ANTI-INFLAMMATORY ACTIVITY OF CRUDE SAPONIN EXTRACTS FROM FIVE NIGERIAN MEDICINAL PLANTS

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Abstact

Crude saponin extracts of five medicinal plants used in the treatment of inflammatory diseases like rheumatoid arthritis, gout and haemorrhoids were screened for anti-inflammatory activity using carrageenan-induced rat paw oedema test. These plants were the whole plant of *Schwenkia americana* Linn (WSA), the rhizomes of *Asparagus africanus* Lam (RAA), the leaves of *Dichrostachys cinerea* Linn (LDC), the stem bark of *Ficus iteophylla* Miq (BFI) and the leaves of *Indigofera pulchra* Willd (LIP). A modify traditional method of crude saponins extraction was used to give the following percentage yields: WSA-2.74%, RAA-3.59%, LDC-1.62%, BFI-0.81% and LIP-1.57% respectively. Thin-layer chromatography was used to identify the type of saponins present in the extracts. The acute toxicity study of the crude saponin extracts in mice gave the following intraperitoneal LD_{50}: WSA-471.2mg/kg, RAA- 1264.9mg/kg, LDC-1264.9mg/kg, BFI-118.3mg/kg and LIP-1264.9mg/kg respectively. The anti-inflammatory study of the extracts showed statistically significant ($P<0.05$) decreases in the rat paw-oedema as compared to the control. The percentage inhibitions of the extracts after four hours were as follow: WSA-61%, RAA-55%, LDC-72%, BFI-66% and LIP-40% respectively. These values were found to be comparable to that of ketoprofen-63%. The study showed that the anti-inflammatory properties attributable to these plants may be due to their saponins contents.

Key words: - *Asparagus africanus, Dichrostachys cinerea, Ficus iteophylla, Indigofera pulchra, Schwenkia americana*, Saponin, Anti-inflammatory activity, Carrageenan, TLC.

Introduction

Inflammatory diseases are a major cause of morbidity world-wide. Non-steroidal anti-inflammatory drugs and steroids are the most common drugs used to treat inflammation. Gastrointestinal side effect is a major side effect associated with the currently available non-steroidal anti-inflammatory drugs which limit their application. This may be contributing to the current move by large proportion of world population towards herbal remedies for the treatment of inflammatory diseases.

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world (Parekh et al., 2005). A number of medicinal plants are used in developing countries for the management of a number of disease conditions including pain and inflammatory conditions. The validation of the folkloric claims of these medicinal plants will provide scientific basis for the conservation of tropical medicinal resources, the deployment of the beneficial ones as phytomedicine in the primary health care and the development of potential bioactive constituents as novel lead compounds or precursors in drug design. One of such phytoconstituents is saponins.

Saponins are heterogeneous group of naturally occurring surface active glycosides produced mainly by plants and also by lower marine animals and some bacteria (Francis et al., 2002). They are composed of triterpenoid or steroid aglycone moiety and complex oligosaccharide substituent. The hydrophilic properties of the sugar part and lipophilic properties of the aglycone part give saponins their amphiphilic or surfactant properties which in turn give rises to their ability to form stable aqueous foams as well as forming complexes with membrane steroids and lipid compounds (Hostettmann and Marston, 1995). The mounting demand for natural products coupled with their physicochemical properties and numerous biological activities has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics and pharmaceutical industries (Guclu-Ustundag and Mazza, 2007). Steroidal saponins are important raw materials for the production of steroidal hormones and drugs (Brain et al., 1968). Saponins are used as immunological adjuvants in the formulation of vaccines due to their immune enhancing properties (Francis et al., 2002). Information on the biological activities of saponins from variety of sources provide lead for the development and design of new drugs. An example is the chemotherapeutic activity of the ginseng

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saponins prompted the development of anticancer drugs (Huang and Qi, 2005) and a new class of HIV drugs called maturation inhibitors (PA – 457) developed from betulinic acid derivatives (Panacos, 2005).

