Medicinal and Aromatic Plant Research Journal
Vol. 3(1), pp. 1-8, February 2015
Research Paper

Comparative studies of the pharmacological activities of *Prosopis africana* fruits and its fraction

J. A. Bosha[1]* • I. U. Asuzu[2]

[1]College of Veterinary Medicine University of Agriculture, Makurdi, Nigeria.
[2]Faculty of Veterinary Medicine University of Nigeria, Nsukka, Nigeria.

*Corresponding author. E-mail: boshajoel@yahoo.com.

Accepted 14th January, 2015

Abstract. The pharmacological effects of the methanolic extract of *Prosopis africana* (Guill and Perr) Taub fruits (PA) and one of its fraction ($FPA_)$ were investigated. The extract was prepared by cold maceration using 80 % methanol at room temperature to obtain a yield of 4.2% w/w dry extract. Nine (9) fractions of the extract were obtained by accelerated gradient chromatography. FPA was most active and was selected for further *in vitro/in vivo* studies with the parent extract. Its yield was 7.3% w/w. The extract (PA) showed 5 spots with R_f values of 0.07, 0.24, 0.39, 0.54 and 0.69. while FPA showed 3 spots with R_f values of 0.19, 0.39 and 0.54. The extract and FPA showed both concentration and time-dependent inhibitions of the intrinsic peristaltic contractions of the isolated rabbit jejunum, as well as induced concentration-dependent inhibitions of the contractions induced by Ach (2.5 µg/ml) on the isolated rabbit jejunum. These inhibitions were reversible after washing. Phytochemical spot tests revealed that PA contained alkaloids, carbohydrates, saponins, tannins, flavonoids, polyuronoids, sterols, terpenes and reducing sugars while FPA contained all constituents of PA except flavonoids, polyuronoids and reducing sugars. The findings of this study have shown that PA and its fraction FPA has good local anaesthetic activities comparable to 2% lignocaine hydrochloride as seen in the guinea pig wheal test and other comparable pharmacological activities like inhibitions of rabbit jejunum contractions, though in varying degree. These may be due to a combination of constituents since both of them showed similar phytochemical constituents with little variation.

Keywords: Methanolic extract, fractions, local anaesthetic, inhibitions*, Prospis africana,* phytochemicals.

INTRODUCTION

Recorded history from ancient Egypt, Assyria, China and India shows that the use of plants for medicinal purposes is the most ancient approach to healing (Trease and Evans, 2009). There is a worldwide “green” revolution which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Schneider, 2004). They are cheaper to produce and affordable, and are more readily available (Oliver-Bever, 1986). In recognition of these noble facts, the World Health Organisation (WHO) in 1991 approved its 44.34 resolution, which encourages all member countries of WHO to promote the use of “traditional, harmless, efficient and scientifically proved remedies”.

Locally, *Prosopis africana* has been used to treat body pains, anxiety and toothache (Iwu, 1993; Adikwu, 1994). The bark and root decoctions are used in treating Trypanosomosis in cattle in central Nigeria (Atawodi et al., 2002). The most popular use of *P. africana* among African tribes especially in Northern Nigeria is as a flavouring agent, a locally fermented condiment in soup (Keay et al., 1964; Adikwu, 1994; Burkil, 1994; Barminas et al., 1998). The gum from the seed has been studied for its bioadhesive activities and found to be good in binding, granulation and disintegration in drug production (Adikwu, 1994; Okoye, 1999; Attama et al., 2005). The fruits are also used by some African tribes as a fish poison (Neuwinger, 2004).

The present study is aimed at conducting a comparative,

systematic scientific investigation into some pharmacological activities of the fruit of *P. africana* and its fraction using both *in vivo* and *in vitro* models. This is necessary because extracts and fractions may have same or different activities based on their phytochemical content (Sofowora, 2009).

MATERIALS AND METHODS

Sample collection and identification

Ripe fruits of P. *africana* were collected from the premises of the University of Agriculture, Makurdi, in Makurdi Local Government Area of Benue State, Nigeria, and were identified by a taxonomist, Mr. Ekuno of the Forestry Department, University of Agriculture, Makurdi. A sample specimen was deposited in the forestry herbarium with voucher number UAM/FHM 10. They were dried in the sun as described by Pamplona-Rogers (2004). The dried fruits were subsequently pulverized using pestle and mortar.

Preparation of extract

Five hundred (500 g) of the dried and pulverized fruits were extracted with 80% methanol, by cold maceration at room temperature (25°C), with intermittent shaking for 48 h. Whatman filter paper No. 1 was used to filter the methanolic extract, which was evaporated to dryness using vacuum rotary evaporator.

