

Original Research Article

Phytochemical composition, and analgesic and anti-inflammatory properties of essential oil of *Chamaemelum nobile* (Asteraceae L All) in rodents

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Abstract

Purpose: To investigate the *in vivo* analgesic and anti-inflammatory activities of essential oil of dried flowers of *Chamaemelum nobile* (Asteraceae L. All) in Swiss mice and Wistar rats, respectively.

Methods: The volatile oil of the dried flowers of *C. nobile* obtained by hydrodistillation was analyzed by gas chromatography-mass spectrometry (GC-MS). Animals were assigned to the following experimental groups: *C. nobile* (180 mg/kg), ibuprofen (100 mg/kg) and vehicle-treated groups. Chemical and thermal pain models were used for the antinociceptive study in mice while fresh egg albumin-induced acute inflammation model in rats was used for anti-inflammatory study of the essential oil.

Results: The most abundant components of the oil were α -bisabolol (50 %) and farnesene (5.35 %). Oral administration of essential oil (180 mg/kg, po) significantly ($p < 0.05$) reduced pain and prevented inflammation in the different test models used in this study.

Conclusion: The essential oil of the dried flowers of *C. nobile* shows *in vivo* analgesic and anti-inflammatory activities in rodents, and therefore, possesses potentials for development into effective analgesic and anti-inflammatory agent.

Keywords: *Chamaemelum nobile*, Asteracea, essential oil, anti-inflammatory, antinociceptive

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INTRODUCTION

Essential oils are complex mixtures of aromatic volatile oily compounds characterized by their odours. These oils are generally less dense compared to water. Although the primary role of essential oil is to protect the plant against bacteria, viruses, fungi and insects, nevertheless,

they are known to exert beneficial effects in aromatherapy [1]. These plant secondary metabolites have been associated with positive pharmacological properties such as sedative, anxiolytic, antioxidant, antiviral and antimicrobial effects [2-4].

Chamomile is known for its therapeutic uses especially for its calming and sleep promoting effects. It is a member of the daisy family (*Asteraceae/Compositae*) which is represented by two main species: German (*Chamomilla-recutita*) and Roman (*Chamaemelum nobile*) Chamomile [5]. *Chamaemelum nobile* (*C. nobile*) is a low-growing perennial plant, with finely divided leaves arranged alternately on the stem. The flower has a yellow central disc surrounded by silvery-white petals which when crushed give an apple-like smell. Of the two common members of this genus, German chamomile has been explored for several biological activities [6], while Roman chamomile (*C. nobile*) known to be common in South Africa is yet to be extensively studied. Roman chamomile is thought to possess carminative, sedative, antimicrobial, antiemetic and antispasmodic properties [7]. Locally, *C. nobile* is used for treatment of arthritis and other inflammatory conditions.

Traditionally, *C. nobile* (*Asteraceae* L. All) is used for the management of pain and inflammation as well as a sedative. The therapeutic properties of *C. nobile* oil are widely attributed to the presence of farnesene and α -pinene, flavonoids, coumarins, terpenoids and mucilage [8] The aim of this study was to investigate the chemical composition, *in vivo* safety, analgesic and anti-inflammatory properties of essential oil from flowers of *C. nobile*. To the best of our knowledge, no study on the acute toxicity *in vivo* analgesic and anti-inflammatory activities of *C. nobile* essential oil has been reported so far.

EXPERIMENTAL

Plant material and chemicals/drugs

C. nobile flower was collected in the Eastern Cape, and was authenticated by Dr Immelman, a taxonomist at KEI Herbarium, Walter Sisulu University, South Africa where a voucher specimen (no. AO2/14024) of the plant material was deposited for future reference.

The following drugs were used for biological studies: Formalin (BDH Chemical Ltd, England) and ibuprofen (Dis-Chem Pharmacies, South Africa).

Essential oil extraction

The plant material was air dried in the laboratory. A Clevenger apparatus was used to extract 300 g of dried *C. nobile* by hydro-distillation for 4 h. The isolated pale blue essential oil was preserved in a tightly-capped bottle until used for experiments. Dichloromethane was used to dilute

oil in a 1: 200 ratio for determination of its composition by gas chromatography and mass spectrometry.