The five medicinal plants used for this study are locally used in traditional medicine as anti-inflammatory agents. *Schwenkia americana* Linn family Solanaceae is a slender erect herb, woody at the base, grows up to 1m tall, common in waste places and widespread in tropical Africa and America. *Asparagus africanus* Lam family Liliaceae (Asparagaceae) is an erect plant that grows up to 1.75m tall with numerous wiry, spiny branches. It is annual from a woody perennial root-stock and generally widespread in the drier parts of tropical Africa. *Dichrostachys cinerea* (Linn) Wright & Arn family Leguminosae-Mimosoideae is a shrub or small tree that grows up to 12m high or 2-5m in the drier areas and is widespread across tropical Africa. *Ficus iteophylla* Miq family Moraceae is a tree that grows up to 12m high, with the trunk about 4m in girth, widespread in the savannah region. *Indigofera pulchra* Willd family Leguminosae-Papilionoideae is a small under shrub or semi-woody erect herb that grows up to 1.5m high widespread in the savannah region of West Africa. These plants provide common remedy for rheumatic pain, arthritic conditions and swellings in Northern Nigeria (Burkill, 1995, 1997, 2000). Some previous studies have reported the anti-inflammatory activities of the crude extracts of these plants (Hassan et al., 2008a; Hassan et al., 2008b; Musa, 2008).

Literature survey shows that *S. americana* contains steroidal saponins (Kapunda and Delaude, 1988) and the isolation of monodesmodic and bidesmodic saponosides from the roots *A. africanus* collected in Ethiopia (Debella et al., 1999). Saponins have also been detected to be present in *D. cinerea* (Kuber and SanthRani, 2009) and also the isolation of free triterpenes such as alpha- amyrin from the plant (Joshi and Sharma, 1974). Some free triterpenes and sterols have been identified in the roots and leaves of different species of Ficus (Lansky et al., 2008) and also in some species of Indigofera (Leite et al., 2006). The anti-inflammatory and other effects of saponins from various plants are well documented (Lacaille-Dubois and Wagner, 1996; Francis et al., 2002). The numerous biological activities associated with saponins have lead to great interest in their characterization and in the investigation of their pharmacological and biological properties. This study was under taken to investigate the anti-inflammatory activity of the crude saponins extracts of the various parts of the above mentioned medicinal plants.

Materials and Methods

Plant Materials

The whole plants of *Schwenkia americana*, the rhizomes of *Asparagus africanus*, the leaves of *Dichrostachys cinerea*, the stem bark of *Ficus iteophylla* and the leaves of *Indigofera pulchra* were collected from Samaru and Basawa villages near Zaria, Kaduna state of Nigeria in the month of August, 2008. They were identified and authenticated in the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where specimens with numbers 532, 900129, 900236, 7167 and 410 respectively have been deposited. The various parts were air-dried under shade and powdered separately using mortar and pestle.

Extraction Procedures

The powdered materials (100g each) were extracted exhaustively with aqueous ethanol (70%). The extracts obtained were concentrated separately under reduced pressure, suspended in distilled water, filtered and partitioned with ethylacetate, then n-butanol. The butanol fractions were further partitioned with 1% potassium hydroxide to remove polyphenolic compounds (Woo et al., 1980). The butanol fractions were then concentrated under reduced pressure, dissolved in methanol and precipitated with diethyl ether (Hostettmann et al., 1991) to give saponins residues which were weighed and coded as WSA for *S. americana*, RAA for *A. africanus,* LDC for *D. cinerea,* BFI for *F. iteophylla* and LIP for *I. pulchra.*

Test for Saponins

The crude saponins extracts were subjected to froth and haemolytic tests (Brain and Turner, 1975).

