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Research Article

IN VITRO ANTIOXIDANT ACTIVITY, *IN VIVO* ANTIPYRETIC AND ANTI-INFLAMMATORY ACTIVITIES OF *PARONYCHIA CAPITATA* L.

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ABSTRACT

Objective: The aim of this study is to evaluate the *in vitro* antioxidant activity of *Paronychia capitata* various extracts, acute toxicity, the *in vivo* antipyretic and anti-inflammatory activities of the n-butanol extract.

Methods: The antioxidant properties were tested using 2,2-diphenyl 1-picrylhydrazyl radical scavenging and β-carotene linoleic acid system, the total phenolic and flavonoid contents were determined. Furthermore, anti-inflammatory activity and antipyretic activity of the n-butanol extract were assessed on male Wistar rats at the dose levels 250, 500 mg/kg body weight, using the egg albumin-induced edema, and brewer's yeast-induced pyrexia method, respectively.

Results: The n-butanol extract of *P. capitata* had considerably the highest antiradical activity with inhibitory concentration 50 value of 8.667 µg/ml.

Conclusion: The experimental data demonstrated that n-butanol extract of *P. capitata* possess significantly remarkable anti-inflammatory and antipyretic activities compared to the standard drugs.

Keywords: Caryophyllaceae, Flavonoid, Inflammation, Fever, *Paronychia capitata*.

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INTRODUCTION

Paronychia is a genus that belongs to the subfamily of *Paronychioideae* [1]. It is represented by five species in the flora of Algeria [2]. *Paronychia capitata* L. known under the name of (Atai el Djebel). The infusion of the aerial parts of the plant has been used in Spanish folk medicine to purify blood, regulate the circulation and treat gout, as well as an agent for dermatitis and as an expectorant [3,4]. It is also used as cholagogue, dermatologic, anti-infective, lithotritic, diuretic, digestive and antihypertensive [5,6]. Although there are no reports of the medicinal uses of *P. capitata*, the aerial parts of *P. argentea* Lam. are used in the Algerian popular medicine for the treatment of renal diseases, diabetes, and as diuretic. This plant is also used to treat stomach ulcers, anorexia, bladder and prostate diseases, and heart pains [7,8]. According to literature survey, no data are available about the therapeutic applications or the biological activity of *P. capitata* in Algeria. Therefore, this study examined the total phenolic and flavonoid contents, the antioxidant activity (AA) and the anti-inflammatory and antipyretic activities of various extracts of *P. capitata* collected from the East of Algeria.

P. capitata Lam. was collected in May 2015, in Batna, East of Algeria. All parts of the plant previously dried and ground into thin powder was macerated twice in 70% ethanol for 48 hrs at room temperature. After filtration and evaporation of the solvent, the resulting aqueous phase undergoes a liquid-liquid extraction with petroleum ether then ethyl acetate, and final n-butanol. Evaporation dry organic solutions raised the following extracts: The petroleum *P. capitata* ether extract, ethyl acetate extract and the butanol extract. Aqueous extract was prepared by boiling the air-dried powdered plant in distilled water for 10 minutes and then cooled to room temperature overnight. The aqueous extract was filtered using Whatman filter paper. The filtrate was concentrated using rotary evaporator and lyophilized.

The total phenolic content of the plant extracts was determined according to the Folin-Ciocalteu method [9]. The results were calculated using the standard calibration curve of gallic acid (0-200 µg/ml) and expressed as micrograms of gallic acid equivalents per milligrams of dry extract (µg GAE/mg of extract). Colorimetric aluminum chloride method was used for flavonoid determination [10]. Total flavonoid contents were calculated from a calibration curve obtained by preparing quercetin solutions in methanol (0-40 µg/ml), and expressed in microgram of quercetin equivalents per milligrams of dry extract (µg QE/mg of extract).

The free radical scavenging activity of the extracts was measured *in vitro* by 2,2- diphenyl 1-picrylhydrazyl (DPPH) essay [11]. The AA of the extracts was expressed as inhibitory concentration (IC50) in (µg/ml) and quercetin was used as reference standard. To assess lipid peroxidation activity of the samples, β-carotene bleaching essay was carried out according to the method described by Kartal *et al.*[12] After the incubation period, the absorbance was measured at 490 nm at 0, 1, 2, 4, 6, 24, 26, 28, and 48 hrs. All determinations were performed in triplicate. The total AA was calculated.

