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Phytochemical and Antibacterial Screening of Leaf and Stem Bark Extracts of Bridelia ferrugina

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Abstract
The search for the presence of bioactive compound in wild plants that are more effective, safe and available is a continued process. In view of that, ethanol leaf and methanol stem bark extract of *Bridelia ferrugina* were screened for phytochemical properties and anti-bacterial potential. The quantitative analysis of both leaf and stem bark reveals the presence of alkaloids (2.07±0.42) (4.27±0.012), glycosides (5.48±0.02) (4.32±0.04), saponins (9.85±0.50) (10.83±0.12), tannins (5.37±0.16) (8.33±0.06) respectively. The stem bark extract contain Flavonoids (11.70±0.54). The Anthraquinone, Cardiac Glycoside, Saponin glycoside and Volatile oils and Steroids were absent. The effect of ethanol leaf and methanol stem extract stem bark extract of *Bridelia ferrugina* on *E. coli, Salmonella typhi, Pseudomonas aeruginosa* and *Streptococcus aureus* were also evaluated. The result obtained revealed that methanol extract was the most effective on *Streptococcus aureus, Salmonella typhi* and *Escherichia coli,* while ethanol extract was most effective on *Pseudomonas aeruginosa*. The result of study was discussed in terms of its medicinal potentials as a basis for its traditional uses in the treatment of diseases.
Keywords: Phytochemical, antibacterial, *Bridelia ferrugina* extracts.

INTRODUCTION

A major disaster for the mortality and morbidity amongst humans and animals are infectious diseases. Emergence of multi-drug resistance, existing anti-bacterial with undesirable effects and restricted anti-bacterial spectrum creates further annoyance diverting attention towards natural antibacterial [1]. Traditionally from prehistoric times, the use of different parts of medicinal plants was practiced to cure specific ailments evidently due to presence of some bioactive compounds like alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins and volatile oils and others [2]. Secondly, Plants are the most important resources on earth [3]. All over the world, several hundreds of plants are good sources of medicinal agents and are used in traditional medicine for many different purposes, including bacterial and fungal infections [4]. Traditionally, the usage of plants in curing illness has deep roots in human history [5], example Chincona spp and quinone have antimalarial effects; hence they are prescribed for many ailments in addition to their antimalarial activity and alkaloids like desrpidine, reserfine and resannamine have been found to have analgesic effect [6]. Plants materials have a wide range of applications in pharmacy, medicine and toxicology [7]. A number of plants used as medicine are either still been used or have provided the chemical models for modern derivatives of their natural products [8].

World Health Organization (WHO) engaged in a study of medicinal plants to determine their effectiveness and best means of preservation. Twenty thousand (20,000) plants species have been identified and with development of organic chemistry some of the active ingredients have been isolated and purified [8].

Bridelia ferruginea belongs to the family *Euphorbiaceae* which is commonly found in savannah regions [9]. The bark, leaves and roots are ingredients of Yoruba (in Nigeria) infusions chiefly administered to children [10]. In Congo, the stem bark is used to relieve toothache and in Ivory Coast for dysentery and diarrhea or as a laxative [11]. The bark is used as antidote against poison and arrow poison [10]. The leaves have been used to treat diabetes, purgative and a vermifuge [12]. In Togo, the roots of the plants are used as chewing sticks and the bark is used for intestinal and bladder disorder remedies as well as skin diseases [13]. Antimicrobial properties of stem bark of *Bridelia ferruginea* against facultative gram negative rods have been reported by [14]. The activities of methanol, petroleum ether and chloroform bark extract of *Bridelia ferruginea* against some potential pathogenic organisms have been extensively investigated [15]; [16]. However reports on bioactive activities of the extracts from roots are widespread. Presently, this study was intented to elucidate the chemical constituent of stem bark and roots with a view of authenticating the plants antimicrobial potentials.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used for this study are of analytical grades

Plant Materials

Leaves and stem bark of *Bridelia ferruginea* (*Kizni*) were obtained from the bush area of Abare village in Anka local government, Zamfara State. The plant specimen was identified and authenticated by a botanist

from botany unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto.