Thin-layer Chromatography

Thin-layer chromatographic (TLC) analyses of the crude saponins extracts were carried out using the method of Stahl (1969). Various solvent systems were used and the developed plates were visualized using Liebermann-Burchard spray reagent and heating at100^{o}C for 10 mins.

Test Animals

Swiss albino mice weighing 17-27 g and Wister rats weighing 150-200 g of both sexes were used for the study. The animals were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in clean animal cages and given standard animal feeds (Grower' mash:

Sander's Feed Ltd, Kaduna-Nigeria) and clean water *ad libitum.* All experiments were carried out according to standard ethics guiding the handling of laboratory animals.

Drugs and Dosages

The following drugs were used: Ketoprofen (Lek, Yugoslavia: 10 mg/kg), 1% suspension of carrageenan (Sigma: 0.1ml/animal), crude saponin extracts, vehicle (Normal saline: - DANA, Nigeria: 1 ml/kg). All test solutions were administered intraperitoneally.

Acute Toxicity Study

This was carried out to determined the LD_{50} using the method described by Lorke, (1983). Thirteen mice were used to determine the LD_{50} of each of the crude saponin extract. In the first phase of each of the study, nine mice divided into three groups each containing three mice, were administered with the crude saponin extracts at doses of 10, 100, 1000 mg/kg body weight intraperitoneally respectively. Depending on the dose that killed the animals, the second phase involving four groups of one animal each were then treated with graded doses lower than the lethal dose per body weight. Signs of toxicity and lethality were observed for 24 hrs at regular intervals. The LD_{50} of the different crude saponin extracts were calculated as the geometric mean of the lowest lethal dose and the highest non-lethal dose.

Carrageenan-induced Rat Paw Oedema Test

The study was carried out according to the method described by Winter et al. (1962). Forty-two rats were divided into seven groups containing six animals each. Five groups were pretreated with the crude saponin extracts: WSA 90 mg/kg, RAA 250 mg/kg, LDC 250 mg/kg, BFI 20 mg/kg and LIP 250 mg/kg respectively. These doses were 20 percent of their calculated LD_{50} (Vongtau at el., 2004) while two groups received ketoprofen (10 mg/kg body weight) as positive control and normal saline (1 ml/kg body weight) respectively to each group. All doses were given intraperitoneally. After 30 mins, 0.1 ml of freshly prepared carrageenan suspension (1%w/v in normal saline) was injected into the sub-plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of vernier caliper at 0, 1, 2, 3 and 4 hours after injection of the carrageenan. Percentage inhibition of oedema was calculated using the following formula:

$$\% \text{ inhibition of oedema} = \frac{\text{Mean oedema increase (Ct)} - \text{Mean oedema increase (Tt)}}{\text{Mean oedema increase (Ct)}} \times 100$$

Where Ct = Control group at time 1, 2, 3 and 4 hours
Tt = Treated groups at time 1, 2, 3 and 4 hours

Statistical Analysis

The data obtained from the carrageenan-induced paw oedema test were expressed as mean ± SEM and analyzed using one way ANOVA followed by Dunnett post hoc t-test for multiple comparisons. P values less than 0.05 ($P<0.05$) were considered to be statistically significant.

Result

WSA and RAA have the highest percentage content of crude saponins while BFI has the lowest value as shown in Table 1. The crude saponin extracts tested positive to the froth and haemolytic tests. The TLC profile of each of the crude saponin extracts showed the presence of three major spots with different R_F values using various solvent systems as shown in Table 2. RAA, LDC and LIP gave similar LD_{50} of 1264.9 mg/kg while WSA and BFI gave LD_{50} of 471.2 and 118.3 mg/kg respectively. The main sign and symptom of toxicity of WSA, RAA and BFI was depression while that of LDC and LIP was excitation as shown in Table 3. The crude saponin extracts produced statistically significant ($P<0.05$) decreases in the carrageenan-induced rat paw oedema at 2 hrs which was the point of maximum oedema production in this study as shown in Table 4. BFI produced the highest percentage inhibition of oedema followed by LDC at doses of 20 and 250 mg/kg respectively which was comparable to ketoprofen (10 mg/kg) at 3 hrs as shown in Table 5.

