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In vivo and *In vitro* Anti-inflammatory and Free Radical Scavenging Activities of Methonolic Extract of Different Parts from *Nauclea vanderguchtii* De Wild *(Rubiaceae)*

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Authors' contributions

This work was carried out in collaboration among all authors. Author CYC carried out all the experiment of the manuscript. Author NEL coordinator, had managed student in technical experiment, had written and read the manuscript. Author BZC senior lecturer lab group. They help to realize the experiment of the manuscript. Author MFAL student lab group. They help to realize the experiment of the manuscript. Author MAJ student lab group. They help to realize the experiment of the manuscript. Author AAGB collaborators, helped to choice the plant of the manuscript. Author DAB supervisor and chief of laboratory, had revised, corrected and read the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Nauclea vanderguchtii (N. vanderguchtii)* species belongs to the family Rubiaceae and it is widely used in traditional Cameroonian medicine against inflammatory diseases such as arthritis, rheumatism and gastric disorders. The present study was aimed to evaluate anti-inflammatory

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effect of a methanol extracts of leaves, stems, roots and barks with multiparametric analyses through *in vitro* assays and an *in vivo* model.
Methodology: Leaves, stems, barks and roots of *N. vanderguchtii* were air-dried and a methanolic extract was further obtained. Red blood cell membrane stabilization and protein denaturation were carried out as screening assays for anti-inflammatory activity in vitro, following by Diphenyl picryl-hydrazyl (DPPH), 2,2'- azino-bis – (3 - ethylbenzothiazoline -6- sulfonique (ABTS) and Ferric Reducing Antioxidant Power (FRAP) antioxidant activity. Furthermore, the anti-inflammatory capacity of leaves and stems methanolic extract was evaluated *in vivo* by carrageenan-induced oedema.
Results: Each part of *Nauclea vanderguchtii*, effectively and significantly stabilized red blood cell membrane. The methanol leaves extract exhibited better effect (53.12%), followed by stems (50.55%), barks (50.98%) and roots (49.6%) compare to an ibuprofen (51.16%), a standard reference drug. Those extracts also inhibited the denaturation of the egg albumin ($P < .05$; $P < .01$). Methanol stem and leaves extracts from plant were the effective scavengers of ABTS - radical (95.92 ± 0.37%), DPPH - radical (91.12 ± 0.13%). FRAP of methanolic extract was concentration-dependent. Moreover, methanol leaves extract of *Nauclea vanderguchtii*, significantly ($P<.01$) prevented paw edema with the maxima 58.97% (200 mg / kg).
Conclusion: This study shows that *N. vanderguchtii* extracts possess significant anti-inflammatory and antiradical activities. These activities are more pronounced in leaves than other parts of plant and justify the traditional use.

Keywords: Nauclea vanderguchtii; anti-inflammatory; in vitro; in vivo; free radical.

1. INTRODUCTION

"Inflammation is the defense response of living tissues to injury, infection or irritation" [1]. Its objective is to recognize and eliminate foreign substances from the body. Inflammation can manifest as various characteristic symptoms such as Calor, Dolor, Rubor, Tumor [2]. "Proteins denaturation is a well-documented cause of inflammation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Inflammatory diseases are associated with the chronique release of cytokines and reactive oxygen species. The reactive oxygen and nitrogen species may further increase the tissue injury" [3]. "There is much evidence shown that the production of reactive species occurs at the site of inflammation and contributes to the tissue damage" [4].

"The are many anti-inflammatory drugs to treat the consequences of inflammation belonging to steroidal or non- steroidal inflammatory drugs. However, studies suggest that these drugs are not free from adverse effects, as they are responsible for gastro intestinal complications such a mucosal damage and bleeding" [5]. "Moreover, non- steroidal inflammation drugs can also cause acute renal failure" [6]. "For these reasons, many researchers have shifted their focus on finding the medicinal plants, which have gotten the anti-inflammatory property and can serve as potential ingredient for future drug development" [7].