Accelerated gradient chromatography

After drying, the methanolic extract was subjected to accelerated gradient chromatography (AGC) to separate the components into various fractions according to Harborne (1991).

Animals and treatment

Guinea pigs (*Carvia porcellus)* and rabbits (*Oryctolagus cuniculus*) were obtained from the laboratory animal facilities of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. All the animals were kept in plastic cages and were supplied with clean drinking water and feed *ad libitum* with standard commercial growers mash (Vital Feeds Jos, Nigeria). The guinea pigs and the rabbit's feeds were also complemented with grasses. Ethical conditions governing the conduct of experiments with life animals were strictly observed (Zimmermann, 1983).

Frogs (*Rana pipiens)* used in this study were obtained from the water tank at Nkrumah Hall, University of Nigeria Nsukka.

Local anaesthetic and ocular effects of the extract (PA) and fraction (FPA)

Local anaesthetic effects of the extract and the fraction were carried out using guinea pigs. The wheal test as described by McLeod (1970) was used. The effects of the extract and fraction on ocular reflexes (corneal, conjunctival and pupilary) were studied following instillation of the extract and fraction into the eye in rabbits. The method of McLeod (1970) was used and only white rabbits were used for the study.

In vitro muscle studies

The *in vitro* studies were carried out on the guinea pig ileum, rabbit jejunum and frog rectus abdominis muscles as described by Perry (1970).

The effect of extract and fraction on acetylcholine (Ach) induced contractions of rabbit jejunum segments were also studied using 2.5 µg/ml of Ach and varying concentrations of the extract and fraction. The amplitude of contractions were measured by standard methods and the degree of effect expressed in percentage.

Phytochemical analysis

Phytochemical spot test were carried out as described by Trease and evans (2009).

Data analysis

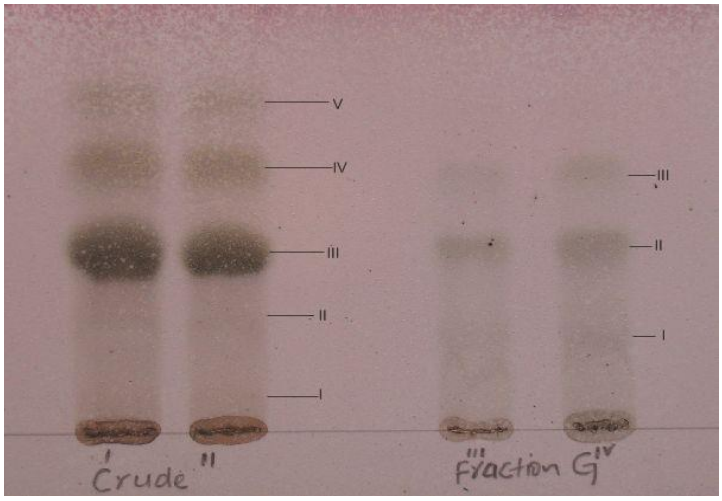
Data from the experiment was analysed using One way analysis of Variance (ANOVA) and Duncan multiple range post-hock at ($P < 0.05$) for significance difference.

RESULTS

The yield of *P. africana* was 4.2% w/w. The methanolic extract of *P. africana* fruit (PA) was golden brown and sticky with sweet aromatic odour. It did not dissolve well in 5% tween 20 but dissolved in distilled water with foaming. The yield of the fraction (FPA) was 7.3% w/w. It was brownish in colour and dissolved well in water with foaming also.

Accelerated gradient chromatography

The solvent system that gave the best separation of the extract was the combination of Chloroform: Ethylacetate: Methanol (1:3:2). Nine (9) different fractions were obtained (F_1-F_9), out of which F_7 showed better activity during preliminary investigation and was selected as FPA for further investigations.



Legend

I and II = PA

III and IV = FRACTION FPA

Figure 1. Chromatogram of PA and FPA.

Table 1. The R_f values of various spots of PA and FPA.

Spot	Rf value	PA	FPA
I	0.07	+	-
II	0.19	-	+
III	0.24	+	-
IV	0.39	+	+
V	0.54	+	+
VI	0.69	+	-

Thin-layer chromatography

The extract (PA) showed 5 spots on the chromatogram while the FPA showed 3 distinct spots. When viewed under the ultraviolet (UV) lamp at 254 and 366 nm, the spots appeared as quenching zones for both the extract (PA) and the fraction FPA (Figure 1). The R_f of the spots for the crude extract as well as the fraction were calculated thus (Table 1).

Local anaesthetic effect of PA and FPA

The local anaesthetic effect of PA and FPA were assessed using the guinea pig wheal test. The result of the test is as shown in Table 2.

