

Evaluation of The Antimicrobial Activity of Saponins-Rich Fraction of *Zanthoxylum zanthoxyloides* Leaf

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ABSTRACT

Zanthoxylum zanthoxyloides has been reported to have several medicinal uses and has also been documented to be rich in saponins. Thus, this study aimed to evaluate the in vitro antimicrobial properties of saponins-rich extract of *Z. zanthoxyloides* leaf. The extraction of saponins from the methanolic extract of *Z. zanthoxyloides* leaf was done using standard procedure. The antimicrobial property of the extract was also tested by agar well diffusion method for activity against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Moraxella catarrhalis* isolated from clinical samples. The susceptibility patterns of test isolates against the saponins-rich fraction were determined at extract concentrations of 100, 125 and 250 mg/ml, respectively. Ciprofloxacin was used as positive control while methanol was used as the negative control. Phytochemical screening of the crude methanol extract of the leaf of *Z. zanthoxyloides* showed the presence of saponins, alkaloids, flavonoids and tannins. The antimicrobial results revealed that the extract had no inhibitory effect on *S. aureus* at all the concentrations but the largest zone of inhibition for the extract was recorded with *K. pneumoniae* and *M. catarrhalis* with mean diameter zone of inhibition of 15.04 ± 0.167 mm at 250 mg/ml. The minimum inhibitory concentration of the extract on *M. catarrhalis*, *S. aureus* and *K. pneumonia* were at 50, 125 and 500 mg/ml, respectively. The apparent antimicrobial activity of the saponins-rich fraction of *Z. zanthoxyloides*, as shown by the results of this research, suggests its potential usefulness in the prevention and treatment of infectious diseases.

Key words: Ciprofloxacin, *Klebsiella pneumonia*, *Moraxella catarrhalis*, phytochemical screening, saponins, *Staphylococcus aureus*.

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INTRODUCTION

Medicinal herbs are plants that contain substances that can be used for therapeutic purposes, some of which are precursors for the synthesis of drugs (Sofowora, 1984). The biomolecules universally present in plants can be classified as primary metabolites which include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophyll, etc, and secondary metabolites, known as the phytochemicals which include saponins, carotenoids, ascorbic acid, tocopherols, alkaloids, flavonoids, terpenoids, phenolics, etc) (Pari and Latha, 2004; Vasu et al., 2009). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves, but recent research demonstrates that many

phytochemicals can protect humans against diseases (Kumar et al., 2009). Historically, plants have played significant roles in health (Vogel and Vogel, 1997). Phytochemical screening has unveiled the chemicals responsible for these functions (Faraz et al., 2003). Structural analysis of these phytochemicals has provided the basis for their phytotherapeutic potencies (Stintzing et al., 2002). This has also increased the relevance of plant in drug developments. While the biological activities of plant extracts and phytochemicals are many and the list is still running, the biological activities most characterized include anti-cancer, anti-obesity, anti-microbial, anti-inflammatory, abortifacient and anti-plasmodial activities (Stintzing et al., 2002). One such plant considered of great importance is

Zanthoxylum zanthoxyloides (Rutaceae), commonly called 'toothache bark' or 'candle wood' (English), orin ata (Yoruba) is widespread in West Tropical Africa occurring in savanna and dry forest and coastal area of Nigeria and mostly in the southern part of the country (Iwu et al., 1999). *Z. zanthoxyloides* contains various secondary metabolites which are known for their diverse biological properties. The plant is known for its antioxidative, anti-inflammatory, anti-sickling, antibacterial, antiviral, antihepatotoxicity, antiallergic, antitumoral and antihypertensive properties (Sofowora et al., 1975; Andersson et al., 1996; Adesina, 2005).

