Study Of Analgesic Activity And Drug-Excipient Compatibility Of Polyherbal Ointment

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Abstract
The definition of pain is a disagreeable sensation induced by harmful or intense stimuli. The aim of this research was to assess the topical analgesic properties of a polyherbal ointment composed of ethanolic extracts of the following herbs: Datura stramonium (seeds), Nerium indicum (leaves), Curcuma longa (rhizome), Acorus calamus (rhizome), and Emblica officinalis (fruits). The analgesic activity of a topical ointment containing essential extracts was assessed in rats through the utilization of the hot-plate method. A sodium salt of diclofenac was used as the standard prescription. For the development of an anti-inflammatory formulation, herbal components such as Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus, and Emblica officinalis were chosen due to their demonstrated efficacy in the treatment of inflammation, as determined by a review of the relevant literature. These plants were integrated into the ointment formulation as a topical delivery system. On the basis of its appearance, pH, spreadability, extrudability, rheological behavior, in vitro release performance, and analgesic study, the ointment was evaluated. Early on, the analgesic properties of the topical preparation were detected, exhibited notable efficacy in comparison to the standard drug diclofenac sodium 90 and 120 minutes after drug administration via hot-plate technique. The stability investigation was conducted at three-month, one-month, and zero-hour intervals; variations in physicochemical properties and other attributes were observed throughout this time period. Over the course of three months, the appearance, color, and odor of the ointment remained unchanged. Utilizing Fourier-transform infrared spectroscopy (FTIR), the compatibility study was evaluated.

Keywords: Analgesic activity, Topical ointment, FTIR, Hot Plate method

INTRODUCTION

Pain is characterized as an adverse affective and sensory encounter that is linked to tangible or possible harm to bodily tissues. In recent decades, common analgesics, including pentazocine and diclofenac, have been utilized extensively. Approximately 30% of patients experience pain relief from these analgesic drugs, particularly opioids and nonopioid analgesic drugs, at most, to the extent of 50%. [1] Moreover, numerous of these medications induce severe adverse effects. Research has indicated that narcotics induce physical dependence, tolerance, and addiction in individuals, whereas nonsteroidal anti-inflammatory medications are commonly associated with gastrointestinal disturbances [2]. Herbal remedies are generally regarded as secure and less detrimental to the human body in comparison to synthetic medications. Labs across the globe are thus involved in the evaluation of botanical species for biological activities that may have therapeutic applications. A primary factor considered in the selection of plants for this study is the therapeutic efficacy as claimed by traditional healers. Herbal remedies are documented in the traditional Indian medicinal system as potential treatments for a wide range of ailments.

Consequently, individuals depend on herbal remedies as an alternative approach to managing pain. In comparison to synthetic medications, herbal remedies are readily available, cost-effective, and have fewer adverse effects [3].

An assortment of physical, chemical, and biological assays are employed in the assessment of the polyherbal anti-inflammatory ointment. Physical tests examine the ointment's appearance, and consistency, whereas chemical tests analyze the active ingredients. In biological testing, animal models are utilized to determine the anti-inflammatory efficacy of the ointment. Furthermore, safety assessments are conducted to ensure that the application of the ointment to the epidermis will not result in any adverse effects.[4,5]

A transient, noxious stimulus of brief duration is administered in these tests; it is detected by free nerve endings and transmitted via conducting neuronal pathways. The hot-plate test method is classified as a supraspinally organized response because it engages upper brain functions.

2. MATERIALS AND METHODS

2.1. Collection of Materials

Emblica officinalis, Datura stramonium, Nerium indicum, Curcuma longa, and Acorus calamus were gathered from the surroundings of the Jaunpur Campus of Veer Bahadur Singh Purvanchal University.

