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Evaluation of the Antibacterial Activity of Seed Coat Extracts of Roselle (*Hibiscus Sabdariffa L.*) on Selected Antibiotic Resistant Bacterial Species

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Abstract: This research study is on the evaluation of the antibacterial activity of Roselle (Hibiscus sabdariffa L.) seed coats using Aqueous, Ethanol, Hexane and Methanol as solvent. it was examined against selected antibiotic resistant bacterial species (Escherichia coli, Staphyloccocus aureus, Streptococcus pneumonia, Klebsiella aerogenes, Salmonella spp, Pseudomonas aeruginosa). The result evaluates the zones of inhibitions shown by each of the extracts against the test organisms which later revealed that at the various concentrations used, none of the seed coat extracts showed any inhibitory properties against the test microorganism. The phytochemical analysis of the seed coat of H. sabdariffa revealed the presence of bioactive compounds such as Alkaloids, carbohydrates, steroids, Cardiac glycosides. The result from the study reveals that the seed coat extracts of Hibiscus sabdariffa does not possess any inhibitory activity against the bacterial species examined. Notwithstanding, other areas should be exploited for its possible application.

Keywords: *Hibiscus sabdariffa*, Seed Coat Extracts, Antibacterial Activity, Phytochemical Analysis

1. Introduction

Antimicrobial treatment has resulted in a dramatic rise of the average human life expectance, notwithstanding these disease-causing microorganism are becoming resistant to drugs which is now of a great public health concern. There is an exponential increase in microbial resistance that has dominated the clinical community for a long time especially among bacterial species which has resulted in high mortality rate [1]. Hospitals have become particularly notorious for spreading lethal infections, according to the most recent report [2] by the United State Centers for Disease Control and Prevention (CDC), Hospital-Acquire Infections (HAI) now affect one in 25 patients, 4 percent is battling an infection picked up in a hospital or other health care facility, this translates to more than 600,000 hospital patients each year. These infections represent a significant proportion of all infectious diseases acquired by human. Microbial development of drugs resistance has resulted in much research, to find new antibiotics to be able to combat this nuisance. The presence and availability of plants on the earth has been of immeasurable benefits to mankind. The natural bioactive products from plants which include: alkaloids, anthraquinones, saponins, tannins, cardiac glycosides, flavonoids among others. The effectiveness of these procedures has been attributed mainly to the presence of active phytochemical or bioactive compounds in plants [3, 4 and 5]. Hibiscus is one of the most common flowering plants grown worldwide. There are more than 300 species of the plant, one of which is Roselle (*Hibiscus sabdariffa* Linn) which is a member of the family Malvaceae [6]. It has been used in folklore medicine as diuretic, laxative, astringent, treatment of cardiac, nervous and cancer [7]. Its calyce and seeds have been documented to have hypotensive effects [6]. This plant is one of such plant with medicinal potential, despite its uses little is still known about the seed coat as most researches focus on the floral while some other parts of the plant such as the seed coats, are not reckoned to be of any real value. This research seeks to know the possibility of the seed coat extract of roselle (*Hibiscus sabdariffa*) tackling the problem of antibiotics resistance among some common pathogenic bacteria.

2. Materials and Methods

2.1. Collection of Plant Sample

Dry seeds of *Hibiscus sabdariffa* were obtained from Jos North Local Government Area of Plateau State Nigeria. The plant seeds used for this research project were identified and authenticated by Professor S.E. Agina of the Department of Plant Science and Technology, University of Jos.

2.2. Test Organisms

The test organisms employed in this study were collected from Our Lady of Apostle Hospital, Jos Plateau State. The clinical bacterial isolates were further checked for their identity, viability and purity at the microbiology laboratory in the University of Jos using standard biochemical test as described by Cheesbrough [8]. The organisms include: *Escherichia coli, Salmonella* spp*, Streptococcus pneumonia, Klebsiella aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa.*

2.3. Sample Preparation

The *Hibiscus sabdariffa* seeds were cleaned by removing stones, plant parts and other debris. The seeds were rinsed with clean water; the rinsed seeds were soaked with a little quantity of water between six [6] to twelve [12] hours to allow for the dehulling of the seed. The resulting powder was stored in an air-tight container until when required for use.