The methanol extract preparation of the powdered root of *Z. zanthoxyloides* containing flavonoids, chelerythrine, berberine and phenol canthine-6-one have been reported to possess strong antibacterial activity (Odebiyi and Sofowora, 1979; Tsuchiya et al., 1996). They have been used as components of antiseptic, antiparasitic and analgesic preparations for managing smallpox, syphilis and related disease conditions (Olatunji, 1983). It is important to note that the presence or absence of any particular bioactive compound depends on the extraction solvent, the plant part used for the extraction, the age of the plant, percentage humidity of the harvested material, time and situation of harvest, extraction procedures and the amount of the samples used (Dai and Mumper, 2010; Gracelin et al., 2013). Extracts obtained with different methods and different solvents from one plant can exhibit different antibacterial effects on a particular bacterium (Nostro et al., 2000). Saponins are natural glycosides of steroid or triterpene which have been shown to exhibit a variety of biological and pharmacological properties including antioxidant, hypolipidemic potentials and inhibition of erythropoiesis in *Rattus norvegicus* (Elekofehinti et al., 2012). However, to the best of our knowledge, there is no information in the literature on the *in vitro* antibacterial effect of saponins from this plant leaf; hence, this study was carried out to evaluate the *in vitro* antimicrobial potentials of saponins-rich fraction from *Z. zanthoxyloides* leaf.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh *Z. zanthoxyloides* leaves were collected in farmland towards the end of harmattan season from Ugbe Akoko in Akoko North-East Local Government Area of Ondo State. It was identified and certified at the herbarium of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko. The leaves were washed with clean water, air-dried until a constant weight was obtained and pulverized by an electric blender into powder form. The powdered plant material was kept in an airtight container and preserved in a cool, dark and dry place

until the extraction commences.

Preparation of Extract

One hundred grams of the powdered leaf was soaked in 500 ml of methanol (95%) for 72 h (3 days), with occasional stirring using a glass rod to ensure proper mixture of the vessel content. The content was then filtered through two layers of cheesecloth. The extract (filtrate) was then concentrated under reduced pressure at 45°C until the extraction solvent was completely removed. A green soluble crude residue was obtained.

Qualitative Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out on the extracts using the standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1998) with minor modification.

Isolation of Saponins from Crude Methanolic Extract of *Z. zanthoxyloides* Leaves

Isolation of saponins-rich fraction was carried out using a procedure described by Abdel-Gawad et al. (1999) and Elekofehinti et al. (2013) with slight modification. The crude extract was partitioned with hexane and water (1:2, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the water layer was concentrated and partitioned between ethyl acetate and n-butanol (1:3, v/v). The butanol fraction was concentrated to obtain crude saponins fraction.

In vitro Antimicrobial Assay

Antibacterial Sensitivity Testing of the Extract

The modified Juliani et al. (2002) agar diffusion technique was employed to determine the antimicrobial activities of the saponins-rich fraction. Different concentrations of the extract, 100, 125, 250 and 500 mg/ml were prepared. About 0.2 ml of the standardized 24 h old culture of the test organisms in the nutrient broth was spread onto sterile prepared nutrient agar plates. These were then allowed to settle. Wells of 6 mm diameter were cut and filled with 0.3 ml of each concentration of the extract and then allowed to diffuse into the medium for 1 h, after which the plates were incubated at 37°C for 24 h. Thereafter, the diameter of the zone of inhibition was measured in millimeters. Ciprofloxacin was used as a positive control while methanol was used as a negative control. The experiment was conducted in triplicate.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is defined as

Table 1. Qualitative phytochemical analysis of methanolic extract of *Z. zanthoxyloides* leaves.

Phytochemicals	Inference
Flavonoids	+
Tannins	+
Saponins	+++
Phenols	++
Alkaloids	+++
Steroids	+
Terpenoids	+
Glycosides	+

Absent (-), highly present (+++), moderately present (++), low (+).

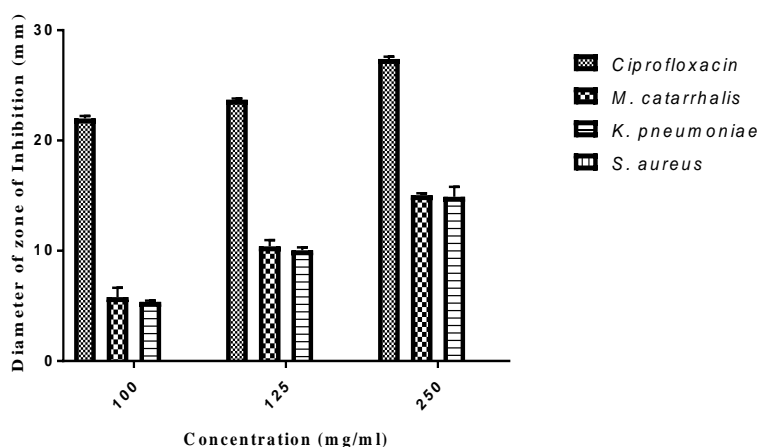


Figure 1. Antimicrobial activity of saponins-rich fraction of *Z. zanthoxyloides* leaf. Bars are means \pm standard error of mean of triplicate determinations (n=3).

the minimum concentration of the extract that will not allow any viable growth or turbidity of the test organism (Cheesbrough, 2004). The Agar dilution technique was used to determine the minimum inhibitory concentration of the extract. The extract was incorporated into Molten Nutrient Agar to make final concentration ranging from 3.1 mg/ml to 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml. The untreated but inoculated agar plates (without saponins-rich fraction) served as controls. Each test organism was radially streaked on the agar plate and incubated at 37°C for 24 h and clearance zones around any of the wells were noted and measured in millimeters. The lowest concentration of the saponins-rich extract that inhibited the growth of the test organisms was recorded as MIC.

