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Full Length Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF MISTLETOE (*AGELANTHUS DODONIESFOLIUS* (DC)) COLLECTED FROM SHEA BUTTER TREE (*VITELLERIA PARADOXA*)

Audu Yunusa[1,2], Shuaibu Babaji Sanusi[2], Alona Cuevas Linatoc[2], Murtala Muhammad Mainassara[2], and Muhammad Ahmad Ibrahim[1]

[1]Department of Biology, Faculty of Applied Sciences and Technology, Ibrahim Badamasi Babangida University Lapai, Niger State

[2]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Educational Hub, 84600 Pagoh, Johor, Malaysia

*Corresponding Author's Email Address: sanusishuaibu@gmail.com

ABSTRACT
The increase in the multi-drugs resistant strains of bacteria is a great concern to public health. For long, various plant-derived compounds have been used as drugs. *Agelanthus dodoneifolius* (DC) refers to '*Kauchi*' in Hausa has been used ethno-botanically by the Hausa and Fulani ethnic group of Northern Nigeria for the treatment of many human and animal diseases. Such diseases include diarrhea, dysentery, and stomach ache. In this study, the phytochemical screening and antibacterial activity of the stem bark and leaves extracts of mistletoe plant (*A. dodoneifolius*) was evaluated. The plant sample was qualitatively screened for phytochemicals using a standard procedure. The antibacterial activity was examined using the standardized suspension method. The phytochemical screening revealed the presence of tannins, steroid, alkaloid, anthraquinones and glycosides. The results of antibacterial activity of the *A. dodoneifolius* showed that *S. aureus* was more susceptible to methanolic extract of stem bark and leaves extracts producing the largest diameter of inhibition zone of (17 mm) and (15 mm) at the concentration of 10 mg/ml respectively. These results validated the traditional use of *A. dodoneisfolius* and more phytochemical screenings are necessary to fully explore this species which may lead to development of a novel antibacterial agent.

Keywords: *Agelanthus Dodoneisfolius,* Antibacterial Activity, Synergetic Effect

1. INTRODUCTION

The use of plant as a source of medicine to treat pathogenic diseases predate history; nearly all culture and civilization from ancient time to present day have used herbal medicine to treat infections (de Boer *et al.,* 2005). The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal or medicinal plant products as sources of novel compounds to fight the ever increasing problems (Adwan *et al.,* 2006). The primary benefits of using plant derivative medicine are that they are relatively safer than synthetic alternative, offering profound therapeutic benefit and more affordable treatment (Amos *et al.,* 2005). African mistletoe, *Agelanthus dodoneifolius* (DC) from Loranthaceae family is a parasitic plant that is found growing on the branches or aerial parts of the host plants (usually tree). Mistletoes are known all over the world to cause damage to the host plants which pose serious threat to plantation by paralyzing cultivated plant and tended plants (Deeni & Sadiq, 2002). In addition, they are agent of diseases and therefore affect the host physiology leading to reduce growth survival and reproduction (Shaw *et al.,* 2004). Despite their destructive nature to their host, the plants are also generally used medicinally for the treatment of various ailments. *A. dodoneifolius* (DC) refers to '*Kauchi*' in Hausa is used ethno-botanically by the Hausa and the Fulani ethnic group of Northern Nigeria in the treatment of many human and animal diseases which include dysentery, diarrhea, stomach ache, and cancer (Deeni & Sadiq, 2002). In Burkina Faso, the decoction of this species have been traditionally used to treat cardiovascular diseases and asthma (Boly *et al.,* 2016; Carré *et al.,* 2014). Pharmacological studies of *A. dodoneifolius* revealed that it possess vast biological properties including anti-plasmodial activity (Builders *et al.,* 2012; Abdullahi *et al.,* 2015; Eyya *et al.,* 2017), Antioxidant (Boly *et al.,* 2016), anti-inflammatory activities (Rainatou Boly *et al.,* 2015), Modulatory activities (Mouithys-mickalad, 2014), Antibacterial Activity (Ndamitso *et al.,* 2013), Relaxant effect (Ouedraogo *et al.,* 2005), Anticonvulsant (Uthman *et al.,* 2015), Antimicrobial properties (Deeni & Sadiq, 2002), analgesic, and anti-pyretic (Abdullahi *et al.,* 2016). Furthermore, the phytochemical analysis of *A. dodoneifolius* have been reported the presence of alkaloids, anthraquinones, phytate, saponins, tannins, glycosides, flavonoids carbohydrates, steroids, oxalate and terpenes, depending on the host trees and solvent used (Deeni & Sadiq, 2002; Ndamitso *et al.,* 2013; Idu *et al.,* 2016). The present study was undertaken to screen the phytochemical and antibacterial activity of the stem bark and leaves extracts of mistletoe plant (*A. dodoneifolius*).