Table 1: Percentage crude saponin yield (%w/w) of the dried plant materials and the result of phytochemical tests for the presence of saponins

Saponin Extract	Yield (%w/w)	Froth Test	Haemolytic Test
WSA	2.74	+	+
RAA	3.59	+	+

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LDC	1.62	+	+
BFI	0.81	+	+
LIP	1.57	+	+

Note:- + = Positive to the test

Table 2: TLC profile of the crude saponin extracts of the five plants under study

Saponin Extracts	Solvent System	No of Spots	Colour of Spots	R_F Values
WSA	EA, MeOH, H_2O (3:2:1)	3	1-3 Green	1- 0.1 2- 0.2 3- 0.4
RAA	EA, MeOH (2:1)	3	1-3 Green	1- 0.2 2- 0.5 3- 0.6
LDC	EA, MeOH, H_2O (6:1:1)	3	1- Yellow 2- 3 Purple	1- 0.4 2- 0.5 3- 0.6
BFI	EA, EtOH (1:1)	3	1-3 Purple	1- 0.3 2- 0.6 3- 0.8
LIP	EA, EtOH (3:1)	3	1- Yellow 2- 3 purple	1- 0.1 2- 0.2 3- 0.4

Note:- EA= Ethylacetae; MeOH= Methanol; H_2O; EtOH= Ethanol

Table 3: LD_{50} of the crude saponin extracts in mice

Saponin Extracts	LD_{50} (ip; mg/kg)	Sign and Symptom of Toxicity
WSA	471.2	Depressed
RAA	1264.9	Depressed
LDC	1264.9	Excited
BFI	118.3	Depressed
LIP	1264.9	Excited

Table 4: The result of anti-inflammatory activities of the crude saponin extracts of the five plants studied

Treatment Groups	Mean Increases in Paw Oedema ± SEM			
	1 hour	2 hours	3 hours	4 hours
Normal saline (10ml/kg)	0.162 ± 0.014	0.238 ± 0.031	0.166 ± 0.032	0.172 ± 0.029
WSA (90mg/kg)	0.114 ± 0.016^{NS}	0.158 ± 0.031*	0.102 ± 0.022^{NS}	0.068± 0.019**
RAA (250mg/kg)	0.096± 0.004**	0.110± 0.010**	0.082 ± 0.011*	0.078± 0.011**
LDC (250mg/kg)	0.076± 0.028**	0.102± 0.029**	0.050 ± 0.026**	0.048±0.019***
BFI (20mg/kg)	0.064±0.008***	0.074±0.011***	0.056 ± 0.021**	0.058±0.012***
LIP (250mg/kg)	0.122 ± 0.009^{NS}	0.146 ± 0.022*	0.108 ± 0.013^{NS}	0.104± 0.013*
Ketoprofen (10mg/kg)	0.056±0.010***	0.106 ± 0.009**	0.080 ± 0.013*	0.066±0.008***
ONE WAY ANOVA	df = 6, 28 F = 6.405 α = P<0.001	df = 6, 28 F = 5.615 α = P<0.01	df = 6, 28 F = 3.467 α = P<0.05	df = 6, 28 F = 6.011 α = P<0.001

Note:- *P<0.05, **P<0.01, ***P<0.001, NS = not significant, n = 5

Table 5: Percentage inhibition of the saponins extracts and ketoprofen on the carrageenan-induced rat paw oedema test

Drug Treatments	Percentage inhibition of oedema (%)			
	1hour	2 hours	3 hours	4 hours

WSA	30	34	39	61
RAA	41	54	51	55
LDC	53	57	70	72
BFI	61	69	66	66
LIP	25	39	35	40
Ketoprofen	65	56	52	63

