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Anti-Inflammatory and Anti-Microbial Effects of *Prosopis Africana* Methanol Stem Back Extract

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Abstract

The anti-inflammatory and anti-microbial effect of *Prosopis Africana* methanol stem bark extract was determined. Results of qualitative phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids, phenols, steroids and terpenoids. The quantitative analysis revealed the quantity of alkaloids (0.11%), tannins (1.92%), phenols (3.77%), flavonoids (0.77%), steroids (0.14%), terpenoids (0.21%) and saponins (4.01%). The anti-inflammatory effect of methanol stem-bark extract of *Prosopis africana* was assessed in male albino rats using paw immersion method. The anti-inflammatory effect produced by methanol stem-bark extract of *Prosopis africana* was found to be significant ($p<0.05$) at the dose of 200mg/kg, which was more effective than other concentrations at time interval of 0-90minutes. The antimicrobial activities of the methanol stem-bark extract of *Prosopis africana* produced the highest zone of inhibition (17mm) on *Pseudomonas aeruginosa* and the lowest zone of inhibition (11mm) on *Staphylococcus aureus*. The minimum inhibitory concentration value of *Prosopis Africana* methanol stem-bark extract against *E.coli,* S. *aureus* and *P. areuginosa* was 2.5mg/ml each compared to *K. pneumonia* which was 5.5mg/ml. Results obtained indicate the antimicrobial effect of the methanol stem-bark extract of *Prosopis Africana* against *K. pneumoniae* was more effective than ampicillin. This work has further supported

the basis for the use of the plant as anti-inflammatory agent in the treatment of various diseases associated with the microorganisms studied.

Keywords: *Prosopis Africana,* anti-inflammatory, antimicrobial, *Pseudomonas aeruginosa, Staphylococcus aureus, E.coli* and *Klebsiella pneumonia*

INTRODUCTION

Medicinal plants continue to be an interesting source of natural products for treating various health conditions. It is estimated that more than 150,000 plant species have been studied, many of which contain valuable therapeutic agents (Nunes *et al*., 2020). Evidence based research supports the medical and pharmacological benefits of plant-derived compounds, with increasing interest in the identification and characterization of bioactive compounds from natural sources (Adebayo *et al.,* 2015).

Inflammatory diseases are considered major threats to human health worldwide (Akhtar, 2022). Inflammation is a ubiquitous process that happens in a disturbed state of homeostasis (Campos *et al*., 2014). Inflammation is usually triggered by damage to living tissues resulting from bacterial, viral, fungal infections, physical agents and defective immune response. (Virshette *et al*., 2019). Inflammation is the body's severe reaction to any kind of damage. Pain, redness, heat or warmth, and swelling are the four primary indicators of swelling. The arterioles in the surrounding tissue dilate when a part of the human body is injured. This results in increased blood flow to the affected area (Sami *et al*., 2021).

The fundamental aim of inflammatory response is to localize and eliminate the harmful agents; secondarily, to remove damaged tissue components to culminate in healing of the affected tissues, organs, or system. The resolution of inflammation is influenced by several anti-inflammatory mediators and the recruitment of monocytes for the removal of cell or tissue debris (Virshette *et al*., 2019). It is possible that the resolution may not occur in the acute phase, thereby turning into a chronic phase. Acute inflammation may be the body's first response to damaging stimuli. The inflammatory response is out of proportion in chronic inflammation, resulting in body harm. Cyclooxygenase (COX) is a major enzyme in the production of prostacyclins, prostaglandins and thromboxanes which play a role in inflammation, pain and platelet aggregation (Pilotto *et al*., 2010). The permeability (pore size) of these arterioles is also increased by vasoactive chemicals, allowing blood cells,

chemical substances, blood proteins and fluid to collect in that area. This fluid buildup produces swelling and can cause discomfort by compressing nerves in the area (Sami *et al.*, 2021).

