



Assessment of Antimicrobial Activities and Toxicological Effects of Green and Red Cultivars of Roselle- *Hibiscus sabdariffa* L

Serifat Olatundun Salami¹ and Anthony Jide Afolayan^{1*}

¹*Department of Botany, Medicinal Plants and Economic Development (MPED) Research Centre, University of Fort Hare, Alice 5700, Eastern Cape, South Africa.*

Authors' contributions

This work was carried out in collaboration between both authors. Authors SOS and AJA did the conceptualisation and formal analysis. Author SOS did the funding acquisition. Author AJA did the investigation. Authors SOS and AJA did the methodology, visualisation, wrote the original draft, wrote, reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The use of synthetic antibiotics has been the major way of curing diseases; however, over-use of antibiotics has led to emergence of multi-drug-resistant strains of several groups of microorganisms. This study aimed at examining roselle extracts for antimicrobial properties with a view to providing the best alternative to the injudicious use of synthetic antibiotics and also examines the toxicological effects of roselle extracts.

Methodology: Ethanolic and aqueous extracts of roselle leaves and calyces were evaluated for antimicrobial activity based on minimum inhibitory concentration (MIC₅₀) using Broth dilution method. The toxicological effects based on LC₅₀ were also evaluated using Brine shrimp- *Artemia salina*. Simple percentage was used to determine the mortality rate of the nauplii while the minimum inhibitory concentrations of the extracts were determined using MINITAB 17 statistical package (P<0.05). Data were expressed as mean ± standard deviation of three replicates.

*Corresponding author: E-mail: aafolayan@ufh.ac.za;

Results: Roselle calyces, pre-flowering green and flowering red exhibited excellent inhibition to bacteria. Calyces had better antimicrobial activities and higher toxicological effects than the leaves. Green roselle at pre-flowering stage and red flowering were good for antimicrobial screening.
Conclusion: Roselle extracts possessed excellent antibacterial and antifungal properties. These can be explored to develop new drugs, which can suppress the resistant strains. All the plant extracts were greater than 1000 mg/mL, which indicates that they were non-toxic to brine shrimp larvae.

Keywords: Anti-bacteria; anti-fungal; toxicological effects; roselle extracts; stages of growth.

ABBREVIATIONS

PrG : Pre-flowering green;
PrR : Pre-flowering red;
FG : Flowering green;
FR : Flowering red;
PoG : Post-flowering green;
PoR : Post-flowering red,
CG : Calyces green;
CR : Calyces red;
Eth : Ethanollic extract;
Aqu : Aqueous extract.

1. INTRODUCTION

New diseases emanate on a daily basis while the old diseases are becoming persistent. The persistence of diseases could partly be due to inability of majority of people to afford modern drugs or because of the resistance developed by the pathogens to synthetic drugs. In the light of the evidence of rapid global spread of resistant isolates, the need to discover new antimicrobial agents is paramount [1]. Thus, the search for new drugs from plants has been receiving interest to combat the drug resistant pathogens and the search for affordable herbal products has become the order of the day.

Roselle is an annual plant, which is popular in folk medicines. Extracts of roselle have shown a broad range of therapeutic effects such as antiobesity [2], antihypertensive [3], anticancer [4] and antibacterial [3]. Calyces are considered the most important part of the plant and used in various researches [5] while leaves which are used as vegetables are rarely used. Sulaiman et al. [6] affirmed that herbal formulation may be obtained from extracts from stem, bark or leaves of various plants. Almost all parts of most medicinal plants are useful.

Many people believe that plants are natural and they can be consumed safely, unfortunately, this has proven otherwise [7]. Secondary metabolites

produced by plants may contain some molecules, which are essential for therapeutic use and sometimes contain some undesirable substances that could be toxic to the body when consumed. Thus, the safety of the consumption of plant extracts is very important in traditional medicine. Determination of the toxicological effects of the leaves and calyces is vital for utilization of roselle in traditional medicine.

Koleva et al. [8] reported that the type of cultivar, environmental conditions and the degree of maturation mainly affects the phytochemicals present in medicinal plants. There may be differences in the antimicrobial activities for different cultivar and at different stages of growth because of different phytochemical contents. Khare et al. [9] stated that different plant parts had different content of bioactive substances while [10] affirmed that there was high correlation between antioxidant activities and their polyphenols. Since there is correlation between the bioactive content and antioxidant activity (which may be due to genotype, age of the plants, environmental conditions or different plant parts), there may be differences in the antibacterial and antifungal activities of the plant. There is little information on the effects of stages of plant growth, different plant parts and cultivars on the antimicrobial activities of roselle. Studying toxicological effects of roselle leaves at different growth stages is novel. This work focused on antimicrobial and toxicological effects of the leaves and calyces of green and red roselle, with a view to finding the effective antimicrobial agent with no or minimal toxicity level for the safety of man and for the development of modern drugs. Objectives of this study are to:

- Examine the effects of green and red cultivars of roselle on their antimicrobial activities.
- Investigate the effects of different plant parts (leaves and calyces) of green and red roselle on their antimicrobial properties.

