Indian Journal of Experimental Biology
Vol. 60, November 2022, pp. 842-850
DOI: 10.56042/ijeb.v60i11.51723

HPLC analysis, acute toxicity and anti-inflammatory effects of *Salix alba* L. barks extracts on experimental animal models

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Received 27 June 2021; *revised 19 March 2022*

The white willow, *Salix alba* L., rich in polyphenols and flavonoids, is traditionally used for its antipyretic, analgesic and anti-inflammatory potential in Algeria. As part of the ethnobotanical survey of medicinal plants in Setif region in Algeria, in the present study, we assessed the safety profile of *S. alba* barks methanol (SAME) and aqueous (SAQE) extracts, their phytoconstituents, and their antioxidant and anti-inflammatory activities. The *in vitro* antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), reducing power and hydroxyl radical tests assays. HPLC analysis identified 16 compounds in each extract with different concentrations. Gallic acid, syringic acid and cinnamic acid were high in the aqueous extract, while the methanol extracts were rich in chlorogeni cacid, catechin, methyl gallate, pyrocatechol, rutin, ferulic acid, naringenin, taxifolin and kaempferol. The extracts showed significant reducing power, DPPH and hydroxyl radical scavenging effects. *In vivo* tests showed a strong effect on carrageenan-induced paw edema after 5 h with an inhibition of 88.62 and 87.56% for SAME and SAQE (500 mg/kg), respectively. The extracts also at 500 mg/kg showed significant inhibition of xylene-induced ear edema of 57.81% with SAME 67.18% with SAQE. The results have shown that the methanol and aqueous extracts of the barks of *S. alba* had no toxic effect on biochemical parameters as well as the organ weights and behaviour. It indicates that *S. alba* could be a promising source of anti-inflammatory agent.

Keywords: Antioxidant, Polyphenols, White willow

Free radicals cause protein, DNA damage and lipid peroxidation. These changes are responsible for several diseases including cancer, cardiovascular diseases, atherosclerosis and inflammatory diseases[1]. Acute inflammation includes a series of events like increased permeability, vasodilation, fluid exudation and migration of leukocytes. In an inflammatory environment, massive quantities of different free radicals were produced by activated neutrophils and macrophages *via* the NADPH oxidase. These free radicals created in inflammation might lead to toxic effects in inflamed site[2]. The human body has anti-free radical defence system which includes antioxidants that can be enzymatic or none enzymatic[3]. Since antiquity, medicinal plants were known as an important source of pharmaceutical agents which can prevent and treat several diseases.. Many properties of plant products are associated with the presence of phenolic compounds, which are essential for the development of plants and play an important role in defence mechanisms. Polyphenols are secondary metabolites of plant that are involved in various physiologic phenomena[4]. They are known for their antioxidant activity due to their hydroxyl groups which give hydrogen to free radicals[5-7]. In addition, polyphenols possess anti-inflammatory properties by modulating the inflammatory cascade[8-9]. However, the popularization of traditional herbal medicine poses many problems, including the lack of sufficient studies on therapeutic properties and toxicity to provide sufficient guarantees as to their rational use.

Salix alba L. (Fam. Salicaceae) locally called "Safsaf abyadh", and commonly, white willow, grows mainly on wet soils in cold and temperate regions[10]. Traditionally, it has been used as antipyretic, analgesic and anti-inflammatory[11,12]. The effectiveness of this plant is mainly due to flavonoids, tannins and other phenolic compounds including salicin, a precursor of salicylic acid[13]. *Salix alba* is shown to recognized 5-HT1D receptors, targets of triptans[14], leading to the hypothesis that it possesses migraine prophylactic activity[15]. White willow contains certain metabolites such as salicin and saliginin, which act as inhibitors of tumour by inducing apoptosis, damaging DNA and by affecting

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cell membranes or denaturing proteins in some human cancers[16].

As part of the ethnobotanical survey of the use of medicinal plants in three localities in Setif region in Algeria, here, we have evaluated the acute toxicity of Algerian *Salix alba* L., determined its chemical composition and elucidated the antioxidant and anti-inflammatory properties of the methanol and aqueous extracts of *S. alba* barks.

