

Original Research Article

Evaluation of antioxidant, anti-inflammatory and analgesic activities of leaves of *Saba senegalensis* (A.DC) Pichon (Apocynaceae).

ABSTRACT:

Aims: To evaluate antioxidant, anti-inflammatory and analgesic activities of extracts of *Saba senegalensis* leaves.

Study design: *In vitro* antioxidant assay and *in vivo* anti-inflammatory, analgesic assay of *Saba senegalensis* extracts.

Place and Duration of Study: *Saba senegalensis* leaves, were collected in the Centre Region of Burkina Faso, in June–July 2015. The experiment were conducted at the department of Medicine and Traditional Pharmacopea- Pharmacy (MEPHATRA-PH) of Institut of Research in Health Science (IRSS).

Methodology: The anti-oedematous tests with carrageenan and the analgesic with acetic acid and and investigate effect on isolated organ were carried out. The standards were acetylsalicylic acid and paracetamol.

Results: After five hours of carrageenan-induced edema test, aqueous decoction (AD) presented better inhibition on all measure. In fact, at the different doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg it presented percentages of inhibitions respectively of 30.81 %, 62.27 % and 72.71 %. For the analgesic test, the hydroethanol macerate (HEM) showed a better pain reduction compared to the AD with a maximum effect of 77.28% at 400 mg/kg. Antioxidant activity with AD and his fractions shows that AD showed a better activity for the DPPH assay with an IC_{50} of $1.74 \pm 0.10 \mu\text{g/mL}$ and a reducing power of $59.53 \pm 2.16 \text{ mmol ET/g Sample}$. For HEM and his fractions, the ethyl acetate fraction ($F_{\text{HEM-AcOEt}}$) showed a better IC_{50} of $0.18 \pm 0.01 \mu\text{g/mL}$ for the DPPH test and dichloromethane fraction ($F_{\text{HEM-DCM}}$) a reducing power agent of $88.88 \pm 2.65 \text{ mmol ET/g Sample}$. All fractions were endowed with antioxidant properties by both methods.

Conclusion: The study findings suggest that the presence of phenolic and terpenoid compounds could explain the antioxidant, anti-inflammatory and analgesic properties of these extracts.

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INTRODUCTION

Oxidative stress is developed as a result of excessive generation of free radicals to that of the physiological requirement of the body. Low levels of antioxidants in the living system assist the development of ageing related ailments including atherosclerosis, cancers, diabetic neuropathy, Alzheimer's disease and inflammatory disorders. Biological macromolecules such as proteins, lipids and DNA are damaged by the deleterious action of free radicals [1].

Inflammation is a fundamental protective response that enables human survival when encountering a microbial invasion or injury, and also maintains the tissue homeostasis under various deleterious surroundings [2]. It is attained by the migration of plasma and white cells comprising monocytes that are locally distinguished into macrophages from blood into wounded tissues. Immune reaction is vital for the body to remove dangerous pathogens and it is categorized by way of an acute inflammation.

Pain is a well-defined as an unwanted corporeal or demonstrative experience and it is also categorized as acute or chronic pain. Pain is also a manifestation of inflammation.

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In inflammation various pro- and anti-inflammatory intermediates are produced comprising cytokines, interleukins, ROS, chemokine's all of them play a serious role in governing the inflammation [3]. Clinically inflammatory disorders are usually managed by steroidal (betamethasone) and the non-steroidal anti-inflammatory drugs (NSAIDs; acetylsalicylic acid). However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. The use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects. In fact, chronic administration of such drugs can cause gastrointestinal ulcers, renal insufficient and cardiovascular disorders [1, 4].

For these reasons, the use of medicinal plants has become the news strategy of several studies to develop and introduce new drugs with greater safety and efficiency. Indeed, medicinal plants contain a large number of bioactive molecules of multiple interests not only in traditional medicine but also in the food and pharmaceutical industry.

