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EXPERIMENTAL PAPER

Anti-inflammatory effects of medicinal plants mixture used by Bedouin people in Saudi Arabia

EMAN ELSHARKAWY[1,2], MONA ELSHATHELY[1,3], GEHAD ABDEL JALEEL[4], HAYA IBRAHIM AL-JOHAR[5]

[1]Northern Border University-ARAR
North Region, Saudi Arabia

[2]Department of Eco physiology
Desert Research Center
Mathef El-Mataria, 15753 Egypt

[3]Department of Biology
Science Faculty for Girls
King Abdullaziz University
Jeddah, Saudi Arabia

[4]Researcher, Pharmacology Department
National Research Center
El-Bohoth St.
Dokki, Saudi Arabia

[5]Saudi Food and Drug Authority
National Drug Control Laboratory
Riyadh, Saudi Arabia

corresponding author: el Sharqawyeman@yahoo.com

S u m m a r y

Due to toxic and adverse side effects of synthetic drugs, traditional herbal medicine has the potential as a source of new bioactive molecules. That is why we investigated this research, searching new anti-inflammatory drugs from plants growing around us. The anti-inflammatory effect of the mixture of different plants used in traditional medicine (*Alkanna tinctoria, Rubia tinctorum* and *Artemisia herba alba*) were studied using carrageenan-induced paw edema (oil mixture extract 100 mg/kg and 200 mg/kg). These material reduces carrageenan-induced inflammation in rats and shows inhibition after 4 h especially at a concentration 200 mg/

kg. Oil mixture extract were investigated by capillary GC and GC–MS in combination with retention indices revealed the presence of about 17 compounds: artemisol, epiglobulol and α-bisabolol are the main constituents as well as volatile oil and other triterpenes: taraxasterol, stigmasterol, ursan-12-ene, olean-12-ene, betulin, germincol, lupeol acetate and lupeol. Other heterocyclic compounds: moropholine and piperidinol were also identified.

Key words: *anti-inflammatory*, *α-bisabolol*, *caryphyllene oxide*, *Alkanna tinctoria*, *Rubia tinctorum*, *Artemisia herba alba*, *carrageenan-induced paw oedema method*

INTRODUCTION

Natural products have been a thriving source for the discovery of new drugs for a long time because of their chemical diversity. With increased use of herbal remedies, traditionally used medicinal plants receive increased attention from scientists and pharmaceutical industry. The newer work on medicinal plants is mostly the rediscovery of traditional effects at cellular and molecular levels. Development of standardized, safe and effective herbal formulations such as multitarget therapeutics and prophylaxis could be a tenable approach for the future. Hundreds of plant metabolites are reported to have many pharmacological activities, although, most of these reports are of academic interest and very few find entry at clinical trials. Compilation of the information would help promote wider acceptance and use of these plant-based drugs in a main stream of medicine [1].

Inflammation is a part of a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joint that is warm to touch, pain joint to its stiffness and loss of joint function. Inflammation is either acute or chronic. Under specific circumstances, it could turn into a chronic state and, subsequently, become a causative factor of the pathogenesis. Inflammation is a self-defence reaction in its first phase, hence it is regarded as a main therapeutic target and often the best choice in the treatment of the disease and alleviation of symptoms [2].

The aim of this review is to discuss a new method of the use of mixture of medicinal plants as a drug used in inflammation. We made a mixture of more than one plant, each having anti-inflammatory effect. We collected them in a same percentage and extracted with olive oil and then studied the anti-inflammatory effect of this mixture, using a method of Bedouin for the treatment of many skin diseases. Subsequently, we studied the anti-inflammatory effect of this mixture. These plants are alkanet (*Alkanna tinctoria*), common madder (*Rubia tinctorum)* and *Artemisia herba alba.*

Alkanet is a plant of the borage family whose roots are used as a red dye. The plant is also known as orchanet, Spanish or Languedoc bugloss. It is native for Mediterranean region. *Alkanna tinctoria* has a bright blue flower. A dark red root of blackish appearance externally but blue-red inside, with a whitish core. The root produces a fine red colouring material which has been used as a dye in the Mediterranean region since antiquity. As a dyestuff, the root is soluble in alcohol, ether and oils but insoluble in water [3].

