Original paper

Study on the Bioactive Constituents and *in vitro* Antioxidant and *in vivo* Anti-inflammatory Activities of Extracts from the Fruits of *Ziziphus Jujuba* Mill. cv. *Jinsixiaozao* Hort

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The aims of this study were to investigate the antioxidant and anti-inflammatory effects of extracts from fruits of *Ziziphus jujuba* Mill. cv. *jinsixiaozao* Hort (ZJMH) and its active chemical constituents. For antioxidant activity, reducing power, DPPH and β-carotene bleaching inhibition assay were used. Ethyl acetate extract exhibited the strongest antioxidant potential in all the models. For anti-inflammatory activity, petroleum ether extract showed the strongest inhibition (32.86%) against carrageenan-induced paw edema, which also displayed potent anti-inflammatory activity against xylene-induced ear edema (41.52%). The contents of phenolic acids, flavonoids, triterpenes, anthocyanins, triterpenoid saponins of different extracts were also observed. Moreover, seven compounds were first isolated from petroleum ether extract and showed considerable anti-inflammatory activity, especially alphitolic acid and stigmasterol. The results showed that ZJMH can be a natural antioxidant and anti-inflammatory agent.

Keywords: *Ziziphus jujuba* Mill. cv. *jinsixiaozao* Hort., chemical constituents, antioxidative activity, anti-inflammatory activity, alphitolic acid, stigmasterol.

Introduction

Inflammation is a protective mechanism of organisms to remove injurious stimuli from the tissues, and is involved in the development of several diseased conditions or disorders. Long-term administration of nonsteroidal anti-inflammatory drugs (NSAIDs) may induce numerous adverse effects, including gastrointestinal ulcers, bleeding, and renal disorders because of their nonselective inhibition of both constitutive cyclooxygenase-1 (COX-1) and inducible (COX-2) isoforms of the enzymes (Pendota *et al*., 2014). Moreover, new clinical and epidemiological data consider the oxidative stress as an important determinant in the development of chronic inflammation (Bekir *et al*., 2013). Natural foods such as fruits and vegetables act as antioxidant and anti-inflammatory agents by quenching free radicals, increasing antioxidant defenses, or inhibiting the release of pro-inflammatory mediators (Chou *et al*., 2012). Studies focused on finding antioxidant and anti-inflammatory agents with selective pharmacology and less toxicity from natural plants have becomes a major focus of scientific research worldwide.

The jujube fruits are known as a predominantly nutritious food due to their biologically active components such as triterpenic acids, flavonoids, phenolic acids, and vitamins (Gao *et al*., 2013). Various parts of the jujube plant have been traditionally used for...
the treatment of different kinds of diseases. The fruits and seeds have been traditionally applied to treat various diseases such as anorexia, lassitude, insomnia, anxiety, loose stools, insufficiency of the spleen, and fatigue in women (Mahajan et al., 2009; Guo et al., 2011; Yu et al., 2012).

Numerous previous studies have focused on different jujube species and revealed that the antioxidant capacities of jujube can be attributed to the high level of polyphenols, flavonoids, and anthocyanins; and this activity differs with cultivars, tissues, ripening stages. The predominant phenolic acid in jujube was shown to be protocatechuic acid, followed by gallic acid, chlorogenic acid and caffeic acid (Zhang et al., 2010; Zoizio et al., 2014). Jujube is usually used as an antidote in a traditional Chinese formula, Shi Zao decoction, to relieve the drastic inflammatory irritant nature of Euphorbia species. Previous study (Yu et al., 2012) demonstrated that the triterpene acids fraction is the most active part causing the inhibitory effects on the inflamed cells after activation by Euphorbia kansui and prostratin.

As we know, previous studies of Ziziphus jujuba Mill. cv. jinxisiaozao J. Hort. (ZJMH), widely cultivated in China, were mainly focused on its polysaccharides. Therefore, the objectives of this study were to investigate the antioxidant and anti-inflammatory capacities and their active constituents (phenolic acids, flavonoids, triterpenes, anthocyanins, triterpenoid saponins) of different extracts. Moreover, the anti-inflammatory activities of the compounds isolated from this fruit were also evaluated.