2. MATERIALS AND METHODS

Plant Samples Collection

The plant parts (stem-bark and leaves) of mistletoe (*A. dodoneifolius)* were freshly obtained from S*hea butter tree (Vitelleria paradoxa)* from Biological garden of Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria**.**

Preparation and Extraction of Plant Material

The collected leaves and stem-bark of *A. dodoneifolius* were thoroughly washed, chopped into smaller pieces, and air-dried at room temperature for three weeks. The dried plant parts were pulverized using mortar and pestle and then sieved to obtain a fine powder. Due to its broad spectrum and relatively non-selective property of extracting, methanol was chosen as the solvent for the extraction. Forty grams (40 g) of the fine powder of

A. dodoneifolius plant parts (leaves and stem bark) were extracted with 400 mL of methanol using a soxhlet extractor for 3 hours, at room temperature. The crude extracts obtained were evaporated to dryness using a water bath, and the dried extracts were weighed into McCartney bottle. The percentage yield of each of the extract was calculated from the respective weights of the extracts using the formula below:

$$\text{Percentage yield}(\%)\frac{\text{weight of crude sample}}{\text{weight of initial sample}}\times 100$$

Phytochemical Analysis of the Leaves and Stem Bark of Mistletoe (*Agelanthus dodoniefolius*)

Phytochemical analysis of the methanol leaves and stem bark extracts of *Agelanthus dodoneifolius* for major constituents including alkaloids, tannins, saponins, steroids, anthraquinones and glycosides was carried out using standard qualitative method as previously described by Trease and Evans (1989).

Source of Test Organisms

The test organisms used in the study including *Staphylococcus aureus*, *Bacillus subtilis, Salmonella typhi*, and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory, Ibrahim Badamasi Babangida University, Lapai. The bacteria isolates were cultured into nutrient agar slants to obtain pure isolates. Prior to inoculation, sterility of the agar slants were tested by incubating them after preparation for 24 hours. The test organisms were separately prepared by sub-culturing the pure isolates in nutrient agar and incubated at 37 °C for 24 hours.

Determination of Anti-bacterial Activity

The antibacterial activity of the plant extracts were screened by the method previously described by Aliyu *et al.* (2009). Few colonies of the test organisms were aseptically suspended in saline solution (0.85% NaCl) and the turbidity was adjusted to 0.5 (1 x 10^8 cells/mL) McFarland standard. The standardized suspension was used to inoculate the surfaces of nutrient agar plate using sterile cotton swab. A cork borer of 6 mm diameter was used to punch the surface of the inoculated nutrient agar thereby producing wells. The 6 mm wells were filled with 0.5 mL of the desired concentration (10, 5, 2.5 and 1.25 mg/mL) of the methanol extracts of stem bark and leaves of *A. dodoneifolius* and standard antibiotic (Ciprofloxacin) (10 mg/mL) which was used as positive control to determine the sensitivity of the test organisms. All the prepared plates were allowed to stand for 1 hour at room temperature for the extracts to diffuse into the agar and then incubated at 37°C overnights for 24 hours. The antibacterial activity was assessed by measuring the diameter of zone of inhibition formed. All the tests were carried out in triplicate and average was calculated.

Determination of Synergistic Effect of Methanolic Leaves and Stem Bark Extract of *A. dodoneifolius*

The synergistic effect of methanolic leaves and stem bark extract of *A. dodoneifolius* was determined using the same method as above by combining both extracts in equal amount.

3. RESULTS

Table 1 showed the percentage yield and physical characteristics of the methanol leaves and stem bark extracts of *A. dodoneifolius*. The leaves methanol extract of *A. dodoneifolius* showed the highest percentage yield (25.35%) followed by stem bark methanol extract of *A. dodoneifolius*. Stem bark methanol extract of *A. dodoneifolius* appeared light green in colour with coarse texture, while leaves methanol extract of *A. dodoneifolius* appeared dark green in colour with sticky texture.

Table 1: Percentage Yield and Physical Characteristics of the Methanol Leaves and Stem Bark Extracts of *A. dodoneifolius*

Plant parts	Solvent	Yield (%)	Texture	Colour
Stem bark	Methanol	17.45	Coarse	Light green
Leaves	Methanol	25.35	Sticky	Dark green

The result of the phytochemical analysis of methanol extracts of *A. dodoneifolius* leaves and stem bark is shown in Table 2. Plant constituents including alkaloids, anthraquinones, steroids, tannins, and glycosides were detected in all the extracts while saponins were not detected (Table 2).

Table 2: Phytochemical Constituents of Methanol Leaves and Stem Bark Extracts of *A. dodoneifolius*

Chemical constituents	Leaves extract	Stem bark extract
Alkaloids	+	+
Anthraquinones	+	+
Saponins	-	-
Steroids	+	+
Glycosides	+	+
Tannins	+	+

Key: + = present; - = absent

Table 3 shows the data obtained from the antibacterial activity of methanol extracts of *A. dodoneifolus* leaves at different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml, and 1.25mg/ml) against the tested organisms. It showed that the extracts exhibit antibacterial activity against *S. aureus*, *B. subtilis*, and *S. typhi P. aeruginosa*. At 1.25mg/ml concentration, except for *S. aureus* all the tested organisms were resistant to the extracts. *S. aureus* was the most sensitive cell with the largest zone of inhibition (17 mm) at 10mg/ml, followed by *B. subtilis* (15 mm) and *P. aeruginosa* (14 mm).

