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Anti-Inflammatory Effect of Methanol Stem Bark Extract of *Parkia Biglobosa, Lannea Humilis* and Ko-888 In Wistar Albino Rats.

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Abstract
Background*: This research strived to establish the anti-inflammatory efficacy of methanol stem bark extract (MSBE) of Parkia biglobosa (PB) and Lannea humilis (LH) and a commercial tonic labeled KO-888 on Wistar albino rats using standard procedures.*
Materials and Methods*: Methanol stem bark extract (MSBE) of Parkia biglobosa (PB) and Lannea humilis (LH) was obtained by subjecting 50:50 dried and pulverized stem barks of both plants to soxhlet extraction. The commercial tonic (KO-888) was obtained from a distributor in Keffi. MSBE and KO-888 were also subjected to antioxidant studies using DPPH and phytochemical evaluations following standard protocols. The animal studies were performed using 30 adult Wistar albino rats induced with acute inflammation using carrageenan. The animal models were divided in groups of 3 rats each as follows; Group I= Untreated, II= MD KO-888 (1 ml/kg), III= HD KO-888 (2 ml/kg), IV= LDE (5 mg/kg), V= MDE (10 mg/kg), VI= HDE (20 mg/kg), VII= Diclofenac (7 mg/kg) and VIII= Normal.*
Results: *The result of the phytochemical screening showed copiously high concentration of alkaloids, glycosides, terpenoids, phenols and tannins in PB and LH as compared to KO-888. KO-888 had higher flavonoid than the extracts. In vitro antioxidant studies showed PB and LH had a high inhibitory activity at the least concentration (25 µg) when compared to KO-888. MSBE of PB and LH showed strong antiulcer potency at a low dose (5 mg/Kg), while that of KO-888 tonic was at a high dose (1 ml/Kg). Oral administration of MSBE and KO-888 was effective in reducing inflammatory edema induced by carrageenan in a dose-dependent manner. However, this anti-inflammatory effect was weak in the initial phase (zero hour) of edema but significant in the late phase third hour.*
Conclusion*: The result of the research establishes the anti-inflammatory efficacy of the plant extract and KO-888 and justifies the local use of the extracts (MSBE) and KO-888 tonic as traditional medicines/remedies. It is recommended that further studies be carried out on the medicinal plant extracts in a bid to isolated and characterize pure compounds for possible drug development.*
Keywords*: Antioxidant, Parkia biglobosa, Lannea humilis, KO-888, Inflammation, Phytochemicals, diclofenac.*

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I. Introduction

Plants have been used as a source of medicines for both humans and animals since time immemorial in crude forms such as decoctions, syrups, powders, infusions and ointments (Tugume and Nyakoojo, 2019). The importance of the study of substances obtained from plants cannot be overstated. Many conventional drugs used in therapy are obtained or conceptualized from plants sources (Egharevba, 2015). In the African sub-region, there is availability of a vast number of naturally occurring medicinal plants such as *Parkia biglobosa* (*PB*), *Lannea humilis* (*LH*), *Ficus thonningii* (Moraceae), etc (Egharevba, 2015).

Parkia biglobosa is a dicotyledonous angiosperm belonging to the family Fabaceae (Caesalpinioideae - Mimosoid clade), in the genus *Parkia* (worldagroforestrycentre.org, 2012). *Parkia biglobosa* is widely acclaimed by the Hausa communities of northern Nigeria for the treatment of diseases such as malaria, diabetes mellitus and pains. The stem barks are boiled in water and taken as a decoction for the treatment of malaria, inflammatory diseases and infections to diarrhea (Asase *et al.*, 2005). The bark soaked in ethanol is also

used in some communities for anti-diarrhoeal properties and as effective anti- snake venoms that protects against neurotoxic, haemotoxic and cytotoxic effects of poisonous snakes (Agunu *et al.,* 2001).