Chronic inflammation plays a role in the burdens associated with pathological conditions in both developed and developing countries, particularly in African countries (Souza *et a*l., 2020). For instance, chronic inflammation is known to play a role in the development of obesity-associated diabetes secondary to insulin resistance. (Zhen *et al.*, 2015). Pain is an unpleasant sensory and emotional feeling accompanying existing or impending tissue damage or referenced to such damage (Swieboda *et al.,* 2013). Non-steroidal anti-inflammatory drugs are commonly prescribed for treatment of pain and inflammatory conditions. However, because many NSAIDs are associated with side effects such as gastrointestinal bleeding and suppressed function of the immune system, attention has been shifted to alternative pharmacotherapies (Adebayo *et al.,* 2015). These nonsteroidal anti-inflammatory drugs have been shown to reduce pain and inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme, thereby reducing the production of prostaglandin (Buhrmann *et al.,* 2011). However, there are medicinal plants with anti-inflammatory therapeutic effects with low or no side effects (Oguntibeju, 2018).

Prosopis africana (Guill & Rich) known as African mesquite, belongs to the family, mimosaceae (Leguminoceae). *Prosopis africana* is a perennial leguminous tree of the sub family *Mimosida*e and is mostly found growing in the savanna regions of Western Africa (Kolapo *et al.,* 2009). It is called kiriya in Hausa. It is very popular for its seeds, which in fermented form, is used as a food condiment. *Prosopis africana* is one of the many species of *Prosopis,* which have been reported to be of medicinal value. The potential uses of its gum for gels is used in tablet formulation in pharmaceutical industries. The plant has anti-tyrosine activity and is also useful in preventing skin whitening or as anti-browning agents (Atawodi *et al.*, 2009). Almost all parts of the tree are used in medicine, the leaves in particular is used for the treatment of headache and toothache as well as in wound care (Ezike, 2010). Leaves and bark are combined to treat rheumatism. Remedies for skin diseases, caries, fevers and eye washes are obtained from the bark. The roots are diuretic and are used to treat gonorrhoea, tooth and stomach-ache, dysentery and bronchitis (Weber *et al.,* 2008). In Mali the leaves, bark, twigs and roots are used to treat and relieve

bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps. In Ghana, boiled roots serve as a poultice for sore throat, root decoction for tooth ache. It is also used as chewing stick by Yorubas in south western Nigeria, bark as a dressing or lotion for wounds or cuts (Ayanwuyi, *et al.,* 2010). *Prosopis africana* is used for menstrual and general body pain in Nupe land in North central Nigeria, (Mann *et al.,* 2003).

This study was therefore carried out to investigate the anti-inflammatory and anti-microbial activities of methanol stem bark extract of *Prosopis africana*.

MATERIALS AND METHODS

Equipments

Water bath (HH-2B-SCIENTIFIC), Weighing balance (scoutpro SPV401,Chaus corporations, pine brook NJUSA), Incubator (Gallen kamp,size 2 model no. lH-100), Rotary evaporator (Decibel DB-3135II) and Electric oven (Warned sp-65G)

Drugs and Chemicals

Aspirin and Ampicilin that were used in this study were of pharmaceutical grade and were obtained from pharmaceutical company, (SKG-PHARMA LIMITED) and (MECUREINDUSTRIES LTD) respectively. Methanol, trichloromethane, formalin, ferric chloride, sodium hydroxide, chloroform, sulphuric acid, meyer's reagent, diethyl ether, muller hinton agar, folin phenol reagent, sodium carbonate solution, acetic acid, ethanol, petroleum ether and concentrated ammonium hydroxide were of analytical grade.

Test Organisms

Bacterial isolates were collected from the Department of Microbiology, School of Life Sciences, Modibbo Adama University Yola, Adamawa State, Nigeria. The microorganisms were stored on nutrient agar at 4°c prior to use. The bacterial isolates are:

Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia and *Pseudomonas aeruginosa*

Animal Source

Thirty (30) male albino rats weighing between 130 ± 10g were purchased from National Veterinary Research Institute (NVRI), Vom, Plateau State.