Material and Methods

Material and reagents The fruits of ZJMH were purchased in Cangzhou City, Hebei Province of China, and identified by Professor Wenyuan Gao (School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China). The voucher specimens were deposited at 4°C in a laboratory of Tianjin University.

Ascorbic acid, gallic acid (Nr. 110831-200803), rutin (Nr. 100080-200707), oleanolic acid, and jujuboside B were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Carrageenan (Nr. C1867), β-carotene (reference standard, purity ≥99.0%), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Linoleic acid, Twain 20, and Folin–Ciocalteu’s reagent were provided by Guangfu Fine Chemical Technology, Tianjin. They were fed with a rodent standard pellet diet with free access to water ad libitum and kept at 25 ± 1°C under a 12 h light/dark cycle condition. The Animal Ethics Committees of the Faculty of Medicine approved all experimental protocols, in accordance with ‘Principles of Laboratory Animal Care and Use in Research’ (Ministry of Health, Beijing, China).

Determination of total polyphenols The total polyphenols (TPs) content in the PE, EA, and n-BuOH extracts of the JZMH was determined using Folin–Ciocalteu’s reagent (Zhang et al., 2013). The TPs content was calculated from the calibration curve of gallic acid ranged from 20 to 100 μg.

Determination of total flavonoids The total flavonoids (TFs) content was measured using the aluminum chloride colorimetric
method outlined by Zhang et al. (2013) with some modifications. The data were expressed as milligram rutin equivalents (RE)/g extract through the calibration curve ranged from 14 – 70 μg.

**Determination of total anthocyanins** The total anthocyanins (TAs) content was determined by the pH differential method (Zhang et al. 2013).

**Determination of total triterpenes** The total triterpenes (TTs) content of different extractions were assessed based on the method of Li et al. (2014) with minor modifications. TTs content was expressed as oleanolic acid equivalents (milligrams of oleanolic acid/g extract) through the calibration curve ranged from 4 to 20 μg.

**Determination of total triterpenoid saponins** Total triterpenoid saponins (TSs) content of samples was analyzed using the method described by Oleszek (2002). TSs content was calculated from the calibration curve ranged from 6.67 to 40 mg and expressed as milligram of jujuboside B equivalents (jujuboside B/mg extract).

**Antioxidant Activity of the different extractions**

**Reducing power** The reducing power of different extracts was investigated largely based on the method optimized by Lue et al. (2010). The reducing power was expressed as the vitamin C equivalents, which were calculated from the calibration curve of vitamin C ranged from 5 to 120 μg.

**DPPH free radical scavenging assay** The ability to scavenge DPPH free radicals of PE, EA, and n-BuOH extracts from ZJMH was evaluated according to the method of Arabshahi-Delouee (2007).

**β-Carotene bleaching inhibition assay** The inhibition activity of β-carotene oxidation by peroxide radicals the different extracts was determined based on a modified procedure by Li et al. (2011b).

**Anti-inflammatory capacity** Xylene-induced mouse ear edema and carrageenan-induced mouse hind paw edema were used to evaluate the acute anti-inflammatory activity according to the method of Huang et al. (2010). The activity of the different extracts and the isolated compounds were carried out separately. Those mice were randomly divided into 17 groups (6 mice in each group). Each extract was administered orally for 5 consecutive days at the dose of 10 mg/kg, while the compounds and the DEX were administered orally for 5 consecutive days at the dose of 10 mg/kg.

**Carrageenan-induced mouse hind paw edema** Edema was induced by injecting carrageenan (1% w/v, 0.1 mL/paw) into the right hind paw of the mice at 40 min after the fifth administration. Paw volume was measured before and after ovalbumin injection after 30 min, 1, 3 and 5 h, using a sliding caliper. The anti-inflammatory effect was evaluated by an increase in paw edema (mm). Swelling inhibition rate was assessed in terms of the mean thickness increase of each paw in comparison with the control group.