2.2. Preparation of Plant Material
To preserve the phytoconstituents, the recently harvested Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits were desiccated at a temperature of 40°C using a heated air grill. The coarse powder resulting from the processing of the desiccated plant components with a Willy machine was then placed in a container that was securely sealed. Each component of the plant was extracted separately using ethanol. By refining, the resulting extracts were concentrated. Pending further utilization, the concentrated extracts were preserved in desiccators.[6,7]

2.3. Formulation of Ointment
The substance of the ointment was manufactured via fusion. The components of the base were consolidated in the basin and subjected to melting at a temperature of 70ºC. The ingredients were chilled while being agitated continuously at a temperature of 70ºC for specific time intervals following melting. The process of ointment formulation involved the trituration of the base material with the active ingredients using a mortar and pestle. The components utilized in the formulation of ointment base and polyherbal ointment are as follows: Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits. The excipients included in the ethanolic extract are as follows: stearic acid (15 g), white wax (2 g), yellow vaseline (8 g), propylene glycol (8 g), methyl paraben (0.2 g), and propyl paraben (0.1 g). Each extract contains 1 g.[8,9,10]

2.4 Assessment of the ointment
The results for pH, color, consistency, state, washability, spreadability, and viscosity indicate that the formulations are skin-compatible. After ointment application, no indications of irritation were detected in albino rodents.[11] The polyherbal ointment was evaluated and found to have the following characteristics: pH: 7.02; spreadability (g cm/sec): 14.36; viscosity (CPS): 3247; washability: acceptable; and extrudability: average.

3.5 Pharmacological Screening
3.5.1 Animals
Adult, healthy albino rodents between 150 and 200 g in weight were utilized in the experiment. The animals were kept at 22–24°C and on a 12-hour light–dark cycle. [12] They were provided with water and standard laboratory feed. Each of the three groups contains albino rodents. The experimental protocols were adhered to in compliance with the ethical standards governing the examination of experimental pain in conscious animals [21]. Every animal experiment was conducted at Deshpande Laboratories Pvt. Ltd. An Institutional Animal Ethics Committee-Certified Drug Testing Laboratory with ISO 9001:2008 Accreditation and Register No. CPCSEA Consensus: 1582/PO/Re/S/11/ CPCSEA.

2.5.2 Goat Skin Diffusion Assay
A razor was used to depilate the epidermis of the goats just prior to slaughter. After being placed in ice-cold saline, the newly cleansed epidermis was transferred to the laboratory.[13] A wide-mouth beaker containing phosphate-buffered saline was used to hold the skin in place, and 2 grams of the final ointment formulation were distributed uniformly across the skin using a spatula. At 30-minute intervals, 200ul of samples were withdrawn from the container, and the OD 450 was calculated and plotted against time. (refer to Table 1 and Figure 1).

2.5.3 Dermal Toxicity
Once daily for seven days, 100 mg of ointment and a blank formulation were applied to depilated rat epidermis.[14] Redness, inflammation, pruritus, and additional observable morphological indicators were detected by two unbiased observers. (Figure 2).

2.5.4Analgesic activity
To determine the analgesic activity of a polyherbal ointment containing extracts of Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus, and Emblica officinalis, the hot-plate method as described by Abd Allah et al. was utilized. A hot-plate test was conducted utilizing an Eddy's hot-plate, which was electronically controlled and maintained at a temperature of 53°C (±0.1°C). In order to measure the basal reaction time, unrestrained rats from each group were placed on the heated plate just prior to the application of the ointment base or drug, which was considered to be zero time. The test group animals were treated with a 100 mg topical ointment applied to the albino rodents' hind paws. The animals in the standard and control groups were similarly applied diclofenac ointment (1.16%) and the ointment base that was used in the test group. Twenty-five minutes subsequent to the application of the drug, any residual ointment on the skin's surface was removed using a cotton swab. The pain threshold of the treated animals was assessed at time intervals of 0.5, 1, 1.5, 2, and 2.5 hours subsequent to the administration of the drug. The duration of latency to elevate and lick the hind limb, as well as attempts to leap from the apparatus, were documented for both the control and drug-treated groups. Thirty seconds was the cutoff time to prevent additional tissue injury from heated plate exposure [15].

2.6 Drug-Excipients Interaction Study
A. Stability studies
For stability investigations, the ointment was observed for a few days to identify any visible physical changes. The ointments shall be exposed to the following conditions: when left open and in the presence of air, and when sealed at

ambient temperature under both light and darkness. Physical and chemical stability assessments were conducted at the outset and during the duration of storage. The physical integrity of the ointment was evaluated under standard room lighting conditions through visual observation. The ointment maintained its tangible appearance throughout the duration of the investigation. The chemical stability of the ointment was assessed through the utilization of an analytical technique that indicates stability.[16]

