

A close-up photograph of a person's open palm holding a large quantity of capsules. The capsules are primarily red and yellow, with some green and brown ones scattered among them. The background is a solid black color.

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Full Length Research Paper

Phytochemical and antimicrobial investigations of different fractions of the methanolic extract of *Corchorus capsularis* leaves

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The purpose of this study was to understand and investigate the phytochemical and antimicrobial effects of different fractions of the methanolic extract of *Corchorus capsularis* leaf. For the extraction of lipophilic compounds with the help of methanol solvent system, cold extraction process was used, where the grinded coarse powder of the leaf was soaked in methanol and kept for four days for proper extraction. After that, the extract was concentrated by using rotatory evaporator and dried in room temperature. Compounds in the extract were separated according to their polarity by using vacuum liquid chromatography (VLC) and solvents of different polarity N-hexane, benzene, diethyl ether, chloroform, dichloromethane. Later, thin layer chromatography (TLC), flavonoid assay, disk diffusion, inhibitory action in nutrient broth was done for different fractions of the methanolic extract. Diethyl ether, chloroform, dichloromethane fractions showed good anti-oxidant property but poor antibacterial activity against different types of microorganism. Chloroform fraction showed good inhibitory activity against *Aspergillus niger* (fungus) in nutrient broth.

Key words: *Corchorus capsularis*, vacuum liquid chromatography (VLC), thin layer chromatography (TLC), disc diffusion method, nutrient broth, Bangladesh.

INTRODUCTION

Bangladesh is known as a growing and populated country where the majority of the community lives below the poverty level, particularly the rural areas. The general

public in Bangladesh is habituated together with the local and indigenous foods. Vegetables are extremely popular in their day-to-day meals (Ullah et al., 2013). Drug

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development from purely natural origins demonstrated that natural products or natural product-derived medication made up of approximately 28% of all fresh chemical entities unveiled to the market. These are typically originated from terrestrial plant life, microorganisms, marine microorganisms, etc. However, till recently, an unimportant part of the plants has been clinically examined for their therapeutic attributes (Quader et al., 1987). Jute (*Corchorus capsularis* L.) is a yearly bast fiber plant. It can be located primarily within Southeast Parts of Asia Just after cotton. Plant seeds of *C. capsularis* L. comprised of corchorin, corchorotoxin helveticoside, cardiac glycosides, corchoroside A and B, olitoriside, erysimoside, strophanthidol glycosides, biosides, oligosaccharide and olitoriside; whilst leaves comprised of saponins, flavonoids, glucoside, capsularin steroids triterpenes and several various secondary metabolites. The pharmacological experiments unveiled the fact that the plant possessed anti-inflammatory, analgesic, antipyretic, cardiac, antioxidant, antimicrobial, insecticidal and several additional pharmacological properties (Al-Snafi, 2013). Jute (white jute, *C. capsularis* L.; nalta jute or tossa jute; Tiliaceae family), known as a fiber plant, is a time-honored medicinal vegetable in North Africa, the Middle and Near East and Southeast Asia (Furumoto et al., 2002). The leaves from *C. capsularis* have been reported to hold demulcent, laxative, appetizer, stimulant and stomachic and its infusion is customarily used to cure constipation, dysentery, fevers, liver problems as well as dyspepsia. Additionally, a decoction of the roots, as well as unripe fruits, has long been used to combat dysentery. The leaves of *C. capsularis* are actually consumed as vegetables in numerous area of the world such as Bangladesh, Africa, Middle East and Southeast Parts of Asia, which include Malaysia, for a long period (Zakaria et al., 2007). In the present study, phytochemical and antimicrobial investigations of different fractions of the methanolic extract of *C. capsularis* leaf were screened. Most jute plantations are in India and Pakistan. Other producing countries are China, Taiwan and Brazil. It contains 60 to 65% cellulose, an amount lower than flax, ramie and cotton. It's woody substance, lignin makes it less durable than other fibers. About 75% of the world's jute is used for coarse woven fabrics, sacking or burlap. It is also used in twines and carpet yarns. White jute is more commonly grown for fiber than the closely related, tussa jute (*Corchorus olitorius*) (Roedlein, 1958). The *C. capsularis* is reported to have cardiogenic, carminative, diuretic, antidiarrhetic, purgative, etc. (Chopra et al., 1958; Satyavathi, 1971; Rao, 1972). Natural products, particularly the ones made from higher plant life, have fascinated researchers from ancient time for their likely beneficial values. Bangladesh is actually an affluent repository of medical plants, a lot of which can be widely used in the Unani, Ayurvedic, herbal and various

traditional techniques of treatments (Ramadevi, 2013; Kaisar et al., 2011). This study reported the phytochemical and antimicrobial investigations of different fractions of the methanolic extract of *Corchorus capsularis* leaves.

MATERIALS AND METHODS

The study of taxon of *C. capsularis*

Jute thrives nicely where the annual rainfall is 1500 mm, with a minimum of 250 mm in each of the months of March, April and May. Optimum necessary temperature is 18-33°C. Jute (*C. capsularis*) is harvested during the wet season. Farming primarily depends on pre-monsoon showers as well as moisture conditions. *C. capsularis* is much more water tolerant and for that reason, it can be typically grown in minimal lands, and in many cases under water logging circumstances (Islam et al., 2013).

Collection of plant and identification

C. capsularis L. was collected from Lalmohan (Ward No. 3) is an Upazila of Bhola District in the Division of Barisal, Bangladesh in the month of January, 2014 and was identified by the taxonomist of Bangladesh National Herbarium, Dhaka, Bangladesh.

Methanolic extraction process

Fresh leaves were collected from the plant and dried under the sun light. Dry leaves were weighed again in electronic balance (SHIMADZU ATX2204) to measure the total weight of the dry leaves. Then, the dry leaves were converted to fine powder by using a blender (Miyako, Japan) then weight of the blended leaves measured and preserved in an air tight container. Methanol (Merck, India) was selected as the solvent for extraction from leaves of *C. capsularis* L. The powdered leaf (325 g) of *C. capsularis* L. was soaked in methanol (~ 970 ml) for 4 days and filtered by using of filter paper Filter paper (Double Rings 100 – 11 cm, HANGZHOU XINHUA PAPER Industry Co. Ltd., China). It was stirred with a clean glass rod to ensure the maximum amounts of constituents present in the blended leaves become soluble into methanol (Merck, Germany). To prevent the evaporation of the solvent and volatile compounds from the medium, the beaker was covered by aluminium foil. The extract was then concentrated with a Buchii rotary evaporator (IKA RV-10, Biometra). An aliquot of the crude methanolic extract (32.5 g) was fractionated by vacuum liquid chromatographic (VLC) technique using silica gel 10 mesh (Merck, Germany) N-hexane (Merck, Germany), benzene (Merck, Germany), diethyl ether (Merck, Germany), chloroform (Merck, Germany), dichloromethane (Merck, Germany) in increasing order of polarity. Two parts were obtained after filtration: residue and filtered part, filtered part was poured into 1000 ml round bottomed flask (Borosil, Japan) and was concentrated with the help of rotary evaporator at low temperature of 45°C and RPM of 120. When evaporation was completed, di-ethyl-ether (Merck, Germany) was used for collecting the extract (Faisal et al., 2016).

