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Phenolic compounds, in vivo anti-inflammatory, analgesic and antipyretic activities of the aqueous extracts from fresh and dry aerial parts of *Brocchia cinerea* (Vis.)

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ABSTRACT

Aerial parts of *Brocchia cinerea* (Vis.) (Asteraceae family) are traditionally used for the treatment of pain, fever and inflammation. The present study aimed to investigate the phenolic profile, the acute oral toxicity, and anti-inflammatory, analgesic and antipyretic activities of the aqueous extracts from fresh (FBC) and dry (DBC) aerial parts of *Brocchia cinerea* (Vis.). Phenolic profile from FBC and DBC was characterized by HPLC-PDA/ESI-MS. The anti-inflammatory, analgesic and antipyretic of both FBC and DBC were evaluated by carrageenan induced paw edema, acetic acid induced writhing and brewer's yeast-induced pyrexia in Wistar rats, respectively. The results achieved showed that thirteen phenolic compounds were detected in the aqueous extracts obtained from the aerial parts of FBC and DBC, highlighting a quite different quantitative profile. The FBC and DBC administrated orally at a dose of 400 mg/kg significantly reduced edema, after 2 h of the injection of carrageenan ($p < 0.001$) with a percentage inhibition of 47.73% and 50.01% respectively. On the other hand the rats treated with DBC at the same dose significantly lowered the writhing induced by the injection of acetic acid (18.52 ± 0.38) with respect to the ones treated with FBC (20.47 ± 0.92). Moreover, a significant reduction in rectal temperature was observed in rats treated with FBC/DBC and at doses of 200 and 400 mg/kg. Furthermore, no acute toxicity symptoms were observed on oral administration of all doses of both FBC and DBC in Wistar rats. The results of the present study indicate that the aerial part of *Brocchia cinerea* extracts exhibit strongly anti-inflammatory, analgesic, and antipyretic properties and they can be potentially used in the treatment of inflammation, pain and fever.

1. Introduction

Since antiquity medicinal plants have been employed for their beneficial properties and their use continuously increases due to their attributed high safety profile, availability and low cost [1]. Medicinal plants still represent a large untapped source of compounds that might serve as lead for the development of novel drugs [2]. However, studies are needed to validate the therapeutic properties and the toxicological safety of plants, in order to ensure their safe use, as well as to provide necessary information for the development of new medicines [3,4]. Various plants exhibit various pharmacological properties, such as, antioxidant, antimicrobial, anti-inflammatory, antipyretic and analgesic

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activities. They are.

used in traditional medicine for treating various diseases (microbial infections, pain, pyrexia, inflammation, etc), which are related to the presence of various bioactive compounds, including polyphenols, terpenes, terpenoids, alkaloids, vitamins, proteins [5,6]. Moreover, inflammation is a physiological process, which protects organisms against microbial infections and acts as a defence mechanism against diseases. It is characterized by pain, heat, swelling, redness and disturbance of physiological functions. Pyrexia or fever is caused by tissue damage, inflammation, infection, graft rejection, malignant tumours and other diseases states. Pyrexia may produce large amounts of pro-inflammatory mediators (IL, TNF-α, interferon and cytokines), which enhance prostaglandin E2 (PGE2) that, in turn, activates the hypothalamus to raise body temperature [7]. Pain is physical discomfort caused by illness or injury [6]. Pain is processed by the central nervous system by specialized neurons called nociceptors, which send pain-related information to the brain in response to noxious stimuli [8]. Acute or severe pain can be induced by the synthesis of prostaglandins, serotonin, and cyclooxygenase COX-1 and COX-2 [9]. In addition, chronic inflammation can cause various diseases, including digestive disorders, neurodegenerative diseases, diabetes, cardiovascular diseases, cancer and autoimmune diseases; for this purpose, the therapeutic strategy to fight inflammation related these diseases, is needed. However, nonsteroidal anti-inflammatory drugs (NSAIDs) commonly used for targeting inflammation, demonstrate serious side effects on human health, such as gastric irritation, gastric ulcers, haemorrhage, liver and kidney toxicities [10]. Scientists have focused their efforts toward to identify effective and safe potent anti-inflammatory drugs from medicinal plants [11].