Effect of PA and FPA on guinea pig ileum

The extract (PA) and its Fraction (FPA) could not exert any measurable effect on the guinea pig ileum but the extract (PA) 1.6 mg/ml inhibited the contraction of the ileum induced by Ach by 42.3% while FPA was able to inhibit the same Ach-induced contraction of the ileum by 9.8% (Table 3).

Effects of PA and FPA on frog *Rectus abdominis*

The administration of Ach 2.5 µg/ml gave no contraction of the frog *Rectus abdominis* muscle, PA 1.6 mg/ml and FPA G 120 µg/ml did not show any measurable activity.

Effect of PA and FPA on rabbit jejunum

Both PA and FPA induced concentration and time-dependent inhibitions of the peristaltic and Ach-induced contractions of rabbit jejunum. They induced a concentration and time-dependent reversible inhibition of the intrinsic contractions of the rabbit jejunum. At 0.2 mg/ml, PA showed no inhibition of the contraction of rabbit jejunum at 1 s but inhibited the contraction by 11.1% at 15 s, 19% at 30 seconds 31% at 45 and 60 s, 33% at 75 s and 39% at 90 s. While FPA at 30 µg/ml inhibited the rabbit jejunum contraction as follows: 0% at 1 s, 5.4% at 15 s, 8.9% at 30 and 45 s, 12.5% at 60 s, 15.9% at 75 s and 19.1% at 90 s (Table 4 and Figure 2).

Both PA and FPA showed concentration-dependent inhibition of contraction induced by acetylcholine (2.5 µg/ml on the rabbit jejunum. At 0.2 mg/ml the Ach-induced concentration was reduced by 25%. At 0.4 mg/ml, the contraction was reduced by 30.0 and 65.0% at 0.8 mg/ml. At the highest concentration of 1.6 mg/ml the contraction was reduced by 63.0%. While FPA at 30 µg/ml reduced the Ach-induced contraction of the rabbit jejunum by 8.6%. At 60 µg/ml, it was reduced by 15.7% and at 120 µg/ml it was further reduced by 27.1%. The highest concentration of FPA 240 µg/ml, showed the reduction of the Ach- induced contraction by 42.9% (Table 5).

Phytochemical spot test

Results of the spot test showed that the methanolic

Table 2. Local anaesthetic effects of PA, FPA and Lignocaine hcl on guinea pig.

Measurement		PA	FPA	Lignocaine hcl
Concentration mg or µg/ml	High	3mg	0.6 µg	1 mg
	Low	1 mg	0.2 µg	0.3 mg
No of negative response in min	High	35/36	14/36	32/36
	Low	29/36	3/36	30/36
% Anaethesia	High	97.22	38.89	88.89
	Low	80.56	8.33	83. 33

Each value was taken in triplicate.

Table 3. Effect of the extract PA and Fraction FPA on Ach-induced contractions of guinea pig ileum.

Concentration of drug/extract	Contraction (cm) Ach alone	Contraction (cm) Ach + PA/FPA	Difference (cm)	% inhibition
Ach 10 µg + PA 1.6 mg	7.5	4.33	3.17	42.3*
Ach 10µg + FPA 120µg	11.83	10.67	1.16	9.8

Table 4. Effect of PA and FPA on rabbit jejunum showing both concentration and time-dependent inhibitions of intrinsic contractions.

Concentration mg/ml or µg/ml	Percentage inhibitions per time in seconds							
	0	1	15	30	45	60	75	90
PA 0.2 mg/ml	0	0	11.1	19.4	30.6	30.6	33.4	39.8*
PA 0.4 mg/ml	0	2.7	20.0	45.9	54.0	62.0	64.9	67.6
PA 0.8 mg/ml	0	9.1	59.1	77.3	81.8	86.4	86.4	100
PA 1.6 mg/ml	0	7.1	57.1	71.4	85.7	100	-	-
FPA 30 µg/ml	0	0	5.4	8.9	8.9	12.5	15.9	19.1
FPA 60 µg/ ml	0	6.3	6.3	10.4	10.4	20.0	25.1	29.5
FPA 120 µg/ ml	0	12.5	12.5	25.0	25.0	31.3	35.4	37.5
FPA 240 µg/ml	0	15.0	32.5	32.5	47.5	52.5	52.5	55.0

Each value was taken in triplicate.

extract of *Prosopis africana* (Guill and Perr) Taub (PA) contained alkaloids, carbohydrates, saponins, tannins, flavonoids, polyuronoids, sterols and terpenes, reducing sugars but no starch. While FPA contained all that the crude extract had except flavonoids, polyuronoids and reducing sugars (Table 6).