Thin layer chromatography (TLC)

Different solvent fractions were analyzed by performing thin layer chromatography (TLC) to determine the composition of each

extract. TLC was done under two solvent systems. Successively, the polarity of the solvent systems was increased to get a clear graph of all the possible compounds present in the extract. The compositions of various solvent systems used for TLC: Intermediate polar solvent: Chloroform 5 ml, ethyl acetate 4 ml, formic acid 1 ml; Polar solvent: Chloroform 6.3 ml, methanol 3.5 ml, distilled water 1 ml; Spotting: It was done with capillary tube in the following sequence. Same solvent was used to prepare samples solutions for same fraction. It was then put into the TLC with the solvent. After the solvent reached the upper limit, it was seen under UV light and was exposed to 10% sulphuric acid solution, dried and then heated to 80-90°C for charring purpose. This helped in the fixation of spot and allowed it to be prominently visible. A second batch was dipped into 0.4% DPPH solution and dried while keeping in a dark place (Saha et al., 2016a).

Anti-oxidant test

Total flavonoid assay

Initially, 1.5 ml methanolic leaf solution (1 mg/ml) of three fractions (dichloromethane, chloroform and diethyl ether) was taken in test tubes and different dilution of standard solution of quercetin (2.5, 5.0, 10, 20, 30 and 40 µg/ml) were added to 10 ml volumetric flask containing 6 ml of deionized water. To the above mixture, 0.45 ml of 5% NaNO₂ was added. After 6 min, 0.45 ml of 10% AlCl₃ was added into the mixture. After 6 min, 6 ml 4% (w/v) NaOH was added and the total volume was made up to 10 ml with distilled water. Finally, 0.6 ml deionized water was added to the sample for 15 min incubation. Then, the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent per g dry weight of sample (Sanadhya et al., 2013).

Anti-microbial assay

The antibacterial activity was carried out by the disc diffusion method using. Three different bacterial strains of Gram positive, different strains of Gram negative bacteria and fungus were used to carry out this assay. In this study, fractions obtained after VLC were tested for antimicrobial activity by disc diffusion method. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium (TECHNO PHARMCHEM, India) uniformly seeded with the test microorganisms. The antibacterial activity was carried out by using 50 µL of suspension containing ~10³ CFU/mL of microorganism spread on nutrient agar medium. A disc was soaked with solutions of 50 µl of test samples and dried placed. Standard antibiotic discs (erythromycin 15 µg/disc) together with blank disks were used as a positive and negative control. Those plates are retained at the lower temperature (4°C) for 24 h to allow for highest diffusion of the test components to the neighboring media. These plates are next inverted as well as incubated at 37°C for 24 h to get the best possible growth of the microorganisms. The test components possessing antimicrobial property restrict microbial growth within the media neighboring the discs and in so doing provide a transparent, distinct area understood to be the zone of inhibition. This antimicrobial actions belonging to the test agent is then simply determined by calculating the dimension of zone of inhibition depicted in millimeter (mm). Petridishes and other glassware were sterilized by autoclaving (Hirayama, Japan) at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 min. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized. After

incubation at 37°C for 24 h, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Saha RK et al., 2016b).

RESULTS AND DISCUSSION

Thin layer chromatography (TLC)

The results obtained after TLC of the fractions obtained from methanolic extract of the *C. capsularis* leaf is shown in Figure 1 and Table 1.

Anti-oxidant tests (total flavonoid assay)

Determination of total flavonoids content of the fractions of methanolic extract of *C. capsularis* (leaves) requires a standard curve which was obtained from a series of different quercetin concentrations.

Anti-microbial assay

Disc diffusion method

After incubation of 24 h at 37°C, Petri-dishes were observed for zone of inhibition for different fractions of methanolic extract of *C. capsularis* leaf. The results are shown in Table 3.

Inhibitory effect in nutrient broth

Absorbance's of the samples, positive control and blank before and after incubation are shown in Tables 4 and 5.

DISCUSSION

TLC plates were seen under UV light and different compound were found to be separated in the plates. Numbers 4 and 5 show some spots in dichloromethane, chloroform and diethyl ether. So, compounds are present in this fraction. Number 3 show spots in N-hexane and benzene after charring done in 10% sulphuric acid solution. Charring with H₂SO₄ in high temperature separated compound transformed into black color. Finally, numbers 1 and 2 show pale yellow color in dichloromethane, chloroform & diethyl ether fractions, which indicate flavonoids present in the fractions. To evaluate the antioxidant activities of VLC fraction of *C. capsularis* (leaves) total flavonoid assay was used. Total flavonoid content of different fractions of *C. capsularis* is