Discussion

WSA and RAA gave the highest percentage content while their TLC profile when sprayed with Liebermann-Burchard reagent showed the presence of green spots indicative of steroidal saponins (Chandel and Rastogi, 1980). The purple spots observed on TLC analyses of LDC, BFI and LIP were suggestive of the triterpenoid saponins. Steroidal saponins have been reported to be found in monocotyledons such as Agavaceae, Dioscoreaceae, Liliaceae and Solanaceae while triterpenoids are more predominate in dicotyledons such as Leguminosae, Caryphyllaceae etc (Guclu-Ustundag and Mazza, 2007). RAA, LDC and LIP gave higher intraperitoneal LD_{50} of 1264.9mg/kg each which indicates that these crude saponins extracts are relatively less toxic and therefore safer than WSA and BFI. LDC and LIP gave relatively similar TLC profile and the same LD_{50} along with the sign and symptom of excitation. This may be due to the fact that the plants from which the crude saponin extracts were obtained belong to the same family (Leguminosae) although from different sub families.

The crude saponin extracts showed significant anti-inflammatory activity in the carrageenan-induced rat paw oedema test. The development of the carrageenan-induced oedema has been shown to be bi-phasic in nature. The first phase of the development has been attributed to the release of histamine, serotonin and kinins while the second phase has been considered to be due to the release of prostaglandins and bradykinins (Larsen and Hanson, 1983). The result showed that LDC (250 mg/kg) and BFI (20 mg/kg) exhibited significant inhibitory activity against the carrageenan-induced oedema at all the phases of inflammation which was observed to be comparable to that produced by ketoprofen (10mg/kg). Generally the results obtained in this work suggest that all the crude saponin extracts tested have significant anti-inflammatory activity that might be mediated through the inhibition of the release and synthesis of the agents that produce inflammations.

Saponins have been reported to possess a wide range of biological activities such as haemolysis, pesticidal, molluscidal, antimicrobial, insecticidal, anthelmintic, analgesic, anti-inflammatory, sedative and antitumor activities (Lacaille-Dubois and Wagner, 1996; Rao and Gurfinkel, 2000). Evidence for the anti-inflammatory properties of saponins has been provided by several studies using different models of inflammation (Capra, 1972; Chandel and Rastogi, 1980; Singh et al., 1992; Gepdiremen et al., 2005; Cheeke et al., 2006). Saponins isolated from the leaves and root extract of *Camellia sinensis* have been shown to inhibit carrageenan-induced rat paw oedema in a dose dependant manner (Sur et al., 2001). Other mode of actions have been used to explain the anti-inflammatory activity of saponins; an example is the anti-inflammatory activity of platycodin D isolated from the roots of *Platycodon grandiflorum* shown to be due to the inhibition of 12-O-Tetradecanoyl-phorbol-13-acetate (TPA)-induced PGE_2 production through the inhibition of COX-2, secondly the hederagenin glycoside from *Kalopanax pictus* was reported to inhibit lipopolysaccharide (LPS)-induced iNOS and COX-2 protein expression which led to the inhibition of nitric oxide (NO), PGE_2 and tumor necrosis factor-α (TNF-α) (Yaun et al., 2006). It has been suggested that the numerous biological activities of saponins were linked to their amphiphilic nature which help in the accomplishment of these activities through their ability to intercalate into the plasma membrane resulting in changes in membrane fluidity that in turn affect membrane function thus eliciting a cellular response.

Conclusion

The results of this study have shown that the crude saponin extracts of the whole plant of *S. americana,* the rhizome of *A. africanus*, the leaves of *D. cinerea*, the stem bark of *F. iteophylla* and the leaves of *I. pulchra* possess some potential anti-inflammatory properties. In line with the general principles however, we suggest the need to further investigate the therapeutic properties of these crude saponins extracts for therapeutic purpose and also to isolate and characterize their saponins.

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