 1.5, 2, 3 ml/kg of essential oils from both plants, while the control group received saline (10ml/Kg) by orogastric route. The treated groups were observed for three hours following administration, and up to 14 days. During this period, mortality rate and all physical and behavioral changes were recorded [19, 20].

2.7. Tail immersion test

Rats were divided into groups of five each. Saline (10ml/kg), paracetamol (100mg/Kg), Tramadol (30mg/kg), methanolic extracts at doses of 300 and 500 mg/kg and essential oils at doses of 0.06 and 0.1 ml/Kg were orally administered.

The anti-nociceptive or the central analgesic activity of the methanolic extracts and essential oils was evaluated by the tail-flick test using thermal stimuli method [21]. About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 55 ± 2 °C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum reaction time was fixed at 16 sec in order to prevent the tissue damage [22].The reading of reaction time was recorded15, 30, 45 and 60 minutes after the administration of products [23]. Percentage analgesia at 30mn was calculated using the following formula [23]:

$$\% \text{ Analgesia} = TL - BL / ML - BL \times 100$$

Where: ML is the Maximum latency or cut off time, TL is the Test latency and BL correspond to the Basal latency or control latency

2.8. Statistical analysis

The results were expressed as the mean ± standard deviation (SD). The statistical significance between control and treated groups were analyzed using ANOVA single factor for multiple comparisons and a value of $p \leq 0.05$ was taken to be significant.

3. RESULTS AND DISCUSSION

3.1. Yields and organoleptic properties of methanolic extracts and essential oils

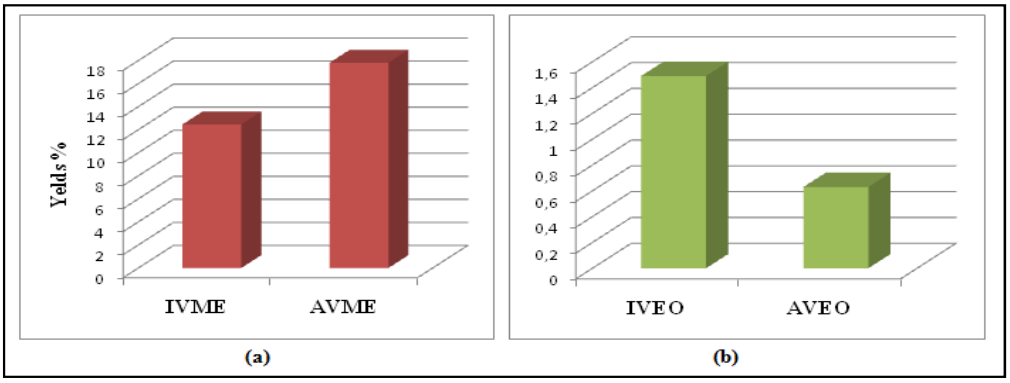

Fig. 1. Extraction yields of methanolic extracts (a) and essential oils (b) obtained from both plants

Methanolic extracts of the two plants have a dark color and strong odor, with a viscous aspect of *A. valentinus* (AVME), which registered the higher yield (17.82%) compared to the methanolic extract of *I. viscosa* (IVM) (12.46%). The yield of IVME obtained in this work is close to that obtained by Gökbulut and his collaborators in 2013[24], but it is lower to that obtained by Laghrifi *et al*. (2013) [25] using absolute methanol. Previous work on the species of the genus *Anacyclus* have registered lower yields varying between 1.12 and 3.72% [26, 27]. Essential oils of both plants have a liquid appearance, yellow color with strong and characteristic odours. In profitability side, essential oil of *I. viscosa* (IVEO) gave the highest proportion with 1.49%, while the essential oil of *A. valentinus* (AVEO) presented 0.63%. This may be due to the climatic conditions of the plant. The yield depends on the geographical origin of the plant, the season of harvest, method and conditions of the extraction. It is only relative [28, 29].

