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Original research paper

Double-blind randomised controlled trial of an emollient cream with and without 1 % supercritical CO_2 extract of *Calendula officinalis* in contact dermatitis

MIA ŠUŠAK CRNČEVIĆ[1]
TONI DURDOV[1]
DORIS RUŠIĆ[1]
LARA RAMIĆ[1]
ANA ĆOSIĆ[1]
JOSIPA BUKIĆ[1,2,*]
STELA JOKIĆ[3]
KRUNOSLAV ALADIĆ[3]

[1] *Department of Pharmacy University of Split School of Medicine, 21000 Split, Croatia*

[2] *Department of Laboratory Medicine and Pharmacy, Faculty of Medicine Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia*

[3] *Faculty of Food Technology Osijek Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia*

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ABSTRACT

This double-blind randomised controlled trial aimed to evaluate the efficacy of an emollient cream with and without 1 % supercritical CO_2 extract of *Calendula officinalis* in contact dermatitis. Twenty healthy volunteers without pre-existing dermatological conditions participated in this single-centre study. Each participant's forearm was divided into three test sites: control (no treatment), placebo (base emollient), and intervention (calendula cream). The study employed random allocation of test sites using Microsoft Excel software. Both investigators and participants were blinded to the treatment assignments. The main outcomes were changes in skin hydration and transepidermal water loss (TEWL), measured with non-invasive probes (Corneometer CM 825, Tewameter TM 300), and erythema measured by Mexameter MX 18. Following irritant exposure, all sites showed increased TEWL and reduced hydration, confirming skin barrier impairment. The intervention site demonstrated significantly greater hydration compared with both the control ($p = 0.017$) and placebo sites ($p = 0.035$) on day 4, with this improvement persisting on day 8 ($p = 0.043$). TEWL values at the intervention site were significantly lower than control on day 3 ($p = 0.022$), indicating faster barrier recovery. No significant differences in erythema were observed between groups, and no adverse events occurred. Results of this study indicate that the addition of 1 % *Calendula officinalis* extract to an emollient cream enhanced skin hydration and accelerated recovery after irritant exposure, suggesting potential benefit in managing contact dermatitis with non-pharmacological skincare formulations.

Keywords: contact dermatitis, calendula, supercritical extraction, transepidermal water loss, skin hydration, skin barrier

INTRODUCTION

Contact dermatitis, either allergic or irritant, is a common skin disease associated with disruption in the skin barrier, which affects patients' quality of life but also impacts their

*Correspondence; e-mail: jbukic@mefst.hr

work capacity. It is assumed that the genetic predisposition has a role in the development of all forms of dermatitis, and on the other hand, environmental factors, especially in occupational settings, are recognised as key contributors (1–3). Interestingly, a well-known cause of allergic contact dermatitis, nickel, despite twenty years of regulation, is still widely used in earrings. A much higher proportion of nickel was found in earrings from Asia (34.5 %) compared to European earrings (11.3 %). These results imply that stricter implementation of existing regulations and consideration of new measures are necessary for better control of allergic contact dermatitis (4). Although prevention methods are proposed in both forms of contact dermatitis, some patients still require topical or systemic therapy, which sometimes causes adverse reactions, and in the case of topical steroids, they are not recommended for long-term use due to safety issues. Therefore, in these patients, only frequent use of moisturisers has been encouraged (5, 6).

According to a review by Rajkumar *et al.*, the skin barrier is not one section of the skin, and it consists of distinct layers: a well-known chemical layer, microbiological layer, physical layer (cells and lipids) and immunologic layer (immune cells), all of which maintain physiologic functions of the skin. Moreover, several studies have demonstrated that moisturisers can improve skin barrier disruption through various mechanisms of action, depending on the specific layer involved (7, 8). However, consensus on which moisturisers or ingredients, such as humectants, emollients, or occlusives, should be the gold standard in contact dermatitis has not been established yet.

Due to existing gaps in the treatment of skin diseases in general, not only contact dermatitis, dietary supplements, natural products, even marine by-products, and other complementary therapies are becoming increasingly popular among affected patients. One of the most prominent examples is vitamin D, whose role is being investigated in numerous skin conditions, and its deficiency is increasingly associated with more severe disease symptoms (9, 10). On the other hand, the popularity of various herbal extracts is also growing, as they have traditionally been used in the prevention or treatment of many different disorders, and research in natural compounds has been following this increasing trend (11). Current evidence suggests that herbal extracts may help prevent and manage contact dermatitis by decreasing inflammation and enhancing antioxidant defences. However, the limited number of clinical trials makes it difficult to establish firm conclusions (12). Therefore, the aim of our study was to evaluate the efficacy of calendula extract in a clinical study conducted in healthy participants with an induced model of irritant contact dermatitis.