Gas chromatography-mass spectrometric (GC-MS) analysis

Analysis by GC-MS (gas chromatography-mass spectroscopy) was performed on an Agilent 5973N GC-MS system operating in EI mode at 70 eV, equipped with a HP-5 MS fused silica capillary system with a 5 % phenylmethylsiloxane stationary phase. A 30 m by 0.25 mm capillary column with a film thickness of 0.25 μ m was used. The column was heated from 70 °C to 240 °C at an incremental rate of 5 °C/min. Highest temperature attained was 450 °C and maintained for 77.25 minutes. The flow rate of the carrier gas, helium, was 1 ml/min at a split ratio of 100:1. Scan time was 78 min with a scanning range of 35 to 450 amu. Kovats indices were determined by the analysis of 1 μ l *C. nobile* diluted in hexane was injected for analysis. A similar procedure was used for *n*-Alkanes (C_8 to C_{30}). The identification of compounds was also based on the Kovats retention indices which were calculated using *n*-alkanes C_8 - C_{20} . Obtained values were then compared with reported values in literature [9,10].

Animals

Wistar rats weighing (150 to 200 g) and Swiss mice weighing 20 to 30 g of both sexes were procured from the South African Vaccine Initiative (Johannesburg). Animals were accommodated in the animal holding facility in the Department of Biological and Environmental Sciences where they had access to food and water *ad libitum* for two weeks before commencement of tests. Experimental animals were fasted overnight, but allowed access to drinking water before the treatment for proper hydration. Ethical approval for the study was granted by the Walter Sisulu University Research Ethical Committee (Ethical clearance no. DVC (AA & R) DRD/SREC: ref no. 03). Studies were carried out in line with the recommendations of the International Guidelines for the use of Animals for Scientific Purposes [11]. In this study, essential oil of *C. nobile* was emulsified with Tween 80 (5 %) before administration to the animals.

Acute toxicity study

Acute toxicity of the essential oil of *C. nobile* was assessed in mice using the oral route (po) according to Lorke's method [12]. This method involves the use of thirteen animals for a rapid

and economic LD₅₀ estimation using oral route. The experimental procedure was divided into 2 phases. In the first phase 9 mice were assigned to 3 groups of 3 rats each for the dose levels of 10, 100 and 1000 mg/kg. In the second phase only one mouse was used per dose level of each of four dose levels: 1000, 1600, 2900 and 5000 mg/kg, respectively. Immediately after treatment, each mouse was placed inside the Plexiglas cage and observed for immediate effects during the first 30 mins and thereafter for 24 h after treatment for lethal effects culminating into death. Animals were monitored for additional 14 days to ascertain the delayed effects of *C. nobile*. LD₅₀ of *C. nobile* essential oil was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to Eq 1.

$$LD_{50} = \sqrt{(A \times B)} \dots \dots \dots (1)$$

where A is the maximum dose producing 0 % death and B is the dose that produces 100 % death [15]. From the results of LD₅₀, the working doses (W) were determined according to Eq 2.

$$W \leq 1/2(LD_{50}) \dots \dots \dots (2)$$

Thermal pain (tail flick) test

In this experiment, mice were randomized into three groups of 6 mice each and treated with: 180 mg/kg of *C. nobile*, 100 mg/kg ibuprofen and 5% tween 80 respectively 1 h before the thermal challenge. Mice were gently restrained while allowing the posterior third of tail to rest over a glass window slit of the Tail Flick meter (Ugo Basile, model 37360) as described previously by Nkeh-Chungag *et al* [13]. Baseline tail withdrawal latencies from the heat source were determined before oral administration of assigned treatment to animals. Tail withdrawal latencies were again determined 1, 2 and 4 h after drug treatment.

Formalin-induced pain test

Prabhu *et al*'s [14] method for the formalin test was used. Mice (n = 6 / group) were selected for the study. The control group (group 1) received the vehicle; group 2 received 180 mg/kg essential oil of the *C. nobile* while group 3 was treated with ibuprofen (positive control) (100 mg/kg) orally, 1h before 0.1 ml of 2.5 % formalin diluted in saline was injected into the mice paws. Animals responded by licking or biting the injected paw. The frequency of licks/bites was computed for the first 5 minutes and then for the next 20 to 30 minutes after formalin injection [15]. Pain inhibition (H) was then calculated using Eq 3.

$$H (\%) = \{1-(T/C)\}100 \dots \dots \dots (3)$$

where T represents the number of times treated mice bit or licked the injected paw, and C is the number of times control mice licked/bit the treated paw.