Collection and Identification of the Plant Stem Bark

The plant stem bark was collected from farms around Modibbo Adama University Yola, Nigeria. The plant stem was identified by a Botanist in the Department of Plant Science, Modibbo Adama University Yola, Adamawa State, Nigeria.

Preparation of the Extract

The plant stem bark was cut using kitchen knife, air-dried at room temperature (26°C) for 2 weeks, after which it was ground to a uniform powder using mortar and pestle. The methanol extracts was prepared by soaking 100g of the dry powdered plant materials in 500ml of methanol at room temperature for 24hours. The extract was filtered after 24hrs, first through a Whatmann filter paper and then through cotton wool. The extracts was concentrated using a rotary evaporator.

Animal Treatment

Thirty (30) male albino rats were used for the study and were allowed to acclimatize in standardized metal cage for 14days. The animals had free access to feeds (vital feeds Jos) and water. The rats were distributed into groups and housed in 5 cages (6 per cage). All animal procedures were in strict accordance with the NIH guide for the care and use of laboratory animals.

Phytochemical Screening

The qualitative phytochemical constituents in the methanol stem extract of *Prosopis africana* were determined as described by (Harbome, 1973). The quantitative analysis was carried out for alkaloids (Harborne, 1973), tannins (Van-Burden and Robinson, 1981), phenols and flavonoids (Boham and Kocipai-Abyazan, 1994), steroids, terpenoids and saponins (Sofowora, 1993).

Experimental Design

Thirty male albino rats were randomly divided into five equal groups (N=6). Group 1 was not given treatment (negative control); group 2 was orally dosed with aspirin aqueous suspension at a dose rate of 100mg/kg body weight as a reference drug (standard control), Group 3, 4, and 5 were orally dosed with 50mg/kg, l00 mg/kg, and 200mg/kg body weight of the methanol extract of *Prosopis africana* respectively. After 30 minutes, Edema was induced on the right hind paw of the rats by subplantar injection of 100µl of formalin (2.5%). The paw edema of the rats was measured at 0, 60 and 90 minutes.

Group	Description Dose (mg/kg) b.w	Reaction time in seconds (minutes)			
		Before	0	60	90
1	Negative control				
2	Positive control (Aspirin) 100				
3	Methanol extract of *P. Africana* 50				
4	Methanol extract of *P. Africana* 100				
5	Methanol extract of *P. Africana* 200				

Anti-Inflammatory Effect

The anti-inflammatory effect of the methanol extract of *Prosopis Africana* was evaluated by immersion of the paw as described by Arzi *et al.,* (2015) and the reading was taken at 0, 60 and 90 seconds.

Determination of Antimicrobial Activity of the Extract

The antimicrobial activity was performed using agar-well diffusion method as described by Campos *et al.,* (2014).

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used. Result was expressed as mean ± SEM. The difference between the means was regarded as significant at $p<0.05$ and the differences of the mean was expressed using SPSS software version 23.

RESULTS

Table 1 shows the result of qualitative phytochemical analysis. The active phytochemical constituents in methanol stem bark of *Prosopis Africana* observed were tannins, saponins, flavonoids, alkaloids, phenols, steroids and terpenoids.

Table1: Qualitative Phytochemical ontent of Methanol Stem Bark Extract of *Prosopis africana*

Secondary Metabolite	Inference
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Phenol	+
Steroids	+
Terpenoids	+

+ = Present

Table 2 shows the quantitative phytochemical content in percentage of each phytochemicals present in the methanol stem bark of *Prosopis african*. Saponin has the highest quantity of 4.01% while steroids has the lowest quantity of 0.14%

Table2: Quantitative Phytochemical Analysis of Methanol Stem Bark Extract of *Prosopis africana*

Phytochemicals	Percentage (%)
Tannins	1.92
Saponins	4.01
Flavonoids	0.77
Alkaloids	0.11
Phenol	3.77
Steroids	0.14
Terpenoids	0.26

Table 3 shows the anti-inflammatory effects of methanol stem bark extract of *Prosopis africana*

at 100mg/kg was comparable to aspirin 100mg/kg at time interval of 0, 60 and 90 minutes.

