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Qualitative phytochemical screening and antibacterial activities of *Basella alba* and *Basella rubra*

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Abstract

The study was carried out on *Basella alba* and *Basella rubra* leaves to further emphasize their importance and utilization as a support for promoting healthy diets in Nigeria. *B. alba* and *B. rubra* leaves were extracted in water:methanol (2:3) by maceration. Phytochemical screening and antibacterial sensitivity of the aqueous methanol extracts of both leafy vegetables were carried out using appropriate reference methods. The antimicrobial activity of the extracts against selected bacteria isolates were evaluated using agar well diffusion method at 30 mg/mL and 35 mg/mL concentrations. The results of the phytochemical screening revealed the presence of saponin, phlobatannin, cardiac glycoside, flavonoid and alkaloid in both extracts. Aqueous methanol extract of *B. rubra* exhibited *in vitro* antibacterial activity against all tested bacteria isolates; *Clostridium sporogenes* (NCIB 532), *Streptococcus faecalis* (LIO), *Bacillus anthracis* (LIO), *Bacillus polymyxa* (LIO) and *Micrococcus luteus* (NCIB 196). *Clostridium sporogenes* (NCIB 532) and *Streptococcus faecalis* (LIO) were resistant to the aqueous methanol extract of *B. alba* with no zone of inhibition shown. Streptomycin was used as the control at 1 mg/mL. The phytochemicals contained in these vegetables possess antimicrobial properties either in isolation or synergistically. Therefore, further research should be done to isolate and evaluate the mode of actions of the active principles that are important for the promotion of good health from these plant varieties.

Keywords: Phytochemical screening; Antimicrobial sensitivity; *Basella alba; Basella rubra;* Streptomycin

INTRODUCTION

Vegetables are important constituent of the human diet across all regions of the world. In most cases, they do not form the bulk of our diet, as do other classes of food, but provides the human body with both nutritive and non-nutritive functions. They provide the body with vitamins and mineral elements, which are essential for sustaining human life [1]. Vegetables are also known to contain non-nutritive substances or phytochemicals, which have the capability to protect human against disease. These phytochemicals are not essential nutrients and thereby are not required by the human body for sustaining life [2]. However, the presence of phytochemicals in vegetables and some other plant species explain the reason for their use in treatment and management of various diseases.

Global prevalence of infectious diseases caused by bacteria remains a major public health problem [3]. Also, the recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents [4]. This in turn has prompted a revival

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in research on the antimicrobial role of plants against resistant strains [5]. World Health Organization (WHO) encourages countries to identify and use important plant for health benefits [6]. Many efforts are continuously been made to discover new antimicrobial compounds from natural sources such as microorganisms, animals, and plants [7].

In recent years, studies on plants with medicinal values have been on the incline because they possess lower toxicity profile when compared to their synthetic counterparts. As such, food based approach and development of new drugs from natural products are considered important interventions in the action plan against chronic diseases affecting humans [8]. These medicinal plants possess diverse biologically active compounds, some of whose use have been proven or have therapeutic potentials for use as antimicrobial, anti-inflammatory, anticancer, antiulcer or hepatoprotective agents among many others. Some of these substances that are able to inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antibacterial drugs.

Nigeria has a wide range of vegetable species that have shown important roles in folklore medicine for the treatment of several diseases [9]. *Basella alba or Basella rubra* belong to the family Basellaceae and are commonly known as the Malabar spinach [10]. *B. alba, is locally called "Amunu-tutu" in southwestern part of Nigeria.* These two herb species, *B. rubra* and *B. alba* differ from each other on the basis of flower and stem colour. *B. alba* has green stem with white flowers while *B. rubra* has red stem and flowers. The anatomical differences in between these two varieties was reported negligible and therefore both species were considered to be the same [11] whereas some other literature refer to the form which has red/purple pigmentation as *B. rubra* and an entirely different species [12,13].

Roots and leaves of *B. rubra* have been used for the removal of after birth, stomach pains and increase milk production [14]. The anticancer, antioxidant and anti-inflammatory activities of *B. alba* was reportedly due to the presence of β sitosterol and lupeol [15,16]. Ethanol extracts of *B. alba* showed inhibitory activities against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* but not against *Candida albicans* [17]. Also, aqueous, ethanol and petroleum ether extracts of *B. rubra* leaves exhibited antibacterial activity against *E. coli*, *Vibrio cholera*, *Staphylococcus aureus* and *Staphylococcus typhi* [18].

However, despite the known benefits of *B. alba* and *B. rubra*, they are still one of the numerous underutilized indigenous vegetables in Nigeria [19]. Also, there is little knowledge of antimicrobial activities of this plant against some specific Gram-positive bacteria. The specific objectives of this study are therefore to screen the aqueous methanol extracts of these *Basella* species for phytochemicals and evaluate their antimicrobial activities against some Gram-positive bacteria.