Brocchia cinerea (*B. cinerea*) commonly known as "*Cotula cinerea*" (Family Asteraceae) is widely distributed in the Sahara desert, especially in South-eastern Morocco and represents an important source of flavonoids, tannins, terpenoids, alkaloids, steroids and cardenolides. These secondary metabolites well-known for their biological properties, such as, antioxidant, antimicrobial, analgesic, and antipyretic [12–14]. *B. cinerea* is extensively used in traditional medicine for the treatment of many diseases e.g. indigestion, colic, rum, cold, digestive troubles, urinary and pulmonary infections, diarrhoea, migraines, coughing, broncho-pulmonary, rheumatoid arthritis, asthma, headache, and fever [15].

Few studies have been published on the in vitro analgesic and antipyretic activities of the alcoholic extracts obtained from *B. cinerea* [16, 17]. The present study aimed to characterize the phenolic compounds and to evaluate the in vivo anti-inflammatory, analgesic, and antipyretic activities of the aqueous extracts obtained from fresh and dry aerial parts of *B. cinerea*, collected in the South-eastern Morocco. To the best of our knowledge, the phenolic composition, and the in vivo anti-inflammatory, analgesic, and antipyretic activities of the aqueous extracts of *B. cinerea* are reported hereby for the first time.

2. Experimental section

2.1. Plant materials

The aerial parts of *B. cinerea* were collected in May 2020, from H'ssia, located in the Alnif area of South-eastern Morocco. The plant was botanically identified by Professors Mohamed Ibn Tattou and Hamid Khmmar at the Department of Botany at the Scientific Institute of Rabat (Morocco). Voucher specimen (RAB 110972) was deposited at the herbarium of the Department of Botany at the Scientific Institute of Rabat (Morocco). Afterward, harvested *B. cinerea* plant was divided into two parts. The first part was dried in the absence of light for 15 days at room temperature, whereas the second part was used immediately for the preparation of the aqueous extracts.

2.2. Sample preparation

A total of thirty-five g of either fresh or dry aerial parts of *B. cinerea* were extracted separately in a Soxhlet extractor with water using the method reported by Alara et al. [18]. A heating mantle was used to reflux the mixture for varied extraction time between 1 and 4 h. After the extraction time has been reached, the extract solution was allowed to cool at room temperature. Afterwards, each extract was filtered through a Whatman nylon filter (Merck Life Science, Merck KGaA, Darmstadt, Germany) and evaporated by a rotary evaporator. The obtained crude extracts were dried and stored at 4 °C until use. The procedure was carried out in darkness and repeated in triplicate. Recovery of phenolic compounds from *B. cinerea* was evaluated after addition of a known amount (50 ppm) of daphnetin and calculated as the mean of three replicates.

2.3. HPLC-PDA/ESI-MS analyses

HPLC analyses of the aqueous extracts from the aerial parts of fresh and dried *B. cinerea* were carried out using a Shimadzu HPLC system (Kyoto, Japan) equipped with a CBM-20A controller, two LC-20 AD dual-plunger parallel-flow pumps, a DGU-20A5R degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an SPD-M20A photo diode array detector, and an LCMS-2020 mass spectrometer, through an electrospray (ESI) source operated in negative ionization mode (Shimadzu, Kyoto, Japan).

Chromatographic conditions: Analyses were carried out on a Ascentis Express C18, 15 cm × 4.6 mm I.D. with particle size of 2.7 μm (Merck Life Science, Merck KGaA, Darmstadt, Germany). The injection volume was 5 μL, mobile phase consisted of water/formic acid (99.9:0.1, *v/v*) (solvent A) and ACN/formic acid (99.9:0.1, *v/v*) (solvent B), the gradient profile was as follows: 0 min, 0% B, 20 min, 20% B, 30 min, 35% B, 40 min, 80% B, 45 min, 100%B. The flow-rate was 1 mL/min and was split to 0.2 mL/min prior to ESI-MS detection.

PDA conditions. The wavelength range was 200–400 nm. Chromatogram was extracted at a wavelength of 330 nm. Time constant was 0.08 s with a sample frequency of 40 Hz.

MS conditions: mass spectral range 100–1200 *m/z*; event time: 0.5 s; nebulizing gas (N_2) flow: 3 L/min; interface temperature: 300 °C; Heat temperature block: 400 °C, DL (desolvation line) temperature: 250 °C; DL voltage 1 V; interface voltage 4.5 kV.