Materials and Methods

Ethnobotanical survey

Ethnobotanical survey was carried out according to Fadil *et al.*[17] with few modifications. The data concerning the use of plants were collected from herbalists, traditional healers and villagers in the region of Setif in north-eastern of Algeria through three survey sites, Setif city, Aïn Oulmene and Bougaa (Fig. 1). In this survey, 77 people (50 men and 27 women) aged between 32 and 64 took part.

Collection of plant material

Salix alba was collected manually on march, 2017 from the region of Bougaa, Setif (northeast of Algeria) and authenticated by Pr H. Laouer (Ferhat Abbas University of Setif, Algeria). A voucher specimen (003/DBEV/UFA/17) was deposited at the Department of Vegetal Biology and Ecology, University of Setif 1, Algeria. The sample was dried in shade then grounded and the obtained powder was stored in the dark until the extraction.

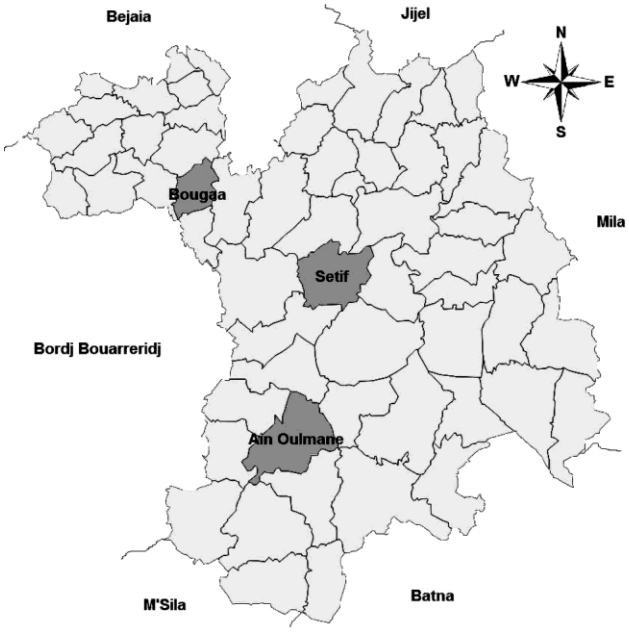

Fig. 1 — Map showing the study area (Setif region)

Preparation of extracts

Two processes of extraction were followed in this work. In the first procedure, the dried powder of bark part of *Salix alba* was extracted by methanol for 7 days then 5 days at 25°C[18]. The excess methanol was evaporated by the rotary evaporation at 45°C until dryness to obtain *Salix alba* methanol extract (SAME). In the second protocol, the powder was extracted at a ratio of 1:10 (w/v) by decoction in distilled water and filtered using a filter paper then concentrated to dryness by allowing it to stand in an oven at 40°C[19].

Phytochemical analysis

Determination of polyphenols and flavonoids amounts

The total content of Phenolic in plant extracts was determined with Folin-Ciocalteu spectrophotometric method[20]. A volume of 200 µL of each *S. alba* extract (SAME, SAQE) was diluted and mixed with 1 mL of Folin-ciocalteu reagent (1/10). After 4 min, 800 µL of Na_2CO_3 (7.5%) were added. After incubation for 2 hours in the dark, the absorbance was measured at 765 nm. Results were expressed as equivalent mg of gallic acid / gram of plant extract. The concentration of flavonoids in bark extract was estimated using $AlCl_3$ reagent[20]. The plant extracts (1 mL) were added to the same volume of a methanol solution containing 2% of $AlCl_3$. The mixture was vigorously shaken then incubated for 10 min at room temperature (25°C). Absorbance was read at 430 nm and results were expressed as mg of quercetine equivalent / g extract (mg QE/g).

Characterization of polyphenols by HPLC

Determinations of polyphenols compounds in aqueous and methanol extracts were performed by HPLC using an Agilent 1260 series[21]. C18 column (4.6 mm × 250 mm i.d., 5 mm) was used for the separation of components. Mixture containing two eluent systems: water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) was used as a mobile phase at a flow rate of 1.0 mL/min and the column temperature of 35°C. The elution gradient applied is as follows: 0 to 5 min (80% A); 5-8 min (40% A); 8 to 12 min (50% A); 12-14 min (80% A) and 14-16 min (80% A). Eluted samples and standards were detected at 280 nm.