- Determine the antimicrobial effects of green and red roselle at different stages of growth.
- Determine the toxicological effects of green and red roselle leaves (different stages of growth) and calyces.

2. MATERIALS AND METHODS

2.1 Plant Material

Plant materials were obtained from MPED experimental plot, Crop Production Research Farm, University of Fort Hare. Green and red cultivars of roselle were harvested at pre-flowering, flowering and post-flowering stages. The leaves at each stage of growth and calyces were oven dried at 40°C to constant weight and blended to powdery form.

2.2 Preparation of Plant Extracts

Distilled water and ethanol were used for extraction. Briefly, 50 g of each of the pulverized plant samples was shaken at 120 rpm for 24 h in a mechanical shaker (Orbital Incubator Shaker, Gallenkamp). The extracts were filtered using Whatman No.1 filter paper placed on Buchner funnel connected to a vacuum pump. The aqueous filtrates were chilled at -40°C in a chiller (Polyscience AD15R-40-A12E, USA) and concentrated to dryness within 24 hours using freeze dryer (Savant vapor trap, RV-T41404, USA). Ethanolic filtrate was concentrated to dryness using a rotary evaporator set at 78°C. After general extraction, the extracts were reconstituted in distilled water (ethanolic extracts were first dissolved in few drops of ethanol for quick solubility) to get a stock solution of 200 mg/mL. Two-fold serial dilutions of the standard drugs (ciprofloxacin (antibacterial)) and nystatin (antifungal) and of the sample extracts were prepared (100, 50, 25 and 12.5 mg/mL) in the respective broth. Note the following acronyms:

PrG = Pre-flowering green; PrR= Pre-flowering red; FG = Flowering green; FR = Flowering red; PoG = Post-flowering green; PoR = Post-flowering red, CG = Calyces green; CR = Calyces red; Eth = Ethanolic extract; Aqu = Aqueous extract.

2.3 Microorganisms Tested

Six bacterial strains were employed to investigate the antibacterial activities of the

extracts, three of these were gram positive: *Streptococcus pyogenes*, *Bacillus subtilis*, and *Staphylococcus aureus*, while three were gram negative: *Escherichia coli*, *Psuedomonas aeruginosa* and *Streptococcus pneumonia*.

Four fungi were also used in this work, these were *Penicillium chrysogenum* *Candida albicans*, *Penicillium aurantiogriseum* and *Candida glabrata*.

2.4 Inoculum Preparation

Bacterial strains were cultured and then sub-cultured in sterile Petri dishes containing nutrient Broth and incubated overnight at 37°C. Bacteria was scooped from the culture using a loop and dissolved in 0.9% saline, the turbidity of this is then compared with 0.5 McFarland to get bacterial concentration almost equal to 1×10^8 CFU/mL. Fungi spore density of about 10^5 spores/mL was prepared using spectrophotometer at 580 nm.

2.5 Agar Dilution Assay

The method described by [11] was used in preparing this assay. Muller Hinton agar was prepared for bacterial study while Sabourand agar was prepared for anti-fungal investigation. Agar of 19 mL was put in the Erlenmeyer flasks and autoclaved at 121°C for 15 min, these were then allowed to stand at 55°C in water bath. One milliliter of each extract serially diluted was mixed with the 19 mL molten agar to give a concentration range of 10 mg/mL (highest concentration) - 0.625 mg /mL (lowest concentration). The agar, which was mixed with plant extract, was poured into each of the Petri dishes, which were already divided into sections to accommodate each bacteria/fungi. The agar was left to cool and solidify. Then, 10 µL of either bacteria or fungal inoculum was poured onto the solidified agar at the corresponding section of the Petri dish to give the desired final inoculum of 1×10^4 CFU/ spot [12]. Ciprofloxacin which was used as positive control for bacteria was in the concentration range of 2 - 64 µg/ mL while Nystatin which was used as positive control for fungal was in the range of 0.5 – 16 µg/mL. The Petri-dishes were placed in an incubator set at 37°C for 24 h for bacterial preparation while fungal preparation were incubated at 30°C for 72 h. Thereafter, the bacteria growth was examined on each Petri dish. The minimum inhibitory concentrations of extracts were determined as the lowest concentration of the

extracts that inhibit the visible growth of the organisms.

2.6 Toxicity Assay

The method described by [13] was employed with little modification. A two-fold serial dilution of both extracts was carried out with the seawater to obtain a concentration range of 0.0625 - 1 mg/mL. Potassium dichromate ($K_2Cr_2O_7$) which was a positive control was also two - serially diluted in seawater to obtain a concentration range of 0.0625 – 1 mg/mL. Seawater was used as negative control.