EXPERIMENTAL

Preparation of extracts. Fresh plants of *B. alba* and *B. rubra* were collected within the environs of Ile-Ife, Osun state, Nigeria and identified at the Ife herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The fresh leaves were air-dried until constant weight at room temperature. The dried samples were pulverized into fine powder. About 169.5 g of *B. alba* or 147.3 g of *B. rubra* powdered material was extracted in water:methanol (2:3) solvent using maceration. The samples were left to extract for 72 h, with constant shaking, using an electrically controlled

mechanical shaker. The solution was filtered using Whatman filter paper (Grade 42) embedded in a funnel. The filtrate was evaporated *in-vacuo* at 45° C to constant weight and kept in a desiccator until needed.

Phytochemical screening of extracts. The aqueous methanol extracts of *B. alba* and *B. rubra* were screened for the presence of secondary metabolites using standard methods [20].

Subculturing of bacterial isolates. The bacterial isolates used were typed culture of National Collection for Industrial Bacteria (NCIB) and Locally Isolated Organisms (LIO). The bacteria isolates are *Clostridium sporogenes* (NCIB 532*), Streptococcus faecalis* (LIO), *Bacillus anthracis* (LIO), *Bacillus polymyxa* (LIO) and *Micrococcus luteus* (NCIB 196). Nutrient broth was used for subculturing of bacterial isolates. Bacterial isolates were inoculated on nutrient broth (Oxoid, Ltd.) and incubated at 37 °C for 18 – 24 hours in Gallenkamp incubator before use.

Determination of antibacterial activity. The antibacterial activity of the extracts against selected bacteria isolates were evaluated using the agar well diffusion method [21]. The medium employed was Mueller-Hinton agar medium. With the aid of a sterile 1 mL pipette, about 0.2 mL of the broth culture of the test organisms was inoculated into 18 mL of Mueller-Hinton agar medium which had already been cooled down to 45^0 C. This was well mixed and poured into previously sterilized petri dishes and allowed to set for 10minutes. Wells of 8 mm size diameter were made with sterile borer into agar plates containing the bacterial inoculum. The wells were made about 5 mm to the edge of the plate and labelled accordingly. Each well was then filled with 0.5 mL volume prepared from the extract. Streptomycin (1 mg/mL) and water:methanol (2:3) solution was used as the positive and negative control respectively. These were introduced into the wells instead of extract for the controls. All the work was carried out under aseptic conditions. The plates were allowed to stand for one hour on the bench to allow proper diffusion of the antimicrobial agents into the medium and then incubated uprightly at 37^0 C for 24 hours. The relative susceptibility of the organisms to the extracts are indicated by clear zones of growth inhibition around the wells. The zone of inhibition was measured and expressed in mm. Tests were carried out in triplicates.

Data analysis. Data were analyzed using computer software SPSS, version 21 with 95% confidence limit. Statistical tests were done using analysis of variance (one-way ANOVA) and $p<0.05$ values were considered to indicate statistically significant differences.

RESULTS AND DISCUSSION

Extraction yield. The percentage yield for the aqueous methanol extracts of *Basella alba* and *Basella rubra* are 5.6% and 4.8% respectively as shown in Table 1. A previous study also reported that the leaf fraction of *B. alba* had an higher percentage yield than *B. rubra* [22].

Phytochemical screening. The aqueous methanol extracts of both *B. alba* and *B. rubra* indicate the presence of saponin, flavonoid, alkaloid, cardiac glycosides and tannin as shown in Table 2. Steroid was present in the aqueous methanol extract of *B. rubra* but not in *B. alba* while xanthoprotein was detected in the aqueous methanol extract of *B. alba* but not in *B. rubra*. Anthraquinone and phlobatannins were not detected in neither the aqueous methanol extracts of *B. alba* nor *B. rubra*. The result of this study correlates with the previous studies on aqueous and methanol extracts of *B. alba* where saponin, tannin and flavonoid were present while anthraquinone, phlobatannin and alkaloids were absent [19]. The absence of alkaloid could be due to the generally low alkaloid content of *Basella* species [22]. In a

similar vein, saponin, tannin and flavonoid were reported present in hydroalcoholic extracts of *B. rubra* leaves while anthraquinone and alkaloids were absent [23]. These secondary metabolites influence the overall biological activity of the plant.

Antibacterial activities. The antibacterial activities of leaf extracts of *B. alba* and *B. rubra* are shown in Table 3. The results showed that *Clostridium sporogenes* and *Streptococcus faecalis* were resistant to the effects of aqueous methanol extracts of *B. alba* at both 30 mg/mL and 35 mg/mL. Meanwhile, the aqueous methanol extracts of *B. rubra* showed inhibitory properties against all tested Gram-positive bacterial isolates. The aqueous methanol extract of *B. alba* had better inhibitory activities on *Bacillus polymyxa* at both 30mg/mL and 35 mg/mL than *B. rubra* as evident in the zones of inhibition ($p<0.05$) while *Micrococcus luteus* was more susceptible to the effects of aqueous methanolic (2:3) extract of *B. rubra* than *B. alba* at both 30 mg/mL and 35 mg/mL ($p<0.05$). There was no significant difference in the activities of both extract on *Bacillus anthracis* ($p>0.05$) at 30 mg/mL and 35 mg/mL respectively as shown in Figure 1. All antibacterial activities were dose-dependent except for the activities of the extracts of both vegetables against *Bacillus anthracis* at the tested concentrations. The negative control, water:methanol (2:3) solution has no antibacterial activity. The result of this study is similar to findings on methanol extract of *Basella alba* where *Micrococus luteus* was susceptible to different concentrations of the extract in a dose-dependent manner [24].

