Analgesic, antipyretic and antibacterial activities of the ethanolic extract of Stem bark of *Buchholzia coriacea* Engl. (*Capparidaceae*)

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**1 ABSTRACT**

The objective of this study was to evaluate the analgesic, antipyretic and antibacterial activities of stem barks ethanol extract of *Buchholzia coriacea*. The Analgesic effect was evaluated by using the acetic acid-induced writhing as well as the pain induced by formaldehyde, while antipyretic activity was evaluated using Brewer's yeast induced pyrexia (*Saccharomyces cerevisiae* 20%). The antibacterial activity was determined by the liquid microdilution method. The results obtained show that the ethanolic extract at the doses of 200 and 400 mg/kg inhibits the pain induced by the acetic acid 0.6 % and by the formaldehyde 2.5 % (p < 0.001) compared to the control group. The ethanolic extract at all used doses reduces significantly (p<0.01) the hyperthermia induced by brewer's yeast (*Saccharomyces cerevisiae* 20 %). Moreover, the ethanolic extract concentrations ranging from 10 to 2000 μg / ml inhibit in vitro the growth of *Pseudomonas aeruginosa* and *Bacillus Subtillis* with Minimum Inhibitory Concentrations (MIC) of 3.25 μg/ml each, *Staphylococcus aureus* and *Escherichia coli* germs with Minimal Inhibitory Concentrations of 6.25 μg/ml. The Minimum Bactericidal Concentrations (MBC) was 3.25 μg/ml for *Pseudomonas aeruginosa* and 6.25 μg/ml for *Bacillus subtiliss*. For *Staphylococcus aureus* and *Escherichia coli*, the MBC was 12.5 μg/ml. These results suggest that the ethanolic extract of stem barks of *Buchholzia coriacea* has analgesic, antipyretic and antibacterial effects. These effects could justify the traditional utilization of stem barks of *Buchholzia coriacea* in the pain and fever treatment, but also against bacteria strains.
INTRODUCTION

The research of the herbal drugs is currently attracting the attention of researchers in Africa and the rest of the world. Efforts one made to explore all the medicinal and food plants on the African flora and world, in order to put them for the benefit of public health. The major stake of today is the persistence of some chronic diseases and the appearance of others not yet controlled (WHO, 2013). The Conventional medicines approved and advised by WHO have side effects and undesirable consequences and to that are added the precariousness and the poverty of the patients deprived of financial means to afford the primary care of health are major dispensaries of public health. Bouquet, (1969); WHO, (2014). The traditional use of medicinal plants is the main source of remedies for people in tropical countries (Bouquet and Jacquot, 1967; Adjanohoum et al., 1988; Bouquet, 1969; Abena et al., 1996; Abena et al. 2003; Agbonon, 2005). The vegetable species, used in traditional medicine in the tropical countries have a priceless value. Buchholzia coriacea (Capparidaceae) is one of the medicinal plants used in Africa (Ghana, Liberia, Cameroun, Democratic Republique of Congo and in Congo,…). This plant is a large 20-25 meter high tree of the primary education tropical forest of Liberia, Nigeria and Basin of Congo where it is known in vernacular language under the name of Songo Kama in laari and ombanda in Téké. Its traditional use is very widespread in the ethnomedicine. (Agbonon et al., 2002; Agbonon, 2002; Fred-jaiyesimi et al. 2011; Adu-Amoah et al., 2014; Felix-Silva et al., 2014). In Nigeria various parts of Buchholzia coriacea are employed in the treatment of fever, malaria, bacterial infections, diabetes, menstrual pains and gastro-intestinal infections. Elsewhere in the African tropical countries, the seeds are employed in the treatment of cough, irregular menstruation, headache, sinusitis and congestion, bronchitis oedema, and as facilitator of childbirth. The seed is also employed as antidiabetic, antihypertensive, antirheumatic, antibacterial, and antiparasitic (Ezekiel and onyeozini, 2009; Ezekiel et al., 2011; Anton et al., 2011; Ayoola et al., 2011; Chen, 2011; Chika et al., 2012; Enechi and Nwodo, 2014). In Congo-Brazzaville the stem barks of the tree are usually used for the treatment of inflammation, pain, microbial infections and against the fever (Malmberg and Yaksh, 1992; Ezekiel and onyeozini, 2009; Ezekiel et al., 2011; Anton et al., 2011; Ayoola et al., 2011; Chen, 2011; Chika et al., 2012; Enechi and Nwodo, 2014). The ethanolic extract of the stem barks of Buchholzia coriacea can reduce the above-mentioned chronic diseases on the long run. However, the needs for administration by the oral route to boost the analgesic, antipyretic, anti-inflammatory activities appears very essential. We report here in the current work the potential analgesic, antipyretic, and antibacterial activities of the stem barks of Buchholzia coriacea in rats and mice to give a scientific base of the traditional use of this plant.