Preparation of Samples

The leaves and stem bark were air dried under shade for 5days and 13days respectively and then Pulverized in a mill and stored in air tight containers and coded accordingly (i.e. leaves and stem barks separately)

Extraction of Plant sample

10 grams of each of the powdered samples were soaked into 100ml distilled water and allowed to stand for 72hrs and the mixture was filtered with Whatsmann No. 1 filter paper. The residues were discarded and the filtrate was used for qualitative screening for phytochemicals. 100grams of each of the samples were soaked into 500 ml of ethanol and methanol respectively and sealed to prevent evaporation and allowed to stand for 72 hrs and then filtered. The filtrates were placed in an oven at 46^0C for 24 hrs.

Phytochemical screening

Phytochemical screening of Leaves and stem bark of *Bridelia ferruginea* (*Kizni*) extracts was performed by standard methods [2][17]

Test organisms

The test organisms used in this study were Bacterial isolates; *Escherichia coli*, *Pseudomonas aureginosa*, *Salmonella typhi* and *Staphylococcus aureus* which were obtained from Usmanu Danfodiyo University Teaching Hospital (UDUTH), Department of Microbiology, Sokoto. Their Purity was confirmed by sub-culturing into nutrient broth incubated at 37°C for 18 hrs. The developed colonies were observed under the microscope after simple staining. Pure culture was kept on agar slopes at 4°C until needed.

Antibacterial Screening

The antibacterial activity of the crude extracts was determined in accordance with agar-well diffusion method as describe [18]. The bacterial isolates were first grown in a nutrient broth for 18 hrs before use and standardized to 0.5 McFarland standards (10^6 $Cfuml^{-1}$), by using inoculation loop. A loopful of colony of test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland standards as described by the National Committee of clinical laboratory (2008). Standard inocula of isolate were swabbed on the surface of the prepared solidified Muller Hinton agar. Well were then bored into the agar using a sterile 6mm diameter court borer. Approximately 0.1 ml of stock solution prepared with DMSO (dimethyl sulphoxide) was introduced into the well. It was allowed to stand at room temperate for 2 hrs and then incubated at 37°C as positive control for bacteria Ciprofloxacin (0.5 mg/ml) was use as positive control. The control was set up in parallel using the solvent that were used to constitute the extract.

Zones of inhibition

ZI was determined by preparing various concentrations of extracts (25mg/ml, 30mg/ml, 60mg/ml, 90mg/ml and 120mg/ml respectively) by using the method [18]. Two hundred microliter of the standardized cell suspensions were spread on a Muller-Hinton agar. Wells were then bored into the agar using a sterile 6mm diameter cork borer. Various concentrations of the crude extracts were dispensed into the wells and properly labeled. The preparations were left to diffuse before incubation. The inoculated plates were incubated at 37°C for 24 hrs. The plates were then observed for clearing around the wells, that is, zones of inhibition.

RESULTS

Table 1. Phytochemical screening of leaf and stem bark extracts of *Bridelia ferruginea*

PHYTOCHEMICALS	OBSERVATIONS	
	LEAF	**STEM BARK**
Alkaloids	++	++
Flavonoids	+	+++
Anthraquinone	ND	ND
Glycosides	++	++
Saponins	++	+++
Steroids	ND	+
Volatile oils	ND	+
Tannins	+++	++
Cardiac glycosides	ND	+
Saponin glycosides	+	+

+++ = presence in appreciable amount, ++ = presence in moderate amount, + = presence in trace amount and ND = not detected

Table 2. Phytochemical constituent of leaf and stem bark extracts of *Bridelia ferruginea*.

	OBSERVATIONS	
PHYTOCHEMICALS	**LEAF**	**STEM BARK**
Alkaloids	2.07 ± 0.42	4.27 ± 0.12
Flavonoids	-	11.70 ± 0.58
Glycosides	5.48 ± 0.02	4.32 ± 0.04
Saponins	9.85 ± 0.50	10.83 ± 0.67
Tannins	5.37 ± 0.16	8.33 ± 0.06

Values are express in mean ± standard deviation.