Nauclea vanderguchtii is a tree grows along streams in the forest as well as in swampy areas. It is found in southern Nigeria, Democratic Republic of the Congo, Cabinda in Angola, Ghana, Liberia, Gabon and in southwestern Cameroon [8]. The bark, leaves and roots are used to treat venereal diseases. Its bark is used to treat arthritis, rheumatism, gastric disorders, also used as a sexual stimulant to regulate the menstrual cycle [8]. Parts of tree are particularly used for the treatment of malaria but also to the management of dysentery, diarrhea, gonorrhea and typhoid [9]. The phytochemical study conducted on the leaves, bark, stems and roots of *Nauclea vanderguchtii* by Nkouayeb et al.[10] led to the isolation of compounds including a citric acid derivative, an alkaloid and triterpenes. Those compounds have been reported to possess anti-inflammatory effect by acting on mediators and/or enzymes of inflammatory. The present study was undertaken to evaluate *in vitro, in vivo* anti-inflammatory and free radical properties of methanolic extracts from different parts of *Nauclea vanderguchtii*.]

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The fresh bark, leaves, stems and roots of *Nauclea vanderguchtii* were collected in Solé (4.589098, 9.801983), a locality in the Littoral region, Cameroon. Authentication was carried out at the National Herbarium (Yaoundé, Cameroon), where a Voucher specimen (N.49316HNC) was deposited. Different parts of the plant were air-dried. 3.5 kg (bark), 1.6 kg (stems), 1.5 kg (leaves) and 1.5 kg (roots) were ground separately into a fine powder. Resulted powder was macerated in methanol 70% (15 L, 10 L, 10 L and 10 L respectively) at room temperature for 72 h. The mixture for each part was filtrated. The filtrate was concentrated at 40◦C under reduced pressure to yield 439.0 g, 34.4g, 110.2 g and 127.2 g respectively, of Nauclea vanderguchii extract which was stored in the refrigerator at 4°C for the duration of our study.

2.2 Animals

In vivo experiments were performed on adult Wistar rats (45-60 days old, 120 ±10 g) bred in the animal facility of the Laboratory of Biology and Physiology of Animal Organisms of the University of Douala. Rats were housed under optimum conditions of temperature (22 ± 2 ◦C) with an alternating cycle (12 h light/dark). Animals were supplied with standard laboratory diet and water. They were divided into 6 groups of 5 rats each for the experiment. All animals were fasted overnight before dosing, tap water being available ad libitum.

2.3 *In Vivo* Anti-inflammatory Effect

”In vivo anti-inflammatory activity of leaves or stems methanol extracts of *N. vanderguchtii* has been studied using the method of” [11]. Acute inflammation was produced in all groups by a single subplantar injection of 0.1 ml of freshly prepared 1% carrageenan in normal saline in the right hind paw of rats. Group I (untreated) served as a control and received by oral route distilled water (10 ml/kg), groups II, III (assays groups) received methanol leaves extract and groups IV, V (assays groups) received stems methanol extract at 100 or 200 mg.kg-1 b.w. Group VI received standard reference drug ibuprofen (200 mg.kg-1 b.w). The extracts and ibuprofen were administered 30 min prior to carrageenan injection. The paw volume of hind was measured before (V0), at 1/2 h, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h (Vt) after carrageenan injection using a plethysmometer. The reduction in edema was calculated by comparison with control. Anti-inflammatory activity was expressed as a percentage reduction of edema compared to control according to the following formula: [(vt - vo) control - (vt - vo) treated] x 100/ (vt - vo) control [11]. where vt is the average volume of each group and vo is the average volume obtained for each group before any treatment.

2.4 *In vitro* Anti-inflammatory Effect

2.4.1 Egg albumin denaturation test

“The inhibition of egg protein denaturation was estimated by the protocols previously described by Mizushima and Kobayashi [11], with slight modification” [12]. A volume of 5 mL of reaction mixture was prepared by adding 0.2 mL of fresh chicken egg albumin to a phosphate buffer solution (2.8 mL, pH 6.4) and 2 ml of extracts concentrations (5, 10, 25, 50, 100, 200 and 500 µg.ml-1). An equal volume of distilled water served as a control. Reaction mixture was incubated at 37°C for 15 min, following by heating at 70°C for 5 min. After cooling, absorbance of reaction mixture was recorded at 660 nm. Ibuprofen was taken as standard. The process was carried out in triplicates. The percentage of inhibition of albumin denaturation was calculated using the following formula: PI = (Abs test/Abs control -1) / Abs control X 100

Where $Abs_{control}$ is the absorbance of control; Abs_{test} is the absorbance of sample extract

