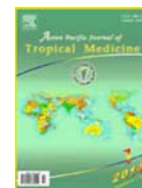Contents lists available at ScienceDirect

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In vivo antiinflammatory activity and chemical composition of *Hypericum scabroides*

Sadia Mansour[1,2*], Noureddine Djebli[1], Esra Eroglu Ozkan[3], Afife Mat[3]

[1]*Pharmacognosy, Api−Phytoteraphy Laboratory, Department of Biology, Faculty of SNV, Mostaganem University, Algeria*

[2]*Molecular Applied Genetic Department, Sciences and Technology Oran University, Algeria*

[3]*Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey*

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ABSTRACT

Objective: To evaluate the methanolic extracts of aerial parts of *Hypericum scabroides* (HSM) (200 mg/kg, *p.o.*) for *in vivo* anti−inflammatory activity.
Methods: The anti−inflammatory activity of HSM was tested in mice weighting (25±5) g. Either vehicle (control group), the methanolic extracts (200 mg/kg) or diclofenac (50 mg/kg), was administered (*p.o.*) for 60 min before an edema was induced in the mice paw by subcutaneous injection of carrageenin. The mouse−paw volume was measured 1 h, 3h and 6 h after injection of carrageenin.
Results: The HSM showed significant reduction of edema in carrageenan induced mice paw edema model at 1 h and 3 h for (78.03±15.54)% and (40.44±16.36)%, respectively. The diclofenac 50 mg/kg exhibited % reduction in paw volume (31.00±11.52)%, (0.80±0.09)% and (9.39±1.99)% after 1 h, 3 h and 6 h, respectively compared to control group. The obtained results revealed that HSM has significant anti inflammatory activity. Furethermore, the chemical composition of HSM was analyzed by using high performance liquid chromatography−diode array dedector. The plant contained pseudohypericin (trace) hypericin (trace), chlorogenic acid (0.014 0±0.000 5)%, rutin (0.005 0±0.000 6)%, hyperoside (0.016±0.005)%, isoquercitrin (0.034 0±0.000 5)% and kaempferol (trace).
Conclusions: The obtained results of the present investigation revealed that methanol extract of *Hypericum scarbroides* has significant anti−inflammatory activity.

1. Introduction

Inflammation or phlogosis is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells, which involves a complex sequence of bio−chemical events closely associated to the pathogenesis of various diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout, migraine[1−3].

Aging, skin disorders, rheumatoid arthritis, atherosclerosis are caused by oxidative stress, occurring when production of reactive oxygen species (ROS)−in the form of hydroxyl radical, superoxide anion, peroxyl radical, singlet oxygen, hydrogen peroxide or ozone, exceeds the antioxidant protective capacity of target cells[4]. Free radicals are the initiators of a redox reaction cascade, resulting in changes of the chemical structure of biological macromolecules, such as proteins, lipids and DNA, or disturbances of human cell metabolism[5], or even tissue injury[6]. Human skin is exposed to both external factors, such as radiation, smoking, pollutants, organic solvents, pesticides[7] and internal ROS products from normal cell metabolism, normal aerobic respiration, stimulated polymorphonuclear leukocytes or macrophages[8,9], that increase the level of oxidative

stress[10,11]. These conditions could delay the healing of skin injuries, such as burns, ulcers, wounds, eczema.

Nowadays, although the synthetic anti inflammatory drugs are dominating the market, the element of toxicity from these drugs cannot be ruled out. Many drugs [both nonsteroidal anti inflamatory drugs (NSAIDs) and corticosteroids] have been developed but their safety profile studies have shown that none of them is clearly safe. Due to adverse reactions of synthetic and chemical medicines *i.e.* causing gastrointestinal irritation and reappearance of symptoms after discontinuation, herbal medicines have made a comeback to improve our basic health needs. Many plants and herbs such as ginger, turmeric and olive oil, have been shown to exhibit potent anti inflammatory effects[12]. Currently available drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are searched every nook and corner of the world. Therefore, drugs lacking those effects are searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigations of the efficacy of plant−based drugs used in the traditional medicine have been paid great attention because they are cheap and have little side−effects[13,14].