Kourounakis et al. [4] also reported that alkannin and shikonin, two natural products isolated from *Alkanna tinctoria* and *Lithospermum erhythrorhizon* (*Boraginaceae*) are used in folk medicine. They are claimed to possess, among other properties, wound healing and anti-inflammatory activities. They were investigated together with structurally related naphthazarin for their *in vitro* antioxidant and hydroxyl radical scavenging activities as well as their *in vivo* anti-inflammatory activity. It was found that all examined compounds significantly inhibited lipid peroxidation of hepatic microsomal membranes *in vitro*, competed with DMSO for free hydroxyl radicals and reduced inflammation (mouse paw edema induced by FCA) very efficiently. The examined compounds proved compounds equal or superior to the common reference for each of these properties. I is concluded that the claimed and/or proven actions of alkannin and shikonin are at least partly attributable to their intervention in free radical processes.

Rubia tinctorum, common madder or dyer's madder, is a plant species belonging to the genus *Rubia*. The plant's roots contain several polyphenolic compounds like 1,3-dihydroxyanthraquinone (purpuroxanthin), 1,4-dihydroxyanthraquinone (quinizarin), 1, 2,4-trihydroxyanthraquinone (purpurin) and 1, 2-dihydroxyanthraquinone (alizarin). The latter one gives its red colour to a textile dye known as Rose madder. It has been used also as a colorant, especially for paint that is referred to Madder lake [5].

The anti-diarrhoeal effect of aqueous extract of *Rubia tinctorum* L. (*Rubiaceae*) roots in rodents was examined. At doses of 300, 600 and 800 mg/kg aqueous extract protected rats, in a dose-dependent fashion, against castor oil-induced diarrhoeal dropping by 37, 59 and 64%, respectively. Furthermore, it has significantly (by 41%) inhibited the gastrointestinal transit of charcoal in mice at 800 mg/kg dose of extract. These data suggest that *Rubia tinctorum* showed antidiarrhoeal activity by inhibiting intestinal motility which was concordant with its use in traditional medicine [6].

Artemisia (*Asteraceae family*, *Anthemideae* tribe) is a shrub growing wild in arid. Common names are wormwood (English), armoise (French), shīeh (Arabic). It has been used in folk medicine since ancient times. Herbal infusions of these species have been used as analgesic, antibacterial, antispasmodic, and haemostatic agents [7]. Historically, *Artemisia* has been a productive genus in the search for new biologically active compounds. Phytochemical investigations have proven that this genus is rich in sesquiterpenes and monoterpenes [8-10].

Artemisia herba-alba (*Asteraceae* family) is a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean region, extending into northwestern Himalayas [11]. It has been used in folk medicine for the treatment of colds, diabetes mellitus, coughing, intestinal disturbances, including intestinal worms, and for the treatment of human and livestock wounds [12, 13].

In present paper, we carry out a detailed investigation on the chemical composition and anti-inflammatory properties of mixture of plants used by some Bedouin people in North Region of Saudi Arabia. We revised most of compounds isolated from these plants which may be responsible for the activity of plants as in table 1.

Table 1.

Review of chemical components of the plants

Scientific name	Arabic name	Family	Chemical components	Biological activity	Reference
Alkanna tinctoria	Aoad Hawa	*Boraginaceae*	1-pulegone (22.27%), 1,8-cineole (13.03%), α-terpinyl acetate (6.87%), and isophytol (6.83%)	1-Antioxidant	2
			2-quinone(naphthoquinone)	2-Antioxidant	14
Rubia tinctoria	Fowh	*Rubiaceae*	1-(purpuroxanthin), 1,4-dihydroxyanthraquinone (quinizarin), 1,2,4-trihydroxyanthraquinone (purpurin) and (alizarin). 2 xanthine, triterpenes, quinines, xantho-rpurin, ruberythric cid,	was used as a colorant, especially for paint	5
			2-purpurin-	Anti-tumor	
Artemisia herba alba	Sheh	*Asteraceae*	verbenol, isabolone oxide (17.55%), farnesene epoxide (17.08%) and β-thujone (6.14%) were the major constituents in the volatile oil of camphor (5.12%), myrtenol (4.19%), fenchol (3.86%), and α-bisabolol oxide (2.99%)	The essential oil showed significant antiproliferative activity against the acute lymphoblastic leukaemia	15

MATERIAL AND METHODS

Plant material

Preparation of plant oil extract

The plant samples were purchased from attar shop. 10 g of each plant were cleaned and ground to fine powder, then immersed in 100 ml of olive oil for 15 days. After 15 days the mixture was filtered, then the filtrate was left to concentrate and kept in dark bottle till used.