Young male adult Wistar albino rats (150-200 g) were used in these experiments. They were housed in plastic cage at temperature of (25±2)°C with a 12 light/dark cycle, respectively, before and during the experiment, and had free access to drinking water. Animals were fed with a standard diet. The oral acute toxicity test was performed using ten healthy rats that were allocated in two groups. From 12 hrs before to 4 hrs after the oral administration, the animals were fasted (animals had water but not food) and weighed. The essay was followed as Organization for Economic Co-operation and Development Guideline 425 [13]. The control group received normal saline 10 ml/kg body weight by gavage, whereas the exposed groups (Groups 1) received 2000 mg/kg *n*-butanol extracts of *P. capitata.* Animals were observed during the first 30 minutes, 2, 4, and 6 hrs after treatment, and daily

thereafter for a total of 14 days in terms of weight loss, mortality, changes in behavior, skin, eyes, and fur.

The anti-inflammatory effect was assessed in acute inflammation method already described by Anosike *et al*. [14] Initially, normal paw thickness of each rat was noted. Inflammation was produced by injection of 0.1 ml of undiluted fresh egg albumin into the sub planter surface of the right hand paw of each rat 1 hr after oral administration of *P. capitata* butanol extract (PCBU 250, 500 mg/kg body weight), or diclofenac (Phamalliance, Ouled-Fayet, Algiers- Algeria) (DICLO; 30 mg/kg body weight). The control group was received 10 ml/kg of normal saline. The paw thickness was measured before injecting egg albumin and after 30, 60, 120, 180, 240, and 300 minutes using Vernier caliper (Shanghai Shenhan China).

Before the start of the antipyretic activity experiment, the animals were divided into four groups, each of five rats. The rectal temperature of each one was recorded using digital thermometer (VEDO Lente- Artsana, Italy) and then pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10 ml/kg) in normal saline [15]. All groups were fasted overnight, after 18 hrs rectal temperature of each rat was recorded. Group I received normal saline (10 ml/kg) as a negative control, Group II received paracetamol (Winthrop Pharma Saidal. Oued-Smar. Algeria) (PARA 150 mg/kg) as a standard drug [16] while the remaining Groups III and IV received 250, 500 mg/kg PCBU, respectively. After drug administration, the rectal temperature was then recorded over 5 hrs.

The data of the *in vivo* activities were analyzed using one-way analysis variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control. All values were expressed as mean±standard error of the mean (n=5). Values were considered statistically significant when p<0.01. The *in vitro* data were reported as mean ± standard deviation of three replicates (n=3). The data were analyzed using one-way ANOVA and Newman-Keuls *post-test*.

The amount of total phenolics varied in different extracts and ranged from 05.140 to 32.238 µg GAE/mg of dry extract. The results indicated that PCBU (32.238±1.404 µg GAE/mg) has the highest phenolic content, and the *P. capitata* ethyl acetate extract (05.140±0.132 µg GAE/mg) has the lowest phenolic contents. However, the highest flavonoid contents (6.077±0.443 µg QE/mg of dry extract) was found in *P. capitata* aqueous extract among the various extracts of *P. capitata* as shown in Table 1.

The extracts of *P. capitata* were screened for their possible AA by two complementary test systems; the DPPH free radical scavenging and the β-carotene/linoleic acid system. For the DPPH radical scavenging activity, all the sample extracts exhibited significant dose-dependent activity. Moreover, the results indicated in Table 1 show that the butanol fraction PCBU has the lowest IC50 (8.667±0.577 µg/ml) among extracts of *P. capitata* which is comparable to the one of standard quercetins. On the other hand, the comparable β-carotene bleaching rates of the positive control butylated hydroxytoluene (BHT) and *P. capitata* different extracts (Table 1). The butanol fraction PCBU shows the maximum inhibition (84.92%) which is close to the positive control BHT.

Acute oral toxicity; during the 14 days treatment, no significant difference (p>0.01) was recorded between the experimental and control group. The safety of the extract is evidenced by the high LD50

Table 1: Total phenolic and flavonoid contents, effects of *P. capitata* L. various extracts and positive control (quercetin, BHT) on the *in vitro* free radical (DPPH) scavenging and in β-carotene/linoleic acid system

EXTRACTS	TOTAL PHENOLIC[a]	TOTAL FLAVONOID[b]	IC50 (µg/ml)	ANTIOXIDANT ACTIVITY (%)
PCBU	32.238±1.404[a]	3.481±0.185[a]	08.667±0.577[a]	84.916±2.401[a]
PCAC	05.140±0.132[c]	2.648±0.754[a]	11.333±0.577[b]	74.082±1.470[b]
PCEP	29.062±0.884[c]	3.113±0.581[a]	24.046±1.000[c]	66.540±5.182[b]
PCAQ	10.199±2.24[b]	6.077±0.443[b]	16.000±0.000[d]	42.852±2.779[c]
QUERCETIN	/	/	1.149±0.0004	/
BHT	/	/	/	99.167±1.056