Antioxidant capacity evaluation

DPPH scavenging assay

Radical scavenging activity of plant extracts against stable $DPPH^{\bullet}$ (2,2-diphenyl-2-picrylhydrazyl

hydrate) following Guemmaz *et al.*[22] protocol. A volume of 50 µL of various dilutions (0-50 mg/mL) from the extract was added to 1250 µL of DPPH solution (0.004% in methanol) and the absorbance was measured at 517 nm after 30 min of incubation in the dark. Butylated hydroxytoluene (BHT) was used as standard.

Determination of reducing power

The Fe^{3+} reducing power of *S. alba* barks extract was evaluated according to Hazra *et al.*[23]. A volume of 400 µL of the diluted extract in distilled water (for SAQE) or methanol (for SAME) was mixed with 400 µL phosphate buffer (0.2 M, pH6.6) and 1% of potassium hexacyanoferrate in solution. After 20 min of incubation at 50°C, 10% of TCA was added and the mixture was centrifuged for 10 min at 3000 rpm. The upper portion of the solution (400 µL) was mixed with 400 µL of distilled water and 80 µL of $FeCl_3$ solution (0.1%) was added. The absorbance was read at 700 nm.

Hydroxyl radical scavenging

The hydroxyl scavenging activity of *S. alba* extract was determined by its ability to compete with salicylic acid for hydroxyl radicals. Following Ates *et al.*[24], the reaction mixture contained, in a last, 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM), 0.3 mL of sodium salicylate (20 mM) and 200 µL of various concentrations of the extract. After incubation for 1 h at 37°C, absorbance was measured at 562 nm.

Animals

The animals used in this study were Swiss albino mice (20-30 g) and Wistar albino rats (150-200 g). Animals were obtained from 'Pasteur Institute of Algeria', Algiers. They were kept in the animal house of the University under favourable conditions (22ºC±3°C, 12/12 h light/dark cycle), and have free access to food and water. They were treated in accordance to guidelines of the Organization for Economic Cooperation and Development for assessing the safety and efficacy of plants based medicines[25] and to the Committee of the 'Association Algérienne des Sciences en Experimentation Animale' under law No. 88-08/1988.

Acute toxicity study

The study of acute oral toxicity of the extracts was evaluated according to the OECD[26] guideline 425. The test used five groups of 5 animals each: Gr. I as control, Gr. II & III received SAQE (*S. alba* bark aqueous extract) (2000 and 5000 mg/kg, respectively); and Gr. IV & V received SAME (*S. alba* bark methanol extract) (2000 and 5000 mg/kg, respectively). All the animals were deprived from food twelve hours before the study and were kept under close observation for 14 days. They were weighed just before the administration of the extract and then on days 7 and 14 after[27].

Biochemical parameters

The blood samples were obtained in heparin tubes from sacrificed animals and the serum was separated by centrifuging at 3000 rpm for 15 min. The biochemical tests are carried out to evaluate liver damage by determining levels of creatinine, aspartate amino transferase (ASAT) and alanine aminotransferase (ALAT), using a semiautomatic Biochemistry Analyzer (Bechman) with standard spectrophotometric diagnostic kits (Spinreact, Spain)[28].

Anti-inflammatory evaluation

Xylene-induced ear edema

In this study, anti-inflammatory effects were assessed using the xylene-induced ear edema test described by Mayouf *et al.*[29]. Animals were orally treated with two doses of *S. alba* extract (250 and 500 mg/kg), indomethacin (50 mg/kg) and distilled water. One hour before the administration of 30 µL xylene to the posterior surface of the right ear. Ear thicknesses were measured using digital calliper two hours after the application of xylene[30]. The difference of thickness between the right and the left ear was determined and the percentage of inhibition of ear edema was calculated as following:

$$\% \text{ inhibition of edema} = 100 \times (\Delta tc - \Delta ts)/\Delta tc$$

where Δtc: difference of the ear thicknesses in the positive control and Δts: difference of the ear thicknesses in the sample (tested group)