2.7 Brine Shrimp (*Artemia salina*) Lethality Assay

Brine shrimp lethality assay has been used as an alternative bioassay technique to screen toxicity of plant extracts [14,15]. Brine shrimp eggs were poured into a beaker containing filtered seawater and put inside illuminated hatching chamber set at 25°C. This was left to stand for 24 hrs after which most of the eggs had hatched into larvae called nauplii. Ten nauplii were transferred into each of the Petridishes that contain serially diluted extract and standard using Pasteur pipettes. The surviving nauplii (which were detected through their movement) were examined and counted in each Petri dish with the aid of a 3X magnifying glass after every 12 h. This was done for 72 h under constant illumination. The set up was replicated for each concentration. Numbers of the survivors and death were recorded. Data were processed at a 95% confidence interval to estimate LC_{50} value. Extract of LC_{50} greater than 1000 μ g/mL was considered non-toxic [14].

2.8 Statistical Analysis

The minimum inhibitory concentrations of extracts which were the lowest concentrations of extracts that inhibit the visible growth of the bacteria and fungi were determined using the MINITAB Release 17 statistical package ($P < 0.05$).

Simple percentage was used to determine the % mortality of the nauplii using the following formular:

$$\text{Mortality\%} = \frac{\text{Total nauplii} - \text{Alive nauplii}}{\text{Total nauplii}} \times 100$$

Data were expressed as mean \pm standard deviation of three replicates of each treatment

and error bars were put on the graphs to show the variation.

3. RESULTS

3.1 Anti-bacterial

The effects of ethanolic and aqueous extracts of roselle are presented in Table 1. In ethanolic extracts, inhibiting activities of both green and red cultivars were excellent especially the calyces, which had the minimum inhibiting concentration (MIC) range of 1.25 – 5 mg/mL. The calyces were potent against all bacteria tested. Overall, the inhibiting capacities of the extracts against all bacteria ranged from MIC 1.25 - > 10 mg/mL. Although all extracts inhibited the growth of *S. pyrogenes*, green calyces had the highest inhibition (1.25 mg/mL) followed by green roselle at the pre-flowering stage, red at flowering stage and red calyces (2.5 mg/mL). The remaining extracts showed moderate inhibition (5 mg/mL) against the bacterium. The red calyces, however, showed the strongest inhibition against *B. subtilis* having a MIC of 1.25 mg/mL, followed by green calyces and pre-flowering green while flowering red was less potent to the bacterium (10 mg/mL). Also both calyces and pre-flowering green showed higher inhibition (2.5 mg/mL) than other extracts against *S. aureus*, while post-flowering stage of the two cultivars had the lowest inhibition of 10. Furthermore, green calyces showed the highest inhibition (1.25 mg/mL) against *K. pneumoniae* followed by red calyces and flowering red (2.5 mg/mL), all other extracts showed moderate inhibition of MIC 5 mg/mL to the bacterium. Red calyces showed highest inhibition against *E. coli* (1.25 mg/mL) followed by green calyces (2.5 mg/mL). Flowering stage of the red roselle and post-flowering stage of both cultivars showed low inhibition (10 mg/mL). Green and red calyces, as well as flowering red, exhibited higher activities (2.5 mg/mL) against *P. aeruginosa* than other extracts, pre-flowering green, however, had the lowest inhibition (10 mg/mL).

In aqueous extracts, apart from pre-flowering green, which had high inhibition against *P. aeruginosa*, all aqueous extracts in all growth stages showed low inhibition with $MIC \geq 10$ mg/mL to all the tested bacteria. All calyces, however, showed excellent activities in both solvents. Cyproflaxin showed higher inhibitory effects against all the bacteria.

Table 1. Minimum Inhibitory Concentrations of roselle extracts on selected bacteria

Growth stage		<i>S. pyogenes</i>		<i>B. subtilis</i>		<i>S. aureus</i>		<i>K. pneumoniae</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
		mg/mL		mg/mL		mg/mL		mg/mL		mg/mL		mg/mL	
		Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu
Pre-flowering	Green	2.5	10	2.5	>10	2.5	10	5	10	10	>10	10	10
	Red	5	10	5	10	5	10	5	10	5	10	5	2.5
Flowering	Green	5	>10	5	>10	5	>10	5	>10	5	>10	5	10
	Red	2.5	10	10	10	5	>10	2.5	10	10	>10	2.5	10
Post-flowering	Green	5	10	5	10	10	10	5	10	10	10	5	10
	Red	5	10	5	10	10	10	5	10	10	>10	5	10
Calyxes	Green	1.25	2.5	2.5	2.5	2.5	5	1.25	2.5	2.5	5	2.5	2.5
	Red	2.5	2.5	1.25	2.5	2.5	2.5	2.5	2.5	1.25	2.5	2.5	2.5
Standard	Ciproflaxin(µg/ml)	64		32		16		8		4		2	

Eth: ethanolic extract, Aqu: Aqueous extracts

Table 2. Minimum Inhibitory Concentrations of roselle on fungi at different stages of growth

