



Faculty of Sciences  
Department of Biological Sciences and Biotechnology

# **SCREENING OF SELECTED EDIBLE WILD FRUITS FROM THE TSWAPONG REGION (BOTSWANA) FOR THEIR ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL**

by

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# TABLE OF CONTENTS

|                                                                                                        |          |
|--------------------------------------------------------------------------------------------------------|----------|
| TABLE OF CONTENTS.....                                                                                 | I        |
| LIST OF FIGURES .....                                                                                  | IV       |
| LIST OF TABLES .....                                                                                   | VII      |
| LIST OF ABBREVIATIONS.....                                                                             | IX       |
| DECLARATION REGARDING THE WORK AND COPYRIGHT .....                                                     | XI       |
| ACKNOWLEDGMENTS .....                                                                                  | XII      |
| DEDICATION .....                                                                                       | XIV      |
| ABSTRACT.....                                                                                          | XV       |
| <b>CHAPTER ONE INTRODUCTION .....</b>                                                                  | <b>1</b> |
| 1.1 BACKGROUND .....                                                                                   | 1        |
| 1.2 PROBLEM STATEMENT.....                                                                             | 2        |
| 1.3 RATIONALE .....                                                                                    | 2        |
| 1.4 HYPOTHESES .....                                                                                   | 3        |
| 1.5 OBJECTIVES OF THE STUDY.....                                                                       | 3        |
| 1.5.1 General objective.....                                                                           | 3        |
| 1.5.2 Specific objectives:.....                                                                        | 3        |
| <b>CHAPTER TWO LITERATURE REVIEW .....</b>                                                             | <b>4</b> |
| 2.1 Edible Wild fruits of Botswana .....                                                               | 4        |
| 2.1.1 Vangueria infausta .....                                                                         | 4        |
| 2.1.2 Ximenia americana L and Ximenia caffara Sond .....                                               | 5        |
| 2.1.3 Grewia Genus.....                                                                                | 5        |
| 2.1.4 Mimusops zeyheri.....                                                                            | 6        |
| 2.2 ANTIOXIDANTS .....                                                                                 | 6        |
| 2.2.1 Edible Wild fruits-antioxidant ( <i>in vitro</i> and <i>in vivo</i> antioxidant potential) ..... | 6        |
| 2.2.2 Evaluation of antioxidant Capacity .....                                                         | 8        |
| 2.2.3 Chemical/biochemical based antioxidant activity assays.....                                      | 8        |
| 2.2.4 Mechanism actions of phenolic acids as antioxidants .....                                        | 8        |
| 2.2.5 Mechanism actions of flavonoids as antioxidants .....                                            | 10       |
| 2.2.6 Health benefits of antioxidants in humans .....                                                  | 13       |
| 2.3 OXIDATIVE STRESS .....                                                                             | 15       |
| 2.3.1 Reactive oxygen species and Reactive Nitrogen species .....                                      | 15       |
| 2.3.2 Oxidative stress on cellular component.....                                                      | 16       |
| 2.4 ANTIOXIDANT ANALYSES OF EDIBLE WILD FRUITS .....                                                   | 17       |
| 2.4.1 <i>In vitro analysis</i> of wild edible fruits.....                                              | 17       |
| 2.4.2 (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay).....                             | 17       |
| 2.4.3 ABTS radical scavenging assay .....                                                              | 18       |
| 2.4.5 Hydrogen Peroxide Scavenging Activity .....                                                      | 18       |

|                                                                                                         |           |
|---------------------------------------------------------------------------------------------------------|-----------|
| 2.5 ANTIMICROBIAL ACTIVITY ASSAY (AGAR WELL DIFFUSION METHOD AND MINIMUM INHIBITORY CONCENTRATION)..... | 19        |
| <b>CHAPTER THREE MATERIALS AND METHODS.....</b>                                                         | <b>20</b> |
| 3.1 SAMPLES .....                                                                                       | 20        |
| 3.2 CHEMICALS .....                                                                                     | 20        |
| 3.3 ANALYTICAL METHODS .....                                                                            | 20        |
| 3.3.1 Sample preparation .....                                                                          | 20        |
| 3.3.2 Extraction of secondary metabolites from edible wild fruits .....                                 | 21        |
| 3.4 QUALITATIVE PHYTOCHEMICAL SCREENING OF EDIBLE WILD FRUITS .....                                     | 21        |
| 3.4.1 Test for Tannins.....                                                                             | 22        |
| 3.4.2 Test for Saponnin.....                                                                            | 22        |
| 3.4.3 Test for Terpenoids (Salkowski test).....                                                         | 22        |
| 3.4.4 Test for Phenols .....                                                                            | 22        |
| 3.5 QUANTIFICATION OF MAIN PHYTOCHEMICAL CLASSES .....                                                  | 22        |
| 3.5.1 Total phenolic content quantification (Folin-Ciocalteu method) .....                              | 22        |
| 3.5.2 Total Flavonoid Content quantification.....                                                       | 23        |
| 3.6 EVALUATION OF ANTIOXIDANT ACTIVITY (DPPH AND ABTS RADICAL SCAVENGING CAPACITY ASSAY) .....          | 24        |
| 3.6.1 DPPH radical scavenging capacity assay .....                                                      | 24        |
| 3.6.2 ABTS radical scavenging capacity assay .....                                                      | 24        |
| 3.7 ANTIMICROBIAL ACTIVITY ASSAY (AGAR WELL DIFFUSION METHOD AND MINIMUM INHIBITORY CONCENTRATION)..... | 25        |
| 3.7.1 Agar well diffusion method.....                                                                   | 25        |
| 3.7.2 Minimum Inhibitory Concentration (MIC) .....                                                      | 26        |
| 3.8 STATISTICAL ANALYSIS .....                                                                          | 27        |
| <b>CHAPTER FOUR RESULTS .....</b>                                                                       | <b>28</b> |
| 4.1 PHYTOCHEMICAL SCREENING .....                                                                       | 28        |
| 4.1.1 Qualitative Analysis .....                                                                        | 28        |
| 4.2 QUANTIFICATION OF MAIN PHYTOCHEMICAL CLASSES .....                                                  | 29        |
| 4.2.1 Total Flavonoids Content .....                                                                    | 29        |
| 4.2.2 Total Phenolic Content .....                                                                      | 31        |
| 4.3 DPPH AND ABTS SCAVENGING ACTIVITY .....                                                             | 33        |
| 4.3.1 ABTS Assay .....                                                                                  | 33        |
| 4.3.2 DPPH assay .....                                                                                  | 35        |
| 4.4 ANTIMICROBIAL ACTIVITY ASSAY (AGAR WELL DIFFUSION METHOD AND MINIMUM INHIBITORY CONCENTRATION)..... | 37        |
| 4.4.1 Agar well diffusion method.....                                                                   | 37        |
| 4.4.2 Minimum Inhibitory Concentration.....                                                             | 38        |
| 4.5 CORRELATIONS .....                                                                                  | 38        |
| <b>CHAPTER FIVE DISCUSSION.....</b>                                                                     | <b>41</b> |
| 5.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS.....                                                             | 41        |

|                                                                                                            |           |
|------------------------------------------------------------------------------------------------------------|-----------|
| 5.2 QUANTITATIVE PHYTOCHEMICAL ANALYSIS.....                                                               | 41        |
| 5.2.1 Total phenolic content .....                                                                         | 41        |
| 5.2.2 Total flavonoid content.....                                                                         | 43        |
| 5.3 EVALUATION OF ANTIOXIDANT ACTIVITY (DPPH AND ABTS RADICAL SCAVENGING<br>CAPACITY ASSAY) .....          | 44        |
| 5.3.1 DPPH scavenging activity .....                                                                       | 44        |
| 5.3.2 ABTS scavenging activity .....                                                                       | 45        |
| 5.4 ANTIMICROBIAL ACTIVITY ASSAY (AGAR WELL DIFFUSION METHOD AND MINIMUM<br>INHIBITORY CONCENTRATION)..... | 46        |
| 5.4.1 Agar well diffusion method.....                                                                      | 46        |
| 5.4.2 Minimum Inhibitory Concentration.....                                                                | 46        |
| 5.5 CORRELATIONS .....                                                                                     | 47        |
| <b>CHAPTER SIX CONCLUSIONS AND RECOMMENDATIONS .....</b>                                                   | <b>48</b> |
| 6.1 CONCLUSIONS .....                                                                                      | 48        |
| 6.2 RECOMMENDATIONS .....                                                                                  | 48        |
| <b>REFERENCES.....</b>                                                                                     | <b>50</b> |
| <b>APPENDICES .....</b>                                                                                    | <b>71</b> |

## LIST OF FIGURES

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 1:</b> Relationship Between Oxidative Stress, Antioxidants and ROS .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 7  |
| <b>Figure 2:</b> An overview of phenolic acids' chemical structure. This figure was adopted from (Al Harthi <i>et al.</i> , 2015) .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 10 |
| <b>Figure 3:</b> Mechanism actions of phenolic acids as antioxidants. This figure was adopted from (Tadapaneni., 2010) .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 10 |
| <b>Figure 4:</b> Chemical structures of main classes of flavonoids. This figure was adapted (Nebo <i>et al.</i> , 2014) .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 12 |
| <b>Figure 5:</b> Mechanism actions of flavonoids as antioxidants. This figure was adapted from (Katz <i>et al.</i> , 2011) .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 13 |
| <b>Figure 6:</b> Diseases Associated with Oxidative .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 15 |
| <b>Figure 7:</b> A summarized flow chart illustrating the Quantification and Qualitative analysis of bioactive active compounds extracted from edible wild fruits .....                                                                                                                                                                                                                                                                                                                                                                                                                                           | 27 |
| <b>Figure 8:</b> Illustrates concentration of total flavonoid content per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of <i>Grewia bicolor</i> , <i>Grewia cana</i> Sond, <i>Vangueria infausta subsp. Infausta</i> (VI), <i>Ximenia caffara</i> S, <i>Mimusops zeyheri</i> , <i>Ximenia americana</i> L, <i>Grewia flava</i> , and standards-based on Quercetin acid equivalent. Bar graphs represent mean $\pm$ standard error (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) amongst the samples within each solvent..... | 30 |
| <b>Figure 9:</b> Illustrates concentration of total phenolic content per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of <i>Grewia bicolor</i> , <i>Grewia cana</i> Sond, <i>Vangueria infausta subsp. Infausta</i> (VI), <i>Ximenia caffara</i> S, <i>Mimusops zeyheri</i> , <i>Ximenia americana</i> L, <i>Grewia flava</i> , and standards based on Gallic acid equivalent. Bar graphs represent mean $\pm$ standard error (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) in the same solvent .....                        | 32 |
| <b>Figure 10:</b> scavenging activity for ABTS per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of <i>Grewia bicolor</i> , <i>Grewia cana</i> Sond, <i>Vangueria infausta subsp. Infausta</i> (VI), <i>Ximenia caffara</i> S, <i>Mimusops zeyheri</i> , <i>Ximenia americana</i> L, <i>Grewia flava</i> , and standards based on Ascorbic acid equivalent. Bar graphs represent mean $\pm$ standard error (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) amongst the samples within each solvent.....                         | 34 |
| <b>Figure 11:</b> Illustrates the scavenging activity for DPPH per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of <i>Grewia bicolor</i> , <i>Grewia cana</i> Sond, <i>Vangueria infausta subsp. Infausta</i> (VI), <i>Ximenia caffara</i> S, <i>Mimusops zeyheri</i> , <i>Ximenia Americana</i> L, <i>Grewia flava</i> , and standards based on Gallic acid equivalent. Bar graphs represent mean $\pm$ standard errors (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) within each solvent.....                              | 36 |

|                                                                                                                                                                                                      |    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure 12:</b> Heat map of Pearson correlation for seven distilled water extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH.....                     | 39 |
| <b>Figure 13:</b> Heat map of Pearson correlation for seven ethanol extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH.....                             | 39 |
| <b>Figure 14:</b> Heat map of Pearson correlation for seven methanol extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH.....                            | 40 |
| <b>Figure 15:</b> Heat map of Pearson correlation for three methanol extracts with respect to antimicrobial activity versus TPC and TFC.....                                                         | 40 |
| <b>Auxiliary Figure 16:</b> Calibration curve of standard Quercetin for determination of TFC.....                                                                                                    | 71 |
| <b>Auxiliary Figure 17:</b> Gallic acid standard calibration curve for quantification of Total Phenolic Content.....                                                                                 | 72 |
| <b>Auxiliary Figure 18:</b> Ascorbic acid standard calibration curve for quantification of ABTS.....                                                                                                 | 73 |
| <b>Auxiliary Figure 19:</b> Quercetin acid standard calibration curve for quantification of DPPH ....                                                                                                | 73 |
| <b>Auxiliary Figure 20:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>S. aureus</i> bacteria (Trial #1).....                 | 89 |
| <b>Auxiliary Figure 21:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>S. aureus</i> bacteria (Trial #2).....                 | 90 |
| <b>Auxiliary Figure 22:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>S. aureus</i> bacteria (Trial #3).....                 | 90 |
| <b>Auxiliary Figure 23:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>S. aureus</i> bacteria (Trial #1).....                 | 91 |
| <b>Auxiliary Figure 24:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>S. aureus</i> bacteria (Trial #2).....                 | 91 |
| <b>Auxiliary Figure 25:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>S. aureus</i> bacteria (Trial #3).....                 | 92 |
| <b>Auxiliary Figure 26:</b> Auxillary Figure Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia americana</i> L for <i>S. aurus</i> bacteria (Trial #1).. | 92 |
| <b>Auxiliary Figure 27:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia americana</i> L for <i>S. aureus</i> bacteria (Trial #2) .....             | 93 |
| <b>Auxiliary Figure 28:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia Americana</i> L for <i>S. aureus</i> bacteria (Trial #3).....              | 93 |
| <b>Antimicrobial Figure 29:</b> activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>E. coli</i> bacteria (Trial #1) .....                            | 94 |
| <b>Auxiliary Figure 30:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>E. coli</i> bacteria (Trial #2) .....                  | 94 |

|                                                                                                                                                                                        |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Auxiliary Figure 31:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia caffra</i> S for <i>E. coli</i> bacteria (Trial #3) .....    | 95 |
| <b>Auxiliary Figure 32:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia americana</i> L for <i>E. coli</i> bacteria (Trial #1) ..... | 95 |
| <b>Auxiliary Figure 33:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia americana</i> L for <i>E. coli</i> bacteria (Trial #2) ..... | 96 |
| <b>Auxiliary Figure 34:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Ximenia americana</i> L for <i>E. coli</i> bacteria (Trial #3) ..... | 96 |
| <b>Auxiliary Figure 35:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>E. coli</i> bacteria (Trial #1) .....    | 97 |
| <b>Auxiliary Figure 36:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>E. coli</i> bacteria (Trial #2) .....    | 97 |
| <b>Auxiliary Figure 37:</b> Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of <i>Mimusops zeyheri</i> for <i>E. coli</i> bacteria (Trial #3) .....    | 98 |

## LIST OF TABLES

|                                                                                                                                               |    |
|-----------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Table 1:</b> Phytochemicals present in seven edible wild fruits: Qualitative Analysis .....                                                | 28 |
| <b>Table 2:</b> Antibacterial activity of methanolic extracts of <i>X. Americana L</i> , <i>X. caffra S</i> and <i>M. zeyheri</i> .....       | 37 |
| <b>Table 3:</b> Minimum Inhibitory Concentration for methanolic extracts of <i>X.americana L</i> , <i>X.caffra</i> and <i>M.zeyheri</i> ..... | 38 |
| <b>Auxiliary Table 4:</b> Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water).....                        | 73 |
| <b>Auxiliary Table 5:</b> Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol) .....                              | 74 |
| <b>Auxiliary Table 6:</b> Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol).....                                | 75 |
| <b>Auxiliary Table 7:</b> Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water).....                        | 76 |
| <b>Auxiliary Table 8:</b> Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol) .....                               | 77 |
| <b>Auxiliary Table 9:</b> Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol) .....                              | 78 |
| <b>Auxiliary Table 10:</b> Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol) .....                               | 79 |
| <b>Auxiliary Table 11:</b> Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol) .....                              | 80 |
| <b>Auxiliary Table 12:</b> Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water).....                        | 81 |
| <b>Auxiliary Table 13:</b> Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol) .....                               | 82 |
| <b>Auxiliary Table 14:</b> Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol) .....                              | 83 |
| <b>Auxiliary Table 15:</b> Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water).....                        | 84 |
| <b>Auxiliary Table 16:</b> Total Phenolic and Flavonoid Content of the wild edible fruits examined per 10 mg dry sample.....                  | 85 |
| <b>Auxiliary Table 17:</b> fruit extracts' DPPH radical scavenging activity compared to that of ABTS per 10 mg of dry sample .....            | 87 |

**Auxiliary Table 18:** below shows the zone of inhibition from the selected plate of Bacterial strains (*S. aureus* and *E. coli*). A= (*X. caffra* S for *S.aureus* bacteria), B=( *X. caffra* S for *E. coli*), C=(Gentamici positive control for *E. coli*), D= (Negative control for *S.aureus*), E= (*X. americana* L for *E. coli* bacterial strain), F=(*Mimusops zeyheri* for *S. aureus* bacterial strain), G= (Gentamicin positive control for *E. coli*), H= (*Ximenia americana* L for *E. coli* bacterial strain), I= (*X. americana* L for *S. aureus* bacterial strain), J= (*X. caffra* S for *S.aureus* bacteria), J= (*Mimusops zeyheri* *E. coli* bacteria), K= (Negative Control for *S.aureus* bacteria)..... 99

## **LIST OF ABBREVIATIONS**

AAE - Ascorbic acid equivalent

ABTS - 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

ANOVA - Analysis of variance

ATP- Adenosine triphosphate

DMSO – Dimethyl sulfoxide

DPPH - 2,2, -diphenyl-1-picrylhydrazyl

DW - Dry Weight

GAE - Gallic acid equivalent

HAT- Hydrogen atom transfer

LOX - Lipoxygenase

MDA- Malondialdehyde

MIC- Minimum inhibitory concentration

MHA - Mueller-Hinton agar

MHB - Mueller-Hinton broth

NADPH- Nicotinamide adenine dinucleotide phosphate hydrogen

NO- Nitric oxide

O<sub>2</sub>·- Superoxide radicals

OD- Optical density

OH·- Hydroxyl radical

PrB - Polyphenol-rich extract

PRx - Peroxiredoxin

QAE - Quercetin acid equivalent

SOD- Superoxide dismutase

TFC - Total flavanoid content

TPC - Total phenolic content

RNS - Reactive nitrogen species

ROS - Reactive oxygen species

YPD - Yeast extract peptone dextrose

ZOI- Zone of inhibition

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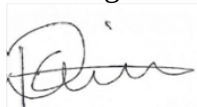
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I, the Candidate's **Primary Supervisor**, hereby confirm that I have inspected the above titled thesis and, to the best of my knowledge, it is based on the original work of the candidate.

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## **DEDICATION**

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To the aspiring young scientists and academics who are working nonstop to find natural compounds from environmental assets in the hopes of curing infectious illnesses. I'm always inspire

## ABSTRACT

In recent years, accumulating studies on traditional edible plants species have shown potential Pharmaceutical and health benefits including antioxidant, anticancer, anti-aging, anti-atherosclerosis, antimicrobial and anti-inflammatory activities. There are limited studies on traditional edible plant species of Botswana. In this study, seven edible wild fruits (*Vangueria infausta*, *Ximenia americana*, *Ximenia caffra*, *Grewia flava*, *Gweria cana* *Grewia bicolor* and *Mimusops zeyheri*) were extracted with ethanol, distilled water and methanol and the pulp extract were investigated for their phytochemical properties, antioxidant and antimicrobial potential. *Ximenia spp.* showed the presence of phenols, saponins, terpenoids and tannins, while *Grewia spp.*, *Mimusops zeyheri* and *Vangueria infausta* showed the presence of phenols, saponins and terpenoids as screening by qualitative method. Methanolic extract of *Ximenia. Caffra* showed significantly higher total flavanoid content (TFC) and total phenolic content (TPC) with  $38.25 \pm 1.00$  quercetin acid equivalent (QAE)/10 mg Dry Weight (DW) and  $233.78 \pm 21.07$  gallic acid equivalent (GAE)/10 mg Dry Weight respectively whereas lowest was recorded in *V. infausta* with  $2.08 \pm 0.29$  QAE/10 mg DW and *G. flava* with and  $4.25 \pm 0.83$  GAE/10 mg DW respectively. Higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) activity was observed in methanolic extract of *X. caffra* ( $91.44 \pm 44$  QAE/10 mg DW and  $67.16 \pm 0.95$  AAE/10 mg DW respectively) whereas the lowest was recorded in ethanolic extract of *G. flava* and distilled water extract of *G. bicolor* ( $13.40 \pm 0.86$  QAE/10 mg DW and  $2.78 \pm 0.50$  AAE/10mg DW respectively). For antimicrobial activity, *X. americana*, *X. caffra* and *M. zeyheri* extracts displayed antibacterial action against *Staphylococcus aureus* and *Escherichia coli* when tested using the well diffusion method and minimum inhibitory concentration respectively whereas no antifungal activity was recorded for the three methanolic extracts. Pearson correlations showed strong positive correlation for DPPH versus TFC, TPC and ABTS versus TFC, TPC, whereas TPC/TFC versus minimum concentration inhibition and zone of inhibition were strongly positive and negative respectively. The findings show the potential use of these fruit pulps for the development of functional ingredients with medicinal benefits.

## CHAPTER ONE INTRODUCTION

### 1.1 Background

Reactive oxygen species (ROS) are generated from cellular oxygen metabolism as well as exogenous sources such as tobacco smoke, pollutants, ultraviolet radiation, and ionization radiation (Adom *et al.*, 2005; Hancock *et al.*, 2001). Free radicals tend to exhibit considerable reactivity when there are unpaired electrons present and if not adequately regulated, they can cause damage to cell membranes, cellular proteins, lipids, and Deoxyribonucleic acid (DNA) (Young and Woodside., 2001; Halliwell., 1996). Oxidative stress occurs when cells are subjected to excessive levels of these unstable ROS. Several studies have described the relationship between increased cellular ROS and the pathogenesis of chronic diseases including cancer, inflammation, cardiovascular diseases, and age-related macular degeneration (Leavy., 2014; Nogueira and Hay., 2013; Sugamura and Keaney., 2011). The process of oxidation in cells is regulated by antioxidants, which delay or prevent cellular oxidation. Antioxidant protection is normally achieved through a balance between pro-oxidants and endogenous and/or dietary antioxidants (Sobha and Andallu., 2013; Hancock *et al.*, 2001). Pathogenesis is most prominent when there is antioxidant depletion, an inadequate supply of dietary antioxidants, and/or faulty antioxidants in cells, which compromise the body's ability to regulate cellular oxidation. Some of the dietary nutrients with significant antioxidant potential are phytochemicals from fruits, vegetables, and cereal grains (Krinsky and Johnson., 2005; Tapiero *et al.*, 2004). The suppression of cell proliferation by phytochemicals found in wild fruits has been linked to a lower risk of cancer, cardiovascular disease, inflammation, cataracts, and macular degeneration. (Sobha and Andallu., 2013; Adom *et al.*, 2005). Wild fruits are wild plants and are often exotic, underutilized, or less known. Many wild fruits are safe to consume, and some have been developed as medicine (Motlhanka *et al.*, 2008). Due to different genotypes and environmental concerns, wild fruits contain rich phytochemicals such as polyphenols, anthocyanin and flavonoids that are known for their antioxidant and antimicrobial activities, hence their role in the management and prevention of diseases associated with oxidative stress (Atawodi *et al.*, 2009; Kyung *et al.*, 2007; Lin *et al.*, 2005). In recent years, studies on wild edible fruits species have been increasing in the world, however, in Botswana there are limited studies on antioxidants and antimicrobials of edible wild fruits species. Therefore, this study is aimed at

screening selected edible fruits of Botswana for their antioxidant and antimicrobial potential. In addition to providing empirical data on Botswana's wild fruits and their health benefits, the study will contribute to the existing body of knowledge.