Analysis

The constituents of the volatile oils extracts were analysed by GC-MS as reported [16]. The GC-MS analyses were carried out on a Shimadzu GC-MS-QP2010 gas chromatography-mass spectrometer equipped with capillary column DB-5-MS Agilent (30 m x 0.25 mm, film thickness 0.25 μm) under following conditions. Helium was used as a carrier gas at a pressure of 81.90 kPa, with flow of 1.33 ml/min; the

temperature in the injector was 250°C; the temperature of the oven progressed from 60 to 240°C to 3°C/min. The ionization mode used was the electronic impact at 70 eV. Later, under the same experimental conditions, each oil was co-injected with a homologous series of linear hydrocarbons (C9-C25)-alltech, to accomplish the calculations of the retention index (RI) of each constituent of the samples applying Van Den Dool & Kratz Equation.

The identification of compounds was performed by analysis and comparison of the mass spectra with database of Wiley 7 library and by comparison of RI with those of the literature [17-24]. The relative quantification of the components of each sample was obtained through the relative area of the peaks in the chromatograms.

Anti-inflammatory method

The method developed by Winter [25] was employed. Albino Wistar rats of either sex (120–130 g) were divided into various groups, each of six animals. Animals were deprived of food for 12 h prior to experiment and only water was given *ad libitum*. First group was used as a control group and received 1 ml of distilled water (10 ml/kg); the second group received indomethacin orally (10 mg/kg) suspended in distilled water. Other groups received test compounds at doses of 100 and 200 mg/kg orally, dissolved in distilled water.

One hour after the administration of the compounds, carrageenan suspension (0.1 ml of 1% *w/v* suspension in 0.9% saline solution) was injected into the subplanter region of left hind paw of animals. Immediately, the initial paw volume was measured using plethysmometer (UGO Basile 21025 Comerio, Italy) before carrageenan injection. Thereafter, the paw volume was measured after 1, 2, 3 and 4 h after carrageenan administration. The difference between initial and subsequent readings gave the change in edema volume for the corresponding time. Edema volume of control (Vc) and volume of treated (Vt) were used to calculate percentage (%) inhibition and (%) edema volume by using following formula:

$\%\ \text{Inhibition} = [1 - (Vt/Vc)] \times 100$

$\%\ \text{Edema volume} = 100 \times (\text{edema volume after drug treatment/Initial volume})$

Approval for this study was obtained from the Ethic Committee of The National Research Centre-Egypt (12311), and in accordance with the recommendations of the proper care and use of laboratory animals.

Statistical analysis

Values were expressed as means ±SD. Comparisons between means were carried out using one way ANOVA followed by least significant difference (LSD) and Tukey multiple comparisons test.

$F1.6=149.5$, $p<0.05$, $p<0.05$ was accepted as being significant in all types of statistical tests. SPSS software (version 17) was used.

RESULTS AND DISCUSSION

Drugs (oil mixture extract) were screened for *in vivo* anti-inflammatory activity by the inhibition of carragenan-induced rat paw edema method at doses of 100 and 200 mg/kg orally. Results are presented in table 3 as percent edema increase at the right hind paw and percent inhibition.

Carrageenin is a useful agent for studying new anti-inflammatory drugs. Carrageenin-induced edema is a non-specific inflammation resulting from a complex of diverse mediators [26]. Edemas of this type are highly sensitive to non-steroidal anti-inflammatory drugs (NSAIDs). This model reliably predicts anti-inflammatory efficacy of the NSAIDs and during second phase it detects compounds which are anti-inflammatory agents as a result of inhibition of prostaglandin amplification [25].