B. Fourier Transform Infrared Spectroscopy Analysis

FTIR spectroscopy was employed in the second phase of the investigation to assess the compatibility between the ointment and the excipients utilized. The FTIR spectroscopy method is utilized to analyze substantial alterations in the location and configuration of the absorbance bands. The plain FTIR spectrum is illustrated in Figure 3. As the second method utilized in our investigation, FTIR spectroscopy was employed to observe the interaction between extracts and excipients.[17] By examining notable alterations in the location and configuration of the absorbance bands, it is possible to deduce the presence or absence of various functional groups on subsequent molecules. The Test Formulation of FTIR spectra and the Test Formulation three months after the FTIR spectra were observed in Figure 4, respectively. Emblica officinalis (fruits), Datura stramonium (seeds), Nerium indicum (leaves), Curcuma longa (rhizome), and Acorus calamus (rhizome) comprise the FTIR spectrum of the active drug-excipient physical composition. All characteristic peaks were preserved in ethanolic extract and excipients such as stearic acid, white wax, yellow vaseline, triethanolamine, propylene glycol, methyl paraben, and propyl paraben. (Figures five and six)

3. RESULT

Table: 1 Permeability Parameters Goat Skin Diffusion Assay of Polyherbal Ointment. percentage (mean±SE), n=3

Time (min)	Results
0	0.06 ± 0.005
30	0.17 ± 0.005
60	0.62 ± 0.02
90	0.81 ± 0.01
120	1.07 ± 0.05
150	1.10 ± 0.02

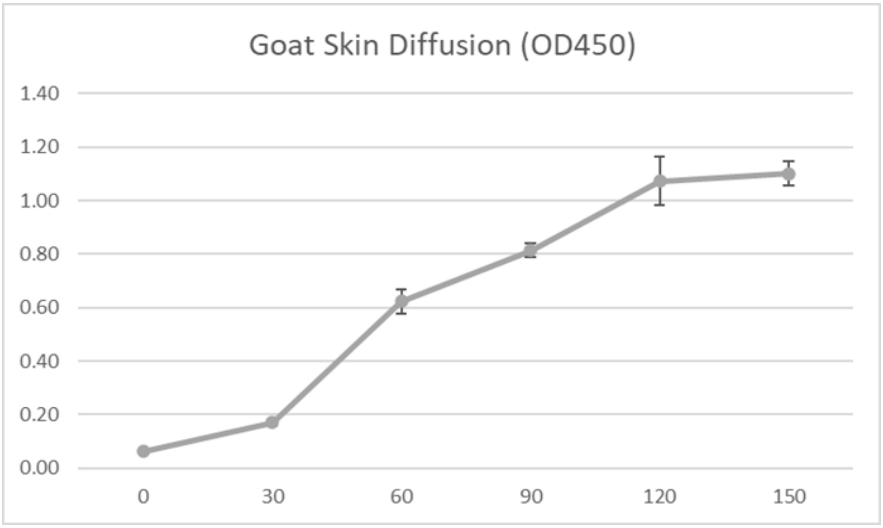

Figure:1 Permeability Parameters Goat Skin Diffusion Assay of Polyherbal Ointment

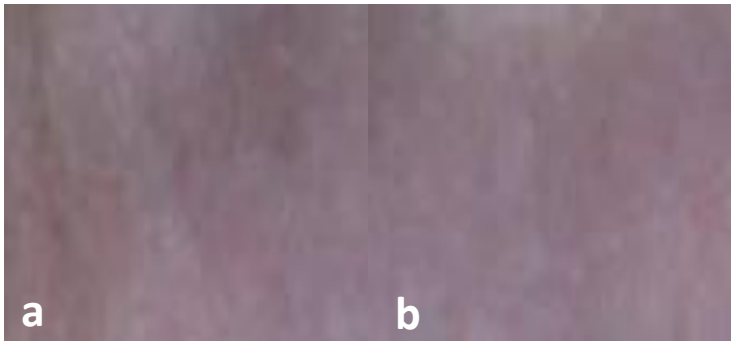

Figure: 2 Images of rat skin treated with test samples applied topically for 7 days, untreated (a), Test (b). No signs of inflammation or irritation were observed**.**