EXPERIMENTAL

Extraction process and analysis

Calendula extract was produced by a supercritical fluid extraction system, as described in published literature (13, 14). In total, 100 g of ground calendula flowers were placed into the extractor vessel, and the obtained extract was collected in weighted tubes. Separator conditions were 25 °C and 15 bar. The conditions of the extraction process were pressure of 30 MPa, 40 °C constant temperature and the flow rate of the mass 1.4 kg h^{-1} during 1 hour. A time of 1 hour is optimal for the extraction of calendula flowers, given that further extraction has a very low yield and is not economical. Headspace solid-phase

microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) were used to perform analysis of the calendula extract.

During the process, volatiles were extracted using a manual SPME fibre (according to the instructions of the manufacturer), coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm; purple hub). The fibre was obtained from Supelco (USA). For HS-SPME, 0.01 g of calendula extract was placed in a 20-mL glass vial sealed with a PTFE/silicone septum. The sample was placed in a PAL 120 RSI autosampler equipped with a heating compartment. The vial was incubated in PAL 120 RS at 40 °C for 15 min to equilibrate, followed by a 45-min headspace extraction. The SPME fibre was retracted into the needle after the sampling, detached from the vial, and injected into the GC-MS (250 °C) for 7 min of thermal desorption directly onto the GC column.

An Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass spectrometer (Agilent Technologies, USA) was used for the analysis of calendula extract. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm; Agilent Technologies). The injector was operated at 250 °C in split mode (1:100), with helium as the carrier gas at a constant flow of 1.0 mL min^{-1}. The oven program started at 70 °C (2 min), ramped at 3 °C min^{-1} to 200 °C, and was held at 200 °C for 18 min. The mass spectrometer operated at 70 eV in scan mode over *m*/*z* 40–400. Injector and detector temperatures were 250 °C and 300 °C, respectively.

The identification of calendula extract compounds was based on comparison with the Wiley 9 (Wiley, USA) and NIST 17 (National Institute of Standards and Technology, USA) mass spectral libraries and literature retention indices calculated using C9–C25 *n*-alkanes. Each calendula extract sample was analysed in triplicate, and a full list of all compounds is available in the Supplementary Material. The GC-MS profile was dominated by sesquiterpenes, particularly γ-cadinene (13.80 %), δ-cadinene (13.57 %), α-muurolene (7.08 %), α-cadinol (6.59 %), and *t*-muurolol (5.50 %), which represented the major constituents.

Clinical study

The clinical study at the University of Split School of Medicine was performed in October 2025. The study was accepted by the Ethics Committee of the University of Split School of Medicine (approval number 2181-198-03-04-25-0084). Furthermore, the research study was conducted according to all existing ethical principles.

Participants in the study were 20 healthy individuals (both male and female), ranging in age from 22 to 35 years. No participant had any pre-existing history of dermatological conditions. Before enrolling, all subjects provided informed consent after receiving a detailed explanation of the research objectives, methodologies, and procedures. Strict exclusion criteria were implemented for the study. Participants were disqualified if they presented with any skin disease, including skin cancer and sun damage, in the intended research region. Furthermore, subjects had to refrain from using several medicines, including those with immunomodulatory effects, drugs which affect histamine and corticosteroids, for a period of 30 days prior to the testing phase and were required to strictly comply with the study protocol. For safety reasons, this research was not appropriate for those who are pregnant, breastfeeding, immunosuppressed, or have a history of photosensitive disorders. The history of allergic or irritant reactions to the components of the tested formulations was also examined.

On each of the participants' forearms, three sites were selected and designated for the irritant dermatitis model, which involved the application of a 1 % (*m*/*m*) aqueous solution of sodium lauryl sulfate (SLS). Following the initial measurement of basal skin parameters, a volume of 60 μL of the SLS solution was applied to a paper patch, as described in official guidelines (15). This patch was then secured under occlusion for twenty-four hours using twelve-millimetre Finn chambers. Participants were instructed to avoid any contact with moisture on the test site during this specified time frame. Once the Finn chambers were removed, the tested skin area was cleansed with water, and the skin parameters were measured again. Selected sites were randomised with Microsoft Excel software to be either a control location, which had no treatment, a placebo location, which had an emollient for the treatment or the intervention location, which had calendula cream for the treatment.