Growth stage		<i>Penicillium chrysogenum</i>		<i>Candida albicans</i>		<i>Candida glabrata</i>		<i>Penicillium aurantiogriseum</i>	
		mg/mL		mg/mL		mg/mL		mg/mL	
		Eth	Aqu	Eth	Aqu	Eth	Aqu	Eth	Aqu
Pre-flowering	Green	>10	>10	< 0.0625	< 0.0625	>10	< 0.0625	< 0.0625	< 0.0625
	Red	>10	>10	< 0.0625	< 0.0625	>10	>10	< 0.0625	10
Flowering	Green	>10	10	< 0.0625	5	>10	10	< 0.0625	< 0.0625
	Red	>10	>10	< 0.0625	< 0.0625	>10	2.5	< 0.0625	< 0.0625
Post-flowering	Green	>10	>10	< 0.0625	1.25	>10	>10	< 0.0625	< 0.0625
	Red	>10	< 0.0625	< 0.0625	2.5	>10	< 0.0625	< 0.0625	2.5
Calyxes	Green	>10	>10	< 0.0625	< 0.0625	>10	>10	< 0.0625	>10
	Red	>10	>10	< 0.0625	1.25	>10	>10	< 0.0625	< 0.0625
Nystatin	µg/ml	16		8		4		2	

Eth: ethanolic extract, Aqu: Aqueous extracts

3.2 Anti-fungal

The activities of the extracts (in both solvents) were in the MIC range of < 0.0625 - >10 mg/mL. The extracts inhibiting capacities were low to *P. chrysogenum* and *Candida glabrata*, the majority of extracts requiring more than the highest concentration (>10 mg/mL), while the extracts showed excellent inhibitory activities to *Candida albicans* and *P. aurantigriseum*, the majority of the extracts had MIC value of <0.0625 mg/mL.

All extracts showed low activity to *P. chrysogenum* having MIC value of >10 mg/mL, except aqueous extracts of red post-flowering which displayed the strongest activity having a MIC of 0.0625 mg/mL. All ethanolic extracts, as well as aqueous extracts of both cultivars at the pre-flowering stage, flowering red and green calyces, showed excellent inhibition against *C. albicans* having MIC value of 0.0625 mg/mL. Flowering green, however, had moderate inhibition with MIC value of 5 mg/mL. Similarly, all ethanolic extracts had the highest inhibition of < 0.0625 mg/mL except post-flowering red which had moderate inhibition of 2.5 mg/mL, pre-flowering red and green calyces had low activities (10 mg/mL and >10 mg/mL respectively). Nystatin had high inhibitory effects against the tested fungi (Table 2).

3.3 Toxicity

3.3.1 Percentage mortality

Ethanolic extracts of flowering red showed the highest lethal capacity (34.16%) followed by red pre-flowering and red post-flowering (29.16%) at the highest concentration of all growth stages. Red, however, showed no mortality (0%) at the flowering stage of the lowest concentration. The highest mortality in green cultivar occurred at the pre-flowering stage of the highest concentration (25.83%). This was just a little bit higher than that at the post-flowering stage of the highest concentration (25%). Lowest mortality occurred at the flowering stage of of green cultivar at concentration level of 0.125 mg/mL.

In aqueous extracts, however, pre-flowering green at the highest concentration showed highest mortality (41.67%) followed by the flowering green of the same concentration (17.25%) while no mortality occurred at flowering and post-flowering stages of green roselle at the highest concentration. Mortality did not occur at the flowering stage of both cultivars at the lowest

concentration. Calyces of both cultivars in both solvents had a complete mortality of 100% at the highest concentration, while potassium dichromate showed the highest mortality along the concentration gradient except the highest concentration which was lower (90%) than calyces. Lowest mortality, however, occurred in red at 0.0625 mg/mL (4.17%) while no mortality occurred at the lowest concentration in green. In aqueous extracts, the extract with the next highest mortality after calyces of 1 mg/mL was red calyces at 0.5 mg/mL (14.17%) while no mortality occurred at the lowest concentration. In green calyces, mortality at 0.5 and 0.0625 mg/mL was 0.83%, this was the next to the highest mortality (100%). No mortality occurred at 0.125 and 0.25 (0%). Potassium dichromate showed the strongest lethal capacity in all concentrations of the aqueous except the highest concentration where calyces gave the highest mortality.

It is noteworthy that LC₅₀ of all the extracts were greater than the highest concentration (1 mg/mL) (Figs. 1 – 4).

3.3.2 Total mortality

The total mortality for the standard was the highest (71%). Of all the extracts, total mortality displayed by the ethanolic extracts was the highest (25%), this was a little bit higher than the aqueous value (24%). The green calyces were also a little lower than red calyces at both solvents (23.8% and 21.8%) for ethanolic and aqueous solvents respectively. In all the growth stages, ethanolic extract of flowering green showed higher total mortality followed by an aqueous extract of pre-flowering red (16.16%) which was a little higher than the ethanolic extract of pre-flowering green (13.33%). Aqueous extracts of flowering red and post-flowering green showed the least total mortality (4.17%), though it was not quite different from post-flowering red (4.31%).