**Xylene-induced ear edema** One hour after the fifth administration, 0.1 mL xylene were applied to the anterior and posterior surface of the right ear of each mouse with the left ear as a control. After mice killed, circular sections were taken using a cork borer with a diameter of 9 mm, and weighed. Swelling degree was calculated by the mean weight increase of each ear, while the inhibition of inflammation in treated mice was calculated in comparison to control mice.

**Statistical analysis** Statistical Product and Service Solutions (SPSS) version 13.0 was used for the statistical analysis of the data of the animal experiments. Results were expressed as mean ± SEM. The data were statistically analyzed by least significant difference (LSD) and Student Newman-Keuls (S-N-K) tests. The results were considered statistically significant at P < 0.05.

**Results and Discussion**

**Phytochemical analysis** Polyphenols, flavonoids, and anthocyanins have been reported to be the most important phytochemicals responsible for antioxidant capacity. They play an important role in counteracting reactive oxygen species (ROS) to minimize molecular damage (Wang et al., 2014). Moreover, flavonoids also have anti-inflammatory effects. In this study, the TPs contents of different extracts from the fruit of ZJM H varied from 8.12 ± 0.37 to 86.50 ± 0.87 as mg of gallic acid/g extract (Table 1), while TTs contents ranged from 84.73 ± 0.82 to 289.28 ± 3.39 as mg of rutin/g extract (Table 1). The TAs content in the n-BuOH fraction was only 4.69 ± 0.05 as mg of cyaniding 3-glucoside equivalents (c-3-gE)/g extract. Both TPs and TAs contents were highest in the EA fraction and lowest in the PE fraction. Caffeic, ferulic, protocatechuic, chlorogenic, and cinnamic acid were the prevailing phenolic acids in jujubes (Wu et al., 2012). The distribution of phenolic acids in the peel, pulp, and seed of jujube fruits were evaluated (Zhang et al., 2010; Wang et al., 2011) and they predominated in fruit peel. Different types of flavonoids such as flavan-3-ols (Gao et al., 2012) and flavonols (Sun et al., 2011) have been found in jujube fruits, with catechin, epicatechin, and rutin as the predominant flavonoids. In addition, flavonoids might vary significantly by species and maturity degree (Guo et al., 2011). For their antioxidant and other physiological activities, the presence of polyphenols in fruits is of great importance to consumers and researchers.

Triterpenic acids are widely distributed in all parts of *Ziziphus* species plants as free acids, triterpene esters, or aglycones (Romero et al., 2010), which have been reported to possess numerous biological activities, including antioxidant and anti-inflammatory activities (Mullauer et al., 2010). In this study, for PE, EA, and n-BuOH extraction, the amount of TTs ranged from 497.49 ± 0.27 to 313.05 ± 1.54 as mg of oleanolic acid/g extract, while the TSs contents varied from 535.06 ± 5.93 to 295.05 ± 1.66 as mg of jujuboside B/g extract. TTs and TSs contents in all extracts of ZJM H were remarkably higher than other constituents, which was consistent with previous findings (Zhang et al., 2013). In order to find the active constituents, 7 compounds (Fig. 1a, b), including 6 triterpenoid acids (compounds 2 – 7) and one steroid (compound 1),
were isolated from the PE fraction for further assessment of in vivo acute anti-inflammation. Up to now, 4 dominating types (lupane, ursane, oleanane, and cenoanthe type) of triterpenic acids have been isolated from the fruits of *Ziziphus* species (Guo et al., 2009, 2011). In this paper, 3 lupane types (betulinic, cenoanthe and alphitolic acid), 2 ursane types (ursolic and ursuran acid) and one oleanane type (oleanenic acid) of triterpenic acids, together with stigmasterol, were all isolated from this jujube cultivar for the first time.

Antioxidant capacity of the different extractions from ZJMH

Many researches have indicated that the antioxidant activity of fruits seems to be influenced by the contents of TPs and TPs levels. Meanwhile, highly significant linear correlations were observed between the polyphenol contents and their antioxidant capacity (Du et al., 2009). To obtain accurate evaluations of antioxidant activities of ZJMH, 3 different assays with various antioxidant mechanisms (reducing power, DPPH, and β-carotene bleaching inhibition assay) were used in this study and significant differences (P < 0.05) were observed among all the extractions.