MATERIALS AND METHODS

4.1 Material plant: This study was undertaken on the barks of trunk of Buchholzia coriacea (Capparidaceae). These barks were collected in a northern district of Brazzaville (Congo), in May 2017, on the basis of geographical co-ordinate of 0532339 UTM of Latitude and 9534483 UTM of Longitude. The collected sample was deposited at the Herbarium of the Department of Botany at the Center of Study on Plant Resources (CERVE) where they were identified and compared to the reference sample registered under the number 2456 at the date of 17 -2-1968. Then, they were dried in the laboratory of Life and Earth Sciences of the School Normal Superior (SNS) at room temperature and away from the sun. After drying, these barks were ground to powder using a mortar. The obtained powder was used to prepare the ethanolic extract. 500 g of powder Buchholzia coriacea trunk bark was
mixed in 5000 ml of 96% ethanol for 72 hours with stirring. After filtration on the hydrophilic cotton and Whatman paper, the filtrate obtained was concentrated using a Rotavapor Buchi R-10. The concentrate was stored in sterile and dry flasks to evaluate the analgesic, antipyretic and antimicrobial effects.

4.2 Animal material: The albino mice Swiss, (males and females) weighting between 20-30 g and Wistar rats (males and females) weighting between 100-200 g obtained from the Faculty of Health Sciences were used. All animals were acclimated during one week before the experiments. They were fed and maintained under standard lighting conditions (12 h light and 12 h dark) at a temperature of 27 ± 1 °C. They were fasted for 24 h before experiments, water was given ad libitum.

4.3 Bacterial strains: In this study, Gram + bacteria (S. aureus and B. subtilissus) and Gram-bacteria (E. coli and P. aeruginosa) were used. These strains were provided to us by the microbiology laboratory (ESTEBA) of the University of Lomé, Togo;

4.4 Acetic acid-induced abdominal writhing in mice: The pain was induced in the mice by using 0.6% acetic acid solution (Elion Itou et al., 2018). The animals were divided into groups of 6 mice each. The aqueous extract of B. coriacea (200 and 400 mg/kg), paracetamol (standard drug, 50 mg/kg) or saline 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.

4.5 Formaldehyde-induced paw licking: The formaldehyde-induced paw licking was studied in rats using the method described by Elion Itou et al., (2014). Different doses of ethanol extract of B. coriacea (200 and 400 mg/kg), Tramadol (standard drug, 10 mg/kg) or saline 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 surface of the right paw by the subcutaneous route. 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 evaluated by the degree of the inhibition of the pain. A central analgesic would inhibit the two phases equally, but a peripheral analgesic would only inhibit the second phase.

4.6 Brewer's Yeast Pyrexia Test: The test was performed according to the method described by Toétino et al., (1963), Narayanan et al., (2000). Rectal temperature of animals was recorded using digital thermometer (Dostmann mark). Pyrexia was induced by injection of 5 mL/kg s.c. of 20 % suspension of Brewer's yeast (Saccharomyces cerevisiae). After 12 h rectal temperature was recorded. After that ethanol extract of B. coriacea (200 and 400 mg/kg), salicylic acid (standard drug, 300 mg/kg) or saline water (control group, 0.5 mL/100 g) were administered orally to groups. Rectal temperature was recorded periodically at 1, 2, 3, 4 and 5 hours after drugs administration (Elion Itou et al., 2018).

4.7 Evaluation of antibacterial effect of ethanol extract of Buchholzia coriacea: This antibacterial activity of the ethanolic extract of B. coriacea was performed according to the method used by Ezekiel and Chika et al. (2012) and Nwachukwu et al. (2014). Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using the microdilution technique with Muller Hinton Broth. The bacterial suspensions were diluted with Müller Hinton broth and distributed in the 96 well plates containing a concentration range of each extract between 10-2000 μg/ml. Inocula as determined by colony counting from control wells without extracts were approximately 10^5 CFU /ml. Plates were incubated at 37 °C for 24 hours. MIC was defined as the minimum concentration of extract for which no visible growth is observed with the naked eye and CMB as the minimum concentration that kills 99.99 % of starting bacterial germs. The CMB was determined by taking 100 μl of suspension
in the wells without visible growth and by seeding nutrient agar. Incubation was at 37 ° C for 48 hours at which colonies were counted.