Table 3: Anti-inflammatory Effect of Methanol Stem Bark Extract of *Prosopis africana*

Groups	Description	Dose (mg/kg) b.w	Before Induction	Reaction Time in Minutes		
				0	60	90
1	Negative control		7.18±0.06[a]	7.126±0.23 [a]	7.36±0.17 [a]	7.38±0.17 [a]
2	Positive control (Aspirin)	100	4.75±0.12	4.92±0.06	5.15±0.15	5.79±0.12
3	Methanol extract of P.africana	50	5.10±0.12	5.37±0.17	5.85±0.08	6.20±0.09 [a]
4	Methanol extractof *P.africana*	100	4.28 ±0.19	4.45 ±0.12	4.53±0. 11	4.58 ±0.10
5	Methanol extractof *P.africana*	200	3.42 ±0.06[b]	3.94 ±0.06^{b}	4.38±0.06	4.45 ±0.12

Results are Mean ± SEM for 5 determinations

a = Significantly increased compared to other groups

b = Significantly decreased compared to other groups.

Table 4 shows the effect of methanol stem bark extract of *Prosopis africana* (l0mg/ml) on some Microorganisms. The highest zone of inhibition (17mm) was on *Pseudomonas aeruginosa* while the lowest zone of inhibition (11mm) was on *Staphylococcus aureus.* The methanol extract inhibited the growth of the entire microorganism used.

Table4: Diameter (mm) of Zone of Inhibition of Methanol Stem Bark Extract of *Prosopis Africana* (l0mg/ml) and Standard Antibiotics on some Microorganisms

Microorganisms	**Zone of inhibition (mm)**	
	Methanol Extract	**Ampicilin**
S.aureus	11.0±0.04[cb]	45.0±1.15^{a}
P.aeruginosa	17.0±0.94[ac]	25.5±1.15
E.coli	11.5 ±0.07[cb]	30.1±1.73
K.pneumoniae	15.5±0.04[a]	15.4±0.58^{b}

Results are Mean ± SEM for 3 determinations

a = Significantly increased compared to other Microorganisms

b = Significantly decreased compared to other Microorganisms

c = Significantly decreased compared to standard drug

Table 5 shows the minimum inhibitory concentration (MIC) values of methanol stem bark extract of *Prosopis africana* on the microorganism used. The minimum inhibitory concentration of 2.5mg/ml was recorded using the methanol extract against *Pseudomonas aeruginosa, Staphylococcus aureus* and *E.coli.*

Table5: Minimum Inhibitory Concentration of Methanol Stem Bark Extract of *Prosopis Africana* (mg/ml).

Microorganism	Methanol Extract
S.aureus	2.5 ± 0.58
P.aeruginosa	2.5 ± 0.58
E.coli	2.5 ± 0.58
K.pneumoniae	5.5 ± 0.15^{a}

a = Significantly increased compared to other concentration

DISCUSSION

Results of phytochemical constituents of *Prosopis Africana* as presented in table 1, shows that the stem-bark of the plant contains alkaloids, tannins, phenols, flavonoids, steroids, glycosides, terpenoids and saponins. These compounds have been selected, classified in appropriate subgroups and the data are reported based on their pharmacological activity in different experimental models (Mona *et al.*, 2014).The presence of this phytochemicals indicate that the stem-bark possess some medicinal properties (Katie *et al.,* 2006). The alkaloids comprise the largest single class of secondary plant substances. They have a remarkable range of pharmacological activity. Several isoquinoline alkaloids (berbamine, berberine, cepharanthine and tetrandine) were examined for anti-infl ammatory activity. They have been shown to be active in different assays as reported by different authors (Atawodi ., 2004 and Kolapo, 2009).