The reducing power of a compound is related to its electron transfer ability. The best antioxidant potential was observed in the EA extract (20.11 ± 0.212 mg of Vc/g extract) (Fig. 2a). The antioxidant potential of PE extract was lowest, with a value of 3.30 ± 0.008 mg of Vc/g extract, which was only about 16.4% of that of the EA extract. The order of antioxidant potency of different extracts from ZJMH in this model is the same as that of the TFs and TPs levels.

The DPPH test is based on the reduction of DPPH solution with the presence of a hydrogen donating antioxidant. The most active part is EA extract with the highest TFs and TPs levels (Fig. 2b). While n-BuOH extract presented similar moderate antioxidant potential as EA extract and PE extract showed the lowest DPPH-scavenging capacity, which contained the lowest TPs and TFs contents. These results are quite coincident with the study of Zhang et al. (2010): jujube cultivars/tissues with more contents of total polyphenols and flavonoids showed much higher DPPH scavenging activity. In our lab’s previous work (Zhang et al., 2015), TFs and TPs were mainly responsible for the DPPH radical-scavenging activity and reducing power of *Ziziphus jujuba* Mill., respectively, which were all affected by TAs, cAMP and cGMP.

Due to the presence of peroxyl free radicals created as a by-

**Table 1.** Content of polyphenols, flavonoids, triterpenes, anthocyanins, and triterpenoid saponins of different extractions of ZJMH (mg/g).

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPs</th>
<th>TFs</th>
<th>TTs</th>
<th>TAs</th>
<th>TSs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>8.12 ± 0.37a</td>
<td>84.73 ± 0.82a</td>
<td>497.49 ± 0.27a</td>
<td>nd</td>
<td>535.06 ± 5.93a</td>
</tr>
<tr>
<td>EA</td>
<td>86.50 ± 0.87b</td>
<td>289.28 ± 3.39b</td>
<td>342.96 ± 1.72b</td>
<td>nd</td>
<td>382.21 ± 2.64b</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>21.59 ± 0.12c</td>
<td>113.69 ± 0.79c</td>
<td>313.05 ± 1.54c</td>
<td>4.69 ± 0.05</td>
<td>295.05 ± 1.66c</td>
</tr>
</tbody>
</table>

TPs, TFs, TTs, TAs, TSs are total polyphenols, flavonoids, triterpenes, anthocyanins, triterpenoid saponins, respectively. Values are expressed as the means of 3 replicates ± SEM. Mean values with different letters (a-c) within the same column are statistically different (P < 0.05). nd means not detected.
phase (1.5 – 2.5 h) involves the liberation of bradykinin. The last phase (2.5 – 6 h), presumably, mediated by prostaglandins, is the most important process in the inflammatory response (Sowemimo et al., 2013). In this phase, the maximal vascular response also reaches its maximum level, which is closely associated with leukocyte migration to the inflamed area. It is well established that prostaglandins, as modulators of inflammatory responses, have a major role in the mechanism (Li et al., 2011a). Cyclooxygenase (COX) is the key enzyme to synthesize prostaglandins from arachidonic acid and the production of prostanoids is through the serum expression of COX-2 by a positive feedback mechanism (Kumar et al., 2013). Non-steroidal anti-inflammatory drugs

![Fig. 1. Isolation flowchart of the PE fraction (a) and chemical structures of compounds (1-7) (b) isolated from the fruits of ZJMH. Fr: fraction; PE: petroleum ether; EtoAc: ethyl acetate; n-BuOH: n-butanol; MeOH: methyl alcohol.](image-url)