Significant anti-inflammatory activity was observed with inhibition in edema of 78% for (100 mg/kg) of extract and 82.5% for 200 mg/kg after 4 h. which is comparable to standard drug indomethacin (89.5%) against paw edema induced by carrageenin as in Figure 1.

Table 2.

Chemical compositions of drug mixture by GC-Maas

No.	Molecular formula	Percentage%	Chemical compound
1	$C_{15}H_{24}$	1.00	Muurolen
2	$C_{15}H_{24}O$	5.09	Artemisol
3	$C_{15}H_{24}$	0.53	α-Cedrene
4	$C_{15}H_{24}$	0.53	Sechyllene
5	$C_{15}H_{24}$	1.00	α-Cubebene
6	$C_{15}H_{24}$	1.00	Isoledene
7	$C_{15}H_{26}O$	0.89	Ledene oxide
8	C15H24O	0.89	Spathulenol
9	$C_{15}H_{24}$	1.48	β-Caryophyllene
10	$C_{15}H_{24}O$	0.65	Caryophllene oxide
11	$C_{15}H_{26O}$	2.1	Epiglobulol
12	$C_{15}H_{24}$	1.48	Isocarophylene
13	$C_{15}H_{24}$	1.26	α-Humulene
14	$C_6H_{13}NO$	3.47	Piperdinol
15	C_4H_9NO	3.47	Moropholin
16	$C_{10}H_8$	1.26	Azuline
17	$C_{15}H_{26}O$	1.95	α-Bisabolol

Table 3.

Anti-inflammatory of drug at different concentration

	% Edema				% Inhibition			
Time(H)	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Control	54.8±2.9	67.1±4.4	79.5±4.1	83±4.7				
Indomethacin	49±4.2	37±4.1*	23±3.0*	8.6±0.9*	10.5	44	70	89
100 mg/kg	54±4	40±3.3*	33.8±2.6*	18±1.7*	.6	40	57.5	78
200 mg/kg	48±3.9	34.6±2.4*	26.5±1.2*	13±1.6*	3.3	43	64.7	82.5

Values are expressed as means ±SEM (n=6).
* Significantly different from control group at $p<0.05$.
[a] Significantly different from indomethacin group at $p<0.05$.

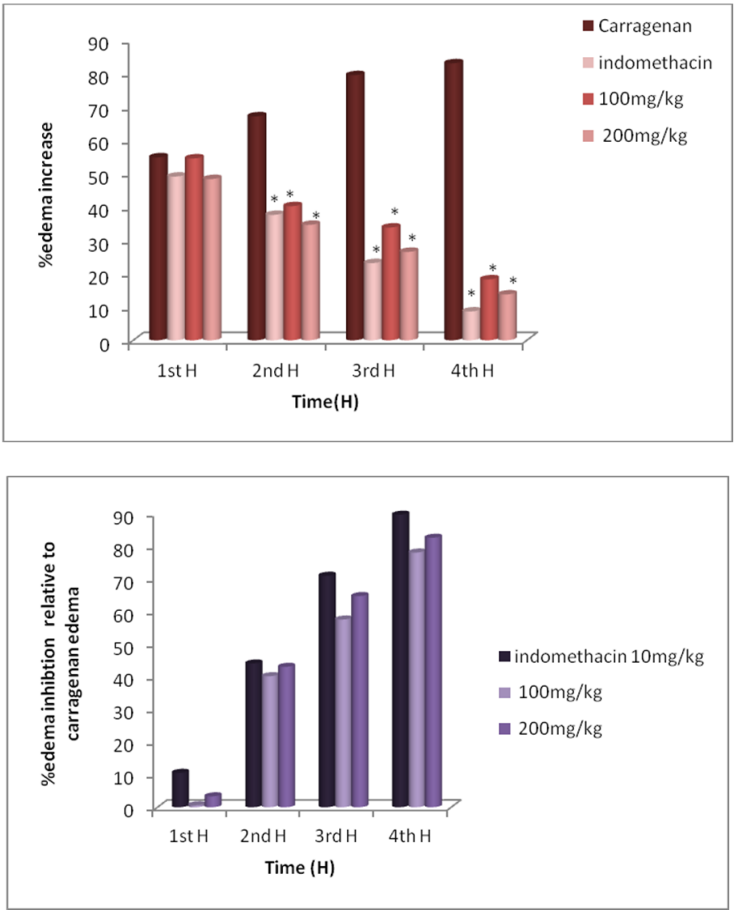

Figure 1.