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Many species of plants are still left unexplored, despite intensive research on various herbal plants. In Burkina Faso, the antioxidant, anti-inflammatory and analgesic properties of plants such as *Dicliptera verticillata* and *Pterocarpus erinaceus* have been shown respectively by Sawadogo et al. (2006) and Ouedraogo et al. (2011) [5, 6]. *Saba senegalensis* (*S. senegalensis*) plant, used in traditional medicine in Burkina Faso, is chosen for the present study for its important medicinal properties. Ethnobotanical survey conducted in 2006 by researchers from the Research Institute for Health Sciences (IRSS) have shown that the leaves of this plant were used for the treatment of various pathology whose parasitosis [7]. According to Nacoulma, (1996) [8] leaves and unripe fruits of the plants would have anti-inflammatory properties. Yougbaré, (2016) [9] has also proven the anti-inflammatory and antioxidant properties of the leafy stems of the plant. The leaves contain constituents such as steroidal terpenes, saponins, flavonoids and polyphenols which were found to be useful for wound healing, in the treatment of inflammation, pain and antioxidant properties [10].

It appears from the perusal of literature that the plant *S. senegalensis* has different important pharmacological parameters. Thus, different extracts and fractions from *S. senegalensis* leaves was evaluated in experimental models *in vitro* and *in vivo* to prove the medicinal properties antioxidant, anti-inflammatory and analgesic.

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2. Materials and Methods

2.1. Chemicals reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl), Methanol, Fe (III), Ammonium acetate buffer (Prolabo, France), Trolox (Fluke, France), Carrageenan (Sigma-Chemical Co.), acetic acid, trichloroacetic acid, ferric chloride, Ketamine, Paracetamol and acetylsalicylic acid (ASA), dimethyl sulfoxide (DMSO), Acetylcholine, Atropine.

2.2. Plant material

Fresh aerial parts of *Saba senegalensis* (leaves), were collected in the Centre Region of Burkina Faso, in June–July 2015. These plants were identified by the plant taxonomist at the herbarium of the National Centre for Scientific and Technological Research (CNRST), where the voucher specimens were kept under the reference numbers No. 00223 HNBU. The leaves of plants were air dried at room temperature, powdered using pestle and mortar and kept in amber colored bottles until use in order to keep all their physicochemical properties.

2.3. Extraction procedure

Fully shade dried aerial parts of *S. senegalensis* were powdered followed by two different extraction: aqueous decoction with water and hydroethanolic macerate (ethanol/eau 8/2).

The aqueous extract collected is subjected to evaporation at low pressure at 35 °C before fractionation. A concentrated portion of the extract obtained following leaching with dichloromethane is retained (F_{AD}-DCM) and the other portion is used for liquid-liquid separation with ethyl acetate. At the end of the separation, the extract with ethyl acetate (F_{AD}-AcOEt) collected is subjected to evaporation at low pressure at 35 °C and the residual aqueous phase (residue). The F_{AD}-DCM, F_{AD}-AcOEt and F_{AD}-Residue fractions are evaporated to dryness and stored for phytochemistry and pharmacology.

After 24 hours of maceration, lixiviation is carried out and then the collected extract is subjected to evaporation at low pressure at 35 °C. A concentrated portion of the extract obtained following extract with dichloromethane was retained (F_{HEM}-DCM) and the other portion was used for liquid-liquid separation with ethyl acetate. At the end of the separation, the extract with ethyl acetate (F_{HEM}-AcOEt) collected is subjected to evaporation at low pressure at 35 °C and the residual aqueous phase (F_{HEM}-Residue) is kept. The F_{HEM}-DCM and F_{HEM}-AcOEt fractions in one part, and the residue in the other part were evaporated to dryness and then preserved for phytochemistry and pharmacology assays.

2.4. Experimental animal

Swiss albino mice of either sex, the same age or 20-24 g weight purchased from animalerie of IRSS (Institut de Recherche en Sciences de la Santé) were used in the present study. All the animals were housed in appropriate cages at standard controlled laboratory condition (21 ± 2°C, 12 h light/dark cycle). Water and food were easily available to all experimental animals during acclimatization period. Food was withdrawn from all animals 17 hrs before starting the experiment. The animals were randomly divided into five groups (n = 6). This study was approved by the ethical committee of IRSS.