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**Table:**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Compounds</th>
<th>Purity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr.G</td>
<td>Oleanonic acid</td>
<td>730</td>
</tr>
<tr>
<td>Fr.H</td>
<td>Ursolic acid</td>
<td>83</td>
</tr>
<tr>
<td>Fr.I</td>
<td>Betulinic acid</td>
<td>272</td>
</tr>
<tr>
<td>Fr.K</td>
<td>Ceanothic acid</td>
<td>30</td>
</tr>
<tr>
<td>Fr.N</td>
<td>Alphitolic acid</td>
<td>230</td>
</tr>
<tr>
<td>Fr.P</td>
<td>PP12 Stigmasterol (90mg)</td>
<td></td>
</tr>
<tr>
<td>Fr.T</td>
<td>PP10 Ursolic acid (730mg)</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- **Peaks:**
  - Fr.3: PP12 Stigmasterol (90mg)
  - Fr.8-15: PP10 Ursolic acid (730mg)
- **Chemical Structures:**
  - **Compound 1 (stigmasterol)**
  - **Compound 2 (oleanonic acid)**
  - **Compound 3 (betulinic acid)**
  - **Compound 4 (ursolic acid)**
  - **Compound 5 (ceanothic acid)**
  - **Compound 6 (alphitolic acid)**
  - **Compound 7 (ursolic acid)**
(NSAIDs) have been reported to inhibit the cyclooxygenase enzyme (Murugan et al., 2013). In this study, the anti-inflammatory effect of the crude extracts from ZJMH in mice was evaluated by an increase in paw edema (mm) and almost all the extracts demonstrated a significant (P < 0.05) inhibition of carrageenan-induced paw edema volume during the 5 hours after drug administration. The PE extract showed the strongest anti-inflammatory potential among the three extracts with a maximum inhibition of 32.86% at the dose of 200 mg/kg, and the EA and n-BuOH extracts with the inhibitions of 19.96% and 19.02% at 5 h, respectively (P < 0.001). Dexamethasone was used as a positive control, and it showed the highest inhibition of 43.5% (P < 0.001) at 5 h. The above results indicated that all the extracts from ZJMH exhibited the highest inhibition at the last phase. This suggested that the anti-inflammatory capacity of the extracts possibly involved these signal pathways which inhibited the prostaglandins production by the inhibition of the action of COX-2.

Xylene-induced ear edema in mice is a preliminary and simple animal model for evaluating potential anti-inflammatory agents, steroids as well as non-steroidal antiphlogistic agents (Sowemimo et al., 2013). Ear edema can cause an acute inflammatory response leading to severe vasodilation and edematous changes of skin partially related with phospholipase A2 (Tong et al., 2014). All the extracts (200 mg/kg) from ZJMH inhibited the ear edema formation significantly (P < 0.05), compared to dexamethasone (10 mg/kg, 52.80%, P < 0.001). The PE and EA extracts (200 mg/kg) significantly inhibited ear edema by 41.52% (P < 0.001) and 25.55% (P < 0.05), respectively. The n-BuOH fraction (200 mg/kg) showed the strongest inhibition of ear edema by 43.51% (P < 0.001). The results from this study indicated that the extracts possibly act the suppression of ear edema by inhibiting the release of PLA2 or antagonize its action. n-BuOH extract showed much
higher inhibition of ear edema than that of paw edema. Maybe, the constituents in n-BuOH extract have higher activity in inhibiting the release of PLA2 than that of COX-2. Although further work is needed to understand the compositions and anti-inflammatory mechanism, the above results still suggested the potential usefulness of the extracts from the fruits of ZJMH in the treatment of sub-chronic inflammation.

**Effects of the compounds on carrageenan-induced mouse hind paw edema and xylene induced mouse ear edema** In order to identify the specific compounds responsible for the observed anti-inflammatory activity in the extracts, 7 compounds were isolated from the PE extract and their anti-inflammatory capacities were assessed by the above 2 animal models. All of them considerably suppressed the edema induced by carrageenan and xylene at 10 mg/kg (Fig. 4, Table 2). In comparison to carrageenan control, the reduction of paw edema by alphitolic acid was durable for 5 h and the inhibition was up to 43.97% (P < 0.001) at 5 h, which showed a similar suppressive effect to dexamethasone (10 mg/kg, 47.63%