Carrageenan induced paw edema in rats

Anti-inflammatory activity was also assessed using carrageenan-induced paw edema assay according to Buodonpri *et al.*[31]. Rats were injected at the sub plantar level with 100 µL of carrageenan suspension (1% in saline) freshly prepared in the right hind paw. Extract (250 and 500 mg/kg in 1 mL H_2O) and Diclofenac (20 mg/kg) or aqueous solution (vehicle control) were orally administered 1h before carrageenan injection. The thickness of the paws was measured by digital calliper, before and after injection of carrageenan at 1, 2, 3, 4 and 5 h). The reduction percentage of edema was calculated as following:

$$\% \text{ inhibition} = 100 \times (V_c - V_t)/V_c,$$

Table 1 — Ethnobotanical survey of medicinal plants uses in anti-inflammatory remedies in Setif region

Family	Species Plant	Local Name	Part Used	Method of use
Capparaceae	*Capparis spinosa*	Kabbar	The bark of the roots	Infusion, Decoction[9]
Zygophyllaceae	*Peganum harmala*	Harmal	Leaves, roots, stems	Cataplasm[7]
Apiaceae	*Thapsia garganica*	Dryas	Root and aerial part	Poudre[7]
Urticaceae	*Urtica dioica*	Horayg	Leaves	Cataplam, infusion, decoction[6]
Equisetaceae	*Equisetum arvense*	*Dhaylḥiṣān*	Aerial part	Infusion, Decoction[6]
Lamiaceae	*Thymus vulgaris*	Zaater	Aerial part	Decoction, tisane, fumigation, powder[4]
Zingiberaceae	*Zingiber officinale*	Zendjabil	Rhizome	Decoction, tisane[8]
Brassicaceae	*Lepidium sativum*	Habrchad	Seeds	Fresh dried[3]
Dioscoreaceae	*Tamus communis*	Karma souda	Rhizome	Cataplam[2]
Salicaceae	*Salix alba*	Safsaf abyadh	Bark	Cataplam, infusion decoction, powder[8]
Malvaceae	*Malva sylvestris*	Khobiza	Leaves, shoots, flowers, fruits	Infusion, decoction[5]
Myrtaceae	*Myrtus communis*	Rayhane	leaves,	Infusion, decoction, Cataplam[3]
Asteraceae	*Inule visqueuse*	Amagramane	Aerial part	Tisane[7]
Moringaceae	*Moringa oleifera*	Mouringa	Leaves	Infusion, Decoction[2]

where V_c is the paw thickness in the control group and V_t is the paw thickness in tested group

Statistical analysis

All results were expressed as means ± SD (*in vitro*) results were expressed as mean ± SEM (*in vivo*) using Graph Pad Prism version 5.03. Analysis of variance (ANOVA) was used to test differences between groups. Differences were considered significant at $P \leq 0.05$.

Results

Ethnobotanical survey of medicinal plants

The informants were asked about the application patterns and the use of medicinal plants in the region in the treatment of inflammations. Fourteen species in 14 families have been reported to treat inflammatory diseases and associated symptoms (Table 1). The most cited species are *Capparis spinosa* (9 citations), *Salix alba* and *Zingiber officinale* (8 citations each).

Acute toxicity of extracts

Clinical signs

The first set of five mice receiving 2000 mg/kg aqueous or methanol extract of *Salix alba* showed that no mouse was killed. No evidence of toxicity was observed during the 14 days observation period. Therefore, the approximate acute lethal dose (LD_{50}) of *S. alba* bark extract in mice has been estimated at more than 2000 mg/kg. The aqueous extract at the dose of 5000 mg/kg showed no symptoms of toxicity on the behavioural responses of the treated mice observed for 14 days. However, mice given 5000 mg/kg methanol extract of *Salix alba* extract exhibited changes in respiratory rate, coat characteristics and mortality were observed during the experimental period in groups mentioned above.

Effect on the body and organ weights

The body weight of mice treated with *Salix alba* bark extract doses (2000 mg/kg and 5000 mg/kg) is presented on Table 2. Results revealed no significant differences. Table 3 shows the relative organ weight of the mice treated with the doses (2000 mg/kg and 5000 mg/kg) of *Salix alba* bark extract for 14 days. In general, no significant difference was noticed in the relative weight of organs.