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2.5. Antioxidant activity

2.5.1. Antiradical activity against

DPPH is a stable free radical most commonly used for the evaluation of radical scavenging potential of extract/fractions of phytomedicine. Procedure according to the method of Ouedraogo et al. (2011) [6]. DPPH radical solution was prepared in methanol (4 mg/50 mL) and 10 mg of samples was added to 1 ml of methanolic solution. We carried out dilution by series in a 96-well microtitre plate. Methanol was used as negative control while Trolox served as standard. The samples and control solutions were incubated in dark for 30 min at controlled temperature (20–25°C). After incubation bleaching of the purple-coloured methanolic solution of DPPH was observed. The absorbance of samples, control and standard were measured at 515 nm using Multiskan Ex (Thermo Electron Corporation) Spectrophotometer standard control. Each determination was carried out in triplicate and the results were expressed as mean values ± standard deviations. The percentage residual DPPH was evaluate on a graph as function of quantity of antioxidant:

$$\% \text{ DPPH} = [(A_{\text{DPPH}} - A_E) / A_{\text{DPPH}}] \times 100$$

Where A_E is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

The extract concentration providing 50% inhibition (IC₅₀), antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration.

2.5.2. Reducing power assay

Reducing power of various extracts and fractions was determined according to a previously described procedure [11, 12]. Various concentrations of sample extracts (0.5 mL) were mixed with 1.25 mL (0.2 M, pH 6.6) sodium phosphate buffer and 1.25 mL of 1% potassium ferricyanide. The mixture was shaken vigorously and then incubated at 50 °C for 30 min. After incubation, 1.25 mL of 10% trichloroacetic acid (TCA) (w/v) was added and then the mixture was centrifuged at 1000 rpm in a refrigerated centrifuge for 10 min. The upper layer (0.625 mL) was mixed with 0.625 mL of deionized water and 0.125 mL of 0.1 % ferric chloride (FeCl₃). The absorbance was measured

spectrophotometrically at 700 nm. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration.

2.6. *In vitro* anti-inflammatory activity

Anti-inflammatory potential of the leaves of *S. senegalensis* by carrageenan-induced paw edema in mice was studied according to the method of [5]. NMRI mice were divided into five groups, each containing six mice and treated in orally administration at doses of 200 mg/kg, 400 mg/kg and 600 mg/kg of AD and HEM. Standard was treated with ASA at dose of 150 mg/kg and negative control with 1% saline. All these doses were administered 1 h earlier before the sub-plantar injection 0.05 mL of carrageenan (1 mL/kg; 1% in saline w/v) in hind paw. Paw volume was measured by plethysmometer (Ugo Basile srl, No 37140) immediately after carrageenan injection (zero hours) and was repeated at 1, 3 and 5 h after induction of inflammation. The average volumes of the right hind paw of each mouse was calculated according to following formula:

Percentage inhibition of edema was calculated for each animal by using the following formula:

$$\% \text{ Inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where V_c and V_t represent average paw volume of control and treated animals respectively.

2.7. Analgesic activity

The peripheral analgesic activity of the test samples was evaluated by acetic acid-induced writhing method [13]. The frequency of abdominal writhing in mice following intraperitoneal injection of acetic acid is observed. For this study, mice ($n = 6$) were divided into five groups. Each mouse was given an injection of 0.6 % acetic acid aqueous (1.0 %, at a dose of 0.1 mL/10 g body weight) into the peritoneal cavity and the animals were then placed in a transparent plastic box. The number of abdominal constrictions was counted for 15 min beginning from 5 min after the acetic acid injection. Mice, standard group was treated with paracetamol (100 mg/kg), control received distilled water 0.1 mL/10 g body weight and other groups were treated with AD and HEM of *S. senegalensis* at doses 100, 200 and 400 mg/kg.

Percentage inhibition of writhing compared to the control was taken as an index of analgesia, and it was calculated using the following formula:

$$\text{Inhibition (\%)} = [(W_c - W_t) / W_c] \times 100\%$$

Here, W_c is the number of writhing responses in the control group, and W_t is the number of writhing responses in the test groups.

2.8. Activity on isolated organ

The principle is described by Chen et al. (2012) [14] and adapted to laboratory of IRSS. It consists of measuring the effect of the extract on the contractile activity of the duodenum isolated from the rat. For this, male or female rats previously fasted for 16 to 18 hours, were anesthetized with ethyl urethane (80 %) at 1 g/kg. The animal is lying on its back on a wooden board to be incised in the abdomen. After opening, the duodenum was removed and immersed immediately in a survival solution, Tyrode composed of (in g) 8 NaCl, 0.2 KCl, 0.2 $CaCl_2$, 0.01 $MgCl_2$, 0.05 NaH_2PO_4 , 1 $NaHCO_3$, 1 Glucose in 1L of distilled water.