Bar diagram showing % inhibition

The analysis of oil extract plants mixture(drug) by GC-MS revealed the presence of about 17 known volatile oil compound (tab. 2). Other triterpene compounds: artemisol, epiglobulol and α-bisabolol are the main constituents of volatile oil. Other triterpenes compounds are: traxasterol, taraxasterol acetate, stigmasterol, ursan-12-ene, olean-12-ene, betulin, lupeol, germanicol and lupeol acetate. Other heterocyclic compounds: moroholine and piperidinol were detected. The compounds identification was performed by the analysis and comparison of the mass spectra with database of Wiley 7 library and by comparison of RI with those of literature [16]. The relative quantification of the components of each sample was obtained through the relative area of peaks in the chromatograms.

Most of identified compounds isolated from the plants found in table 2 show anti-inflammatory effect of compounds like α-bisabolol, caryophllene oxide and traxasterol.

Plant essential oils are typically composed of volatile aromatic terpenes and phenylpropanoids. These lipophilic volatiles freely cross cellular membranes and have various ecological activities, like plant-insect interactions. The sesquiterpene (E)-β-caryophyllene is a major plant volatile found in large amounts in the essential oils of many different spice and food plants, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) and black pepper (*Piper nigrum* L.). In nature, β-caryophyllene is usually found together with small quantities of its isomers (*Z*)-β-caryophyllene [(*Z*)-BCP or isocaryophyllene] and α-humulene (formerly α-caryophyllene) or in a mixture with its oxidation product, BCP oxide).

Jurg et al. [27] show that the essential oil component (*E*)-BCP selectively binds to the CP5, 940 binding site (i.e. THC binding site) in the CB_2 receptor, leading to cellular activation and anti-inflammatory effects. Oral (*E*)-BCP inhibits carrageenan-induced edema in wild type mice. To obtain *in vivo* evidence of the anti-inflammatory effects induced by (*E*)-BCP *in vitro*, they examined the effectiveness of orally administered (*E*)-BCP in wild-type mice ($Cnr2^{+/+}$) and CB_2 receptor-deficient ($Cnr2^{-/-}$) mice. (*E*)-BCP (5 and 10 mg/kg) dosed orally significantly inhibited carrageenan-induced paw edema in wild-type mice by ≈70% and 50%, respectively (somewhat unexpectedly, the lowest dose of (*E*)-BCP was most effective in this experiment because no anti-inflammatory effect could be observed with the two lower doses of (*E*)-BCP in $Cnr2^{-}$ mice, the anti-inflammatory effects of (*E*)-BCP in wild-type mice show that this natural product exerts CB_2 receptor-dependent cannabimimetic.

In our study, the β-caryophylene and its derivatives are found in large amount (1.49 %), so we suggest that β-caryophyllene, α-bisabolol and azuline are responsible for anti-inflammatory activity. There are many reviews that agree with this results.

Much of the effectiveness of chamomile is due to its well-documented anti-inflammatory properties, which involve the nervous system at various levels. In studies, using experimentally-induced gastritis and other mucous membrane inflammations, chamomile consistently demonstrates a quick and prolonged anti-inflammatory effect. Studies have also shown that individual anti-inflammatory constituents of chamomile, including azuline, chamazuline, bisabolol and matricine, each have their own distinct mode of action. Some are more powerful but

perform for a shorter time, while others are milder but exhibit activity for a long period of time [28-32].

An overview of the traditional importance of anti-inflammatory effects, triterpenoids in pharmacognosy give rather poor results unless saponins are included. Without them, plant terpenoids with a lupane skeleton, lupeol is the simplest one and recently has been reported as the active principle of *Crataeva religiosa* when it was tested on the habitual models of inflammatory response, it showed moderate activity on the carrageenan-induced rat hind paw and rat pleurisy [33].

