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DOI: https://doi.org/10.64388/IREV10I2-1722295

Phytochemicals Analyses and Antimicrobial Properties of Hibiscus Sabdariffa Flower Extracts on Selected Microbial Organisms (Bacteria and Fungi)

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Abstract- ***The rise in prevalence of antimicrobial resistance has led to continuous search for effective antimicrobial agents from medicinal plants. Hibiscus sabdariffa, a known medicinal plant has shown to contain antimicrobial properties in preliminary studies. This study accessed the phytochemical components and antimicrobial activities of Hibiscus sabdariffa flower against selected bacterial and fungal organisms. The agar well diffusion method was used to check for antimicrobial activity. The MIC, MBC, and MFC were also determined. The results of the study showed that while the aqueous and the ethanol extracts of H. sabdariffa contained richly flavonoids, saponins and oxalates, but in moderate amounts tannins, alkaloids, hydrogen cyanide, phenols and others, the ethanol extracted more components. Significant antimicrobial activity was observed against all the tested bacterial strains ($p < 0.04$) and against Aspergillus fumigatus and Aspergillus flavus (aqueous extract) ($p < 0.05$), but no significant activity was seen against Candida albicans and Fusarium species. No significant differences were found between the aqueous and the ethanol extracts for all organisms ($p > 0.05$) excluding S. aureus, P. aeruginosa and A. flavus ($p < 0.035$). There was no significant differences between the mean MIC of the extracts and the control antibiotics ($p = 0.382$). However, a significant difference was found in the mean MIC values against the fungal strains ($p = 0.00018$). In conclusion , this study showed that Hibiscus sabdariffa contains antimicrobial activities which may serve as a promising natural source of antimicrobial agents for effective antimicrobial resistance.***

Keywords: antimicrobial activity, antimicrobial resistance, phytochemical analysis, hibiscus sabdariffa.

I. INTRODUCTION

Hibiscus sabdariffa is a perennial shrub which is widely distributed across tropical and sub-tropical regions of Africa, Asia, and the Caribbean[1]. It is mainly cultivated and harvested for its nutritional, culinary, and medicinal purposes, alongside other purposes. It contains bioactive compounds such as anthocyanins, flavonoids, phenolic acids, tannins, saponins, alkaloids, organic acids, and polysaccharides[2].

Microbial infections is a leading cause of disease burden worldwide[3]. Inclusive are ocular infections such as bacteria conjunctivitis, fungal keratitis, and endophthalmitis etc which represent a substantial public-health concern[4]. Bacterial conjunctivitis alone accounts for about 135 million cases out of 10000 in the US annually[5]. Although many cases are self-limiting, complications arising from poor management including corneal ulcers and vision loss can occur, particularly in children, the elderly, and immunocompromised individuals[6]. This burden unfortunately is intensified by antimicrobial resistance (AMR). According to WHO (2020), drug-resistant infections could cause an estimated 10 million deaths annually by 2050, with rising resistance reported among priority ocular pathogens[3]. Despite the documented antimicrobial potential of H. Sabdariffa, there is limited comparative evidence on the efficacy of the aqueous and ethanol extracts against clinically important bacterial and fungal pathogens, and relative to conventional agents.

This study therefore investigated the phytochemical constituents and antimicrobial activities of H. sabdariffa flower against selected bacterial and fungal ATCC strains. The specific objectives were:

1. To compare the activity of the different extracts (ethanol and aqueous) against selected the bacterial species.
2. To compare the activity of the different extracts against the selected fungal species.
3. To compare the activity of the extracts with the conventional antimicrobial agents-ciprofloxacin and fluconazole.

II. MATERIALS AND METHODS

This study is a laboratory-based experimental study which investigated the phytochemical constituents and antimicrobial activities of Hibiscus sabdariffa flower extracts against selected bacterial and fungal species.

Plant Collection and Authentication

Fresh Hibiscus sabdariffa flowers were collected from a local horticultural farm in Elele, Rivers State, Nigeria, and was authenticated by a pharmacognosist in the Department of Pharmacy, Madonna University, Nigeria. The plant materials were washed with clean water and sun-dried. It was then blended using sterile electric blender (IndiaMART), sieved through a 1-mm mesh, and stored in airtight plastic containers until extraction.

Extraction of Plant Material

The extraction of the plant material involved two extraction methods.