Statistical Analysis

All analyses were carried out in triplicate and data were expressed as mean \pm standard error of mean (SEM). The data were statistically analyzed using Student's t-test and differences were considered statistically significant at $p < 0.05$ using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

The extraction yield obtained from the cold maceration of 100 g of plant leaf powder using methanol was 8.2%. Qualitative phytochemical analysis of the methanolic extract of *Z. zanthoxyloides* leaf revealed the presence of flavonoids, tannins, saponins, phenols, alkaloids, and steroids and triterpenes. Among the test results, alkaloid test displayed strong color, suggestive of extremely high content of alkaloids; followed by saponins, phenols, and steroids and tannins with good color production. Flavonoid test produced just enough color change to infer positive, indicative of low flavonoids content in the sample. Table 1 shows the results for qualitative phytochemical analysis of the methanolic extract of *Z. zanthoxyloides* leaf. Figure 1 revealed that the saponins-rich fraction of *Z. zanthoxyloides* leaf has a significant antibacterial effect on the two strains of the gram-negative bacteria (*K. pneumoniae* and *M. catarrhalis*). The result of the antibacterial susceptibility testing showed that all the strains of the three pathogens (*S. aureus*, *K. pneumoniae* and *M. catarrhalis*), were highly susceptible to ciprofloxacin. The acceptable standard diameter

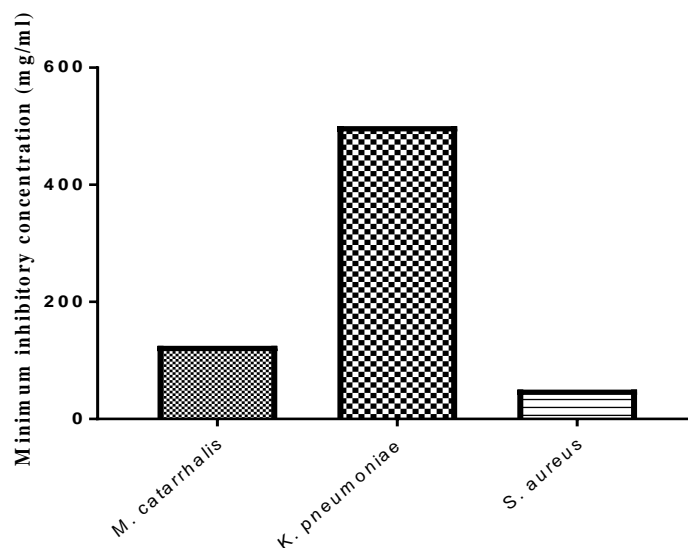


Figure 2. MIC of saponins-rich fraction of *Z. zanthoxyloides* leaf against selected organisms.

zone of inhibition for sensitive organisms for this antibiotic is >21mm. However, for the plant extract, the average zones of inhibition observed against *K. pneumoniae* and *M. catarrhalis* at concentration of 100, 125 and 250 mg/ml were 5.80 ± 0.84 , 10.04 ± 0.26 and 15.04 ± 0.17 mm, respectively. The MIC shown in Figure 2, ranged from 50 mg/ml to 500 mg/ml.

DISCUSSION

The extraction of saponins from the leaf of *Z. zanthoxyloides* using methanol yielded 8.2% w/w of the dried powdered extract. This is close to the value earlier reported by Kosh-Komba et al. (2017), which was 7.5% w/w. However, this result contrasts the reports of Adegbolagun and Olukemi (2010) and Ogwal-Okeng et al. (2003), which recorded higher values (16.7 and 10.5%, respectively) for the extraction of the root bark. This difference could be attributed to the method of isolation, solvent used for extraction and the part of the plant used. Qualitative phytochemical screening of the crude methanol extract in this study showed the presence of alkaloids, saponins, flavonoids, phenolics, tannins, steroids, glycosides. This is in agreement with previous works carried out on the leaf and root of *the plant* (Kosh-Komba et al., 2017; Adesina, 2005; Chaaib, 2004). These classes of phytochemicals are known to possess a variety of biological activities including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, and anticancer activities (Navarro et al., 2001; Frédérich et al., 2002; Amić et al., 2003; Cushine and Lamb, 2005; Souto et al., 2011). Plants are important sources of antimicrobial compounds and have huge therapeutic potential without any side effects as often seen with synthetic antimicrobial drugs (Singh et