3.2. Acute toxicity

Oral administration of different doses of methanolic extracts (from 300 to 2500 mg/Kg) and essential oils (from 0.3 to 3 ml/Kg) of *I. viscosa* and those of *A. valentinus* did not change the behavior of animals and did not cause any death during the observation period. The LD50 is assumed therefore higher than 2500 mg/Kg for the methanolic extracts and 3ml/kg for essential oils. Hammouchi [30] by conducting investigations and ethnobotanical studies on food, aromatic condimental, medicinal and toxical plants in Morocco reported that *I. viscosa* is a non-toxic medicinal plant, while *A. valentinus* is a medicinal plant, aromatic, condiment and spice considered.

All extracts can be considered to be non-toxic up to the doses tested. This test was important to define the doses for pharmacological activity and it is also a preliminary step to value medicinal plants.

3.3. Effect of methanolic extracts and essential oils on tail immersion

The results of the central analgesic action exhibited by studied plant extracts are shown in figures 2 and 3.

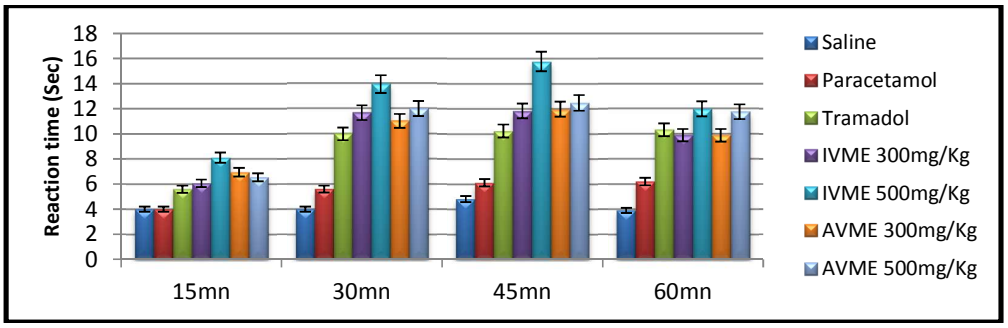

Fig.2. Reaction time of saline, standard drugs and methanolic extracts of *I. viscosa* and *A. valentinus* in tail immersion test on rats

The tail immersion method indicated that the central analgesic effect of the methanolic extracts of plants tested was significant and dose dependent as revealed by the increased reaction time after giving thermal stimulus to the rats.

For the tested drugs, analgesic action peaking at 45 min and the highest activity was recorded to IVME at 500mg/Kg with a latency time of 15.76 ± 0.03 Sec, followed by AVME at 500mg/Kg with a reaction time of 12.47 ± 0.034 Sec.

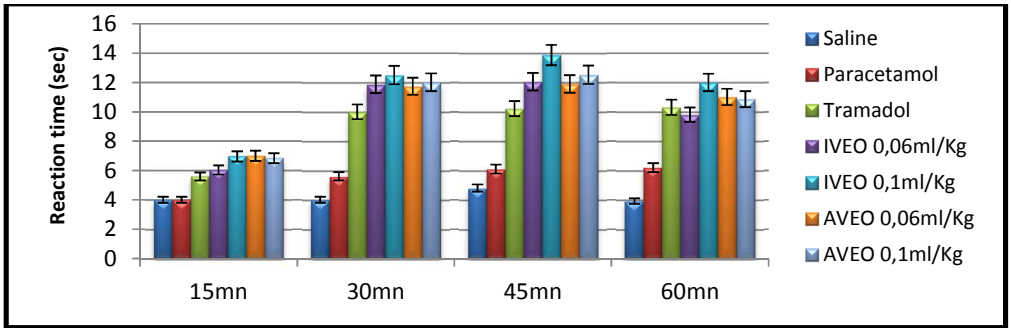

Fig.3. Reaction time of saline, standard drugs and essential oils of *I. viscosa* and *A. valentinus* in tail immersion test on rats

The analgesic activity was observed 30 min and up to 45mn after the oral administration of the essential oils obtained from *I. viscosa* and *A. valentinus* (60 and 100 µl/kg) which was practically higher than that seen with tramadol. This was evidenced by an increase in the latency of the avoidance response from 6.034 ± 0.030 to 12.05 ± 0.036, 6.956 ± 0.032 to 13.87 ± 0.052, 6.99 ± 0.026 to 11.9 ± 0.036 and 6.84 ± 0.033 to 12.52 ± 0.024 Sec for IVEO (0.06ml/Kg), IVEO (0.1ml/Kg), AVEO (0.06ml/Kg) and (AVEO) 0.1ml/Kg respectively.