Quantitative determination was carried using calibration curves of the following standards, namely 4-*O*-Caffeoylquinic acid (y = 5593.9x-23310; R^2 =0.9985), Apigenin-6,8-C-diglucoside (y = 4915x+ 105.2; R^2 =0.9995), Luteolin-7-*O*-glucoside (y = 4130.5x+436.0; R^2 =0.9999) and Kaempferol-3-*O*-glucoside (y = 3481.0x+5372.4; R^2 =0.9991) (Merck Life Science, Merck KGaA, Darmstadt, Germany). Standard calibration curves were prepared in a concentration range 100–5000 mg/L with five different concentration levels. Each analysis was performed in triplicate.

2.4. Experimental animals

Wistar rats weighing 180–200 g of either sex were gathered from the animal house facility of the Faculty of Sciences (Moulay Ismail University), Meknes, Morocco. The animals were kept under standard environmental conditions and provided food and water. They were housed in clean cages at room temperature 26 ± 2 °C, and light/dark cycle (12 h/12 h). Except of the antipyretic activity and the acute toxicity assay, the animals were withdrawn from food 12 h before the start of the experience. The experimental protocol was carried out as per the rules and regulations of the Animal Ethics Committee of Moulay Ismail University, Morocco.

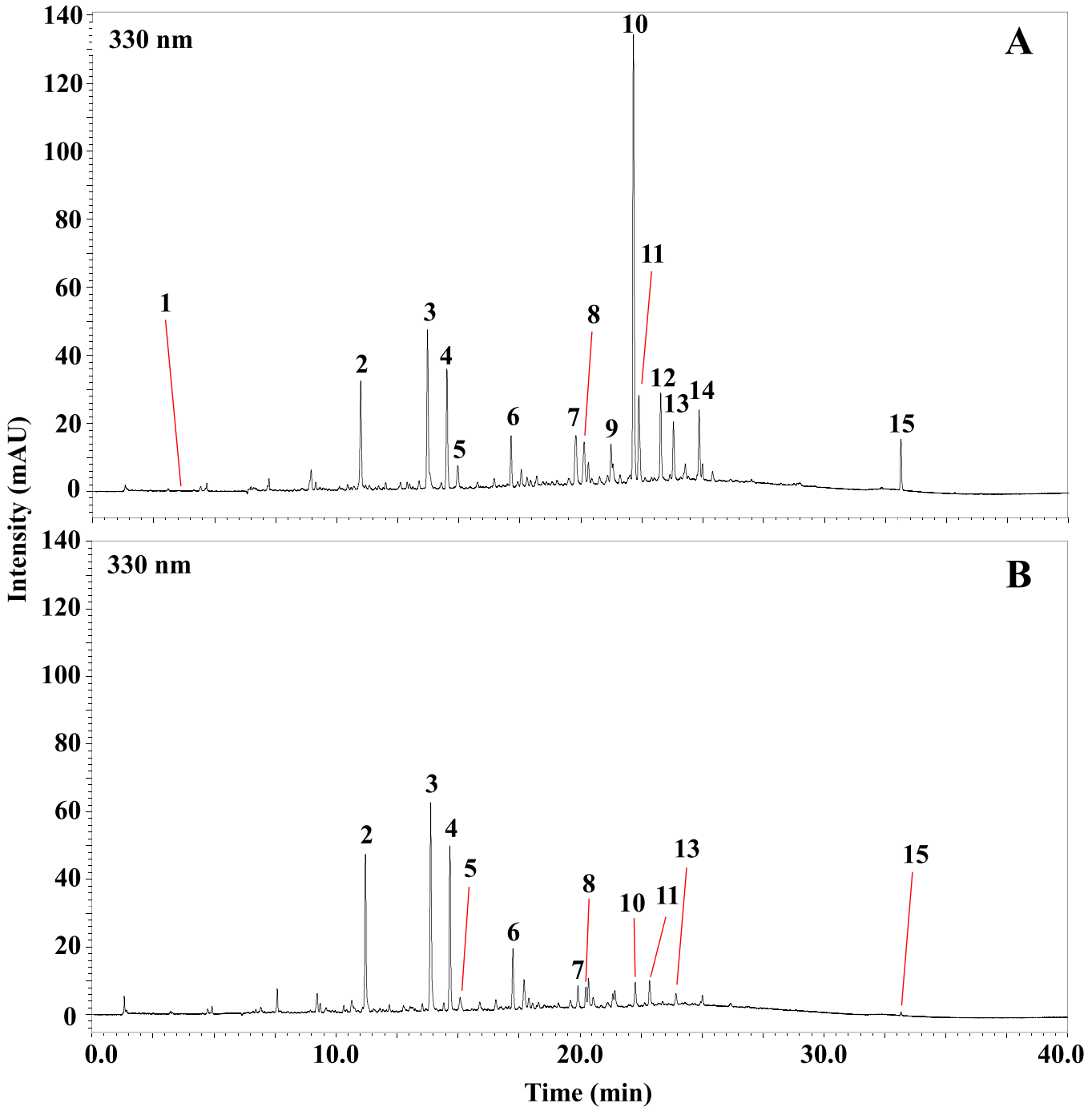

Fig. 1. Characterization of phenolic compounds (λ = 330 nm) in aqueous extract of both fresh (A) and dried (B) *B. cinerea* through HPLC-PDA/ESI-MS. For peak identification, see Table 1.

2.5. Acute toxicity

Wistar rats were subjected to evaluate the acute toxicity of the aqueous extracts of *B. cinerea* according to established guidelines [19]. The animals were divided into nine groups of five individuals and fasted for 12 h before the experience. Wistar rats were Orally administered with the aqueous extracts from both fresh and dry aerial parts (FBC and DBC) separately at different doses (200, 400, 600 and 800 mg/kg). Control animals received only distilled water and kept at the same environmental conditions. The animals were observed for any behavioural changes for 1 h intervals from 12 h and daily for 14 days.

2.6. Biological activity

2.6.1. Anti-inflammatory activity