DISCUSSION

The methanolic extract of *P. africana* fruits (PA) in this study showed a yield of 4.2% w/w while the fraction(FPA) had a yield of 7.3% w/w they were both brownish in colour with sweet aromatic odour. They both dissolved in distilled water with foaming, but did not dissolved well in 5% tween 20. The dissolution of PA in water makes it easier for it to be used in stunning fish in tropical Africa (Jonathan et al., 2004; Neuwinger, 2004). Different parts of *P. africana* like stem bark and fruit pods are used in dropping in stagnant waters during the dry season to catch fish (personal communication). This toxic effect is more on cold blooded animals especially fishes due to the presence of saponins (Bruneton, 1994). This may explain why humans who eat fish stunned by PA do not stand the risk of being poisoned (Bosha and Asuzu, 2011a). Since saponins are present in both the PA and Fraction FPA, it is possible that saponins may be responsible for some of its action.

Out of the nine fractions obtained from the chromatography of PA, only fraction F_7 (FPA) was used

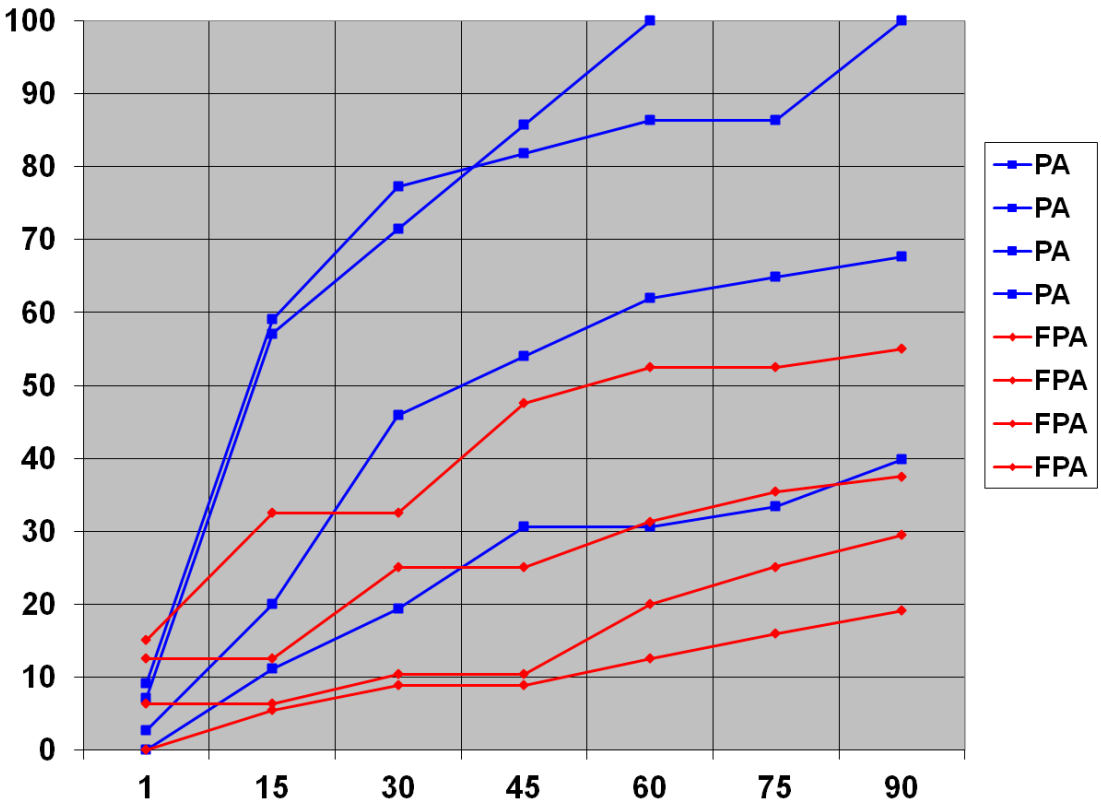


Figure 2. Graph of PA and FPA activity on rabbit jejunum.

Table 5. Effect of PA and Fraction (FPA) on Ach – induced contractions of rabbit jejunum.