The duodenum segment about 2-3 cm long is cut and then the two ends carefully attached. One end of the segment is attached to a support rod and the other to an isotonic transducer using a wire which

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is itself connected to the recorder via an amplifier. It is placed in an isolated organ tank containing Tyrode (at 37 °C; pH 7.4) and oxygenated (95 % O₂ - 5 % CO₂)

A period of 60 min of stabilization was observed and Tyrode was renewed every 15 min. The effect of the aqueous extract is tested by administering increasing concentrations in cumulative mode in the tank. The amplitude of the contractions obtained in the presence of the aqueous extract is appreciated compared to those obtained with the reference substances. The effect of atropine is tested in the curative mode by adding a concentration of 5.10⁻⁵ mg/mL in the single-concentration tank of the aqueous extract.

The percentage amplitude variation of the basic contractions in the presence of extract was calculated according to the following formula:

$$AV (\%) = (Y1/Y2) \times 100$$

With: Y1 = Height reached in the presence of the extract and Y2 = Height of the basic contractions

2.9. Statistical Analysis

Results were expressed as mean ± E.S.M. The analysis of the results of the *in vitro* tests was carried out on the basis of statistical processing using Graph Pad Prism software version 5.0. The comparison of the different groups was carried out using One way ANOVA, followed by the Dunett multiple comparison test (treated groups vs control). The differences were considered to be statistically significant at p < 0.05 compared to the control.

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3. RESULTS

3.1. *In vitro* antioxidant activities

3.1.1. DPPH radical scavenging activity

DPPH radical scavenging activity of all the extract/fractions from *S. senegalensis* leaves is given in Figs. 1-4.

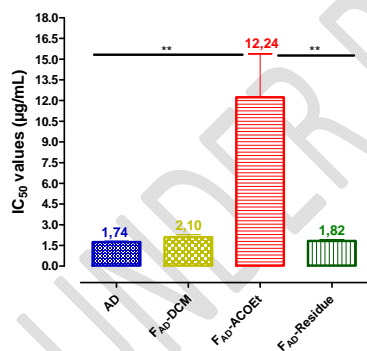


Fig. 1. IC₅₀ of AD and fractions from *S. senegalensis* leaves.

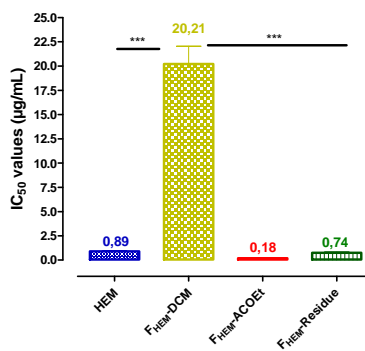


Fig. 2. IC₅₀ of HEM and fractions from *S. senegalensis* leaves.

Results are mean ± E.S.M, n = 6, **p<0.01, *** p<0.001 compared all pairs vs column (one way ANOVA analysis followed by Dunnett's test)

F_{AD}-ACOEt deliberated the highest scavenging activity (IC₅₀ = 12.24 ± 5.42 µg/mL) among the extract/fractions of the leaves of *S. senegalensis*. The IC₅₀ value recorded for DPPH radical scavenging of Trolox was 0.043 ± 0.004 µg/mL. Based on the IC₅₀ values for DPPH radical scavenging the extract/fractions of *S. senegalensis* leaves can be arranged in order of F_{AD}-ACOEt >

$F_{AD-DCM} > F_{AD-Residue} > AD$. The extract/fractions exhibited a dose dependent response for the DPPH radical scavenging activity (Fig. 1-2).

3.1.2. Reducing power assay

The results of the present study showed the dose dependent response for reducing power of the various extracts/fractions of *S. senegalensis* leaves and the results were expressed as trolox equivalent mmol/g sample. **Figs. 3** and **4** respectively illustrate the reducing effect of the ferricyanide complex of Fe^{3+} to the ferrous (Fe^{2+}) of extracts/fractions from *S. senegalensis* leaves.