The anti-inflammatory activity of the aqueous extracts of *B. cinerea* was assessed according to the method described by Winters et al. [20]. Wistar rats were randomly divided into six groups of five animals each. The first group of animals considered as a positive control was treated orally with indomethacin (10 mg/kg body weight). The second group considered as a negative control has received only distilled water. The other four groups have received orally, and separately DBC and FBC with doses that range from 200 and 400 mg/kg body weight. After 1 h, animals were injected with 0.1 mL of carrageenan (1%) into the plantar

Table 1
HPLC-PDA-ESI-MS phenolic fingerprint of both fresh and dried *B. cinerea* extracts. Column: Ascentis Express C18, 15 cm × 4.6 mm, 2.7 μm d.p. (ESI, negative ionization mode; in-source secondary fragment ions are reported). Results are expressed as mg/kg ± S.D. (n = 3).

N.	t_R (min)	UV_{max} (nm)	$[M-H]^-$	Tentative identification	*B. cinerea* fresh (mg/kg)	*B. cinerea* dried (mg/kg)	Ref.
1	3.59	283	191	Quinic acid	Nq	Nq	–
2	10.98	296_{sh}, 323	353, 189, 137	5-*O*-Caffeoylquinic acid	853.39 ± 14.36	1044.58 ± 25.48	–
3	13.72	296_{sh}, 324	353, 189, 137	3-*O*-Caffeoylquinic acid	1139.54 ± 31.27	1453.89 ± 37.87	–
4	14.52	296_{sh}, 324	353, 189, 137	4-*O*-Caffeoylquinic acid	859.89 ± 29.00	1060.90 ± 40.68	Std*
5	14.97	285	431, 378	Unknown	–	–	–
6	17.15	270_{sh}, 334	593, 269	Apigenin-6,8-C-diglucoside	404.35 ± 3.86	409.37 ± 5.99	[25; Std*]
7	19.80	343	609, 285	Luteolin-2"-*O*-hexosyl-6-C-hexoside	790.69 ± 26.79	262.36 ± 2.11	[26]
8	20.15	334	543	3,4-diferuloylquinic acid	Nq	Nq	–
9	21.24	344	579, 285	Luteolin-2"-*O*-pentosyl-6-C-hexoside	441.76 ± 12.35	Nd	[26]
10	22.16	265_{sh}, 347	447, 285	Luteolin-7-*O*-glucoside	4633.42 ± 20.22	271.91 ± 6.50	[27; Std*]
11	22.39	267_{sh}, 335	527, 285	Kaempferol-3-sulfate-7-*O*-hexoside	1157.86 ± 100.38	242.19 ± 5.11	[27]
12	23.28	296_{sh}, 324	515, 137	3,4-*O*-diCaffeoylquinic acid	761.93 ± 14.32	Nd	Std*
13	23.80	330	625	Unknown	–	–	–
14	24.86	296_{sh}, 324	515, 137	3,5-*O*-diCaffeoylquinic acid	621.95 ± 35.40	Nd	[26]
15	33.13	350	359	Trihydroxy-trimethoxy-flavone	447.70 ± 4.08	Nq	[27]

Values are expressed as the mean; S.D. (n = 3); Nd: not detected; Nq: not quantified; *Confirmed by co-standard injection.

surface of the left hind paw. The paw edema volumes were measured using a plethysmometer (Ugo Basile, Milan, Italy) before the injection of carrageenan. Subsequently, paw volumes were again measured at hourly intervals for five hours.