Acute inflammation test (fresh egg albumin model)

Male Wistar rats were divided into 3 groups (n = 6) and treated as follows: group I (control group) - vehicle; group II - with ibuprofen (100 mg/kg, *po*) and group III - essential oil of *C. nobile* (180 mg/kg, *po*). One h later, 0.1 ml fresh egg albumin was injected into the left hind paw of all rats [16]. The diameter of injected paws was measured at 0, 1, 2, 3, 4 and 5 h using a Vernier caliper (model YT-7201, Poland) after fresh egg albumin injection. The change in the paw size of the rat was determined by subtracting the paw diameter, at the various times from the baseline measure of the paw diameter.

Statistical analysis

Data was analyzed using Graphpad Instat® (version 5). ANOVA followed by Dunnett's test. Was performed to compare results from the different treatment groups at indicated times. Results were expressed as mean ± SEM. *P* < 0.05 was considered statistically significant.

RESULTS

Phytochemical composition of essentials oil

Table 1 shows the chemical composition of the essential oil of *C. nobile*. Hydrodistillation of *C. nobile* flower yielded 1.3 % blue coloured, aromatic smelling essential oil. Eighteen volatile compounds were identified in the essential oil of *C. nobile* with alpha-bisabolol being the most abundant quantitatively (> 50 %) with a KI of 1740 and a RT of 33.29 s. The second most abundant compound was farnesene (5.35 %) with a KI of 1564 and RT of 33.29 s, respectively, followed by spathulenol (2.56 %) which had a KI of 1640 and a RT of 26.03 s (Table 1). The rest of the compounds occurred in relatively smaller amounts (< 1 %). Figure 1 shows chemical structures of major compounds identified in the oil.

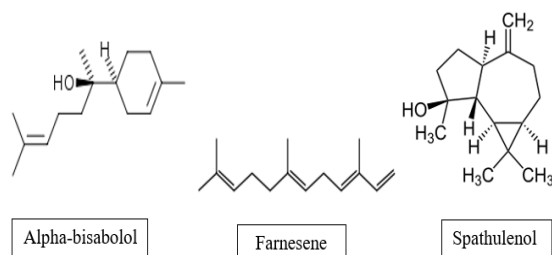


Figure 1: Chemical structures of the major compounds identified in the essential oil of *C. nobile*

Acute toxicity study

In the first phase acute toxicity testing using Lorke’s method, doses up to 1000 mg/kg did not cause mortality when administered via the oral route. Similarly no mortalities were observed in mice during the second phase when animals received 1000, 1600, 2900 and 5000 mg/kg. Furthermore, no changes were noted in the animals’ behavior and physical appearance 30 minutes after oral administration of extracts and up to 14 days later. Thus the LD₅₀ was estimated as greater than 5000 mg/kg, *po* (Table 2).

Effect of *C. nobile* essential oil on thermal pain in mice

Results showed that pre-treatment with 180 mg/kg of *C. nobile* significantly (*p*<0.05) increased the tail flick latency in the second hour compared to control. Ibuprofen, however, increased tail flick latency throughout the period of experimentation though these effects were only significant during the first two hours after oral administration of the drug compared to the vehicle group (Figure 2).

Table 2: Acute toxicity test of the essential oil of *C. nobile* in mice

Dose (mg/kg)	Death pattern after 24 h
Phase 1	
10	0/3
100	0/3
1000	0/3
1000	0/3
Phase 2	
1600	0/1
2900	0/1
5000	0/1
LD ₅₀	≥5000 mg/kg, <i>po</i>

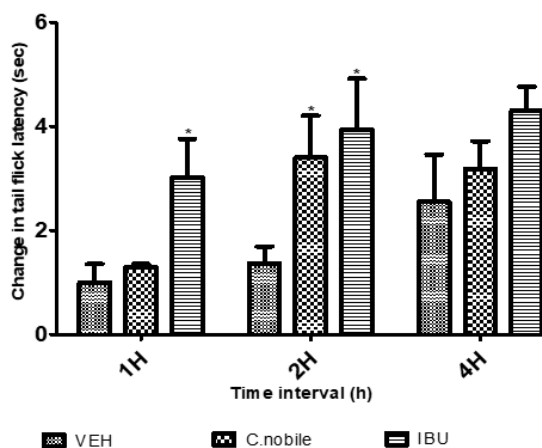


Figure 2: Effect of *C. nobile* essential oil on thermal pain in mice. Each bar represents Mean ± SEM of change in tail flick latency. VEH, *C. nobile*, and IBU represent vehicle, *C. nobile* essential oil (180 mg/kg), and ibuprofen (100 mg/kg) respectively. All treatments were by the oral route; **p* < 0.05, statistically compared to vehicle.