Concentration of PA/FPA	Contraction Ach alone (cm)	Contraction Ach+ PA or FPA (cm)	Difference (cm)	% inhibition
PA 0.2 mg/ml	3.50	2.64	0.86	25
PA 0.4 mg/ml	3.50	2.45	1.05	30
PA 0.8 mg/ml	3.50	1.23	2.27	65
PA 1.6 mg/ml	3.50	1.30	2.20	63
FPA 30 µg/ml	3.50	3.20	0.30	8.6
FPA 60 µg/ml	3.50	3.95	0.55	15.70
FPA 120 µg/ml	3.50	2.55	0.95	27.10
FPA 240 µg/ml	3.50	2.0	1.50	42.90

Each value was taken in triplicate

for the comparative studies because of its better activities during preliminary trials The spots obtained for both PA and its fraction (FPA) shared similar properties when viewed under the UV lamp at 254 nm. The spots (5) for PA and (3) for the fraction (FPA) showed quenching zones which were not visible at 366 nm. Two spots from the fraction and 2 spots of PA shared common characteristics including their R_f values which were the same (Table 2 and Figure 1). The R_f values of the spots from the fraction (FPA) were 0.186, 0.386 and 0.543 while those of the PA were 0.07, 0.24, 0.386, 0.543 and 0.686. Similarity in these spots especially 0.386 and 0.543 may explain the similarity in the actions of PA and its fraction FPA.

Ocular experiments did not give positive results with either the extract (PA) or fraction (FPA). The action of local anaesthetics on the autonomic nervous system is usually qualitative, that is, “all or none” (Shetty and Anika, 1982). PA and its fraction did not show any loss of eye reflex or change in ocular blood vessels, but rather PA at 1 mg/ml showed local anaesthetic effect comparable to 2 % Lignocaine Hcl at same concentration (81 and 89%) respectively. While FPA at a lower concentration (0.6 µg/ml) showed less local anaesthetic effects (39%)

Table 6. Phytochemical analysis.

Phytochemical	PA	FPA
Alkaloids	++	++
Carbohydrates	+++	++
Saponins	++	+
Tannins	++	++
Flavonoids	++	-
Polyuronoids	+	-
Streols/Terpenes	++	++
Reducing sugar	++	-
Starch	-	-

+++ = Abundant; ++ = Moderately present; + = Low concentration; - = Absent

compared to PA and 2% Lignocaine HCL (Table 2). Considering the fact that PA is still in its crude form (Bosha and Asuzu, 2011a; Bosha and Asuzu, 2011b), and FPA is used here in a very low concentration (µg/ml). If a linear measurement is therefore adapted, it will be apt to say that both have good local anaesthetic potentials. The duration of activity was also comparable to 2% Lignocaine Hcl as it acted over 30 min (Cuminis et al., 2007; Bosha et al., 2010; Bosha and Asuzu, 2011b). This showed that both PA and FPA contain some pharmacological principles which have actions on the peripheral nerves observed as local anaesthesia in guinea pig. These pharmacological principles are the phytochemicals like alkaloids, saponins, tannins, steroids etc (Trease and Evans, 2009). Both PA and FPA have shown great potentials of a clinically useful local anaesthetic considering that they were used in the crude form (Bosha et al., 2010; Bosha and Asuzu, 2011b). Local anaesthetics are sodium channel blockers (Wood et al., 2004; Rogers et al., 2006; Cumminis et al., 2007). They prevent or relieve pain by interrupting nerve conduction through binding to specific receptor sites within the pore of sodium channel and block sodium ion movement preventing the large transient increase in permeability of excitable membrane to sodium ion.This action is due to direct interaction with voltage-gated sodium channels (Budsberg, 2005; Cumminis, 2007). Voltage-gated sodium channels are important in electrogenesis and nerve impulse conduction and are target for important clinically relevant analgesics such as Lidocaine (Wood et al., 2004; Rogers, 2006; Cumminis et al., 2007; Bosha and Asuzu, 2011b).

The extract (PA) and fraction (FPA) could not exert any measurable effect on the guinea pig ileum alone. However, PA inhibited the contraction induced by Ach (10 µg/ml) by 42.3% when the former was introduced to the tissue bath and Ach added to it without washing, while the fraction inhibited Ach (10 µg/ml) induced contractions by 9.8% when used similarly (Table 3). The inhibitory activity of PA here were greater than that of FPA. This may be due to concentration difference, since PA is in mg/ml and FPA is in µg/ml. Another source of difference may be their phytochemical composition which is similar except some varialtions in flavonoids, polyuronoids and reducing sugars which are absent in FPA. The inhibitory effect observed here may be due to a combined effect of the above phytochemicals. PA and FPA did not show any measurable effects on frog *Rectus abdominis* muscle. This means they both have no effect on skeletal muscles. Both PA and Fraction FPA induced concentration-dependent inhibition of the intrinsic contractions of the rabbit jejunum. The inhibitions also increased with time (Table 4, Figure 2). This points to the fact that as the concentration of PA was increased the effect on the tissue (inhibition of contraction) also increased and the duration of the onset of inhibitory effect was shortened. The tissue therefore completely ceased to contract before 90 seconds as seen in the 0.8 and 1.6 mg/ml which ceased at 75 and 45 s respectively (Table 4 and Figure 2). Fraction FPA also behaved in a similar manner to PA (Table 4, Figure 2). As the concentration of Faction FPA increased the level of inhibition of the intrinsic contractions of the rabbit jejunum increased with time and concentration.