The % inhibition of anti-inflammatory activity was calculated from the following formula:

$$\%\ \text{inhibition of edema} = \frac{(Vt - V0)\ \text{control rats} - (Vt - V0)\ \text{treated rats}}{(Vt - V0)\ \text{control rats}} * 100$$

where V_0 and V_t are the paw volume of rats before and after carrageenan administration respectively.

2.6.2. Analgesic activity

The analgesic activity of FBC and DBC was evaluated using acetic acid induced writhing test following the method previously described by Koster [21]. Briefly, Wistar rats were randomly divided into six groups of five animals per group. The first group of animals was assigned as positive control, and orally treated one with standard drug aspirin, at the dose of 100 mg/kg body weight; the second group assigned as a negative control, and treated orally with distilled water, whereas the other groups have received orally doses that range between 200 and 400 mg/Kg of DBC and FBC. After one hour of treatment, the acetic acid (0.6%, 10 mL/kg, i.p.) was administered to each rat to induce abdominal contraction known as writhing [22]. After 5 min of injection, the number of abdominal contractions was counted over a period of 30 min. The percentage inhibitions of abdominal writhing were calculated according to the formula given below:

$$\%\ \text{inhibition} = (1\text{-}\frac{We}{Wc}) * 100$$

where W_e is the mean of contractions count in animals treated with DBC, FBC, or aspirin, and W_c is the mean of contractions count in the negative control animals.

2.6.3. Antipyretic activity

The antipyretic activity was assessed by using Brewer's-yeast-induced pyrexia model in rats [23]. Before inducing pyrexia, the initial rectal temperature was recorded using a digital thermometer. Pyrexia was induced by subcutaneously injecting a 15% aqueous solution of Brewer's yeast at 10 mL/kg in the back below the nape of the animal's neck. All groups were fasted overnight but allowed free access to water, and after 18 h, the rectal temperature of each rat was recorded. An induction of pyrexia was confirmed by the elevation of temperature more than 0.5 °C [24]. Wistar rats were randomly divided into six groups of five animals. The control group received oral distilled water. Paracetamol (100 mg/kg, orally) was used as a reference standard drug, while DBC and FBC extracts solutions were fed orally at 200 or 400 mg/kg. Rectal temperatures were recorded at 0, 1, 2, 3, and 4 h post treatment.

2.7. Statistical analysis

The results obtained were presented as the mean ± SEM (standard error of mean). Data were analysed using one-way analyses of variance (ANOVA) followed by Dunnet's multiple comparison post hoc test. GraphPad Prism Software 5.0 (GraphPad Software, La Jolla, CA, USA) was used and the significant differences were measured at *$p < 0.05$, * *$p < 0.01$, and * **$p < 0.001$ as compared to standards.

3. Results and discussion

3.1. Determination of phenolic compounds in the aqueous extracts of *B. cinerea* by HPLC-PDA/ESI-MS

The characterization of the phenolic compounds occurring in the aqueous extracts of fresh and dried *B. cinerea* aerial parts, at maximum conditions of Soxhlet extraction, were carried out by HPLC-PDA/ESI-MS. As shown in Fig. 1, a total of thirteen different phenolic compounds were detected in both extracts of *B. cinerea*. As reported in Table 1, from both fresh and dried *B. cinerea*, different classes of phenolic compounds were characterized, including flavonoids (apigenin-6,8-*C*-diglucoside, luteolin-2"-*O*-hexosyl-6-C-hexoside, luteolin-2"-*O*-pentosyl-6-*C*-hexoside, luteolin-7-*O*-glucoside, kaempferol-3-sulfate-7-*O*-hexoside and trihydroxy-trimethoxy-flavone) and phenolic acids (quinic acid, 3,4-diferuloylquinic acid, and caffeoylquinic acid derivatives). Three out of them, namely 4-*O*-Caffeoylquinic acid, apigenin-6,8-*C*-diglucoside and luteolin-7-*O*-glucoside were unequivocally identified by co-standard injection; the rest of the phenolic compounds was identified by combined information coming from UV–vis, MS and

Table 2
Anti-inflammatory effect of the fresh and dry aerial parts of *B. cinerea* extracts on carrageenan-induced paw edema in rats.