## **1.2 Problem Statement**

In the past, Botswana, like most African countries, relied heavily on edible wild fruit species to treat diseases and illnesses. In the 21<sup>st</sup> century, people increasingly turn to technology and biotechnology for medicines, with the majority relying on pharmacies and hospitals to obtain their medications. Moreover, there has been inadequate scientific research done on edible wild fruits in terms of isolating and characterizing their bioactive compounds. As public concerns grow about the safety of artificial dietary supplements, it becomes imperative to examine organic alternatives to synthetic antioxidants, such as plant-derived antioxidants and antimicrobials.

The goal of this project was to screen edible wild fruits for their antioxidant and antimicrobial potential, a prospective nutraceutical that has a significant tie to Botswana. The findings will help researchers better understand how indigenous fruits can be used in the management of oxidative stress-related chronic illnesses; this endorses the development and commercialization of indigenous plants as nutraceuticals, which will have a significant influence on Botswana's Agriculture and Economy sectors.

## **1.3 Rationale**

The increasing body of evidence suggests that edible wild fruits can help to protect against oxidative stress. The protective role of wild edible fruits is associated with high levels of phytochemicals and antioxidants. Despite agricultural advancement and people heavy reliance on crop plants, the habit of consuming wild fruits has not entirely disappeared. In fact, numerous studies have documented its nutritional and health benefits. In Botswana, wild edible plant species play a distinctive, biologically diverse, and extensive role. A close relationship exists between the phytochemical composition, antimicrobial activity of edible wild fruits and their efficacy in treating oxidative stress-related diseases. It also aimed to reduce dependency on synthetic antioxidants by demonstrating that edible wild fruits have antioxidant potential as well as their importance in diets. Edible wild fruits were selected based on their ability to scavenge free radicals in our bodies based on traditional uses, beliefs, and literature review.

#### **1.4 Hypotheses**

Edible wild fruits of Botswana are a good source of secondary metabolites and can act as antioxidant and antimicrobial agents in managing oxidative stress.

#### **1.5 Objectives of the study**

##### **1.5.1 General objective**

To screen seven edible wild fruits from the Tswapong region for their antioxidant and antimicrobial potential.

##### **1.5.2 Specific objectives:**

- To extract crude secondary metabolites from the pulp of the seven edible fruits
- To qualitatively determine phytochemicals of the pulp extracts
- To quantify total phenolic content and total flavonoid content of the pulp extracts
- To examine biochemical antioxidant activities of the pulp extracts using DPPH and ABTS assays
- To examine antimicrobial activity of the pulp extracts

## CHAPTER TWO LITERATURE REVIEW

### 2.1 Edible Wild fruits of Botswana

In Botswana, hundreds of native fruit tree species exist, many of which are important locally despite being obscure globally. These trees are used primarily for food and medicine, but also for fuel, craft, or monetary items, and many wild fruits harvested from the wild in Botswana, include *Sclerocarya birrea*, *Ziziphus mucronata*, *Vangueria infausta*, *Mimosopos zeyheri*, *Adenosoni adigitata*, *Ximenia spp.* and *Grewia spp.* that can support nutritional needs within rural communities. It is estimated that low crop yield occurs every 3 years on average in Botswana, emphasizing how vital edible wild fruits are for the survival of Botswana people. Projects for domesticating these fruits could have a profound impact on local people's livelihoods and nutrition (Badimo *et al.*, 2015; Motlhanka *et al.*, 2008; Amarteifio and Mosase., 2006; Mojeremane and Tshwenyane., 2004; Rossiter *et al.*, 1997).

However, despite the worsening global food situation, it is worth highlighting that indigenous people in African countries like Botswana live off wild fruit (Badimo *et al.*, 2015; Marumo *et al.*, 2009).

#### 2.1.1 *Vangueria infausta*

*Vangueria infausta subsp. infausta* (VI) known as wild medlar or African medlar in English and mmilo in Setswana is a fruit whose plant parts are prized for their medicinal effects in numerous cultures, and it's mostly found in Madagascar, Tanzania, Kenya, Botswana, and Southern and Central Mozambique. (Maroyi., 2018; Chiau *et al.*, 2013). It is a shrub to small plant that grows primarily on rocky slopes in Botswana. The fruit has a substantial vitamin C content and is usually consumed when ripe and the root extracts, leaves, fruits, seeds, and barks are mostly used to treat malaria, pneumonia, parasitic worms, and chest pains (Setshogo and Fenter., 2003). Phytochemicals that have been identified from this plant include alkaloids, anthraquinones, coumarins, glycosides, polyphenols, saponins, secoiridoids, steroids, tannins, and terpenoids (Maroyi., 2018; Ketlhoilwe and Jeremiah., 2016; Mbukwa *et al.*, 2007). Researchers have examined *V. infausta* leaves methanol extract for its antioxidant activity and it was concluded that the extracts exhibited hepatoprotective and pancreatic protective properties (Sanad., 2020).

### **2.1.2 *Ximenia americana* L and *Ximenia caffara* Sond**

*Ximenia americana* and *Ximenia caffara* also known as sour plum in English or Moretlologa wa pudi and moretlologa wa kgomo respectively in Setswana from the family Olacaceae. These are two distinct tree species native to Botswana as well as other regions of Africa. The size difference distinguishes them from each other. The roots, fruits, barks, and leaves are used to treat diseases like malaria, intestinal worms, stomach aches, bilharzia, fever, leprosy, chest pains, headaches, coughs, an antidote to poison, infertility, and inflamed joints. The species blooms in November. The oil collected from the seeds has been scientifically proven to have an anti-proliferative effect on human cells (Caco-2) and human embryonic kidney (HEK-293) (Neelo *et al.*, 2015; Majinda and Motswaledi., 1998). A report by (Mølgaard *et al.*, 2001) highlighted that some countries, including Ethiopia, Kenya, Malawi, Mozambique, Namibia, Somalia, Tanzania, South Africa, and Zimbabwe, commonly use *X. caffra* to treat parasites like tapeworms, ectoparasites, and schistosomes.

### **2.1.3 *Grewia* Genus**

*Grewia* genus is a large angiosperm family with around 400 flowering plant species, trees, and shrubs found across the sub-tropical and coastal areas. As outlined in other publications, it was noted that *Grewia avellana* Heirn (Marago-a-bahumagadi in Setswana and Velvet bushwillow in English), *Grewia schinzii* K.Schum (Mokgomphate in Setswana and Rough leaved in English), *Grewia bicolor* (mogwana in Setswana and bastard brandy bush in English), *Grewia flava* (moretlwa/moseme in Setswana and velvet raisin in English) and *Grewia asiatica* L (phalsa in English), as distinct species based on the form of the leaf shape, arrangement of tertiary veins and quantity of flower stalks (Lamola *et al.*, 2017; Badimo *et al.*, 2015; Bartsch., 2000). Local people harvest different parts of these plants for medicinal purposes. Commercially accessible goods manufactured from these plants can be found in health food stores and businesses that sell organic products from the wild. These plant's fruits and barks are often used to cure a variety of ailments which include skin illness, hypertension, ulcers, and diarrhea, as well as for food security (Lamola., 2015; Lamola *et al.*, 2017; Ullah *et al.*, 2012). Phytochemicals that have been identified during qualitative phytochemical screening include alkaloids, flavonoids, saponins, tannins, anthraquinones, and reducing sugars (Gololo *et al.*, 2016; Lamola *et al.*, 2017). A chemical analysis of *G. asiatica*'s acetone extract from Gwalior India revealed that its antifungal and antioxidant properties are strong as indicated by (Goswami *et al.*, 2018).

#### **2.1.4 *Mimusops zeyheri***

Known as mompudu in Setswana and red milkwood in English, it is a midsized tree that grows up to 15 m tall. It has thick leathery leaves that are glossy dark green on top and lighter green on the bottom (Daniel and Shimane., 2011). It is a plant that may be found in most of Southern Africa and Tanzania. The pulp from the fruit may be made into a dry pulp known as "sesema." It is also fermented in some regions to make native fruit liquor. Conventional healers mostly utilize the plant's roots and leaves for treating wounds, candidiasis, gonorrhea, inflammation, bleeding gums, tuberculosis, and ulcers (Omotayo *et al.*, 2020; Semenya *et al.*, 2012).

### **2.2 Antioxidants**

Antioxidants are described as any chemicals that significantly protect the body against the toxic effects of free radicals (**Fig. 1**) (Antolovich *et al.*, 2002). The presence of antioxidant compounds, particularly those with a phenolic structure, is believed to play a very imperative role in preventing oxidative stress in the body (Pisoschi *et al.*, 2021). Different antioxidants act at different levels: they may prevent the initiation of chain reactions by removing free radicals (preventative antioxidants), they may scavenge free radicals generated in chain reactions, thereby interrupting the chain sequence (chain breaking) and they may remove peroxides, thereby preventing the further generation of ROS. They further help to prevent detrimental effects brought about by oxidants which can either be enzymatic or non-enzymatic driven (Birben *et al.*, 2012; Cao *et al.*, 2004).

#### **2.2.1 Edible Wild fruits-antioxidant (*in vitro* and *in vivo* antioxidant potential)**

Numerous experiments, particularly on animals and cell lines, have been conducted to assess the antioxidants activity of edible wild fruits. A study carried out by (Masisi *et al.*, 2021) demonstrated that ethanol *Grewia flava* extracts displayed cytoprotective qualities against H<sub>2</sub>O<sub>2</sub> induced HeLa cells. The MTT test was used to assess the cytotoxic effects of water and acetone extracts of *Grewia flava* leaves on Vero monkey kidney cell lines. It was concluded that the extracts of *Grewia flava* were less harmful to Vero cells as compared to the positive control doxorubicin. The edible wild fruit *Grewia flava* proved to be non-toxic, and the chemicals in the fruits are effective against intestinal infections (Lamola., 2015). It is noteworthy that (Mongalo *et al.*, 2017) also observed that the 15 acetone extracts of selected medicinal plants under investigation were shown to have significant antimicrobial action against the chosen microbes

and were less toxic to Vero kidney monkey cells. According to *in vitro* and animal investigations on nutraceutical properties of *Vangueria infausta subsp. infausta* (VI) carried out by (Maroyi., 2018), it was established that the fruits have antibacterial, anti-inflammatory, and antioxidant properties. In accordance with (Taneva *et al.*, 2016), rosehip (*Rosa canina L.*) fruit extracts were analyzed, and their antioxidant activity and polyphenol content were assessed *in vitro*. The findings indicated that the examined extracts might be used as a natural source of antioxidant chemicals that have therapeutic effects.

The impact of polyphenol-rich extract (PrB) from *Vaccinium angustifolium* (wild blueberries) was evaluated on adult mice (3-4 months old) whose brains were induced with oxidative stress. The brain supernatant was evaluated for its antioxidant potential using the FRAP test as well as amounts of ascorbic acid, malondialdehyde, and reduced glutathione present. It was concluded that PrB treatment reduced MDA levels in the brain while increasing ascorbate levels. (Papandreou *et al.*, 2009).

The antioxidant activities of selected 7 wild edible fruits were investigated *in vitro* in this study using different methods which included DPPH, ABTS, TPC, TFC, and cell-based assay.

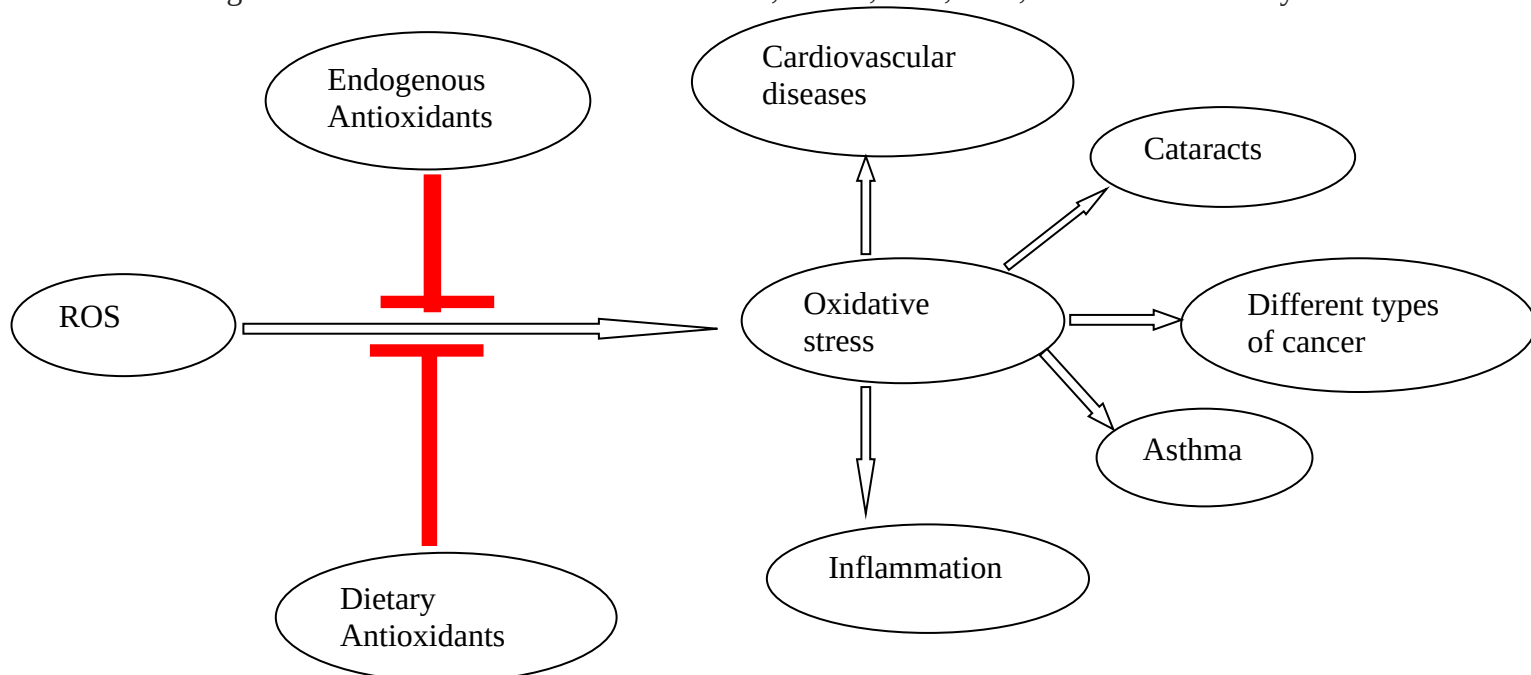


**Figure 1:** Relationship Between Oxidative Stress, Antioxidants and ROS

### 2.2.2 Evaluation of antioxidant Capacity

Antioxidant capacity is the overall ability of organisms or food to catch free radicals and prevent their harmful effects. Antioxidant activity is measured using three different types of assays: chemical/biochemical assay: (2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), Ferric reducing antioxidant power (FRAP), Hydrogen peroxide scavenging activity (Halvorsen *et al.*, 2002), animal model assay and cell-based assay (Leong and Shui., 2002). Animal models and human experiments provide the most accurate results; nevertheless, these are costly and require a lengthy time to perform and are not appropriate for the first antioxidant assessment of food extracts and supplements. The chemical assay is the most frequently used method because of its low cost and its simplicity (Moon and Shibamoto., 2009; Wolfe and Liu., 2007). Nevertheless, the results are dubious when it comes to estimating antioxidant capacity *in vivo* due to the use of non-physiological pH and temperature in the chemical experiment and the fact that the antioxidant compound's absorption and metabolism are excluded (Frankel and Meyer., 2000).

### 2.2.3 Chemical/biochemical based antioxidant activity assays

Several techniques have been developed for determining the antioxidant content of foods and chemicals. These assays use a variety of methods for measuring antioxidant activity, including single electron transfer (ET), metal chelating, reducing power, and hydrogen atom transfer (HAT). Each approach has its own set of benefits and disadvantages, therefore it's critical to assess antioxidant activity using a variety of methods for measuring antioxidant activity (Magalhães *et al.*, 2008; Pérez-Jiménez *et al.*, 2008; Aruoma., 2003). On a general basis, the tests mainly focus on assessing the ability to scavenge particular biological reactive oxygen species (ROS) or nitrogen-oxygen species (NOS) from stable non-biological radicals, as well as an assessment of overall reduction capacities and this, include 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), Ferric reducing antioxidant power (FRAP), Hydrogen peroxide scavenging activity (Burawat *et al.*, 2016; Shahidi and Zhong., 2015; She *et al.*, 2010; Koleva *et al.*, 2001).

### 2.2.4 Mechanism actions of phenolic acids as antioxidants

Phenolics and polyphenolics are presented by a very large group of natural products which include quinones, flavones, tannins, and flavonoids. Phytochemicals like phenolic acids have a

diverse range of powerful antioxidative effects and are prevalent in many widely consumed plant-based foods (Mattila and Kumpulainen., 2002). They are secondary metabolites present in almost all fungi and plants produced via shikimic acid and they are divided into two major groups namely: hydroxybenzoic acids and hydroxycinnamic acids (Khanam *et al.*, 2012). The configuration of phenolic compounds, particularly the benzene ring, the amount, and location of OH groups (**Fig. 2**), determines their capacity or effectiveness to act as antioxidants.

A phenolic compound's antioxidant action might be mediated by either a hydrogen atom transfer (HAT) action or single-electron transfer (SET) via proton transfer (**Fig. 3**) (Balasundram *et al.*, 2006) and electron transfer in a series of proton losses (Lee *et al.*, 2020). The hydrogen atom from the phenolic antioxidant by HAT mechanism can be added to the free radicals thus making them be more stable (Zeb., 2020). The efficacy of a powerful antioxidant by single-electron transfer mechanism is determined by transferring one electron to decrease the oxidative rate on a molecule containing carbon, radicals, and oxygen. A phenolic compound donates an electron to a free radical and forms an anion, which then transfers an electron to create a stable molecule via the sequential proton loss electron transfer (SPLET) process (Ojha *et al.*, 2018).

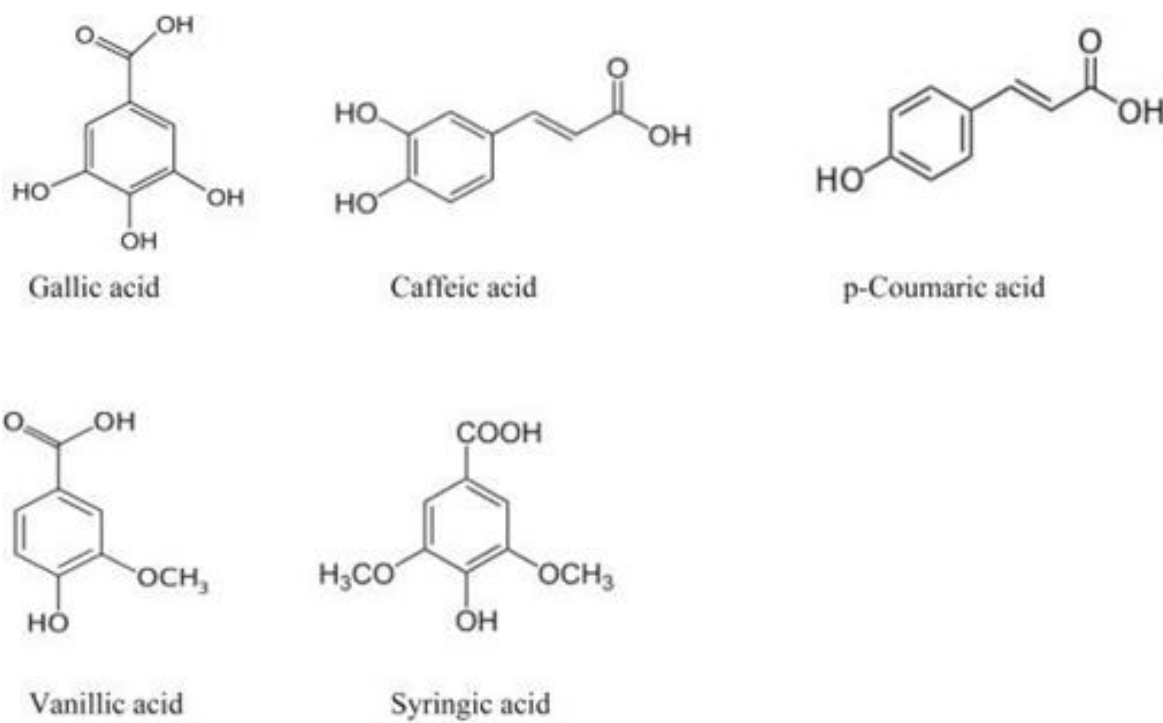


**Figure 2:** An overview of phenolic acids' chemical structure. This figure was adopted from (Al Harthi *et al.*, 2015)

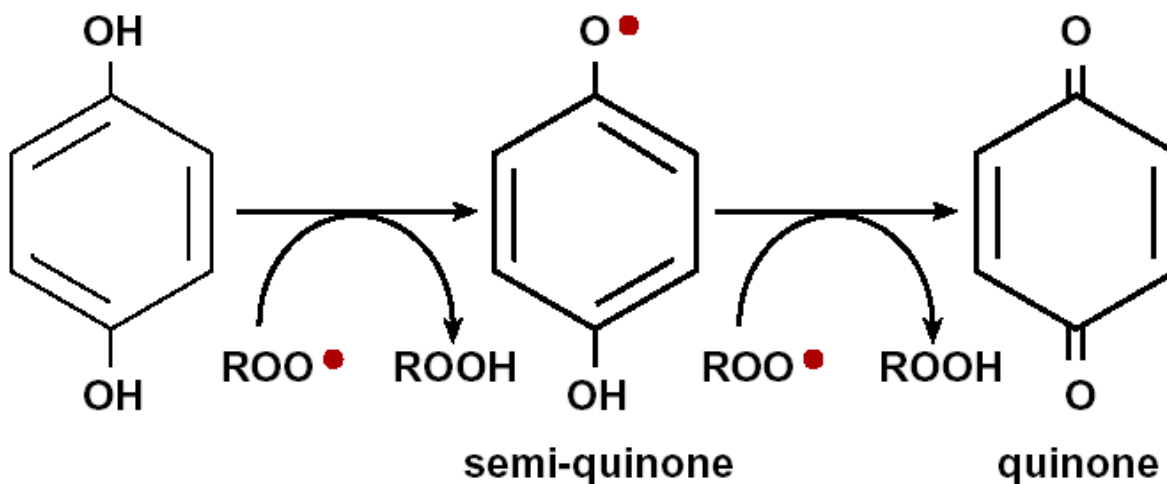


**Figure 3:** Mechanism actions of phenolic acids as antioxidants. This figure was adopted from (Tadapaneni., 2010)

### 2.2.5 Mechanism actions of flavonoids as antioxidants

Flavonoids are polyphenolic secondary metabolites widely dispersed throughout the plant kingdom and originate in substantial levels in commonly consumed fruits, vegetables, wines, grains, and beverages (Panche *et al.*, 2016; Manach and Donovan., 2004). The flavonoid family is divided into several sub-groupings; the six main classes are flavonols, flavones, flavan-3-ols, isoflavones, flavanones, and anthocyanidins (**Fig. 4**) (Kurutas., 2015). Flavonoid antioxidant activity is determined by their chemical structure, which is based on the phenol hydroxyl, and the ability to stabilize the resultant phenoxyl radicals by hydrogen bonding or prolonged hydrogen delocalization (**Fig. 5**). The antioxidant activity of functional hydroxyl groups in antioxidants is mediated by their capacity to bind metal ions and scavenge free radicals. Metal chelation might be critical in preventing the formation of radicals that could harm target biomolecules. (Chen *et al.*, 2014).