Table 1: Elution time, Kovat index and composition of essential

Compound	KI	Elution time (s)	Composition (%)
Camphene	953	10.502	0.03
Benzaldehyde	961	10.594	0.04
Cymene	1011	13.198	0.08
Limonene	1031	13.398	0.25
Camphor	1144	17.319	0.09
Endo-Borneol	1165	18.178	0.24
Terpinen-4-ol	1177	18.388	0.09
α-Terpeneol	1185	18.364	0.07
Pulegone	1237	20.144	0.29
1-Methyl-naphthalene	1289	26.846	0.09
β-Selinene	1436	27.247	0.12
Farnesene	1458	26.033	5.35
E-Nerolidol	1564	28.714	0.33
Spathulenol	1640	29.266	2.56
α-Bisabolol	1740	33.285	50.03
Perhydrofarnesyl	1843	34.994	0.12
Ambrettolide	1927	43.990	0.26
Tetracontane	4000	49.990	0.16

Table 3: Antinociceptive effect of orally administered *C. nobile* essential oil on formalin-induced pain

Treatment	Neurogenic phase (0-5 min)			Inflammatory phase (20-30 min)	
	Dosage (mg/kg, p.o)	No of licks/bites	Inhibition (%)	No. of licks/bites	Inhibition (%)
Control	Normal saline	16.7±1.40	-	14.7±2.4	-
Ibuprofen	100	10.2±1.19	39	7.3±1.15*	50
<i>C. nobile</i>	180	13.5±1.38	19	5.5±0.76**	63

Values are mean ± SEM. (n = 6), *p < 0.05), **p < 0.01 compared with control

Formalin-induced pain

Normally, formalin-induced pain occurs in two phases. The first 5 min period after formalin injection is described as the neurogenic phase while from 20 to 30 min is known as the second/late or inflammatory phase. Neither ibuprofen nor the essential oil of *C. nobile* affected the neurogenic phase of formalin-induced pain compared to control as the number of licks/bites remained relatively high in both groups. In the inflammatory phase however, ibuprofen (7.3±1.15 vs 14.7±2.4; p < 0.05) and *C. nobile* (5.5±0.76 vs 14.7±2.4; p < 0.01) significantly inhibited paw licking/biting compared to the control.

Anti-inflammatory activity

The anti-inflammatory activity of *C. nobile* was demonstrated by reduction in paw diameter in Wistar rats. As presented in Figure 3, egg albumin induced a time-dependent increase in paw diameter in control animals though this increase was greatly reduced in treated animals.

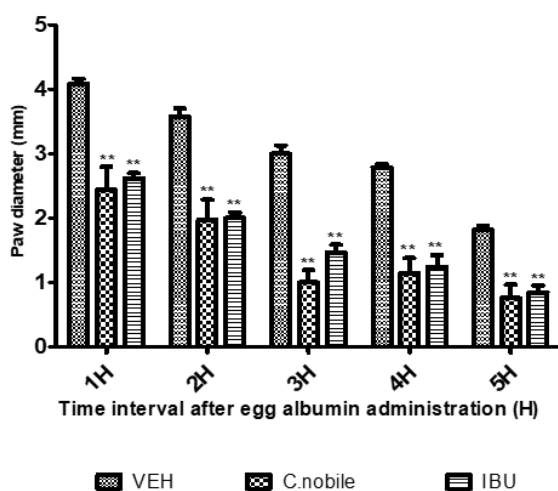


Figure 3: Effect of *C. nobile* essential oil on egg albumin-induced paw oedema in rats. **Note:** Each bar represents Mean ± SEM of variation in paw oedema size. VEH, *C. nobile*, and IBU represent vehicle, *C. nobile* essential oil (180 mg/kg), and ibuprofen (100 mg/kg), respectively. All treatments were through the oral route; **p < 0.01, statistically compared to vehicle (ANOVA, Dunnett's)

The peak of albumin inflammation was noted at 1h post treatment. The anti-inflammatory effect of *C. nobile* was very similar to that of ibuprofen. Both *C. nobile* and ibuprofen significantly (p < 0.01) inhibited the egg induced paw inflammation in rats from the 1st h to the 5th h post treatment.