Lannea humilis is a deciduous shrub growing up to 3m tall (Burkil, 2004), occasionally becoming a tree with a flat or spreading crown that can grow up to 5m. The plant has a thick bole; it sometimes forms thickets (Ruffo *et al.,* 2002). The plant is gathered from the wild in heavy, compact and hard sandy soils as a local source of food, medicines, fibre and wood. A decoction of the stem bark of this plant is utilized in the treatment of sickness; hack, bodily torments intense looseness of the bowels, cholera, and asthma (Burkil, 2004; Ruffo *et al.*, 2002).

KO-888 is a commercial (sold in Nigeria and other African countries) blend of carefully selected potent herbs and roots that have been scientifically proven to prevent and cure common ailments.

KO-888 is brewed from the sap found in the *Aloe ferox* (*A. ferox*) plant which is grown in the Western Cape of South Africa. The use of KO-888 improves overall health and immune system and is used for the treatment of arthritis, gout, fibrosis, diabetes, migraines, insomnia, gall and kidney stones, throat infections, heart burn colic, stomach ulcers, bladder infections, haemorrhoids, shingles, constipation, osteoporosis, tonsillitis, eczema, hypertension, conjunctivitis and many other ailments (Personal Contact, 2020).

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli (Kumar *et al*., 2013). It is a protective reaction of body's cells to injury or infections and allergic or chemical irritation. It is characterized by certain inflammatory features which are redness, pain, swelling, heat, and loss of function because of the blood vessels dilation that leads to the increase of blood flow in that area, thus resulting in the migration of immune cells like neutrophils and macrophages, along with the fluids causing edema toward the inflamed regions. The process of inflammation is quite complex, initiated by several factors which include molecules that ranges from bacteria to chemical and therefore results in cellular trauma or death. Tissue injury induced by this trauma results in the inflammatory mediators release including reactive oxygen species (ROS) like superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), nitric oxide and cytokines (Kumar *et al*., 2017; Maier & Chan, 2002). Immune system disorders have been linked to increased expression of pro-inflammatory mediators, including cytokines, NADPH oxidase, NF kappa B, myeloperoxidase, and Inos (Rahman *et al*., 2004).

Pharmacological and physiological constituents of some herbal medicines are known to regulate and modulate various functions of inflammatory response in the body either directly or indirectly (Valko *et al*., 2007). Therefore, the need to develop newer and safer anti-inflammatory drugs still exists. In general, medicines derived from plants are perceived to be safer than their synthetic equivalents (Shaw *et al.,* 2012).

II. Materials and Methods

Equipment/Instruments

The major instruments used for this study include; colorimeter, rotary evaporator, soxhlet extractor, distillation set, spectrophotometer (Jenway, Uk), weighing balance (vickas ltd, England), Refrigerator (Thermocool, England), micropipette (Jenway, UK) and Vernier caliper.

Drugs and Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade.

Collection of Plant material

Fresh stem bark of *Parkia biglobosa* (PB) and Lannea humilis *(*LH) were obtained from Katakpa village in Toto Local Government Area in Nasarawa State, Nigeria. The plant was identified by a Taxonomist at the Herbarium section, Department of Plant Science and Biotechnology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

Preparation of Plant Extract

The stem bark of PB and LH were chopped and air-dried at ambient temperature for 2 weeks, after which they were pulverized to uniform powder using an aluminum electronic blender. The dried and powdered stem bark of PB and LH (500 g each) were extracted using soxhlet apparatus. The solvent was evaporated using a rotary evaporator and dried in an oven at 40°C. The dried extract was transferred into a vial and kept in a refrigerator until use. Tonic KO-888 was obtained from a commercial distributor in Keffi.

Experimental Animals

30 healthy, adult Wistar albino rats of either sex weighing 200 – 300g were used. The experimental animals were acclimatized to the laboratory conditions for 14 days as required by the Organisation for Economic Co-operation and Development (OECD) guidelines. The animals were housed in cages under standard husbandry condition (at temperature of 26 ± 2 ^{o}C, relative humidity 45-55 % and alternate cycle of 12 hours of dark and light) with free access to commercial pellet laboratory diet and water ad-libitum throughout the experimental period. All the procedures in the experiment were carried out according to the Guide for the

Care and Use of Laboratory Animals set by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

A pilot study was conducted and the results evaluated to ascertain successful inducement of acute inflammation before commencement of this study.