Synthetic and natural antioxidants are of particularly importance in maintaining the oxidative stress level under the critical point in human organism. Previous *in vitro* and *in vivo* studies reported the antioxidant capacity of several species of medicinal plants[15,16], acts at cellular level, through cell growth stimulation, membrane potential stabilizing or at molecular level, through ROS scavenging, lipid peroxidation, *etc*[17]. These roles have been attributed, in part, to their biological active constituents, such as liposoluble and watersoluble vitamins (E and C, respectively) and polyphenolic substances[18].

As plants produce a significant amount of antioxidants to prevent oxidative stress, they represent a potential source of new compounds with antioxidant activity[19].

Some of these plants are those of the *Hypericum* genus, (Clusiaceae Lindley, syn. Guttiferae A.L. de Jussieu). They include a large number of species distributed worldwide[20]. Several of the botanical species belonging to the genus are used in folk medicine and among them *Hypericum perforatum* L. (*H. perforatum*), also named St. John's Wort, is especially known as a traditional remedy for the treatment of melancholia, abdominal and urogenital pain and ulcerated bums[21,22]. Over the last two decades, it has been demonstrated that *H. perforatum* is effective as an antidepressant[23,24], an antiviral[25], and an antimicrobial[26].

The drug contains peculiar chemical constituents such as naphthodianthrones (hypericins), acylphloroglucinols

(hyperforin), flavonol glycosides (quercetin derivatives) and biflavones and all the metabolites seem to contribute to its pharmacological activity[27]. Current use of *H. perforatum* is mainly for the treatment of mild to moderate depression[21,28], and drug extracts for antidepressant applications have become increasingly popular.

During the recent years, several phytochemical studies on other species of *Hypericum* have also been performed, leading to the isolation of new molecules, some having biological activity.

These studies suggested that some species such as *H. perforatum* possess anti−inflammatory and anti−viral activity, which could be potentially used to alleviate conditions like inflammatory bowel disease, diarrhea, and respiratory infection[29−32].

Besides *H. perforatum*, other species of the *Hypericum* genus are being studied to identify their constituents as well as anti−inflammatory, anti−proliferation, and anti−microbial activities[33−35]. Among these species, a more lipophilic fraction of *Hypericum gentianoides* methanol extract rich in acylphloroglucinols, was found to have potent inhibitory effect on LPS−induced macrophage production of prostaglandin E2[36].

In this study, an attempt has been made to investigate another species of *Hypericum*−−*Hypericum scabroides* (*H. scabroides*). It aims to evaluate the anti−inflammatory activity of the methanol extract of aerial parts of *H. scabroides*.

2. Materials and methods

Fresh aerial parts of *H. scabroides* (Clusiaceae Lindley, syn. Guttiferae A.L. de Jussieu) for the proposed work were collected from East Anatolia, namely, Erzincan−Kelkit, 15 km to Kelkit, 1 550 m and were authenticated. Vouchers were deposited in the Herbarium of Istanbul University, Faculty of Pharmacy (ISTE 85343).

All the experiments were carried out using Swiss albino mice (24−30 g). All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee (1205/c/08/CPCSEA, 21.04.08). The animals were housed in polypropylene cages and maintained under standard laboratory conditions. Animals were housed at a temperature of (24±2) ℃ and relative humidity of 60%−70%. They were fed with a standard diet and water was given *ad libitum*. All experiments were conducted after overnight fasting but there was free access to water. The mice were devided into 3 groups, a minimum of six animals were used in each group.

The following chemicals procured from various sources were used in this investigation *i.e.* carrageenan, Brewer's

yeast (Sigma Aldrich, Bangalore, India), petroleum ether, chloroform, ethyl acetate, methanol, acetic acid (Rankem, New Delhi, India), carboxymethyl cellulose (CMC, Loba Chemie, Mumbai, India) and diclofenac sodium (Akums Drugs and Pharmaceuticals, Delhi, India) were used during the experimental protocol.

2.1. Chemical agents

Hypericin, chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, kaempferol, quercetin, amentoflavon, hyperforin, $AlCl_3$ and D–Galactose were obtained from Sigma–Aldrich (Taufkirchen, Germany). Pseudohypericin was obtained from PhytoPlan (Heidelberg, Germany). Milli–Q ultrapure water was obtained from Millipore (Billerica, MA), high–performance liquid chromatography grade acetonitrile, methanol, ethylacetate and sodium dihydrogen phosphate dihydrate were obtained from Merck (Darmstadt, Germany) and ortho–phosphoric acid 85% was obtained from Fluka (Steinheim, Switzerland).