2.4. Aqueous Extraction

The cold maceration method was employed in the extraction. 50g of the powdered seed coat was transferred into a 500ml beaker. 250ml of sterilized distilled water was added and the mixture was left for 48 hours and intermittently shaken every three hours. This was then heated in a water bath (at 60°C) and filtered. Hot water was continuously added to the residue and subsequently filtered. The procedure was repeated three times and the filtrate was then evaporated to dryness using the water bath and the percentage yield determined. It was kept in the refrigerator (at 4°C) until it was required for use.

2.5. Ethanol, Methanol and Hexane Extraction

Fifty (50g) of the seed coat powder were weighed and transferred into a 500ml conical flask and soaked with 250ml of each of the solvents mentioned above separately. This was then allowed to stand for 72 hours and intermittently shaken every 3 hours on a mechanical shaker. It was then filtered while the residue rinsed in three parts. The filtrates obtained were then evaporated to dryness on a water bath (at 60°C) after which their weight yield determined. The extracts were kept in the refrigerator for phytochemical and anti-bacterial assay.

2.6. Phytochemical Screening

The phytochemical analysis of the aqueous, ethanol, methanol and Hexane extracts of the seed coats of the plant was carried out to determine the presence or absence of alkaloids, saponins, tannin, flavonoids, carbohydrates, cardiac glycosides, Anthraquinones and other bioactive ingredient using standard procedures according to Sofowora [9 and 10].

2.7. Preparation of Seed Coat Extract Concentration

A 4g of each of the extracts were dissolved separately in 10ml of sterilized distilled water to give various concentrations of 400 mg/ml (stock extract) which was serially diluted to give different concentrations of 200gm/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively. The tubes containing the different concentration of the extracts were labeled accordingly and the concentrations were used for the sensitivity test while a gentamycin antibiotic was used as the standard drug control.

2.8. Source of Antibiotic

The antibiotic disc used for the screening of resistant contains known concentration of various antibiotics: Septrin (30µg); Chloramphenicol (30µg); Sparfloxacin (10µg); Amoxacillin (30µg); Augmentin (30µg); Gentamycin (10µg); Perfloxacin (30µg); Tarivid (10µg); Streptomycin (30µg); and Ciprofloxacin (10µg).

2.9. Screening for Resistance Among Clinical Bacterial Isolates

The antibacterial susceptibility test for the detection of resistant microorganisms within the bacterial isolates was determined using the Agar disc diffusion method as described by Cheesbrough [7]. The inoculum size of each bacterial isolate was standardized by picking 5 isolated colonies of overnight bacterial growth on Agar plates. Isolated colonies were suspended in 5ml of normal saline to give a turbidity equivalent of 0.5 Mcfarland turbidity standards. A sterilized cotton swab was dipped into the suspension; the excess was drained before inoculating on the surface plate of the Mueller Hinton agar. The antibiotic discs were then placed firmly with sterilized forceps on the plate, incubated at 35^0c for 18-24 hours after which the inhibition zones were measured in diameters (mm) using a transparent ruler. All tests were done in duplicate with the result compared and recorded.

2.10. Determination of Antibacterial Activity of Crude Seed Coat Extracts

The agar well diffusion method was used in assessing the antibacterial susceptibility of the seed coat extracts. Mueller Hinton agar was prepared based on the manufacturer's instruction. 1ml of each standardized test organism was inoculated into each sterilized Petri dish and 20ml of the prepared media was poured to each, it was swirled for homogeneity and allowed to solidify. Each plate was labeled accordingly. Five wells of 9mm in diameter were made in each plate aseptically using a sterilized cork borer. A volume of 1ml of each extract was transferred into each of the

peripheral wells, while the standard drug gentamycin (40mg/ml) was introduced into separate Petri dishes to serve as control. The plates were then incubated at 35^0c between 18-24 hours. At the end of the incubation period, the susceptibility of the test organism to the extract was assessed by measuring the diameters of zones of inhibition to the nearest millimeter, and was compared.