Table 2: Analgesic activity of Polyherbal Ointment by hot plate method

Treatment	**0** Minute	**30** Minute	**60** Minute	**90** Minute	**120** Minute	**150** Minute
Control	4.23±0.31	3.83±0.45	3.6 ±0.5	4.34±0.32	4.51±0.32	4.75±0.44
Polyherbal ointment	4.52±0.29	5.17±0.31	6.6±0.9*	9.37±0.37**	9.26±0.29**	7.56±0.19*
Diclofenac Gel	4.66 ±0.35	6.33±0.33	9.2±0.8**	10.67±0.42**	11.46±0.45**	10.6±0.51**

n=6;*p<0.05,**p<0.001values are expressed as mean±SEM.One-way ANOVA followed by Dunnett's t-test; all the groups are compared with control.SEM: Standard error mean, *Polyherbal ointment*

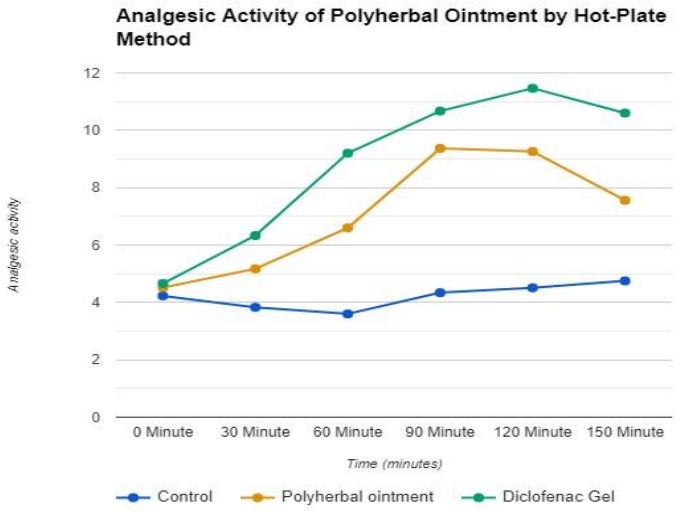

Figure-3: Analgesic activity of Polyherbal Ointment by hot plate method

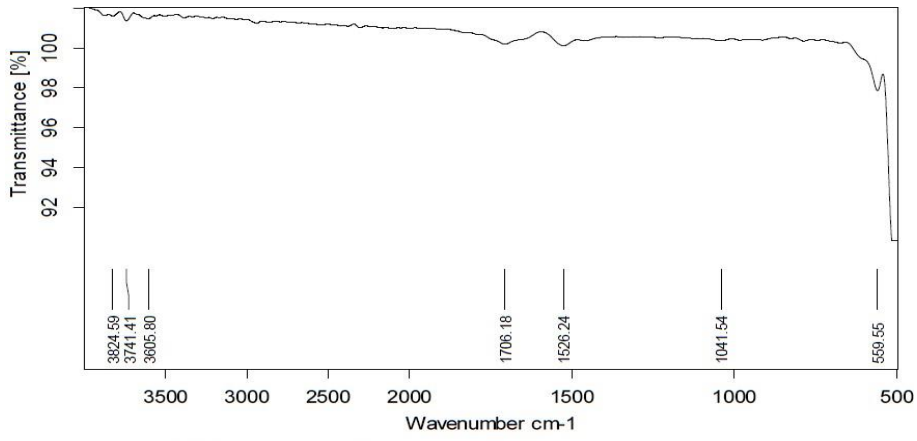

Figure: 4 FTIR Spectra of Blank Formulation

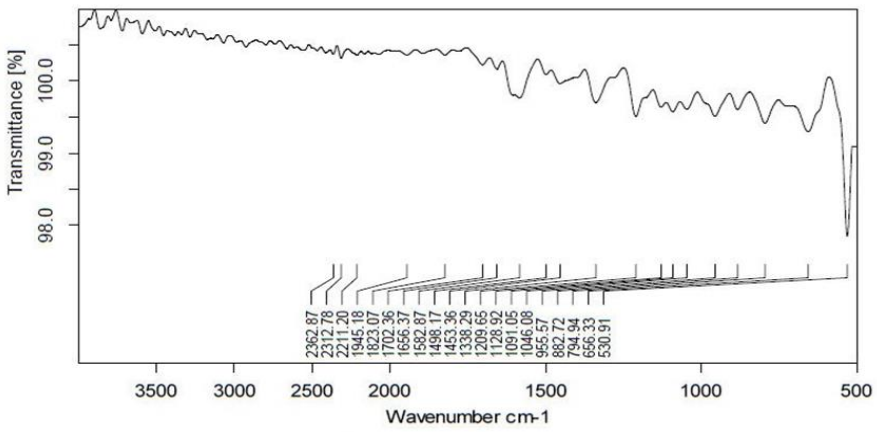

Figure: 5 FTIR Spectra of Polyherbal ointment test Formulation

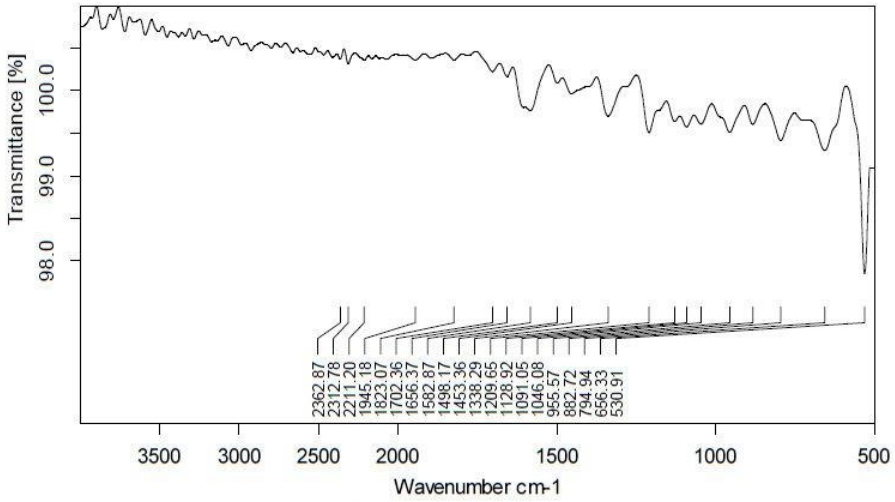

Figure: 6 FTIR Spectra of Polyherbal ointment test Formulation after 3 months

4: DISCUSSION

The central analgesic activity of the polyherbal ointment is determined using the hot-plate test [18]. The findings indicated that the most effective application of the ointment was achieved through topical administration at 90 and 120 minutes. The hot-plate method is regarded as a selective screening technique for substances that bind to the opioid receptor [19]. The action of volatile ointment has been ascribed to the synergistic influence of its active and inactive constituents in numerous scientific investigations. Pharmacokinetics and bioavailability of the active compounds may be influenced by the inactive compounds [20]. Furthermore, establishing a correlation between the composition of an ointment and its biological activity is challenging because the components work synergistically [21]. Thus, it would appear that the analgesic effect of polyherbal extract essential ointment is primarily attributable to the synergistic effect of the entire plant. Both the latency time and