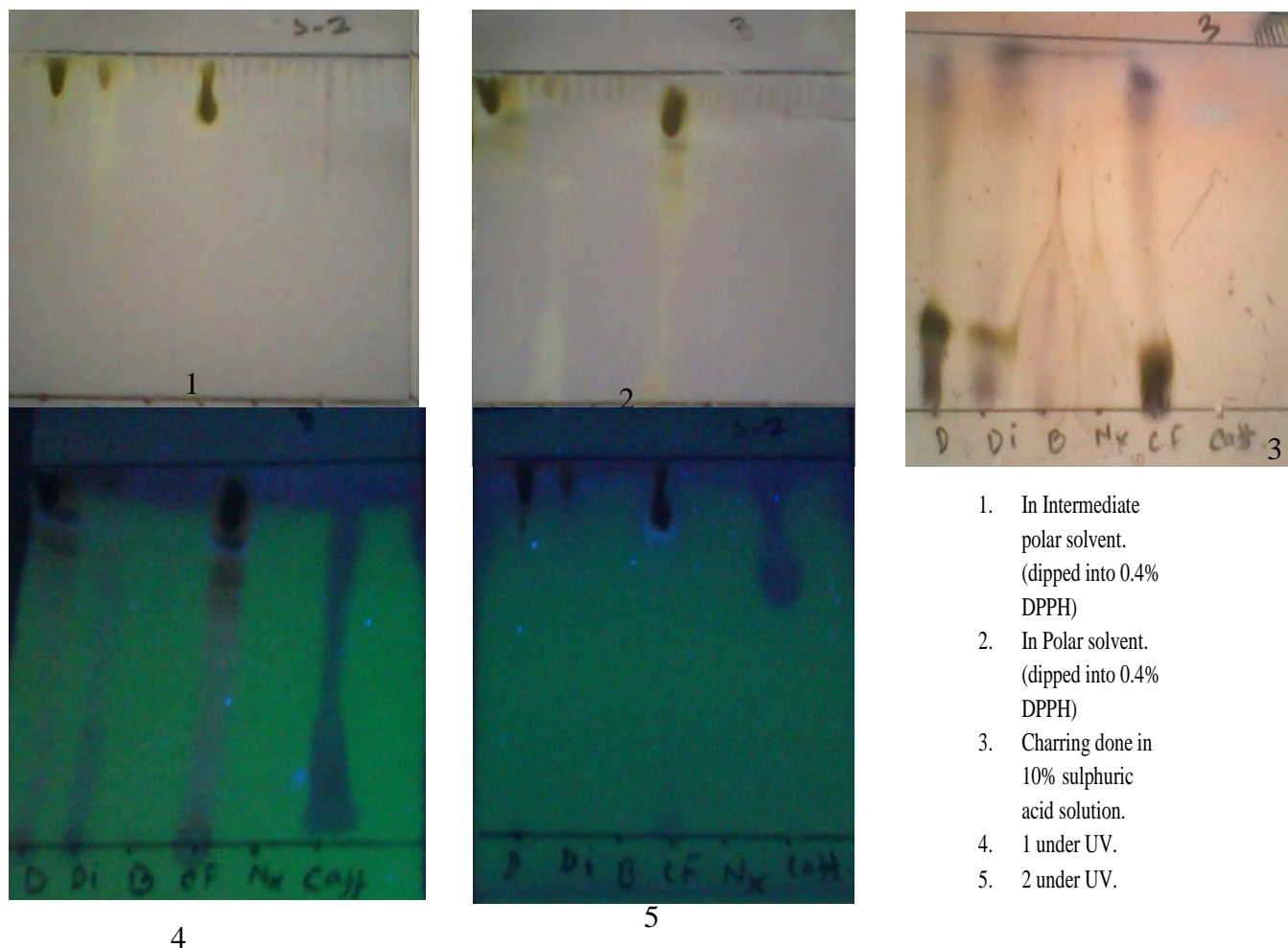


Figure 1. TLC results of the fractions. Numbers 1 and 2: Showing pale yellow color in dichloromethane, chloroform and diethyl ether fractions, which indicate flavonoids are present in the fractions. Number 3– Showing spots in N-hexane and benzene after charring done in 10% sulphuric acid solution. Numbers 4 and 5- Showing spots in dichloromethane, chloroform and diethyl ether. So, compounds are present in this fraction.

Table 1. Absorbance of different concentrations of quercetin.

Concentration of quercetin (µg/ml)	Absorbance (415 nm)
2.5	0.0365
5	0.0417
10	0.0521
20	0.0735
30	0.0906
40	0.1127

shown in Table 2. Antimicrobial screening leaves of *C. capsularis* showed activity against Gram positive bacteria (*Staphylococcus aureus*) and fungi (*A. niger*) as shown in Table 3 and Figures 2 to 5. The positive control used in

this study was erythromycin. The results showed possible benefits of VLC fraction of *C. capsularis* (leaves) as an antiviral therapeutics. Total flavonoid assay, disk diffusion and inhibitory effect in nutrient broth were done for

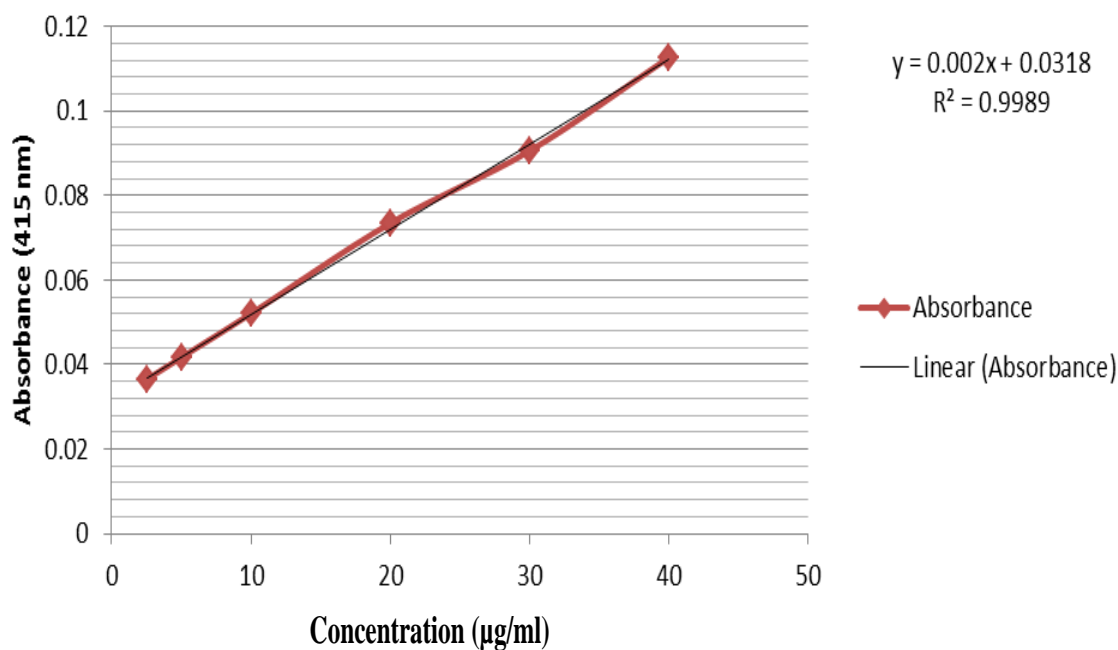


Figure 2. Concentration versus absorbance graph of quercetin.

Table 2. Total flavonoid content of different fractions of *C. capsularis* leaf.