During their first measurement, participants received instructions on how to properly apply the calendula extract cream and placebo product. Each participant was provided with a single sample of the emollient and a single sample of calendula cream. The emollient, commercially available for *ex tempore* products in pharmacies, consisted of supportive skin-care ingredients without pharmacological effect (aqua, petrolatum, cetearyl alcohol, paraffinum liquidum, Ceteareth-20, propylene glycol, benzyl alcohol, citric acid, sodium citrate), and the calendula cream was prepared as 1 % (*m*/*m*), extract and cream respectively. On testing days, participants were specifically instructed to abstain from applying any cosmetics to the target area. The emollient and cream samples were weighed both at the beginning and at the conclusion of the study in order to confirm adherence to the study protocol.

During the study, 5 mg cm^{-2} of the cream or emollient was applied to the designated forearm twice daily, ensuring a minimum interval of eight hours between applications. On days when measurements were taken, the first application of the cream or emollient was performed by the examiner immediately after the skin parameters were recorded. The second application, along with all applications on non-measurement days, was carried out by the subjects themselves following the provided instructions. To guarantee the accuracy of the TEWL measurement, the final cream application had to occur at least twelve hours before the scheduled measurement time.

Three key skin parameters associated with skin barrier function were assessed using the MPA6 device (Courage + Khazaka GmBH, Germany), TEWL, with the Tewameter TM 300 probe (Courage + Khazaka GmBH), providing values expressed in g m^{-2} h^{-1}. Furthermore, the level of skin hydration was quantified using the Corneometer CM 825, while skin erythema (redness) was evaluated with the probe Mexameter MX 18.

The study lasted a total of nine days, during which seven separate measurements were taken. On Day 0, baseline skin parameter values were recorded, followed by the application of the Finn chamber containing SLS. On Day 1, after the chamber was removed, skin barrier damage values were measured, and the treatment intervention began. Recovery values for the skin on both forearms were subsequently measured on Days 2, 3, 4, and 8. Conversely, Days 5, 6, and 7 involved the application of therapy only, with no additional measurements performed.

Data analysis included a two-way ANOVA test for repeated measures, and the *post hoc* Bonferroni test was subsequently employed to compare values both across the different groups at specific time points and between distinct time points for each individual group. All statistical computations were carried out using the IBM SPSS Statistics software (version 25). Statistical significance was defined as $p < 0.05$. Results were consistently displayed as the mean ± standard deviation (SD), unless noted otherwise.

RESULTS AND DISCUSSION

Demographic data

In total, 20 participants were included in the study. Most of the participants were women (17, 85.0 %), with only 3 participants being men (15.0 %). The oldest participant was 35, and the youngest one was 22, with the median age being 24.50 (IQR 7). The majority of the participants were non-smokers (16, 80 %), with 4 participants smoking at the time (20 %).

Hydration

Comparison of the hydration values at the start of the study across three different locations on patients' arms showed the same baseline hydration values (39.83 ± 7.51 AU). The combined effect of location of measurement and time on hydration value was significant (p = 0.008, ANOVA for repeated measures, Greenhouse-Geisser correction), which means that hydration values changed differently between 3 locations (control, placebo and intervention) over the time of the study.

As seen in Fig. 1. hydration values decrease in all groups after SLS irritation with value on the third day of the study being significantly lower than the baseline value on control location (39.83 ± 7.51 *vs.* 31.16 ± 10.81 AU, baseline and day 3, control location, p = 0.034), but the difference was not significant on placebo location (39.83 ± 7.51 *vs.* 32.23 ± 11.50 AU, baseline and day 3, placebo location, p = 0.250), and intervention location (39.83 ± 7.51 *vs.* 32.57 ± 12.06 AU, baseline and day 3, intervention location, p = 0.538).

On day 4 of the study, the intervention location had significantly higher hydration values than both the control location (35.11 ± 17.38 *vs.* 24.48 ± 14.17 AU, intervention and control location, p = 0.017) and the placebo location (35.11 ± 17.38 *vs.* 27.45 ± 14.89 AU, intervention and placebo location, p = 0.035). After eight days of the study, hydration values were still significantly higher in the intervention location compared to the control location (30.47 ± 15.82 *vs.* 25.63 ± 12.54 AU, intervention and control location, p = 0.043.