4. DISCUSSION

The result of the current study supports the use of roselle for the treatment of bacteria and fungi diseases. It also established the fact that roselle leaves (at various stages of growth) and calyces were non-toxic to *Artemia salina*. From this study, it was found that both calyces strongly depressed the growth of all the bacteria in both ethanol and water. Sulaiman et al. [6] reported that *Hibiscus sabdariffa* was active against *Escherichia coli*, *Klebsiella pneumonia*,

Staphylococcus aureus and *Pseudomonas aeruginosa*. The excellent inhibitory activity may be the reason why calyces are commonly used in researches instead of leaves. This finding is contrary to the report of [16] that leaves have large number of secondary metabolites as compared to other parts of the plants and the concentration of secondary metabolites is more in leaves. The calyces suppressed the growth of both gram positive and gram negative; thus, the compounds in the calyces may be isolated and used in pharmaceutical industries to develop broad-spectrum bacterial drugs. Although all the extracts inhibited *S. pyrogenes*, green calyces gave a strongest inhibition against the bacteria, likewise the pre-flowering green and flowering red. The extracts may therefore, be utilized in the preparation of a new drug to combat the

diseases caused by *S. pyrogenes*. In the same vein, red calyces strongly inhibited the growth of *B. subtilis* while green calyces and pre-flowering green also inhibited well. These three extracts can be suggested for use in developing new drugs against *B. subtilis*. Similarly, both calyces and pre-flowering green inhibited *S. aureus* growth while post-flowering stage of the two cultivars gave the lowest inhibition. Therefore, roselle calyces and green roselle at the pre-flowering stage may be explored to cure the diseases caused by *S. aureus*. However, roselle at the post-flowering stage may not be fit to use to develop drugs against the diseases caused by this bacterium. Although all ethanolic extracts depressed the growth of *K. pneumoneae*, green calyces, red calyces and flowering red excellently inhibited the growth of the bacteria. Thus, these

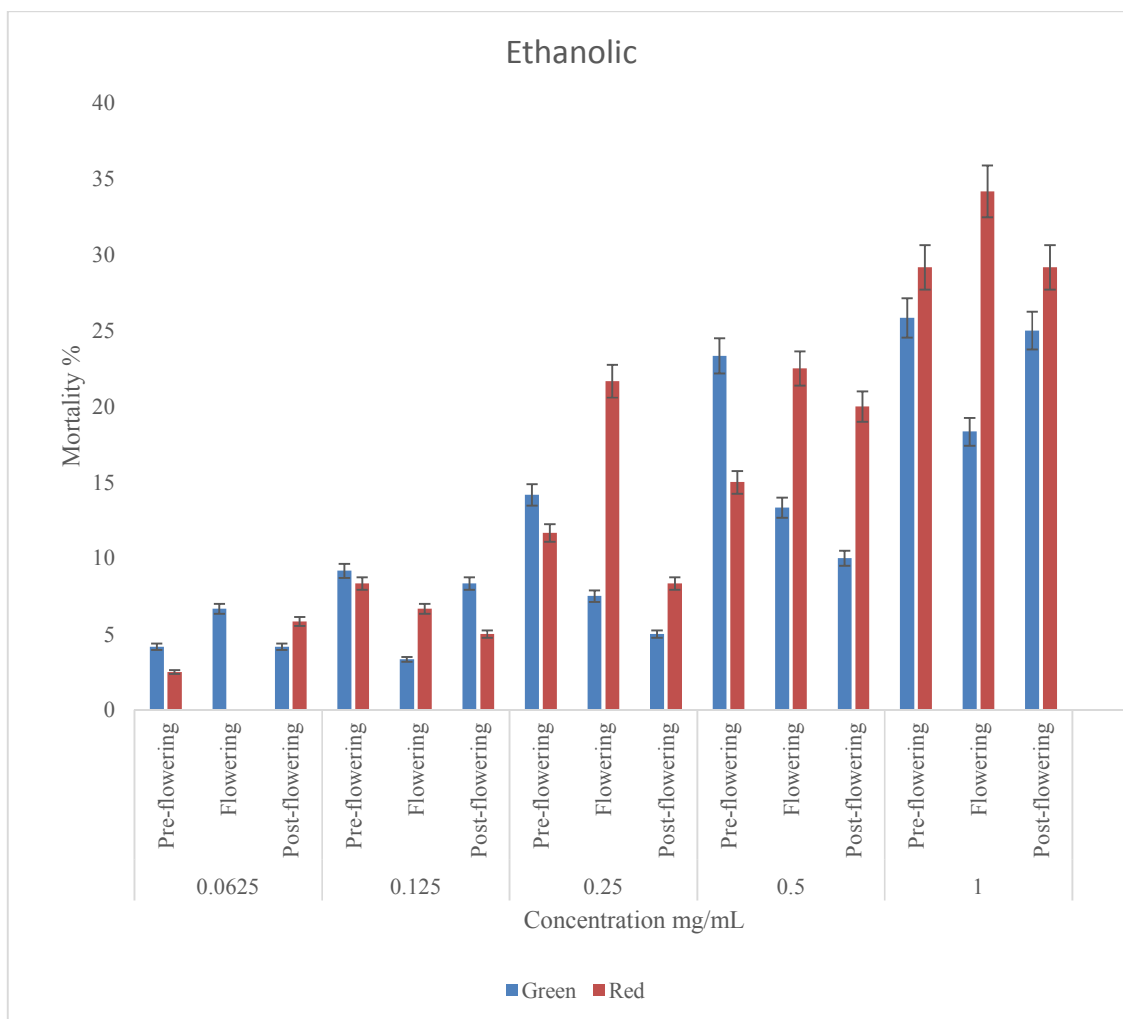


Fig. 1. Percentage mortality of *Artemia salina* nauplii in graded concentrations of ethanolic extracts of roselle at different growth stages

