

ORIGINAL CONTRIBUTION

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Antioxidant property, anti-inflammatory and analgesic effects of aqueous extracts of two onion bulbs varieties (*Allium cepa* L.)

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Abstract

Background: oxidative stress and inflammation remain the main health problems often linked to degenerative diseases. The aim of this study was to evaluate antioxidant, analgesic and anti-inflammatory properties of onion growth in Cameroon.

Methods: Aqueous extracts were produced from two varieties of onion (Violet of galmi; Goudami) and were quantified for their polyphenols content. Concentrations of extracts (0.1, 0.5, 1, 1.5 mg/mL) were also prepared, their in vitro antioxidant properties evaluated by using standard methods. Anti-inflammatory effect of onion extracts was assessed by applying carrageenan sub plantar test to Wistar rats, but acetic acid test was used to study analgesic effect.

Results: The polyphenols content and antioxidant activities of onion extracts changed significantly ($p < 0.05$) with variety and increased ($p < 0.05$) with concentration. Total phenolic (range 58.03–67.43%), Total Reducing power (range 0.27–0.94%), DPPH radical scavenging (range 37.33–202.08%) were higher in Violet of galmi extracts, while Ferrous ion chelating power (range 23.15–97.42%) was higher in Goudami extract. The two varieties of onion extracts reduce ($p < 0.05$) rat paw edema in a dose dependent manner compared with negative control group. Violet of Galmi variety (1.5 mg/kg) reaches 100% of inhibition after 2 h whereas the Goudami variety (1.5 mg/kg) reaches 100% of inhibition after 3 h. In terms of analgesic activity, aqueous extract of the two varieties of onion exhibited analgesic effect characterized by reduction in writhes. Analgesic activity increased significantly ($p < 0.05$) with concentration of onion but Violet of galmi extracts has also shown more efficacy than Goudami extracts.

Conclusion: The present findings suggest that aqueous extract of violet of galmi and Goudami bulb onions have antioxidant activity and protective effects against inflammation and pain.

Keywords: Onion, Aqueous extract, Antioxidant, Analgesic, Anti-inflammatory

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Background

Onion (*Allium cepa* L.) is a biannual herbaceous plant largely produced for its bulb, the most consumed vegetable in the world [1]. It is generally used as a condiment to increase the flavor of foods, but can also be eaten raw. It is preferred to other condiments for cooking food like fish and meat [2]. In Cameroon, onion plays a central role in the daily food menu of people with an annual consumption of about 90,000 tons/year. As food ingredient, onion contribute not only to the food sensory properties, but also to the nutritional properties. In fact, the bulb is source minerals and vitamins such as Calcium, vitamin C, Potassium, etc. [3, 4]. Beyond their nutritional and seasoning contribution to food, onion is also growth for its medicinal properties. A local survey made prior to this study revealed that onion protects against caught, arthritis due to meat consumption, reduce pains and limit aging. Such observations suggest the potential role of onion against inflammation, and oxidation. From the above derived the central question of this study: what are the anti-inflammatory, analgesic and antioxidant properties of onion extracts?

Literature reported several studies on anti-inflammatory and antioxidant properties of onion extracts [5–8]. Many reported works were done on the white variety of onions. However, there are different varieties of onion depending on the climate such as in fairly arid, steppe, dry mountain slopes, rocky sites, cold and dry summer vegetation [9]. In Cameroon, there are two particular varieties based on the color of the flesh: white and violet. The violet locally called violet of galmi is the most used variety for heath problem. In addition, the extract is generally administered to population at varying doses (0.1–1.0 mg/kg body weight) depending on the traditional therapist. This suggest that the variety and doses are important variable that may affect the properties of the onion which need to be investigated. The objective of this study was to study the effect of variety and doses on the antioxidant, anti-inflammatory and analgesic properties of aqueous of onion bulb.