**Statistical analysis:** All values were expressed as mean ± standard error of mean (SEM).

5 RESULTS

5.1 Effect of ethanolic extract of *B. coriacea* on the pain induced by the 0.6 % acetic acid: The administration of 0.6 % acetic acid intraperitoneally caused abdominal writhing (Fig.1). The results obtained show that paracetamol (50 mg / kg) and the ethanolic extract (200 and 400 mg / kg) significantly reduce (p <0.05 and p <0.001) the number of abdominal writhing (Figure 1). The number of writhing developed by the mice is 54.6 ± 1.57 and 42 ± 2.98 for the ethanolic extract (200 and 400 mg / kg), 43.20 ± 1.93 for paracetamol and 72.2 ± 6.599 for the control group. In addition, the ethanolic extract (200 and 400 mg / kg) and paracetamol inhibit the pain induced by acetic acid (Figure 2). These inhibitions were 24.37 and 41.82 % for the ethanolic extract and 40.27 % for the paracetamol (reference molecule).

![Figure 1](image.png)

**Figure 1:** Effects of the extract ethanolic of stem barks on abdominal writhing induced by acetic acid in mice. * p < 0.05, *** p < 0.001 (ANOVA) versus control group. Para=paracetamol. B.C = *Buchholzia coriacea*
5.2 Effect of ethanolic extract of *B. coriacea* on formaldehyde oedema: The effect on the pain induced by formaldehyde 2.5% in neurogenic and inflammatory pain responses are presented in figure 3 and 5. The results in neurogenic and inflammatory pain responses show that the ethanolic extract (200 and 400 mg/kg) of *B. coriacea* as well as the tramadol (standard drug, 10 mg/kg) significantly reduce (p<0.001) the number of licking and biting the legs of the animals treated compared to the control group (figures 3 and 5). In addition, the tramadol® and the ethanolic extract (400 mg/kg) protect better the animals against and inflammatory pain responses compared to the extract ethanolic at the dose of 200 mg/kg (figures 4 and 6). For neurogenic pain response, these inhibitions were of 32.39; 13.52 and 19.13 respectively for the tramadol and the extract ethanolic at the doses of 200 and 400 mg/kg (figure 6).

5.3 Antipyretic effect of the ethanolic extract: Table 1 shows the results of the effect of the ethanolic extract (200 and 400 mg/kg) on pyrexia induced by Brewer’s yeast (*Saccharomyces cerevisiae*). These results show that the ethanolic extract (200 and 400 mg/kg) does not significantly (p>0.05) reduce hyperthermia from 1 hour to 2 hours of observation compared the control group. However, from the 3 hours to 5 hours, this extract significantly (p<0.05 and p<0.01) inhibits the hyperthermia induced by a 20% suspension of Brewer’s yeast (*Saccharomyces cerevisiae*) compared to the control group. In addition, aspirin (standard drug, 300 mg/kg) significantly (p<0.01) reduces hyperthermia from 2 hours to 5 hours compared to the control group.

5.4 Antimicrobial effect of the ethanolic extract: Table 2 shows the in vitro effect of the ethanolic extract of stem bark of *B. coriacea* at a concentration of 2000 μg / ml on the growth of *S. aureus*, *P. aeruginosa*, *B. subtillus* and *E. coli*.  

![Figure 2. Analgesic effect of the ethanolic extract of stem barks of *B. coriacea* on pain induced by acetic acid in mice. Para=paracetamol. B.C = *Buccholzia coriacea***](image)
They show that at this concentration, this extract strongly inhibits the growth of *P. aeruginosa* and *B. subtilis* compared to *S. aureus* and *E. coli*. This inhibition is demonstrated by low Minimal Inhibitory Concentrations (MIC) as well as low Bactericidal Minimal Concentration (BMC). These MIC and CMB were 3.25 and 3.25 µg/ml for *P. aeruginosa*; 3.25 and 6.25 µg/ml for *B. subtilis*. However, *S. aureus* and *E. coli* have the same CMI and CMB values of 6.25 and 12.25 µg/ml respectively.