Addition of Ach in the presence of PA inhibited the contraction induced by Ach concentration-dependently. PA at 0.2 mg/ml reduced the contraction induced by Ach by 25%. At 0.4 mg the contraction was reduced by 30.0%, 65.0% at 0.8 mg/ml and 63.0% at 1.6 mg/ml (Table 5). Addition of Ach (2.5 µg/ml) in the presence of fraction FPA without washing showed a concentration-dependent percentage inhibition of Ach-induced contractions as observed in PA (Table 5).

In this study, it was noted that both PA and Fraction FPA were able to inhibit the normal intrinsic peristaltic contractions of the rabbit jejunum as well as the contractions induced by Ach in a concentration as well as time- dependent manner. It was also observed that higher concentrations of PA (0.8 and 1.6 mg/ml) were able to completely inhibit the intrinsic contractions of the rabbit jejunum at 75 and 45 s respectively (Table 4, Figure 2). This shows concentration-dependent effect. These inhibitions bring about delay in passage of content in the intestine. It is stated that this help in producing bulk and can be used to suppress appetite and treat obesity (Adikwu, 1994). Bosha and Asuzu (2011a) opined that the delay is responsible for the antidiarrhoeic effect of *P. africana* and may be due to the presence of tannins and other phytochemicals in the extract. Tannic acid is a constituent of most commercial anti-diarrhoea compounds (Brander and Pugh, 1971). The inhibitions by both PA and Fraction FPA were reversible. This was observed as washing of the tissue reversed the inhibitory action and the tissue returned to normal peristaltic movements. These inhibitions are thought to be anti-muscarinic,since contraction of the jejunum is believed to be through muscarinic receptors, and could be blocked by atropine and related compounds (Sanni et al., 2005).

Drugs mainly prevent contraction in one of the following

ways: 1 By acting at the terminal node of motor fibril to prevent conduction of action potential like local anaesthetics. 2 By preventing the synthesis of Ach as in botulinum toxin or 3 By preventing Ach from attaching to membrane to initiate depolarization, a curare-like action (Graham, 1971). The inhibitory action of PA and Fraction FPA is likely due to a local anaesthetic mechanism. As local anaesthetics prevent increase in the permeability of exitable membrane to sodium ion (Na^+) the threshold for electrical excitability decreases, the rate of rise of action potential (A.P) declines, impulse conduction slows and the safety factor for conduction decreases and propagation of AP and nerve conduction eventually fails. This can result in reduced motility of the jejunum. Many workers have also reported the mechanism of action of local anaesthetics as sodium channel blocking (Sindrup and Jensen, 1999; Catterall and Mackie, 2001; Budsberg, 2005; Rogers et al., 2006; Cummins et al., 2007). They stated further that various pain conditions can be treated with sodium channel blockers. Administration of acute inflammatory mediators including prostaglandins E_2 (PGE_2), serotonin and carrageenan modulate their activity.

The phytochemical spot tests of PA and FPA revealed that PA contained alkaloids, carbohydrates, saponins, tannins, flavonoids, polyuronoids, sterols and terpenes and reducing sugars, while FPA contained all that PA had except flavonoids, polyuronoids and reducing sugars (Table 6). Most of these chemical constituents have been known to be associated with particular pharmacological activities (Okoli et al., 2006; Bose et al., 2007; Rahman et al., 2007; Marchioro, et al., 2005). Terpenoids, steroids, flavonoids and tannins have been found to be responsible for analgesic, anti-inflammatory and antipyretic activities (Bruneton, 1994; Dhanabal et al., 2007; Owu et al., 2008; Wendel, et al., 2008). Flavonoids in addition are said to have enzyme inhibitory activities as well as antibacterial, antiviral, and hepatoprotective actions. They also have antioxidant effect. That means they attack free radicals. The analgesic effect ascribed to PA and FPA in this study may be mediated by one or more of the other constituents. Since FPA did not show presence of flavonoids polyuronoids and reducing sugars, their activities may be due to the presence of their other constituents namely, tannins, saponins, terpenes, sterols and alkaloids.