Table 2 — Effect of the extracts of *Salix alba* on body weight

Days	Control	SAQE 2 g/kg	SAQE 5 g/kg	SAME 2 g/kg
1 day	27.8±0.665	26±0.634^{ns}	28±0.896^{ns}	28.4±0.511^{ns}
7 days	30.8±0.584	29.8±1.360^{ns}	31.2±0.972^{ns}	28.8±1.598^{ns}
14 days	32.2±0.375	30.8±1.116^{ns}	33.4±0.929^{ns}	30±1.143^{ns}

Table 3 — Effects of extract of *Salix alba* on organ weights

Organs	Cont.	SAQE 2 g/kg	SAQE 5 g/kg	SAME 2 g/kg
Heart	0.176±0.009	0.154±0.008^{ns}	0.154±0.013^{ns}	0.164±0.016^{ns}
Lungs	0.256±0.018	0.216±0.02^{ns}	0.206±0.022^{ns}	0.212±0.010^{ns}
Liver	1.802±0.073	1.744±0.142^{ns}	1.436±0.045^{ns}	1.782±0.208^{ns}
Kidney	0.458±0.037	0.462±0.046^{ns}	0.458±0.031^{ns}	0.444±0.029^{ns}
Spleen	0.226±0.014	0.186±0.015^{ns}	0.186±0.015^{ns}	0.448±0.079^{ns}

Effect of extracts on biochemical parameters

Assessment of liver and kidney function is essential to assess the possible toxic effects of extracts and drugs[32].The effects of acute administration of *Salix alba* on biochemical parameters are presented in Fig. 2. No statistically significant difference in liver function parameters such as creatinine, ASAT and ALAT was observed.

Characterization of phytoconstituents

Total polyphenols and flavonoids contents

The quantitative determination of total polyphenols and flavonoids in *Salix alba* bark extracts are shown in Table 4. The content of total polyphenols in the extracts was higher in the SAME (656.95±0.14 mg EGA/g E) than in the SAQE (440.12±0.14 mg EGA/g E).

HPLC analysis

The chromatograms the *Salix alba* aqueous and methanol extracts are presented in Fig. 3. The

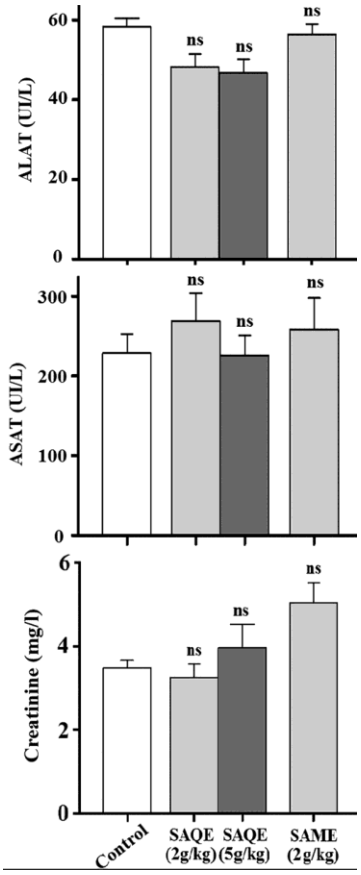

Fig. 2 — Biochemical parameters of control and mice treated with *Salix Alba* measured during the acute toxicity. [Values are expressed as mean ± SEM (n = 5)]. ns: no significant]

Table 4 — Yields of extraction and total polyphenols and flavonoids contents of *Salix alba* methanol (SAME) and aqueous (SAQE) extracts

Extracts	Yield of extraction (%)	Polyphenols (µg GAE/mg of extract)	Flavonoids (µg QE/mg extract)
SAME	40.03%	656.95 ± 0.14	16.07 ± 0.01
SAQE	8.76%	440.12 ± 0.14	21.16 ± 0.01

[GAE, gallic acid equivalent; QE, quercetin equivalent]

aqueous extract showed high concentrations of gallic acid, syringic acid, and cinnamic acid and the methanol extract was rich in chlorogenic acid, catechin, methyl gallate, pyrocatechol, rutin, ferulic acid, naringenin, taxifolin and kaempferol. It is interesting to mention that coffeic acid, rutin, ellagic acid, coumaric acid, vanillin, kaempferol were not found in the aqueous extract, while, coffeic acid, ellagic acid, coumaric acid and vanillin, were not found in the methanol extract.