**Table 2.** Effects of the different fractions and compounds from ZJMH on xylene-induced mouse ear edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Swelling (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>--</td>
<td>8.40 ± 2.25</td>
<td>--</td>
</tr>
<tr>
<td>DEX 1</td>
<td>10</td>
<td>3.97 ± 0.57***</td>
<td>52.80***</td>
</tr>
<tr>
<td>PE</td>
<td>200</td>
<td>4.91 ± 1.14***</td>
<td>41.52***</td>
</tr>
<tr>
<td>EA</td>
<td>200</td>
<td>6.59 ± 1.77*</td>
<td>21.55*</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>200</td>
<td>4.75 ± 1.43***</td>
<td>43.51***</td>
</tr>
<tr>
<td>Control 2</td>
<td>--</td>
<td>5.90 ± 0.93</td>
<td>--</td>
</tr>
<tr>
<td>DEX 2</td>
<td>10</td>
<td>2.82 ± 0.58***</td>
<td>52.26***</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>10</td>
<td>3.99 ± 1.19**</td>
<td>32.46**</td>
</tr>
<tr>
<td>Oleanonic acid</td>
<td>10</td>
<td>5.45 ± 2.38</td>
<td>7.60</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>10</td>
<td>4.62 ± 0.78</td>
<td>21.78</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>10</td>
<td>4.78 ± 0.95</td>
<td>18.93</td>
</tr>
<tr>
<td>Ceanothic acid</td>
<td>10</td>
<td>4.40 ± 0.95*</td>
<td>25.45*</td>
</tr>
<tr>
<td>Alphitolic acid</td>
<td>10</td>
<td>2.98 ± 0.63***</td>
<td>49.55***</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>10</td>
<td>3.98 ± 0.65**</td>
<td>32.48**</td>
</tr>
</tbody>
</table>

DEX, dexamethasone. Results are expressed as the mean ± SEM (n = 6); *, P < 0.05; **, P < 0.01; ***, P < 0.001 was compared to the control groups; 1, the control and positive groups for the different fractions; 2, the control and positive groups for the compounds.

**Fig. 4.** Anti-inflammation effects of the compounds (1-7) isolated from ZJMH on carrageenan-induced mouse hind paw edema. DEX, dexamethasone. All the compounds and the DEX were administered orally for 5 consecutive days at the dose of 10 mg/kg. Results are expressed as the mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 was compared to the control group.
P < 0.001). Stigmasterol, oleanonic acid, betulinic acid, and ceanothic acid, also inhibited the carrageenan-induced paw edema obviously (P < 0.05) at 1 and/or 5 h, respectively. For the xylene induced mouse ear edema assay, the inhibition rates of alphitolic acid, ursolic acid, stigmasterol and ceanothic acid (10 mg/kg) were up to 49.55% (P < 0.001), 32.48% (P < 0.01), 32.46% (P < 0.01), and 25.45% (P < 0.05), respectively.

In general, alphitolic acid exhibited the strongest inhibition against acute inflammation induced by carrageenan and xylene, which was similar to that of dexamethasone. The inhibitions of stigmasterol, ceanothic acid, and ursonic acid were remarkably stronger than other compounds from ZJMH. Previous studies demonstrated that the sterols and triterpenoids had anti-inflammatory activities by inhibiting the nuclear factor kappa-B (NF-κB), or suppressing the secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and keratinocyte-derived chemokine (KC) (Li et al., 2012). Stigmasterol is a common phytosterol widely distributed in fruits and vegetables and it exhibits a pronounced anti-inflammatory effect through the down-regulation of inducible nitric oxide synthase (iNOS) expression leading to prostaglandin E₂ (PGE₂) suppression, and a dramatic suppression of the level of protein and mRNA expression of these enzymes (Pandith et al. 2013). The topical anti-inflammatory activity of alphitolic acid against 12-O-tetradecanoylphorbol-13-acetate (TPA) induced mouse ear edema was with a potency comparable to that of indomethacin as shown by Aguirre et al. (2006). Oleic acid is commonly known for its anti-inflammatory effects as well as various other pharmacological properties. Betulinic acid has displayed anti-inflammatory action through attenuating the expression of TNF-α, transforming growth factor-β1 (TGF-β1) and iNOS or inhibiting activation of NF-κB and NF-κB-regulated gene expression (Nader et al., 2012). Ursolic acid is commonly seen as an anti-inflammatory agent by inhibition of TNF-α, IL-5, IL-13, IL-17, IL-8 secretion, inhibition of COX-2 transcription, release of macrophage migration inhibitory factor, and down-regulation of enzymes required for the permeation of lymphocytes (Li et al., 2012). The anti-inflammatory effect of jujube demonstrated that the triterpene acids fraction was the predominant effective part of jujube (Yu et al., 2012). Moreover, 7 triterpenoid acids including those we isolated in our work are likely be active compounds concerned with their pronounced inhibitory action on the activated inflammatory cells. In accordance with previous findings, PE extraction from fruits of ZJMH markedly suppressed the inflammation induced by carrageenan and xylene. Furthermore, the 6 triterpenoid acids and one steroid might very well account for it involving one or more of the above mechanisms.