Fractions	Amount (ml)	Absorbance (415 nm)	Conc.Form Std. Curve	Flavonoid Content (mcg Quercetin Equivalents/g of sample)
Dichloromethane (1 mg/ml)	1.5	0.037	2.6	3900
Chloroform (1 mg/ml)	1.5	0.038	3.1	4650
Diethyl ether (1 mg/ml)	1.5	0.045	6.6	9900

Table 3. Results of disc diffusion method.

Microorganisms		Sample names with zone of inhibition (mm)				Positive Control (Erythromycin 15 µg disc)
Positive strain	Negative strain	DCM	CF	DIE	NHX	
<i>Staphylococcus aureus</i> (+)		8	-	-	NT	9
<i>Shigella boydii</i> (-)		7	-	-	NT	20
<i>Saccharomyces cerevisiae</i> (yeast)		7	-	-	NT	22
<i>Aspergillus niger</i> (Fungus)		-	8	-	NT	24
<i>Salmonella typhi</i> (-)		7	-	7	-	18
<i>Vibrio mimicus</i> (-)		7	-	-	-	20
<i>Candida albicans</i> (yeast)		7	7	-	-	26
<i>Staphylococcus saprophyticus</i> (+)		-	7	-	NT	NT
<i>Beta-hemolytic streptococci</i> (+)		-	7	-	NT	NT

DCM- Dichloromethane, CF- Chloroform, DIE - Diethyl ether, NHX- N-hexane, NT – Not tested. Sample disc size = 6 mm. The diameters of zones of inhibition (mm) are expressed as mean ± SD (n=3); a diameter less than 7 mm was considered inactive.

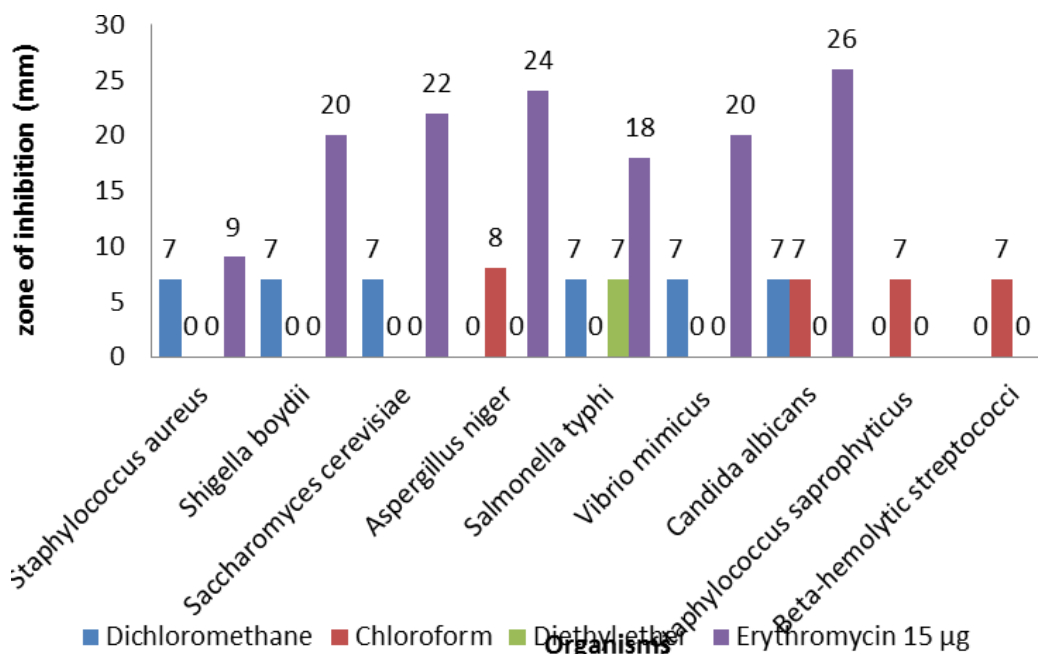


Figure 3. Zones of inhibition of different fractions of methanolic extract of *C. capsularis* (leaves) against various microorganisms.

Table 4. Absorbance of the samples, positive control and black before incubation.

Microplate well series	Positive control	Negative control	20 µl	10 µl	5 µl	2.5µl
Series-1	0.352	0.19	0.137	0.137	0.132	0.141
Series-2	0.333	0.244	0.13	0.14	0.135	0.131
Series-3	0.285	0.178	0.13	0.133	0.12114	0.125
Average	0.323333	0.204	0.132333	0.136667	0.12938	0.132333

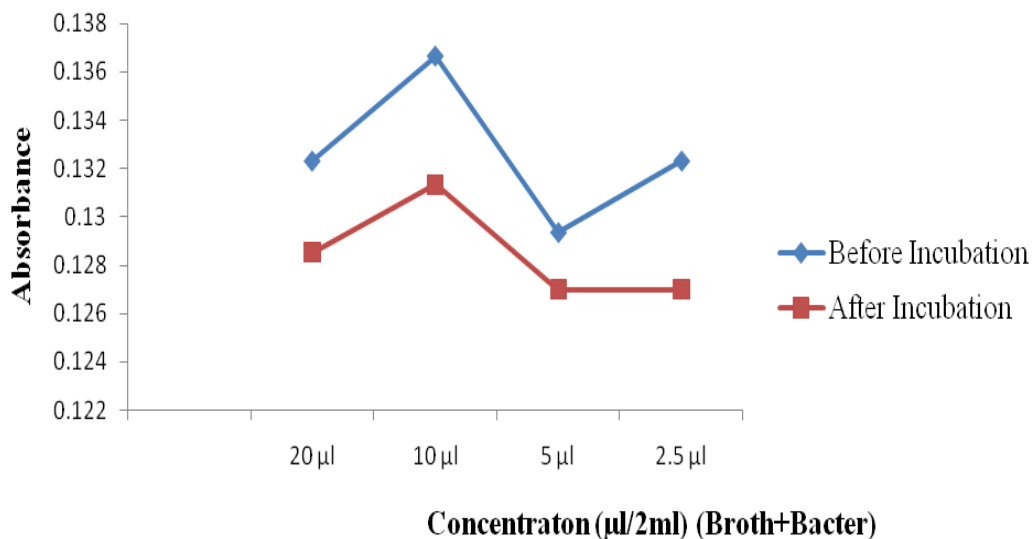


Figure 4. Concentration vs. absorbance of the samples before and after incubation.

Table 5. Absorbance of the samples, positive control and black after 24 h incubation.