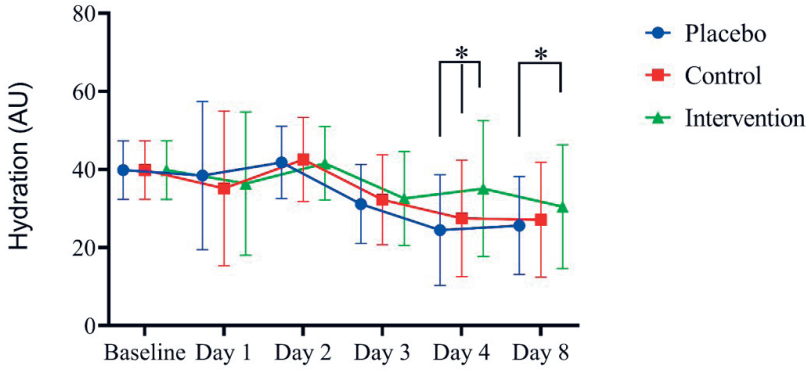

Fig. 1. Changes in hydration values between the calendula extract, control, and placebo groups. * Statistically significant, N = 20.

TEWL

Measurements on all studied locations on participants' skin had the same baseline TEWL values (14.19 ± 3.81 g m^{-2} h^{-1}). TEWL values on all locations increased after SLS irritation and were significantly lower on day 3 on control location (14.19 ± 3.81 *vs.* 36.70 ± 11.22 g m^{-2} h^{-1}, baseline and day 3, control location, $p < 0.001$), placebo location (14.19 ± 3.81 *vs.* 34.41 ± 12.80 g m^{-2} h^{-1}, baseline and day 3, placebo location, $p < 0.001$) and intervention location (14.19 ± 3.81 *vs.* 30.65 ± 8.77 g m^{-2} h^{-1}, baseline and day 3, intervention location, $p < 0.001$).

After the initial increase, TEWL values recovered at all locations. There was a significant decrease of TEWL values on control location (36.70 ± 11.22 g m^{-2} h^{-1} *vs.* 24.68 ± 13.25 g m^{-2} h^{-1}, day 3 and day 8, control location, $p = 0.001$), placebo location (34.41 ± 12.80 g m^{-2} h^{-1} *vs.* 23.12 ± 11.23 g m^{-2} h^{-1}, day 3 and day 8, placebo location, $p < 0.001$) and intervention location (30.65 ± 8.77 g m^{-2} h^{-1} *vs.* 20.44 ± 5.82 g m^{-2} h^{-1}, day 3 and day 8, intervention location, $p = 0.002$). The only difference in TEWL values between the locations was found on day 3 when the intervention location had significantly lower TEWL values compared to the control location (30.65 ± 8.77 g m^{-2} h^{-1} *vs.* 36.70 ± 11.22 g m^{-2} h^{-1}, control and intervention group on day, $p = 0.022$) as described in Fig. 2.

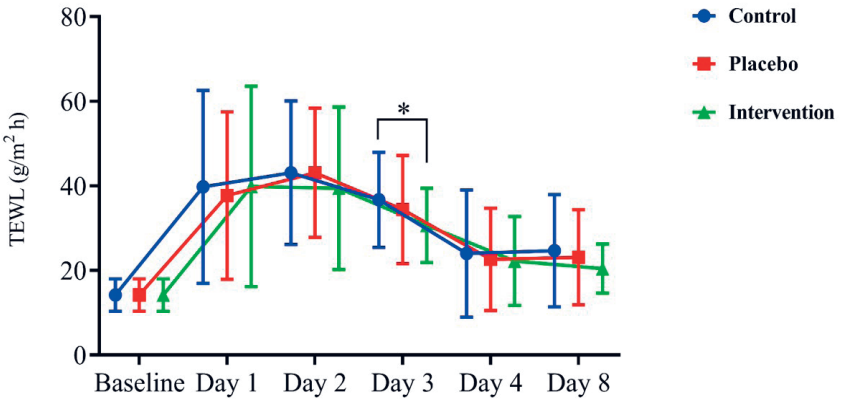

Fig. 2. Changes in TEWL values between the calendula extract, control and the placebo group. * Statistically significant, $N = 20$.