2.2. Extraction and preparation of sample

The dried aerial parts of *H. scabroides* were macerated with methanol (100 mL) (3×24 h) and this procedure was repeated twice at room temperature. The extract was filtered and dried under reduced pressure at a temperature below 45 °C. The crude methanol extract was lyophilized and stored at −20 °C for biological investigations[37–39].

2.3. Inflammatory paw edema test in mice

In this part of the experiment, the anti–inflammatory activity of the methanolic extract was investigated on carrageenan–induced inflammatory paw edema[40].

The methanolic extracts of aerial parts of *H. scabroides* was dissolved and dispersed in physiological saline (0.09%) and administrated by orally for pretreated group of mice at 200 mg/kg dosage. Physiological saline (0.09%) was given to the control group at the same volume as vehicle. One hour after administration, 0.1 mL of 0.5% carrageen solution was injected into the footpad of the hind paws of each mouse in all groups. Prior to carrageenan injection, the mice′ paw volume was measured with a plethysmometer.

Increasing of carrageenan induced inflammatory paw volume was measured at 1, 3, and 6 h over the injection. The anti–inflammatory activity of *H. scarbroides* extracts was compared with that of 50 mg/kg diclofenac.

The percentage inhibition of the inflammation was calculated from the formula: inhibition% $= (D-D_t)/D_0 \times 100$, where D is the diameter of injected paw, D_0 is the average inflammation (hind paw edema) of the control group of mice at a given time 0; and D_t is the average of diameters of hind paw edema of the drug treated (*i.e.* extract or reference diclofenac) mice at the same time[41].

2.4. Acute toxicity

Methanolic extracts of aerial parts of *H. scabroides* in 200 mg/kg doses was administered to the mice orally (*p.o.*). All animals were observed for 24 h after drug administration.

2.5. Statistical analysis

The observations were expressed as mean±SD. The difference in response to test drugs was determined as a percentage. $P<0.05$ was considered significant when compared to the control group. All statistical calculations were analysed using One–way ANOVA followed by multiple comparison tests.

3. Results

3.1. Acute toxicity

After oral administration of the methanolic extracts of aerial parts of *H. scabroides* 200 mg/kg doses, no mortality was recorded for all animals observed 24 h after drug administration.

3.2. Chemical compounds

Chemical compounds of methanolic extract of *H. scabroides* represented in Table 1, revealed presence of some compounds in traces (pseudohypericin, hypericin, kaempferol). Furthermore, Chlorogenic acid, Rutin, Hyperoside and Isoquercitrin percentage values of methanolic extracts of aerial parts of *H. scabroides* varied between 0.014% and 0.034%.

3.3. Liquid chromatography

The liquid chromatography chromatograms of the methanolic extract of *H. scabroides* were showed in Figure 1. At 590 nm, the phytochemical investigation in the present study revealed the presence of pseudohypericin and hypericin. Moreover at 360 nm, the presence of chlorogenic

Table 1

% Value of chemical compounds of HSM (Mean±SD; n=3).

Compounds	Retention time (min)	Calibration equation values	Linear regression(r^2)	% Value of HSM
Pseudohypericin	4.86	$y=2.582269e{+}007x{+}1741.874$	0.9998	Trace
Hypericin	13.93	$y=6.03411e{+}007x{+}297.2292$	0.9999	Trace
Chlorogenic acid	4.33	$y=5110294x{+}1490.398$	0.9999	0.0140±0.0005
Rutin	8.89	$y=1.383368e{+}007{+}5188.182$	0.9999	0.0050±0.0006
Hyperoside	10.19	$y=2.849917e{+}007x{+}526.7023$	0.9999	0.0160±0.0005
Isoquercitrin	10.75	$y=1.671137e{+}007x{-}3712.788$	0.9999	0.0340±0.0005
Quercitrin	14.41	$y=1.205178e{+}007{-}3518.974$	0.9999	Not determined
Kaempferol	17.09	$y=5.183916e{+}007x{+}4373.856$	0.9999	Trace
Quercetin	17.84	$y=3.688175e{+}007x{+}18905.43$	0.9999	Not determined
Amentoflavon	20.27	$y=2.207879e{+}007{+}772.0972$	0.9996	Not determined
Hyperforin	27.75	$y=6212343x$	0.9997	Not determined