Microplate well series	Positive control	Negative control	20 μ l	10 μ l	5 μ l	2.5 μ l
Series-1	0.682	0.105	0.136	0.133	0.130	0.134
Series-2	0.617	0.113	0.123	0.133	0.131	0.128
Series-3	0.676	0.103	0.126	0.128	0.120	0.119
Average	0.658333	0.107	0.128533	0.131333	0.127	0.127

**Figure 5.** Comparison between positive and negative control before and after incubation.

different fractions of the methanolic extract of the leaf. It is shown by this research that there is not much to do in the antimicrobial section of different fractions of methanolic extract of the *C. capsularis* leaf.

Conclusion

The results of the preliminary studies indicated phytochemical and antimicrobial activities of *C. capsularis* leaves. Thus, the plant warrants further investigation to isolate the active constituents responsible for these activities.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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Full Length Research Paper

Anti-inflammatory and analgesic effects of leaves of *Chromolaena odorata* L. (King and Robinson)

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This study was to evaluate anti-inflammatory and analgesic effects of the aqueous extract of leaves of *Chromolaena odorata* (Asteraceae) collected in Brazzaville-Congo. Acute inflammation was induced by using the carrageenan and formaldehyde models, and chronic inflammation by the cotton pellet induced granuloma model. Analgesic effect was evaluated by using the acetic acid-induced writhing, the pressure induced by the analgesimeter as well as the pain induced by formaldehyde. The results obtained show that aqueous extract (400 and 800 mg/kg) inhibits the edema induced by the carrageenan and formaldehyde. Moreover, this extract at the doses used (400 and 800 mg/kg) significantly inhibits granuloma fabric induced by cotton pellet. In addition, aqueous extract (400 and 800 mg/kg) inhibits significantly the pain induced by the three methods used. In conclusion, aqueous extract of *C. odorata* has anti-inflammatory and analgesic effects. These observations justify the traditional use of this plant in the treatment of inflammatory pathologies and the pain.

Key words: *Chromolaena odorata*, anti-inflammatory, analgesic.

INTRODUCTION

In Africa, therapeutic properties of the medicinal plants had been known for several years. However, few medicinal plants were studied scientifically at the

moment. In Africa, about 80% of the populations use them in the treatment of several pathologies (Etchike et al., 2011; Epa et al., 2015). In spite of the development

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of synthetic drugs, the vegetable drugs in his various forms occupy a special place. Moreover, WHO recognize the traditional medicine like one of the essential elements of primary care of health: «*save the plants which save the life*» such is a WHO slogan which summarizes his strategy in this domain (WHO, 2014). So, to find a simple methodology of evaluation of effectiveness and security of medicinal plants is significant. Vegetable plant in these different origins contributes to the discovery of new drug used in the treatment of several inflammatory and analgesic pathologies (Abena et al., 1996). Inflammation is one reactional phenomenon operated by the body every time the integrity of its morphological and biological constants is threatened. Inflammation is not synonymic of an infection, but the infection can be the cause of an inflammation (Bokia, 2016). It can present diverse symptoms such as the edema, the pain and the heat or fever. Inflammation is always accompanied by pain, so the evaluation of the pain treatment constitutes a real major stake in public health. So, searching new anti-inflammatory and analgesic drugs is necessary. *Chromolaena odorata* is a medicinal plant used in Congolese traditional medicine. The juice of the leaves is used to cure the wound (Bissangou and Ouamba, 1997). The previous studies undertaken in other countries give it some pharmacological properties such as: analgesic, anti-inflammatory and antipyretic (Owoyele et al., 2008), antibacterial (Etchike et al., 2011; Agban et al., 2013), anti-oxidative (Kavitha et al., 2013) and antifongic (Kra et al., 2009). This is the reason why anti-inflammatory and analgesic effects of leaves of *C. odorata* (Asteraceae) collected in Brazzaville-Congo in this study were investigated..

MATERIALS AND METHODS

Plant

The leaves of *C. odorata* were collected in Brazzaville (February, 2014). Botanical identification of the plant material was done by Dr. A. Mousamboté, botanist systematist at the Institute of Rural Development and confirmed at the herbarium where the samples of *C. odorata* were compared with the reference samples (number 1183/07/1965). After identification, the plant material was dried and pulverized. 250 g of powder of leaves were mixed with 2500 ml of distilled water in a heating balloon. The mixture was boiled for 15 min. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60°C). The aqueous extract obtained was kept for the experiments.

Animals

Albino rats (150 to 200 g) and albino mice (20 to 30 g) of either sex obtained from the Faculty of Science and Technology of Marien NGOUABI-University were used. They were fed with a standard feed and water *ad libitum*. They were acclimatized during one week before experimentation and were housed under standard conditions (12 h light and 12 h dark) and at the temperature of $27 \pm 1^\circ\text{C}$. The rules of ethics published by the International Association for the Study of Pain (Zimmermann, 1983) have been considered.

Evaluation of anti-inflammatory effect of aqueous extract of *C. odorata*

Carrageenan induced inflammation

Method described by Elion Itou et al. (2014) was used. The animals were divided into groups of 5 rats each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), diclofenac (standard drug, 5 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups, 1 h prior to the local injection of carrageenan (0.05 ml, 1%) into the plantar aponeurosis. Edema was measured by using pletysmometer (Ugo Basile, Italy) at 1/2, 1, 2, 3, 4, 5, 6 and 24th h. The anti-inflammatory effect is evaluated by the inhibition of edema (Elion Itou et al., 2014).

Formaldehyde induced paw inflammation

The rat paw edema was induced with formaldehyde 2.5% (0.2 ml/rat). The animals were divided into groups of 5 rats each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), Tramadol (standard drug, 10 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups, 1 h prior to the local injection of formaldehyde into the plantar aponeurosis. Edema was measured by using pletysmometer (Ugo Basile 7140, Italy) at 1, 2, 3 and 4th hour after formaldehyde administration (Mbiantcha et al., 2010). The anti-inflammatory effect is evaluated by calculating the inhibition of edema.

Cotton pellet induced chronic inflammation

The effect of aqueous extract of *C. odorata* on granuloma formation was studied according to the method described by Elion Itou et al. (2014). 100 mg of cotton pellet were sterilized at 60°C during 24 h and placed interscapular region of rat after ether anesthesia and incision. Distilled water (control group), diclofenac (standard drug) and aqueous extract of *C. odorata* (400 and 800 mg/kg) were administered orally during seven (7) days. On the eighth day, the cotton pellet was removed, cleared of from all adhering tissue and dried at 60°C for 24 h and weighed. The anti-inflammatory effect was given by the inhibition (I) of the granuloma formation (Elion Itou et al., 2014):

$$I = (B - A) / A \times 100$$

where *A* = weight of cotton pellet before implantation (100 mg); *B* = weight of dried cotton pellet after implantation.