Materials and methods

Sample preparation and extraction procedure

The Goudami (white variety) and Violet of Galmi (violet variety) onions were collected from a research farm of the Agronomy and Development Research Institute (ADRI) in Meskine-Maroua, Cameroon. The samples were assigned a voucher specimen number PCG/H/2019/2020/004. Sample was freed of damaged onions and foreign matters and sliced using a stainless-steel knife. The slices were ground into paste in an electric grinder (Culatti, Polymix, France) and the paste mixed with water (1/10 g/mL) for 48 h extraction in a soxhlet apparatus. The extracts were filtered and concentrated to vacuum under reduced pressure in a rotary

evaporator followed by dehydration at 40 °C in an electric oven. The extract powders of both onion varieties (white and violet) were then used to prepare onion extract concentrations 0.1, 0.5, and 1.5 mg/mL by dissolving a mass of powder in water.

Determination of some bioactive compound content of onions extracts

The amount of total phenolic compound in onion extract was determined with Folin–Ciocalteu reagent, according to the method of Gao et al. [10] described by Nguimbou et al. [11]. Briefly, 20 µL of extract solution (10 mg/mL) was added to a mixture of 200 µL of Folin–Ciocalteu reagent and 1380 µL of distilled water followed by thorough mixing. After 3 min, 400 µL Na₂CO₃ (20%) was added. The mixture was allowed to stand for 20 min at 40 °C with intermittent shaking. The absorbance was measured at 760 nm using a spectrophotometer (Spectronic Genesys 2PC, USA). The total phenolic content was determined as milligrams of gallic acid equivalent per 100 g of dry matter, using an equation obtained from the standard gallic acid (0.2 g/L) calibration graph. Flavonoid content (equivalent of mg quercetin per 100 g of dry matter) was determined as described by Mimica-Dukic et al. [12] and Vitamin C level was evaluated using the method described by Evered [13].

Determination of antioxidant properties of onions extracts

The reducing power of onion extract was measured according to the method described by Duh et al. [14], and compared to 0.1, 0.5 and 1.5 mg/mL of ascorbic acid. DPPH Free radical scavenging was determined following Okada et al. [15] and results compared to Trolox solution (0.1, 0.5, 1.5 mg/mL). Ferrous ion chelating power was measured according to the method of Suter and Richter [16], EDTA (0.1, 0.5 and 1.5 mg/mL) was used for comparison. The standard solutions were formulated using methanol (95%).

Evaluation of in vivo analgesic and anti-inflammatory activities of onion extract

Experimental animals and treatments

The experimental procedures described below were approved by the institutional animal ethical committee of Higher Technical Teachers' Training College of Ebolowa, University of Yaounde I. Healthy male Wistar rats and albino mice were procured from the animal house of Faculty of Sciences, University of Yaounde I, Cameroon. Animals were subjected to a four-day acclimatization period during which the rats were fed standard diet (Cassava starch 60%, Sucrose 5%, Casein 10%, Tournesol oil 10%, salt mixture with starch 5%, Cellulose 5%, Vitamin mixture 4% and mineral mixture

1%). The animals had free access to water and diet, housed in individual cages located in an animal room set at $25 \pm 2^\circ\text{C}$ temperature, 55% relative humidity in a 12 h light/dark cycle.

Anti-inflammatory test

Anti-inflammatory property of onion extract was tested following the Carrageenan-induced paw edema in rats [17]. Wistar rats (weighing 135–150 g, 3 months' rats) ($n = 54$) were divided into nine groups with six rats in each (Table 1). Six groups of animals received different doses of aqueous extract of onion (0.1, 0.5 and 1.5 mg/kg) intraperitoneally (I.P) half an hour before carrageenan injection. Animals in the negative control group received normal saline, while animals in the positive control group were administered 1 mg/kg of Celecoxib, the standard anti-inflammatory drug and another group given 1 mg/kg of dexamethasone. Paw edema was induced by sub-plantar injection of 50 μl 1% (w/v) solution of sterile carrageenan in saline to the right hind paw [17]. Paw volume was measured plethysmographically according to Jung-Chun et al. [17]; before (0 h) and at 0.5, 1, 2, 3 and 4 h after induction of inflammation. The mean increase in paw volume was measured hourly and at every time point, the percentage inhibition of edema was calculated:

$$\% \text{Inhibition} = \frac{V_{\text{Test}} - V_{\text{Control}}}{V_{\text{control}}} \times 100$$

Where; V_{Test} = Mean increase in paw volume of test group and V_{control} = Mean increase in paw volume for the control group.