Apart from their significant antioxidant activity, flavonoids can activate a variety of antioxidants and defensive genes through nuclear transcription factors and blocking biochemical pathways of inflammatory agents (Salaritabar *et al.*, 2017). Several flavonoids, including pigenin, luteolin, quercetin, genistein, and kaempferol, have been shown to inhibit the growth and production of vascular endothelial growth factor (VEGF) in ovarian cancer cells *in vitro* and to improve the defense mechanism against breast cancer and lung cancer, particularly in smokers. (Romagnolo and Selmin., 2012; Skowrya., 2014).

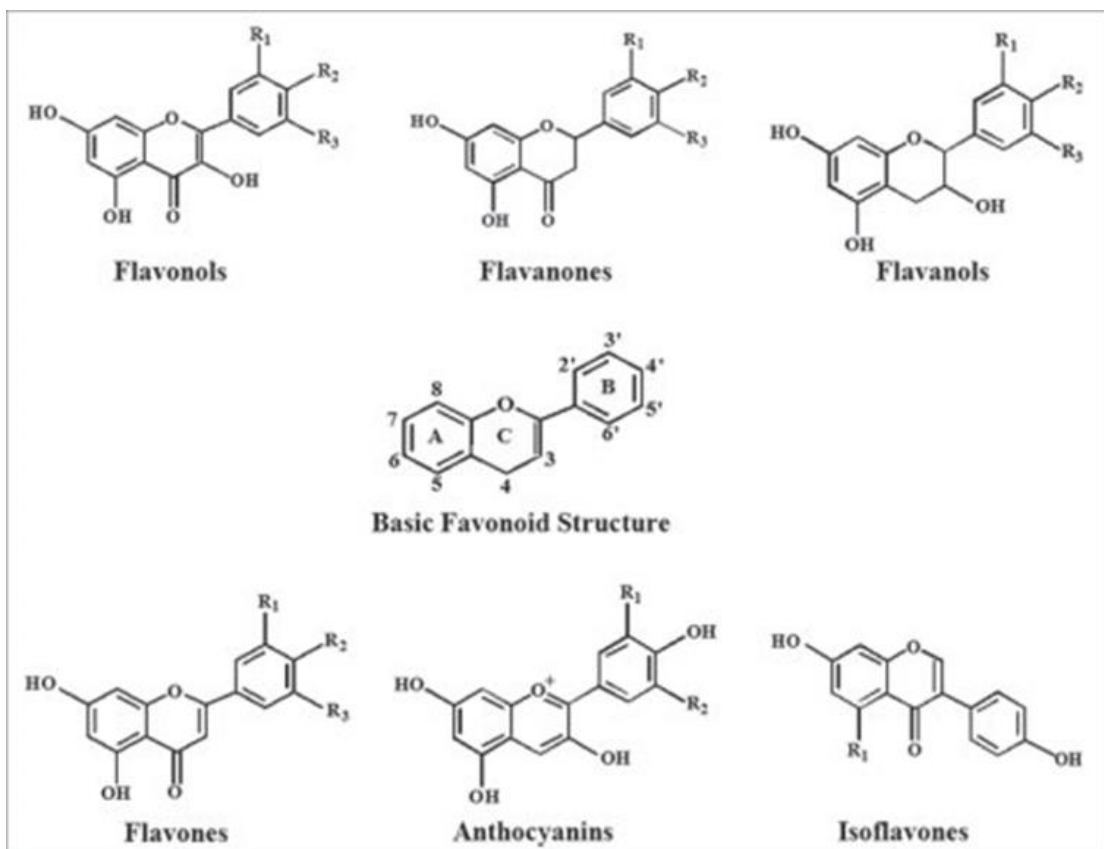


**Figure 4:** Chemical structures of main classes of flavonoids. This figure was adapted (Nebo *et al.*, 2014)

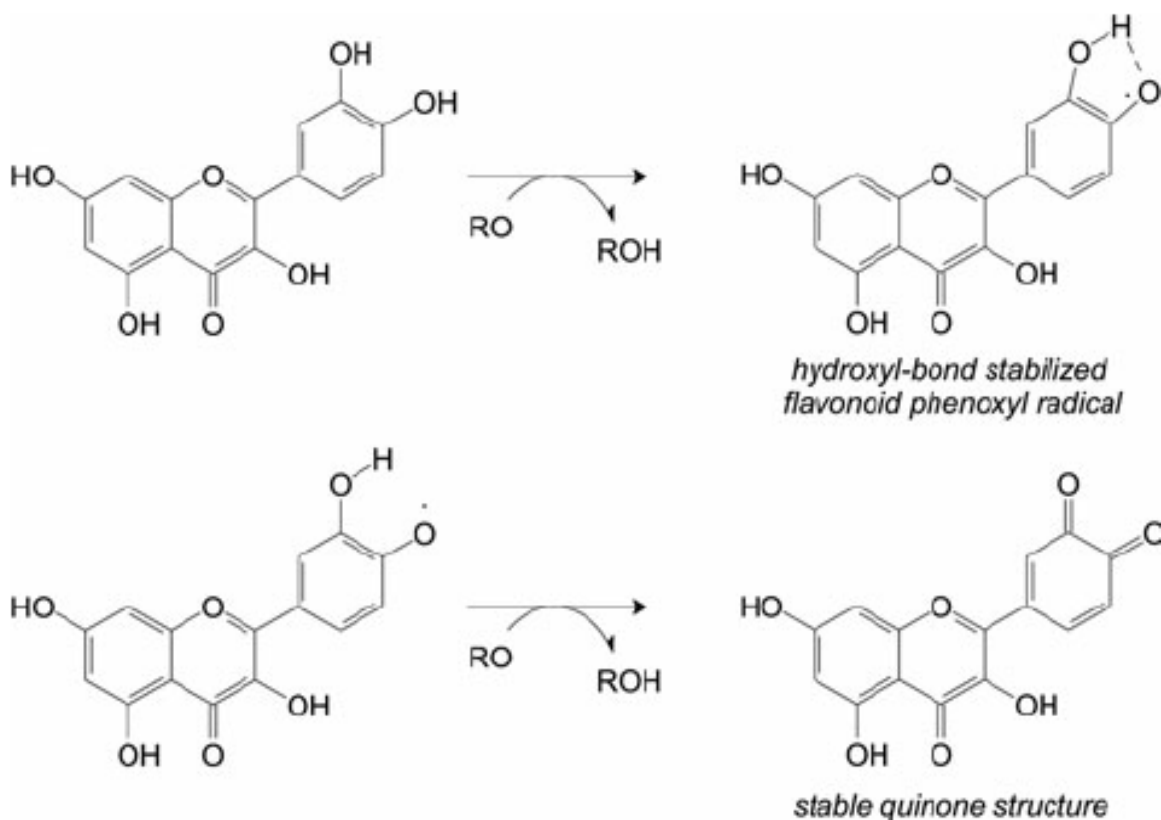


**Figure 5:** Mechanism actions of flavonoids as antioxidants. This figure was adapted from (Katz *et al.*, 2011)

### 2.2.6 Health benefits of antioxidants in humans

Phytochemicals present in fruits are thought to contribute to the health benefits of fruit, offering protection against disease and enhancing longevity. It has been shown, for instance, that high polyphenol intake reduces mortality rate in humans (Joseph *et al.*, 2016; Ivey *et al.*, 2015; Tresserra-Rimbau *et al.*, 2014). In addition, flavonoids intake is also linked to a lower risk of cardiovascular disease and dementia (Commenges *et al.*, 2000; Hertog *et al.*, 1993). Toxicological concerns about synthetic antioxidants have led to increasing popularity of natural antioxidants such as phenols and flavonoids. (Shahidi and Ambigaipalan., 2015). Eating fruits and vegetables may also reduce the dependency syndrome associated with refined foods.

## 2.3 Oxidative stress

Oxidative stress refers to a condition that occurs when free radicals are present in excess in the body, causing oxidative stress on lipids, membranes, proteins, and DNA. It can be measured as an increase in GSH/GSSG (glutathione/glutathione disulfide) in the plasma (Farías *et al.*, 2017; Jones., 2006). Oxidants and free radicals (mainly formed in the mitochondria) have sparked a huge interest in the field of biological science because of their fundamental role seen in physiological conditions as well as their inference on numerous wide ranges of diseases (Lushchak., 2014). Oxidative stress has been associated with diseases like diabetes, tumor (Al-Abrash *et al.*, 2000), Alzheimer's disease (Smith *et al.*, 2000), autism (James *et al.*, 2006), lung and kidney malfunctions, Parkinson's disease, cardiovascular diseases (Rahman *et al.*, 2012) various cancers (colorectal, prostate, breast, lung, bladder cancers) (Phaniendra *et al.*, 2015) (Fig. 6).

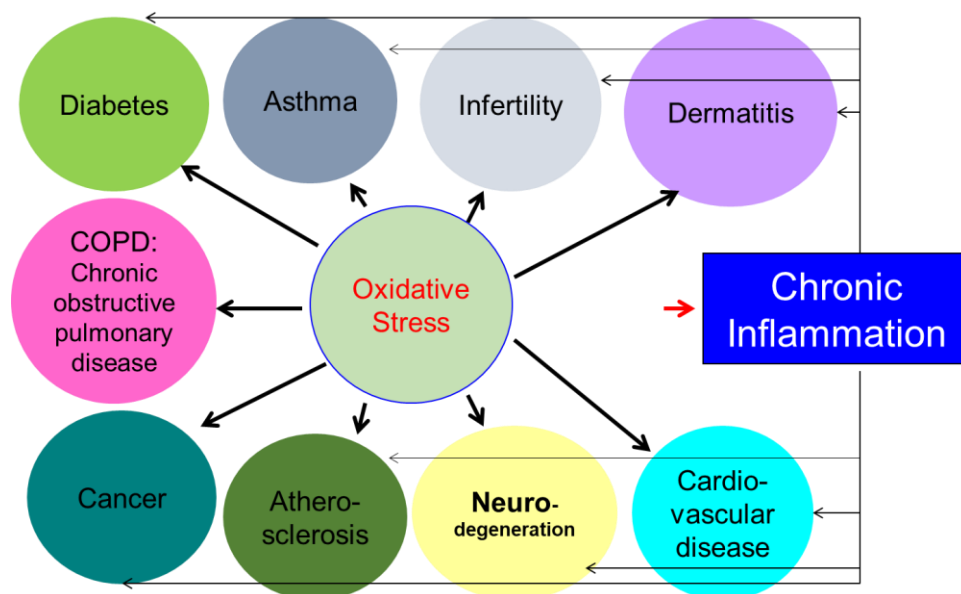


**Figure 6:** Diseases Associated with Oxidative

### 2.3.1 Reactive oxygen species and Reactive Nitrogen species

Free radicals from nitrogen and oxygen are quite volatile, and reactive (Poljšak and Dahmane., 2012). The main assumption that they damage chemical pathways indiscriminately and, thereby,

causing cell death. However, it has now been fully established that ROS may also function as signaling molecules for both physiological and pathologic conditions (Finkel., 1998). These benefits include activating transcription factors of a nucleus, gene expression, signal transduction, and preventing unfavorable aftermath of tumor cells. Furthermore, superoxide radicals ( $O_2^-$ ) responsible for cell development can also attack pathogens stimulating the response of biological inflammatory signals. The signaling molecule Nitric Oxide (NO.) responsible for organ and cellular function is essential for vasodilation and can help enhance the response of the immune system (El-Bahr., 2013; Fang *et al.*, 2002)

Free radicals can cause oxidative stress, a harmful process that can harm all cellular components when present in high doses making cells become free radicals themselves resulting in clinical symptoms (Phaniendra *et al.*, 2015; Fang *et al.*, 2002). They can either be endogenous or exogenous (Birben *et al.*, 2012). Additionally, endogenous oxidation can either be free radicals where a single electron is accepted/donated from other molecules and can exist on their own or non-radicals whereby they are produced from accepting two electrons and their interaction with transition metal can convert them to radical products (Abaturov *et al.*, 2014). Though non-radical species are not free radicals, they play a significant role in the production of free radicals in living organisms (Phaniendra *et al.*, 2015).

Exogenous ROS/RNS are mainly formed due to external environmental factors such as smoking, alcohol consumption, unhealthy diet, physical exercises, and drugs which in turn are metabolized into free radicals inside the body (Liguori *et al.*, 2018). The regular redox mechanism is greatly altered by free radicals that are generated from organic and non-organic molecules that have an unequal number of electrons thus making them extremely reactive and detrimental to vital biological molecules such as proteins, lipids, and nucleic acids required for the synthesis of Deoxyribonucleic acid (DNA). Consequently, this increases the level of oxidative stress in an organism and limits other systems of antioxidant enzyme activity such as catalase (CAT) and glutathione peroxide (GPx) (Phaniendra *et al.*, 2015; Sen *et al.*, 2010).

### **2.3.2 Oxidative stress on cellular component**

When cells are exposed to high levels of oxidative stress, they activate numerous biochemical events in a cell. These cellular events may include DNA damage, apoptosis, and increased proliferation finally leading to the death of most or all the cells. Moreover, the production of

hydroxyl radicals (HO•) or oxido-iron (IV) radicals induces the susceptibility of lysosomes to oxidative stress, and when in high concentrations this may lead to the rupture of lysosomes releasing harmful contents to the cytosol causing cellular damage and consequently apoptosis and necrosis (Kurz *et al.*, 2006). The DNA oxidative modifications are primarily generated by free radicals which are associated with risks of proliferation of cancer cells, disruption of normal synthesis of DNA, and inactivation of significant proteins needed for antioxidant activity (Kryston *et al.*, 2011; Risom, *et al.*, 2005).

## **2.4 Antioxidant analyses of edible wild fruits**

### **2.4.1 *In vitro* analysis of wild edible fruits**

Compounds and complex samples can be assessed for their antioxidant properties using various techniques. Most employed two chemical parameters that are used to evaluate the antioxidant potential of different wild edible fruits include electron transfer (ET) and hydrogen atom transfer (HAT). In the HAT method, researchers can test whether an antioxidant compound can scavenge ROS (e.g., peroxy radicals) by hydrogen donation. ORAC and TRAP assays are examples of HAT tests. Numerous research has been reported on the suppressive ability of different berries against cytochrome P450 (CYP isozymes). Raspberry inhibited CYP1A2, CYP2D6, and CYP3A4 *in vitro* to a little extent (Langhammer and Nilsen., 2014). Cranberry suppressed the activity of CYP2C9 and intestinal CYP3A4/5 *in vitro* but had no impact *in vivo*. (Ngo *et al.*, 2009; Ngo *et al.*, 2010).

A study by (Babu *et al.*, 2013) revealed that at doses of 2, 4, 6, 8, and 10 mg/mL, the *triphala* extract was able to inhibit the DPPH free radicals by 16.46, 29.27, 52.10, 70.24, and 84.35% respectively by means of hydrogen donating activity. Based on a study by (Uchôa *et al.*, 2016), it was verified that methanolic extract of *Ximenia Americana* L. stem bark at a concentration of 100 µg/mL indicated a very high DPPH radical scavenging activity surpassing percent inhibition of 90 %.

### **2.4.2 (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay)**

The DPPH technique is the most used test for determining plant extracts' ability to scavenge free radicals. The DPPH assay is a free stable radicle that measures the activity of antioxidants. The DPPH reacts with a hydrogen donor which is an antioxidant or reducing compound. It is formed

upon absorption of hydrogen from an antioxidant and the reduced molecular forms DPPH by indicating color changes from purple (strong absorption) to pale yellow depending on the number of electrons accepted. Therefore, the antioxidant concentration effect can easily be evaluated by following the decrease of ultraviolet absorption at 517 nm. Trolox is mostly used as a standard antioxidant (Kasote *et al.*, 2019; Skowrya., 2014). Since the radical molecules are stable and don't have to be produced in another experiment, the assay done with a methanol DPPH solution is regarded as reliable, simple, and precise for evaluating the antioxidants' radical scavenging ability (Kedare and Singh., 2011).

#### **2.4.3 ABTS radical scavenging assay**

The ABTS assay measures ABTS<sup>•+</sup> radical cation induced by metmyoglobin and hydrogen peroxide. This assay measures radical scavenging activity by electron donation and when combined with an antioxidant assay, it provides a comprehensive analysis of a test sample's antioxidant activity. Ferryl myoglobin is formed from myoglobin and hydrogen peroxide. The ferryl myoglobin radical can oxidize ABTS to generate radical cation ABTS<sup>•+</sup> that is green in color and can be measured by absorbance. Antioxidants suppress this reaction by electron radical scavenging activity and inhibiting the formation of colored ABTS radicals. The concentration of antioxidants in a sample is inversely proportional to ABTS<sup>•+</sup> radical formation and absorbance (Rajurkar and Hande., 2011; Zheleva-Dimitrova *et al.*, 2010).

In a study by (Adebiyi *et al.*, 2017), the results revealed that *Grewia carpinifolia* stem extracts were shown to have a 100% scavenging activity of the ABTS<sup>•+</sup> radical, which is comparable to the standard at the maximum concentration of 1 mg/mL. This demonstrates that *Grewia carpinifolia* extract has a strong capacity to neutralize the ABTS radicals.

#### **2.4.5 Hydrogen Peroxide Scavenging Activity**

Although hydrogen peroxide does not react with most physiologically essential compounds, it is an intracellular precursor of hydroxyl radicals, which are hugely harmful to cells (Goud *et al.*, 2008). Thus, the antioxidant capacity of the extracts can be measured by their ability to scavenge hydrogen peroxide. Research led by (Sivasankari *et al.*, 2013) showed all the *Ehretia laevis roxb* had the ability to scavenge hydrogen peroxide. The methanol extract had the best hydrogen peroxide scavenging efficacy of the four *Ehretia laevis roxb* extracts investigated (IC<sub>50</sub>: 14.860.28 g/mL). The scavenging activity of *Triphala* recorded an IC<sub>50</sub> of 16.63 ± 2.01 µg/m as

documented by (Babu *et al.*, 2013). This might be due to the existence of phenolic groups, which can transfer electrons to hydrogen peroxide, neutralizing it and converting it to water (Bhaskar and Balakrishnan., 2009; Umamaheswari and Chatterjee., 2008).

## **2.5 Antimicrobial activity assay (Agar well diffusion method and Minimum Inhibitory Concentration)**

Antibiotic resistance has increasingly become a problem in recent years due to the increase in infections and its prevalence. As a result of multidrug resistance and its consequences, this has made the use of natural alternative remedies more popular. Higher plants may yield antimicrobials with new mechanisms of action from natural products (Kavitha and Padma., 2011; Machado *et al.*, 2003; Austin *et al.*, 1999). While the mechanisms of antimicrobial activity of polyphenolic compounds have not yet been fully elucidated, some studies have found that this activity is associated with alterations in intracellular functions caused by the hydrogen bonding between phenolics and enzymes in cells. Microbes lose their intracellular constituents due to these mechanisms, thus causing cell wall integrity to be compromised hence inhibiting microbial growth instantly (Bouarab-Chibane *et al.*, 2019). For *In vitro* antimicrobial activity, an extract can be assessed using a variety of laboratory methods. Among the most popular and fundamental methods are disk-diffusion and broth/agar dilution. (Balouiri *et al.*, 2016). Antimicrobial agents are classified by their MIC as low concentrations that impede the noticeable growth of microbes after overnight incubation (Merkl *et al.*, 2010). A report by (Ogbonna *et al.*, 2003) stated that *X. Americana* has effective antibacterial effects against *Proteus vulgaris*, *Bacillus subtilis*, *E. coli*, and *Salmonella typhi*.

## CHAPTER THREE MATERIALS AND METHODS

### 3.1 Samples

Edible wild fruits including *Vangueria infausta subsp. Infausta* (VI), *Ximenia americana* L, *Ximenia caffara* S, *Grewia flava*, *Grewia cana* Sond, *Grewia bicolor* and *Mimusops zeyheri* were handpicked from the Tswapong area in the Central District of Botswana (22°39'55"S 27°13'12"E). The samples were hand separated to get the fleshy pulp for use throughout the study.

Edible wild fruits were selected based on the following criteria:

1. Plant species must be readily available in the research region for easy collection of samples for analysis.
2. In the research area, plant species should normally be frequently utilized to treat human diseases.
3. Phytochemical classes with known antioxidant activity should also be found in plant species belonging to families that have historically been demonstrated to possess them.

### 3.2 Chemicals

Sodium carbonate and Potassium Persulfate were purchased from Sigma-Aldrich Company (St. Louis, Missouri, United States), 2,2- Diphenyl-1- picrylhydrazyl (DPPH), Aluminium chloride, Ascorbic acid, absolute Ethanol, Methanol, Folin-Ciocalteu reagent, (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), reference standards (Gallic acid, Quercetin), Mueller-Hinton were purchased from Rochelle Chemicals & Lab Equipment (Johannesburg, 2180, South Africa) and distilled water. Chloroform, Mueller-Hinton agar, Mueller-Hinton broth, yeast extract peptone dextrose, DMSO, Sulfuric acid, and ferric chloride were purchased from Sigma-Aldrich (Burlington, Massachusetts, USA). Germanticin and Cycloheximide were purchased from Glentham Life Sciences Ltd (Corsham SN13 9SW United Kingdom).

### 3.3 Analytical Methods

#### 3.3.1 Sample preparation

The fleshy pulp from seven samples was frozen prior to freeze-drying using a Freeze Dryer (VirTis Benchtop 3.3 ES, Pennsylvania, United States of America). Dried samples were then ground into fine powder by using a coffee grinder, then a mortar and pestle were used to get an

even-sized particle. The fine powder was stored at -80°C before analysis in zip-lock bags to keep secondary metabolites from degrading. A summarized flow chart (**Fig. 7**) illustrates the quantification and qualitative analysis of bioactive active compounds extracted from edible wild fruits.