Anti-inflammatory Activity of MSBE and KO-888 on Carrageenan-induced Paw Oedema

The animals were divided into eight (8) groups of three (3) rats each as follows;

Group I= Untreated,
II= MD KO-888 (1 ml/kg),
III= HD KO-888 (2 ml/kg),
IV= LDE (5 mg/kg),
V= MDE (10 mg/kg),
VI= HDE (20 mg/kg),
VII= Diclofenac (7 mg/kg)
VIII= Normal.

The animals were pre-treated (except Group 1and Group VIII) with MSBE 1 hour before the administration of carrageenan. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of the rats. The different groups were treated with MSBE (5, 10 and 20 mg/kg), KO-888 (0.43 ml/kg), diclofenac (7 mg/kg) dissolved in normal saline and the paw volume was measured at 0 hour, 1 hour and 3 hour after carrageenan injection using a vernier caliper.

The anti-inflammatory activity (%) was: (1-D/C) x100

Where, D represents the percentage difference in paw volume after treatment agents were administered to the rats and C represents the percentage difference of volume in the control groups.

Statistical analysis

All results obtained from this research were analyzed and presented as mean ± SD and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 26. One-way analysis of variance (ANOVA) was used. Differences in mean were considered to be statistically significant at ($p \leq 0.05$).

III. Results

Qualitative and Quantitative Phytochemical Screening of PB, LH and KO-888.

The result of the Qualitative Phytochemical Screening (Table 1) between PB, LH and KO-888 showed presence of terpenoids, glycosides, alkaloids, flavonoids, tannins and phenols in copious quantity in PB and LH respectively.

Table 1: Qualitative Phytochemical screening of PB, LH and KO-888

Phytochemicals	PB	LH	KO-888
Tannin	++	++	++
Saponin	+	++	++
Flavonoid	++	++	+
Steroid	+	++	_
HCN	+	++	_
Glycoside	+++	++	_
Alkaloid	+++	+++	+ +
Phenol	++	++	-
Terpenoid	+++	+++	-

Keys; + means present, ++ means Abundant, +++ means Very Abundant while – means Absent Results.

Results are expressed in Mean ±SD (n=3). Mean values with different letters as superscripts down the column are considered significant at P<0.05

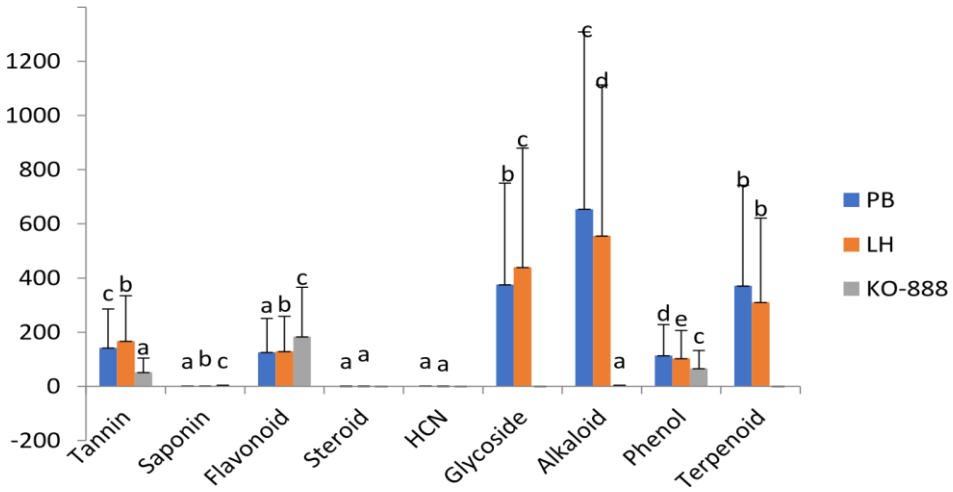

Figure 1: Quantitative Phytochemical screening of PB, LH and KO-888

In-vitro DPPH Radical Scavenging properties of PB, LH and KO-888.

