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Full Length Research Paper

Activity of saponin fraction of *Anisopus mannii* against some pathogenic microorganisms

A. B. Aliyu[1]*, A. M. Musa[2], M. S. Abdullahi[3], M. A. Ibrahim[4], M. B. Tijjani[5], M. S. Aliyu[5] and A. O. Oyewale[1]

[1]Department of Chemistry, Ahmadu Bello University, Zaria-Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria.
[3]National Research Institute for Chemical Technology-Basawa, Zaria-Nigeria.
[4]Department of Biochemistry, Ahmadu Bello University, Zaria-Nigeria.
[5]Department of Microbiology, Ahmadu Bello University, Zaria-Nigeria.

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Partially purified saponin fraction was obtained from column chromatography of crude saponin precipitate of the aerial part of *Anisopus mannii*, and subjected to antimicrobial susceptibility study against pathogenic microorganisms using the disc diffusion technique. The result of the antimicrobial activity indicated by zone of inhibition of growth ranged from 13.0 to 22.2 mm. The fraction was more potent on *Escherichia coli* (22.2 mm) and least on *Klebsiella pneumoniae* (13.0 mm). The saponin fraction showed the lowest minimum inhibitory concentration (MIC) value of 30 mgml^{-1} and minimum bactericidal concentration (MBC) value 40 mgml^{-1} on *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, E. coli, Shigella dysentriae* and *Pseudomonas aeruginosa.* The spectra of the activity exhibited by the partially purified saponin signify the potential of saponin from *A. mannii* for the development therapeutic agents against these pathogenic microorganisms.

Key words: *Anisopus mannii*, saponin fraction, antimicrobial activity, pathogenic microorganisms.

INTRODUCTION

All over the world, infectious disease is the number one cause of death accounting for approximately one-half of all the deaths in tropical countries (Iwu et al., 1999). This perhaps may be attributed to the increasing incidence of multiple resistance in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Afolayan and Aliero, 2006). The situation has resulted in the emergence of hitherto unknown diseases causing microbes that pose enormous public health concern (Iwu et al., 1999). The spectrum of emerging and re-emerging infectious diseases spells out the need to identify new and complementary antimicrobial agents to combat these unusual pathogens and plant natural products are an obvious source (Williams, 2006). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic protypes (Maurer-Grimes et al., 1996; Rabe and van Staden, 1997; Afolayan, 2003).

Anisopus mannii N.E. Br. (Asclepaidaceae) is a glabrous twining shrub with leaves petiolate, elliptic, ovate and shortly cuspidate at apex up to 15 cm or more long and 12 cm broad, and the stem twining to a height of 3.7 to 4.6 cm (Hutchinson and Dalziel, 1963). The plant is known as “kashe zaki” (Hausa) meaning “destroying sweetness”. It is a familiar herb in the traditional medicinal preparations in Northern Nigeria, where a decoction of the leaves is used as remedy for diabetes mellitus, diarrhea and pile. Phytochemical and antimicrobial screening, hypoglycemic effects as well as the toxicological studies of the stem aqueous extracts of *A. mannii* has been reported (Sani et al., 2009a; b; 2010). The proximate composition, mineral elements, antinutrional factors and *in vitro* antioxidant activity of aerial part of the plant were also reported (Aliyu et al., 2009; 2010). Chemical constituents such as

*Corresponding author. E-mail: aliyubabando@gmail.com. Tel: +2348057371917.