Tannins apart from complexing with proteins (which is used in leather industry) is used internally as an anti-diarrhoeic and most commercial anti-diarrhoea agents contain tannic acid (Brande and Pugh, 1971). It also has antibacterial and antifungal activities as well as enhancing tissue regeneration (Bruneton,1994; Awe, et al., 1999; Olaniyi, 2000). Saponins are used by plants for defence against fungi and other microbes. They are hemolytic and increase permeability of red blood cells and result in loss of haemoglobin (Bruneton, 1994). They are toxic to cold blooded animals especially fishes and most plants that contain them are used as fish poisons. The use of *P. africana* in catching fish may also be as a result of the presence of saponnis (Bruneton, 1994; Neuwinger, 2004). Saponins also have analgesic, anti-inflammatory and anti-oedematic properties. Alkaloids occur in various forms in plants and are localized in peripheral tissues. They mainly protect plants against predators and are intermediate metabolites of storage substances. They also act as growth regulators. Many of them such as morphine and atropine have various clinical uses. They have analgesic, anti-inflammatory, antibiotic and anti-tumour activities (Bruneton, 1994).

In conclusion the methanolic extract of *P. africana* fruits (PA) and its fraction FPA has demonstrated high level of local anaesthetic effect in guinea pig comparable to 2% Lignocaine hcl. They both inhibited Ach-induced contractions in the gut smooth muscle of the guinea pig ileum and rabbit jejunum. The inhibition in the rabbit jejunum was both concentration and time-dependent. These actions are strongly suspected to be mediated through anti-muscarinic activity and by the phytochemicals present in them. Despite their phytochemical variation, all the pharmacological activities present in PA were also found in the fraction FPA It is believed that a combination of these phytochemicals were responsible for the observed activities of the two compounds. More studies should be carried out to establish the mechanism of action of the effects of PA and FPA both *in vivo* and *in vitro* and the identification of the active principles responsible for these actions.

ACKNOWLEDGEMENT

The first author acknowledges the support of the university of Agriculture Makurdi by granting him study leave to conduct the research**.**

REFERENCES

Adikwu MU (1994). Physico-chemical properties and pharmaceutical application of Prosopis africana (Guill & Perr) Taub. gum. Ph.D Thesis Department of Pharmaceutics University of Nigeria, Nsukka (unpublished) pp. 55-70.

Attama AA, Nnamani PO, Okorie O (2005). Effect of pH and Ionic Strength on the bioadhesive properties of Prosopis africana gum. J. Pharm. Bioresour. 2(2):141-145.

Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyika EO, Anigo KM, Abu EA, James DB, Njoku G C, Sallau AB (2002). Indegenous Knowledge system for treatment of Trypanosomosis in Kaduna State of Nigeria. J. Ethnopharmacol. 79(2):279-82.

Awe SO, Makinde JM, Olajide OA (1999). Cathartic effect of the leaf extract of Vernonia Amygdalna. Fitoterapia 70:161-165.

Barminas JT, Maina HM, Ali J (1998). Nutrient Content of *Prosopis africana* seed plant. Food Hum. Nutr. 52(4):325- 328.

Bose A, Mondal S, Gupta JK, Gosh T, Dash GK, Si S (2007). Analgesic, anti-inflamatory and antipyretic activities of the ethanolic extract and its fractions of *Cleome rutidosperma Fitoterapia* 78:515-520

Bosha JA, Asuzu IU (2011a). Pharmacological activities of Prosopis africana fruits. Lambert Academic Publishing GmbH & Co. Dudweiler Landstr. Saarbrucken, German. pp. 65-70.

Bosha JA, Asuzu IU (2011b). Some aspect of the pharmacology of the

methanolic extract of *Prosopis africana* Fruit (Guill and peer) Taub. Trop. Vet. 29(3):1-11.

Bosha JA, Asuzu IU, Onyeyili PA (2010). Pharmacological effects of a fraction of the methanolic extract of *Prosopis africana* fruit (Guill and per) taub. Niger. Vet. J. 31(3):235-241.

Brander GC, Pugh DM (1971). Veterinary Applied Pharmacology and Therapeutics.2nd ed.Bailliere Tindall,London. pp. 271-279.

Bruneton J (1994). Pharmacognosy, phytochemistry, medicinal plants 2nd ed. Lavoisier publishing, New York. pp. 1-880

Budsberg CS (2005). Pain management. In: Textbook of Veterinary Internal Medicine. Diseases of the dog and cat Ettinger, J. S. and Feldman, E. C. (Eds.), Elsevier Saunders 1(6):22-24.

Burkil HM (1994). The useful plants of West Africa 3rd ed. *Royal botanic gardens* Kew 4:751-753.

Catterall W, Mackie K (2001). Local anaesthetics. In: Goodman and Gilman's The pharmacological basis of therapeutics 10th ed. Hardman, J. G. and Limbird, L. E. McGraw-Hill. pp. 367-380.

Cummins TR, Sheet PL, Waxman SG (2007). The roles of sodium channels in nociceptoin: Implications for Mechanisms of pain. Pain 131:243-57.