### **3.3.2 Extraction of secondary metabolites from edible wild fruits**

The crude extracts from the samples were extracted under low light intensity to prevent excessive light, which could alter the quality of the bioactive compounds. Appropriately 10 mg of each ground sample were immersed in various 100 mL solvents in 100 mL conical flasks (absolute methanol, absolute ethanol, and distilled water). Absolute methanol and ethanol extracts were then stored in the dark (cupboard) at room temperature for 24 hours with frequent agitation. For distilled water extracts, they were covered with aluminum foil and then stored in a 4°C cold room to prevent microbial contamination since water is a good medium for their survival, and most of the microbes don't survive at very low temperatures. The extracts were then filtered through Whatman No.3 filter paper, afterward, the filtrates were re-suspended with their respective solvents then kept in the dark at room temperature for 24 hours then filtered again in a clean beaker through Whatman No.3 filter paper and this was repeated 3 times to ensure maximum collection of bioactive compounds. Subsequently, the solvents from the crude extracts were evaporated using a rotary evaporator (Sanjing chemglass Jiangsu, China) then placed in the fume hood (Escoglobal, Changi South Sheet 1, Singapore) for 1 hour (to limit the chances of microbial growth in the extracts) to remove any solvent left behind from the rotary evaporator, also to obtain pure and concentrated bioactive compounds. The fume hood (Escoglobal, Changi South Sheet 1, Singapore) vertical sash was covered with aluminum foil to prevent light (due to bioactive compounds being light sensitive). Dried crude extracts were then collected in dark vials (to prevent bioactive degradation from light) then and stored at -80°C until needed for analysis. The biochemical test samples were reconstituted in absolute methanol with a concentration of 1 mg/mL.

### **3.4 Qualitative phytochemical screening of edible wild fruits**

Phytochemical screening in seven aqueous extracts were performed using standard procedures to identify the chemical compounds such as tannins, saponnin, terprnoids, and phenols as described by (Rajesh *et al.*, 2014) with modifications.

#### **3.4.1 Test for Tannins**

About 1 mL of each extract in a test tube was boiled in 20 mL of water and then filtered through Whatman No.3 filter paper. After adding 3 drops of 2% ferric chloride using a dropper, red or blue hues could be observed, indicating tannins were present. Measurements were carried out in triplicates for each experiment.

#### **3.4.2 Test for Saponin**

In a water bath, 5 mL of the extract was boiled in 20 mL of distilled water and then filtered through Whatman No.3 filter paper, followed by a mixture of 10 mL of the filtrate and 5 mL of distilled water, which was then vigorously shaken using vortex Stuart™ Scientific SA8 vortex mixer, Sigma-Aldrich, (Burlington, MA, United States) for a stable persistent foam. In addition, 3 drops of olive oil were added to the frothing and shaken vigorously, then the emulsion formed confirmed the presence of saponins. Measurements were carried out in triplicates for each experiment.

#### **3.4.3 Test for Terpenoids (Salkowski test)**

The terpenoids in the extract were detected by mixing 5 mL of each extract with 2 mL of chloroform and 3 mL of concentrated H<sub>2</sub>SO<sub>4</sub> as an interface layer. The reddish-brown coloration of the interface indicated the presence of terpenoids. Measurements were carried out in triplicates for each experiment.

#### **3.4.4 Test for Phenols**

To 1 mL of the crude extract, 2 mL of distilled water was added, thereafter 0.5 mL of 60 g/L Sodium Carbonate and 0.5 mL of 10× fold Folin Ciocalteu's reagent (dissolved in distilled water) were added next. The presence of phenols was indicated by the formation of a blue or green color. Measurements were carried out in triplicates for each experiment.

### **3.5 Quantification of main phytochemical classes**

#### **3.5.1 Total phenolic content quantification (Folin-Ciocalteu method)**

Total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu method (She *et al.*, 2010) with minor modifications. Appropriately, 200 µL of diluted extracts were separately mixed with 1.5 mL of freshly made 10-fold diluted Folin-Ciocalteu reagent (dissolved in distilled water). After 15 minutes of incubation in a dark at room temperature, the mixture was neutralized with freshly prepared 1.5 mL of 60 g/L Sodium Carbonate. The absorbance of the

mixture was measured at 725 nm with a Thermo Scientific™ SPECTRONIC™ 200 Spectrophotometer (Madison, United States of America) against a blank of distilled water after 90 minutes' reaction in the dark after observing the blue color of the samples. Gallic acid, a phenolic chemical (100, 300, 400, 500, 600, 800, 900, 1000 µg/mL) was used to prepare standard curve from a stock of concentration 1 mg/mL prepared in absolute methanol. Thereafter, 200 µL of adequately diluted Gallic acid was mixed with 1500 µL of freshly made 10- fold diluted Folin-Ciocalteu reagent, after 15 minutes, Sodium Carbonate (60 g/L) was added to the mixture to neutralize it. The solution was then properly mixed with a vortex Stuart™ Scientific SA8 vortex mixer, Sigma-Aldrich, (Burlington, MA, United States) for homogeneity then stored at room temperature in the dark for 90 minutes before recording the absorbance at 725 nm with a Thermo Scientific™ SPECTRONIC™ 200 Spectrophotometer (Madison, United States of America) against a blank of distilled water. The TPC was calculated with the formula:  $y = mx + c$ , where x= concentration of the sample, y= absorbance of sample, m and c were derived from the standard curve. The results were expressed as milligrams of Gallic Acid Equivalent (GAE) per milligram of Dry Weight of the sample (mg GAE/mg DW). All measurements were conducted in triplicates.

### **3.5.2 Total Flavonoid Content quantification**

The TFC was determined by the colorimetric technique for Aluminum chloride adapted from a protocol described by (Chlopicka *et al.*, 2012) with modifications. For the preparation of 10% Aluminum Chloride, 10 g of Aluminum Chloride was dissolved in 100 mL of distilled water in a 100 mL volumetric flask. For the preparation of the Quercetin acid standard curve, 3 mg of quercetin was mixed with 3 mL of absolute ethanol to prepare a stock solution of 1 mg/mL. The working dilutions were prepared over a concentration range in order to produce differently concentrated solutions (100, 200, 300, 400, 500, 600, 700, 800µg/mL). Separately, 3 mL of the diluted standard solutions were mixed with 3 mL of 10% Aluminum Chloride (1:1 ratio). The absorbance of the reaction mixtures was measured at 415 nm after 30 minutes of incubation period at room temperature against a blank of distilled water with a Thermo Scientific™ SPECTRONIC™ 200 Spectrophotometer (Madison, United States of America). Similarly, for estimation of the flavonoid content of crude extracts, the same procedure was followed. The TFC was calculated with the formula similar to that of TPC. The results were expressed in milligrams

of Quercetin Acid Equivalent (QAE) per milligram of Dry Weight of the sample (mg QAE/mg DW). All measurements were conducted in triplicates.

### **3.6 Evaluation of antioxidant activity (DPPH and ABTS radical scavenging capacity assay)**

#### **3.6.1 DPPH radical scavenging capacity assay**

The capacity of the samples to scavenge radicals using the stable free radical DPPH was used to assess the antioxidant activity of the edible wild fruits, as described by (She *et al.*, 2010) with slight modifications. The DPPH stock solution of 0.3 mM prepared in absolute methanol in 100 mL conical flasks covered with aluminum foil (due to its sensitivity to light) at room temperature was used to prepare 0.07 mM of DPPH radical solution in absolute methanol. Aliquots of 0.1 mL reconstituted crude extracts alongside control (0.1 mL absolute methanol with no crude extract) were treated with 3.9 mL of 0.07 mM DPPH radical solution in test tubes then incubated for 30 minutes at room temperature in the dark. The absorbance at 524 nm was used to monitor the reduction in DPPH radical concentration using a Thermo Scientific™ SPECTRONIC™ 200 Spectrophotometer (Madison, United States of America) against a blank of absolute methanol. Measurements of all samples were performed under dim light. The standard curve for Quercetin acid was prepared with concentrations range of 100, 300, 400, 500, 600, 800, 1000 µg/mL. The DPPH activity was calculated with an equation generated from the standard curve with the formula  $(Ab-Ba) = mx + c$  where x= concentration of the sample, Ab=absorbance of control, Ba= absorbance of sample, m and c were derived from the standard curve. The results were expressed as mg QAE/mg of Dry Weight sample. All measurements were conducted in triplicates.

#### **3.6.2 ABTS radical scavenging capacity assay**

The ABTS cation radical scavenging assay was based on a previous report by (Koleva *et al.*, 2001). To make the ABTS cation chromophore, 7 mM ABTS was mixed with 2.4 mM potassium persulfate (1:1) in distilled water to make stock solution. From then on, the stock solution was stored at room temperature for 16 hours in the dark before use. In order to obtain an absorbance at 734 nm of  $0.7 \pm 0.02$ , the stock solution was diluted with distilled water before the assay using a Thermo Scientific™ SPECTRONIC™ 200 Spectrophotometer (Madison, United States of America). The Ascorbic acid standard was prepared from a variety of concentrations ranging from 100, 200, 300, 400, 500, 600 µg/mL. After mixing 100 µL of each concentration standard

with 3900  $\mu$ L of ABTS solution and incubating for 30 minutes in the dark at room temperature, absorbance at 735 nm was measured against a blank of distilled water. Under the same parameters, the total antioxidant capacity of crude extracts was estimated relative to the reactivity of Ascorbic acid as a reference, and the findings were represented as mg AAE/mg DW. The diluted reconstituted extracts were treated in the same way. The ABTS activity was calculated with the formula: **(Ab-Ba) = mx + c** where x= concentration of the sample, Ab=absorbance of control, Ba= absorbance of sample, m and c were derived from the standard curve. All measurements were conducted in triplicates for each experiment.

### **3.7 Antimicrobial activity assay (Agar well diffusion method and Minimum Inhibitory Concentration)**

#### **3.7.1 Agar well diffusion method**

For the antimicrobial activity, fruit extracts with satisfactory antioxidant activity were selected along with the best solvent. From the preliminary experiments, methanolic extracts of *Ximenia americana* L, *Ximenia caffara* S and *Mimusops zeyheri* were selected for antimicrobial activity due to their significantly higher antioxidant activity, TFC, and TPC as compared to other fruits. An *in vitro* antimicrobial test using agar well diffusion was conducted on the selected methanolic extracts against pathogenic microbes following a procedure outlined by (Rakholiya *et al.*, 2014). The microorganisms used in the test were *Staphylococcus aureus*, *Escherichia coli* for antibacterial activity whereas for antifungal activity *Rhizopus oryzae* and *Aspergillus niger* were used. Antibacterial and antifungal susceptibility tests were conducted using Mueller-Hinton agar (MHA) and yeast extract peptone dextrose (YPD) respectively. The media were prepared and autoclaved then poured aseptically in sterile petri dishes. To obtain a concentration of 10 mg/mL, the 3 methanolic extracts, there were reconstituted in 10% dimethyl sulfoxide (DMSO). A Mueller-Hinton broth (MHB) was used to culture bacteria and fungi for 18 hours before being incubated in a VWR 3500 Incubating Orbital Shaker (12620-948, VWR International, Radnor Township, Pennsylvania, USA) at 160 rpm at 37°C and 30°C respectively. Thereafter, microbial suspensions were diluted with MHB to obtain an absorbance of 0.4 at 600 nm. The microbe cells were then plated aseptically into the agar media using spread plate technique. A sterile pipette tip of 8 mm diameter was used to punch the wells on the agar after which the bottom end of the well was covered with a bit of the agar to avoid the extract from overspilling underneath the media. Afterwards, 100  $\mu$ L of the extracts were introduced into the wells of the YPD and MHA after

which the wells were sealed with their respective agar. Gentamicin antibiotic 200 µg/mL was used as a positive control for bacterial samples and MHB used as a negative control. Cycloheximide 200 µg/mL was used a positive control for fungal samples whereas negative controls were the same as those used in antibacterial tests. A 24 hours' incubation using (Lamworld Technologies PTY LTD Gaborone, Botswana) incubator was performed on the plates at 37°C and 30°C for bacteria and fungi respectively. All measurements were performed in triplicates, along with positive and negative controls. Antimicrobial activity was determined by measurement of the inhibition zones (millimetres) with a 30 cm ruler.

### **3.7.2 Minimum Inhibitory Concentration (MIC)**

The MICs were only performed based on the specimens that showed a zone of inhibition of >15 mm for the crude extracts following procedure outlined by (Rakholiya *et al.*, 2014). To determine the MIC of fruit extracts, a micro broth dilution procedure was used in sterile 96 well surface treated tissue culture plates (VWR North American, Radnor, PA USA). In each well, 150 µL of MHB was added followed by 20 µL of each varying concentration of fruit extracts (10, 5, 2.5, 1.25, 0.625 mg/mL) in decreasing sequence then lastly 30 µL of microbe suspension. Each well reached a final capacity of 200 µL. Each test set had 2 control wells which were comprised of positive control (Muller-Hinton broth, microbe suspension, and Gentamicin antibiotic 200 µg/mL) for bacteria and negative control (Muller-Hinton broth, DMSO, and microbe suspension). A 24 hours incubation using (Lamworld Technologies PTY LTD Gaborone Botswana) was performed on the plates at 37°C. Absorbance was recorded at 600 nm using a 96-microplate reader (Thermo Scientific, Multiskan FC, Shanghai, China). The MIC was calculated using an equation:  $y = mx + c$  where  $y=50\%$ ,  $m$ , and  $c$  were obtained after performing regression analysis and  $x = IC_{50}$ . All experiments were done in triplicates.

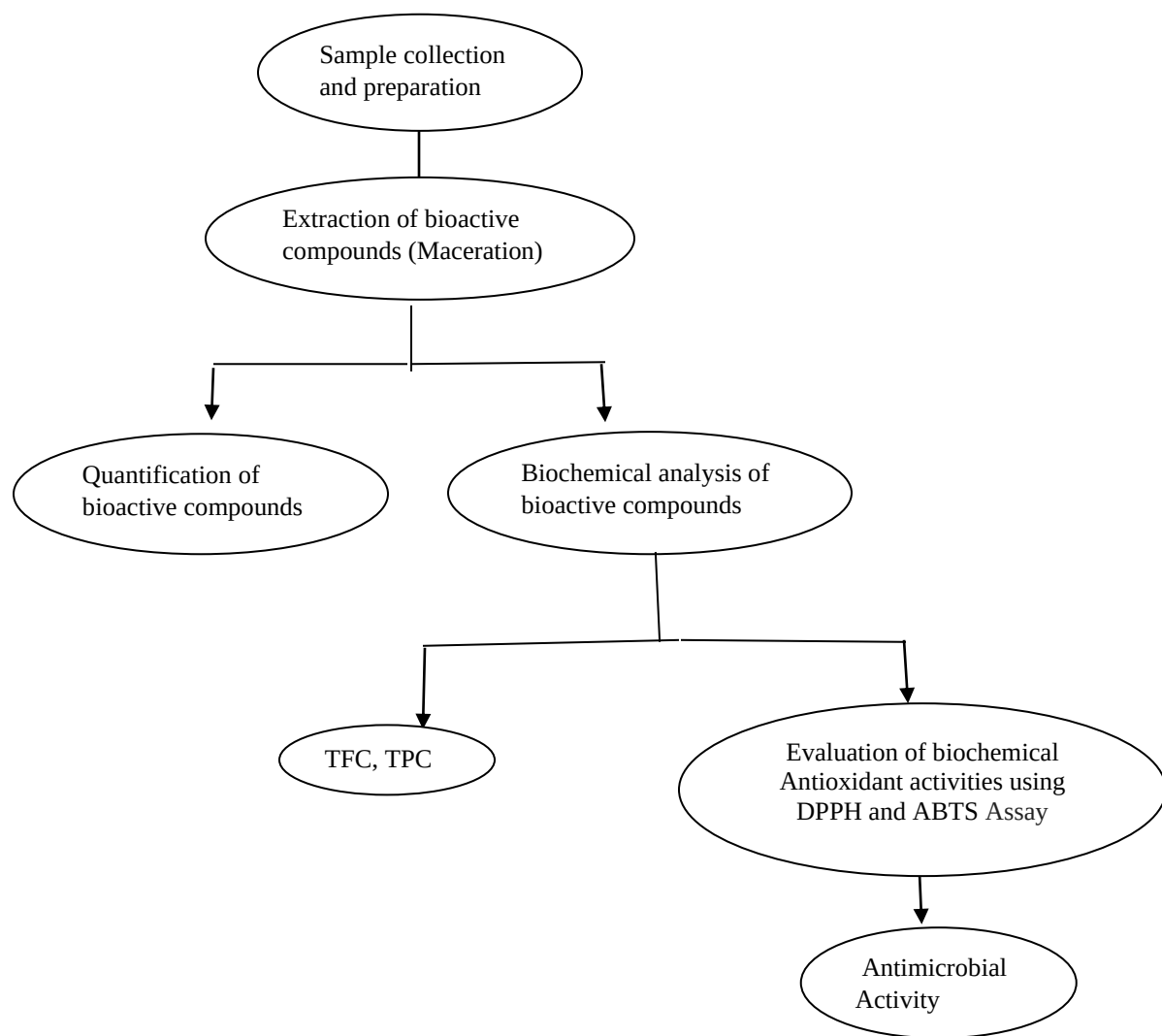


**Figure 7:** A summarized flow chart illustrating the Quantification and Qualitative analysis of bioactive active compounds extracted from edible wild fruits

### 3.8 Statistical analysis

Results were subjected to a one-way analysis of variance (ANOVA) by GraphPad Prism 7.05 statistical software. Values are presented as mean  $\pm$  standard error of 3 replicates (n=3). Statistical significance of the difference among means was estimated at  $P < 0.05$ , using Tukey-Kramer's multiple range test. Graphical representation of data was done using OriginPro 2022. Regression analysis was performed on DPPH, ABTS, TPC, MIC, and TFC.

## CHAPTER FOUR RESULTS

### 4.1 Phytochemical Screening

#### 4.1.1 Qualitative Analysis

As shown in (Table 1), tannins were absent in edible wild fruits for all the solvents with an exception noticeable in *Ximenia caffara S* and *Ximenia americana L*. Terpenoids were present in almost all the solvents for all the samples except for distilled water extracts of *Grewia bicolor*, *Gweria cana Sond*, *Mimusops zeyheri*, *Grewia Flava* and ethanolic extract of *Grewia flava*. Phenols were found in almost all methanolic and ethanolic solvent-extracted samples except for ethanolic extract of *Grewia flava* and distilled water extracts of *Ximenia caffara S*, *Mimusops zeyheri* and *Ximenia americana L*. Saponins were present in all solvents for *Ximenia americana L* and *Ximenia caffra S* but abscent in *Grewia bicolor*, *Vangueria infausta*, *Grewia flava* and *Grewia cana Sond* for all the solvents with an exception noticeable in methanolic *Grewia bicolor* and *Vangueria infausta*.

**Table 1:** Phytochemicals present in seven edible wild fruits: Qualitative Analysis

| Sample  |            | <i>Grewia bicolor</i> |   |   | <i>Grewia cana Sond</i> |   |   | <i>Grewia flava</i> |   |   | <i>Ximenia caffara S</i> |   |   | <i>Ximenia americana L</i> |   |   | <i>Mimusops zeyheri</i> |   |   | <i>Vangueria infausta</i> |   |   |
|---------|------------|-----------------------|---|---|-------------------------|---|---|---------------------|---|---|--------------------------|---|---|----------------------------|---|---|-------------------------|---|---|---------------------------|---|---|
| Solvent |            | M                     | E | D | M                       | E | D | M                   | E | D | M                        | E | D | M                          | E | D | M                       | E | D | M                         | E | D |
| 1       | Tannins    | -                     | - | - | -                       | - | - | -                   | - | - | +                        | + | + | +                          | + | + | -                       | - | - | -                         | - | - |
| 2       | Phenols    | +                     | + | - | +                       | + | - | +                   | - | - | +                        | + | + | +                          | + | + | +                       | + | + | +                         | + | - |
| 3       | Saponins   | +                     | - | - | -                       | - | - | -                   | - | - | +                        | + | + | +                          | + | + | +                       | + | - | +                         | - | - |
| 4       | Terpenoids | +                     | + | - | +                       | + | - | +                   | - | - | +                        | + | + | +                          | + | + | +                       | + | - | +                         | + | + |

(+ shows the phytochemical class is present, - shows the phytochemical class is not present)

(M- Methanol, E- Ethanol, D- Distilled water)

## 4.2 Quantification of main phytochemical classes

### 4.2.1 Total Flavonoids Content

The total flavonoids content of extracts in all the seven edible wild fruits were calculated using Quercetin as a reference and represented as equivalents (mg QAE/mg) (**Fig. 8**). The antioxidant concentrations of the extracts for all the solvents were calculated using the regression equation  $R^2 = 0.9682$ ,  $y = 0.0002x + 0.0035$  as shown in appendix (**Auxiliary Fig. 16**). *Ximenia caffara* S, *Ximenia americana. L* and *Mimusops zeyheri* showed significant higher values ( $P < 0.0001$ ) respectively for ethanol, methanol and water extracts followed by *Grewia spp.*, while *Vangueria infausta* showed the lowest TFC value (**Fig. 8**).

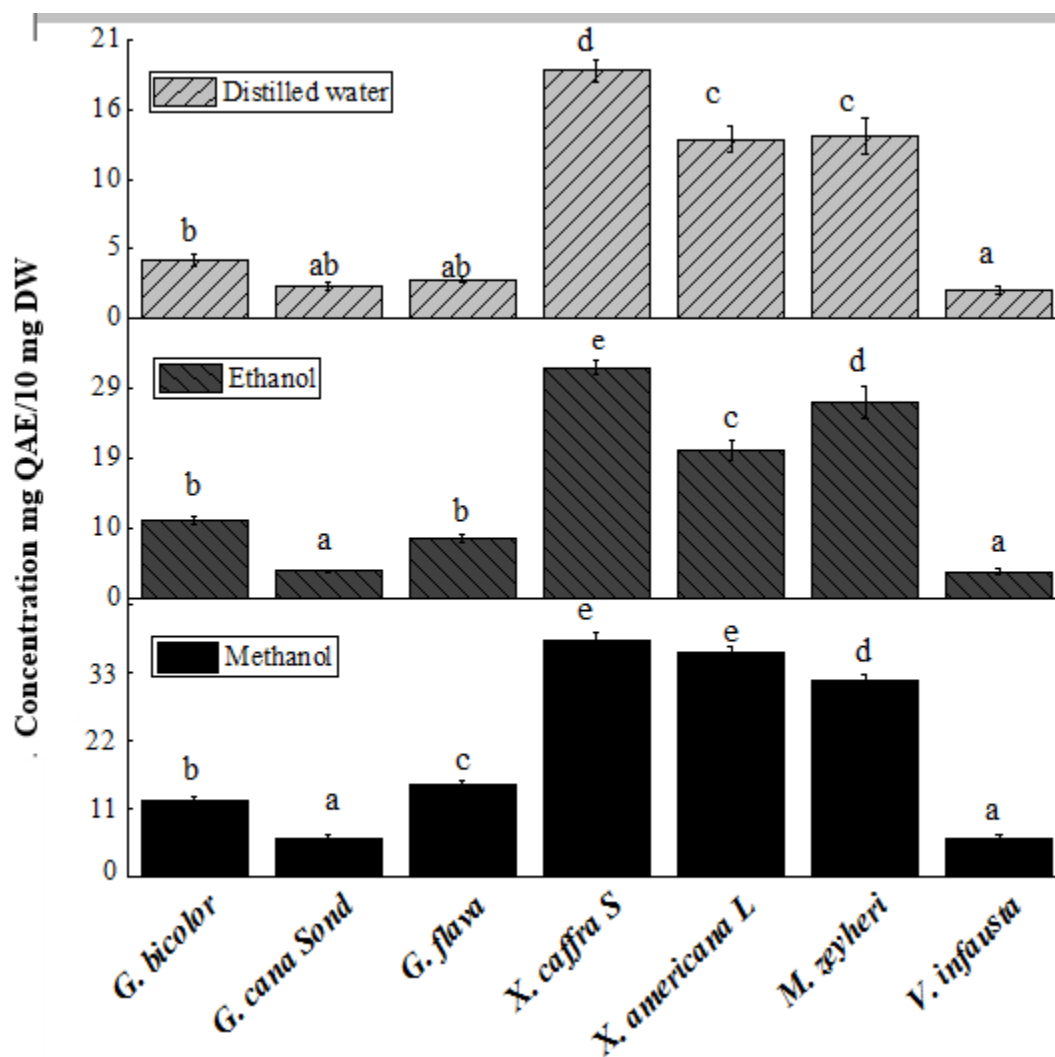


**Figure 8:** Illustrates concentration of total flavonoid content per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of *Grewia bicolor*, *Grewia cana Sond*, *Vangueria infausta subsp. Infausta* (VI), *Ximenia caffra S*, *Mimusops zeyheri*, *Ximenia americana L*, *Grewia flava*, and standards-based on Quercetin acid equivalent. Bar graphs represent mean  $\pm$  standard error (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) amongst the samples within each solvent

#### 4.2.2 Total Phenolic Content

In terms of Gallic acid equivalents, the concentration of the methanol, ethanol, and distilled water extracts were determined with a folin-Ciocalteu assay (**Fig. 9**) to construct a standard curve equation of  $y = 0.0005x - 0.0089$  and a regression line of  $R^2 = 0.9955$  and expressed as equivalents (mg GAE/ mg) as shown in appendix (**Auxiliary Fig. 17**).