Treatments	Mean paw volume (mL)						% of inhibition 5 h
Control	0 h	1 h	2 h	3 h	4 h	5 h	
	0.48 ± 0.15	0.61 ± 0.71	0.69 ± 0.66	0.75 ± 0.46	0.81 ± 0.06	0.86 ± 0.21	-
Indomethacin (10 mg/kg)	0.47 ± 0.24	0.52 ± 0.09	0.56 ± 0.41***	0.60 ± 0.16**	0.63 ± 0.19**	0.65 ± 0.44***	55.26
FBC (200 mg/kg)	0.52 ± 0.96	0.59 ± 0.27	0.64 ± 0.59	0.68 ± 0.04**	0.73 ± 0.31***	0.76 ± 0.57**	36.84
FBC (400 mg/kg)	0.49 ± 0.52	0.56 ± 0.62	0.60 ± 0.39***	0.64 ± 0.12**	0.67 ± 0.27***	0.69 ± 040**	47.73
DBC (200 mg/kg)	0.50 ± 0.41	0.57 ± 0.48	0.62 ± 0.07	0.66 ± 0.08**	0.70 ± 0.35**	0.73 ± 0.17**	39.47
DBC (400 mg/kg)	0.47 ± 0.12	0.53 ± 0.55	0.57 ± 0.61***	0.61 ± 0.32**	0.64 ± 0.20***	0.66 ± 0.34***	50.01

Values are expressed as the mean ± SEM (n = 5),
FBC: Fresh aerial parts of B. cinerea; DBC: Dry aerial parts of B. cinerea.
** $p < 0.01$,
*** $p < 0.001$ are considered significant, compared to the control and indomethacin

literature data [25–27]. This is the first working reporting the phenolic composition of *B. cinerea* extracts. As internal standard, daphnetin, a phenolic compound not occurring in *B. cinerea* aerial parts, was employed. Considering its very similar chemical structure, it was assumed that similar recoveries could be attained for all phenolic compounds contained in the samples and a value as high as 85% demonstrated the exhaustiveness of the extraction method.

Regarding quantification, 3-*O*-Caffeoylquinic acid (1453.89 ± 37.87 mg/kg), 4-*O*-Caffeoylquinic acid (1060.90 ± 40.68 mg/kg), and 5-*O*-Caffeoylquinic acid (1044.58 ± 25.48 mg/kg) were the most abundant polyphenolic compound in dried *B. cinerea*, while luteolin-7-*O*-glucoside (4633.42 ± 20.22 mg/kg), kaempferol-3-sulfate-7-*O*-hexoside (1157.86 ± 100.38 mg/kg) and 3-*O*-Caffeoylquinic acid (1139.54 ± 31.27 mg/kg) were the most abundant in fresh *B. cinerea*. Indeed, the amount of apigenin-6,8-*C*-diglucoside detected in the fresh *B. cinerea* (404.35 ± 3.86 mg/kg) was quite similar to that of dried *B. cinerea* (409.37 ± 5.99 mg/kg). Notably, quinic acid and 3,4-diferuloylquinic acid were detected in traces in the both extracts of *B. cinerea*.

3.2. Acute toxicity

The obtained results revealed that both FBC and DBC at the dose levels (200, 400, 600 and 800 mg/kg) did not cause any acute oral toxicity effects and mortality in the Wistar rats. Markouk et al. reported that the ether, ethyl acetate, and n-butanol extracts of *B. cinerea* administrated orally at a dose up to 10 mL/kg to Wistar rats did not result any acute toxicity [14].

3.3. Anti-inflammatory activity

The results of this pharmacological test are summarized in Table 2. Notably, a significant reduction.

of edema was observed under both aqueous extracts from fresh (FBC) and dry (DBC) aerial parts of.

B. cinerea at a dose of 400 mg/kg, after 2 h of the injection of carrageenan ($p < 0.001$). These both FBC and DBC showed the percentage of inhibition of 47.73% and 50.01% at 5 h after the injection of carrageenan respectively, compared with the standard drug indomethacin (55.26%). Both FBC and DBC administrated orally at a dose of 200 mg/kg reduced edema at 3 h after carrageenan injection, with the percentage of inhibition of 36.84% and 39.47% respectively, at the 5 h after the injection of carrageenan.