Alkaloids protect against chronic diseases. Saponins protect against hyper cholesterolemia and has antibiotic properties. Steroids and triterpenoids was shown to be analgesic. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains was reported by Oguntibeju *et al.*, 2008 and Zhen *et al.*, 2015). Flavonoids could be extremely helpful as they possess anti-allergic, anti-inflammatory, antiviral and antioxidants activities (Hossain *et al.,* 2013 and Sandoval *et al.,* 2002).

The quantitative estimation of primary metabolites revealed that the methanol stem bark extract of *Prosopis africana* contained various phytochemicals (Table-2). The tannins content 1.92%, saponins content 4.01%, flavonoids content 0.77%, alkaloids content was 0.11%, phenol content 3.77%, steroid content 0.14% and terpenoids content 0.26%. Saponins has the highest value. Saponins posses antimicrobial activity in cold blooded animals. Saponins are also used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory activity and weight loss (Manickam and Veerabahu, 2014). Phenol has the second highest value. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bacterisides are compared (Akinyeye *et al.,* 2014).

The Methanol stem bark extract of *Prosopis africana* exhibited potent anti-inflammatory activity at the dose level of 50, 100 and 200mg/ml, in this study. It is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. The anti-inflammatory effect produced by methanol stem bark extract of *Prosopis africana* was more significant at high dose of 200mg/ml, which was more effective than aspirin (Positive control) and other concentrations. Thus, it implies that *Prosopis africana* stem bark can be used as a pain reliever. The decoction of the stem bark is claimed to have pain relieving properties in general body pain and menstrual pain by the Nupe people of Nigeria (Mann *et al*., 2003). This anti-inflammatory and analgesia carried out by *Prosopis africana* could be attributed to its rich chemical constituents like steroids, flavonoids, saponins, glycosides, tannins, phenols, terpenoids and alkaloids in the stem bark. Secondary metabolites such as flavonoids are known to target prostaglandins which are involved in pain perceptions (Chakraborty *et al*., 2004). Hence the presence of flavonoids may be contributory to the analgesic activity of the *Prosopis Africana* stem bark. Various chemicals such as alkaloids, tannins, saponnins, cyanoglycosides, terpenoids, oleic and stearic acids which are naturally present in plants have been implicated in the conferment of antimicrobial activities on the plant containing them (Abdelrahman, 2003). In the carrageenan-induced inflammatory assay, the extract showed significant anti-inflammatory activity ($P<0.001$) from the third hour (Ayanwuyi *et al,* 2010).

The antimicrobial activities of the methanol stem bark extract of *Prosopis Africana* produced the highest zone of inhibition (17mm) on *Pseudomonas aeruginosa* and the lowest zone of inhibition (11mm) on *Staphylococcus aureus.* The methanol extract exhibited

inhibitory activity on *Klebsiella pneumonia* more than the antibiotic (ampicillin). The results from this work revealed that *Prosopis Africana* had inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.* Previous report have demonstrated that among the most commonly known pathogens that cause infectious disorders of the skin is *Staphylococcus aureus* (Hailu *et al.,* 2005). Bacterial pathogens produce pain by directly activating sensory neurons that modulate inflammation. Thus, the facts that the methanol stem bark extract of *Prosopis Africana* exhibited activity against *Staphylococcus aureus* might justify the use of *Prosopis Africana* stem bark locally for the treatment of skin disorder.

The extract was evaluated for its minimum inhibitory concentration against *E. coli, S. aureus, P. areuginosa* and *K. pneumonia.* The minimum inhibitory concentration value for methanol extract of *Prosopis Africana* stem bark against *E. coli, S. aureus* and *P. areuginosa*was 2.5mg/ml each. While that of *K. pneumonia* is 5.5mg/ml, suggesting that very small amount of the extracts are required to inhibit the growth of the bacteria.

Conflict Of Interest

The authors declared there is no conflict of interest

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