*Ximenia caffra* S, *X. americana*. L and *Mimusops zeyheri* showed a significant higher ( $P < 0.0001$ ) TPC respectively for all the solvents (**Fig. 9**) compared to the rest of the extracts. *Grewia flava* indicated low TPC for methanolic and ethanolic extracts followed by *Vangueria infausta* subsp. *Infausta* (VI). *Grewia bicolor*, *Grewia cana* Sond *Vangueria infausta* subsp. *Infausta* (VI) and *Grewia flava* showed the lowest ( $P < 0.0001$ ) TPC for all the solvents (**Fig. 9**) as compared to all the extracts.

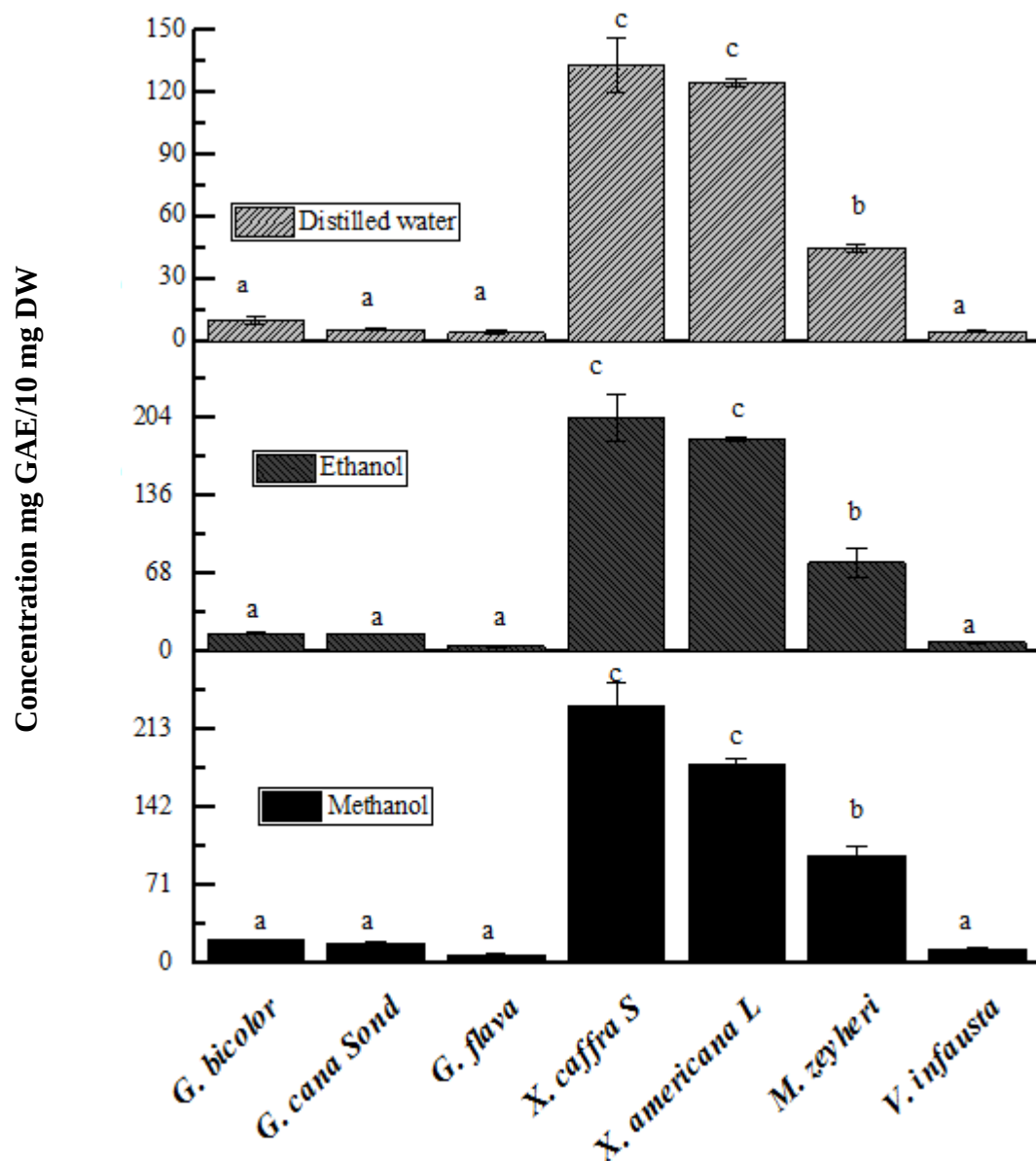


**Figure 9:** Illustrates concentration of total phenolic content per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of *Grewia bicolor*, *Grewia cana Sond*, *Vangueria infausta subsp. Infausta* (VI), *Ximenia caffra S*, *Mimusops zeyheri*, *Ximenia americana L*, *Grewia flava*, and standards based on Gallic acid equivalent. Bar graphs represent mean  $\pm$  standard error (n=3). Different superscripts indicate significant differences (P < 0.05, Tukey-Kramer's range test) in the same solvent

### 4.3 DPPH and ABTS scavenging activity

#### 4.3.1 ABTS Assay

The antioxidant potency of Ascorbic acid (standard) was employed as a reference in this assay, which evaluated the relative potential of antioxidants to scavenge the  $\text{ABTS}^{\cdot+}$  radical. As a result, this standard curve was used to accurately predict the antioxidant capacity of crude extracts and it indicated a significantly favorable linear  $R^2 = 0.9428$  and linear equation  $y = 0.0009x + 0.0074$  as shown in appendix (**Auxiliary Fig. 18**). The results are presented as (mg AAE/mg) (**Fig. 10**).

Methanolic, ethanolic and distilled water extracts of *X. caffara* S, and *X. americana* L showed a significant higher ( $P < 0.0001$ ) ABTS activity when compared to other extracts (**Fig. 10**). This was followed by distilled water and ethanolic extracts of *Grewia cana* Sond showing significant low ABTS activity whereas *Vangueria infausta* subsp. *Infausta* (VI) showed significant lower ABTS activity for methanol extracts as compared to other fruit extracts (**Fig. 10**).

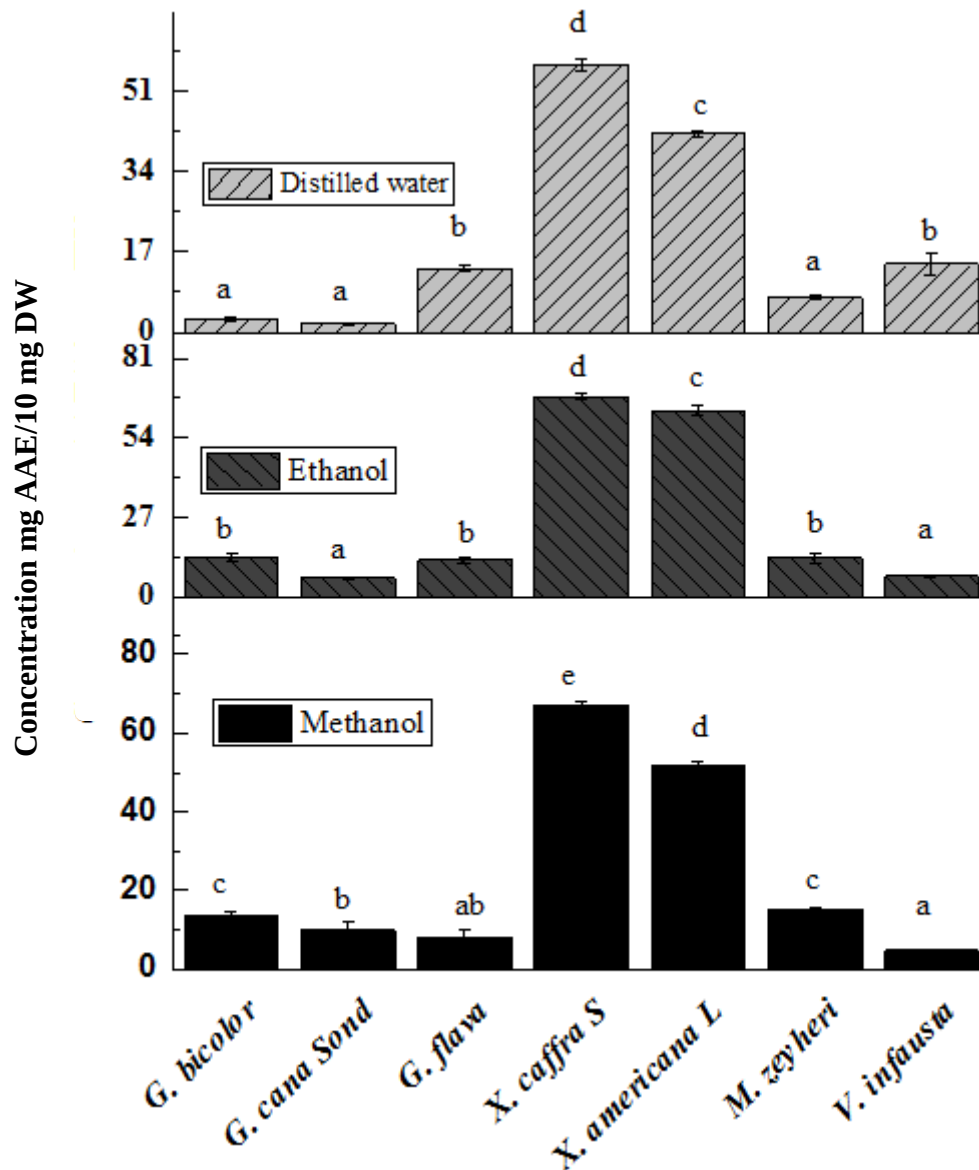


**Figure 10:** scavenging activity for ABTS per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of *Grewia bicolor*, *Grewia cana Sond*, *Vangueria infausta subsp. Infausta* (VI), *Ximenia caffra S*, *Mimusops zeyheri*, *Ximenia americana L*, *Grewia flava*, and standards based on Ascorbic acid equivalent. Bar graphs represent mean  $\pm$  standard error (n=3). Different superscripts indicate significant differences ( $P < 0.05$ , Tukey-Kramer's range test) amongst the samples within each solvent

#### 4.3.2 DPPH assay

The regression equation  $R^2 = 0.9882$  and linear equation  $y = 0.001x - 0.0607$  as shown on the appendix was used to calculate the concentration of antioxidants from the samples (**Auxiliary Fig. 19**) from the appendix. The DPPH scavenging ability of the fruit extracts was presented as milligrams of Quercetin acid equivalent (QAE) per milligram (mg QAE/mg) (**Fig. 11**).

The extracts for *Ximenia. caffara S*, *Ximenia americana L* and *Mimusops zeyheri* had significant higher DPPH scavenging activity respectively while *Grewia flava*, *Grewia bicolor* and *Vangueria infausta subsp. Infausta (VI)* had the lowest DPPH scavenging activity for all the 3 solvents ( $P < 0.0001$ ) (**Fig. 11**).

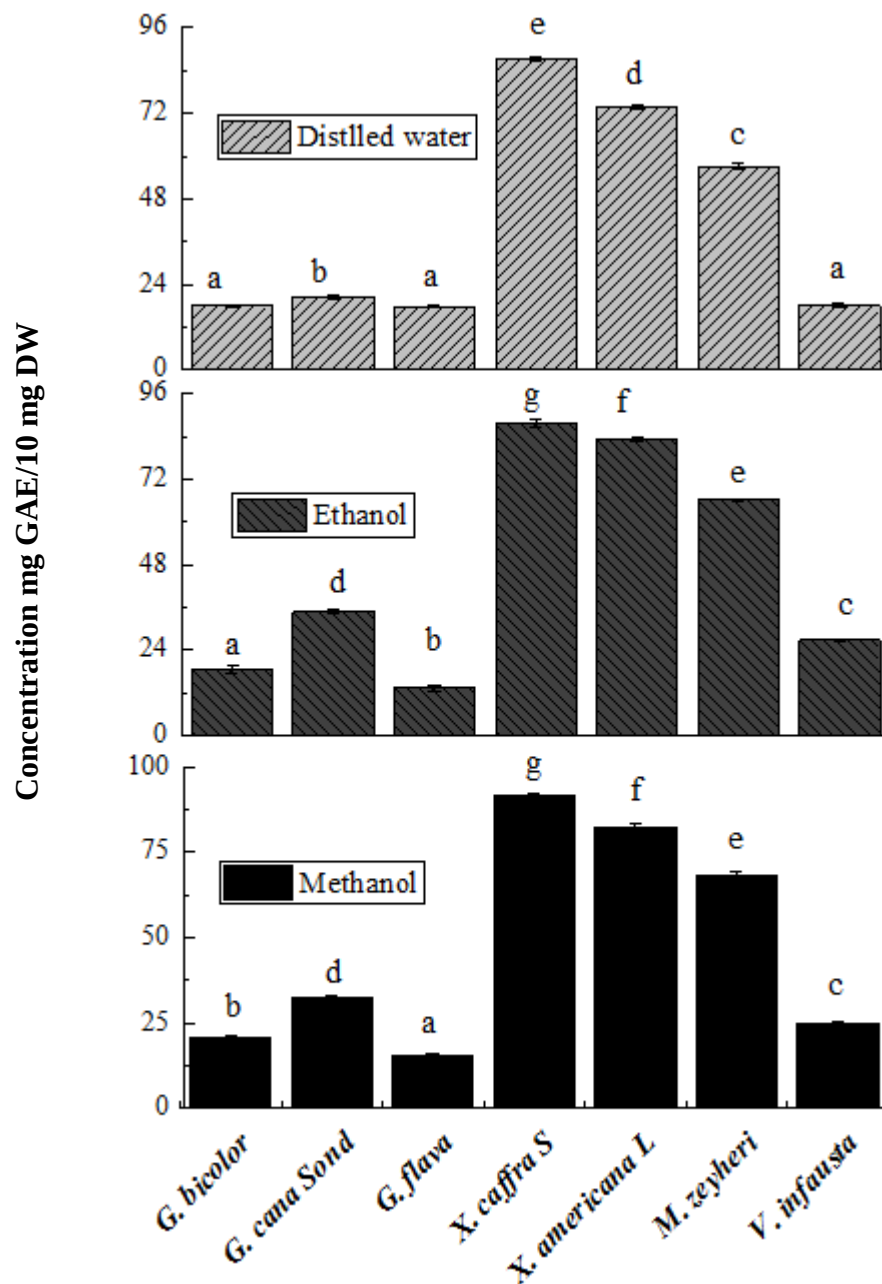


**Figure 11:** Illustrates the scavenging activity for DPPH per 10 mg Dry Weight of methanolic, ethanolic and distilled water extracts of *Grewia bicolor*, *Grewia cana Sond*, *Vangueria infausta* subsp. *Infausta* (VI), *Ximenia caffra S*, *Mimusops zeyheri*, *Ximenia Americana L*, *Grewia flava*, and standards based on Gallic acid equivalent. Bar graphs represent mean  $\pm$  standard errors (n=3). Different superscripts indicate significant differences (P < 0.05, Tukey-Kramer's range test) within each solvent

#### 4.4 Antimicrobial activity assay (Agar well diffusion method and Minimum Inhibitory Concentration)

##### 4.4.1 Agar well diffusion method

The antimicrobial activity against specific microorganisms tested was confirmed. Methanolic extracts of *X. americana* L, *X. caffra* S and *M. zeyheri* were tested for the antimicrobial activity on two bacteria, *E. coli* and *S. aureus* and two fungi, *R. oryzae* and *A. niger* using agar well diffusion method based on their satisfactory antioxidant activity from preliminary analysis (DPPH, ABTS, TFC, TPC) along with the best solvent. The antibacterial properties of methanolic extracts of edible wild fruits against microorganisms are shown in (**Tables 2**). All methanolic fruit extracts showed promising antibacterial activity against *S. aureus* and *E. coli* bacteria (**Table 2**). In *S. aureus* and *E. coli* bacteria, the Gentamicin positive control produced inhibition zones of  $41.67 \pm 1.53$  and  $26.00 \pm 1.00$  mm respectively while there was no zone of inhibition for negative control for both bacterial strains, whereas the fruit crude extracts of *X. americana* L, *X. caffra* S and *M. zeyheri* displayed an inhibition zones of  $27.33 \pm 2.08$ ,  $30.67 \pm 1.53$  and  $19.67 \pm 0.58$  mm for *E. coli*, respectively whereas for *S. aureus* a record of  $29.33 \pm 1.53$ ,  $34.33 \pm 1.53$  and  $20.67 \pm 1.53$  mm for *X. americana*, *X. caffra* and *M. zeyheri* was observed respectively (**Table 2**). The two fungi, *R. oryzae* and *A. niger* tested showed no zone of inhibition for all the extracts.

**Table 2:** Antibacterial activity of methanolic extracts of *X. Americana* L, *X. caffra* S and *M. zeyheri*

| Edible wild fruit sample | Zone of Inhibition (mm) |                    |
|--------------------------|-------------------------|--------------------|
|                          | <i>E. coli</i>          | <i>S. aureus</i>   |
| <i>X. americana</i> L    | $27.33 \pm 2.08^d$      | $29.33 \pm 1.53^d$ |
| <i>X. caffra</i> S       | $30.67 \pm 1.53^d$      | $34.33 \pm 1.53^d$ |
| <i>M. zeyheri</i>        | $19.67 \pm 0.58^b$      | $20.67 \pm 1.53^b$ |
| Positive Control         | $26.00 \pm 1.00^c$      | $41.67 \pm 1.53^c$ |
| Negative Control         | $0 \pm 0.00^a$          | $0 \pm 0.00^a$     |

Values are presented as the mean  $\pm$  standard error of 3 replicates (n=3) at (P<0.0001)

#### 4.4.2 Minimum Inhibitory Concentration

Minimum inhibitory concentrations are the lowest concentrations of antimicrobial agents that prevent the observable microbial activity of a microorganism after overnight incubation (Brady *et al.*, 2006). In light of the results of the study (**Table 3**), *E. coli* and *S. aureus* strains were inhibited at a minimum concentration of  $4.86 \pm 0.76$  and  $3.56 \pm 0.60$  mg/mL respectively for *X. caffra* when compared to *X. americana* and *M. zeyheri* with a record of  $7.51 \pm 0.51$  and  $10.20 \pm 0.38$  mg/mL for *E. coli* respectively,  $6.37 \pm 0.34$  and  $8.83 \pm 0.25$  mg/mL for *S. aureus* respectively. As a result of the tests, aqueous extracts are valid in their ability to inhibit the tested microbes (**Table 3**).