Concentrations of found components are given in Table 5. HPLC analysis proved that several phenolic compounds were found, although the concentrations differ between the two extracts.

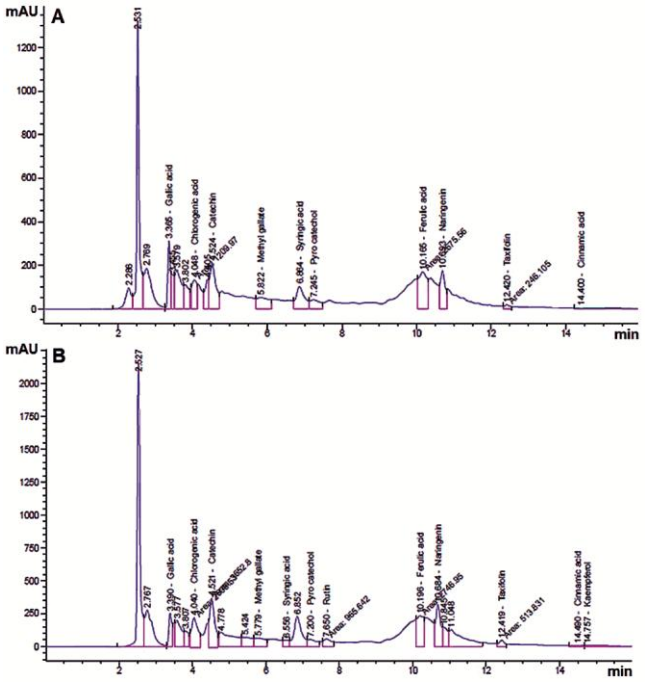

Fig. 3 — HPLC chromatograms of *Salix alba* (A) aqueous; and (B) methanol extracts

Table 5 — Phytoconstituents concentrations in aqueous (SAQE) and methanol (SAME) extracts of *Salix alba*

	Rt (min)	Conc. (µg/g)	
		SAQE	SAME
Gallic acid	3.302	7490.65	6465.33
Chlorogenic acid	4.094	5543.23	12367.34
Catechin	4.530	24313.09	36957.53
Methyl gallate	5.731	1291.05	1373.63
Syringic acid	6.580	3660.90	1205.61
Pyrocatechol	7.272	5163.03	5923.67
Rutin	7.564	0.00	7278.06
Ferulic acid	10.152	5306.63	5448.23
Naringenin	10.251	4917.95	8858.23
Taxifolin	12.472	2033.16	4244.94
Cinnamic acid	14.388	330.71	152.59
Kaempferol	14.755	0.00	1455.98

[Rt, retention time; Conc, concentration]

In vitro antioxidant activity

DPPH radical scavenging activity

The highest radical scavenging activity was presented by SAME with IC_{50} = 6.7±0.001 µg/mL, followed by SAQE 15.2±0.003 µg/mL). The two extracts are significantly more active than the BHT, the standard compound which showed an IC_{50} of 87±0.003 µg/mL.

Reducing power

The two extracts exhibit high reducing power with IC_{50} of 6.51 µg/mL for SAQE and 30.6 µg/mL for SAME, compared to BHT (IC_{50} = 14.84±0.4 µg/mL).