the effect of the ointment as measured by the heated plate varied in a time-dependent fashion (Table 2). No statistically significant antinociceptive effect was detected at either the 0-minute or 30-minute mark following the application of the polyherbal ointment. The findings revealed that the application of topical ointment to animals for 60 minutes significantly prolonged the basal reaction time. In contrast, standard diclofenac ointment induced a potent analgesic response in animals exposed to stimuli for 60 (9.2± 0.8), 90 (10.67±0.42), and 120 (11.46±0.45) minutes, and significantly prolonged the basal reaction time in comparison to the control group.

The corresponding FTIR spectra of the polyherbal ointment are depicted in Figures 4,5 and 6. Figure 4 depicts the Fourier Transform Infrared (FTIR) Spectra of the Blank Formulation. Figure 5 illustrates the FTIR Spectra of the polyherbal ointment test formulation. Lastly, figure 6 presents the FTIR Spectra of the polyherbal ointment test formulation three months later. Similar peaks were observed in Figures 5 and 6, spanning the range of 530.91 cm^{-1} to 2362.87 cm^{-1}. The consistency of the peaks three months after formulation indicates that the polyherbal ointment is stable.

5: CONCLUSION

A potent analgesic activity was observed in a polyherbal ointment containing ethanolic extracts of Datura stramonium (seeds), Nerium indicum (leaves), Curcuma longa (rhizome), Acorus calamus (rhizome), and Emblica officinalis (fruits). When comparing individual ointments to polyherbal ointment, a synergistic effect was observed, which could potentially offer therapeutic benefits in the context of analgesia. The results obtained from this research indicate that the polyherbal ointment formulation exhibits potential as a viable substitute for traditional analgesic medications in the context of pain management. Additionally, research is required to identify the phytoconstituents and associated mechanisms that are accountable for their analgesic properties.