**Table 3:** Minimum Inhibitory Concentration for methanolic extracts of *X. americana* L, *X. caffra* and *M. zeyheri*

| Specimen              | <i>E. coli</i> (IC <sub>50</sub> mg/mL) | <i>S. aureus</i> (IC <sub>50</sub> mg/mL) |
|-----------------------|-----------------------------------------|-------------------------------------------|
| <i>X. americana</i> L | $7.51 \pm 0.51^b$                       | $6.37 \pm 0.34^b$                         |
| <i>X. caffra</i> S    | $4.86 \pm 0.76^a$                       | $3.56 \pm 0.60^a$                         |
| <i>M. zeyheri</i>     | $10.20 \pm 0.38^c$                      | $8.83 \pm 0.25^c$                         |

Values are presented as the mean  $\pm$  standard error of 3 replicates (n=3) at (P<0.0001)

#### 4.5 Correlations

In order to determine whether phytochemicals contributed to the antioxidant activity in ABTS and DPPH assays, the positive correlations between the TPC/TFC and antioxidant scavenging assays were compared along with antimicrobial activity. In this study, there is a high positive correlation amongst zone of inhibition, TPC, TFC, ABTS assay, and DPPH assay. The correlation for TFC versus ABTS, TPC versus ABTS, TPC versus DPPH, and TFC versus DPPH for methanol, ethanol and distilled water extracts ranged from 0.73 to 1 as depicted in (**Fig. 12, 13, 14**) whereas a negative correlation for minimum inhibitory concentration ranged from -0.97 to -0.99 indicating significantly strong correlation whereas for zone of inhibition a positive correlation of 1 was recorded (**Fig. 15**)

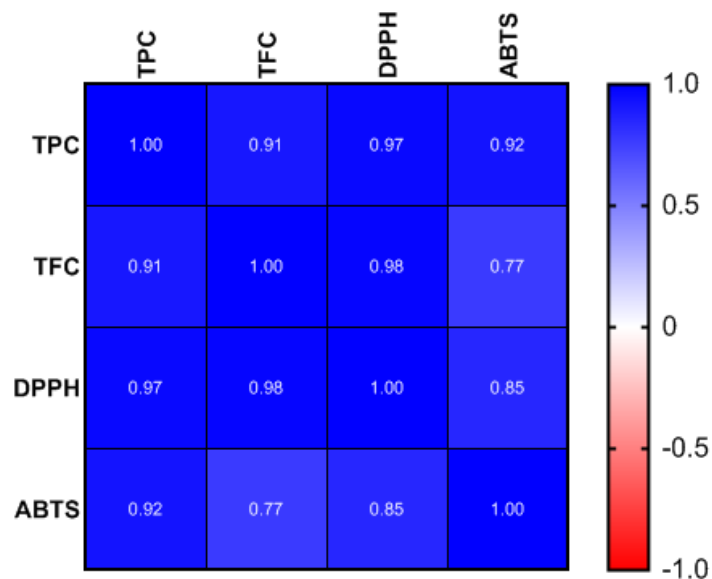


**Figure 12:** Heat map of Pearson correlation for seven distilled water extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH

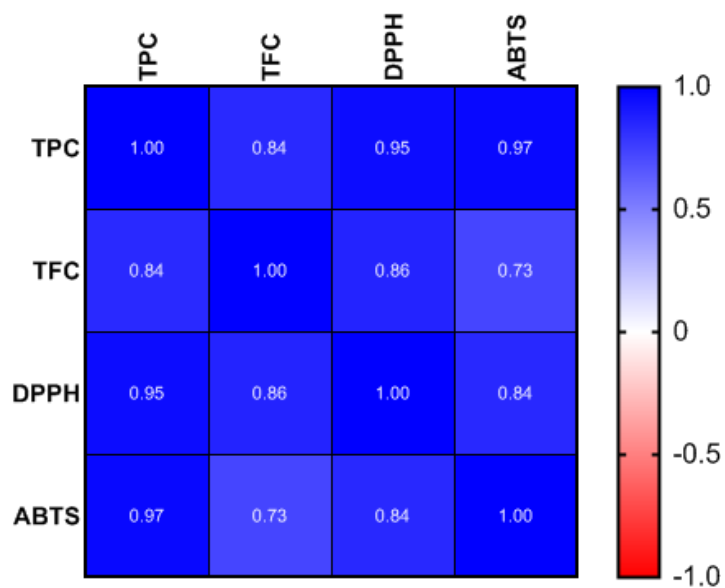


**Figure 13:** Heat map of Pearson correlation for seven ethanol extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH

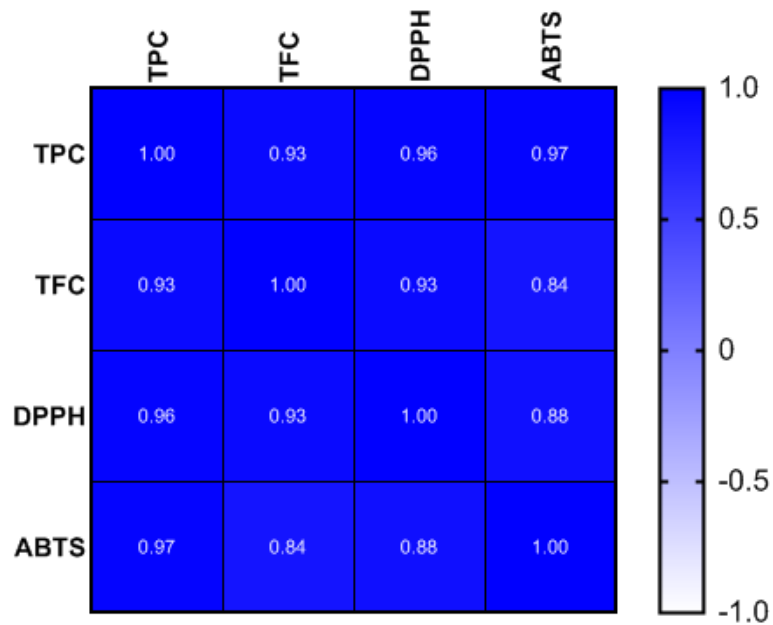


**Figure 14:** Heat map of Pearson correlation for seven methanol extracts with respect to TPC versus DPPH, TPC versus ABTS, TFC versus ABTS, and TFC versus DPPH

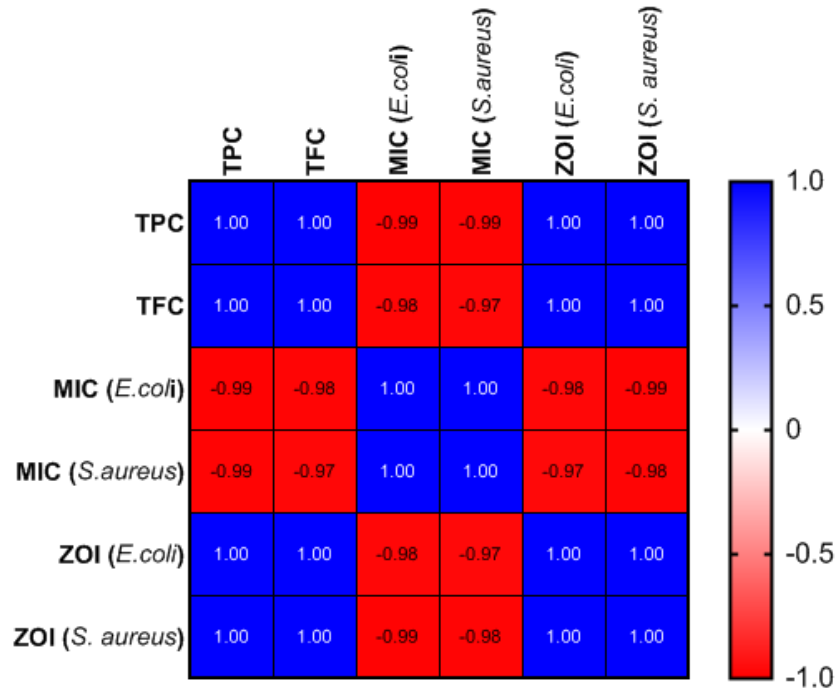


**Figure 15:** Heat map of Pearson correlation for three methanol extracts with respect to antimicrobial activity versus TPC and TFC

## CHAPTER FIVE DISCUSSION

The present study's findings indicated that the wild edible fruits of the target species have antioxidant activity. These observations may justify their usage in traditional medicine and encourage its use as a health nutritional supplement.

### 5.1 Qualitative phytochemical analysis

In a similar study by (Jacob *et al.*, 2021; Geyid *et al.*, 2005) it was reported that methanolic *Ximenia caffra* S revealed the presence of tannins, saponins, flavonoids, phenols which is in agreement with the present study. Likewise, in different research by (Khanal *et al.*, 2016) methanolic extracts of both *Grewia asiatica* and *Grewia optiva* showed the presence of tannins, flavanols, terpenoids, steroids, alkaloids and saponins and this is consistent with the outcomes of methanolic *Grewia bicolor*. Furthermore, *M. zeyheri* and *V. infausta subsp. Infausta* (VI) displayed the presence of phenols, flavones, and saponins which was inline with a study by (Monyela., 2021; Abosi *et al.*, 2006). According to the present results, water had poor ability to extract most of the phytochemicals as compared to methanol and ethanol. Methanol is an excellent solvent for extracting polyphenols, tannins, flavones, and anthocyanins (Masuku *et al.*, 2020; Daniel and Topo., 2012; Handa., 2008; Lapornik *et al.*, 2005; Masoko *et al.*, 2005;) which is also evident from the results obtained showing methanol being able to extract most of the bioactive active compounds during qualitative analysis. It implies that they are more efficient in degrading un-polar cell walls and seeds, which causes the release of anthocyanins and other polyphenols from cells (Lapornik *et al.*, 2005) Plants generally produce different types of secondary metabolites with which plant pathogens can be effectively suppressed (Monyela., 2021; Geyid *et al.*, 2005).

### 5.2 Quantitative phytochemical analysis

#### 5.2.1 Total phenolic content

In this study, the Folin Ciocalteu test was used, which relies on electron transfer from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes in an alkaline solution to create blue compounds that can be measured spectrophotometrically (Dai and Mumper., 2010). *Grewia flava*, *Grewia Cana Sond* and *Grewia bicolor* had a significant lower recorded TPC in the present study for distilled water extracts. This might be because the qualitative analysis of these species revealed that they were devoid of saponins, phenols, tannins, and terpenoids. On

the contrary a study conducted by (Masisi *et al.*, 2021; Zia-Ul-Haq *et al.*, 2013) showed that ethanol-water (60:40, v/v), dionized water, 70 % acetone, ethyl acetate and Dichloromethane extractes *Gweria flava*, *Gweria bicolor*, and *Grewia asiatica* exhibited a significant higher TPC comparable to those of present results. The methanolic extracts of *Vangueria infausta subsp. Infausta* (VI) pulp studied by (Mausse *et al.*, 2021) recorded lower TPC ( $0.528 \pm 0.03$  EAG mg/g) whereas for *M. zeyheri* its TPC was lower which ranged from 1.58 to 2.47 mg/g as indicated by (Monyela., 2021) compared to the results of the present study. The varying findings might be due to different extraction procedures, environmental condition, geno and phenotypes characteristics and solvents and thus, it requires more research at different levels.

The data analysis indicated that amongst the edible wild fruit extracts examined, *X. caffra* S and *X. american* L exhibited a significantly higher TPC when compared to the other fruits in the present study. Similar investigations on the TPC of fruit extracts of *Ximenia* species in Zimbabwe and South Africa have found significant higher TPC when compared to the other fruits (Masuku *et al.*, 2020; Munodawafa *et al.*, 2010; Ndhlala *et al.*, 2008). Another reason for their high TPC in the present study could be attributed to the view that saponins, terpenoids, phenols and tannins were all present from qualitative analysis hence contributing to their higher phenolic content. Another study led by (de Menezes *et al.*, 2019) also identified flavonoids, glycosides, tannins, phenolic acids, terpenoids and quinones for *Ximenia* species which is agreement with the results recorded in the present study.

Even though all methanolic extracts gave a significant higher TPC in this experiment, a study by (Masisi *et al.*, 2021) indicated that water extracts of *Gweria flava* and *Gweria bicolor* recorded a significantly higher TPC than methanolic extracts, this is due to different extraction methods. But that's not the case in this present experiment since all distilled water extracts recorded the lowest TPC, and this present experiment was in line with a study carried out by (Lapornik *et al.*, 2005). Microorganisms may be a chief factor in the low TPC recorded in this experiment for distilled water extracts, as water is a better medium for microorganisms' survival than ethanol and methanol. Nonetheless, phenolic components have an antimicrobial effect as documented by (Cueva *et al.*, 2010; Nohynek *et al.*, 2006; Puupponen-Pimiä *et al.*, 2001), hence this theory is less plausible. The enzyme polyphenol oxidase, which degrades polyphenols in water extracts

but is inactive in methanol and ethanol, provides a more appropriate explanation for the decline in TPC of the distilled water fruit extracts (Zhang *et al.*, 2001).

### 5.2.2 Total flavonoid content

The TFC recorded in this study for absolute methanolic *Xamenia* extracts was significantly higher as compared to *Ximenia parviflora* Benth. var. *parviflora* and *X. americana* as reported by (Lamien-Meda *et al.*, 2008; Sánchez-Gutiérrez *et al.*, 2018) with  $0.30 \pm 0.03$  mg QE/g FW and  $0.25 \pm 0.01$  mg CE/g respectively. TFC for all the absolute methanolic extracts for *Gweria* species in the current study were found to be higher than those of *G. asiatica* ( $4.81 \pm 0.4$  mg QE/g) and averaged *G. coriacea* ( $5.72 \pm 1.72$  mg QU/g FW) as reported by (Malela *et al.*, 2016; Qamar *et al.*, 2020) whereas for *M. zeyheri* recorded TFC which ranged from 0.012 to 1.15 mg/g which was lower than the recorded results (Monyela., 2021). When fruit samples are kept in a freezer before biochemical analysis, this leads to greater extraction efficiency of phenolics due to increased plant cell breakage during ice crystal formation and melting (Asami *et al.*, 2003). Hence this might be attributable to the premise that the fruit samples for the current study were stored at  $-80^{\circ}\text{C}$  before analyzing them. Environmental effects, mature stage of the fruit and preparation techniques could have attributed to the differences in TFC of the mentioned fruits. Additional studies are required to confirm such speculations.

There is noticeable variation in terms of TPC and TFC amongst edible wild fruits as evidenced by the results obtained from this research. Overall, TPC is higher than TFC for all the fruit extracts in all solvents in the present study. The present study's findings are consistent with those reported by (Song *et al.*, 2018; Wong *et al.*, 2014). Although all fruits were collected from the same place, the substantial variance is thought to be related to genetic make-up, stage of fruit ripening and the ability of the fruit to adapt to ecological parameters while synthesizing phytochemicals. The content and quantity of secondary metabolites, as well as the nutritive value of fruits and vegetables, are heavily influenced by genetic and environmental variables (Rana *et al.*, 2019; Shan *et al.*, 2019; Contessa *et al.*, 2013).

### 5.3 Evaluation of antioxidant activity (DPPH and ABTS radical scavenging capacity assay)

#### 5.3.1 DPPH scavenging activity

According to the findings of this experiment, all fruit extracts successfully decreased the DPPH radicals. For *M. zeyheri*, a satisfactory 56% radical scavenging activity was recorded by (Abdelmohsen *et al.*, 2020) which is inline with the recorded results. The fruit *X. caffra* S and *X. american* L had a significant higher DPPH radical scavenging activity for all the solvents repectively. Remarkably, these results agree with the ones reported by (Mapunda and Mligo., 2019) who recorded a significant higher radical scavenging activity of 96.70 % at a dosage of 10 µg/mL for *X. caffra* as compared to other fruits investigated ( $P < 0.05$ ). These two fruits also contained saponins, terpenoids, phenols and tannins which explains their high DPPH activity. *Grewia flava* and *Vangueria infausta* subsp. *Infausta* (VI) species had significant lower scavenging activities which might be attributed to the low TPC and TFC and absence of tannins and saponins for all the solvents (except methanolic extracts) and absense of terpenoids and phenols in ethanol and distilled water extracts as compared to other fruit extracts in the experiment. Incongruent with the conclusions reached by (Masisi *et al.*, 2021; Maroyi., 2018; Lamola., 2015), *Grewia* species and *Vangueria madagascariensis* exhibited a significant stronger radical scavenging activity. Numerous factors, such as the kind of polarity of the solvents employed, extraction method, extraction time, temperature, the location where the plant material was obtained, and harvest seasons, may impact the number of bioactive chemicals and antioxidant capabilities of the plant (Abubaka and Haque., 2020; Yao *et al.*, 2012). These factors might account for the disparities in the results of different research conducted.

Of the seven edible wild edible fruits analyzed, methanol extracts had the highest radical scavenging activity for all the edible wild fruit extracts followed by ethanol and lastly distilled water. The analyses are in conformity with the results documented by (Masuku *et al.*, 2020; Daniel and Topo., 2012) who found that using methanol as an extraction solvent yielded significant high DPHH scavenging activity. For all extracts, distilled water extracts showed the lowest antioxidant activity, possibly due to minimal secondary metabolites in most water extracts results of qualitative analysis. Previously published findings by (Majhenič *et al.*, 2007; Parekh *et al.*, 2006; Clarkson *et al.*, 2004; Karaman *et al.*, 2003;) are in agreement with our study findings showing that water is ineffective in extracting phytochemicals from medicinal plants when compared to organic solvents like methanol. To begin with, water is incapable of extracting

lipophilic phytochemicals that need methanol extraction. Secondly, it's conceivable that the low antioxidant activity of distilled water extracts might be because they weren't processed using traditional techniques, which frequently entail boiling for many hours (Clarkson *et al.*, 2004; Koenen., 2001).

### 5.3.2 ABTS scavenging activity

In general, all solvent extracts for the DPPH assay displayed higher antioxidant activity than that of the ABTS assay. The results generated in this experiment were found to be consistent with those published by (Moonmun *et al.*, 2017; Amensour *et al.*, 2010) who stated that various chemical processes involved in the experiments, as well as varied chemical characteristics of the radicals, may explain the differences in the behavior of the extracts in ABTS and DPPH assays. On the contrary, research by (Adebisi *et al.*, 2017) indicated that the *Grewia carpinifolia* extracts' ability to scavenge the DPPH radical was noticeably lower than that of ABTS<sup>+</sup>. This supports the assumption that plants with DPPH scavenging capabilities may not be able to prevent the ABTS radical due to differences in solubility and preparation methods (Ercisli *et al.*, 2007).

The *Ximenia* species have been found to have substantial antioxidant activity as reported by (Ndhlala *et al.*, 2008) which is inline with the recorded results of the present study. There were several significant polyphenolic compounds found within the *Ximenia* extracts which included catechin, hyperoside, isoquercitrin, and procyanidin B1. The flavonoid hyperoside, which is mostly present in *Hypericum perforatum L.*, has a wide range of biological activities. One of the most crucial benefits is the alleviation of oxidative stress brought on by reactive oxygen species as reported by (Park *et al.*, 2016). According to (Villaño *et al.*, 2007), it has been concluded that procyanidins B1 and B2 are amongst the most effective scavenging molecules. Therefore, its speculated that fruits with higher antioxidant activity in the present study might have been attributed to the availability of these specific compounds.

## **5.4 Antimicrobial activity assay (Agar well diffusion method and Minimum Inhibitory Concentration)**

### **5.4.1 Agar well diffusion method**

Numerous investigations have suggested that phytoconstituents extracts have antimicrobial effects against poison-causing microorganisms such as *Staphylococcus aureus* and *Listeria monocytogenes* (; Chun *et al.*, 2005; Lin *et al.*, 2005; Akiyama *et al.*, 2001; Cowan., 1999). According to the results, three selected methanolic extracts showed a satisfactory inhibition zone against two strains of bacteria. The fruits found in Tswapong region appear to be as powerful as Gentamicin as an antibacterial treatment, indicating that their usage in the food and pharmaceutical sectors might be promising. The three selected methanolic extracts possessed greater potency against *S. aureus* than *E. coli* as shown in the present study. The majority of scientists agree that plant extracts are more effective against gram-positive bacteria than gram-negative bacteria (Chanda *et al.*, 2010; Baravalia *et al.*, 2009; Parekh and Chanda., 2008; Parekh and Chanda., 2007; Rios and Recio., 2005). This might be due to the notion that gram-negative bacterial cells have a complex and multilayered wall (characterized by lipopolysaccharides) that restricts elements such as artificial and natural antibiotics from passing through the membrane into the cell as compared to gram-positive bacteria (Rakholiya *et al.*, 2014). Our results show that there was no antifungal activity for the three methanolic extracts. According to (Maikai *et al.*, 2009) the results revealed that plant extracts at higher concentrations (2 g/3 mL) exhibited antifungal activity against *Solanum trilobatum*. The concentration crude extracts of the present study were 1 mg/10 mL which shows low concentration which could give a better explanation as to why there was no antifungal activity recorded. Another reason could be that the extracts were in their crude form comprised of many chemical constituents as compared to the positive control which is in its purest form. It may be possible to isolate purer chemicals with higher antimicrobial activity by further purifying the fruits extracts (Maikai *et al.*, 2009).

### **5.4.2 Minimum Inhibitory Concentration**

From the results obtained, *X. caffra* S revealed to be a better antibacterial agent followed by *X. americana* L and lastly *M. zeyheri*. The fruits found in Tswapong region appear to be as powerful as Gentamicin as an antibacterial treatment. According to (James *et al.*, 2007), aqueous methanolic extracts of *X. americana* roots, stem barks, and leaves inhibited the growth of *S. aureus*, *Klebsiella pneumoniae*, and *Shigella flexneri* pathogens. These effects were attributed to

the presence of carbohydrates, glycosides, flavonoids, and tannins in these extracts. As demonstrated by (Monik *et al.*, 2015), ethanolic extracts of *X. americana* leaves with erythromycin combined exhibited synergistic antibacterial activity against *Staphylococcus aureus*, which is attributed to high content of phenolic contents, which inhibits membrane penetrability, resulting in improved bactericidal activity. This trend is also noticeable in the present study. Additionally, it has been noted that tannins have been discovered to generate irreversible compounds with proline-rich proteins, which inhibits the creation of cell proteins for bacteria hence hindering their survival (Devi *et al.*, 2014). Furthermore, with impetigo contagiosa patients, (Sharquie *et al.*, 2000) reported an 81.3% cure rate using crude tea ointment containing tannic acid. *In vitro* and *in vivo* studies have shown isoquercitrin isolated from *Ximenia* spp. to have a better bioavailability than quercetin and to have a range of biological activities such as anticarcinogenic properties against oxidative stress, cancer, cardiovascular disease, diabetes, and allergy responses (Valentová *et al.*, 2014). Thus it can be safe to speculate that the three studied wild fruits might have this chemical compound. Further studies are needed to validate such speculation.

## 5.5 Correlations

The antioxidant and antimicrobial activity of phenolic compounds correlates positively with its total phenolic content, according to recent studies (Tshikalange *et al.*, 2016; Zhang and Wang., 2009; Samarth *et al.*, 2008; Kouri *et al.*, 2007; Djeridane *et al.*, 2006; Capecka *et al.*, 2005; Payet *et al.*, 2005). According to the results in this study, TFC and TPC may have contributed 73-98% antioxidant activity on scavenging antioxidant radicals (ABTS and DPPH). These findings imply that various phenolics are in charge of neutralizing various reactive oxygen species. Furthermore, it may be deduced that the antioxidant activity of edible wild fruit extracts may not be caused by these compounds alone and could also be influenced by the existence of some specific active phytochemical compounds. Plant-based antibacterial compounds are predicted to halt the growth of microorganisms and inhibit subsequent infection (Farag *et al.*, 2015) The orientation of phenolic compounds' hydroxyl groups affects the antimicrobial activity of the plant. Thus, the impact will be greater the more phenolic components the extract has (Demir and Ağaoğlu., 2021; Pandino *et al.*, 2011) which is evident in the present study. Positive and negative correlation for zone of inhibition and MIC respectively were recorded suggesting that the higher TFC/TPC the higher antimicrobial activity.

## CHAPTER SIX CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

A successful extraction of phytochemicals from seven fruits from the Tswapong region and their identification of their chemical components were obtained. According to the findings of the present study, the examined fruit samples have significant antioxidant characteristics, notably phenolic and flavonoid components, and may scavenge free radicals. A high phenolic and flavonoid content of *Mimusops zeyheri*, *Ximenia americana* and *Ximenia caffra* species found in Tswapong region has been demonstrated to be responsible for its antioxidant properties, which are attributed to the presence of phytochemicals and that might also explain their better antibacterial properties. The fruit's beneficial properties are not limited to treating health conditions, but they could also contribute to preventing degenerative conditions. Excellent correlation found in ABTS-TPC, ABTS-TFC, DPPH-TPC and DPPH-TFC suggest that phenols and flavonoids are the main components responsible for the antioxidant potential in the tested extracts. Methanol solvent extraction were shown to be better in terms of secondary metabolites and antioxidant capacities. The high values of TPC, TFC, ABTS, DPPH and presence of all secondary metabolites make *Mimusops zeyheri*, *Ximenia americana* and *Ximenia caffra* methanol extract preferable among the studied wild fruits for antimicrobial assay using the bacterial and fungal cells. The model studies revealed growth inhibition of *S. aureus* and *E. coli* which was at the same level and better than commercial antibiotic Gentamicin. Excellent correlation found in growth inhibition-TPC suggest that phenols are the main components responsible for the antibacterial potential in the three extracts. Finally, MIC showed *X. caffra* to be a better antibacterial agent followed by *X. americana* and *M. zeyheri*. Therefore, further studies are required to unfold the mechanism. In conclusion *Mimusops zeyheri*, *Ximenia americana* and *Ximenia caffra* exhibited excellent antioxidant and antibacterial properties and can be suggested as functional food ingredients in lowering the risk of free radical mediated by bacteria. However, their bioavailability needs to be further confirmed.