Erythema

Erythema values were the same on control, placebo and intervention location at the start of the study (199.73 ± 42.12 AU). There was no difference in changes of erythema values over the period of the study between different locations ($p = 0.252$, ANOVA for repeated measures, Greenhouse-Geisser correction).

Erythema values soared after SLS exposure in all locations. Erythema values increased significantly on control location (199.73 ± 42.12 *vs.* 292.12 ± 49.04 AU, baseline and day 3, control location, $p < 0.001$), placebo location (199.73 ± 42.12 *vs.* 289.97 ± 53.89 AU, baseline and

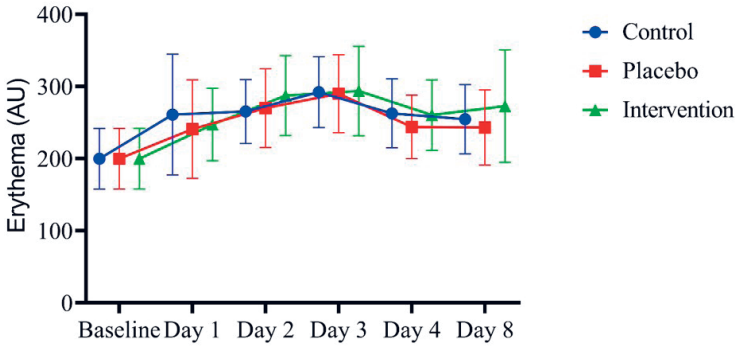

Fig. 3. Changes of erythema values between calendula extract, control and placebo group ($N = 20$).

day 3, placebo location, $p = 0.001$) and intervention location (199.73 ± 42.12 *vs.* 293.77 ± 62.09 AU, baseline and day 3, intervention location, $p < 0.001$). Erythema values slowly decreased after that time point with no significant differences, as seen in Fig. 3.

The results of our study showed that after induced irritation, skin hydration decreased and TEWL values increased at all measurement sites, confirming the impairment of the skin barrier function. However, the intervention site demonstrated faster recovery and significantly higher hydration compared to the control and placebo sites, indicating the effectiveness of the applied calendula formulation. These findings highlight the importance of this research in understanding skin barrier repair mechanisms and developing more effective formulations which could be used long-term.

A decrease in TEWL after use of calendula extract in the form of a cosmetic product was observed in the study by Bikiaris *et al.*, where calendula was compared to a widely used extract of *Centella asiatica*, well known for barrier repair properties. Calendula serums led to a decrease in TEWL, but this effect was weaker compared to the reduction measured for *Centella asiatica* serum. According to the authors, this difference could be attributed to distinct mechanisms of action or specific interactions between calendula extract and the skin barrier (16). This mechanism was proposed in s 2018 study by Ho Kang *et al.*, who concluded that methanol calendula extract acts on the skin barrier through multiple mechanisms – it provides antioxidant protection against ultraviolet-induced damage, restores structural proteins (collagen and laminin), and reduces their degradation by inhibiting matrix metalloproteinase enzymes. In doing so, it strengthens the dermoepidermal junction, enhances skin elasticity and integrity, and helps maintain its protective function. However, this study was conducted in human fibroblasts and clinical trials are still needed to confirm the proposed mechanisms of action (17).

In general, there is a lack of studies on calendula effects in contact dermatitis, but this herbal extract has been researched in other skin conditions. For instance, Ngan *et al.* compared the efficacy of Bao Yuan Gao (traditional Chinese herbal ointment) and calendula cream in patients with radiation dermatitis. Results of this study showed that Bao Yuan Gao demonstrated greater improvement in erythema and skin moisture measurements compared to calendula ointment. However, the aforementioned study has several limitations; for instance, the exact concentration or analysis of calendula extract used in the

cream was not disclosed in the manuscript (18). Moreover, calendula extract has been investigated extensively for its wound healing potential in several recent studies (19–21). However, all of these studies were conducted in animal models (mice and rats), so future studies are needed to confirm possible wound healing effects in humans.

Similarly, the predominant compounds in our extract, the sesquiterpenes γ-cadinene and δ-cadinene, have been investigated in animal models and have shown promising antifungal activity as well as wound-healing and photoprotective potential (22). Clinical studies on isolated compounds are lacking, and future research should compare the effects of these sesquiterpenes with those of the whole calendula extract in skin diseases.