Table 3: Antibacterial Activity of Methanol Stem Bark Extracts of *A. dodoneifolus*

Test organisms	Diameter of zones of inhibition (mm)							
	Conc. (mg/ml)				Control			
	10	5	2.5	1.25	10	5	2.5	1.25
S. aureus	17	14	10	6	23	20	18	0
B. subtilis	15	13	9	0	21	18	12	0
S. typhi	10	8	7	0	16	13	0	0
P. aeruginosa	14	12	9	0	20	15	12	0

Key: 0 = No zone of inhibition, Control = Ciprofloxacin

The result of antibacterial activity of methanol extracts of *A. dodoneifolus* stem bark tested against *S. aureus*, *B. subtilis*, and *S. typhi P. aeruginosa* at different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml, and 1.25mg/ml) is presented in Table 4. It was shown that except for *S. aureus* all the tested organisms were resistant to the extracts at 1.25mg/ml concentration. The largest zone of inhibition (15 mm) was observed in *S. aureus* at 10mg/ml, followed by *B. subtilis* (12 mm).

Table 4: Antibacterial activity of methanol stem bark extracts of *A. dodoneifolus*

Test organisms	Diameter of zones of inhibition (mm)							
	Conc. (mg/ml)				Control			
	10	5	2.5	1.25	10	5	2.5	1.25
S. aureus	15	11	6	4	23	20	18	0
B. subtilis	12	9	5	0	21	18	12	0
S. typhi	9	8	3	0	16	13	0	0
P. aeruginosa	5	5	4	0	20	15	12	0

Key: 0 = No zone of inhibition, Control = Ciprofloxacin

The result of synergetic effect of methanol extracts of leaves and stembark of *A. dodoneifolus* tested against *S. aureus*, *B. subtilis*, and *S. typhi P. aeruginosa* is represented in Table 5. The mixed extract exerted highest antibacterial activity against *S. aureus* (25 mm), followed by *B. subtilis* (18 mm) and *P. aeruginosa* (15) at 10mg/ml.

Table 5: Synergistic Effect of Methanol Leaves and Stem Bark Extracts of *A. dodoneifolus*

Test organisms	Diameter of zones of inhibition (mm)							
	Conc. (mg/ml)				Control			
	10	5	2.5	1.25	10	5	2.5	1.25
S. aureus	25	18	13	11	23	20	18	0
B. subtilis	18	16	9	9	21	18	12	0
S. typhi	12	9	0	0	16	13	0	0
P. aeroginosa	15	14	12	4	20	15	12	0

Key: 0 = No zone of inhibition, Control = Ciprofloxacin

4. DISCUSSIONS

Several investigators had reported that plants contain antibacterial or antimicrobial substances (Adetutu *et al.,* 2011; Anibijuwon *et al.,* 2012; Etim *et al.,* 2012; Noumedem *et al.,* 2013; Habtamu & Melaku, 2018). The result obtained from this study showed that the methanolic extract of *A. dodoneifolius* exhibited antibacterial properties against both Gram-positive and Gram negative bacteria at varying degree. There was however, more activity against the Gram positive organism than the Gram negative. It was suggested by Pelczar *et al.* (1993) that the difference in susceptibility of Gram positive and Gram-negative bacteria to various antibacterial agents perhaps depends on structural differences in their cell walls. Examples are the quantity of peptidoglycan, presence of receptors and activity of autolytic enzymes that determined the penetration, binding and activity of the antimicrobial agents. The striking difference in the effects of the extracts on the bacteria is a suggestive of the activity against cell wall components of the organisms. The findings showed that *S. typhi* was the most resistant bacteria among the tested organisms. It might be due to the fact that *S. typhi* possesses a mechanism for detoxifying the active constituents in the extract. Some bacteria are known to possess mechanism by which they convert substances that inhibit their growth to non-toxic compound. The antibacterial effects observed on the isolates is believed to be due to the presence of alkaloids, anthraquinones, glycosides, steroid, and tannins which have been shown to possess antibacterial properties (Cowan, 1999). Results of the phytochemical analysis revealed the presence of these compounds in the extracts of *A. dodoneifolius*. A further research has as well attributed the observed antibacterial effect of plant extract to the presence of these phytochemical compounds (Nweze *et al.,* 2004).

5. Conclusion

African medicinal plants have been screened for their in-vitro antibacterial activities. The extracts displayed antibacterial activity and thus, validate the ethno-medicinal uses of mistletoe in the treatment of bacterial infection. The result obtained on the phytochemical analysis of the plant species provides preliminary information of the plant and also suggest the type of plant active constituents that may be responsible for the biological activities exhibited by the plant extracts. However more work needs to be carried out to determine the chemistry of the particular active principle and the effect on the organism at these concentrations.

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