**Figure 3.** Effect of the extract ethanolic of *B. coriacea* on the number of licking and biting the legs after formaldehyde administration during the neurogenic pain response. *** p<0.001 significant different (Student t-test) versus control group. B.c = Buccholzia coriacea

**Figure 4.** Analgesic effect of the extract ethanolic of *B. coriacea* in neurogenic pain response induced by formaldehyde 2.5 %
Figure 5: Effect of the extract ethanolic of *B. coriacea* on the number of licking and biting the legs after formaldehyde administration during the neurogenic pain response. *** p<0.001, significant different (Student t-test) versus control group. Tra=tramadol. B.c = *Buchholzia coriacea*

Figure 6: Analgesic effect of the extract ethanolic of *B. coriacea* in inflammatory pain response induced by formaldehyde 2.5 %
Table 1. Effect of the ethanolic extract of stem barks of *B. coriacea* on the hyperthermia induced by of Brewer’s yeast (*Saccharomyces cerevisiae*) in rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Control group (0.5 ml/100g)</td>
<td>37.0 ± 0.22</td>
</tr>
<tr>
<td>Aspirin 300 mg/kg</td>
<td>37.36 ± 0.08</td>
</tr>
<tr>
<td><em>B. coriacea</em> 200 mg/kg</td>
<td>37.42 ± 0.037</td>
</tr>
<tr>
<td><em>B. coriacea</em> 400 mg/kg</td>
<td>37.44 ± 0.07</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of temperature. **p<0.01 significant different versus control group. ns= no significant value p>0.05; 

Table 2. Antimicrobial effect of the ethanolic extract of stem barks of *B. coriacea* 

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Control (NaCl 0.09 %)</th>
<th>Muller Hinton</th>
<th>Extract ethanolic of the barks (10 à 2000 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC and BMC</td>
<td>MIC and BMC</td>
<td>MIC</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.00</td>
<td>0.00</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.00</td>
<td>0.00</td>
<td>3.25</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0.00</td>
<td>0.00</td>
<td>3.25</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.00</td>
<td>0.00</td>
<td>6.25</td>
</tr>
</tbody>
</table>

6 DISCUSSION

The aim of this work was to study the analgesic, antipyretic, and antimicrobial effects of the ethanolic extract of *B. coriacea* bark. Analgesic effect of this ethanolic extract was tested on pain induced by acetic acid 0.6%. Stimulation of prostaglandin receptors by intra-peritoneal injection of acetic acid 0.6% causes a so-called somatic local pain driven by the painful fibres, which results in abdominal contractions in contrast to a visceral pain of diffuse nature (Kosher *et al.*, 1959, Abena *et al.*, 1993, Abena *et al.*, 2003, Chen 2011, Alexander *et al.*, 2012, WHO 2014). This test makes it possible to evaluate the analgesic properties of natural substances of the ethanolic extract of the bark of *B. coriacea*, as reported by Wolf (2010 and 2011); Talbot *et al.*, (2010) and Talbot *et al.* (2011). The ethanolic extract at doses of 200 and 400 mg / kg administered orally and used as a reference product also significantly reduced abdominal cramped to 43.2 (40.27%) compared with the control that developed 72.2 abdominal cramp (figure 1). The analgesic properties of this extract could be attributed to the presence of the chemical compounds (saponosides, flavonoids, tannins, sterols and terpenoids, anthocyanins) that would be responsible for these analgesic effects (Epa *et al.*, 2015). These same observations were made by Sinatra *et al.*, (2005) for chemicals of other plants and other animals. This ethanolic extract could act by inhibition of prostaglandin biosynthesis as reported by Aronoff *et al.* (2006) who showed the mechanisms of inhibition of prostaglandin H2 synthetases.