extracts can be useful in preparing new drugs to treat pneumonia disease. Red calyces demonstrated strong capacity in depressing the growth of *E. coli* while the bacterium was resistant to extracts from green calyces, red roselle at flowering stage and post-flowering stage of the two cultivars. To control food and water borne diseases caused by *E. coli* red calyces may be explored to prepare drugs against *E. coli* while red roselle at flowering stage and roselle at post-flowering stage may not be fit for use against the bacterium. Reports of previous works also confirmed that roselle extracts exhibited antibacterial activity against *E. coli* [17,11]. Extracts from both calyces and red roselle at flowering stage exhibited strong inhibition against *P. aeruginosa* than other extracts can be effective in combating the diseases caused by this organism. The present study agrees with the findings of Unuofin et al. [13,6] that roselle is a better inhibitor of *P. aeruginosa* than streptomycin. It is noteworthy that pre-flowering green, which had shown excellent inhibition against all gram-positive

bacteria, showed passive activity against the gram-negative bacteria. Thus, pre-flowering green can be excellent inhibitors of gram-positive bacteria but not effective against gram-negative bacteria. This report supports the previous findings that plants extracts were more active against gram-positive bacteria than gram-negative type [18,19]. All these findings corroborate the report of [3] who affirmed that roselle possessed excellent antibacterial properties. It is worthy of note that roselle at the post-flowering stage is not good for bacteria control, therefore, matured leaves may not be effective for the treatment of bacterial diseases.

All bacteria were resistant to aqueous extracts at all growth stages except *P. aeruginosa* that was inhibited by red roselle at the pre-flowering stage. Aqueous extracts of plants showed low or no antimicrobial activity [20,19]. Water is, thus, not the most effective solvent for extracting the bioactive compounds from plants This result agrees with the findings of [12].

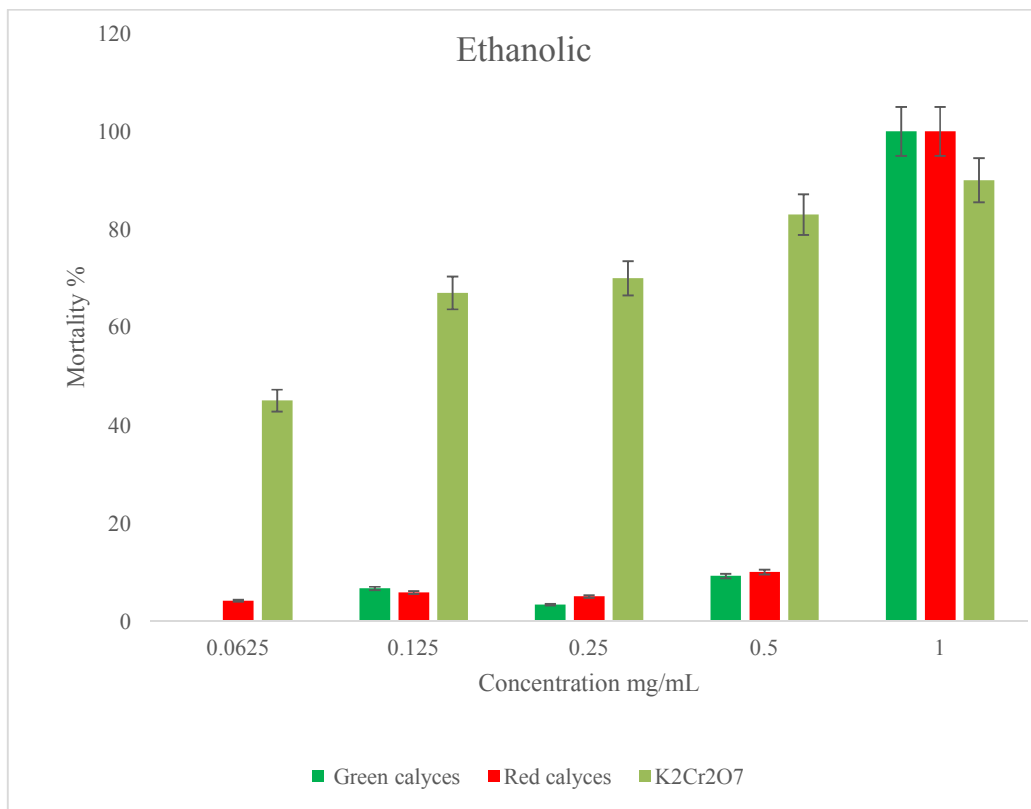


Fig. 2. Percentage mortality of *Artemia salina* nauplii in graded concentrations of ethanolic extracts of roselle calyces and standard