3.4. Percentage analgesia

Table 1. Percentage analgesia of plants extracts and standard drugs

Groups		%analgesia
Standard drugs	Paracetamol	13.33
	Tramadol	50
IVME	300mg/Kg	64
	500mg/Kg	83
AVME	300mg/Kg	58.58
	500mg/Kg	66.66
IVEO	0.06ml/Kg	65.58
	0.1ml/Kg	70.9
AVEO	0.06ml/Kg	64.58
	0.1ml/Kg	66.75

The percentages analgesia of methanolic extracts and essential oils of *I. viscosa* and *A. Valentinus* were higher than that of tramadol and paracetamol (table 1). The highest percentage was registered for IVME at 500 mg/Kg (83%).

In fact, *I. viscosa* is more active than *A. valentinus.* They nevertheless present a central analgesic activity higher than that of Tramadol (30 mg/kg). This activity began at 30minutes after the administration of the drugs tested, which indicates a very rapid absorption from the gastrointestinal tract [31]. In contrast to peripheral analgesics, drugs which act mainly centrally, such as tramadol are actives on central nervous system [32]. It means that the analgesic effect of all extracts tested is due to the activation of the opioid receptor stimulation.

The strong analgesic activity of *I. viscosa* and *A. valentinus* in this study is often attributed to the phytochemicals presents in the extracts such as flavonoïds, phenolic acids and terpenoïds by inhibiting prostaglandins synthesis and central nervous system [33].

The antinociceptive effect of methanolic extracts and essential oils tested are consistent with those reported for the effects of extracts from medicinal plants especially that belonging to the family of Asteraceae such as *Matricaria aurea* [34] and *Ageratum fastigiatum* [35]. In fact, other studies describe the analgesic effects of medicinal plants [31, 36, 37].

4. Conclusion

The results of this study demonstrate potent central analgesic activity of methanolic extracts and essential oils obtained from *I. viscosa* and *A. valentinus* against thermal stimulus pain. Further, works like isolation, structural elucidation and screening of above active principles need to be done to pin the activity of this drug. Combination of these natural products with conventional drugs may also be done.

Acknowledgments
The authors are thankful to all the individuals and institutions who made this survey possible.

Declaration of interest: The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Souza V. C. and Lorenzi H., 2005. Botânica sistemática: guiailustrado para identificação dasfamílias de Angiospermas da flora brasileira, baseadoem APG II. Instituto Plantarum, Nova Odessa.

2. Guignard J.L., 1994**.** Abrégé Botanique, 9ème Ed. 204.

3. Bssaibis F., Gmira N., Meziane M., 2009. Activité antibactérienne de *Dittrichia viscoa* (L.) W. Greuter. Rev. Microbiol. Ind. San et Environn., 3 (1) : 44-55.

4. Boungab K., Tadjeddine A., Belabid L., Fortas Z., Lazrag F., 2011. Antifungal activity of extracts of *Anacyclus valentinus* L. on phytopathogenic fungi. In the proceedings of the 4th International Conference on Alternative Methods in Crop Protection. Evolution of French and European regulatory frameworks. Innovative new means and strategies, New Century, Lille, France. 230- 238.

5. Máñez S., Hernández V., Giner R-M., Ríos J-L., Recio M.C., 2007. Inhibition of pro-inflammatory enzymes by inuviscolide, a sesquiterpene lactone from *Inulaviscosa*.Fitoterapia 78: 329–331.