DISCUSSION

A blue color essential oil was isolated from *C. nobile* harvested in the Eastern Cape of South Africa. This oil showed both analgesic and anti-inflammatory properties in experimental models used. The identified major compound (alpha-bisabolol) has been reported to have anti-inflammatory and other therapeutic activities [17]. The chemical composition of *C. nobile* resembles the chemical composition of *M. chamomilla* (*Matricaria chamomilla*), but of chemotype c [18].

Acute toxicity study gives clues on the range of doses that can be toxic to animals and, could also be used to estimate the therapeutic index of drugs and xenobiotics. Pharmacological substances with LD₅₀ lower than 5 mg/kg body weight are considered highly toxic; those with LD₅₀ between 5 mg/kg and 5000 mg/kg body weight are classified as moderately toxic; while those with LD₅₀ greater than 5000 mg/kg body weight are described as being non-toxic [19]. Results from the present study showed that LD₅₀ of *C. nobile* essential oil is greater or equal to 5000 mg/kg (LD₅₀ ≥ 5000 mg/kg), implying that *C. nobile* essential oil has very low toxicity profile.

The tail flick test is based on the ability of the animal to withdraw its tail due to the increasing intensity of a thermal stimulus, is sensitive to opioid analgesics which act centrally [20] but not NSAIDs. In this study however, the essential oil of *C. nobile* conferred analgesic protected against thermal pain while the analgesic effects of ibuprofen lasted only during the first two hours of the study. Thermal stimulus is generally transmitted from the periphery to the spinal cord via C 1 fibres [21]. This suggests that essential oil of *C. nobile* may possibly inhibit transmission of pain to the spinal cord by preventing

transmission of nerve impulses in the C-fibres. To further confirm the analgesic effect of the essential oil of *C. nobile* against other pain stimulus, we used formalin-induced pain model.

Formalin test which is predominantly used in rat and mice involves a moderate continuous pain generated by tissue injury [22]. Animals respond to formalin injection by licking or biting the injected paw. Animal response in this model generally occurs in two phases. The neurogenic or early phase and the second or inflammatory phase of the formalin test are characterized by periods of high frequency of licking and/or biting of injected. The two phases were separated by a 15 min period of relative calm when the animals rested. The neurogenic phase is believed to be caused by the activation of C-fibres due to peripheral stimulation, while inflammatory pain seems to be predominantly caused by the combination of inflammatory reactions in the peripheral tissue and dorsal horn of the spinal cord [15]. This study showed that the essential oil of *C. nobile* inhibited pain induced by formalin in the first phase better than ibuprofen (a known NSAID) thus confirming that fact that the first phase of this model is not sensitive to NSAIDs [23]. The essential oil of *C. nobile* demonstrated analgesic effects in the second phase of the formalin test indicating that it may act by inhibiting inflammatory reactions in peripheral tissues or attenuated the functional changes expressed in the dorsal horn of the spinal cord. To elucidate the possible anti-inflammatory effect of *C. nobile* essential oil, the egg albumin inflammatory test was performed.

Egg albumin induced inflammation is a biphasic process characterized by the initial release of serotonin, histamine and bradykinins between (0-2 h after albumin injection followed by the release of chemical mediators and pro-inflammatory cytokines IL 1β and TnF- α [24]. The release of chemicals and pro-inflammatory cytokines is responsible for the prolonged manifestations of inflammation which may last for up to 6 h [25]. The anti-inflammatory effects of *C. nobile* in this study, was significantly higher from 1 – 5 h implying that *C. nobile* possibly inhibited all the stages of inflammation.

CONCLUSION

The chemical composition, antinociceptive and anti-inflammatory activities of *C. nobile* have been reported. The three main compounds in *C. nobile* essential oil were α -bisabolol, farnesene and spathulenol. Biological tests showed that the essential oil of *C. nobile* is not toxic on acute use and possesses both analgesic and anti-

inflammatory properties, thus validating the use of the plant for alleviating inflammation-related diseases in South African traditional medicine.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Benedicta N Nkeh-Chungag, Adebola O Oyedeji, Opeoluwa O Oyedeji and CR Constance. Rusike conceived and designed the study. Olukayode O Aremu and Charlotte M Tata carried out the laboratory work, collected and analyzed the data. The manuscript was proof-read by all the authors and approved for publication.

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