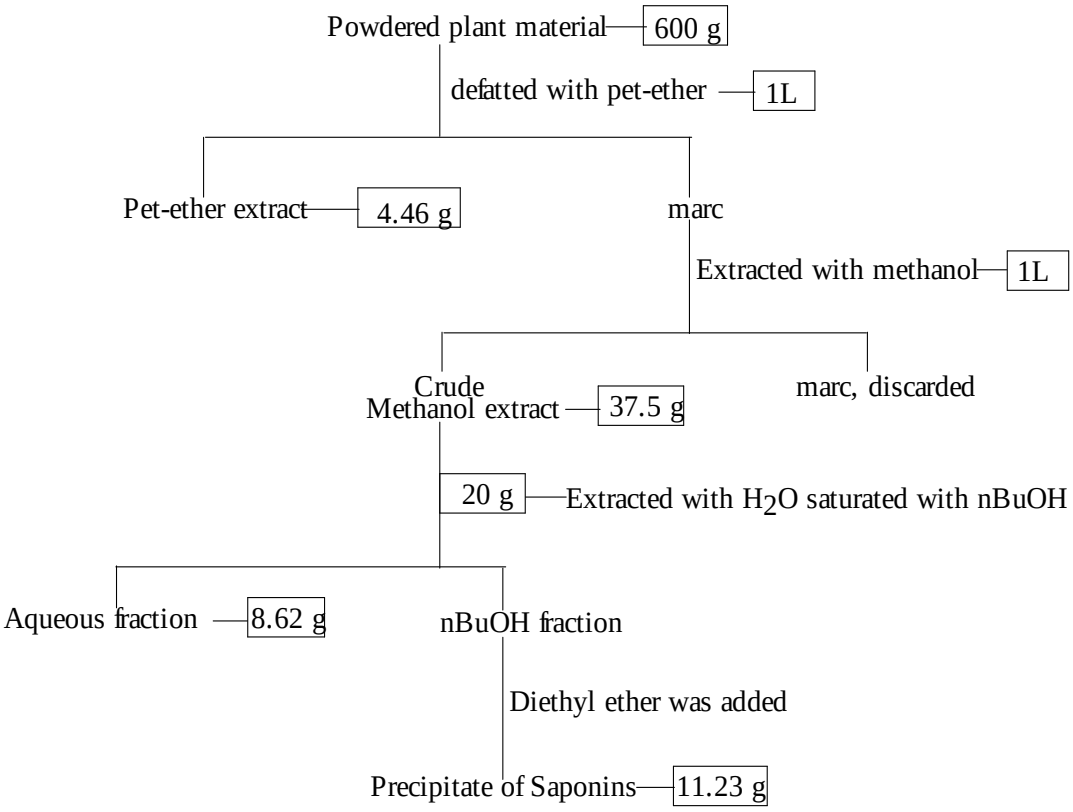

Figure 1. Fractionation method of precipitating saponins from plants, adapted from Hostettmann et al. (1991).

1,7-naphthyridine alkaloid- named anisopusin, 5α-hydroxy-lup-20(29)-en-3β-yl eicosanoate, [6]-gingerdione, [6]-dehydrogingerdione and ferulic acid were isolated from dichloromethane fraction of acetone extract of the stem bark of *A. mannii* (Tsopmo et al., 2009). Plants belonging to Asclepiadaceae family were found to be rich in cardinolides (Warashina and Noro, 1994) and saponin glycosides (Ye et al., 2000). Saponins chemically consist of fat-soluble nucleus (aglycone) that is either a triterpenoid (C-30) or steroid (C-27) attached with one or more sugar side chains (glycone) at different carbon sites of the aglycone. They have characteristic surface active properties and form foamy solutions in water. Phytochemicals whose chemical properties are known can be useful in the remedy of microbial infections. In the last few years, so much phytochemical screening have been reported from different cultures on the activity of plants crude extracts against numerous microorganisms. (Sánchez-Medina et al., 2001; Jeevan Ram et al., 2004; Nanasombat and Lohasupthawee, 2005; Akinyemi et al., 2006; Kuete et al., 2007; Abu Shanab et al., 2008; Tijjani et al., 2009).

However, in our continued search for active therapeutic constituents from Nigerian medicinal plants; this paper reports the activity of partially purified saponin fraction of *A. mannii* against some human pathogenic microorganisms.

MATERIALS AND METHODS

Sample collection and preparation

Whole plant of *A. mannii* N.E. Br. (Voucher No. 217) was collected in February, 2009 at Samaru along Giwa road in Zaria Kaduna State. The plant was taxonomically authenticated and specimen of the sample deposited at the Herbarium, Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. The aerial part of the plant was air dried for two weeks and pulverized to powder using pestle and mortar.

Extraction and preparation of saponin fraction

The sample (600 g) was extracted with methanol (1 L) by cold extraction for two weeks. The extract was filtered using Whatman filter paper No. 2, and concentrated on a rotary evaporator (Büchi Rotavapor R-124) at 45ºC to give 37.5 g of the crude methanol extract.