Analgesic property

The Acetic Acid-induced Writhing Test was used for the determination of analgesic properties of the extract according to Rokia et al. [18]. In the procedure, acetic acid solution (1% v/v) prepared in distilled water was

administered intraperitoneal to the experimental animals to create pain sensation. Albino mice (weighing 20 – 30 g) were randomly divided into height groups of 6 individuals each including 6 test samples, 1 positive and 1 negative control groups as describe above. The positive control was made of diclofenac sodium (10 mg/kg) administered 15 min prior to acetic acid injection. Thirty minutes after oral administration of extracts and control solutions, animals received intraperitoneal administration of 1% (v/v) acetic acid solution at the dose of 0.1 mL/10 g. Five minutes later, the animals were placed on an observation table, observed individually for counting the number of writhing made in 15 min. When writhing was not complete, it was considered half-writhing, two half-writhing being counted as one full writhing.

Statistical analysis

The data reported in the tables and figures were carried out in triplicate or more replicate determinations. All data were expressed as mean \pm standard deviation and were statistically analyzed using one-way analysis of variance (ANOVA). When statistical differences were found, the Duncan's Multiple Range test was applied in order to classify samples at the significant level of 5%. Stat graphics Program (Statically Graphics Educational, version 6.01992 Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis.

Results

Polyphenol content and antioxidant properties of onion extracts

Table 2 showed the total phenolic content of onion extracts ranging 58.03% (Goudami) to 67.43% (Violet of Galmi). Similarly, the flavonoid content was high in variety Violet of Galmi (38.12%). In contrary the tannins and Vitamin C contents were highly represented in Goudami variety (17.13% and 6.02% respectively). The antioxidant properties were equally significantly ($p < 0.05$) influenced by the variety of onion extracts (Tables 3, 4 and 5). Violet of galmi extracts had the strongest total

Table 1 Experimental designs for in-vivo animal models of inflammation

Groups	Experimental design (rat paw edema)	Experimental design (writhing test)
Group 1	Negative control	Negative control
Group 2	Celecoxib (1 mg/Kg)	Diclofenac (1 mg/Kg)
Group 3	Dexamethasone (1 mg/kg)	/
Group 4	Violet of galmi (0.1 mg/Kg)	Violet of galmi (0.1 mg/Kg)
Group 5	Violet of galmi (0.5 mg/Kg)	Violet of galmi (0.5 mg/Kg)
Group 6	Violet of galmi (1.5 mg/Kg)	Violet of galmi (1.5 mg/Kg)
Group 7	Goudami (0.1 mg/Kg)	Goudami (0.1 mg/Kg)
Group 8	Goudami (0.5 mg/Kg)	Goudami (0.5 mg/Kg)
Group 9	Goudami (1.5 mg/Kg)	Goudami (1.5 mg/Kg)

Table 2 Antioxidant content of aqueous extract of onion bulb

Parameters (%)	Onion variety	
	Violet de galmi	Goudami
Total phenolics	67.43 ± 1.64 ^b	58.03 ± 1.52 ^a
Flavonoids	38.12 ± 0.31 ^b	24.11 ± 1.12 ^a
Tannins	7.21 ± 0.15 ^a	17.13 ± 2.23 ^b
Vit C	4.13 ± 0.16 ^a	6.02 ± 0.15 ^b

Means ±SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

reducing power and radical scavenging activity compared to Goudami extracts which had a highest ferrous ion chelating power (Table 5). A dose-response relationship was also found in all antioxidant activities effect tests; thus, the effect increased ($p < 0.05$) as the concentration increased for each individual onion extracts.