Compatibility analyses between plant extracts and excipients were conducted in our research employing Fourier Transform Infrared Spectroscopy techniques. These techniques offer valuable insights into the compatibility between drugs and excipients. The stability of the polyherbal ointment is demonstrated by the absence of peak changes in FTIR spectra three months after formulation.

6: REFERENCE

1. Hanson GR, Venturelli PJ, Fleckenstein AE. Drugs and Society. 10th ed. Boston, Massachusetts, USA: Jones and Bartlett; 2009.
2. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol2014;4:177.
3. Makhija IK, Richard L, Kirti SP, Saleemullah K, Jessy M, Annie S. Sphaeranthus indicus: A review of its chemical, pharmacological and ethnomedicinal properties. Int J Pharmacol 2011;7(2):171-9.
4. Nayak S, Behera SK, Misra MK. Ethno-medico-botanical survey of Kalahandi district of Orissa. Indian J TraditKnowl2004;3:72.
5. Kirtikar KR, Basu BD. Indian Medicinal Plant, II. Allahabad: Lalit Mohan Basu Publication; 1935. p. 1347.

6. J Mishra, AK Dash, DK Dash .Nature's Drug Store: The Free Tree of India: World Journal of Pharmacy and Pharmaceutica, volume -2,2013,page 4778-98 .
7. Chopra RN, Chopra IC, Handa KL, Kapur LD. Chopras Indigenous Drug of India. Calcutta, India: U.N. Dhur and Sons; 1958. p. 601.
8. Samuel JK, Andrews B. Traditional medicinal plant wealth of Pachalur and hamlets Dindigul district, Tamil Nadu. Indian J TraditKnowl 2010;9(2):264-70.
9. Wabale AS, Petkar AS. Ethnomedicinal plants used against jaundice by the tribals of Akole Taluka (MS). J Phytol Res 2005;18(2):259-26.
10. Dennis SG, Melzack R. Comparison of phasic and tonic pain in animals. Adv Pain Res Ther 1979;3:747-60.
11. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: An overview. Pain 1985;22(1):1-31.
12. AK Dash, J Mishra, DK Dash -Antidiabetic activity and modulation of antioxidant status by Ocimum canum in streptozotocin-induced diabetic rats: European Scientific Journal February 2014 edition vol.10, No.6
13. Chhetri HP, Yogol NS, Sherchan J, Anupa KC, Mansoor S, Thapa P. Formulation and evaluation of antimicrobial herbal ointment. Kathmandu Univ J Sci Eng Technol 2010;6(1):102-7.
14. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16(2):109-10.
15. Abdallah FI, Dawaba HM, Mansour A, Samy AM. Evaluation of the anti- inflammatory and analgesic effects of piroxicam loaded microemulsion in topical formulations. Int J Pharm Pharm Sci 2011;3(2):66-70.
16. Giordano, F., Bettinelli, G.P. 1988. An experimental and theoretical approach to the analysis of pharmaceutical binary systems. Journal of Pharmaceutical and Biomedical Analysis, 6, 951–955. https://doi.org/10.1016/0731-7085(88)80115-8.
17. Joshi, B.V., Patil, V.B., Pokharkar, VB. 2002. Compatibility studies between carbamazepine and tablet excipients using thermal and nonthermal methods. Drug Development and Industrial Pharmacy, 28, 687–694. https://doi.org/10.1081/DDC120003860.
18. Ghori SS, Ahmed MI, Arifuddin M, Khateeb MS. Evaluation of analgesic and anti inflammatory activities of formulation containing camphor, menthol and thymol. Int J Pharm Pharm Sci 2016;8(1):271-4.
19. Sarmento-Neto JF, Nascimento LG, Felipe CF, Sousa de DP. Analgesic potential of essential oils. Molecules 2016;21(20):1-29.
20. Svoboda KP, Deans SG. Biological activities of essential oils fromselected aromatic plants. Acta Hortic 1995;390(1):203-9.
21. Tadrent W, Bachari K, Kabouche Z. Comparative compositions and antibacterial activity of the essential oils of Anthemis nobilis L. and Anthemis mixta L. (Asteraceae). Int J Pharm Pharm Sci 2016;8(7):457-9.