2.4.2 Red blood cell membrane stabilization test

Initially, blood samples were taken from rat and immediately shifted into the heparinized tubes. “The tubes were washed three times with normal saline. After centrifugation (3000 rpm, 5 min), red blood cell layer were collected and diluted with isotonic buffer solution (10 m M sodium phosphate buffer, pH 7.4) to make 2% v/v suspension” [13, 14]. After that, reaction mixture (4.5 ml) was prepared by adding test sample (1 ml) of varying strengths (5, 10, 25, 50, 100, 200 and 500 µg.ml-1), red blood cells suspension (2% v/v, 0.5 ml), 1 ml of sodium phosphate buffer 10 mM, pH 7.4 to 2 ml solution of hypotonic sodium chloride (NaCl) (0.25% w/v). Subsequently, reaction mixture was incubated

(56 °C, 30 min) in a water bath, following by centrifugation (5000 rpm, 10 minutes) at room temperature. Clear supernatant was collected, and absorbance of the released hemoglobin was recorded at 540 nm using a spectrophotometer. Ibuprofen was considered as the standard, while phosphate buffer solution was taken as control [15]. The experiment was carried in triplicate. Membrane stabilization percentage was determined as follow: PI= (100- Abs $_{test}$- Abs $_{standard}$/Abs $_{control}$) x 100 [15].

Where Abs $_{control}$ is the absorbance of control; Abs $_{test}$ is the absorbance of sample extract, Abs $_{standard}$ is the absorbance of standard.

2.5 *In vitro* Antioxidant Activity

2.5.1 DPPH radical - scavenging assay

The DPPH scavenging property of methanolic extracts of parts of N vanderguchutii was carried out as described by Liyana-Pathirana and Shahidi [16]. A volume of 1 ml of DPPH solution (5 mg DPPH in 40 ml methanol), was mixed with 1ml of various concentrations (5, 10, 25, 50, 100, 200 and 500 µg.ml-1) of methanol extract of different parts of plant. The mixture was incubated at room temperature for 30 min in dark conditions. The reduction of the DPPH free radical was measured at 517 nm by a spectrophotometer. The experiment was replicated in three independents assays. Ascorbic acid was used as the positive controls. The ability to scavenging the DPPH radical was calculate using the following formulae: DPPH radical scavenging activity (%) = [(OD $_{control}$ - OD $_{assay}$) / DO $_{control}$] x 100 where OD $_{control}$ is an optic density of control, OD $_{assay}$: optic density of sample.

2.5.2 ABTS radical-scavenging assay

To determine ABTS radical scavenging power, the method of Zou et al. [17] was adopted. The stock solution included 2.45 mM potassium persulfate solution and 7mM ABTS solution. Those two solutions were stocked in dark conditions during 16 h before used. The working solution was then prepared by mixing the two stock solutions. The resulting solution was then diluted by tampon buffer (0.2 M, pH 7.4) containing 150 mM of NaCl to obtain an absorbance of 1.5 at 734 nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1ml) were allowed to react with 2.5 ml of ABTS solution and the absorbance was recorded at 734 nm after 7 min using the spectrophotometer. The ABTS scavenving capacity of extract was compared with that of trolox and percentage inhibition was calculated as ABTS scavenging activity (%) = (Abs $_{control}$ – Abs $_{assay)}$ / Abs $_{control}$ x 100

Where Abs $_{control}$ is the absorbance of ABTS radical + methanol; Abs $_{assay}$ is the absorbance of ABTS radical + sample extract/ standard

2.5.3 Ferric reducing antioxidant power

Method prescribed by Oyaizu [18] was used to determine the reducing antioxydant power of methanol extract of different part of the plant. 1.0 ml of different concentrations of extract was mixed with 2.5 ml of phosphate buffer (0.2M; pH: 6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5ml of trichloroacetic acid (10%) was added and centrifuged for 10 min. From the upper layer, 2.5ml solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%). Absorbance of all the sample solutions was measured at 700 mm. Ascorbic acid is used as a positive control.

2.6 Statistical Analysis

The data were expressed as a mean ± SD. Statistical analysis was performed, using GraphPad Prism 5. software. One-way Analysis of variance (ANOVA) followed by the Bonferroni post-field test was used to compare the means of the treated groups with that of the control group. Value less than 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Anti-Inflammatory Effect of *Nauclea Vanderguchtii* Extracts

