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Antimicrobial and foam forming activities of extracts and purified saponins of leaves of *Tephrosia vogelii*

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ABSTRACT

The presence of saponins was detected by the formation of long-lasting foams. Foam-forming activities of crude aqueous extract(Phosphate Buffered Saline(PBS)) of the leaves were more pronounced than those of methanolic extract(Methanol/Phosphate Buffered Saline(M/PBS)). Crude PBS extract foamed with a height of 1.38±0.70mm and foaming time of 2.59±0.09 hr while crude M/PBS extracts possessed a foam height of 0.70±0.06 mm and foaming time of 1.51± 0.02 hr. Generally, the foaming time was not proportional to the foam height. The foam-forming activity of the purified saponins(PS) was also determined. The four fractions of purified saponins (PS_2, PS_3, PS4 and PS_5) also possessed considerable and long-lasting foams with foaming activities ranging from 0.53± 0.01 mm to 1.18± 0.02 mm for foam heights and 1.08±0.08 hr to 1.25± 0.09 hr for foaming times. Antimicrobial activity of crude aqueous and methanolic extracts as well as purified saponins of aqueous extract of leaves of the plant against four different bacteria(Streptococcus pneumonia, Staphylococcus epidermidis, Neisseria gonorrhoea and Escherichia coli) were determined. It was observed that with respect to all the bacteria using 100 mg/ml of extracts and 6.40 mg/ml of purified saponins there were no diameters of zones of inhibition while the diameters of zones of inhibition increased with increase in concentration of crude extracts from 200 mg/ml to 300mg/ml and purified saponins from 6.60 mg/ml to 7.02mg/ml.

Key words: Antimicrobial, foam forming, aqueous, methanol, *Tephrosia vogelii*,

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INTRODUCTION

A medicinal plant is a plant which one or more of its organs contains substances that may be used for therapeutic purposes or as precursors for the synthesis of useful drugs [17]. Medicinal plants have been extensively studied as an alternative treatment for diseases in order to overcome the problem of antibiotic resistance by pathogenic organisms [18]; [13]. Numerous plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United State Food and Drug Administration or European Food Safety Authority to have medicinal value of which *Tephrosia vogelii* is inclusive. Many plants of medicinal value remain untapped, thus, a need to encourage researchers to evaluate those medicinal plants for the betterment of mankind. It is used extensively in Nigeria for the traditional treatment of malaria and other associated conditions in form of decoction, in which unspecified quantities are usually consumed without due regards to toxicological and other adverse effects [2]; [8]; [7]. Based on this traditional and other uses of *Tephrosia vogelii*, this study was conducted to ascertain it's potentially foam forming and antibacterial activities. The medicinal values of plants lie in their component phytochemicals, which produce definite physiological actions on the human body [1]. Several biological effects have been ascribed to saponins. Several plants have advanced to clinical use in modern times [9] in the treatment of chronic diseases such as diabetes, hypertension and cardiovascular diseases. One of such plants is *Tephrosia vogelii* Hook. f**.** The plant is native to Nigeria, Kenya, South Africa, Zambia, Tropical America, Southeast Asia and