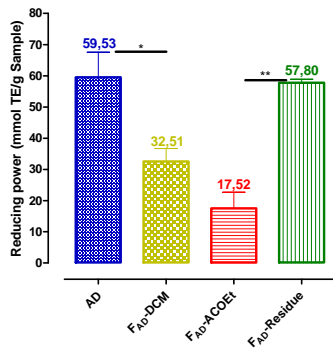


Fig. 3. Reducing power of AD and fractions from *S. Senegalensis* leaves

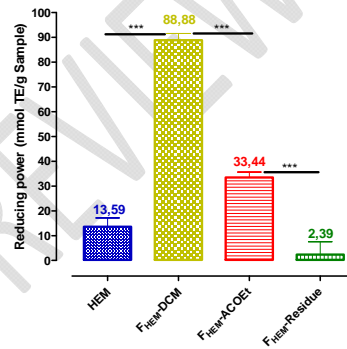


Fig. 4. Reducing power of HEM and fractions from *S. Senegalensis* leaves

In this study the FHEM-DCM at 88.88 mmol TE/ g concentration exhibited the highest reducing power. The reducing power of the extract/fractions at 1 mg/mL can be arranged in order of $F_{HEM-DCM} > AD > F_{AD-Residue} > F_{HEM-AcOEt} > F_{AD-DCM} > F_{AD-AcOEt} > HEM > F_{HEM-Residue}$ mmol trolox equivalents/g sample.

3.2. Anti-inflammatory activity

The anti-oedematous effect of aqueous decoction and hydroethanol macerate has been evaluated at doses of 200 400 and 600 mg/kg. The edema inhibition percentages and volumes are shown respectively in the Table 1 and in the Figs. 5-6.

Table 1. Effect of oral administration of AD and HEM from *S. senegalensis* leaves on carrageenan-induced hind paw edema.

Doses (mg/kg b.w)		Inhibition (% b.w)		
		1H	3H	5H
AD	200	18.85	29.08	30.81
	400	28.03	37.64	62.27
	600	36.97	46.21	72.71

	200	27.29	30.27	34.18
HEM	400	35.48	62.74	62.60
	600	38.95	45.41	70.62

Fig. 5 and 6 show the evolution of edema (mL) according to AD, HEM extracts and the standard acetylsalicylic acid on the paw as a function of time (h).

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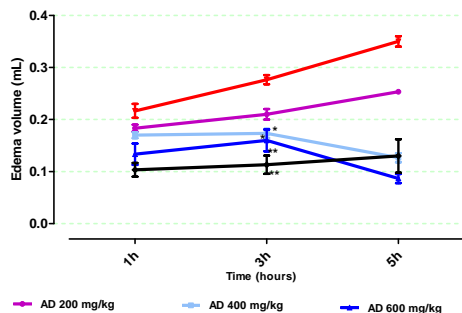
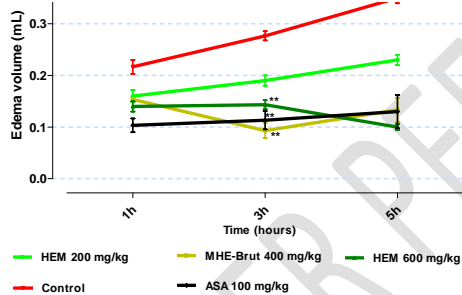


Fig. 5. Effect of AD at different concentrations and AAA on carrageenan-induced hind paw edema

Fig. 6. Effect of HEM at different concentrations and AAA on carrageenan-induced hind paw edema



Values are mean \pm S.E.M. $n = 6$. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ compared with control normal group (one way ANOVA analysis followed by Dunnett's test).

DA and MHE showed inhibition dose-dependent (Table 1). The results of the i.p administration of extracts have similar inhibition percentages to the different hours of measurement of the paw volume. For example, inhibition percentages of 72.71% and 70.62% respectively for AD and MHE at the dose of 600 mg/kg at the fifth hour.

3.3. Analgesic activity

The results of the analgesic effect of the aqueous decoction and the hydroethanolic extract are recorded in the Table 2 below.

Table 2. Analgesic effect of AD and HEM from *S. senegalensis* leaves

Extract	Doses	Number of	Inhibition (%)
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	(mg/kg b.w)	contortions	
Control	---	66.33 ± 1.50	---
	100	41.17 ± 2.13***	37.94
	200	32.83 ± 1.94***	50.50
AD	400	17.33 ± 1.36***	73.87
	---	67.50 ± 1.04	---
	100	30.67 ± 1.86***	54.57
HEM	200	21.50 ± 2.07***	68.15
	400	15.33 ± 1.36***	77.28