### 6.2 Recommendations

Based on the results obtained from the present study, *Mimusops zeyheri*, *Ximenia americana* and *Ximenia caffra* are source of antioxidants, antibacterial and secondary metabolites, and can be regarded as a functional food ingredient. However, there are still additional investigations required to validate the antioxidant and antibacterial potential of these extracts. In order to

confirm the unidentified antioxidant activity contributors in *Mimusops zeyheri*, *Ximenia americana* and *Ximenia caffra*, further HPLC-MS/MS analyses are needed. Furthermore, cell lines and cellular based antioxidant activity assay are useful in investigating the antioxidant mechanism of wild fruits associated phenolic compounds in cell culture assays.

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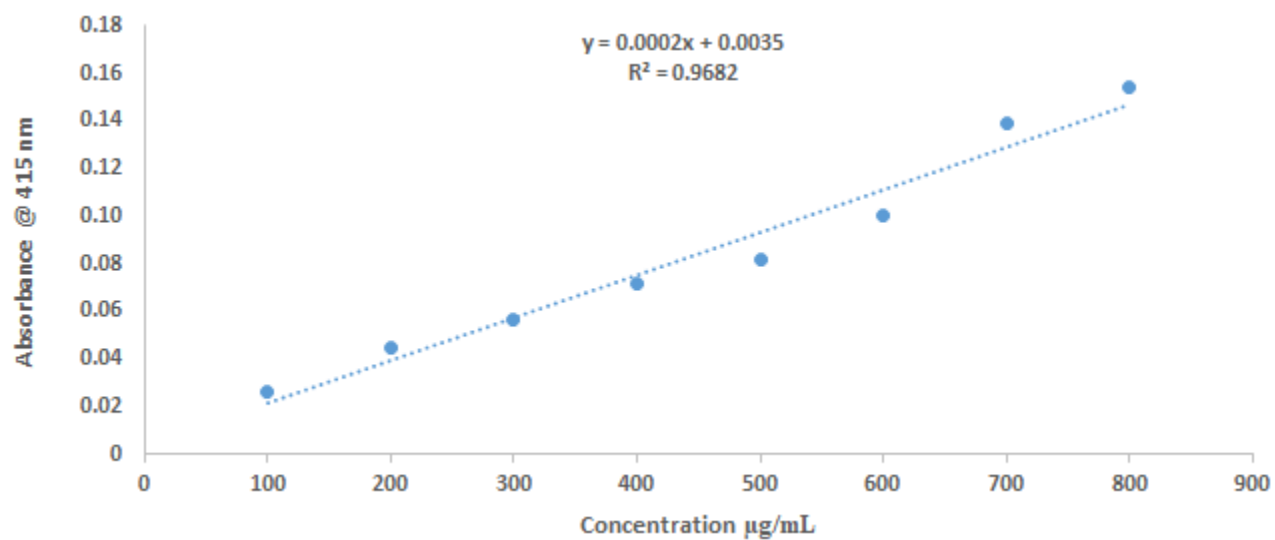
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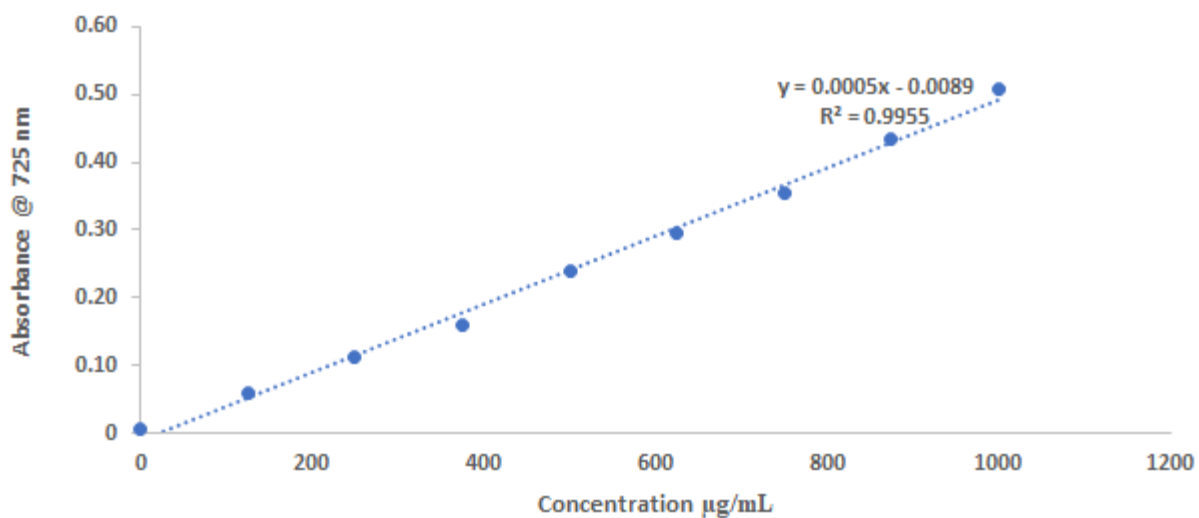
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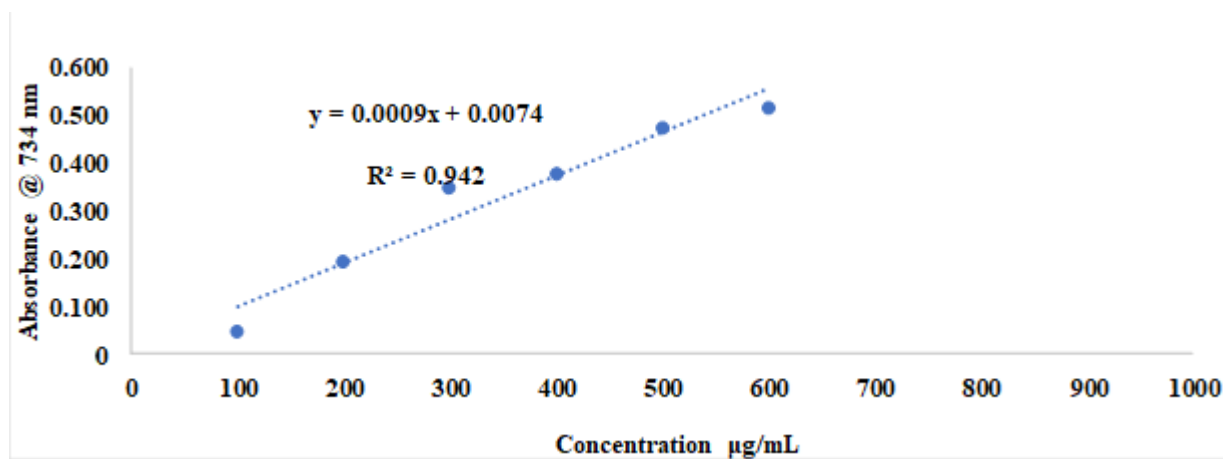
## APPENDICES



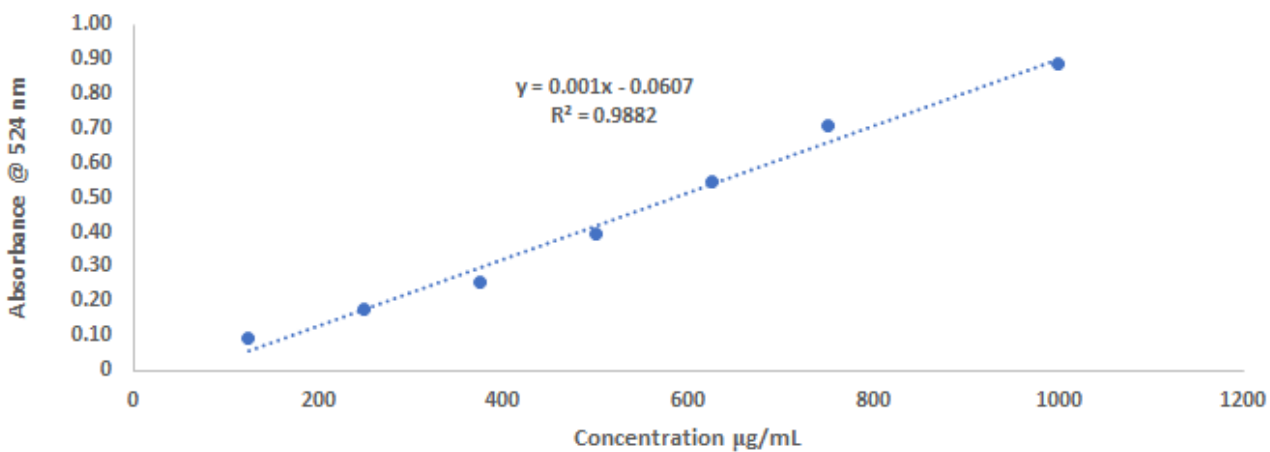
**Auxiliary Figure 16:** Calibration curve of standard Quercetin for determination of TFC



**Auxiliary Figure 17:** Gallic acid standard calibration curve for quantification of Total Phenolic Content



**Auxiliary Figure 18:** Ascorbic acid standard calibration curve for quantification of ABTS



**Auxiliary Figure 19:** Quercetin acid standard calibration curve for quantification of DPPH

**Auxiliary Table 4:** Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water)

| Number of families                                | 1          |                    |                  |         |                  |     |
|---------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| Number of comparisons per family                  | 21         |                    |                  |         |                  |     |
| Alpha                                             | 0.05       |                    |                  |         |                  |     |
| Tukey's multiple comparisons test                 | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond | -2.700     | -4.346 to -1.054   | Yes              | ***     | 0.0010           | A-B |

|                                                          |         |                  |     |      |         |     |
|----------------------------------------------------------|---------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | -0.2000 | -1.846 to 1.446  | No  | ns   | 0.9995  | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -69.20  | -70.85 to -67.55 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -39.30  | -40.95 to -37.65 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -55.80  | -57.45 to -54.15 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 0.03333 | -1.613 to 1.679  | No  | ns   | >0.9999 | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | 2.500   | 0.8540 to 4.146  | Yes | **   | 0.0020  | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -66.50  | -68.15 to -64.85 | Yes | **** | <0.0001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -36.60  | -38.25 to -34.95 | Yes | **** | <0.0001 | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -53.10  | -54.75 to -51.45 | Yes | **** | <0.0001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | 2.733   | 1.087 to 4.379   | Yes | ***  | 0.0009  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -69.00  | -70.65 to -67.35 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -39.10  | -40.75 to -37.45 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -55.60  | -57.25 to -53.95 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 0.2333  | -1.413 to 1.879  | No  | ns   | 0.9987  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 29.90   | 28.25 to 31.55   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 13.40   | 11.75 to 15.05   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 69.23   | 67.59 to 70.88   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -16.50  | -18.15 to -14.85 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 39.33   | 37.69 to 40.98   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 55.83   | 54.19 to 57.48   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 5:** Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol)

|                                                   |            |                    |                  |         |                  |     |
|---------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                         | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>           | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                      | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>          | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond | -11.43     | -13.60 to -9.269   | Yes              | ****    | <0.0001          | A-B |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | -3.867 | -6.031 to -1.702 | Yes | ***  | 0.0004  | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -70.53 | -72.70 to -68.37 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -47.40 | -49.56 to -45.24 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -61.50 | -63.66 to -59.34 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 5.600  | 3.435 to 7.765   | Yes | **** | <0.0001 | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | 7.567  | 5.402 to 9.731   | Yes | **** | <0.0001 | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -59.10 | -61.26 to -56.94 | Yes | **** | <0.0001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -35.97 | -38.13 to -33.80 | Yes | **** | <0.0001 | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -50.07 | -52.23 to -47.90 | Yes | **** | <0.0001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | 17.03  | 14.87 to 19.20   | Yes | **** | <0.0001 | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -66.67 | -68.83 to -64.50 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -43.53 | -45.70 to -41.37 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -57.63 | -59.80 to -55.47 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 9.467  | 7.302 to 11.63   | Yes | **** | <0.0001 | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 23.13  | 20.97 to 25.30   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 9.033  | 6.869 to 11.20   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 76.13  | 73.97 to 78.30   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -14.10 | -16.26 to -11.94 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 53.00  | 50.84 to 55.16   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 67.10  | 64.94 to 69.26   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 6:** Analyzed DPPH One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol)

|                                                   |             |                    |                  |         |                  |     |
|---------------------------------------------------|-------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                         | <b>1</b>    |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>           | <b>21</b>   |                    |                  |         |                  |     |
| <b>Alpha</b>                                      | <b>0.05</b> |                    |                  |         |                  |     |
| Tukey's multiple comparisons test                 | Mean Diff.  | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value | A-B |
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond | -16.30      | -18.45 to -14.15   | Yes              | ****    | <0.0001          |     |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | -8.333 | -10.48 to -6.188 | Yes | **** | <0.0001 | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -69.17 | -71.31 to -67.02 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -47.67 | -49.81 to -45.52 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -64.70 | -66.85 to -62.55 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 5.000  | 2.854 to 7.146   | Yes | **** | <0.0001 | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | 7.967  | 5.821 to 10.11   | Yes | **** | <0.0001 | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -52.87 | -55.01 to -50.72 | Yes | **** | <0.0001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -31.37 | -33.51 to -29.22 | Yes | **** | <0.0001 | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -48.40 | -50.55 to -46.25 | Yes | **** | <0.0001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | 21.30  | 19.15 to 23.45   | Yes | **** | <0.0001 | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -60.83 | -62.98 to -58.69 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -39.33 | -41.48 to -37.19 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -56.37 | -58.51 to -54.22 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 13.33  | 11.19 to 15.48   | Yes | **** | <0.0001 | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 21.50  | 19.35 to 23.65   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 4.467  | 2.321 to 6.612   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 74.17  | 72.02 to 76.31   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -17.03 | -19.18 to -14.89 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 52.67  | 50.52 to 54.81   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 69.70  | 67.55 to 71.85   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 7:** Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water)

|                                         |            |                    |                  |         |                  |  |
|-----------------------------------------|------------|--------------------|------------------|---------|------------------|--|
| <b>Number of families</b>               | 1          |                    |                  |         |                  |  |
| <b>Number of comparisons per family</b> | 21         |                    |                  |         |                  |  |
| <b>Alpha</b>                            | 0.05       |                    |                  |         |                  |  |
|                                         |            |                    |                  |         |                  |  |
| Tukey's multiple comparisons test       | Mean Diff. | 95.00% CI of diff. | Below Threshold? | Summary | Adjusted P Value |  |

|                                                          |        |                  |     |      |           |     |
|----------------------------------------------------------|--------|------------------|-----|------|-----------|-----|
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond        | 1.000  | -2.012 to 4.012  | No  | ns   | 0.906917  | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | -11.66 | -14.67 to -8.652 | Yes | **** | <0.000001 | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -53.81 | -56.82 to -50.80 | Yes | **** | <0.000001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | 2.050  | -0.9615 to 5.062 | No  | ns   | 0.297955  | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -39.33 | -42.34 to -36.32 | Yes | **** | <0.000001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | -10.61 | -13.62 to -7.602 | Yes | **** | <0.000001 | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | -12.66 | -15.67 to -9.652 | Yes | **** | <0.000001 | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -54.81 | -57.82 to -51.80 | Yes | **** | <0.000001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | 1.050  | -1.962 to 4.062  | No  | ns   | 0.886601  | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -40.33 | -43.34 to -37.32 | Yes | **** | <0.000001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | -11.61 | -14.62 to -8.602 | Yes | **** | <0.000001 | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -42.14 | -45.15 to -39.13 | Yes | **** | <0.000001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | 13.71  | 10.70 to 16.72   | Yes | **** | <0.000001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -27.67 | -30.68 to -24.66 | Yes | **** | <0.000001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 1.050  | -1.962 to 4.062  | No  | ns   | 0.886601  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 55.86  | 52.85 to 58.87   | Yes | **** | <0.000001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 14.47  | 11.46 to 17.48   | Yes | **** | <0.000001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 43.19  | 40.18 to 46.20   | Yes | **** | <0.000001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -41.38 | -44.39 to -38.37 | Yes | **** | <0.000001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | -12.66 | -15.67 to -9.652 | Yes | **** | <0.000001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 28.72  | 25.71 to 31.73   | Yes | **** | <0.000001 | F-G |

**Auxiliary Table 8:** Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol)

|                                                   |            |                    |                  |         |                  |     |
|---------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                         | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>           | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                      | 0.05       |                    |                  |         |                  |     |
| Tukey's multiple comparisons test                 | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond | 7.013      | 3.639 to 10.39     | Yes              | ****    | <0.0001          | A-B |

|                                                          |         |                  |     |      |         |     |
|----------------------------------------------------------|---------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | 6.533   | 3.159 to 9.907   | Yes | ***  | 0.0002  | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -54.75  | -58.12 to -51.38 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | 0.1533  | -3.221 to 3.527  | No  | ns   | >0.9999 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -49.80  | -53.17 to -46.42 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 0.9633  | -2.411 to 4.337  | No  | ns   | 0.9516  | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | -0.4800 | -3.854 to 2.894  | No  | ns   | 0.9987  | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -61.76  | -65.14 to -58.39 | Yes | **** | <0.0001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -6.860  | -10.23 to -3.486 | Yes | ***  | 0.0001  | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -56.81  | -60.18 to -53.44 | Yes | **** | <0.0001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | -6.050  | -9.424 to -2.676 | Yes | ***  | 0.0004  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -61.28  | -64.66 to -57.91 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -6.380  | -9.754 to -3.006 | Yes | ***  | 0.0002  | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -56.33  | -59.70 to -52.96 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | -5.570  | -8.944 to -2.196 | Yes | ***  | 0.0009  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 54.90   | 51.53 to 58.28   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 4.953   | 1.579 to 8.327   | Yes | **   | 0.0028  | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 55.71   | 52.34 to 59.09   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -49.95  | -53.32 to -46.58 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 0.8100  | -2.564 to 4.184  | No  | ns   | 0.9787  | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 50.76   | 47.39 to 54.13   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 9:** Analyzed ABTS One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol)

|                                                   |            |                    |                  |         |                  |     |
|---------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                         | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>           | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                      | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>          | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Grewia cana</i> Sond | 3.810      | 0.1043 to 7.516    | Yes              | *       | 0.0421           | A-B |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | 8.860  | 5.154 to 12.57   | Yes | **** | <0.0001 | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -53.48 | -57.18 to -49.77 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -1.667 | -5.372 to 2.039  | No  | ns   | 0.7210  | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -38.00 | -41.71 to -34.29 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 5.523  | 1.818 to 9.229   | Yes | **   | 0.0024  | A-G |
| <i>Grewia cana</i> Sond vs. <i>Vangueria infausta</i>    | 5.050  | 1.344 to 8.756   | Yes | **   | 0.0053  | B-C |
| <i>Grewia cana</i> Sond vs. <i>Ximenia caffara</i> S     | -57.29 | -60.99 to -53.58 | Yes | **** | <0.0001 | B-D |
| <i>Grewia cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -5.477 | -9.182 to -1.771 | Yes | **   | 0.0026  | B-E |
| <i>Grewia cana</i> Sond vs. <i>Ximenia americana</i> L   | -41.81 | -45.52 to -38.10 | Yes | **** | <0.0001 | B-F |
| <i>Grewia cana</i> Sond vs. <i>Grewia flava</i>          | 1.713  | -1.992 to 5.419  | No  | ns   | 0.6968  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -62.34 | -66.04 to -58.63 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -10.53 | -14.23 to -6.821 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -46.86 | -50.57 to -43.15 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | -3.337 | -7.042 to 0.3690 | No  | ns   | 0.0907  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 51.81  | 48.10 to 55.52   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 15.48  | 11.77 to 19.18   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 59.00  | 55.29 to 62.71   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -36.33 | -40.04 to -32.63 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 7.190  | 3.484 to 10.90   | Yes | ***  | 0.0002  | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 43.52  | 39.82 to 47.23   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 10:** Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol)

|                                                   |            |                    |                  |         |                  |     |
|---------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                         | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>           | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                      | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>          | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana</i> Sond | 0.4667     | -25.30 to 26.24    | No               | ns      | >0.9999          | A-B |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i>      | 7.800  | -17.97 to 33.57  | No  | ns   | 0.9372  | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -186.8 | -212.6 to -161.0 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -61.20 | -86.97 to -35.43 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -168.3 | -194.1 to -142.6 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 11.27  | -14.50 to 37.04  | No  | ns   | 0.7446  | A-G |
| <i>Gweria cana</i> Sond vs. <i>Vangueria infausta</i>    | 7.333  | -18.44 to 33.10  | No  | ns   | 0.9523  | B-C |
| <i>Gweria cana</i> Sond vs. <i>Ximenia caffara</i> S     | -187.3 | -213.0 to -161.5 | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -61.67 | -87.44 to -35.90 | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana</i> Sond vs. <i>Ximenia americana</i> L   | -168.8 | -194.6 to -143.0 | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana</i> Sond vs. <i>Grewia flava</i>          | 10.80  | -14.97 to 36.57  | No  | ns   | 0.7775  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -194.6 | -220.4 to -168.8 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -69.00 | -94.77 to -43.23 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -176.1 | -201.9 to -150.4 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 3.467  | -22.30 to 29.24  | No  | ns   | 0.9990  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 125.6  | 99.83 to 151.4   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 18.47  | -7.303 to 44.24  | No  | ns   | 0.2495  | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 198.1  | 172.3 to 223.8   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -107.1 | -132.9 to -81.36 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 72.47  | 46.70 to 98.24   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 179.6  | 153.8 to 205.4   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 11:** Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison

test (Methanol)

|                                                     |            |                    |                  |         |                  |     |
|-----------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                           | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>             | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                        | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>            | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana</i> Sond   | 2.800      | -22.39 to 27.99    | No               | ns      | 0.9997           | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i> | 8.133      | -17.06 to 33.33    | No               | ns      | 0.9172           | A-C |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -213.7 | -238.9 to -188.5 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -76.93 | -102.1 to -51.74 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -160.1 | -185.3 to -134.9 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 13.13  | -12.06 to 38.33  | No  | ns   | 0.5800  | A-G |
| <i>Gweria cana</i> Sond vs. <i>Vangueria infausta</i>    | 5.333  | -19.86 to 30.53  | No  | ns   | 0.9887  | B-C |
| <i>Gweria cana</i> Sond vs. <i>Ximenia caffara</i> S     | -216.5 | -241.7 to -191.3 | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -79.73 | -104.9 to -54.54 | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana</i> Sond vs. <i>Ximenia americana</i> L   | -162.9 | -188.1 to -137.7 | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana</i> Sond vs. <i>Grewia flava</i>          | 10.33  | -14.86 to 35.53  | No  | ns   | 0.7932  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -221.9 | -247.1 to -196.7 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -85.07 | -110.3 to -59.87 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -168.3 | -193.5 to -143.1 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 5.000  | -20.19 to 30.19  | No  | ns   | 0.9919  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 136.8  | 111.6 to 162.0   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 53.60  | 28.41 to 78.79   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 226.9  | 201.7 to 252.1   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -83.20 | -108.4 to -58.01 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 90.07  | 64.87 to 115.3   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 173.3  | 148.1 to 198.5   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 12:** Analyzed TPC One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water)