3. Results and Discussion

The result obtained during the screening of multidrug resistant microorganism using the antibiotic disc is presented in Table 1.

Table 1. *Screening for Multidrug resistant Organism (MDR).*

MICRO ORGANISMS	TYPE OF ANTIBIOTICS									
	SXT	CH	SP	CPX	AM	AU	CN	PEX	OFX	S
E. coli	+	+	-	-	-	-	+	-	-	-
S. Pneumoniae	-	+	-	+	-	-	+	-	-	-
S. aureus	+	-	+	+	-	-	+	+	+	-
K. aerogenes	-	-	+	+	-	-	+	+	+	-
Salmonella sp	-	-	-	+	-	-	+	-	+	-
p. aeruginosa	-	-	+	+	-	-	+	-	+	-

Septrin(SXT 30µg), Chloramphenicol (CH 30µg), Sparfloxacin (SP 10µg), Ciprofloxacin (CPX 10µg), Amoxacillin (AM 30µg), Augmentin (AU 30µg), Gentamycin (CN 10µg), Pefloxacin (PEF 30µg), Tarivid (OFX 10µg), Streptomycin (S 30µg).
+ = Sensivity
- = Resistant

The result in Table 2 shows the presence and absence of phytochemical bioactive compounds that are present in the seed coat of Hibiscus *sabdariffa.* From the table of results, it reveals that alkaloids were present in all of the extracts while steroids were present in the hexane, ethanol and methanol extracts but absent in the aqueous extracts. All the other bioactive components were absent.

Table 2. *Phytochemical constituents of Aqueous, Ethanol, Hexane and Methanol extracts of seed coat of Hibiscus sabdariffa.*

PHYTOCHEMICALS	ASCE	ESCE	HSCE	MSCE
Alkaloids	+	+	+	+
Saponins	-	-	-	-
Tannins	-	-	-	-
Flavonoids	-	-	-	-
Steroids	-	+	+	+
Anthraquinones	-	-	-	-
Cardiac glycosides	-	-	+	+

ASCE (Aqueous seed coat extract), ESCE (Ethanol seed coat extract), HSCE (Hexane seed coat extract), MSCE (Methanol seed coat extract)
- = Absent
+ = Present

The presence of alkaloids in trace quantity was observed based on the phytochemical analysis. Rhoade [11] reported that alkaloids act on diversity of metabolic system in human and other animals, almost uniformly invoking a bitter taste. Suffredini *et al* [12] reported that there is a correlation between compounds isolated from plants and their biological activity. The absence of flavonoids could have accounted for the inactive of the seed coat as several researches have reported it to possess antimicrobial properties due to their ability to form complex with extracellular and soluble protein of microbial [13, 14 and 15]. [16] Also noted that isoflavone compounds have shown antimicrobial activity against the wide range of gram-positive bacteria, fungi and viruses. Tannins form complex with microbial adhesions, enzymes, cell wall envelopes and transport proteins leading to inactivation of these proteins, thereby inhibiting microbial activity. Several scientific evidences show that these compounds have been effective against filamentous fungi, yeast, bacteria and viruses [17,14 and 18]. The non-presence of these compounds may have affected the antibacterial activity.