Dhanabal SP, Raja MM, Ramathan M, Suresh B (2007). Hypoglycemic activity of *Nymphaea stellata leaves* ethanolic extract in alloxan induced diabetic rats. *Fitoterapia* 98:288-291.

Graham JDP (1971). Pharmacology for medical students. 2nd ed. Oxford University press, London. pp. 68-73.

Harborne JB (1991). Phytochemical methods. A guide to modern Techniques of Plant analysis, 2nd ed Chapman and Hall London. pp. 1-236.

Iwu MM (1993). Handbook of African Medicinal Plants. CRC Press Boca Raton. p. 48.

Jonathan GC, Robert AB, Stephen GW, Noel LO (2004). Naturally Occuring Fish Poisons from Plants. J. Chem. Educ. 81(10):1457-1461.

Keay RWJ, Onochie CFA, Stanfield DP (1964). Nigerian Trees Dept. of Forest Research Ibadan. National Press Ltd Apapa, 2:107-115.

Marchioro M, Blank MA, Mourao RHV, Antoniolli AA (2005). Antinociceptive activity of the aqueous extract of *Erythrina velutina leaves. Fitoterapia.* 76:637-642.

Mcleod LJ (1970). Pharmacological Experiments on intact preparations. Churchill Livingstone pp. 34-36.

Neuwinger HD (2004). Plants used for poison fishing in Tropical Africa. Toxicon 44:417-430.

Okoli CO, Akah PA, Egbuniwe ON (2006). Analgesic activity of the Leaf extracts of *Culcasisa scandeus* P. beauv. Indian J. Exp. Biol. 44:422-424.

Okoye IE (1999). Pilot Scale-up Application of gum from *Prosopis africana* family Mimosaceae (Guill& Perr) Taub as a binder in Tablet Technology. PhD. Thesis Dept. of Pharmaceutical Technology, University of Nigeria, Nsukka pp. 50-55. (Unpublished).

Olaniyi AA (2000). Principles of drug quality assuarance and pharmaceutical analysis. Mosuro publishers. pp. 341- 431.

Oliver-Bever P (1986). Medicinal Plant in Tropical West Africa., Cambridge University Press, London. p.1.

Owu BU, Ben EE, Antai AB, Ekpe EA, Udia PM (2008). Stimulation of gastric acid secretion and intestinal motility by *Vernomia amygdalina* extract. *Fitoterapia* 79:97-100.

Pamplona-Rogers GD (2004). Encyclopedia of Medicinal Plants. Pamplona-Rogers(Ed) Editorial Safeliz Spain. pp. 15-443.

Perry WLM (1970). *Pharmacological*. *Experiments on Isolated Tissues*. Staff Deparment of Pharmacology University of Edinburg. Churchill Livingstone pp. 38-58.

Rahman MS, Sadhu SK, Hasan CM (2007). Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. *Fitoterapia* 78:552-555.

Rogers M, Tang L, Madge DJ, Stevens EB (2006). The role of sodium channels in neuropathic pain. Semin. Cell Dev. Biol. 17(5):571-81

Sanni S, Onyeyili PA, Shamaki UB (2005). Responsiveness of duodenal smooth muscle to histamine and carbachol following Escherichia coli infection in Chickens. Veterinarski Arhiv 75:423-429.

Schneider E (2004). Encyclopedia of Medicinal Plants. Pamplona-Rogers(Ed) Editorial Safeliz Spain. p. 19.

Shetty SN, Anika SM (1982). *Laboratory Manual of Pharmacology and Toxicology*. Fourth Dimension Publishers Enugu. pp. 8,43-47,56-59,70-82.

Sindrup HS Jensen TS (1999). Efficacy of pharmacological treatments of neuropathic pain; an update and effect related to Mechanism of drug action. Pain 83:389-400.

Sofowora A (2009). Medicinal Plants and Traditional Medicine in Africa: In Trease and Evans Pharmacognosy . 14th ed. W B Saunders Coy, USA pp. 600-650.

Trease EC, Evans WC (1996). Pharmacognosy. 14th ed. W B Saunders Coy, US pp. 365-650.

Wendel GH, Maria AM, Guzman J, Giordano O, Pelzer LE (2008). Antidiarrhoeal activity of dehydroleucodine isolated from *Artemisia douglasiana. Fitoterapia* 79:1-5.

Wood JN, Boorman JP, Okuse K, Baker MD (2004). Voltage-gated sodium channels and pain pathways. J. Neurobiol. 61**(**1):55-71.

Zimmermann M (1983). Ethical guidelines for investigations of Experimental pain in Conscious Animals. Pain. 16(2):109-110.