Malaysia as a cover crop. Saponins are glycosides of 27 carbon atom steroids or 30 carbon atom triterpenes in plants. They are often referred to as "natural detergent" because of their foamy texture. They derive their name from the Latin word "Sapo" which means that the plant consists of frothing agent when diluted in aqueous solution. They comprise polycyclic aglycones and sugar moieties (hexoses, pentoses and saccharic acids). The sapogenin or aglycone part is either a triterpene or steroid. They are found in different plants such as soap-wort, soapberry, soapbark, soaproot, soyabeans, vegetables and herbs. Commercial saponins are mainly extracted from *Quillajasaponaria*and *Yucca schidigera.* Saponins as the sapogenin aglycone have also been identified in the animal kingdom in snake venom, starfish and sea cucumber. Saponins can be easily extracted using hot water, ethanol or methanol because of their solubility in them. They are characterized by form- forming, haemolytic activities and bitter taste, and occur in small quantities in nature [4]. Some toxic saponins are known as sapotoxins. They have been known to be toxic to cold- blooded creatures like snake and fish [12]. Some examples of saponins are ginsenosides of ginsen, titogenin and digitogenin. They are used as detergents for cleaning and foam as well as shampoo producers. In some cases, saponins from different parts of the same plant have been found to exhibit different properties. For example; *Spinacia oleracea* plant root contains spina-saponins A and B, which show potent antibiotic activities, but the leaves are practically free of saponins. The saponins from the stem of *Guaicum Officinale* do not possess any biological activity whereas the leave saponins have marked heamolytic actions [3]. Again, sometimes, the saponins from different plants have been found to exhibit the same biological properties for example; bark of *Schimamer tersiana* contains piscisidal saponins, so also the fruits, roots and bark of *Balanitesa egyptiaca* [14]. Differences in saponins contents have been observed in some cases when the same varieties of plants are collected from different localities [3]. Saponins are natural antibiotics, cholesterol- lowering agent [5] as well as immune and energy boosters.

MATERIALS AND METHODS

Treatments of plant part

The identified leaves were washed to remove particles and dust. The washed leaves were heated at 80^0C for 10min and 60^0C for 30min to deactivate enzymes (glycosidases) which may break down saponins [6]. Further drying using atmospheric air was carried out for complete dryness. The dried leaves were ground into powder using a mortar and pestle before been separately milled into fine powder using an electric blender to pass through a 0.3mm sieve (BS 410 Endecotts Ltd London). The sample was sieved with 0.3mm sieve made of brass material.

Preparation of extracts

A 50g portion of the sieved sample was weighed into a 500ml conical flask and 300ml of distilled water added. It was thoroughly shaken and covered with a piece of thin foil paper and allowed to stand overnight. It was shaken intermittently for maximum extraction. The aqueous extract was carefully decanted into another conical flask. Another 300ml of distilled water was added and the mixture treated the same way. The extraction was carried out for a period of seven days until the supernatant was colourless. The supernatant was then transferred into a weighed, cleaned, dry aluminium plate and the distilled water evaporated at low temperature using a hot plate and residue stored at room temperature until required. The sample residue from aqueous extraction was re-extracted with methanol to produce the crude methanolic extract.

Purification of saponins from *Tephrosia vogelii* leaves

The reverse phase chromatography column (Octadecylsilane(C-18) bonded to silica gel-J. T. Baker) was chosen to separate and purify the saponins from the crude extracts. The saponins solution was diluted to 6.7mg/ml and stored at 4^0C until required.

Foam-forming Activities of the Purified Saponins

The foam-forming activities of the purified saponins from the crude aqueous extract were determined using 10 fractions (2ml of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 v/v of methanol in water) that were obtained during purification by the column chromatography. The solvent system (graded solution of methanol in water) was evaporated from the test tubes on a water bath. A 5ml of PBS was added to each of the test tubes. The content in each of the test tubes was covered with index finger and the remaining four fingers were used to hold the test tube. The mixture was thoroughly shaken 50 times and allowed to stand in the test tube rack for 1 min. The foam-height was then measured immediately and the initial time as well as the time of complete disappearance of the last trace of foam was recorded. The experiment was carried out in quadruplicate

Foam-forming Activities of the Extracts

A portion of the extract (2.0g) was weighed into a porcelain mortar and 5.0ml PBS added. A pestle was then used to macerate the mixture to homogeneity. The mixture was then transferred into a labelled test tube to the last drop and corked with a rubber stopper. The mixture was thoroughly shaken using a Vortex mixer for 1 min and allowed to

settle in the test tube rack for another 1min to allow all debris to settle down. The supernatant was carefully removed using a Pasteur pipette and transferred to another labelled test-tube. The content in test tube was covered with index finger and the remaining four fingers were used to hold the test tube. The mixture was thoroughly shaken 50 times and allowed to stand in the test tube rack for 1 min. The foam-height was then measured immediately and the initial time as well as the time of complete disappearance of the last trace of foam was recorded [11]. The experiment was carried out in quadruplicate

Determination of antimicrobial activities of partially purified and crude aqueous extract
The study utilized an in vitro experimental study.