An overview on the traditional important taraxasterol acetate from the Ayurvedic drug *Echinops echinatus* (*Asteraceae*) reached 63% inhibition after 4 h, but at a high dose regime (200 mg/kg, *p.o.)* and this activity was not much improved when this product was given *i.p.* (68% inhibition) [34].

The same type of triperpenes have been found in the flowers of some *Asteraceae* plants: taraxasterol in *Cynara scolymus* and faradiol in *Chrysanthemum morifolium*. Both of these substances were examined for inhibitory activity against TPA-induced ear oedema in mice. The compounds were applied 30 min before TPA treatment and showed a strong inhibition of edema (IDs0=0.3 rag/ear for taraxasterol and 0.2 mg/ear for faradiol). In comparison with standard drugs, the triterpenes were similar in potency to indomethaein (inhibitors than hydrocortisone (IDs0 = 0.03 mg/ear).Ds0 – 0.3 mg/ear), although all of them were less effective [35].

From the analysis of drug, traxasterol, lupeol acetate, butlin and lupeol are major constituents of the drug, we suggest also that volatile oil is responsible for the anti-inflammatory effect of drug under study. Therefore, it is safe to use this drug by Bedouins in North region of Saudia Arabia.

CONCLUSION

The present study suggests that the plants mixture of *Alkanna tinctoria*, *Rubia tinctorum* and *Artemisa alba herba* could be a potential source of natural anti-inflammatory properties that could have great importance in the inhibition of inflammation.

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DZIAŁANIE ANTYOKSYDACYJNE MIESZANKI ROŚLIN LECZNICZYCH UŻYWANEJ PRZEZ BEDUINÓW W ARABII SAUDYJSKIEJ

EMAN ELSHARKAWY[1,2], MONA ELSHATHELY[1,3], GEHAD ABDEL JALEEL[4], HAYA IBRAHIM AL-JOHAR[5]

[1]Northern Border University-ARAR
North Region, Saudi Arabia

[2]Department of Eco physiology
Desert Research Center
Mathef El-Mataria, Egypt

[3]Department of Biology
Science Faculty for Girls
King Abdullaziz University
Jeddah, Saudi Arabia

[4]Researcher, Pharmacology Department
National Research Center
El-Bohoth St.
Dokki

[5]Saudi Food and Drug Authority
National Drug Control Laboratory
Riyadh, Saudi Arabia

Autor, do którego należy kierować korespondencję: el Sharqawyeman@yahoo.com

Summary

Ze względu na toksyczne i niepożądane działania leków syntetycznych, zioła stosowane w tradycyjnej medycynie są potencjalnym źródłem nowych związków biologicznie czynnych. Celem prowadzonych badań jest poszukiwanie nowych leków przeciwzapalnych na bazie roślin rosnących wokół nas. Działanie przeciwzapalne mieszanki ziół używanych w medycynie tradycyjnej (*Alkanna tinctoria, Rubia tinctorum* and *Artemisia herba alba*) sprawdzano w teście karageninowym obrzęku łapy szczura po podaniu wyciągu olejowego w dawce 100 mg/kg i 200 mg/kg masy ciała. Badany materiał redukował stan zapalny u szczurów wywołany karageniną po 4 godzinach zwłaszcza w stężeniu 200 mg/kg. Wyciąg olejowy badano za pomocą kapilarnej chromatografii genowej w porównaniu ze współczynnikiem retencji. Stwierdzono obecność około 17 składników: artemisol, epiglobulol i α-bisabolol są głównymi składnikami, a także olejek eteryczny i inne triterpeny: taraksasterol, stigmasterol, ursan-12-en, olean-12-en, betulin, germinkol, octan lupeolu i lupeol. Zidentyfikowano także inne związki heterocykliczne: morofolinę i piperidinol.

Słowa kluczowe: *działanie przeciwzapalne*, *α-bisabolol*, *tlenek kariofilenu*, *Alkanna tinctoria*, *Rubia tinctorum*, *Artemisia herba alba*, *test obrzęku łapy szczura indukowanego karageniną*