Table 3. Antibacterial activity of ethanol and methanol extracts of leaf and stem bark of *Bridelia ferruginea*

Clinical isolates	**Diameter of zone of inhibition/extracts (mg/ml)**					
	25	30	60	90	120	Control
***E. coli* (LE)**	-	-	9	9	12	17
***Pseu. a.* (LE)**	-	-	9	11	16	10
***S. typhi* (BE)**	-	8	8	9	17	20
***S. aureus* (BE)**	9	10	10	13	15	18
***E. coli* (LM)**	8	10	10	12	15	15
***Pseu. a.* (LM)**	8	9	12	14	15	11
***S. typhi* (BM)**	-	8	10	12	16	15
***S. aureus* (BM)**	8	10	12	13	21	20

The diameters of zone of inhibition (mm) are expressed as mean (n=3). LE = leaf ethanol, LM = leaf methanol, BE = Stem bark ethanol, BM = Stem bark methanol, *S. aureus* = *Streptococcus aureus*, *S. typhi* = *Salmonella typhi*, *E. coli* = *Escherichia coli*, *Pseu. a.* = *Pseudomonas aeruginosa*

Discussion

The phytochemical screening of the leaf and stem bark extracts of *Bridelia ferruginea* (*Kizni*) in table 1.0 revealed the presence of alkaloids (leaf and stem bark), flavonoids (stem bark), glycosides (leaf and stem bark), saponins (leaf and stem bark), tannins (leaf and stem bark), saponin glycosides (leaf and stem bark), steroids (stem bark) and cardiac glycosides (stem bark). The presence of alkaloids, saponins, tannins and steroids in the stem bark of the plant, shows that the extract is of pharmacological importance.

These classes of compounds especially alkaloids, flavonoids, saponins are known to be biologically active and therefore aid antimicrobial activities of plant. This result is in line with the work of Kolawale *et al*., (2006) [19], who recorded that antidiabetic activities of these plant extracts could be due to the presence of tannins, alkaloids and steroids as revealed in the phytochemical screening (Kolawale *et al*., 2006) [19]. These compounds have curative activity against several pathogens (Usman *et al.,* 2009) [20]. According to Encerta, (2007) [21], all alkaloids contain quinine, morphine and resperine which are used for malaria, pain relief and valuable tranquilizers respectively. Alkaloids are one of the largest groups of phytochemicals in plants, have amazing effects on humans and have led to the development of powerful pain killer medications (Kam and Liew, 2002) [22]. The leaf and stem bark extracts have also been reported to reduce plasma glucose levels in diabetic patients [15] [19]. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [23]. The plant can also be used in the brewing industries in clarifying beer and wine because of the presence of tannins.

Table 3 shows the effects of *Bredilia ferruginea* stem bark extracts on some pathogenic microorganisms, i.e., *E. coli*, *S. aureus*, *S. typhi* and *P. aureginosa*. The result revealed that the methanol extract was most effective than the ethanol extract on *S. aureus* and *S. typhi*. This finding are in greement with Gunnar *et al*., (1999) [24], who reported that different extracts of plants show different antimicrobial activities on an organism. This implies that antimicrobial activities of a substance are may be concentration dependent, which is concordance with the report of Oboh and Abulu, (1997) [25] that, antimicrobial activity is a function of the active ingredient reaching an organism (Oboh and Abulu, 1997) [25]. The result obtained from this study tend to suggest that extract from the stem bark of *Bridelia ferruginea* could be used in the treatment of food borne diseases, cough, black tongue, boils and wound infections. It could also be suggested however, that further research should be conducted to confirm the promising results demonstrated in this study.

Phytochemical analysis in leaf and stem bark extracts of *Bridelia ferruginea* using methanol extraction revealed flavonoids, saponins, tannins, glycosides and alkaloids in higher concentrations which may indicates the medicinal potentials of the plant especially antibacterial and antifungal activities. Steroids, cardiac glycosides and saponin glycosides were detected in trace amounts, while volatile oils and anthraquinone were not detected.

It also indicates that the chosen method for extraction of the phytochemicals was good and reliable.

Conflicts of interest
I Rabiu Umar Aliyu Wasagu declare that they have no conflict of interest

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