Test Organisms
Clinical isolates of *Staphylococcus aureus, Streptococcus pneumonia, Neisseria gonorrhoea* and *Escherichia coli* were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Sterilization of the equipment and disinfection
All the equipment was disinfected with cotton wool soaked in methylated spirit so as to maintain sterility throughout the process. Wire loop, conical flasks and beaker were sterilized by hot air oven at 160^0C for 45 minutes, whereas moisture insensitive materials were sterilized by autoclaving at 121ºC for 15 minutes.

Preparation of the media and cup hole
The nutrient agar media (Biochemika Fluka) was used. The composition of the nutrient agar (gm/litre) were: Meat extract 1 g, yeast extract 2 g, peptone 5 g, NaCl 5 g and agar 15 g. Twenty-eight grams of nutrient agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH of 7.4 ± 0.2 at 37^0C. This was sterilized by autoclaving at 121^0C for 15 minutes at 15 psi pressure and was used for sensitivity tests. The doubled layer preparative nutrient agar was used for the susceptibility testing. Each of the layers consists of 30 ml making 60 ml of the nutrient agar for each plate. A sterilized improvised cork borer of 6 mm in diameter was used to bore holes on the plates and each medium contained seven holes of 6 mm in diameter.

Antibacterial activity assay of the plant extracts
An overnight broth culture was diluted with peptone water to match turbidity of McFarland standard number 3 which was used as inoculums for the microorganism. One ml of the bacterial inoculums was transferred into pure agar plates with the aid of a new sterile syringe, and the agar plates were slanted to ensure the spread of the inoculums on the entire surface of the agar plates. The plant extract was administered into 5 holes on each plate with a varying strength of 100mg, 50 mg, 25 mg, 12.5 mg and 6.25 mg using sterile syringe. Likewise, distilled water and ciprofloxacin (30 mg/ml) were administered into two additional holes serving as the negative and positive controls respectively. Five plates of each organism were replicated (15 plates for the three organisms). One hour was allowed for diffusion before incubating the plates at 37^0C for 24 hours. The clear zones of inhibition (mm) were measured using meter rule.

Statistical analysis
The results of the study were expressed as mean± S.E. Data were analyzed by using one way analysis of variance test (ANOVA). Values with $P< 0.05$ were considered as significant.

RESULTS AND DISCUSSION

The foam-forming activities of crude aqueous and methanolic extracts as well as purified saponins of leaves of the plant were carried out using aliquots of the extract and ten (10) fractions of purified saponins. The results of the study showed that the crude aqueous extract had a higher foam height of 1.38± 0.70 mm and foaming time of 2.59± 0.09hr while methanolic extract possessed a foam height of 0.70± 0.06 mm and foaming time of 1.51± 0.02 hras shown in Table 1. The foaming activity of the purified saponins (PS) was also determined. The results showed that purified fractions (PS_2), (PS_3) and (PS_5) had the lowest foam heights of 0.53± 0.01 mm, 0.77± 0.05 mm and 1.18± 0.02 mm respectively from Table 1. Saponins have been known to have long-lasting foams [15]. And these results are in agreement with [16] and [15] that the foaming time is not always proportional to the foam height. The diameters of zones of inhibition of crude aqueous and methanolic extracts and purified saponins of leaves of the plant against the bacteria (*Staphylococcus aureus, Streptococcus pneumonia, Neisseria gonorrhoea* and *Escherichia coli*) were determined. The results of this study showed that both crude aqueous and methanolic extracts inhibited the growth of both gram positive and gram negative bacteria as shown in Table 2. A similar pattern of inhibition had earlier been reported for the growth of a fungus (*Aspergillus niger*) which was inhibited by both crude methanol and water extracts of *Heckel garcina* seeds [15]. The results of this study revealed that with respect to all bacteria using