al., 2011). In this study, selected bacteria (*S. aureus*, *K. pneumoniae* and *M. catarrhalis*) were tested for susceptibility to impaired growth and/or death due to saponins-rich fraction of *Z. zanthoxyloides* leaf by agar diffusion technique. The results revealed that the saponins-rich fraction has a significant antibacterial effect on the two strains of the gram-negative bacteria (*K. pneumoniae* and *M. catarrhalis*), but has no effect on the gram-positive bacteria (*S. aureus*). One of the possible reasons for the lack of anti-bacterial effect of this saponins-rich fraction on *S. aureus* may be the weakness of the active antibacterial agent of the extract on the bacterium. Another factor that may affect the antibacterial effects of an extract of a plant is the extraction method and solvent type (Chua, 2013). The result of the antibacterial susceptibility testing showed that all the strains of the three pathogens (*S. aureus*, *K. pneumoniae* and *M. catarrhalis*), were highly susceptible to ciprofloxacin. The acceptable standard diameter zone of inhibition for sensitive organism for this antibiotic is >21mm (NCCLS, 1993). However, for the plant extract, the average zone of inhibitions observed against *K. pneumoniae* and *M. catarrhalis* at a concentration of 100, 125 and 250 mg/ml were 5.80 ± 0.84 , 10.04 ± 0.26 and 15.04 ± 0.17 mm, respectively. The low values recorded for the plant extracts may be attributed to the fact that the extracts are in crude form and contain very small amounts of the bioactive compounds. All the same, several workers have reported bioactivity of crude extracts of many other medicinal plants within such range of diameter zone of inhibitions (Ilori et al., 1996; Ogbeche et al., 1997; Akinyemi et al., 2005). Some saponins appear to exhibit antimicrobial activity against gram-negative bacteria and no activity against gram-positive bacteria as in the saponins tested in this study. A similar

observation was reported by Morrissey and Osbourn (1999). They noted that the saponins isolated from orchid tree (*Bauhinia variegata* L.) bark exhibited greater antibacterial activity for gram-negative bacteria than gram-positive bacteria at concentrations ranging from 2.5 to 10 mg/ml. Conversely, saponins isolated from Soapnut pericarps (*Sapindus mukurossi*, which grows abundantly in China and Japan) showed moderate antibacterial activity against gram-positive bacteria, while no activity was observed against gram-negative bacteria (Tanaka et al., 1996). Another study proved that saponins of *Sorghum bicolor* have an inhibitory effect on gram-positive bacteria but not on gram-negative one and fungi (Soetan et al., 2006), whereas, saponins from *Anabasis articulata* exhibited a potent antimicrobial activity against gram-negative and gram-positive bacteria and *Candida albicans* (Maatalah et al., 2012). Extraction procedures for saponins, undoubtedly affect their antibacterial activity. Rakhimov et al. (1996) and Morrissey and Osbourn (1999), noted that fat-free extracts from the Hong Kong orchid tree (*Bauhinia variegata* L.) bark were more active than high-fat extracts against gram-positive bacterial strains such as *S. aureus*. However, against gram-negative bacterial strains such as *Escherichia coli*, the fat-free extracts exhibited either similar or less activity than the high-fat extracts. The specific mode of action for the antibacterial activity of saponins against both gram-negative and gram-positive bacteria is not yet clear. One report suggests that the aglycone moiety of saponins is the antibacterial determinant and that the sugar moiety is not critical for antimicrobial efficacy (Avato et al., 2006), while another study (Mandal et al., 2005) reported that saponins hydrolyzed by bacterial enzymes to its corresponding aglycone resulted in decreased antibacterial activity. As with hemolytic activity, the antibacterial activity of saponins is likely affected by many factors such as the aglycone, number, position and chemical structure of sugar side chains (Potter et al., 1993; Rakhimov et al., 1996).

CONCLUSION

Based on the results obtained from this study, it is evident that the saponins-rich fraction of *Z. zanthoxyloides* leaf has potential for biological applications as an antimicrobial agent against *K. pneumoniae* and *M. catarrhalis*. This gives credence to the use of this plant in the treatment of diseases associated with these pathogens. Although the saponins-rich fraction did not measure up with the standard antibiotic, ciprofloxacin, further purification may scale up its potencies. However, further *in vivo* and toxicity studies are required for far-reaching conclusions to be drawn.

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