6. Lassouani Tadjeddine Aicha, Kambouche Nadia, MedjdoubHouria, Meddah Boumediène, Dicko Amadou et al., 2013. Antidiabetic effect of *Anacyclus valentines* L. aqueous extract in normoglycemic and streptozotocin induced-diabetic rats. American Journal of Phytomedicine and Clinical Therapeutics. AJPCT, 5 :424-43.

7. Hernández, V.M., R. Carmen, M. Salvador, M.G. Rosa and L.R. José, 2007. Effects of naturally occurring dihydro flavonols from *Inula viscosa* on inflammation and enzymes involved in the arachidonic acid metabolism. *Life Sci.,* 81: 480–488

8. Hamzi S. & Belhadj K. 2008**.** Contribution à l étude de l'activité antifongique des polyphénols extraits de deux plantes médicinales (*Anacyclus valentinus* l et *Anacyclus pyréthum*).

9. IASP, 2010. Pain Terminology. http://www.iasp-pain.org/AM/Template.cfm?Section=Pain_ Definitions & Template=/CM/HTML Display. Cfm & Content ID=1728#Pain (Accessed on 8 December.

10. Bonica JJ., 1979. The need of a taxonomy. Pain 6(3): 247-248.

11. Raj PP., 2007. Toxonomy and classification of pain. In: Kreitler S, Beltrutti D, Lamberto A, Niv D, editors. The handbook of chronic pain. New York: Nova Biomedical Books, p. 41-56.

12. Besra, S.E., R.M. Sharma. and A. Gomes, 1996. Anti- inflammatory effect of petroleum ether extract of leaves of Litchi chinensisGaertn (Sapinadaceae). J. Ethnopharmacol: 54 1-6.

13. Bannwarth B, Pehourcq F., 2003. Pharmacologic basis for using paracetamol: pharmacokinetic and pharma-codynamic issues (in French). Drugs 63, 5–13.

14. AbuhamdahSawsan, Abuhamdah Rushdie, Al-Olimat Suleiman and Chazot Paul, 2013. Phytochemical Investigations and Antibacterial Activity of Selected Medicinal Plants from Jordan. *European Journal of Medicinal Plants,* 3(3) :394 – 404.

15. HemamaliniK K.. Naik Om Prasad, Ashok P., 2010. Anti inflammatory and analgesic effect of methanolic extract of *Anogeissusacuminata*leaf*Int J Pharm Biomed Res*., *1*(3) :98-101.

16. Atik Bekkara Fawzia, Benhammou Nabila and Kadifkova Panovska Tatjana, 2008. Biological activities of the essential oil and ethanolic extract of Inulaviscosa from the Tlemcen region of Algeria. AFS, 30 (3) :132 – 139.

17. European Pharmacopeia (4th edition). Council of Europe: Strasbourg Cedex, France, 2002; pp.1 83-1 84.

18. Falleh H., Ksouri R., Chaieb K., Karray-Bouraoui N., Trabelsi N., Boulaaba M. and Abdelly C., 2008. Phenolic composition of *Cynaracarduncul*us L. organs, and their biological activities. Compt. Rend. Biol. Vol. 33: 372-379.

19. Khan H, Saeed M, Gilani AUH, Khan MA, Dar A, Khan I, 2010. The antinociceptive activity of Polygonatumverticillatum rhizomes in pain models. J Ethnopharmacol, 127(2):521 –527.

20. Kangralkar V. A, Biradar Sandeep, Mandavkar Yuvaraj, Thakur Megha, Chougule Nilesh, 2010. Anti-inflammatory, Antiarthritic, Analgesic and Anticonvulsant Activity Of Cyperus Essential Oils. *Int J Pharm PharmSci.,* 2 (4) :112-115.