Mean±SD (n=3). [a]Expressed as microgram of gallic acid per milligram dry extract, [b]Expressed as microgram of quercetin per milligram dry extract. Means in the same column sharing different letters are significantly different P<0.05, BHT: Butylated hydroxytoluene, DPPH: 2,2-diphenyl 1-picrylhydrazyl, SD: Standard deviation, IC50: Inhibitory concentration, *P. capitata*: *Paronychia capitata*, PCEP: *P. capitata* ether extract, PCAC: *P. capitata* ethyl acetate extract, PCAQ: *P. capitata* aqueous extract

Table 2: Anti-inflammatory effect of diclofenac and the n-butanol extract of *P. capitata* against egg albumin-induced paw oedema in rats

PAW THICKNESS±SEM (mm)							
TREATMENT	**DOSE (mg/kg)**	**30 minutes**	**1 hr**	**2 hrs**	**3 hrs**	**4 hrs**	**5 hrs**
Control	10 ml/kg	9.340±0.145	7.678±0.134	6.534±0.151	6.196±0.109	4.936±0.158	4.070±0.077
DICLO	30	6.418±0.157***	5.340±0.121***	4.492±0.193***	3.402±0.097***	3.120±0.138***	2.710±0.187***
PCBU	500	7.314±0.185***	6.074±0.106***	4.714±0.237***	3.958±0.181***	2.996±0.214***	3.358±0.197**
PCBU	250	7.604±0.036***	6.198±0.066***	4.712±0.210***	3.904±0.095***	3.352±0.182***	3.366±0.228*

All values were expressed as mean±SEM (n=5). *Significantly different from the control at the corresponding time P<0.01, *P. capitata*: *Paronychia capitata*, SEM: Standard error of the mean, PCBU: *P. capitata* butanol extract, ** moderately significant compared with the control P<0.01, *** highly significant compared with the control P<0.001.

Table 3: Antipyretic activity of n-butanol extract of *P. capitata* and paracetamol on yeast induced pyrexia in rats

RECTAL TEMPERATURE (°C) BEFORE AND AFTER TREATMENT								
TREATMENT	**0 hr**	**18 hrs**	**18 hrs 30 minutes**	**19 hrs**	**20 hrs**	**21 hrs**	**22 hrs**	**23 hrs**
Control	36.70±0.341	38.10±0.161	38.10±0.161	38.10±0.161	38.00±0.162	38.00±0.171	38.00±0.171	37.90±0.172
Paracetamol	36.54±0.258	37.88±0.222	37.74±0.224 ns	37.50±0.164 ***	37.20±0.176***	36.92±0.196***	36.76±0.206***	36.60±0.230***
PCBU500 mg/kg	36.26±0.304	37.95±0.189	37.84±0.112 ns	37.60±0.148**	37.20±0.189***	37.00±0.216***	36.68±0.228***	36.44±0.290***
PCBU250 mg/kg	36.24±0.299	37.60±0.158	37.64±0.156 ns	37.44±0.160 ns	37.18±0.168*	37.02±0.180**	36.82±0.228**	36.70±0.225**

All values were expressed as mean±SEM (n=5). *Significantly different from the control at the corresponding time P<0.01, SEM: Standard error of the mean, *P. capitata*: *Paronychia capitata*, PCBU: *P. capitata* butanol extract, ** moderately significant compared with the control P<0.01, *** highly significant compared with the control P<0.001.

value of the butanol extract (>2000 mg/kg). The investigation of the anti-inflammatory activity of n-butanol extract of *P. capitata* L. was assessed using egg albumin induced paw edema method. The study showed that the extract had a significant effect on inflammation and reduced clearly the swelling. As shown in Table 2, the n-butanol fraction evoked a non-dose related anti-inflammatory activity and inhibited significantly the increase of paw edema from 30 minutes to 5 hrs. The maximum inhibitory effect (96.63%) was recorded at the dose 500 mg/kg of the n-butanol extract after 5 hrs. The effect of the *n*-butanol fraction on yeast-induced pyrexia is presented in Table 3. Treatment with *n*-butanol extract of *P. capitata* revealed a significant decrease ($p<0.01$) in rectal temperature between 2 and 5 hrs after administration, in a dose-dependent manner when compared to the control The antipyretic effect was observed from the first hour at the dose of 500 mg/kg and paracetamol (150 mg/kg), which was further maintained for 5 hrs. However, at the 250 mg/kg dose of *n*-butanol extract of *P. capitata* showed a moderate antipyretic activity after 2 hrs.

The results of this study proved the antioxidant anti-inflammatory and antipyretic activities of *P. capitata* extracts. Moreover, this study indicates that this plant may be useful in the protection against inflammatory diseases. However, phytochemical study is necessary to identify the active principles and exact mechanisms of action.

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