Table 1: Quantity and percentage yield of aqueous methanol extracts of *Basella alba* and *Basella rubra*

Plant species	Quantity of powdered material	Quantity of aqueous methanol extracts (% Yield w/w)
B. alba	169.5 g	9.4 g (5.6% w/w)
B. rubra	147.3 g	7.1 g (4.8% w/w)

Table 2: Phytochemical screening of aqueous methanol extracts of *Basella alba* and *Basella rubra*

Phytochemicals	*Basella alba*	*Basella rubra*
Saponin	+	+
Cardiac glycosides	+	+
Phlobatannin	-	-
Steroid	-	+
Xanthoprotein	+	-
Anthraquinone	-	-
Tannin	+	+
Flavonoid	+	+
Alkaloid	+	+

Key: + sign indicates presence and – sign indicates absence

Table 3: Antibacterial activity of aqueous methanol extracts of *Basella alba* and *Basella rubra*

Bacteria	Zone of Inhibition (mm)				
	BA (30 mg/mL)	BR (30 mg/mL)	BA (35 mg/mL)	BR (35 mg/mL)	Streptomycin (1 mg/mL)
Bacillus anthracis (LIO)	20 ± 0.08	20 ±0.08	26 ± 0.07	26 ± 0.05	20 ± 0.04
Clostridium sporogenes (NCIB 532)	-	22 ± 0.05	-	24 ± 0.06	25 ± 0.03
Bacillus polymyxa (LIO)	22 ± 0.06	18 ± 0.03	28 ± 0.12	20 ± 0.10	15 ± 0.07
Micrococcus luteus (NCIB 196)	20 ± 0.09	28 ± 0.10	24 ± 0.05	38 ± 0.09	20 ± 0.06
Streptococcus faecalis (LIO)	-	30 ± 0.09	-	32 ± 0.05	23 ± 0.04

*Mean ± S.D. (n=3)

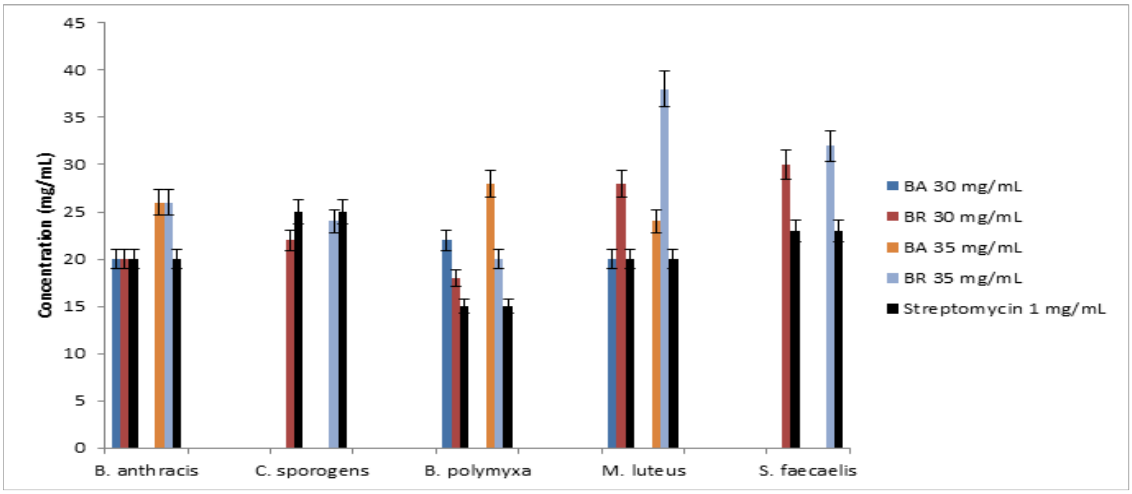

Figure 1: Antibacterial sensitivity of the aqueous methanol extracts of *Basella alba* and *Basella rubra*

The antimicrobial effect of the aqueous methanol extracts against these selected bacteria may be due to the ability of the extraction solvent to extract some phytochemicals reported to exhibit antimicrobial properties such as tannin [25], alkaloid [26], flavonoid [27] and other secondary metabolites which were present in the phytochemical studies done on the aqueous methanol extracts *B. alba* and *B. rubra*.

Although, the results of the antibacterial activities obtained for the *Basella* species compared weakly to the standard drug, Streptomycin ($p<0.05$), they have shown to be good candidates for antimicrobial studies. Therefore, more efforts should be made to isolate, characterize and evaluate the mode of actions of the active compounds responsible for the antimicrobial activities of *Basella* species.

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