21. Dykstra LA, Woods JH. A., 1986. Tail withdrawal procedure for assessing analgesic activity in Rhesus monkeys. Journal of pharmacological Methods, 15:263-26

22. Kumar Subedi Nirmal, Abdur Rahman S. M., and Ahsanul Akbar Mohammad, 2016. "Analgesic and Antipyretic Activities of Methanol Extract and Its Fraction from the Root of Schoenoplectusgrossus," Evidence-Based Complementary and Alternative Medicine, vol. 2016, Article ID 3820704, 8 pages,

23. Sook-Ha Fan, Noraisah Akbar Ali, and Dayang Fredalina Basri, 2014. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercusinfectoria (Olivier) in Rats," Evidence-Based Complementary and Alternative Medicine, vol., Article ID 976764, 6 pages,

24. Gökbulut A, Özhan O, Satilmis B, Batçioglu K, Günal S, Şarer E., 2013. Antioxidant and antimicrobial activities, and phenolic compounds of selected inula species from Turkey. *Natural Product Communications*. 8(4):475–478.

25. Laghrifi Khadija, Mostafa El Idrissi, Youssef Makoudi, RachadAlnamer, 2013. *In Vitro* Antibacterial Activity Of The Methanolic And Ethanolic Extract Of *Inula Viscosa* Used In Moroccan Traditional Medicine. World Journal Of Pharmacy And Pharmaceutical SCIENCES, 2 (5) : 3963-3976.

26. Hamimed S., 2009. *Caractérisation chimiquedes principes à effet antidermatophyte des racines d'Anacyclus pyrethrum* L. Département de chimie, faculté des sciences exactes. Université Mentouri Constantine, Algérie.

27. Sujith K, Ronald D, Suba V., 2011. Antioxidant activity of ethanolic root extract of Anacyclus pyrethrum, International Research Journal of Pharmacy; 222-226.

28. Smith RL, Cohen SM, Doull J, Feron VJ, Goodman JI, et al., 2005. A procedure for the safety evaluation of natural flavour complexes used as ingredients in food: essential oils. *Food Chemestry. Toxicol.,* 43: 345-363.

29. Side Larbi K, Meddah B, Tir Touil Meddah A and Sonnet P., 2016. The antibacterial effect of two medicinal plants inulaviscosa, anacyclusvalentinus (asteraceae) and their synergistic interaction with antibiotic drugs. J. Fundam. Appl. Sci., 8(2):244-255.

30. Hmmouchi M., 1997. Plantes alimentaires, aromatiques, condimentaires, médicinales et toxiques au Maroc. In : Heywood V.H. (ed.), Skoula M. (ed.). Identification of wild food and non-food plants of the Mediterranean region. Chania : CIHEAM, p. 89-108 (Cahiers Options Méditerranéennes; n. 23)

31. Naveed Muhammad, Muhammad Saeed and HaroonKha., 2012. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC Complementary and Alternative Medicine,

32. Shreedhara C. S., Vaidya V. P., Vagdevi H. M., Latha K. P.,. Muralikrishna K. S, and Krupanidhi A. M., 2009. "Screening of *Bauhiniapurpurea* Linn. For analgesic and anti-inflmmatory activities," *Indian Journal of Pharmacology*., 41(2):75–79.

33. Mahmoud Bahmani, Hedayatollah Shirzad, Maedeh Majlesi, Najmeh Shahinfard, Mahmoud Rafieian-Kopaei, 2014. A review study on analgesic applications of Iranian medicinal plants. Asian Pac J Trop Med; 7(Suppl 1): S43-S53.

34. Esam QNAIS, 2011. The analgesic effect of the ethanolic extract of *Matricaria aurea.* Turk J Biol 35 (2011) 347-352.

35. Glauciemar, D. V., Orlando V. S., Celia H. Y and Maria A. C., 2009. Chemical composition and Antimicrobial Activity of Essential oils of *Ageratum fastigiatum* (Asteraceae). Records of Natural Products Journal. 3(1): 52-57.

36. Gulnur, T., Merve, M., Erdem, Y., Mustafa, A., 2004. Main Flavonoids of Tilia argentea DESF. ex DC. Leaves. Turk. J. Chem., 28:745-749.

37. Bhujbal SS. Chitlange SS, Suralkar AA, Shinde DB,. Patil MJ. 2008. Anti- inflammatory activity of an isolated flavonoids fraction from Celosia argentea Linn. J Med Plants Res., 2:52-54.