The present results have several limitations worth mentioning. Primarily, it was conducted in a single centre, which may limit the generalizability of the results. Future research should include multiple centres and a more diverse population in terms of race and ethnicity to enhance external validity. Another limitation is the relatively small sample size. Nevertheless, this study was conducted in human participants and designed as a randomised controlled trial, using objective outcome measures, a major strength that adds robustness to our findings. Despite these limitations, our promising results may encourage further large-scale investigations on *Calendula officinalis*.

CONLUSIONS

Calendula officinalis is a herbal ingredient of growing dermatological interest due to its possible wound healing, anti-inflammatory and antioxidant properties. In our study, we compared the effect of 1 % *Calendula officinalis* supercritical CO_2 extract (most abundant compounds the sesquiterpenes γ-cadinene and δ-cadinene) in emollient cream to an emollient formulation in SLS-induced contact dermatitis. Our results showed that the calendula cream significantly improved skin hydration and accelerated barrier recovery following irritant exposure, as indicated by lower TEWL values. The use of objective, non-invasive outcome measures represents a key strength of this randomised controlled trial.

Supplementary Material is available upon request.

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Authors contributions. – Conceptualisation, M.Š.C. and K.A.; methodology, J.B. and S.J.; validation, D.R. and A.Č.; formal analysis, T.D. and L.R.; investigation, M.Š.C. and J.B.; resources, K.A. and A.Č.; data curation, T.D. and D.R.; visualisation, T.D. and A.Č.; writing, original draft preparation M.Š.C. and L.R.; writing, review and editing J.B. and S.J.; supervision, K.A.; project administration, J.B. All authors have read and agreed to the published version of the manuscript.

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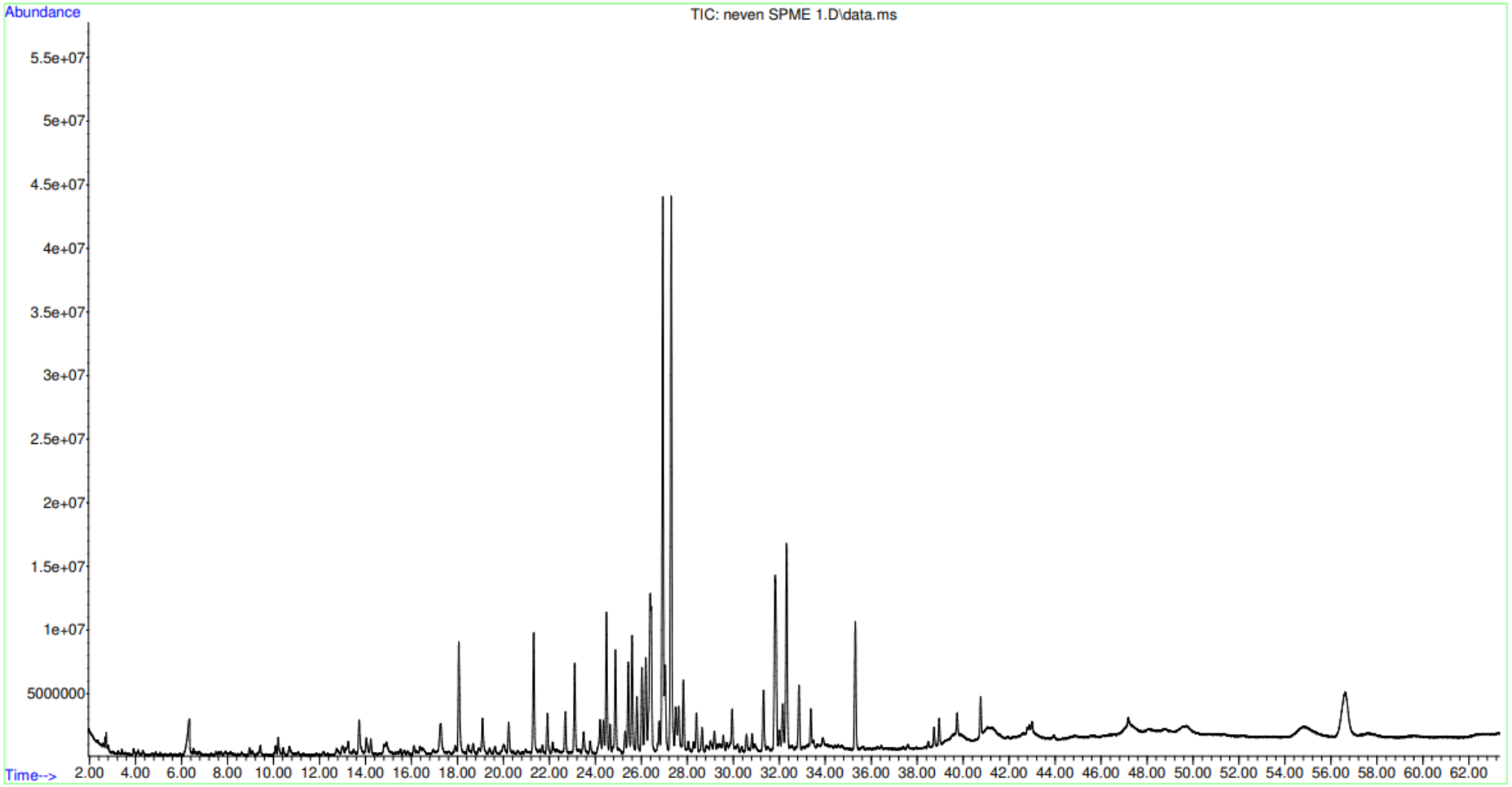
Supplementary material