Table 3. *Antibacterial activity of aqueous, ethanol, methanol and hexane extracts seed coats of H. sabdariffa on the test organisms.*

Conc. of extracts (mg/ml)	25	50	100	200	400	Gentamycin
Test organism	**Zones of Inhibition (mm)**					
Aqueous						
Escherichia coli	0.00	0.00	0.00	0.00	0.00	15
Salmonella spp	0.00	0.00	0.00	0.00	0.00	17
S.Pneumoniae	0.00	0.00	0.00	0.00	0.00	16
Klebsiella aerogenes	0.00	0.00	0.00	0.00	0.00	14
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	16
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	15
Ethanol						
Escherichia coli	0.00	0.00	0.00	0.00	0.00	15
Salmonella spp	0.00	0.00	0.00	0.00	0.00	17
S.Pneumoniae	0.00	0.00	0.00	0.00	0.00	16
Klebsiella aerogenes	0.00	0.00	0.00	0.00	0.00	14
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	16
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	15
Methanol						
Escherichia coli	0.00	0.00	0.00	0.00	0.00	15
Salmonella spp	0.00	0.00	0.00	0.00	0.00	17
S. Pneumoniae	0.00	0.00	0.00	0.00	0.00	16
Klebsiella aerogenes	0.00	0.00	0.00	0.00	0.00	14
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	16
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	15
Hexane						
Escherichia coli	0.00	0.00	0.00	0.00	0.00	15
Salmonella spp	0.00	0.00	0.00	0.00	0.00	17
S.Pneumoniae	0.00	0.00	0.00	0.00	0.00	16
Klebsiella aerogenes	0.00	0.00	0.00	0.00	0.00	14
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	16
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	15

The antimicrobial of quinines as reported by Cowan [14] which involves the forming of complex with microbial

protein, cell wall, polypeptides and membrane-bound proteins could also have affected the inhibitory activity. Many studies have suggested that secondary bioactive components in plants such as tannins, terpenoids, flavonoids, steroids, saponins exhibit antimicrobial activity.

The aqueous, ethanol, methanol and hexane extract of the seed coat of *H. sadariffa,* from table 2 shows the antibacterial activity at various concentration of 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml. The organisms *S. aureus, E. coli, P. aeruginosa, K. aerogenes, Salmonella spp and S. pneumoniae* shows growth of bacterial activity, as none of extracts was able to inhibit the growth of the test organisms.

This present finding is in contrast with [19] reported that aqueous extract of *H. sabdariffa* seeds were found to have inhibitory effects against *Salmonella* spp, *Shigella* spp and *Enterobacter* spp. Also [20] in Ivory coast stated in his finding that seeds oil of *H. sabdariffa* using butylated hydroxyl toluene (BH), 2,2-Biphleny-1-Picryhydrozyl (DPPH) as organic solvent exhibited high antimicrobial activity against *S. aureus, Aspergillus fumigatus* and *Trichophyton mentagrophytes*. However, this research finding reveals that none of the seed coats extracts at various concentrations exhibited any antibacterial activity against all the test organisms which include both gram-positive and gram-negative bacteria. This agrees with Khalid [21] 2015 who reported that the seeds from methanolic extracts of *H. sabdariffa* did not show any activity against both bacterial strains*(Bacillus substilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa)* and two fungal *(Candida albican , Aspergillus niger)* strains examined. This investigated plant part (seed coat) of *H. sabdariffa* did not show any strong antibacterial action against the test organism, nevertheless this result do not mean the absence of phytochemical components in the above plant part rather their presence might not be sufficient enough to produce any inhibitory effect as they are in the crude form or the few that are present might not be the specific ones with antibacterial properties.

4. Conclusion

Plants have served as a remedy to this due to the fact that most antibiotic that are made are produced from synthesis chemical materials which could caused further harm to the human system. Plants have bioactive component and *Hibiscus sabdariffa* is one of such a great potential. The research investigated the antibacterial activity of seed coat extracts of *Hibiscus sabdariffa* on some selected antibiotic resistant bacterial species using water, ethanol, hexane, methanol as solvent. At the end of this research experiment, it was observed that none of the seed coat extracts of *Hibiscus sabdariffa* at the various concentrations used produced any inhibitory effect against the test organism examined as this could be lack of essential phytochemicals or in little proportions.

Recommendation

The *Hibiscus sabdariffa* coat might also have other medicinal value; hence there is a need for further studies - the need to identify with specificity, which plants bioactive component has antibacterial activity and at what quantity to produce such effect.

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