The methanol crude (20 g) was suspended in water saturated with n-butanol in a separatory funnel. The n-butanol portion separated and collected from the aqueous portion. Diethyl ether (200 ml) was added to the n-butanol portion in a pre-weighed container, and crude saponin was precipitated (11.23 g) (Hostettmann et al., 1991). The scheme is as shown in Figure 1.

Table 1. Results of antimicrobial activity of partially purified saponin fraction of *A. mannii.*

	Zone of inhibition (mm)		
Test organisms	**PPSF (100 mgml^{-1})**	**Ampiclox 75 µg**	**Streptomycin 30 µg**
S. aureus	21.8	20	27
S. pyogenes	21.0	0	22
C. ulcerans	20.0	NT	NT
B. subtilis	12.0	30	30
S. dysentriae	19.5	NT	NT
E. coli	22.2	14	0
P. aeruginosa	22.0	NT	NT
K. pneumonia	13.0	0	20
N. gonorrhoeae	0	NT	NT
C. albicans	19.0	NT	NT

PPSF: Partially purified saponins fraction, NT: Not tested.

Percent recovery of crude saponin was calculated as: weight of crude saponin/weight of crude methanol extract × 100%.

Preliminary fractionation of crude saponins

Column chromatography of the saponin fraction was carried out using silica gel (60, 0.04-0.063 mm). Saponin fraction (1 g) was dissolved in minimum amount of methanol and the solution was adsorbed on about 4 g of silica gel and allowed to dry. The adsorbed silica was then applied to the top of the column. A gradient elution of solvent systems with $CHCl_3$: MeOH (10:1) gradually introducing (5:1) and finally introducing (1:1) (Debella et al., 1999).

One hundred and twenty fractions of 5 ml volume were collected and analyzed by thin layer chromatography (TLC) on pre-coated plates using $CHCl_3$:MeOH (2:1) as the mobile phase. The components were visualized under visible and UV-light (254 and 366 nm), plates were then sprayed with 50% sulphuric acid in methanol and heated at 100℃ for 1 to 2 min. From TLC results, fractions F1-44, F45-83 and F84-120 were pooled according to their R_f-values. The combined fractions were allowed to dry in pre-weighed containers to afford the saponin sub-fractions A-I, B-II and C-III. Partially purified fraction A-I (0.47 g) was used for the activity studies against some clinical pathogenic isolates from humans.

Chemical test on the fraction

Partially purified fraction I was subjected to frothing, hemolysis, shinoda and ferric chloride tests (Sofowora, 1993).

Test organisms

The strains of organisms namely: *Escherichia coli, Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Neisseria gonorrhea, Salmonella typhi, Pseudomonas aeruginosa* and *Candida albicans* used in this study were clinical isolates obtained from the Department of Microbiology Ahmadu Bello University Teaching Hospital (ABUTH) Shika.

The isolates were purified on nutrient agar (OXOID) plates and characterized using standard microbiological and biochemical procedures as described by Cowan and Steel (1974) and Macfadden (1977).

Determination of antibacterial activity

The disc diffusion method was used (Nostro et al., 2000). Stock solution (100 mgml^{-1}) of the saponin fraction was prepared using DMSO. Disc (6 mm diameter) were prepared using Whatman filter paper and sterilized by autoclaving. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (OXOID) surface and impregnated with 15 µl of stock solutions (300 µgdics^{-1}). Antibiotic discs of ampiclox (Ranbaxy Laboratory Ltd. India) (75 µg dics^{-1}) and streptomycin (Shijiazhuang Pharm-Group, Zhongnuo Pharmaceutical Co. Ltd. China) (30 µg dics^{-1}) were used as positive control while the diluent (DMSO) without any fraction was used as negative control. The plates were incubated at 37℃ for 24 h. All tests were performed in duplicate and the antimicrobial activity was expressed as the mean diameter of inhibition zones (mm) produced by the fraction.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was carried out using micro broth dilution in accordance with National Committee for Clinical Laboratory Standards (NCCLS), 2002. Serial dilution of the least concentration of the extract that showed activity was prepared using test tubes containing 9 ml of double strength broth. The tests tubes were inoculated with the suspension of the standardized inocula and incubated at 37℃ for 24 h. MICs were recorded as the lowest concentration of extract showing no visible growth of the broth.

Determination of minimum bactericidal concentration (MBC)

Minimum bactericidal concentration was determined by aseptically inoculating aliquots of culture from MIC tubes that shows no growth, on sterile nutrient agar plates and incubating at 37ºC for 48 h. MBC was recorded as the lowest concentration of extract showing no bacterial growth.