For ethanol extraction, Soxhlet extraction method was used. 50 g of the grounded flower sample was extracted with 150 mL of 95% ethanol using a Soxhlet apparatus at the boiling point of ethanol (78 °C). The solvent was then removed using a rotary vacuum evaporator at 50 °C, and the crude ethanol extract was obtained.

Cold maceration was used for the aqueous extraction, 50 g of the grounded flower sample sample was soaked in 150 mL of sterile distilled water (1:3, w/v) for 24 h using. The mixture was then filtered through sterile cheesecloth, and the filtrate was concentrated with a rotary vacuum evaporator at 60 °C to obtain the crude aqueous extract.

Phytochemical Screening

Phytochemical analyses of the ethanol and aqueous extracts was carried out immediately to determine the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terponids, hydrogen cyanide, and organic acids using the standard procedures described by Harborne (1998) and Trease and Evans (2002).

Test Microorganisms used

The antibacterial and antifungal activities of the extracts were accessed against American Type Culture Collection (ATCC) reference strains consisting of four bacterial species: Pseudomonas aeruginosa (ATCC 27853), Klebsiella pneumoniae (ATCC 700603), Streptococcus pneumoniae (ATCC 49619), and Staphylococcus aureus (ATCC 25923), and four fungal species: Aspergillus flavus (ATCC 204304), Aspergillus fumigatus (ATCC 204305), Candida albicans (ATCC 10231),and Fusarium solani (ATCC 36031). The ATCC strains were obtained from New Concept Analytical and Environmental Laboratory, Obinze, Imo state, Nigeria. The viability of the organisms was first confirmed using standard microbiological procedures before antimicrobial susceptibility testing was done.

Culture Media and Inoculum Preparation

Mueller–Hinton Agar (MHA) was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 min. The sterile medium was then poured into 8 sterile petri dishes and allowed to solidify. Bacterial suspensions were standardized to the 0.5 McFarland turbidity standard, while fungal suspensions were standardized using a haemocytometer.

Antimicrobial Susceptibility Testing

The antibacterial and antifungal activities of the ethanol and aqueous extracts were evaluated using the agar-well diffusion technique as described by Valgas et al. (2007). 0.1 mL of the standardized microbial suspension was evenly inoculated on the

surface of the sterile Mueller–Hinton agar plates using a sterile cotton swab to obtain a uniform lawn culture. 6mm wells were aseptically bored into the agar using a sterile cork borer, the extract solutions and control solutions were dispensed into the respective wells. The plates were allowed to stand for 30 min to facilitate diffusion before incubation at 37 °C for 24 h. The zones of inhibition (mm) was determined by measuring the diameter of antimicrobial activity observed.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of each extract was determined using 2.5mg/mL, 3.0mg/mL, 4.0mg/mL, and 5.0 mg/mL concentrations. 100mL of the standardized inoculum were added to each test tube containing the extract(s) Negative control tubes contained nutrient broth and the test organism only, while positive control tubes contained nutrient broth and extract without the organism. After incubation at 37 °C for 24 h, the tubes were examined for turbidity. The MIC was recorded as the lowest concentration that completely inhibited visible microbial growth (Cheesbrough, 2006).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC were determined by subculturing the aliquots from MIC tubes showing no visible growth onto freshly prepared sterile nutrient agar plates without antimicrobial agents. Following incubation at 37 °C for 24h, the lowest concentration that produced no microbial growth was recorded as the MBC or MFC, respectively (Cheesbrough, 2006).

Equipments used

The study utilized standard microbiological and analytical equipment, including heating mantle (Searchtech Instrument, China); analytical balance (FA2104, S. Mettler, China); autoclave (Searchtech Instrument, China); sterile electric blender (IndiaMART); UV–Visible spectrophotometer (Searchtech Instrument, China); centrifuge (Searchtech Instrument, China); water bath (Techmel and Techmel, USA); turbidimeter (Xinru Instrument and Meters Co. Ltd., China); electrothermal oven (Searchtech Instrument, China); laboratory dry oven (Searchtech Instrument, China); muffle furnace (Searchtech Instrument, China); water distiller (Searchtech Instrument, China);; incubator (Searchtech Instrument, China); Mueller–Hinton Agar; Whatman filter papers (Sigma-Aldrich) and standard laboratory glassware, including Petri dishes, volumetric pipettes, test tubes, and sterile forceps.