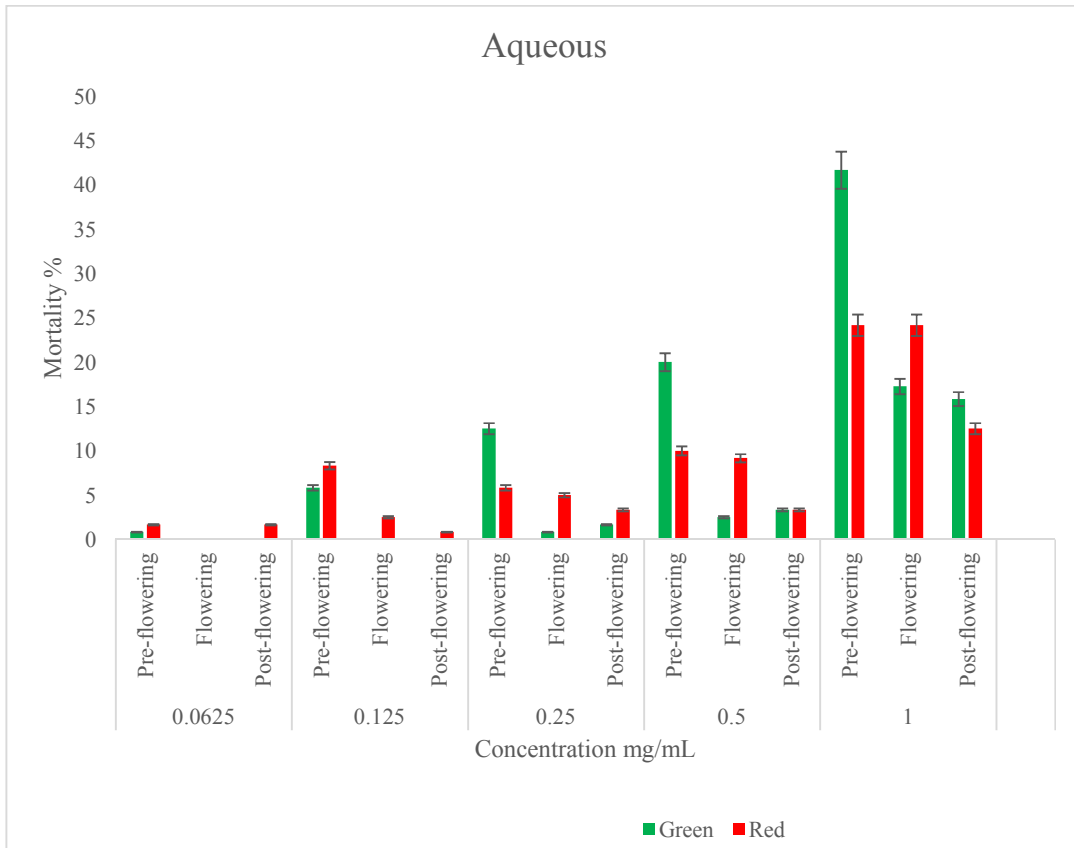


Fig. 3. Percentage mortality of *Artemia salina* nauplii in graded concentrations of aqueous extracts of roselle at different growth stages

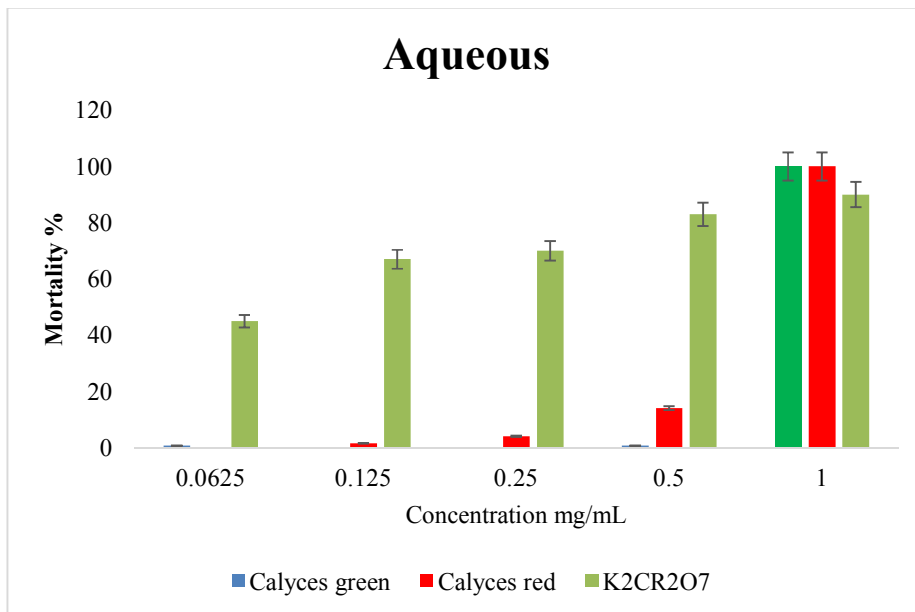


Fig. 4. Percentage mortality of *Artemia salina* nauplii in graded concentrations of aqueous extracts of roselle calyces and standard