3.1.1 Effect of *N. vanderguchtii* methanol extracts on rat red blood cell membrane stabilization

Inflammation is a complex series of functionally protective events and responses that develop when an organism is injured by mechanical or chemical agents [19]. “Stabilization of red blood cell membranes from hypotonicity-induced lysis is regarded as proxy for this lysosomal membrane stabilization that results in anti-inflammatory activity of drugs” [1]. Extracts from

different parts of plant tested at seven different concentrations (5, 10, 25, 50, 100, 200 and 500 µg.ml-1) showed red blood cell membrane stabilization percentage which were different depending of plant part (Fig. 1). Leaves methanolic extract provided the best membrane stabilization at all concentrations. "One vital step in controlling inflammatory responses is the stabilization of the lysosomal membrane of activated neutrophils, which limits the release of lysosomal constituents (protease and bactericidal enzymes) that are responsible for tissue inflammation and damage" [20]. "Red blood cell and lysosomal membranes have similar components, so drug stabilization of the red blood cell membrane may also inhibit destruction of lysosomal membrane" [21].

Overall, the membrane stabilization percentages of the extract of the different parts increase notably with concentration of the extracts. The maximum percentage inhibition was 53.12% for leaves methanolic extract at 500 µg.ml-1 when compared to control group, followed by bark extract (50.98%), stems extract (50.55%) and finally roots extract which exhibited weak inhibition (49.6% at 500 µg.ml-1). The reference medicinal product, ibuprofen, induced a maximum stabililization of 51.16% at 500 µg. ml^{-1}.

3.1.2 Effect of *Nauclea vanderguchtii* methanolic extracts on chicken egg albumin denaturation

The Fig. 2 shows the effects of methanolic extracts from the leaves, stems, roots and bark of *N. vanderguchtii* on egg albumin denaturation. Denaturation of proteins is a well-documented cause of inflammation, it is a process in which proteins lose their tertiary and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. Extracts of *N. vanderguchtii* inhibited albumin denaturation in a concentration-dependent manner. The methanol leaves extract of *N. vanderguchtii* at all concentrations showed significantly (*P< 0.5),* the higher percentage of inhibition compare to other parts and ibuprofen used as standard. These percentages ranged from 33.20% (5 µg.ml-1) to 48.80%

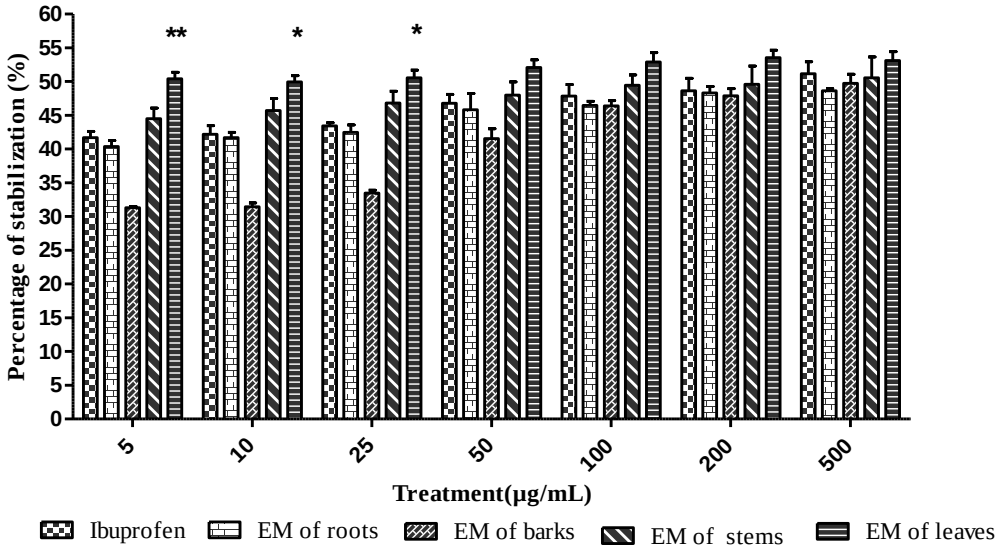

Fig. 1. Percentage of membrane stabilization of rat red blood cells at different concentrations of *Nauclea vanderguchtii* extracts

*Each bar represents the percentage of stabilization of each extract or standard at different concentrations. * P < .05 and ** P < .01 significant and very significant difference from control. n =3*