Statistical Analysis

Data were analyzed using the SPSS version 23. Descriptive statistics were expressed as mean ± standard deviation. Independent sample t-test was used to test the study hypotheses, while one-way ANOVA was used to compare the MIC values among the ethanol extract, aqueous extract, and control groups. Statistical significance was set at $p \leq 0.05$ with a confidence interval of 95%.

III. RESULTS AND DISCUSSIONS

TABLE 1: Phytochemical analysis, revealing the bioactive compounds found in hibiscus sabdariffa flower, showing ethanol extract had higher concentrations in the active ingredients – saponin, flavonoid, and oxalate

Parameter	Aqueous	Ethanol
Flavonoid, µg/g	52.94	705.88
Saponin, µg/g	75.95	5553.68
Oxalate, g/100g	112.50	225.00
Phenol, mg/g	0.17	1.21
Alkaloids, µg/g	0.25	0.83
Hydrogen Cyanide, mg/kg	50.94	13.77
Tannins, %	0.18	0.47
Steroids, mg/100g	1.78	14.16
Cardiac Glycosides,%	6.66	6.94

Tepernoid, mg/100g	1.28	15.12
Phytates, %	0.97	1.39

Table 2: Antibacterial activity of Ethanol and aqueous extracts on bacterial test organisms. All tested isolate showed significant antibacterial activity with P value (<0.05)

Organism	Extract Type	Mean(mm)	SD	t-value	p-value
Staphylococcus Aureus	Ethanol	18.75	6.78	-3.45	0.04
	Aqueous	8.75	1.98	-8.12	<0.01
Streptococcus Pneumonia	Ethanol	11.00	11.00	-13.2	<0.001
	Aqueous	12.38	12.38	-6.85	<0.01
Pseudomonas Aeruginosa	Ethanol	9.50	3.42	-18.5	<0.001
	Aqueous	5.50	3.11	-16.2	<0.001
Klebsiella Pneumonia	Ethanol	7.13	2.36	-5.20	<0.01
	Aqueous	5.25	2.22	6.10	<0.01

Table 3: Antifungal activity of Ethanol and aqueous extracts on fungi test organisms showing Aspergillus Fumigatus and aqueous extract of Aspergillus flavus with p-value(<0.05) while the other fungi organisms show p-value (>0.05)

Organism	Extract Type	Mean(mm)	SD	t-value	p-value
Aspergillus flavus	Ethanol	7.50	3.32	-2.12	0.12
	Aqueous	3.75	2.06	-3.87	0.030
Aspergillus Fumigatus	Ethanol	7.25	2.22	-6.12	0.008
	Aqueous	6.75	0.96	-7.45	0.005
Candida Albicans	Ethanol	4.25	2.63	-2.87	0.06
	Aqueous	5.00	2.16	-1.95	0.14
Fusarium Spp.	Ethanol	13.75	3.20	0.52	0.64
	Aqueous	9.50	7.05	-0.89	0.44

Table 4: Shows no significant difference between the antibacterial and antifungal effects of the ethanol and aqueous extracts on the tested organisms except in Staphylococcus aureus, Pseudomonas Aeruginosa, and Aspergillus Flavus.

Organism	Comparison	t-value	p-value
Staphylococcus aureus	Ethanol vs Aqueous	3.82	0.03
Streptococcus pneumonia	Ethanol vs Aqueous	-0.94	0.42
Pseudomonas Aeruginosa	Ethanol vs aqueous	8.00	<0.01
Klebsiella Pneumonia	Ethanol vs aqueous	3.00	0.10
Aspergillus Flavus	Ethanol vs Aqueous	3.64	0.035
Aspergillus Fumigatus	Ethanol vs Aqueous	0.73	0.51
Candida Albicans	Ethanol vs Aqueous	-0.83	0.46
Fusarium Spp.	Ethanol vs Aqueous	1.42	0.25

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DOI: https://doi.org/10.64388/IREV10I2-1722295

Table 5: Shows significant differences between the antibacterial and antifungal effects of the ethanol and aqueous extracts and the control antimicrobials on the tested organisms except in Fusarium Spp, Candida albicans, and Aspergillus Flavus (ethanol) where there is no significant differences.