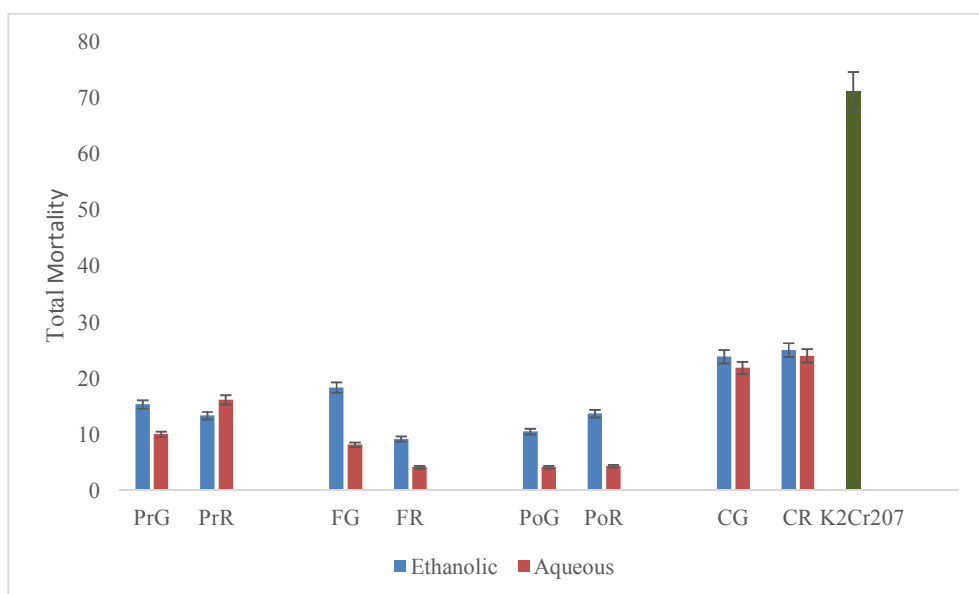


Fig. 5. Effects of roselle extracts at different stages of growth, calyces and $k_2Cr_2O_7$ on total mortality of *Artemia salina* nauplii

PrG = Pre-flowering green; PrR= Pre-flowering red; FG = Flowering green; FR = Flowering red; PoG = Post-flowering green; PoR = Post-flowering red, CG = Calyces green; CR = Calyces red

Penicillium chrysogenum was resistant to all ethanolic and aqueous extracts except post-flowering red. This implies that roselle cannot be a suitable plant for combating the diseases caused by this fungus. However, *Candida albicans* was susceptible to all ethanolic extracts and most aqueous extracts. Hence, roselle calyces and leaves at any stage of growth are excellent material, which can be used to develop a new drug against *Candida albicans*. *Candida glabrata* was strongly resistant to all ethanolic extracts as well as most aqueous extracts but aqueous extracts of pre-flowering green and post-flowering red excellently inhibited the growth of the fungus. This suggests that ethanol is not a good solvent for extracting plant substances for controlling *C. glabrata*, however, water can be a good solvent to extract useful compounds from green roselle at pre-flowering stage and red roselle at post-flowering stage. *P. aurantiogriseum* was susceptible to all extracts (ethanolic and aqueous) except red roselle at pre-flowering stage and green calyces. Thus, roselle can be a good agent for combating *P. aurantiogriseum*. The fungus was resistant to aqueous extracts of green calyces and pre-flowering red, therefore unsuitable for use against the fungus.

According to [14], plant extracts having LC_{50} value greater than $1000 \mu\text{g/mL}$ (1 mg/mL) is considered non-toxic to *Artemia salina*; plant

extracts which have LC_{50} value equal or greater than $500 \mu\text{g/ml}$ (0.5 mg/mL) but not up to 1 mg/mL were considered weak toxic while plant extracts which have LC_{50} values less than 0.5 mg/mL were considered toxic. Based on Meyer's toxicity index, no extracts were toxic to *Artemia salina* nauplii because LC_{50} values for all the extracts were greater than the highest concentration (1 mg/mL). Hence, the extracts are safe to *Artemia salina*. It is worthy of note that ethanolic and aqueous extracts of both calyces at the highest concentration gave complete mortality to the nauplii. This suggests that calyces at 1 mg/mL in both solvents are not suitable for *Artemia salina*, lower concentrations are however safe. Potassium dichromate was lethal along concentration gradient while control was 100% non-toxic as no nauplii died throughout the 72 h.

5. CONCLUSION

The study established the fact that roselle possessed anti-bacterial and anti-fungal properties and also showed that roselle was non-toxic to *Artemia salina* and may thus be safe for human consumption.

Roselle calyces, green roselle at pre-flowering stage and red roselle at flowering stage were found to be highly potent against the tested

bacteria and fungi while roselle at post-flowering stage may not be active for bacterial control as the organisms were resistant to the treatments.

Penicillium chrysogenum and *Candida glabrata* were resistant to almost all the roselle treatments, therefore, roselle may not be effective for combating these fungi while *Candida albicans*, *Penicillium aurantigriseum* and all tested bacteria were susceptible to almost all the treatments; roselle may thus be a good plant to explore to develop drugs against these organisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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