Anti-inflammatory properties of onion extracts

Carrageenan-induced oedema is a biphasic response as illustrated on negative control curve of the Fig. 1. From the Figure, it appears that onion extracts significantly ($p < 0.05$) reduced rat paw oedema at 1st and 4th hour after administration, but the efficacy of these extracts increased with the dose. On the other hand, the variety of onions significantly influenced ($p < 0.05$) the oedema (Table 6). In this respect, Violet of galmi variety had the highest effect, irrespective of the dose of extracts. In this vein, we observed 30 min after carrageenan injection that all the extracts inhibited inflammation. The extract which reached 100% inhibition was Violet of Galmi variety dose 1.5 mg/kg after 2 h whereas the Goudami variety (1.5 mg/kg) reaches 100% of inhibition after 3 h.

Analgesic property of onion extract

In the acetic acid induced writhing models, Fig. 2 illustrated that both Goudami and Violet of galmi extracts were shown significant ($p < 0.05$) reduction in the number of writhes. The number of writhing was more in the Goudami extracts with various doses of 0.1, 0.5 and 1.5 mg/kg than Violet of galmi extracts. In

Table 3 Total Reducing Power of aqueous extracts of onions with standard Ascorbic Acid

Concentration (mg/mL)	Onion variety		Ascorbic acid
	Violet de galmi	Goudami	
0.1	0.37 ± 0.04 ^b	0.27 ± 0.03 ^a	0.48 ± 0.01 ^c
0.5	0.48 ± 0.06 ^b	0.35 ± 0.04 ^a	0.69 ± 0.02 ^c
1	0.87 ± 0.07 ^b	0.65 ± 0.15 ^a	0.98 ± 0.02 ^c
1.5	0.94 ± 0.11 ^b	0.71 ± 0.05 ^a	1.08 ± 0.02 ^c

Means ±SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

Table 4 DPPH radical scavenging of aqueous extracts of onions with standard Trolox

Concentration (mg/mL)	Onion variety		Trolox
	Violet de galmi	Goudami	
0.1	45.51 ± 0.11 ^b	37.33 ± 2.14 ^a	86.62 ± 1.42 ^c
0.5	124.67 ± 2.01 ^b	63.72 ± 1.11 ^{a2}	153.71 ± 2.04 ^c
1	188.12 ± 1.21 ^b	147.17 ± 3.82 ^a	289.24 ± 3.22 ^c
1.5	202.08 ± 1.01 ^b	165.11 ± 2.14 ^a	312.01 ± 2.13 ^c

Means ±SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

addition, the number of writhing was higher at lower doses, irrespective of the variety. It was observed that the extracts were not more potent than Diclofenac sodium.

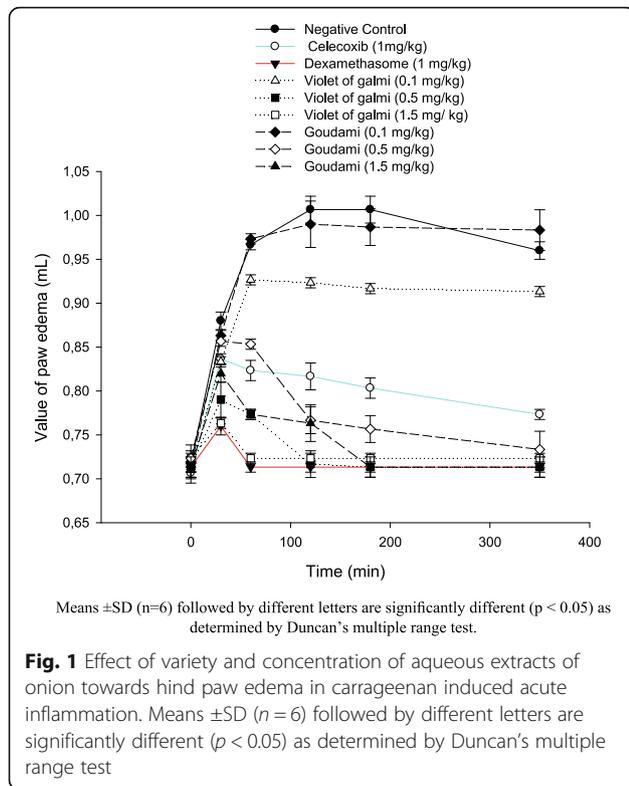
Principal Component Analysis (PCA) of the properties of onion extracts