Comment [W18]: CONTRACTIONS

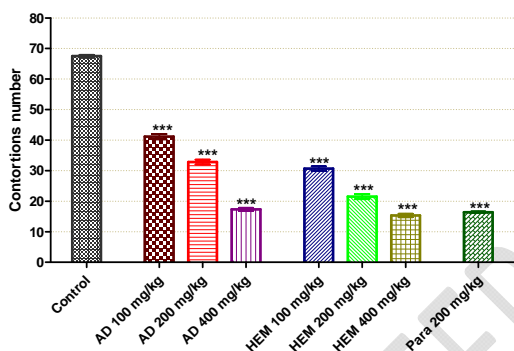


Fig. 7. Analgesic effect of AD and HEM from *S. senegalensis* leaves on contortions number induced by acetic acid

Values are mean ± S.E.M. n = 6. ***: P<0.001 indicate significance compared with control normal group (one way ANOVA analysis followed by Dunnett's test)

Antipyretic effect of various extracts/fractions of the leaves of *S. senegalensis* was assessed in mice. AD at 400 mg/kg exhibited strong antipyretic activity and decreased the number of contortions from 66.33 ± 1.50 to 17.33 ± 1.36 after administration to mice. The decrease of the number of contortion concentration dependent from 66.33 ± 1.50 to 15.33 ± 1.36 was recorded for HEM at 400 mg/kg after administration to mice (Fig. 7).

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3.4. Activity on isolated organ

Additional cumulative concentrations of decoction in the vat induced an increase in the amplitude of the basic contractions of the isolated duodenum. The following table gives the percentage of this amplitude variation.

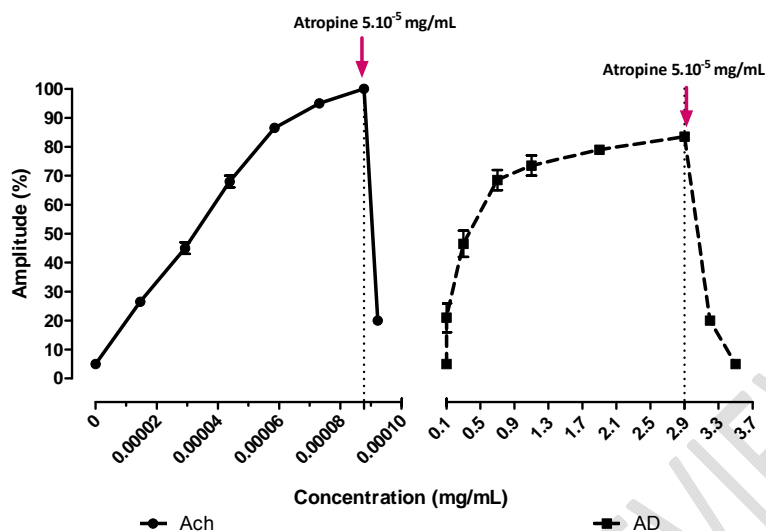


Fig. 8. Recording the effect of atropine on contractions of isolated rat duodenum induced by Acetylcholine and different concentrations of aqueous decoction of *S. senegalensis*

Acetylcholine and AD of *S. senegalensis* resulted in a concentration-dependent increase the amplitude of spontaneous contractions of the isolated rat duodenum. This effect is inhibited by atropine, an acetylcholine antagonist.

4. DISCUSSION

The antioxidant activity of the *S. senegalensis* extract was evaluated *in vitro* by using the DPPH and FRAP tests. In methanol, DPPH occurs as a free radical of purple color that becomes weak after acquiring the proton of the antioxidant. Thus, measuring the reduction of the color intensity of the methanolic DPPH solution can be used to evaluate the ability of antioxidants to give the proton. In this study, for AD and its fractions, AD showed maximal radical-scavenging activity with the lowest IC₅₀ value (1.74 µg / mL), followed by the residual fraction (1.82 µg/mL) with in the order of IC₅₀ DA < F_{AD}-Residue < F_{AD}-DCM < F_{AD}-AcOEt (Fig. 1). As for the hydroethanolic extract and its fractions, the AcOEt fraction showed a better free radical elimination activity with the lowest IC₅₀ value (0.18 µg/mL) followed by the residual fraction (0.74 µg/mL), in the order of IC₅₀ F_{HEM}-AcOEt < F_{HEM}-Residue < F_{HEM}-DCM (Fig. 2)

HEM exhibited good scavenging ability which may be due to donation of an electron or hydrogen to stabilize DPPH free radicals. In addition, in the present study, *S. senegalensis* extracts showed a powerful antioxidant activity on the FRAP assay which measures the ability to reduce the ferricyanide complex of Fe³⁺ to the ferrous (Fe²⁺) form through donating a hydrogen atom. Antioxidant potential of a plant extracts might be due to its richness in polyphenols and flavonoids [14, 15]. These bioactive compounds are known by their redox properties and chemical structure, which might play an important role in chelating transition metals and scavenging free radicals [16].