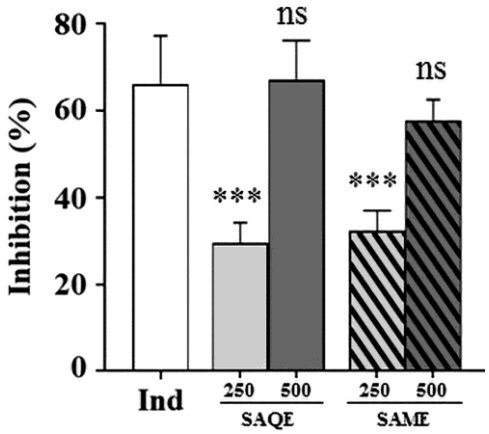

Fig.4 — Effects of *Salix alba* aqueous extract (SAQE: 250 and 500 mg/kg) and methanol extract (SAME: 250 and 500 mg/kg) on xylene induced auricular edema in mice after 2 h. [Values are means ± SEM (n = 6). Ind: Indomethacin (50 mg/kg). Ns, not significant; ****P* <0.001 compared to indomethacin]

Hydroxyl radical scavenging

The elimination of $OH^{\bullet}$ is vital for the living systems[33]. The SAQE exhibited the highest $OH^{\bullet}$ scavenging effect. IC_{50} values of SAQE, SAME were 60±0.01 µg/mL and 386±0.03 µg/mL, respectively. The hydroxyl radical removal activity of the SAQE extract was greater than the ascorbic acid (standard) with IC_{50} = 101.43±0.01 µg/mL.

Acute anti-inflammatory activity

Effects on xylene-induced ear edema

Xylene-induced edema is an acute experimental inflammatory model, that frequently evaluate the anti-inflammatory effects of natural products and has a good predictive value in anti-inflammatory screening[34]. The activity of *S. alba* extracts on xylene-induced ear edema is shown in Fig. 4. Treatment of mice with doses of 250 and 500 mg/kg of aqueous extract reduced the swelling by 39.06 and 67.18%, respectively. On the other hand, when the activity of methanol extract was analyzed, we observed that the dose of 250 mg/kg reduced ear edema by 32.4%, while the dose of 500 mg/kg showed a reduction of 57.81%. These results demonstrate that the effect of aqueous extracts is similar to that of indomethacin (50 mg/Kg) inhibited the edema by 66.24 %.

Carrageenan induced paw edema inhibition

Subplantar injection of carrageenan in rat resulted in a time dependent increase in paw thickness from 1 h to 5 h after carrageenan administration in the control group. The effect of methanol and aqueous extracts of *S. alba* on carrageenan-induced edema was shown in Fig. 5. A group of doses of SAQE and SAME

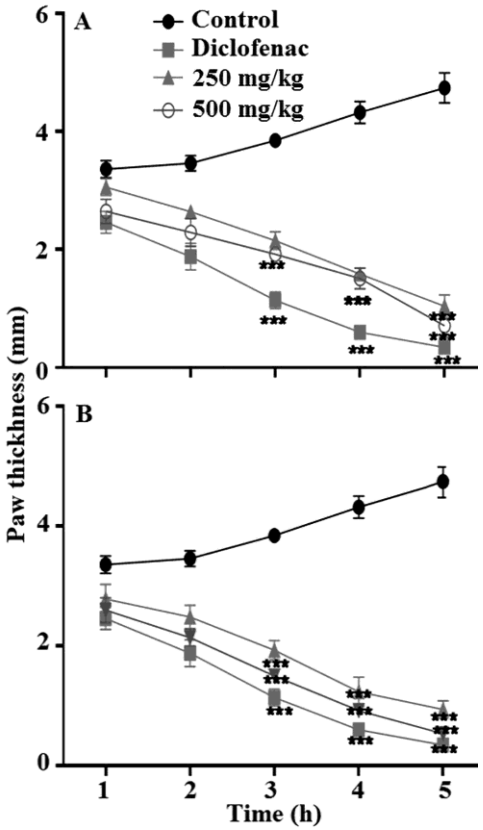

Fig. 5 — Effect of various doses of *Salix alba* (A) aqueous extract (SAQE); and (B) methanol extract (SAME) on carrageenan induced edema in Wistar rats. [Results are expressed as mean ± SEM (n=6). Ns, no significant; ****P* <0.001 compared to positive control]

(250 and 500 mg/kg) gave a significant reduction in paw edema induced by carrageenan with time at 4 and 5 h (*P* <0.001). Oral administration of Diclofenac (20 mg/kg) showed a clear inhibition of the inflammatory response in rats compared to the control (*P* <0.001).