PCA is based on the correlation between the properties of the extracts, from which virtual axes linearly correlated to existing variables are generated. Based on this, 2 principal components were revealed following the execution of PCA of the data obtained as indicated in Fig. 3. The correlations between variables were also generated (Table 7). As shown in Table 7, oedema inhibition was positively correlated to flavonoids ($r = 0.89$; $p < 0.05$), vitamin C ($r = 0.87$; $p < 0.05$) and total phenolic ($r = 0.92$; $p < 0.05$) contents; while number of writhes was negatively correlated to flavonoids ($r = -0.89$; $p < 0.05$) and total phenolic ($r = -0.88$; $p < 0.05$) contents. Figures 3 showed the location of the individual drugs or treatments on the F1 x F2 plan representing respectively 92.39% of the contributions to the axis. Based on the positions of the treatment, Goudami (0.1 mg/kg) and Violet of galmi (0.1 mg/kg) were characterized by high number of writhes and high paw edema volume; the Violet of galmi (1.5 mg/kg) and Goudami (1.5 mg/kg) which had high level of flavonoids, total phenolic compound, tannin and vitamin C were characterized by significant inhibition of oedema and writhing.

Table 5 Chelating Power of aqueous extracts of onions with standard EDTA

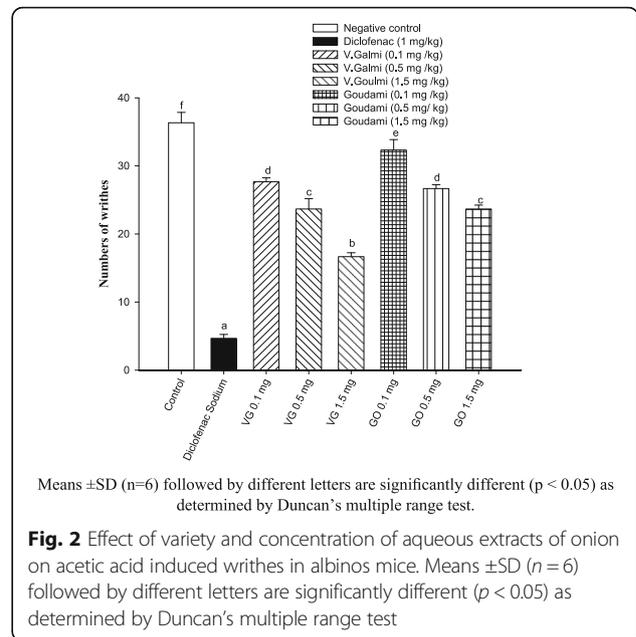
Concentration (mg/mL)	Onion variety		EDTA
	Violet de galmi	Goudami	
0.1	23.15 ± 1.31 ^a	47.16 ± 2.14 ^b	47.51 ± 1.12 ^c
0.5	47.12 ± 2.42 ^a	74.25 ± 1.32 ^b	84.76 ± 2.03 ^c
1	73.55 ± 1.23 ^a	96.51 ± 2.22 ^b	97.75 ± 1.12 ^b
1.5	74.58 ± 2.15 ^a	97.42 ± 1.67 ^b	99.08 ± 1.21 ^b

Means ±SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test



Discussion

Phenolic compounds are reported as potential antioxidant [7]. Onion was reported source of antioxidant including phenolic. The phenolic content in onion extracts were comparable to reported data. But the levels are lower compared to many plant extracts including *Lens cunalaris* (21.9 mg/100 g DM), *Phaseolus vulgaris* (18.8 mg/100 g DM), *Vigna radiate* (17 mg/100 g DM) [19]. Generally, the phenolic content of foods is known to have a positive effect on health, especially antioxidant and antimicrobial properties [14]. As other authors have observed [10, 11, 14], the significant correlations