Organism	Comparison	t-value	p-value
Staphylococcus aureus	Ethanol vs Control	-3.45	0.04
	Aqueous vs Control	-8.12	<0.01
Streptococcus pneumoniae	Ethanol vs Control	-13.2	<0.001
	Aqueous vs Control	-6.85	<0.01
Pseudomonas aeruginosa	Ethanol vs Control	-18.5	<0.001
	Aqueous vs Control	-16.2	<0.001
Kliebsiella pneumonia	Ethanol vs Control	-5.20	<0.01
	Aqueous vs Control	-6.10	<0.01
Aspergillus flavus	Ethanol vs Controls	-2.12	0.12
	Aqueous vs Controls	-3.87	0.030
Aspergillus fumigatus	Ethanol vs Controls	-6.12	0.008
	Aqueous vs Controls	-7.45	0.005
Candida albicans,	Ethanol vs Controls	-2.87	0.06
	Aqueous vs Controls	-1.95	0.14
Fusarium Spp.	Ethanol vs Controls	0.52	0.64
	Aqueous vs Controls	-0.89	0.44

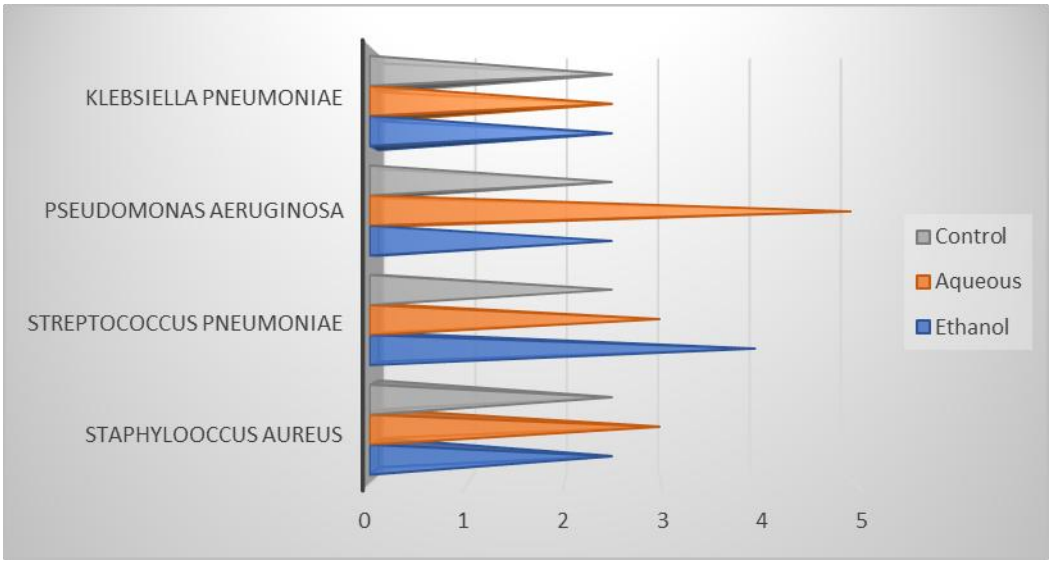

FIG 1: Minimum inhibitory concentration and Minimum bactericidal concentration of Ethanol & Aqueous extract of hibiscus sabdariffa flower and the control antibiotics against bacteria organisms

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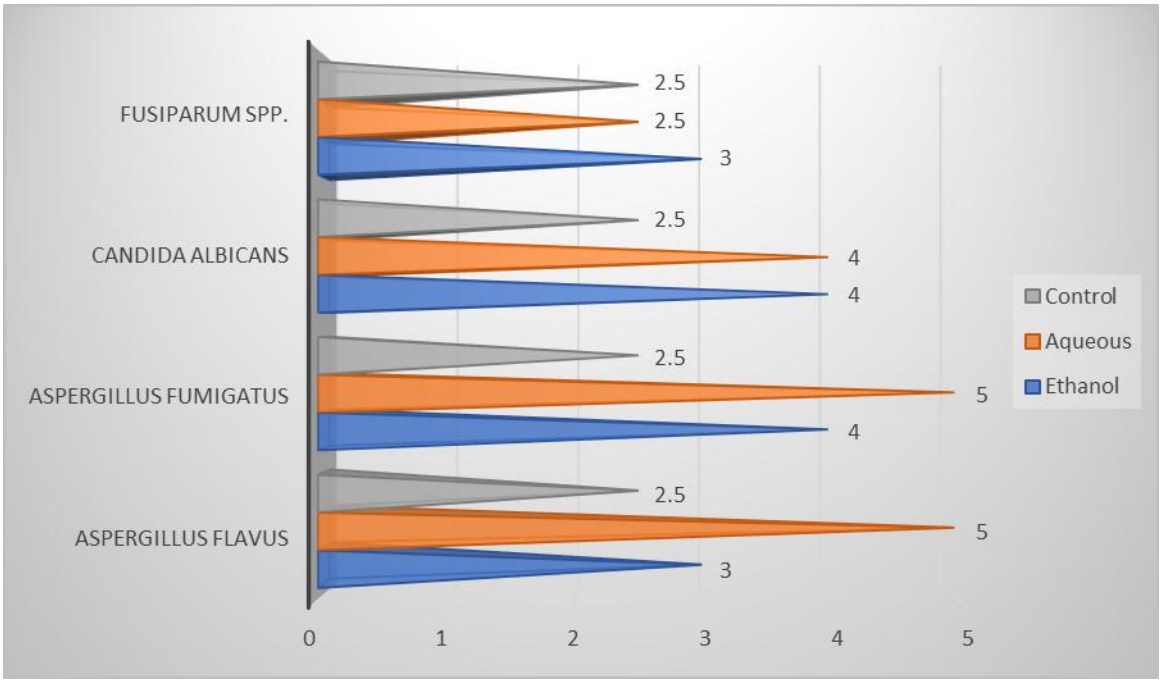