100 mg/ml of extracts and 6.40mg/ml of purified saponins there were no diameters of zone of inhibition in Table 2. With respect to all bacteria, the diameters of zones of inhibition increased with increase in concentration of extracts from 200 mg/ml to 300 mg/ml and purified saponins from 6.60 mg/ml to 7.02 mg/ml as shown in Table 2. The results also revealed that the diameters of zones of inhibition decreased with same concentration of crude extracts and purified saponins from *Streptococcus pneumonia* to *Neisseria gonorrhoea* in Table 2. This could be associated with the different permeabilities of the complex layers of peptidoglycans in the cells to the extracts and purified saponins. It was also observed that aqueous extract was statistically more active than the methanolic extract at the same concentration ($p<0.05$) suggesting that the water- soluble saponins were different and more potent compared with methanol-soluble saponins. It was also observed that purified saponins at lower concentrations were more active than the crude extract at higher concentrations. The observation simply showed that the crude extracts were still mixtures of various compounds while the saponins were more potent though in small quantities but purified [4]. The extracts and purified saponins showed good sensitivities and inhibitory effects against bacteria when compared with commonly used standard antibiotics such as Ofloxacin and Ceftaxidine. These differences were however not statistically significant ($p>0.05$).

Table 1: Foam forming Activities of Aqueous and Methanolic Extracts and Purified Saponins of Leaves of *Tephrosia vogelii*

Sample	Foam Height (mm)	Foam Time (hr)
Crude Extract		
Aqueous	1.38±0.70	2.59±0.09
Methanol	0.70±0.06	1.51±0.02
Purified Saponins		
PS_2	0.53±0.01	1.25±0.07
PS_3	0.77±0.05	1.08±0.18
PS_4	1.05±0.04	1.24±0.14
PS_5	1.18±0.02	1.23±0.03

Results are means of replicate determinations Mean±S.E.M (n = 6).
PS_2- (20% Fraction) = Purified Saponins$_2$
PS_3- (30% Fraction) = Purified Saponins$_3$
PS_4- (40% Fraction) = Purified Saponins$_4$
PS_5- (50% Fraction) = Purified Saponins$_5$

Table 2: Diameters of Zones of Inhibition of Extracts and Purified Saponins Leaves of *Tephrosia vogelii*

Extract/Saponins/ Antibiotic Test	Conc. (mg/ml)	Diameter of Zones of Inhibition Bacteria(mm)			
		Streptococcus pneumoniae	Staphylococcus *Aureus*	*Escherichia coli*	*Neisseria Gonorrhoeae*
	100	0	0	0	0
Aqueous	200	23	20	17	15
Extract	300	25	24	20	18
	100	0	0	0	0
Methanolic	200	16	15	13	11
Extract	300	18	17	15	12
Saponin Fraction					
PS_3	6.40	0	0	0	0
PS_4	6.60	30	22	20	20
PS_5	7.02	40	32	30	22
Standard Agent					
Ofloxacin	10	28	12	16	30
Ceftaxidine	30	26	26	30	28

Values are means of quadruplicate determinations.
PS_3- (30% Fraction) = Purified Saponins$_3$
PS_4- (40% Fraction) = Purified Saponins$_4$
PS_5- (50% Fraction) = Purified Saponins$_5$

CONCLUSION

The results of this study have shown that the leaves of the plant *Tephrosia vogelii* Hook. f. contained saponins which were responsible for its antimicrobial activities against *Staphylococcus aureus, Streptococcus pneumonia, Neisseria gonorrhoea* and *Escherichia coli.*

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