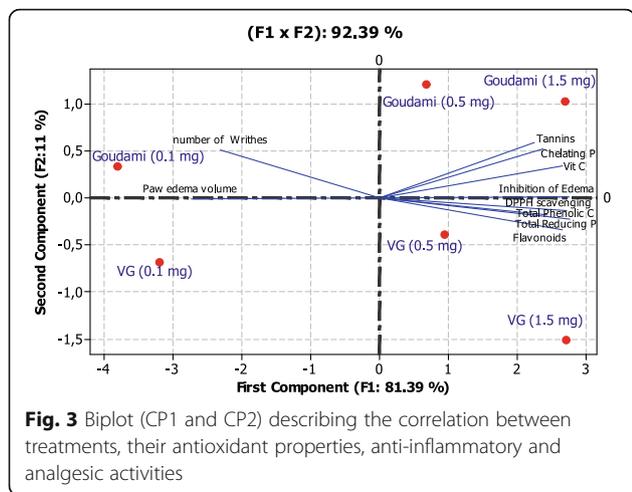
illustrated in this study between the different phenolic compounds and total reducing power, radical scavenging activity and ferrous ion chelating power confirm that they would be responsible for the antioxidant properties of onion extracts.

Phenolic compounds, are also known to exert preventive activity against infectious and degenerative diseases, inflammation and allergies via antioxidant, antimicrobial and proteins/enzymes neutralization/modulation mechanisms [20]. Carrageenan sub plantar test was used to evaluate anti-inflammatory property of aqueous onion extract. The first phase of Carrageenan-induced edema is mediated through the release of histamine, serotonin and kinins whereas, the second phase is related to the release of prostaglandin and slow reacting substances which peak at around 3 h [21]. The results reported here showed that onions bulb contain anti-inflammatory

Table 6 Effect of onion extract on inhibition percentage in albinos rat edema induced by carrageenan

Treatments	Inhibition percentage (%) at different time				
	30 min	60 min	120 min	180 min	350 min
Negative control	59.52 \pm 0.86 ^a	65.07 \pm 1.16 ^a	65.07 \pm 1.15 ^a	72.98 \pm 1.72 ^b	77.18 \pm 1.99 ^b
Celecoxib (1 mg/kg)	81.58 \pm 1.67 ^b	83.47 \pm 1.78 ^c	83.47 \pm 1.45 ^c	86.30 \pm 1.76 ^c	93.84 \pm 1.23 ^c
Dexamethasone (1 mg/kg)	93.44 \pm 1.18 ^e	100 \pm 0.15 ^e	100 \pm 0.86 ^e	100 \pm 0.11 ^e	100 \pm 0.57 ^e
V.Galmi (0.1 mg/kg)	82.60 \pm 1.39 ^b	69.47 \pm 1.19 ^b	69.47 \pm 1.99 ^b	70.87 \pm 1.01 ^b	71.32 \pm 1.39 ^a
V.Galmi (0.5 mg/kg)	89.20 \pm 1.48 ^d	91.56 \pm 1.58 ^d	91.56 \pm 0.80 ^d	100 \pm 0.25 ^e	100 \pm 0.51 ^e
V.Galmi (1.5 mg/kg)	94.46 \pm 1.41 ^e	100 \pm 0.52 ^e	100 \pm 0.41 ^e	100 \pm 0.22 ^e	100 \pm 0.57 ^e
Goudami (0.1 mg/kg)	78.96 \pm 1.54 ^b	63.54 \pm 0.29 ^a	63.54 \pm 1.22 ^a	61.66 \pm 1.77 ^a	71.94 \pm 1.91 ^a
Goudami (0.5 mg/kg)	81.52 \pm 1.24 ^b	81.99 \pm 1.72 ^c	81.99 \pm 1.16 ^c	95.37 \pm 1.14 ^d	98.15 \pm 0.80 ^d
Goudami (1.5 mg/kg)	85.03 \pm 0.95 ^c	91.57 \pm 1.52 ^d	91.57 \pm 1.31 ^d	100 \pm 0.42 ^e	100 \pm 0.11 ^e

Means \pm SD (n = 6) followed by different letters in the same column are significantly different ($p < 0.05$) as determined by Duncan's multiple range test



bioactive compounds which can be used in the treatment of acute inflammation. According to some authors [22, 23] the presence of triterpenic compound in some plants may explain their anti-inflammatory activity. The significant and positive correlation ($r = 0.89$; $p < 0.05$) observed between flavonoids content and edema inhibition percentage indicated that flavonoids would also be responsible for anti-inflammatory activity of these onions. In this vein, it was proven that flavonoids express anti-inflammatory properties by which they inhibit the proliferation and activity of lymphocytes [24].