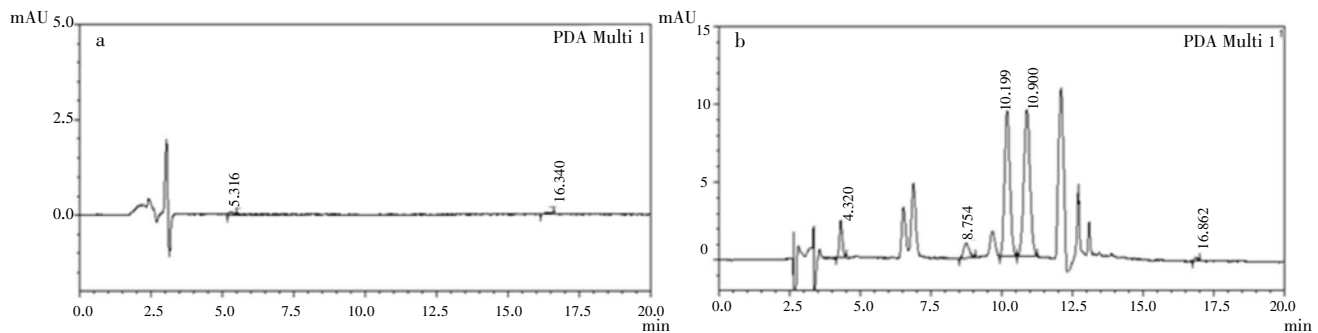HSM: Methanolic extracts of aerial parts of *H. scabroides*.

Figure 1. Liquid chromatography chromatogram of methanolic extract of *H. scabroides*.

Pseudohypericin (1), hypericin (2) in the methanolic extract of *H. scabroides* at 590 nm (a). Chlorogenic acid (1), rutin (2), hyperoside (3), isoquercitrin (4), kaempferol (5) in the methanolic extract of *H. scabroides* at 360 nm (b).

acid, rutin, hyperoside, isoquercitrin and kaempferol was revealed.

3.4. Anti–inflammatory activity

Percentage increase in paw volume was significantly reduced after 1 h pretreatment with *H. scabroides* in treated mice compared to controls. This reduction was maintained for 3 h after pretreatment. The values in 6 h were similar among three groups.

According to diclofenac sodium antiinflammatory effect, the diclofenac sodium group shows an important diminution in percentage increase in paw volume induced by carrageenan (Table 2).

Table 2

Effect of *H. scabroides* methanol extract (aerial parts) on carrageenan induced paw volume.

Treatment	Doses (mg/kg)	Percentage increase in paw volume induced by carrageenan		
		1 h	3 h	6 h
Controls		52.84±9.64	36.97±6.88	22.94±9.89
H. scabroideses	200	8.55±3.74[***]	21.71±5.97[***]	20.16±5.50
Diclofenac	50	36.40±16.66[**]	36.18±0.95[***]	20.80±7.54

Values are expressed in mean±SEM (n=6); [**]: $P{<}0.001$, [***]: $P{<}0.0001$.

The anti–inflammatory activity of methanolic extract of *H. scabroides* was very important during the three post injection hours as it shown in Figure 2. However, this activity was similar as the diclofenac one at the sixth hour of experimentation.
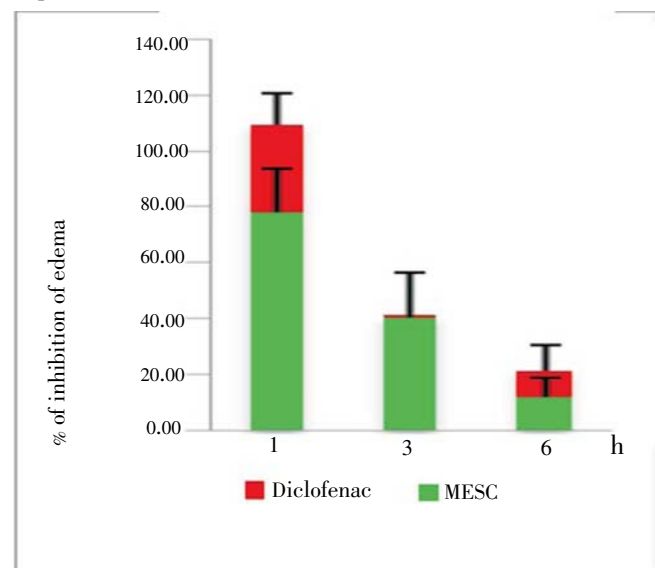

Figure 2. Percentage inhibition of edema by methanol extract of *H. scabroides* (aerial parts) at different time intervals determined with diclofenac sodium as reference.