6.1 Effect on pain induced by formaldehyde at 2.5% in rats: The analgesics act either at the peripheral level (paracetamol) or at the central level (morphine). Thus, the effect of the ethanolic extract was evaluated on formaldehyde-induced central type pain. Indeed
the pain induced by formaldehyde is biphasic. The first so-called neurogenic phase (between 0 and 10 minutes) is due to the direct stimulation of afferent fibers C which transmit painful impulses to the central nervous system. The so-called inflammatory phase (between 10 and 30 minutes) is controlled by inflammatory mediators that are: serotonin, histamine, bradykinin, NO, and prostaglandins (Sudo et al., 2015). The effect of the ethanol extract on 2.5% formaldehyde-induced pain was evaluated on neurogenic pain (Figures 3 and 5) and inflammatory pain (Figure 4 and 6). At this level, the results obtained show that the ethanolic extract of *Buccholzia coriacea* as well as tramadol significantly reduce (p < 0.001) the number of licking and biting of the paws of treated animals compared to the control group (Figures 3 and 5). On the other hand, tramadol and ethanolic extract (400 mg / kg) better protect the animals against neurogenic pain compared to the 200 mg / kg dose with respective inhibition percentages of 32.39; 19.13 and 13.52% from 0 to 10 min (FIG. 10) on the one hand and 38.03 respectively; 47.51 and 5.65% from 10 to 30 minutes (Figure 6). The analgesic effect of the ethanolic extract was evaluated at the peripheral level (with 0.6% acetic acid) and at the central level (with formaldehyde). At the peripheral level, the trauma caused by acetic acid causes the release of histamine, serotonin, bradykinin, prostaglandins, cytokines, ATP and K + and H + ions (Guirimand, 2003, Marieb and Hoehn, 2010). The ethanolic extract (200 and 400 mg / kg) opposes this chemical-type pain such as paracetamol. This suggests that it interferes with or inhibits the release of these allogeneic mediators. The fact that the ethanolic extract inhibits it as tramadol (reference molecule) neurogenic and inflammatory pain suggests that it could act as tramadol.

### 6.2 Effect of the ethanolic extract on the hyperthermia induced by a yeast suspension *Saccharomyces cerevisiae* at 20% on hyperthermia.

The hypothalamus is a center of thermostat regulation of the body, in case of infection, macrophages produce Interleukin 1 (IL1) which will transit to the hypothalamus producing E2 prostaglandins which induce an increase of body temperature (chills, peripheral vasoconstriction) as reported by Aronoff et al., (2006). This result suggests that ethanol extract could lower yeast-induced hyperthermia in rats by blocking the synthesis of prostaglandin E2 in the hypothalamus that induces an increase in body temperature (chills, peripheral vasoconstriction) as indicated Aronoff et al., 2006. These effects could be attributed to flavonoids and tannins would be responsible for their analgesic, antipyretic antibacterial and anti-inflammatory properties. Our results show that the ethanolic extract at doses of 200 and 400 mg / kg had a significant antipyretic effect. This extract reduces the rectal hyperthermia caused by a 20% suspension of yeast *Saccharomyces cerevisiae* in rats from the 2nd hour until the 5th hour at doses of 200 mg / kg and from the third hour at the dose of 400 mg / kg (Table 1), which suggests that the ethanolic extract could lower the temperature of the rats by blocking the synthesis of prostaglandins of the hypothalamus. The flavonoids and tannins revealed in this extract would be responsible for the antipyretic effects.

### 6.3 Effect of the ethanolic extract on the three bacterial strains.

The ethanolic extract showed a positive activity on *Pseudomonas aeruginosa* with a minimum inhibitory concentration of 3.125 μg / ml and a bactericide of 3.125 μg / ml and on *Bacillus subtillus* with a minimum inhibitory concentration of 3.125 μg / ml and a bactericidal concentration of 6.25 μg / ml but low bacteriostatic and bactericidal activity on *Staphylococcus aureus* and *Escherichia coli* with minimum inhibitory and bactericidal concentrations of 6.25 and 12.5 μg / ml respectively (Table 2). These bacteriostatic and bactericidal activities of the ethanolic extract would be attributed to its phytochemical constituents, namely free anthraquinones and their heterosides, flavonoids as demonstrated by Anton and Duquenois (1968); Onyekaba et al., (2011); Chika et al. (2012). These results are similar to those of Ulewitch and Tobias. (1999);
Tanamoto and Azumi (2000); Van Beden et al. (2007) who worked on the seeds of B.coriacea and those of Onyekaba et al. (2011); Chika et al. (2012) on the leaves of B coriacea. At the same time, Anton and Duquenois (1968) have shown that the anthracene derivatives and flavonoids contained in Cassia extracts are responsible for this activity. It would therefore be logical to think that the anti-bacterial activity of the ethanolic extract of the B. coriacea trunk bark of this study, would therefore be due not only to anthraquinones but also to tannins, phenolic products and terpenes.

7 REFERENCES


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