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Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*

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ABSTRACT

Aim of the study: This present study was carried out to evaluate the anti-inflammatory and analgesic effects of 85% ethanol extract (EE) of *Commiphora myrrha* and its different fractions partitioned with petroleum ether extract (EPE), ethyl acetate extract (EEA), *n*-butanol extract (EBu), and the water extract (ECY). Moreover, the chemical constituents in EPE were analyzed and identified by UPLC–QTOF/MS/MS.
Materials and methods: The anti-inflammatory activities were investigated by utilizing the paw edema mice induced by formalin. In addition, we determined the levels of PGE_2 in the edema paw. While the analgesic activity was examined against thermally and chemically induced nociceptive pain in mice, using the acetic acid and hot-plate test methods. The effects of the administration of dolantin or indomethacin were also studied for references. The components in EPE were analyzed by the ultra-performance liquid chromatography coupled with mass spectrum.
Results: In the anti-inflammatory test, EE inhibited the development of paw swelling induced by formalin significantly. The pharmacological activities of the petroleum ether fraction (EPE) were stronger than the EE extract and other fractions at the dose of 100 mg/kg, and furthermore significantly decreased the levels of inflammatory factor PGE_2 in the edema paw tissue at the fourth hour after formalin injection. It has been also shown that the ethanol extract (EE) significantly reduced acetic acid-induced writhing response in mice at the dose of 200 mg/kg, and 100 mg/kg. The petroleum ether fraction (EPE) showed significant analgesic activity in the model at the dose of 100 mg/kg ($p < 0.01$), and the ethyl acetate fraction (EEA) exhibited less analgesic activity ($p < 0.05$). All test samples showed no significant analgesic activity on the hot plate pain threshold in mice. The UPLC–MS/MS chromatogram analysis of EPE stated that EPE contains the ingredients of sesquiterpenes, diterpenes, and diterpenic acids. Moreover, seven main compounds were identified.
Conclusion: These data demonstrated that the EE and EPE posses analgesic and anti-inflammatory activities and may support the fact the traditional application of this herb in treating various diseases associated with inflammatory pain.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressant drugs, which have been used usually in the relief of inflammatory diseases by the people of the world for a long time. However, these drugs were often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcers (Corley et al., 2003). Recently, many natural medicines derived from plants, marine organisms, etc. were considered as the effective and safer for the treatment of various diseases including inflammation and pain (Sheir et al., 2001).

Commiphora myrrha (Nees) Engl. belongs to Commiphora genus in the family Burseraceae, which is a small tree or a large shrub which found in abundance in the dry and arid regions of Ethiopia and Somalia (the largest producers and exporters of myrrh) and to some extent in northern Kenya (Baser et al., 2003). Myrrh, as a traditional natural medicine, is an aromatic gum resin, which was the plant stem resinous exudate of different *Commiphora* species. It has many medicinal powers and has been used to treat various diseases, such as amenorrhea, ache, dysmenorhhea, tumors, fever, stomach complaints, gall bladder, chest ailments, snake and scorpion bites,

Abbreviations: EE, ethanol extract; EPE, petroleum ether extract; EEA, ethyl acetate extract; EBu, *n*-butanol extract; ECY, water extract; UPLC–MS, ultra-performance liquid chromatography coupled with mass spectrum.

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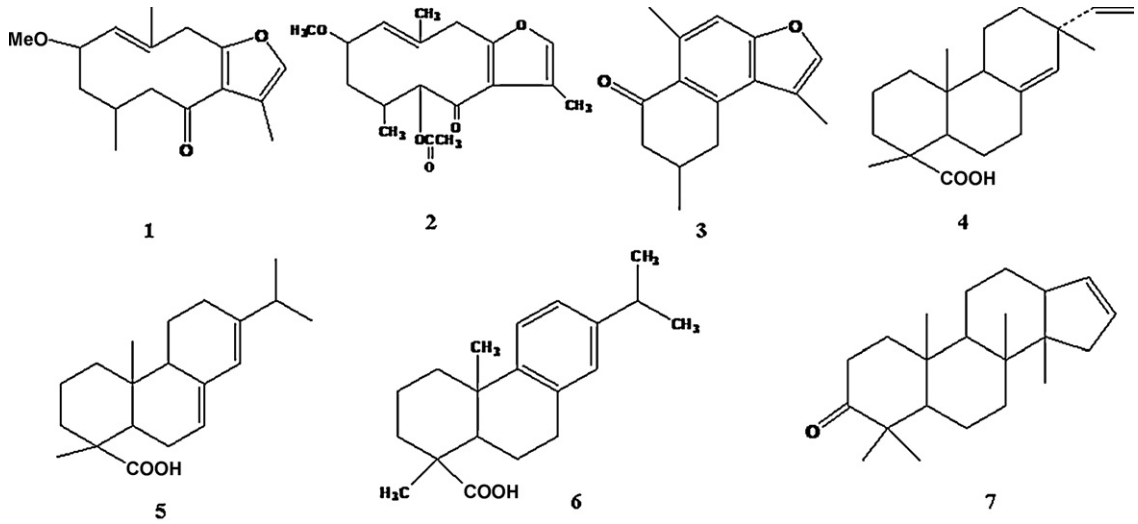