No	Compound	Rt	RI	Calendula (%)
1.	2-Methylbutanoic acid	3.40	845	0.18
2.	Pentanoic acid	3.92	876	0.17
3.	(*E*)-2-Methylbut-2-enoic acid	4.11	886	0.20
4.	ALPHA-THUJENE	4.88	934	0.06
5.	.alpha.-Pinene	5.07	942	0.04
6.	Hexanoic acid	6.34	981	1.67
7.	Octanal	6.78	1004	0.16
8.	D-Limonene	7.63	1037	0.10
9.	1,8-Cineole	7.72	1038	0.08
10.	Benzeneacetaldehyde	8.12	1050	0.15
11.	.gamma.-Terpinene	8.62	1065	0.12
12.	4-Thujanol;	8.96	1073	0.17
13.	Heptanoic acid	9.44	1079	0.37
14.	Linalool	10.09	1102	0.26
15.	Nonanal	10.21	1107	0.46
16.	Benzeneethanol	10.69	1117	0.37
17.	Borenol	12.75	1171	0.27
18.	Menthol	13.01	1177	0.33
19.	4-Terpineol	13.15	1183	0.16
20.	Octanoic acid	13.26	1185	0.40
21.	2,6-Dimethyl-3,7-octadiene-2,6-diol	13.73	1219	1.12
22.	(*E*)-Citral	16.95	1274	0.24
23.	Thymol	18.07	1296	3.13
24.	Carvacrol	18.47	1306	0.28
25.	α-Cubebene	20.23	1352	0.85
26.	α-Copaene	21.32	1378	3.10
27.	β-Bourbonene	21.69	1387	0.25
28.	β-Cubebene	21.92	1391	1.04

29.	α-Gurjunene	22.70	1410	1.12
30.	*trans*-Caryophyllene	23.10	1421	2.30
31.	β-Copaene	23.49	1432	1.05
32.	(-)-3,5-Cadinadiene	24.21	1448	1.97
33.	α-Humulene	24.36	1455	3.39
34.	cis-Muurola-4(15),5-diene	24.64	1462	2.67
35.	γ-Muurolene	25.30	1477	2.27
36.	Germacrene D	25.43	1480	2.99
37.	*trans*-β-Ionone	25.81	1484	2.10
38.	epi-Bicyclosesquiphellandrene	26.03	1488	2.83
39.	.alpha.-Muurolene	26.19	1497	7.08
40.	γ-Cadinene	26.78	1513	13.80
41.	Cubebol	26.94	1517	2.23
42.	δ-Cadinene	27.03	1520	13.57
43.	Dihydroactinidiolide	27.30	1528	1.34
44.	Cadina-1,4-diene	27.50	1533	1.41
45.	α-Cadinene	27.83	1540	2.18
46.	α-Calacorene	28.05	1545	0.28
47.	Viridiflorol	29.73	1590	1.38
48.	α-Cadinol	31.50	1640	6.59
49.	β-Eudesmol	32.01	1652	1.36
50.	t-Muurolol	32.15	1656	5.50
51.	Oplopanone	34.72	1726	3.20
52.	Nonadecane	40.39	1895	1.64

[**]- correct isomer is not identified


Abundance
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5e+07
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