Evaluation of analgesic effect of aqueous extract of *C. odorata*

Acetic acid-induced abdominal writhing in mice

The pain was induced in the mice by using 0.6% acetic acid solution (Sawadogo et al., 2011; Elion Itou et al., 2014). The animals were divided into groups of 6 mice each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups, 1 h prior to the local injection of acetic acid (10 ml/kg, IP). 5 min after acetic acid injection, the number of abdominal writhing made by each mouse was recorded during 20 min. The analgesic effect was given by the inhibition (I) of the abdominal writhing (Elion Itou et al., 2014).

Analgesymeter test

The nociceptive response to pressure was measured as described

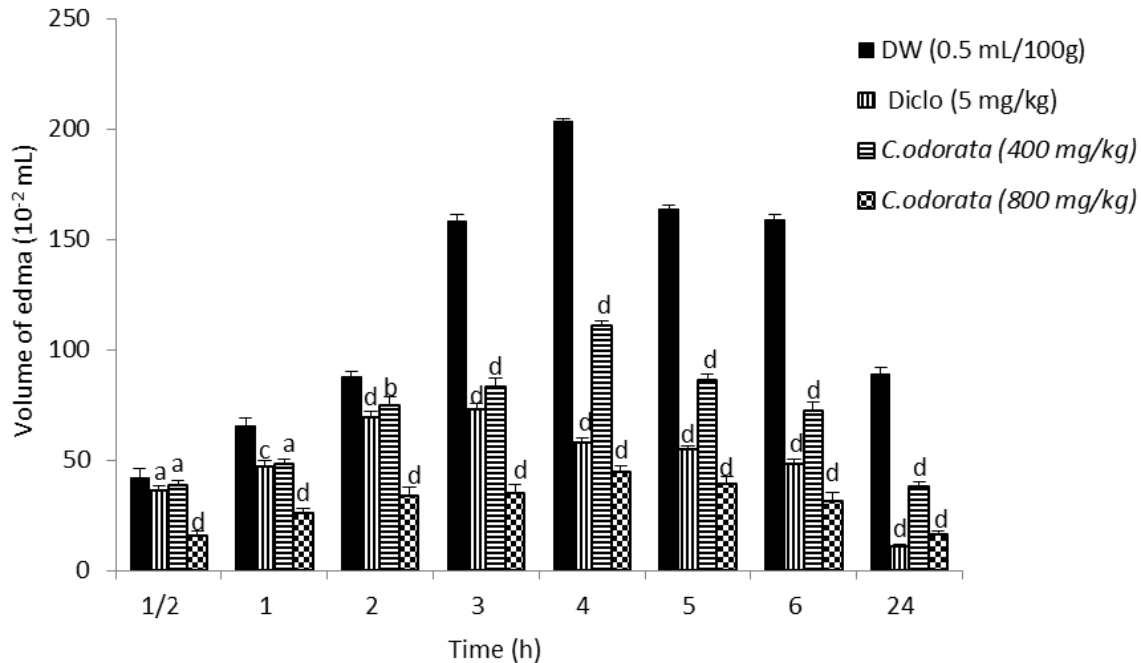


Figure 1. Effect of the aqueous extract of *Chromolaena odorata* on edema induced by carrageenan in rat. Each value represents the mean \pm ESM; a= $p>0.05$; b= $*p<0.05$; c= $**p<0.01$; d= $***p<0.001$ (Student t-test), versus control group. DW: Distilled water, Diclo: diclofenac, *C. odorata*: *Chromolaena odorata*.

by Mbiantcha et al. (2010) using an analgesimeter (Ugo Basile, Italy). This instrument generates a linearly increasing mechanical force or pressure on the dorsal surface on the rat's hind paw. The nociceptive threshold was the point where the animal withdraws its paw. The animals were divided into groups of 6 rats each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups 1 h before the measure of nociceptive threshold. After determination of the nociceptive threshold, the reaction time was calculated (Elion Itou et al., 2014).

Formaldehyde-induced paw licking

The formaldehyde-induced paw licking was studied in rat using the method described by Freitas et al. (2013) and Sudo et al. (2015). The animals were divided into groups of 5 rats each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), Tramadol (standard drug, 10 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups, 1 h prior to the local injection of formaldehyde subcutaneous tissue of the plantar surface of the right paw. Immediately, animals were placed in various cages to observe the noxious effects. The frequency that the animal licks or bites its paw was monitored over 0 to 10 min for neurogenic pain response and 10 to 30 min for inflammatory pain response. The analgesic effect was given by the inhibition of the pain.

Statistical analysis

All values were expressed as mean \pm standard error of mean (SEM). Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p<0.05$

RESULTS

Anti-inflammatory effect of aqueous extract of *C. odorata*

Effect on carrageenan edema

Subplantar injection of the carrageenan produced an inflammatory edema which increased gradually with a maximum between at the 4th hour after injection (Figure 1). Aqueous extract of *C. odorata* (400 and 800 mg/kg) induced significant ($p<0.05$, $p<0.01$ and $p<0.001$) anti-inflammatory effect that increased gradually and reached a maximum (56.98 and 81.75%) at the 24th hour, respectively (Figure 2). Diclofenac (5 mg/kg) shows anti-inflammatory effect ($p<0.01$ and $p<0.001$) with a maximum (80.07%) at the 6th hour (Figures 1 and 2).

Effect on formaldehyde edema

The results are shown in Table 1. Aqueous extract of *C. odorata* (400 and 800 mg/kg) has no reduced the edema evolution at the 1 and 4th hour ($p>0.05$). However, aqueous extract (400 and 800 mg/kg) induced significant ($p<0.05$, $p<0.01$ and $p<0.001$) anti-inflammatory effect that increased between 2 and 3th hour after the injection of carrageenan. Tramadol (10 mg/kg) shows anti-inflammatory effect ($p<0.001$) and was maintained all along the experiment with a maximum of 70.03% at the