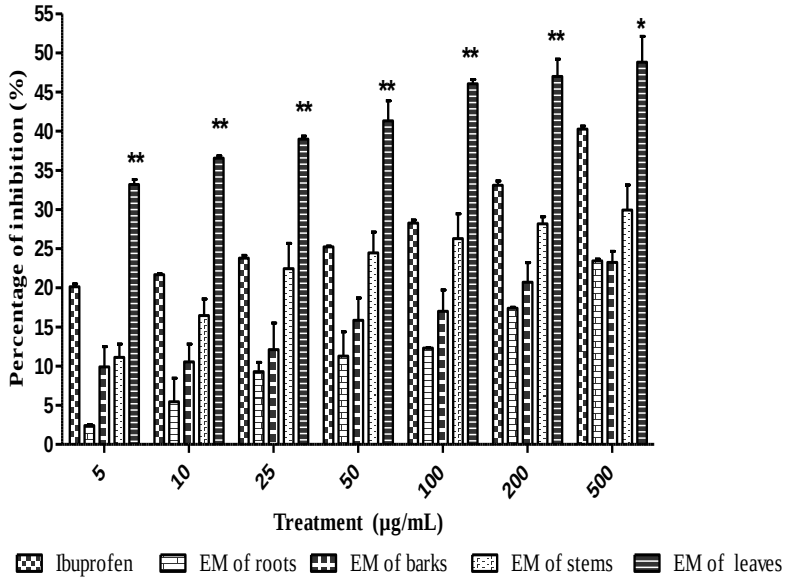

Fig. 2. Percentage of inhibition of egg albumin denaturation at different concentrations of *Nauclea vanderguchtii* extracts

*Each bar represents the percentage of inhibition of each extract or standard at different concentrations. * P < .05 and ** P < .01 being respectively the significant and very significant difference from ibuprofen*

(500 µg.ml-1) for leaves extract, while ibuprofen inhibition percentages ranged from 20.13% (5 µg.ml-1) to 40.26% (500 µg.ml-1). Methanol extracts from stems, roots and bark of *N. vanderguchtii* showed weak inhibitions than ibuprofen. Their maximum inhibition percentages were 29.92%, 23.44% and 23.20%, respectively at 500 µg.ml-1 compared to control. "The prevention of albumin denaturation in response to heat treatment at the physiological pH (pH: 6.2–6.5) is among one of attributes of non-steroidal anti-inflammatory drugs" [22]. "The change in disulphide, hydrogen, hydrophobic and electrostatic bonding are possibly one of mechanism involved in protein denaturation" [23]. "Indeed, alkaloids are able to interact with the aliphatic region around the lysine residue of the albumin protein and thus prevent protein denaturation" [24].

3.2 *In Vivo* Anti-inflammatory Effect of *N. vanderguchtii* Extracts

3.2.1 Effect of *N. vanderguchtii* methanol extracts on carrageenan-induced inflammation

The extracts that revealed the best results in vitro were evaluated in vivo on carrageenan-induced inflammation model in rats.

Table 1 shows the effect of *N. vanderguchtii* methanol extracts on carrageenan test, it appears that, the methanol leaves extract of *N. vanderguchtii* exhibited inhibition of swelling of 58.97% at 200 mg.kg-1 after thirty minutes (*P* < *.01*) compare to control. The stems methanolic extract of *N. vanderguchtii* caused of 42.3% and 46.15% reduction at 100 mg.kg-1 and 200 mg.kg-1 respectively after 30 minutes. Ibuprofen used as standard also inhibited very significantly paw edema with high inhibition percentages during the first phase of inflammation with a maximum of 80.77% at 30 min.

"Carrageenan-induced inflammation is a common in vivo model for the determination of active anti-inflammatory agents that resemble the inflammatory process found in humans" [24]. Intraplantar injection of 1% carrageenan induced an acute and gradual increase in the volume of the injected paw. This edema is the result of a sequential action and incorporates several inflammatory mediators whose release constitutes a triphasic event. "The first phase lasts about an hour after induction and is attributed to the release of histamine and serotonin, the second phase that goes from the second to the third hour is related to the release of kinins and, the third phase starting three hours after the injection of carrageenan is due to the synthesis of prostaglandins and leukotrienes" [25]. "These mediators increase the permeability of capillaries in the region. As a result, the exudate escapes from the bloodstream to the

interstitial space. This exudate is the cause of localized edema, which compresses the nerve endings and thus causes a painful sensation. The thickness of the edema reaches its maximum 6 hours after injection" [2]. In our results, maximum inhibition was obtained thirty minutes following dosing. "The obtained results indicating that, these extracts would have anti-inflammatory activity by inhibiting the release of inflammatory mediators namely serotonin and histamine. Histamine is the main mediator in the initial phase, increasing vascular permeability and acting on microcirculation" [26]. "Serotonin induces edema and plasma extravasation during acute inflammation, is mainly released by mast cells and platelets" [27].