MESC: methanol extract of *H. scabroides*.

4. Discussion

The present experimental investigation revealed that methanol extract of aerial parts of *H. scabroideses* possessed significant anti inflammatory activity in experimental animals at a dose of 200 mg/kg.

Carrageenan–induced hind paw edema is the standard experimental model of acute inflammation. Moreover, the experimental model exhibits a high degree of reproducibility[42]. It has a biphasic effect. The first phase is due to release histamine and serotonin (5–HT) (0–2 h), while plateau phase is maintained by a kinin like substance (3 h) and a second accelerating phase of swelling is attributed to PG release (>4 h)[43].

Methanolic extracts of aerial parts of *H. scabroides* produced significant inhibition of carrageenan–induced paw edema. The inhibition was more important than that of the standard drug––diclofenac sodium. There are several mediators or multi processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis[44]. On preliminary phytochemical screening methanolic extracts of aerial parts of *H. scabroides* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception[45]. Indeed, the occurrence of flavonoids in the methanolic extract from the aerial parts of some species of *Hypericum* is well documented. The content of these metabolites is reported to be high in the flowers during the budding stage, immediately before flowering (up to 12%/dry weight)[46]. Moreover, Umek *et al.* reported that the rutin content in *H. perforatum* is positively correlated with the altitude of the locality where the plant grows. The flavonol quercetin and its glycoside derivatives, rutin, hyperoside, isoquercitrin, quercitrin, and the biflavones 13,118–biapigenin and amentoflavone are described as the most common compounds in the drug extracts. Recently, a detailed chemical study by on–line coupling of high–performance liquid chromatography with UV, nuclear magnetic resonance and mass spectrum[47], allowed the determination of two other flavonoids in *H. perforatum*: the quercetin–glucuronide and the quercetin arabinoside. Our phytochemical study of *H. scarboides* revealed presence of some of these compounds (isoquercitrin, hyperoside, rutin and chlorogenic acid), which could be involved for anti–inflammatory activity of the methanolic extract. Furthermore, different phytochemicals produces have been found to have a broad range of activities, which may help in protection against chronic diseases[48]. These compounds are known to be biologically active. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and have led to the development of powerful pain killer medications[49]. It should be noted that the anti inflammatory activities of many plants have been attributed to their triterpene or flavonoid contents[50]. It has been also demonstrated that various flavonoids (such as rutin, quercetin, and luteolin), biflavonoids, and triterpenoids (such as ursolic acid) produced significant antinociceptive and/or anti–inflamatory activities[50,51]. The effective anti–inflammatory activity of ethanolic extract of aerial parts of *H. scarbroides* is due to the higher concentration of bioactive phytoconstituents[52]. Hence, the presence of flavonoids may be contributory to the anti inflammatory activity of methanolic extracts of aerial parts of *H. scabroides*. Although the exact nature of the anti–inflammatory activity mechanisms of the phytoconstituents have not been elucidated, the results of the present study were validated from a preclinical point–of–view, and the popular use of this medicinal plant in the treatment of inflammatory diseases. These studies are valuable for identifying lead compounds for anti inflammatory drugs, keeping in mind the side–effects of NSAIDs and corticosteroids. Further, human studies are needed to prove the safety and efficacy of long term administration of methanol extract of *H. scarboides* as potential anti–inflammatory agent in routine clinical practice.

Finally this study showed that methanol extract of *H. scarbroides* contains several phytochemical compounds (pseudohypericin, hypericin, chlorogenic acid, rutin, hyperoside, isoquercitrin, and kaempferol) with different percentages. These compounds may be involved for the anti inflammatory activity and protective effect of the methanolic extracts of aerial parts of *H. scabroides* against inflammation process particularly during the first and third hours of inflammation so it could be used for clinical practices.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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