Carrageenan-induced paw edema is a frequently test used method to assess anti-inflammatory activity of composites or natural products. The injection of carrageenan into the subplanar surface of right hind paw of mice has induced edema which is measurable and whose evolution is determined according to the dose and the time of products administration.

The inflammatory response induced by carrageenan is considered to be a biphasic model [12, 17], which leads to the essential characteristics of inflammation, oedema, hyperalgesia, and erythema. As shown in Figs 5-6 during the initial phase (0-1 h) activated mast cell release histamine, bradykinin and serotonin which mediates the increased synthesis of prostaglandins from surrounding tissues of the injured site. During the 2nd phase (1-6 h), characterized by the elevated level of prostaglandins mediated by the elevated release of leukotrienes and bradykinin. During this phase the cyclooxygenase-2 (COX-2) converts arachidonic acid into prostaglandins which is a key factor of inflammation maintenance. In this experiment carrageenan induced edema in the hind paw of rats was inhibited all time by the extracts although the inhibition is more intense at 5 h. AD significantly inhibited the edema among 3 h - 5 h in the order of 62.27% and 72.71% respectively at 400 and 600 mg / kg.pc (Table 1). This inhibition is similar to that of the MHE in the order of 62.60% and 70.62% respectively at doses of 400 and 600 mg / kg.pc (Table 1). These results indicate that the phytochemical constituents present in MHE and AD inhibit the inflammatory mediators of the initial phase and the late phase of carrageenan-induced inflammation. Moreover phytochemical screening of AD and HEM has also shown the presence of flavonoids, tannins, sterols and terpenoids. Many studies are available which analgesic, anti-inflammatory and antioxidant.

The standard also had a highly anti-inflammatory potential after 2, 3 and 4 hours of injection of carrageenan into paw of mice. The acetylsalicylic acid (ASA), as the other NSAIDs, targets the COX-2 enzyme, thereby inhibiting the formation of paw edema. The anti-inflammatory results obtained with MHE and AD in both phases can be attributed to the action of anti-inflammatory agents such as sterols and terpenoids [18]. Steroids are established anti-inflammatory agents that inhibit the production of prostaglandins, not only by inducing the biosynthesis of the phospholipase A2 inhibitor, but also by increasing the level of cyclooxygenase/PGE-isomerase [19, 20]. The anti-inflammatory effects obtained by the MHE and AD extracts during the initial phase could be attributed to the presence of flavonoids and other constituents [9, 21]. Flavonoids are naturally occurring molecules with antioxidant, cytoprotective, and anti-inflammatory actions. However, the anti-inflammatory activities of these chemical components were not well defined [10, 22, 23]. Our studies are in agreement with Majid et al. (2015) [22] who obtained the same anti-inflammatory results on *Euphorbia dracunculoides*. As for Vezza et al. (2016) [10], they showed that flavonoids are effective in the treatment of intestinal inflammations. Indeed flavonoids have shown efficacy on experimental models and their mechanisms of action are similar to those described for drugs currently used in human therapy. They are known for their anti-inflammatory activity because of their effect on the metabolism of arachidonic acid and the release of histamine. They inhibit the production of prostaglandins and the cyclooxygenase expression [24]. Flavonoids also act on the expression of adhesion molecules and pro-inflammatory cytokines by various mechanisms, including the inhibition of transcription of nuclear factor by the inhibition of kinases involved in signal transduction [25]. Fisetin, kaempferol, myricetin, quercetin, and rutin five flavonoids, inhibited IgE or phorbol-12-myristate 13-acetate and calcium ionophore A23187 (PMACI)-mediated histamine release in RBL-2H3 cells. Also, these inhibited elevation of intracellular calcium. Gene expressions and secretion of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, and IL-8 were assessed in PMACI-stimulated human mast cells (HMC-1) [26]. The pharmacological actions of these flavonoids suggest their potential activity for treatment of allergic inflammatory diseases through the down-regulation of mast cell activation. As the coumarins and their derivatives have shown shrinking, anti-viral, anti-inflammatory and antioxidant effects, as well as the antimicrobial properties [27].