3.3 Antioxidant Activity

Moussaid et al. [28] reported that, direct relation was observed between antioxidant and anti-inflammatory activity with extracts having good antioxidant activities shown to also alter the inflammation response. Antioxidant potential of plant extracts can be evaluated by employment widely accepted techniques like: DPPH, FRAP and ABTS.

3.3.1 DPPH radical scavenging activity

"DPPH is a stable, nitrogen centered free radical which produces deep purple color in methanol solution. Furthermore, it's simple, quick and accurate for measurement of the radical scavenging capabilities of experimental extracts" [29]. "the principle of these essays is based on the reduction of purple coloured methanolic DPPH solution in the presence of hydrogen donating antioxidant by the formation of yellow coloured DPPH" [29]. "The DPPH free radical scavenging test of the methanolic extracts of *N. vanderguchtii* was assessed (Fig. 3). The DPPH scavenging power of methanolic extracts increases significantly for concentration-dependent manner. It is also known that, the more antioxidant present in the extract, the more DPPH reduction will occur" [28]. The leaves methanolic extract of *N. vanderguchtii* showed higher activityof 91.12 ± 0.13% (IC_{50} of 3.86 µg/ml); than stems methanolic of (77.87 ± 1.07%) (IC50 of 11.67 µg/ml). The scavenging power of standard drug ascorbic acid was 96.07 ± 0.65% (IC50=3.15 µg/ml). In our study, extract of *N. vanderguchtii* scavenging power can be attributed to the presence of active compound in plant.

3.3.2 ABTS radical scavenging activity

The principle of ABTS assay is based on the inhibition of the absorbance of radical cation, ABTS, which is a characteristic wavelength at 734mn by antioxidants. The principle behind the technique involves the reaction between ABTS and potassium per sulphate to produce the ABTS radical cation (ABTS+) which is a blue-green chromogen. In the presence of antioxidant, reductant, the coloured radical is converted back to colourless ABTS. In our finding presented in Fig. 4, The stems methanolic extract of *N. vanderguchtii* is the effective scavengers of ABTS radical (95.92 ± 0.37%) followed by the leaves methanolic extract with scavenging power of 87.59 ± 2.68%), suggesting the production of ABTS radical cation. Positive control, Trolox showed the highest ability to scavenging ABTS radical of (97.86 ± 0.58%). The inhibitory concentrations (IC50) value of the leaves methanolic extract and stems methanolic extract of *N. vanderguchtii* were 7.56 µg.ml-1 and 6.95 µg.ml-1 respectively, while that of Trolox was 4.77µg.ml-1.

3.3.3 Ferric Reducing Power (FRAP)

"The ability of methanolic extracts of plant to reduce Fe 3+ in to Fe 2+ is shown in Fig. 5. For evaluation of total antioxidant power of experimental extract, Ferric Reducing Antioxidant Power (FRAP) assay is usually performed. This assay estimates the electron donating capacity of any compound based on the reduction of ferric ion into ferrous ion" [31]. "The reducing power of the methanol extracts of *N. vanderguchtii* was tested in vitro and the results obtained showed that leaves and stem extracts showed significant effect to reduce Fe 3+ in a concentration-dependent manner. we have noticed that at 500mg/mL, leaves methanolic extract is more active than the stems methanolic extract. The IC50 value of the methanolic extract of *N. vanderguchtii* is 178.5 µg.ml-1 and 179 µg.ml-1 respectively for leaves and stems, while that of ascorbic acid is 29.95 µg.ml-1, indicating that the antioxidant compounds are electron donors and reduce to oxidized intermediate of lipids peroxidation process, so that they can act as primary and secondary antioxidants" [32,33].

Table 1. Effect of methanolic extracts from *Nauclea vanderguchtii* on carrageenan-induced edema