FIG 3: Minimum inhibitory concentration and Minimum fungicidal concentration of Ethanol extract, Aqueous extract of hibiscus sabdariffa flower and control antifungal against fungal organisms.

IV. DISCUSSION OF FINDINGS

- The ethanoic and aqueous extracts of hibiscus sabdariffa flower contains several bioactive compounds with antimicrobial activity against the microbial strains used in this study. The phytochemical analyses of both the ethanoic and aqueous flower extracts revealed the presence of Flavonoid, Saponin, Terpenoid, Phenol, alkaloid, Cardiac glycosides, Tannin, Steroids, Hydrogen Cyanide, Oxalate and Phytate (Table1). Hibiscus sabdariffa flower is very rich in flavonoids, saponins and oxalates with the ethanol extract having more yield. This may be attributed to its alcohol content which causes lower surface tension there by enabling higher penetration into plant tissue [7].
- The ethanoic and aqueous extracts of hibiscus sabdariffa flower exhibited significant antibacterial effects on each bacterium strain with P- value (<0.05) (Table 2) and against Aspergillus Fumigatus (p = 0.008 & p= 0.005) and Aspergillus flavus (aqueous extract) (p= 0.03) (Table 3). On comparison, the effect of ethanoic and aqueous extract on the general spectrum of organisms revealed significant difference between both extracts P= 0.03, P= 0.01 & p= 0.035 on staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus flavus respectively with ethanol showing better effectiveness, but not on other microbial strains.
- The control antibiotics gave significant differences in antibacterial activity against both ethanol and aqueous extract, with P value <0.01 revealing its broad spectrum of activity, high level of organism susceptibility and its current use as conventional treatment of bacterial infections. However, the control antifungal showed significant differences in only the ethanol and aqueous extract against Aspergillus Fumigatus (P= 0.008; P=0.05), and the aqueous extract against Aspergillus flavus (P=0.03), but not in the rest of the fungi strains. This may be due to the low concentration of the extracts used as well as the complex cell walls of fungi.
- The mean MIC between the aqueous extract, ethanol extract and the control antibiotics were not statistically significant. The ethanol extract =2.9mg/dl, the aqueous = 3.4mg/dl, and the control antibiotics = 2.5mg/dl with P value =

0.382; while the mean MIC between the aqueous extract, ethanol extract and the control antifungal were of high significance. The ethanol extract = 3.5 mg/dl, the aqueous = 4.8mg/dl, and the control antibiotics = 2.5mg/dl with P value = 0.00018. Their respective values also denote the order of superiority in antibacterial efficacy, with the control antifungal having the most significant antifungal activity, followed by the ethanol extract and the aqueous extract.

V. CONCLUSION

This study reveals the therapeutic potential of hibiscus sabdariffa flower as a potent and alternative source of antibacterial agent in treating bacterial infections but further studies involving higher concentrations needs to be carried out to prove its effectiveness an alternative and accessible antifungal therapy.

ACKNOWLEDGMENT

I sincerely appreciate my supervisors; Prof. N. C. Ikoro, Prof. A. U. Megwas and Dr. Azuamah for their unwavering support.

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