Acetic acid induced writhing test is a model of visceral pain which is a very sensitive test for analgesic drugs [25]. It clearly indicated that aqueous extract of Violet of galmi exhibited more degree of anti-inflammatory

activity than Goudami extract. The dose dependent inhibition of acetic acid induced writhing by the extracts indicated a peripheral effect and it is suggestive of the dose dependent manner of medicinal plants extracts in the treatment of pain and inflammation [26, 27]. The efficacy of most herbal preparations is attributed to the presence of various bioactive compounds in combination, thus the significant and negative correlations ($r = -0.87$, $r = -0.89$; $p < 0.05$) observed respectively between total phenolic compound, flavonoids content and number of writhes indicated that phenolic compound like flavonoids would be responsible of writhing inhibition. The inhibition of writhing shows that the extracts may have central effects on the nervous system, since central nervous system depressants have been known to inhibit or reduce the number of writing in acetic acid pain models [28, 29].

Conclusion

Finally, it appears from this study that variety of onion had appreciable influence on antioxidant compounds of their aqueous extract. Carrageenan test and acetic acid test indicate a strong anti-inflammatory and analgesic effect of aqueous extract of onion. Analgesic and anti-inflammatory activities of onion extracts increased significantly with concentration but Violet of galmi extracts has shown more efficacy than Goudami extracts. The varieties of onion growth in Maroua, Cameroun are a good source of antioxidant compounds and can be used for medicinal application. However, the mechanism underlying the analgesic and anti-inflammatory effects of these onions is still needed to be determined.

Table 7 Correlation between antioxidant activities, phenolic compound, anti-inflammatory and analgesic activities

Variables	Total Reducing P.	DPPH scavenging	Chelating P.	Edema Inhibition	number Writhes	Paw edema volume	Flavonoids	Tannins	Vit C	Total Phenolic C.
Total Reducing P	1									
DPPH scavenging	0.74	1								
Chelating Power	0.54	0.77	1							
Edema Inhibition	0.75	0.97	0.82	1						
number Writhes	-0.81	-0.76	-0.41	-0.72	1					
Paw edema volume	-0.75	-0.97	-0.81	-0.99	0.71	1				
Flavonoids	0.81	0.96	0.65	0.89	-0.89	-0.88	1			
Tannins	0.68	0.61	0.89	0.72	-0.44	-0.72	0.52	1		
Vit C	0.80	0.81	0.92	0.87	-0.64	-0.86	0.75	0.96	1	
Total Phenolic C.	0.84	0.97	0.72	0.92	-0.88	-0.91	0.99	0.63	0.83	1

Abbreviations

DPPH: 2,2-Diphenyl – 1-Picrylhydrazyl; EDTA: Ethylene diamine tetraacétique; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; I.P: Intraperitoneally; V_{Test} : Mean increase in paw volume of test group; V_{control} : Mean increase in paw volume for the control group; ANOVA: Analysis of variance; PCA: The Principal Component Analysis

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Authors' contributions

MANG YANNICK DIMITRY conceived and designed the study, carried out the experiments, analyzed and discussed the data and wrote the manuscript. PANYO'O AKDOWA EMMANUEL and DJIOGUE MANEJO JOSIANE EDITH have been involved in revising this manuscript critically for important intellectual content. ABDOU BOUBA Armand, NJINTANG YANOU Nicolas and MBOFUNG Carl Moses were the major contributors in conceiving and designing the experiment. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The experimental procedures described below were approved by the animal ethical committee of the University of Yaounde I, Cameroon.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest related to the publication of this study.

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