Treatment	Doses (mg/Kg)	edema (∆V) in mL						
		30 min	1 h	2 h	3 h	4 h	5 h	6h
Distilled water	-	0.25 ± 0.01	0.29 ± 0.01	0.3 ± 0.01	0.32 ± 0.02	0.3 ± 0.03	0.28 ± 0.02	0.26 ± 0.02
Ibuprofen	200	0.08 ± 0.02 (80.77) **	0.06 ± 0.02 (68.07) **	0.08 ± 0.02 (63.91) **	0.12 ± 0.02 (43.65) **	0.13 ± 0.03 (31.36) *	0.14 ± 0.03 (26.32)	0.12 ± 0.02 (25.77)
N. vanderguchtii (Leaves)	100	0,05 ± 0.03 (58.97) **	0.09 ± 0.05 (52.1) **	0.13 ± 0.06 (42.11) *	0.12 ± 0.06 (41.27) **	0.13 ± 0.06 (35.59) *	0.14 ± 0.05 (27.19)	0.12 ± 0.06 (24.74)
	200	0.05 ± 0.01 (58.97) **	0.08 ± 0.02 (57.98) **	0.12 ± 0.04 (44.36) **	0.13 ± 0.05 (39.68) **	0.13 ± 0.04 (35.59) *	0.13 ± 0.05 (31.58)	0.12 ± 0.03 (28.86)
N. vanderguchtii (Stems)	100	0.07 ± 0.05 (42.3) **	0.12 ± 0.03 (39.5) *	0.16 ± 0.04 (27.07)	0.16 ± 0.04 (23.41)	0.15 ± 0.04 (22.88)	0.15 ± 0.03 (21.93)	0.13 ± 0.04 (21.65)
	200	0.07 ± 0.02 (46.15) **	0.12 ± 0.03 (37.82) *	0.14 ± 0.04 (35.34) *	0.15 ± 0.05 (27.78) *	0.15 ± 0.02 (25.42)	0.14 ± 0.04 (24.56)	0.12 ± 0.04 (23.71)

*Each value represents the average of the variation in the volume of paw edema ∆V (mL) ± ESM, n = 6. The values in parentheses represent the percentage of inhibition. * P < .05 and ** P < .01 being respectively the significant and very significant difference from contro*

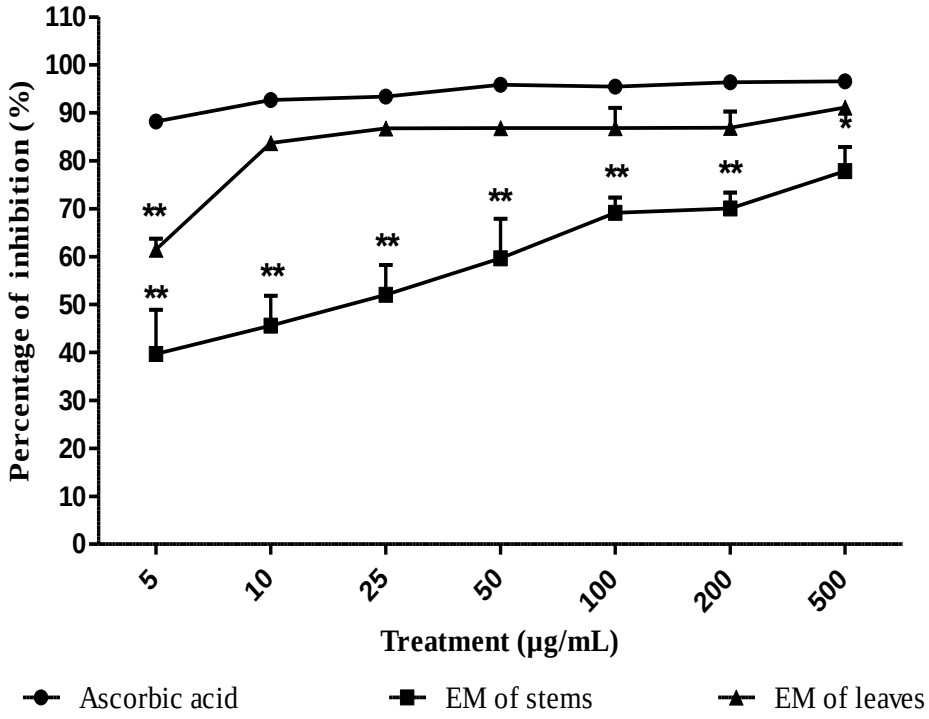

Fig. 3. DPPH free radical scavenging activity of *Nauclea vanderguchtii*

*Each point represents the percentage of inhibition of each extract or standard at different concentrations, ** p < .01 very significant difference from control, n=3*