Discussion

The ethnobotanical survey showed the use of several plants to treat inflammatory diseases in Algeria. Species use depends on the region surveyed. In fact, Mayouf *et al.*[29] showed that Salicaseae fimily is represented in the region of Tebessa (northeast of Algeria) by *Populus nigra* rather than *Salix alba*. According to Karbab *et al.*[28] this family is not represented in the mountainous region of Djebel Zdimm (north of Setif). This could be due to the absence of *Salix alba* in this region.

The content of polyphenols in *Salix alba* extracts as observed in the present study is higher than that of Piątczak *et al.*[35]. Further, Pop *et al.*[36] found that *Salix alba* extract was rich in eriodictiol, isorhamnetin

and Na-salicilate. The phenolic compounds found by Maistro *et al.*[37] were salicylic acid, salicin, salidroside, saligenin, tremulodin, salicoylsalicin, salicortin and tremulacin. Although other flavonoids were not identified in the current study. This may be due to the different method used for extraction and identification of compounds[38]. In addition, the difference between the previously reported results and the current findings may be due to the effect of the growing environment that would affect the chemical composition of the extracts[39].

Antioxidant compounds are generally in the phenolic form[40]. The methanol and aqueous extracts of *S. alba* barks showed high DPPH scavenger effect even at low concentrations. Polyphenols are abundant in our extracts. It is presumed that phenolic compounds can act in the same way as reductones by giving electrons and reacting with free radicals to convert them into more stable products and terminating the free radical chain reaction[41]. The aqueous extract of bark had a reducing power effect greater than the methanol extract. The SAQE has a significant reducing power; it probably has various mechanisms such as the prevention of the initiation of the chain, the decomposition of peroxides, the capacity of reduction and the radical scavenging. The power reduction test measures the ability of an antioxidant to donate electrons[42].

The reduction of hydroxyl radicals may be due to the presence of phenolic compounds capable of giving hydrogen in the extracts. Scavenging of hydroxyl radical is an important antioxidant activity because of the very high reactivity of OH radical, responsible for lipid peroxidation and considerable biological damage[43]. *Salix alba* bark aqueous removed the hydroxyl radicals and prevented their reaction better than standard ascorbic acid. The IC_{50} of the *S. alba* extracts was higher than that found by Gligoric *et al.*[44].

The results of anti-inflammatory activity clearly showed that *S. alba* extracts possessed significant antiphlogistic action against edema induced xylene. Our results are comparable to those found by Zhao *et al.*[45]. In Carrageenan-induced paw edema, which is a time-dependent biphasic inflammatory reaction, various inflammatory mediators participate in its development[46]. The first phase of inflammation (0-1 h) is attributed to the release of histamine, serotonin and bradykinin, and their action on vascular permeability[47]. The later phase 1-5 h) is due to the over production of prostaglandin and oxygen-derived free radicals in tissues[48]. In this study, extracts of *S. alba* barks exerted a significant inhibitory effect on the development of rat paw edema in the last phase i.e after 3 hours of carrageenan injection. Khayyal *et al.*[49] found that, the aqueous extract of *Salix alba* bark was at least as effective as acetyl salicylic acid (ASA) in reducing inflammatory exudates, inhibiting leukocytic infiltration, preventing the rise in cytokines and suppressing prostaglandins. They also found that this extract was more effective than ASA in suppressing leukotrienes.

Finally, extracts from *Salix alba* barks are important antioxidants and anti-inflammatory effects and are safe to use because they have no toxic effects on behaviour, corporal weight and organ relative weights of animals administered by relatively high doses of these extracts (more than 2 g/kg). This extract is therefore relatively non toxic or having low toxicity[20].

Conclusion

Both, the aqueous and methanol extracts of the white willow *Salix alba* barks have been shown to contain considerable quantity of polyphenols and flavonoids. The study of acute toxicity that these extracts have demonstrated their safety. Furthermore, these extracts exhibit high *in vitro* antioxidant potentials. *In vivo* experiments showed that *S. Alba* possesses an anti-inflammatory property even in xylene-induced ear edema or carrageenan-induced pow edema. These findings substantiate the traditional use of *Salix alba*.

Conflict of Interest

Authors declare no competing interests.

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