In our study, n-BuOH fraction was the most potent in xylene-induced ear edema assay. Previous studies showed that alphitolic, betulinic, and oleanonic acids have also been isolated from the n-BuOH extract of the fruits of other Ziziphus species. And other triterpenoid acids (such as 3-O-trans-p-coumaryl alphitolic acid, 2-O-trans-p-coumaryl alphitolic acid, 3-O-cis-p-coumaryl alphitolic acid, betulonic acid, maslinic acid, and 3-O-trans-p-coumaryl maslinic acid), flavonoids (like rutin, quercetin 3-O-robinobioside, and 3′,5′-di-C-glucose), and 1-O-β-glucopyranosyl-(25,35,4R,9E)-2-{(2R)-2-hydroxyecosanoylamo]-9-tetradecone-1,3,4-trirole were also found in this fraction (Yagi et al., 1978a; b; Pawłowska et al., 2009; Yu et al., 2012; Guo et al., 2014). Some have been found to be excellent anti-inflammatory agents. Betulonic acid exhibited potent inhibitory effects on NO and PGE₂ production in mouse macrophages (RAW 264.7) stimulated with bacterial lipopolysaccharide (LPS) (Reyes et al., 2006). Just like betulinic and ursolic acids, maslinic acid exhibited anti-inflammatory effects by primarily acting on the expression of IP-10 gene (one of the pro-inflammatory marker genes), which plays an important role in inflammation (Mueller et al., 2013). Rutin could reduce neutrophil infiltration to inflamed tissues by suppressing the β2-integrin expression in the carrageenan-induced rat pleurisy model (Suyenaga et al., 2014). Furthermore, a series of dammarane saponins, including jujuboside A, C, D, E, and A₁, have been isolated from the n-BuOH extract of seeds of Ziziphus jujube Mill. and all of them showed considerable lipoxygenase-inhibiting activity (Yu et al., 2014). Previous studies suggested n-BuOH fraction in our study may contain alphitolic, betulinic, and oleanonic acids as well as all above active constituents. This could be one reason that the n-BuOH fraction exhibited the most potent anti-inflammatory activity in xylene-induced ear edema. The compositions and anti-inflammatory mechanisms of n-BuOH fraction are worth further investigation.

**Conclusion**

This paper is the first to report the presence of stigmasterol, oleanonic acid, betulinic acid, ursolic acid, ceanothic acid, alphitolic acid and ursonic acid in the fruit of ZJMH. The *in vitro* antioxidative activity and *in vivo* anti-inflammatory capacity of different fractions and all isolated compounds were assessed. The results reveal that ZJMH can be exploited as an economic dietary source for its natural antioxidant and anti-inflammatory agents as important to human health. Further studies on the active constituents and anti-inflammatory mechanisms of the plant and fruits of *Zizyphus* genus are undergoing.

**Reference**


Biological Phytochemical Study of Jujube Fruits

Chen, T., 102, 1233-1240.