Moreover, inflammatory reactions occur as a physiological barrier against infection and healing of damaged tissue by removing the stimuli. When the cell tissues are injured, the inflammatory mediators including prostaglandins, histamines, serotonin, cytokines, bradykinin, reactive oxygen species (ROS) and leukotrienes are produced by macrophages and neutrophils, leading to vasodilation and permeability of the capillaries and increased blood flux to the injured area [5,28]. Currently, non-steroidal anti-inflammatory drugs (NSAIDS) are the most widely used to manage fever, pain and inflammation. These drugs are often associated with side effects, including kidney disorders and gastrointestinal ulcers, due to the inhibition of the protein responsible for maintaining tissue integrity; cyclooxygenase 2 (COX2). For this reason, a growing interest of the use of bioactive compounds from herbal medicines as an alternative are recommended. In Africa continent, a study reported that more than 400 plant species belonging to 80 families have been explored to process in *vitro* anti-inflammatory activity [29]. Indeed, our study showed that the B. *cinerea* exhibit powerful anti-inflammatory activity, which may be due to the presence in its extracts of bioactive compounds including luteolin, apigenin, kaempferol and caffeoylquinic acid derivatives, which are known as a potent anti-inflammatory effect [30,31].

Table 3
Effects of dry and fresh aerial part of *B. cinerea* extracts on acetic acid induced writhing inhibition.

Treatment	No. of writhings	% Writhing inhibition
Control	38.57 ± 0.95	–
Aspirin (100 mg/kg)	14.53 ± 0.62***	62.33
FBC (200 mg/kg)	26.17 ± 0.85**	32.14
FBC (400 mg/kg)	20.47 ± 0.92**	45.51
DBC (200 mg/kg)	23.36 ± 0.49**	43.15
DBC (400 mg/kg)	18.52 ± 0.38***	50.71

Values are expressed as the mean ± SEM (n = 5),
** $p < 0.01$,
*** $p < 0.01$ as compared to control

3.4. Analgesic activity

The analgesic activity of both fresh and dry aerial parts of *B. cinerea* extracts was evaluated by using writhing test, and obtained results were compared with aspirin as the reference standard and presented in Table 3. Our findings showed that the administration of fresh and dry extracts of *B. cinerea* at the doses of 200 and 400 mg/Kg to the rats had significant effect in reducing the writhing induced by the injection of 0.6% acetic acid. The extract of DBC presents the higher writhing inhibition percentage with 43.15% and 50.71% at doses of 200 mg/kg and 400 mg/kg, respectively; while the use of FBC extract induces a writhing inhibition percentage of 32.14% and 45.51% after using the doses of 200 mg/kg and 400 mg/kg, respectively. In a study conducted previously with *B. cinerea* harvested from Zagora region (Morocco), the authors showed that ethyl ether, ethyl acetate, and *n*-butanol extracts induce a constriction inhibition percentage of 62.49%, 50%, and 40.21%, respectively [24]. Pain is usually induced by endogenous inflammatory mediators, which elevate the stimuli of peripheral nerve results in activation of nociceptors. The intraperitoneal injection of acetic acid induces inflammatory pain by impelling capillary permeability and releasing many substances that excite pain nerve endings such as, histamine, bradykinins, serotonin and prostaglandins (PGE2 and PG2α) from arachidonic acid through cyclooxygenase (COX)

Table 4
Antipyretic activity of the fresh and dry aerial part of *B. cinerea* extracts using Brewer's-yeast-induced pyrexia method.

Treatment	Rectal temperature before yeast injection (°C)	Rectal temperature at yeast injection and after treatment (°C)				
		0 h	1 h	2 h	3 h	4 h
Control (Distilled water)	37.11 ± 0.21	37.91 ± 0.14	38.08 ± 0.30	38.17 ± 0.25	38.19 ± 0.43	38.22 ± 0.20
Paracetamol (100 mg/kg)	36.93 ± 0.16	37.93 ± 0.09	37.77 ± 0.13	37.70** ± 0.44	36.66 ± 0.48***	36.62 ± 0.24***
FBC (200 mg/kg)	7.08 ± 0.20	38.05 ± 0.07	38.01 ± 0.71	37.92* ± 0.43	37.75 ± 0.21**	37.74 ± 0.25**
FBC(400 mg/kg)	37.15 ± 0.25	38.03 ± 0.26	37.92* ± 0.42	37.84** ± 0.13	37.72 ± 0.14**	37.60 ± 0.42*
DBC(200 mg/kg)	36.87 ± 0.13	37.95 ± 0.09	37.86** ± 0.01	37.81** ± 0.26	37.75 ± 0.25**	37.73 ± 0.26**
DBC(400 mg/kg)	37.20 ± 0.02	38.01 ± 0.12	37.82** ± 0.35	37.77 ± 0.33**	36.92 ± 0.38***	36.86 ± 0.41***