RESULTS

The result of preliminary fractionation of the crude saponins extract of *A. mannii* has yielded a partially

Table 2. Results of MIC and MBC of partially purified saponin fraction of *A. mannii* ($mgml^{-1}$).

Test organisms	MIC	MBC
S. aureus	30	40
S. pyogenes	30	40
C. ulcerans	30	40
B. subtilis	40	50
S. dysentriae	30	40
E. coli	30	40
P. aeruginosa	30	40
K. pneumonia	40	50
N. gonorrhoeae	NT	NT
C. albicans	40	50

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, NT: Not tested

purified saponin fraction (PPSF) (0.47 g), which upon further chemical test confirmed the presence of saponins and absence of flavonoids. The fraction exhibits antimicrobial activity on the test organisms producing zones of inhibition ranging from 13.0 to 22.2 mm. (Table 1). The activity was more prominent on *E. coli* (22.2 mm) and least on *K. pneumoniae* (13.0 mm). The partially purified saponin fraction showed the lowest minimum inhibitory concentration (MIC) value of 30 mg/ml and minimum bactericidal concentration (MBC) value 40 $mgml^{-1}$ on *S. aureus, S. pyogenes, C. ulcerans, E. coli, S. dysentriae* and *P. aeruginosa. S*imilarly, the MIC value of 40 $mgml^{-1}$ and MBC of 50 $mgml^{-1}$ were exhibited on *Bacillus subtilis, K. pneumoniae* and *C. albicans*. Only *Neisseria gonorrhoeae* was resistant to the PPSF (Table 2).

DISCUSSION

The fractionating method of precipitating saponins as reported by Hostettmann et al. (1991) was found to be effective, because of the percent recovery (56.15%) of the crude saponins extract. Saponins are glycosides of triterpenes, steroids or steroidal alkaloids; they are regarded as natural antimicrobial compounds with very diverse biological activities whose roles in food, animal feedstuffs and pharmaceutical applications have been useful to man (Oleszek and Marston, 2000). Saponins have been implicated as bioactive antibacterial agents of plants containing them (Mandal et al., 2005; Manjunatha, 2006). The partially purified saponin fraction (PPSF) of *A. mannii* has demonstrated interesting activity especially on *E. coli* and *P. aeruginosa*; these are microorganisms that cause infections which are difficult to combat due to multi-drug resistance (Salie et al., 1996; Afolayan and Aleiro, 2006). Saponin obtained from *Sorghum bicolor* was found to possess antibacterial property against gram positive but not gram negative bacteria (Soetan et al., 2006). Another study indicates the antibacterial sensitivity of saponins more on gram negative than gram positive (Morrissey and Osbourn, 1999). In contrast, Hassan et al. (2010) reported the of saponin-rich extract of guar meal having more activity on gram positive than gram negative bacteria. It is however interesting that the PPSF had inhibitory effects on both gram positive and gram negative bacteria and including a fungus *Candida albicans*. Although variations in the chemical nature of the saponins from different plants reported in the literature, may perhaps explain the variation in activity against various pathogens. The PPSF showed strong potency (MIC 30 $mgml^{-1}$ and MBC 40 $mgml^{-1}$) on *S. aureus,* which has been implicated in several human and animal infections.

The significance of this study is the inhibition of microbial growth by the partially purified saponin fraction (PPSF) (Table 1). This is indicative of potential utility of the saponins of *A. mannii* in the development of antibacterial therapeutic agents. Saponins have been typically observed to have higher or lower antibiotic activity (Farnsworth, 1966). However, our findings are rather consistent with the higher antimicrobial potency which agree with previous study of antibacterial activity of saponin from *Gymnema sylvestre* (Gopiesh khanna and Kannabiran, 2008)-a plant of the Asclepiadaceae family. Saponin compounds in plant are believed to naturally protect it against attack from pathogens, which would account for their antimicrobial activity (Osbourn, 2003). The mode of action of antibacterial effects of saponins may involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium (Killeen et al., 1998).

Conclusion

The antimicrobial potential of saponin fraction of *A. mannii* was evaluated and this could be a basis for further purification of pure component (s), so that the activity can

be ascertained for potential utility as therapeutic agents against microorganisms.

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