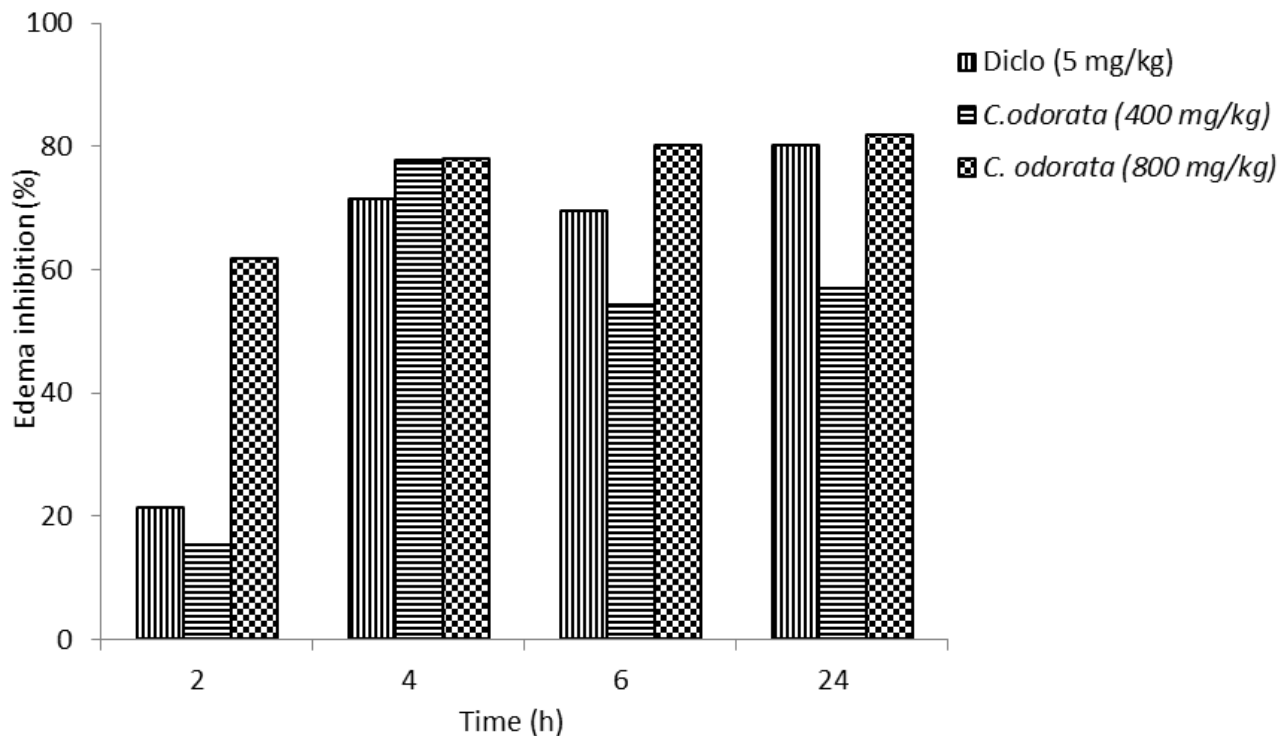


Figure 2. Anti-inflammatory effect of the aqueous extract of *C. odorata* on edema induced by carrageenan in rat. Diclo: diclofenac, *C. odorata*: *Chromolaena odorata*.

Table 1. Effect of the aqueous extract of *Chromolaena odorata* on edema evolution induced by formaldehyde 2.5 %.

Parameter	Doses	Edema volume (10^{-2} ml)				Inhibition (%)	
		1 h	2 h	3 h	4 h	2 h	4 h
Control group	0.5 ml/100 g	174.2± 1.74	218.2± 4.56	263.6± 2.83	280±2.21	-	-
Tramadol	10 mg/kg	73± 6.26***	83.6± 3.90***	79 ±5.20***	69.4± 2.58***	61.68	75.21
<i>Chromolaena odorata</i>	400 mg/kg	173± 1.81 ^{ns}	206.2±1.01 *	249.8± 1.56**	278.8± 2.78 ^{ns}	5.49	0.42
	800 mg/kg	160± 6.68 ^{ns}	204.8± 1.93*	245.6±1.80***	272.8± 3.12 ^{ns}	6.14	2.57

Each value represents the mean ± ESM; ***p<0.001 (Student t-test), versus control group.

4th hour after the injection of carrageenan.

Effect on cotton pellet granuloma

The effect of the aqueous extract of *C. odorata* (400 and 800 mg/kg) as well as diclofenac on the granuloma is shown in Table 2. They show that the aqueous extract of *C. odorata* (400 and 800 mg/kg) as well as diclofenac induced significant ($p<0.001$) the formation of cotton pellet granuloma compared to the control group. Diclofenac and aqueous extract (800 mg/kg) better inhibited the formation of cotton pellet granuloma than

aqueous extract (400 mg/kg) with a maximum of 49.59 and 49.84%, respectively against 19.38% for aqueous extract (400 mg/kg).

Analgesic effect of aqueous extract of *C. odorata*

Effect on the pain induced by the acetic acid 0.6%

The results of the effect of the aqueous extract of *C. odorata* on abdominal cramps are shown in Table 3. Aqueous extract of *C. odorata* at doses used significantly reduced ($p<0.001$) the number of abdominal writhing

Table 2. Effect of aqueous extract of *Chromolaena odorata* on granuloma weight.

Parameter	Doses	Granuloma weight (mg)	Inhibition (%)
Control group	0.5 ml/100 g	95.48±1.74	-
Diclofenac	5 mg/kg	48.13±1.38***	49.59
<i>Chromolaena odorata</i>	400 mg/kg	76.97±1.24***	19.38
	800 mg/kg	47.89±2.14***	49.84

Each value represents the mean ± ESM; ^{ns}p>0.05; *p<0.05; **p<0.01; ***p<0.001 (Student t-test), versus control group.

Table 3. Effect of aqueous extract of *Chromolaena odorata* on abdominal writhes induced by 0.6 % acetic acid solution in mice

Parameter	Doses	Number of abdominal writhes	Inhibition (%)
Control group	0.5 ml/100 g	106.66±2.01	-
Paracetamol	50 mg/kg	100.16±1.01**	6.09
<i>Chromolaena odorata</i>	400 mg/kg	74.40±5.23***	30.24
	800 mg/kg	70.76±4.52***	33.65

p<0.01; *p<0.001 (Student t-test). Each value represents the mean ± ESM; versus control group .

compared to the control group. The effect observed with the aqueous extract at doses used is definitely better than paracetamol used as standard drug with a difference of inhibition of 24.15% at the dose of 400 mg/kg and 27.56% at the dose of 800 mg/kg of the aqueous extract of *C. odorata*.

Effect on the pain induced by the analgesimeter

The effect of the aqueous extract of *C. odorata* on the pain induced by the analgesimeter is as shown in Figure 3. It shows that the aqueous extract at doses used as well as paracetamol (used as standard drug) significantly increases (p<0.001) the reaction time of the animals against the pain compared to the control group. It is also noted that the effect obtained at the dose of 800 mg/kg of the aqueous extract is more significant than that obtained with the aqueous extract at 400 mg/kg and paracetamol.