The *in vitro* Antioxidant Scavenging properties of *PB*, *LH* and KO-888 compared to the standard antioxidant (Ascorbic acid) using 2, 2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) showed that the methanol extract of *PB*, *LH* and KO-888 tonic varied in free radical scavenging activity. Unlike *PB* whose scavenging activity decreased with increase in concentration, *LH* and KO-888 activities were the reverse (their scavenging activity increased with increase in concentration). Therefore, *PB* - *LH* mix can be said to complement each other and act synergistically in exerting their pharmacological properties. KO-888 on the other hand, showed similar effect as *LH*. The pharmacological effect of KO-888 could be attributed to free radical scavenging of the tonic considering the fact that it is used over a long period of time in high concentration (30ml – 120ml per day) depending on the target ailment e.g. diabetes, ulcer, high blood pressure, hypercholesterolemia, etc. (Personal Contact, 2021).

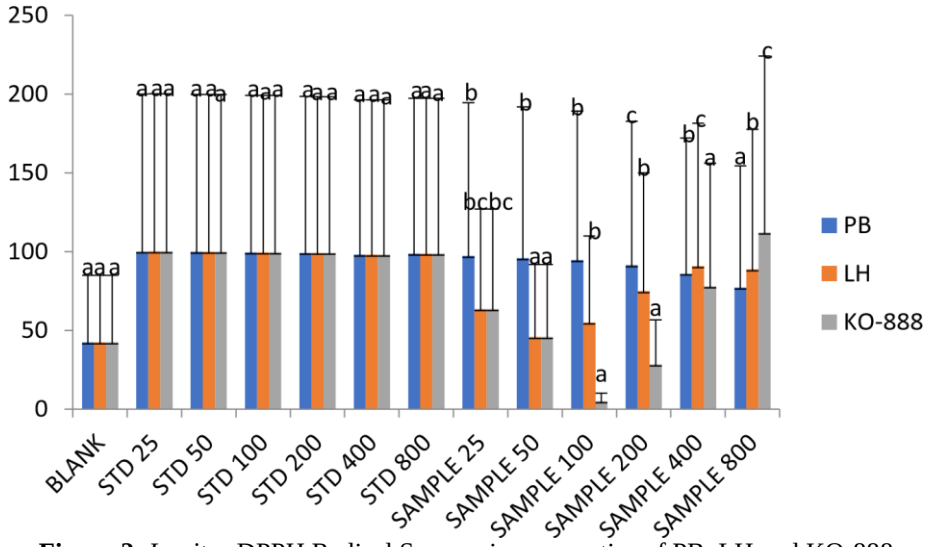

Figure 2: *In-vitro* DPPH Radical Scavenging properties of PB, LH and KO-888

Anti-Inflammatory Activity of MSBE of PB and LH and KO-888

Figure 3: showed the anti-inflammatory activity of MSBE and KO-888 on rats' paw. Results in the first hour after induction of edema showed significant decrease in oedema volume indicating the anti-inflammatory potency of MSBE and KO-888. Same was observed in the 3rd hour but was significantly higher compared to the untreated and normal groups respectively.