Values are expressed as the mean ± SEM, (n = 5),
* $p < 0.05$,
** $p < 0.01$, and
*** $p < 0.001$ are considered significant, compared to the control

enzymes [32]. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 (PGE2) receptor by picking up and transmitting the pain and injury messages through the nervous system to the brain and cause visceral writhing stimuli in rat. The inhibition of prostaglandin synthesis is remarkably efficient as an anti-nociceptive mechanism in visceral pain [33]. Aspirin used in this test as a positive control is a nonsteroidal anti-inflammatory drug (NSAIDs) that has analgesic, antipyretic, and anti-inflammatory effects by performing its action via inhibition of prostaglandin synthesis by inhibiting COX-1 and COX-2. Therefore, the results of this study suggest that analgesic activity of both fresh and dry aerial parts of *B. cinerea* may be linked to the alteration of the prostaglandin biosynthesis by inhibiting cyclooxygenase (COX).

3.5. Antipyretic activity

The antipyretic activity of fresh and dry aerial part extracts of *B. cinerea* was evaluated by using two different concentrations (200 and 400 mg/kg) and compared with that of the standard paracetamol (100 mg/kg), with the obtained results presented in Table 4. These results showed that the rectal temperature of the control group was highly raised and reached 38.22 ± 0.20 °C after 4 h of the yeast injection. However, the administration of FBC and DBC extracts had significant antipyretic activity after 4 h of extracts administration ($p < 0.05$). Fever decreased from 38.22 ± 0.20 °C in control group to 37.74 ± 0.25 °C and 37.60 ± 0.42 °C in groups treated with 200 and 400 mg/kg of FBC extract, respectively. The administration of DBC extract reduced fever from 38.22 ± 0.20 °C in control group to 37.73 ± 0.26 °C and 36.86 ± 0.41 °C in groups treated with 200 and 400 mg/kg respectively. Moreover, a previous study conducted on *B. cinerea*, harvested from the South of Morocco, showed that the ethyl ether and ethyl acetate extracts from *B. cinerea* reduced the fever by 89.43% and 90.12%, respectively, while the butanol extract was ineffective [17].

Fever leads to increased production of pro-inflammatory mediators such as cytokines (interleukin 1β, α, β) and tumor necrosis factor α (TNFα), which stimulate the synthesis of prostaglandin E2 (PGE2), triggering the hypothalamus to raise the body temperature [34]. The significant reduction rectal temperature in rats by using fresh and dry aerial parts of *B. cinerea* extracts may be due to blocking the cyclooxygenase activity and reducing the prostaglandin synthesis [35]. Moreover, the antipyretic effects of *B. cinerea* might be attributed to the presence of pharmacologically bioactive metabolites.

4. Conclusions

The obtained results have shown that both fresh and dry *B. cinerea* collected from Morocco (South-eastern Morocco) did not exhibit any signs of toxicity or mortality in Wistar rats. Interestingly, they displayed remarkable in vivo anti-inflammatory, analgesic and antipyretic effects. These effects might be related to the secondary metabolites of *B. cinerea*, including luteolin and apigenin derivatives, trihydroxy-trimethoxy-flavone, kaempferol and caffeoylquinic acid derivatives, by inhibiting the proinflammatory mediators and signalling pathways. However, supplemental studies are required to isolate by preparative LC selected fractions, or even individual bioactive compounds from the extracts, in order to correlate with the biological activities for the management of several pathologies related to inflammation, fever and pain.

CRediT authorship contribution statement

Nisrine Chlif: Conceptualization, Methodology, Investigation, Writing – original draft. **Aziz Bouymajane:** Supervision. **Yassine Oulad El Majdoub:** Investigation. **Mohammed Diouri:** Conceptualization, Supervision. **Fouzia Rhazi Filali:** Conceptualization, Supervision. **Amar Bentayeb:** Investigation. **Ammar B. Altemimi:** Writing – review & editing. **Luigi Mondello:** Supervision, Project administrator. **Francesco Cacciola:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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