Effect on the pain induced by formaldehyde in rat

The effect on the pain induced by formaldehyde 2.5% in neurogenic and inflammatory pain responses are presented in Table 4. On neurogenic and inflammatory pain responses, the results obtained show that the aqueous extract of *C. odorata* as well as tramadol

significantly reduce (p<0.001) the frequency of licking and biting the legs of the animals compared to the control group. In addition, the tramadol and aqueous extract (800 mg/kg) inhibits better the animals against neurogenic and inflammatory pain (Table 4).

DISCUSSION

Inflammation was evaluated by using the method of the acute inflammation induced by carrageenan and formaldehyde as well as the chronic inflammation induced by cotton pellet. Carrageenan is a mucopolysaccharide administered under plantar in rats which causes an acute inflammation which is traduced by edema (Elion Itou, 2010). Edema induced by the carrageenan is biphasic. First phase (1 h) is mediated by the inflammatory mediators (serotonin and histamine) and the second phase (2 h after carrageenan administration) is mediated by prostaglandins, products of cyclooxygenase (COX) (Perianayagam et al., 2006; Sudipta et al., 2011). Aqueous extract of *C. odorata* (400 and 800 mg/kg) inhibits the evolution of edema with a maximum of inhibition of 4 h after administration of the carrageenan compared to the control group. Moreover, aqueous extract at doses used is also opposed to acute inflammation induced by formaldehyde in rat. In addition, aqueous extract of *C. odorata* (400 and 800 mg/kg)

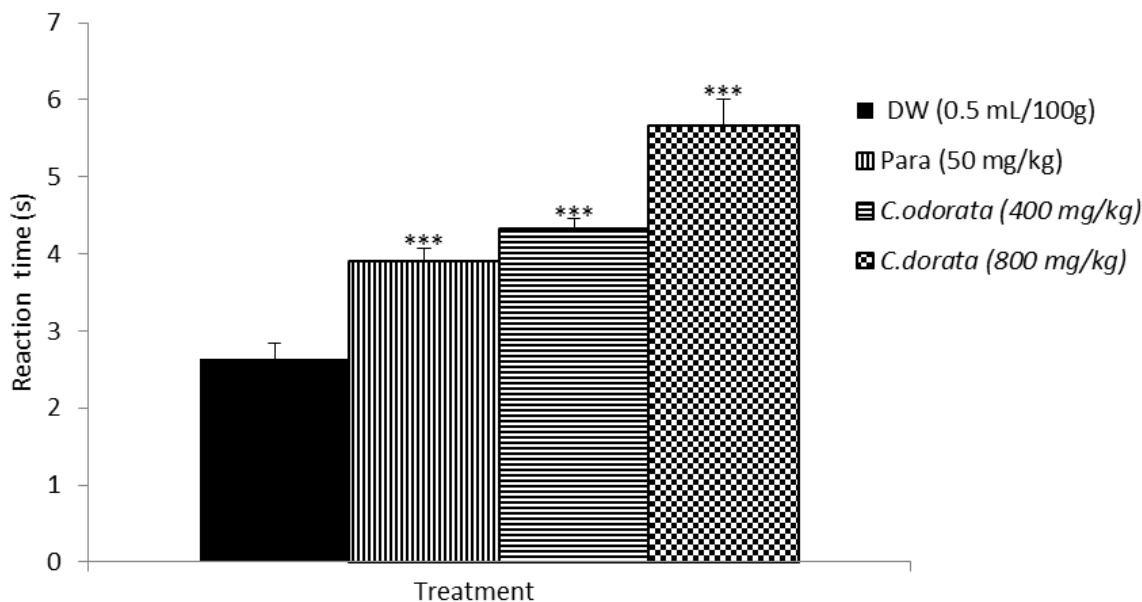


Figure 3. Analgesic effect of aqueous extract of *C. odorata* on pain induced by analgesymeter in rat. *** $p < 0.001$; (Student t-test). Each value represents the mean \pm ESM; versus control group. DW: Distilled water, para: paracetamol, *C. odorata*: *Chromolaena odorata*.

Table 4. Effect of aqueous extract of *Chromolaena odorata* on neurogenic and inflammatory pain response induced by formaldehyde in rat.

Parameter	Doses	Neurogenic pain response		Inflammatory pain response	
		Frequency of legs licking and of biting (0-10 min)	Inhibition (%)	Frequency of legs licking and of biting (10-30 min)	Inhibition (%)
Control group	0.5 ml/100 g	26 \pm 0.63	-	61 \pm 4.27	-
Tramadol	10 mg/kg	5.8 \pm 0.91***	77.69	7.2 \pm 0.27***	88.19
<i>Chromolaena odorata</i>	400 mg/kg	17 \pm 1.04***	34.61	35.6 \pm 0.73***	41.63
	800 mg/kg	8.8 \pm 0.58***	66.15	22.8 \pm 2.35***	62.62

*** $p < 0.001$; (Student t-test). Each value represents the mean \pm ESM; versus control group.

inhibits the formation of granuloma fabric compared to the reference group. These results suggest that the aqueous extract of *C. odorata* (400 and 800 mg/kg) has an anti-inflammatory effect. The inflammation can appear by various symptoms such as the edema (tumefaction or tumour), pain and heat or the fever. It is always accompanied by pain. This is why the evaluation of the analgesic effect is necessary. The analgesic effect of the aqueous extract was evaluated at the peripheral and central level. At the peripheral level, aqueous extract of *C. odorata* (400 and 800 mg/kg) showed an inhibiting effect on the pain induced by acetic acid and the analgesymeter, at the central level; it is opposed to the pain induced by formaldehyde like tramadol (standard drug). These results suggest that aqueous extract of *C.*

odorata (400 and 800 mg/kg) has an analgesic effect. The phytochemical study of the extract revealed the presence of saponins, alkaloids, cardiotonic-heterosids, steroids and terpenoids as well as tannins (Bokia, 2016). The presence of the flavonoïds could explain the anti-inflammatory effect (Bagli et al., 2004; O'Leary et al., 2004), flavonoïds and alkaloids would explain the analgesic effect (Borgi et al., 2007; Sudo et al., 2015).

Conclusion

This study shows that the aqueous extract *C. odorata* has anti-inflammatory and analgesic effects. This justifies the traditional use of this plant in the treatment of the

inflammation and the pain. This study deserves to be completed in order to clarify the mechanisms of action.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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