The decoction of *S. senegalensis* resulted in a concentration-dependent increase in the amplitude of spontaneous contractions of the isolated rat duodenum. This effect is inhibited by atropine, an acetylcholine antagonist (Fig. 8). The extract could therefore contain cholinomimetic substances. The anti-inflammatory effect of pharmacological substances with cholinergic action is described by several authors

The cholinergic anti-inflammatory pathway is a neural mechanism that inhibits pro-inflammatory cytokine release via signals that require the vagus nerve and $\alpha 7$ receptors [28-30]. Initial experiments established that acetylcholine attenuates the production of TNF, IL-1b, IL-6 and IL-18 by human macrophages at the post-transcriptional stage [31, 32]. The molecular link between the brain and the immune system in the cholinergic anti-inflammatory pathway is the nicotinic acetylcholine receptor subunit $\alpha 7$. Nicotine, the prototypical nicotinic acetylcholine receptor agonist, attenuates TNF release in LPS-stimulated human macrophages. Endotoxaemic $\alpha 7$ knockout mice develop significantly increased TNF levels in serum, spleen and liver as compared with wild type mice, indicating that the cholinergic anti-inflammatory pathway exerts tonic inhibition of cytokine production and functions as

Comment [W20]: PROINFLAMMATORY

an essential regulator of inflammation via $\alpha 7$ (Wang, 2003). This cholinergic antiinflammatory mechanism could be related to DA of *S. senegalensis* leaves. The presence of phytochemical groups that inhibit inflammation may explain this effect.

In acetic acid induced writhing test, DA and HEM exhibited significant results (Fig. 7). At 100, 200 and 400 mg/kg doses the abdominal constrictions were reduced to 37.94 %, 50.50 % and 73.87 % ($p < 0.001$) for DA and 54.57 %, 68.15 % and 77.28 % for HEM as shown in Table 2. Furthermore, the acetic acid-induced writhing test is an efficient analgesic model for screening metabolites against visceral inflammatory pain in mice [33]. Injection of acetic acid into the peritoneal cavity of mice induces a response characterized by contraction of the abdominal muscles accompanying an extension of the forelimbs and elongation of the body. These symptoms are believed to be mediated by the prostaglandin pathways [34]. Acetic acid causes abdominal constrictions by releasing certain endogenous chemical substances like histamine, serotonin, bradykinin and different prostaglandins which stimulate the pain sensitive neurons. These neurons are sensitive to pain relieving drugs like non-steroidal anti-inflammatory drugs (NSAIDs) and narcotics [18, 35].

In the present study, *S. senegalensis* extract produced peripheral analgesic activity in mice and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. These extracts, AD and HEM are rich in phenolic compounds; mainly flavonoids and phenolic compounds [21]. Flavonoids are known for their anti-inflammatory activity due to their influence on the metabolism of arachidonic acid and histamine release. Such metabolites can inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase cyclooxygenase, and phospholipase A2 [15, 36]. Such arachidonic acid inhibition by inflamed cells could reduce endoperoxides, prostaglandins, prostacyclin, and thromboxanes from the lipoxygenase pathway as well as hydroperoxy- and hydroxyeico-satetraenoic acids and leukotrienes from the cyclooxygenase pathway [37, 38].

5. CONCLUSION

The findings of this study support the view that medicinal plants are promising sources of potential antioxidants, anti-inflammatory and analgesic agents that may be effective for therapy of human diseases. Phytochemical screening of the aqueous decoction and hydroethanolic macerate of leaves of *S. senegalensis* revealed a rich variety of antioxidant, anti-inflammatory and analgesic compounds present in leaves in particular saponins, flavonoids, tannins and sterols terpenoids. The results presented here should encourage the use of these plants for medicinal health and nutraceutical applications, due to their antioxidant and anti-inflammatory properties.

ETHICAL APPROVAL

The laboratory experimentation was carried out according to the experimental protocols validated by the MEPHATRA-PH laboratories and meeting the international standards in this field (guidelines established by the European Union on the protection of animals, CCE Conseil 86/609). These experiments were carried out on the mouse and did not concern in any case the human subject. These protocols are ethical to experiment on laboratory animals.

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