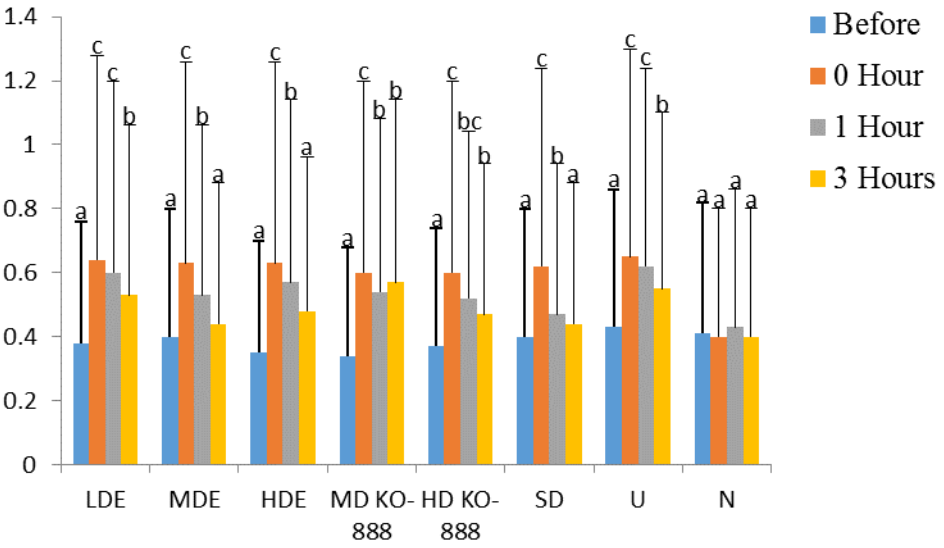

Figure 3: Anti-inflammatory Activity of MSBE and KO-888

IV. Discussion

The results of the phytochemical examination of the MSBE of *PB* and *LH* and KO-888 as shown in Table 2 indicated the availability of some bioactive components such as alkaloids, terpenoids, tannins, saponins, flavonoids, glycosides, steroids, phenols and hydrogen cyanide in varying quality and quantities. The same was present in KO-888 except steroid, hydrogen cyanide and glycoside. These results are in agreement with those of (Achika *et al.*, 2017) which showed the availability of these phyto-components for *LH*. These Phytochemicals or bionutrients plays an important role in the prevention of chronic diseases like diabetes, cancer, coronary heart disease and in the healing of wounds, serve as immunity potentiating agents and as neuro-pharmacological agents. Flavonoids which are normal anti-cancer agents are acquired chiefly from plants, and are utilized for the treatment of degenerative diseases (Ali *et al.*, 2008). The copiously high concentration of these phytochemical constituents in the stem bark of *LH* and *PB*, some of which are bioactive confirms the ethno-medicinal uses of these plants in the treatment of diseases such as stomach and/or ectopic ulcers, diabetes, inflammation and others.

The results of the *in vitro* DPPH free radical scavenging activity of *PB, LH* and KO-888 showed varying or differential antioxidant scavenging capacity when compared with standard antioxidants and with each other. Compared to the standard (Vitamin C), the DPPH radical scavenging activity of *PB* at lowest dilution of 25% was in close range of activity with the standard but *LH* had a significantly ($P < 0.05$) lower inhibitory properties compared with the standard antioxidant and with that of *PB*. KO-888 had no inhibitory activity at the least dilution.

Oral administration of MSBE and KO-888 was effective in reducing inflammatory edema induced by carrageenan in a dose-dependent manner. However, this anti-inflammatory effect was weak in the initial phase (zero hour) of edema but significant in the late phase third hour. Carrageenan-induced rats paw edema involves many mediators that induce the inflammatory response in two different phases (Sufian *et al*., 2017). The initial phase lasts approximately 1 hour 30 minutes after the injection of the carrageenan agent is attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability. A late phase, is the result of the overproduction of prostaglandins in the tissues, mediated by cyclo-oxygenase (COX) and which can continue beyond 3 hours after injection of the carrageenan (Huang *et al*., 2006). The inhibition of edema in the treated group was observed at the 3rdhour after carrageenan injection. This suggests that the inhibitory action of the extracts would be exerted more on the action of prostaglandins or cyclooxygenases (COX 1 and COX 2) responsible for the biosynthesis of prostaglandins. Carrageenan-induced edema is sensitive to cyclo-oxygenase (COX) inhibitors and lipo-oxygenase inhibitors (Traoré *et al*., 2019). Several studies reported the anti-inflammatory properties of phenolic compounds (flavonoids, tannins) and triterpenes through their inhibitory actions against prostaglandins release and natural factor-kappa B (NF-κB) activation (Adnan *et al*., 2020).

V. Conclusion

The present study demonstrated that the MSBE and KO-888 possess anti-inflammatory effects which may probably be related to the anti-inflammatory or free radical scavenging activity of the phytochemical constituents reported. This study justifies the traditional claims on folk medicine reported within and outside Nasarawa State, Nigeria.

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