1. 2 - methoxy - 8, 12 - epoxygermacra-1 (10), 7, 11 – trine – 6 - one
2. 2 – methoxy – 5 - acetoxy - furanogermacr -1 (10) – en – 6 - one
3. myrrhone; 4. sandaracopimaric acid; 5. abietic acid; 6. dehydroabietic acid;
7. mansumbinone

Fig. 1. The chemical structures of seven compounds. (**1**) 2-Methoxy-8,12-epoxygermacra-1 (10) 7,11-trine-6-one, (**2**) 2-methoxy-5-acetoxy-furanogermacr-1 (10)-en-6-one, (**3**) myrrhone, (**4**) sandaracopimaric acid, (**5**) abietic acid, (**6**) dehydroabietic acid, (**7**) mansumbinone.

and skin infections in ancient India, China, Rome, Greece, and Babylon (El Ashry et al., 2003; Shen and Lou, 2008). Especially, the myrrh was a common analgesic and has been used to clean wounds and sores for more than 2000 years, until the European discovered the morphine.

Previous investigations have revealed that myrrh contained about 3–8% essential oil, 30–60% water-soluble gum and 25–40% alcohol-soluble resins (Tucker, 1986). Phytochemical investigation previously resulted in the isolation and identification of many metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives from *Commiphora* species (El Ashry et al., 2003; Hanus et al., 2005; Ahmed et al., 2006; Shen et al., 2007; Shen and Lou, 2008; Su et al., 2008a). The furanosesquiterpenoids were rich in the essential oil of the exudates, and about 20 furanosesquiterpenoids have been isolated or analyzed (Massoud, 2001; Zhu et al., 2003).

The crude extracts and some constituents of the myrrh exhibited diverse biological activities, such as cytotoxic, anesthetic, anti-inflammatory, and antimicrobial effects, and so on (Massoud et al., 2004; Lukas et al., 2005; Omar et al., 2005; Nomicos, 2007). Tipton et al. (2003) reported the cytotoxicity and anti-inflammatory effects of myrrh oil (MO) on human gingival fibroblasts and epithelial cells *in vitro*. The MeOH extract and the EtOAc fraction of *Commiphora wightii* were found to demonstrate significant inhibition of NO formation in lipopolysaccharide (LPS)-activated murine macrophages J774.1 *in vitro* (Meselhy, 2003). The results of our recent study showed that the myrrh extract exhibited significant antidysmenorrheic activity (Wang et al., 2009), inhibition of the isolated uterus contraction, aromatase inhibitory activity (Su et al., 2008b), and as well as protection of human umbilical vein endothelial cell (HUVEC) damage induced by H_2O_2 (Su et al., 2008b).

In the present work, the anti-inflammatory and analgesic activities were evaluated for the 85% ethanol extract and different fractions of *Commiphora myrrha*. The anti-inflammatory activities were investigated by utilizing the paw edema mice induced by formalin, and the levels of PGE_2 were determined at the same time. In addition, the analgesic activity was examined against thermally and chemically induced nociceptive pain in mice, using the acetic acid and hot plate test methods. The main constituents in petroleum ether extract (EPE) were analyzed by the ultra-performance liquid chromatography coupled with mass spectrum (UPLC–MS) to elucidate the possible active compounds responsible for the anti-inflammatory and analgesic effect.

2. Materials and methods

2.1. Plant materials

The resin obtained from Nanjing Medicinal Material (Co., Nanjing, China) was identified as resin derived from *Commiphora myrrha* (Nees) Engl. by Prof. Jin-Ao Duan (Department of Chinese Medicine, Nanjing University of Chinese Medicine, Nanjing, China). Moreover, the sample was collected from Guangdong in China. The voucher specimen (no. NJUTCM0812) were preserved in Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Chinese Medicine.

2.2. Drugs and chemicals

Meperidine hydrochloride injection (no. 050310) was obtained from Qinghai Pharmaceutical Co., Ltd., in China. Acetic acid (no. T20080825) was purchased from Shanghai Chemical Company (Shanghai, China). Formaldehyde (no. 010818) was obtained from Beijing Chemical Company (Beijing, China). Indomethacin was obtained from Shanxi Yunpeng Pharmaceutical Group Co., Ltd. (no. A090604).

Acetonitrile for UPLC analysis was of HPLC grade and purchased from Tedia Fairfield, OH, USA. Formic acid was of AR grade and from the Shanghai Reagent Company of China. Deionized water for UPLC analysis from the Millipore water purification system (Millipore, Milford, MA, USA) and filtered with 0.22 μm membranes.

The purity of seven marker standards, including 2-methoxy-8,12-epoxygermacra-1 (10), 7,11-trine-6-one (**1**), 2-methoxy-5-acetoxy-fruranogermacr-1 (10)-en-6-one, (**2**) myrrhone (**3**), sandaracopimaric acid (**4**), abietic acid (**5**), dehydroabietic acid