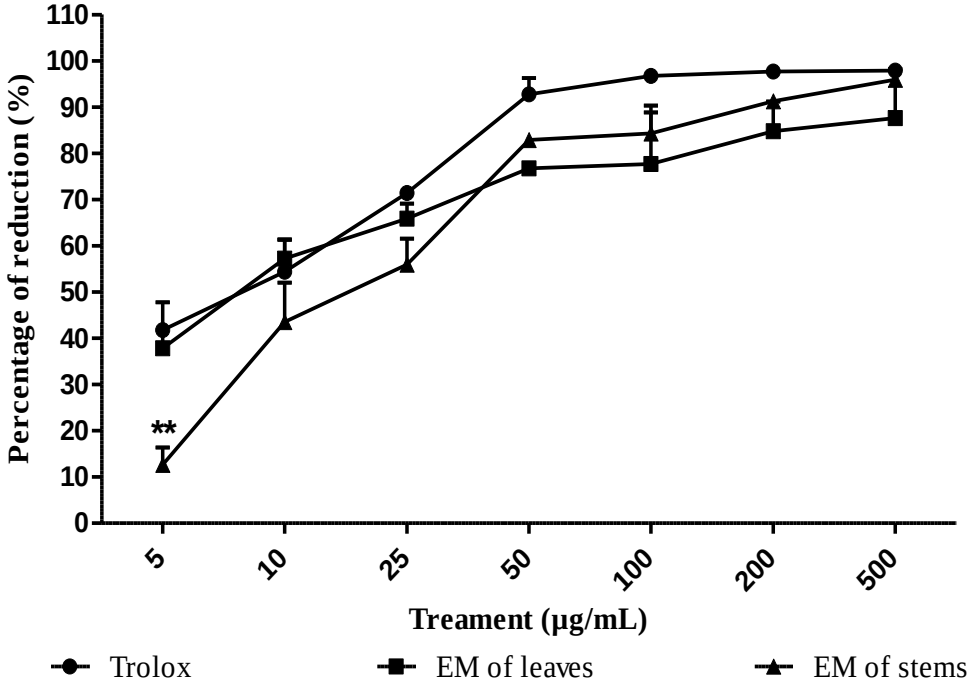

Fig. 4. ABTS free radical scavenging activity of *N. vanderguchtii*

Each point represents the percentage of inhibition of each extract or standard at different concentrations, n=3

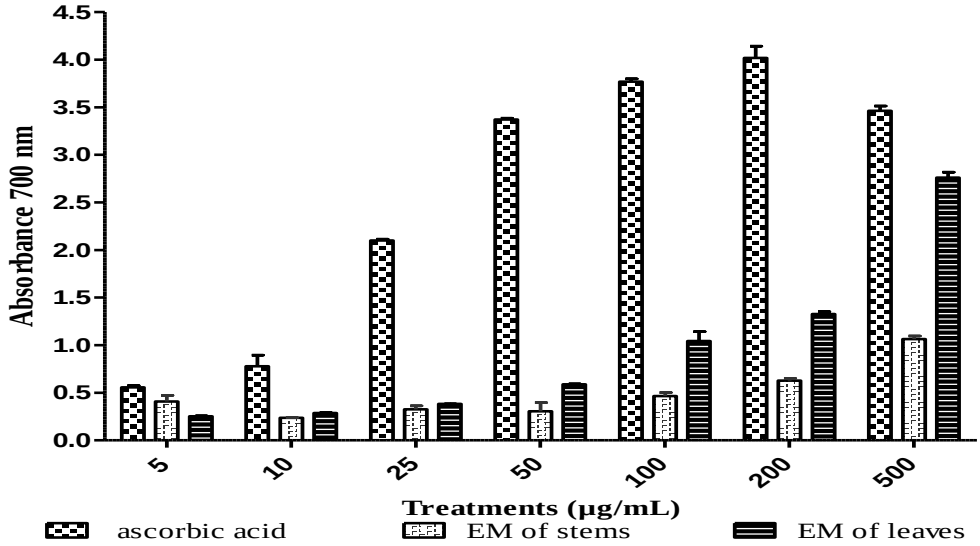

Fig. 5. Effect of extract *N. vanderguchtii* on Ferric Reduction Antioxidant Power
Each bar represents the percentage of inhibition of each extract or standard at different concentrations

4. CONCLUSION

The extracts of the different parts of Nauclea vanderguichtii have effects against edema of the rat's paw that could be explained by their ability to inhibit the denaturation of proteins or to protect the membrane of red blood cells, this effect would be supported by the anti-radical effect of the plant.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that ‘’The experiments were conducted in accordance with prior authorization for the use of laboratory animal obtained from the Cameroon National Ethical Committee (Ref N° CEI-2020 /0758)’’.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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