|                                                     |            |                    |                  |         |                  |     |
|-----------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                           | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>             | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                        | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>            | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana</i> Sond   | 4.267      | -10.03 to 18.57    | No               | ns      | 0.9411           | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i> | 5.267      | -9.034 to 19.57    | No               | ns      | 0.8597           | A-C |

|                                                          |        |                  |     |      |         |     |
|----------------------------------------------------------|--------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S       | -122.9 | -137.2 to -108.6 | Yes | **** | <0.0001 | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -35.13 | -49.43 to -20.83 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia Americana</i> L     | -114.8 | -129.1 to -100.5 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 5.867  | -8.434 to 20.17  | No  | ns   | 0.7930  | A-G |
| <i>Gweria cana</i> Sond vs. <i>Vangueria infausta</i>    | 1.000  | -13.30 to 15.30  | No  | ns   | >0.9999 | B-C |
| <i>Gweria cana</i> Sond vs. <i>Ximenia caffara</i> S     | -127.2 | -141.5 to -112.9 | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -39.40 | -53.70 to -25.10 | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana</i> Sond vs. <i>Ximenia americana</i> L   | -119.1 | -133.4 to -104.8 | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana</i> Sond vs. <i>Grewia flava</i>          | 1.600  | -12.70 to 15.90  | No  | ns   | 0.9997  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -128.2 | -142.5 to -113.9 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -40.40 | -54.70 to -26.10 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -120.1 | -134.4 to -105.8 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | 0.6000 | -13.70 to 14.90  | No  | ns   | >0.9999 | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 87.80  | 73.50 to 102.1   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 8.133  | -6.167 to 22.43  | No  | ns   | 0.4872  | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 128.8  | 114.5 to 143.1   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -79.67 | -93.97 to -65.37 | Yes | **** | <0.0001 | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 41.00  | 26.70 to 55.30   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia Americana</i> L vs. <i>Grewia flava</i>       | 120.7  | 106.4 to 135.0   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 13:** Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Ethanol)

|                                                     |            |                    |                  |         |                  |     |
|-----------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                           | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>             | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                        | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>            | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana</i> Sond   | 6.867      | 3.712 to 10.02     | Yes              | ****    | <0.0001          | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i> | 6.883      | 3.729 to 10.04     | Yes              | ****    | <0.0001          | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S  | -21.07     | -24.22 to -17.91   | Yes              | ****    | <0.0001          | A-D |

|                                                          |         |                  |     |      |         |     |
|----------------------------------------------------------|---------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>        | -16.23  | -19.39 to -13.08 | Yes | **** | <0.0001 | A-E |
| <i>Grewia bicolor</i> vs. <i>Ximenia americana L</i>     | -9.783  | -12.94 to -6.629 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 2.267   | -0.8876 to 5.421 | No  | ns   | 0.2470  | A-G |
| <i>Gweria cana Sond</i> vs. <i>Vangueria infausta</i>    | 0.01667 | -3.138 to 3.171  | No  | ns   | >0.9999 | B-C |
| <i>Gweria cana Sond</i> vs. <i>Ximenia caffara S</i>     | -27.93  | -31.09 to -24.78 | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana Sond</i> vs. <i>Mimusops zeyheri</i>      | -23.10  | -26.25 to -19.95 | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana Sond</i> vs. <i>Ximenia americana L</i>   | -16.65  | -19.80 to -13.50 | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana Sond</i> vs. <i>Grewia flava</i>          | -4.600  | -7.754 to -1.446 | Yes | **   | 0.0029  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara S</i>   | -27.95  | -31.10 to -24.80 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -23.12  | -26.27 to -19.96 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana L</i> | -16.67  | -19.82 to -13.51 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | -4.617  | -7.771 to -1.462 | Yes | **   | 0.0029  | C-G |
| <i>Ximenia caffara S</i> vs. <i>Mimusops zeyheri</i>     | 4.833   | 1.679 to 7.988   | Yes | **   | 0.0019  | D-E |
| <i>Ximenia caffara S</i> vs. <i>Ximenia americana L</i>  | 11.28   | 8.129 to 14.44   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara S</i> vs. <i>Grewia flava</i>         | 23.33   | 20.18 to 26.49   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana L</i>   | 6.450   | 3.296 to 9.604   | Yes | **** | 0.0001  | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 18.50   | 15.35 to 21.65   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana L</i> vs. <i>Grewia flava</i>       | 12.05   | 8.896 to 15.20   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 14:** Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Methanol)

|                                                     |            |                    |                  |         |                  |     |
|-----------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                           | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>             | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                        | 0.05       |                    |                  |         |                  |     |
| Tukey's multiple comparisons test                   | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana Sond</i>   | 6.083      | 3.787 to 8.380     | Yes              | ****    | <0.0001          | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i> | 6.583      | 4.287 to 8.880     | Yes              | ****    | <0.0001          | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara S</i>  | -25.92     | -28.21 to -23.62   | Yes              | ****    | <0.0001          | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>   | -19.42     | -21.71 to -17.12   | Yes              | ****    | <0.0001          | A-E |

|                                                          |        |                   |     |      |         |     |
|----------------------------------------------------------|--------|-------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -23.75 | -26.05 to -21.45  | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | -2.583 | -4.880 to -0.2867 | Yes | *    | 0.0232  | A-G |
| <i>Gweria cana</i> Sond vs. <i>Vangueria infausta</i>    | 0.5000 | -1.797 to 2.797   | No  | ns   | 0.9869  | B-C |
| <i>Gweria cana</i> Sond vs. <i>Ximenia caffara</i> S     | -32.00 | -34.30 to -29.70  | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -25.50 | -27.80 to -23.20  | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana</i> Sond vs. <i>Ximenia americana</i> L   | -29.83 | -32.13 to -27.54  | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana</i> Sond vs. <i>Grewia flava</i>          | -8.667 | -10.96 to -6.370  | Yes | **** | <0.0001 | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -32.50 | -34.80 to -30.20  | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -26.00 | -28.30 to -23.70  | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -30.33 | -32.63 to -28.04  | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | -9.167 | -11.46 to -6.870  | Yes | **** | <0.0001 | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 6.500  | 4.203 to 8.797    | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 2.167  | -0.1300 to 4.463  | No  | ns   | 0.0703  | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 23.33  | 21.04 to 25.63    | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | -4.333 | -6.630 to -2.037  | Yes | **** | 0.0002  | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 16.83  | 14.54 to 19.13    | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 21.17  | 18.87 to 23.46    | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 15:** Analyzed TFC One-way Ordinary ANOVA Tukey's multiple comparison test (Distilled water)

|                                                     |            |                    |                  |         |                  |     |
|-----------------------------------------------------|------------|--------------------|------------------|---------|------------------|-----|
| <b>Number of families</b>                           | 1          |                    |                  |         |                  |     |
| <b>Number of comparisons per family</b>             | 21         |                    |                  |         |                  |     |
| <b>Alpha</b>                                        | 0.05       |                    |                  |         |                  |     |
| <b>Tukey's multiple comparisons test</b>            | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |     |
| <i>Grewia bicolor</i> vs. <i>Gweria cana</i> Sond   | 1.767      | -0.2199 to 3.753   | No               | ns      | 0.0968           | A-B |
| <i>Grewia bicolor</i> vs. <i>Vangueria infausta</i> | 2.267      | 0.2801 to 4.253    | Yes              | *       | 0.0210           | A-C |
| <i>Grewia bicolor</i> vs. <i>Ximenia caffara</i> S  | -14.07     | -16.05 to -12.08   | Yes              | ****    | <0.0001          | A-D |
| <i>Grewia bicolor</i> vs. <i>Mimusops zeyheri</i>   | -9.233     | -11.22 to -7.247   | Yes              | ****    | <0.0001          | A-E |

|                                                          |         |                  |     |      |         |     |
|----------------------------------------------------------|---------|------------------|-----|------|---------|-----|
| <i>Grewia bicolor</i> vs. <i>Ximenia americana</i> L     | -9.000  | -10.99 to -7.013 | Yes | **** | <0.0001 | A-F |
| <i>Grewia bicolor</i> vs. <i>Grewia flava</i>            | 1.433   | -0.5533 to 3.420 | No  | ns   | 0.2434  | A-G |
| <i>Gweria cana</i> Sond vs. <i>Vangueria infausta</i>    | 0.5000  | -1.487 to 2.487  | No  | ns   | 0.9732  | B-C |
| <i>Gweria cana</i> Sond vs. <i>Ximenia caffara</i> S     | -15.83  | -17.82 to -13.85 | Yes | **** | <0.0001 | B-D |
| <i>Gweria cana</i> Sond vs. <i>Mimusops zeyheri</i>      | -11.00  | -12.99 to -9.013 | Yes | **** | <0.0001 | B-E |
| <i>Gweria cana</i> Sond vs. <i>Ximenia americana</i> L   | -10.77  | -12.75 to -8.780 | Yes | **** | <0.0001 | B-F |
| <i>Gweria cana</i> Sond vs. <i>Grewia flava</i>          | -0.3333 | -2.320 to 1.653  | No  | ns   | 0.9967  | B-G |
| <i>Vangueria infausta</i> vs. <i>Ximenia caffara</i> S   | -16.33  | -18.32 to -14.35 | Yes | **** | <0.0001 | C-D |
| <i>Vangueria infausta</i> vs. <i>Mimusops zeyheri</i>    | -11.50  | -13.49 to -9.513 | Yes | **** | <0.0001 | C-E |
| <i>Vangueria infausta</i> vs. <i>Ximenia americana</i> L | -11.27  | -13.25 to -9.280 | Yes | **** | <0.0001 | C-F |
| <i>Vangueria infausta</i> vs. <i>Grewia flava</i>        | -0.8333 | -2.820 to 1.153  | No  | ns   | 0.7768  | C-G |
| <i>Ximenia caffara</i> S vs. <i>Mimusops zeyheri</i>     | 4.833   | 2.847 to 6.820   | Yes | **** | <0.0001 | D-E |
| <i>Ximenia caffara</i> S vs. <i>Ximenia americana</i> L  | 5.067   | 3.080 to 7.053   | Yes | **** | <0.0001 | D-F |
| <i>Ximenia caffara</i> S vs. <i>Grewia flava</i>         | 15.50   | 13.51 to 17.49   | Yes | **** | <0.0001 | D-G |
| <i>Mimusops zeyheri</i> vs. <i>Ximenia americana</i> L   | 0.2333  | -1.753 to 2.220  | No  | ns   | 0.9995  | E-F |
| <i>Mimusops zeyheri</i> vs. <i>Grewia flava</i>          | 10.67   | 8.680 to 12.65   | Yes | **** | <0.0001 | E-G |
| <i>Ximenia americana</i> L vs. <i>Grewia flava</i>       | 10.43   | 8.447 to 12.42   | Yes | **** | <0.0001 | F-G |

**Auxiliary Table 16:** Total Phenolic and Flavonoid Content of the wild edible fruits examined per 10 mg dry sample

| Solvent used for extraction | Edible wild fruit sample | Total Phenolic Content (mg GAE/ 10 mg DW) | Total Flavonoid Content (mg AAE/10 mg DW) |
|-----------------------------|--------------------------|-------------------------------------------|-------------------------------------------|
|                             | <i>Grewia bicolor</i>    | 20.05 ±0.12 <sup>b</sup>                  | 12.35 ±0.74 <sup>b</sup>                  |
|                             | <i>Grewia cana</i> Sond  | 17.25 ±0.95 <sup>a</sup>                  | 6.25 ±0.50 <sup>a</sup>                   |
|                             | <i>Grewia flava</i>      | 6.91 ±0.70 <sup>a</sup>                   | 14.92 ±0.76 <sup>c</sup>                  |

|                        |                                               |                            |                          |
|------------------------|-----------------------------------------------|----------------------------|--------------------------|
| <b>Methanol</b>        | <i>Ximenia caffra S</i>                       | 233.78 ±21.07 <sup>c</sup> | 38.25 ±1.00 <sup>e</sup> |
|                        | <i>Ximenia americana L</i>                    | 180.18 ±6.00 <sup>c</sup>  | 36.08 ±1.04 <sup>e</sup> |
|                        | <i>Mimusops zeyheri</i>                       | 96.98 ±9.46 <sup>b</sup>   | 31.75 ±1.00 <sup>d</sup> |
|                        | <i>Vangueria infausta subsp.Infausta (VI)</i> | 11.91 ±0.90 <sup>a</sup>   | 6.25 ±0.50 <sup>a</sup>  |
| <b>Ethanol</b>         | <i>Grewia bicolor</i>                         | 16.31 ±0.31 <sup>a</sup>   | 10.52 ±0.53 <sup>b</sup> |
|                        | <i>Grewia cana Sond</i>                       | 15.85 ±0.58 <sup>a</sup>   | 3.65 ±0.23 <sup>a</sup>  |
|                        | <i>Grewia flava</i>                           | 5.05 ±0.50 <sup>a</sup>    | 8.25 ±0.50 <sup>b</sup>  |
|                        | <i>Ximenia caffra S</i>                       | 203.11 ±20.53 <sup>c</sup> | 31.58 ±1.04 <sup>e</sup> |
|                        | <i>Ximenia americana L</i>                    | 184.65 ±1.80 <sup>c</sup>  | 20.30 ±1.38 <sup>c</sup> |
|                        | <i>Mimusops zeyheri</i>                       | 77.51 ±13.11 <sup>b</sup>  | 26.75 ±2.29 <sup>d</sup> |
|                        | <i>Vangueria infausta subsp.Infausta (VI)</i> | 8.51 ±1.01 <sup>a</sup>    | 3.63 ±0.38 <sup>a</sup>  |
| <b>Distilled water</b> | <i>Grewia bicolor</i>                         | 10.11 ±1.50 <sup>a</sup>   | 4.35 ±0.48 <sup>a</sup>  |
|                        | <i>Grewia cana Sond</i>                       | 5.85 ±0.83 <sup>a</sup>    | 2.42 ±0.29 <sup>ab</sup> |
|                        | <i>Grewia flava</i>                           | 4.25 ±0.83 <sup>a</sup>    | 2.92 ±0.29 <sup>ab</sup> |
|                        | <i>Ximenia caffra S</i>                       | 133.05 ±13.17 <sup>c</sup> | 18.42 ±0.76 <sup>d</sup> |
|                        | <i>Ximenia americana L</i>                    | 124.91 ±1.90 <sup>c</sup>  | 13.35 ±0.95 <sup>c</sup> |
|                        | <i>Mimusops zeyheri</i>                       | 45.25 ±1.72 <sup>b</sup>   | 13.58 ±1.26 <sup>c</sup> |
|                        | <i>Vangueria infausta</i>                     | 4.85 ±0.70 <sup>a</sup>    | 2.08 ±0.29 <sup>b</sup>  |

|  |                             |  |  |
|--|-----------------------------|--|--|
|  | <i>subsp. Infausta (VI)</i> |  |  |
|--|-----------------------------|--|--|

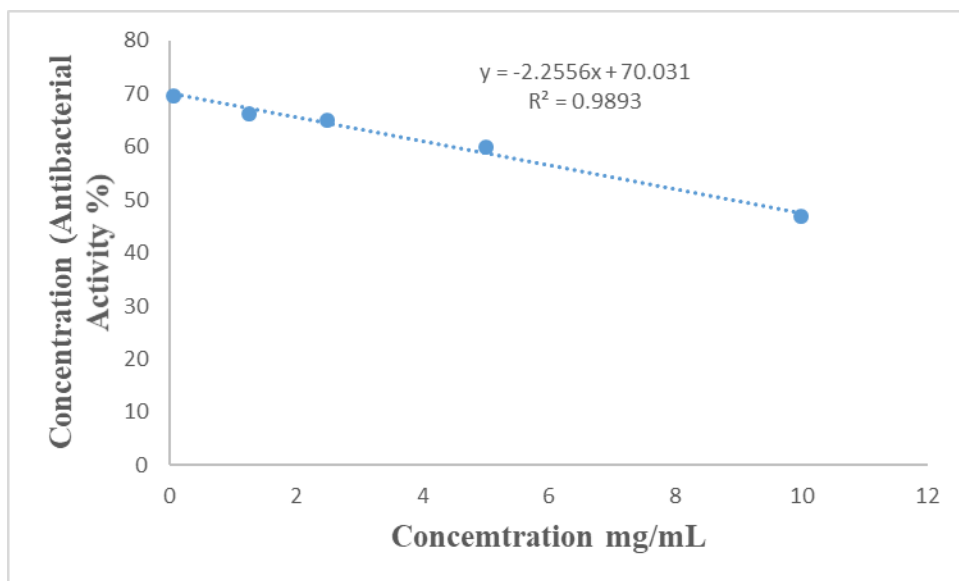
Values are presented as the mean  $\pm$  standard error of 3 replicates (n=3). Same subscripts in the same column show no significant difference at  $P > 0.05$  (Tukey-Kramer's multiple range test) within each solvent.

**Auxiliary Table 17:** fruit extracts' DPPH radical scavenging activity compared to that of ABTS per 10 mg of dry sample

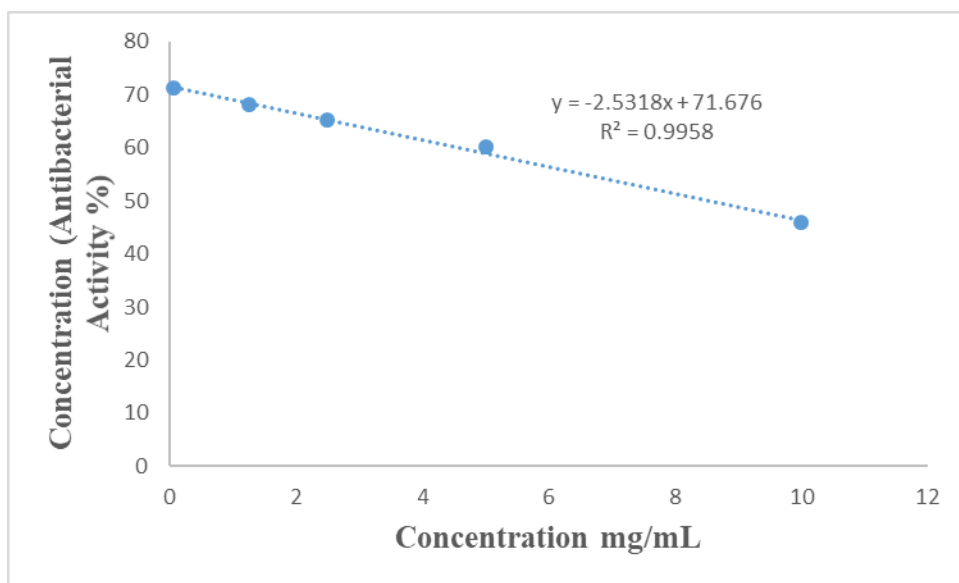
| Solvent used for extraction | Edible wild fruit sample | DPPH Concentration (mg QAE/10 mg DW) | ABTS Concentration (mg AAE/10 mg DW) |
|-----------------------------|--------------------------|--------------------------------------|--------------------------------------|
|                             | <i>Grewia bicolor</i>    | 20.90 $\pm$ 0.25 <sup>a</sup>        | 13.68 $\pm$ 1.00 <sup>c</sup>        |
|                             | <i>Grewia cana Sond</i>  | 32.34 $\pm$ 0.47 <sup>d</sup>        | 9.88 $\pm$ 2.36 <sup>a</sup>         |
|                             | <i>Grewia flava</i>      | 15.30 $\pm$ 0.96 <sup>b</sup>        | 8.16 $\pm$ 1.84 <sup>ab</sup>        |
|                             | <i>Ximenia caffra S</i>  | 91.44 $\pm$ 0.68 <sup>g</sup>        | 67.16 $\pm$ 0.95 <sup>e</sup>        |
|                             | <i>Ximenia</i>           | 82.40 $\pm$ 1.17 <sup>f</sup>        | 51.69 $\pm$ 1.00 <sup>d</sup>        |

|                        |                                                          |                               |                                |
|------------------------|----------------------------------------------------------|-------------------------------|--------------------------------|
| <b>Methanol</b>        | <i>americana L</i>                                       |                               |                                |
|                        | <i>Mimusops zeyheri</i> ,                                | 68.30 $\pm$ 0.99 <sup>e</sup> | 15.35 $\pm$ 0.64 <sup>c</sup>  |
|                        | <i>Vangueria infausta</i><br><i>subsp. nfausta (VI)</i>  | 24.77 $\pm$ 0.46 <sup>c</sup> | 4.83 $\pm$ 0.25 <sup>b</sup>   |
| <b>Ethanol</b>         | <i>Grewia bicolor</i>                                    | 18.40 $\pm$ 1.07 <sup>a</sup> | 13.35 $\pm$ 1.57 <sup>b</sup>  |
|                        | <i>Grewia cana Sond</i>                                  | 34.70 $\pm$ 0.47 <sup>d</sup> | 6.45 $\pm$ 0.36 <sup>a</sup>   |
|                        | <i>Grewia flava</i>                                      | 13.40 $\pm$ 0.86 <sup>b</sup> | 12.50 $\pm$ 1.09 <sup>ab</sup> |
|                        | <i>Ximenia caffra S</i>                                  | 87.57 $\pm$ 1.25 <sup>g</sup> | 68.21 $\pm$ 1.22 <sup>e</sup>  |
|                        | <i>Ximenia</i><br><i>americana L</i>                     | 83.10 $\pm$ 0.38 <sup>e</sup> | 63.26 $\pm$ 1.61 <sup>d</sup>  |
|                        | <i>Mimusops zeyheri</i>                                  | 66.07 $\pm$ 0.26 <sup>f</sup> | 13.31 $\pm$ 1.64 <sup>c</sup>  |
|                        | <i>Vangueria infausta</i><br><i>subsp. Infausta (VI)</i> | 26.74 $\pm$ 0.51 <sup>c</sup> | 6.92 $\pm$ 0.30 <sup>b</sup>   |
| <b>Distilled water</b> | <i>Grewia bicolor</i>                                    | 17.90 $\pm$ 0.40 <sup>a</sup> | 2.78 $\pm$ 0.50 <sup>a</sup>   |
|                        | <i>Grewia cana Sond</i>                                  | 20.60 $\pm$ 0.60 <sup>b</sup> | 1.78 $\pm$ 0.08 <sup>a</sup>   |
|                        | <i>Grewia flava</i>                                      | 17.87 $\pm$ 0.36 <sup>a</sup> | 13.40 $\pm$ 0.62 <sup>b</sup>  |
|                        | <i>Ximenia caffra S</i>                                  | 87.10 $\pm$ 0.58 <sup>e</sup> | 56.59 $\pm$ 1.22 <sup>d</sup>  |
|                        | <i>Ximenia</i><br><i>americana L</i>                     | 73.70 $\pm$ 0.42 <sup>d</sup> | 42.11 $\pm$ 0.66 <sup>c</sup>  |
|                        | <i>Mimusops zeyheri</i>                                  | 57.20 $\pm$ 0.95 <sup>c</sup> | 7.35 $\pm$ 0.46 <sup>a</sup>   |
|                        | <i>Vangueria infausta</i><br><i>subsp. Infausta (VI)</i> | 18.10 $\pm$ 0.61 <sup>a</sup> | 14.45 $\pm$ 2.36 <sup>b</sup>  |

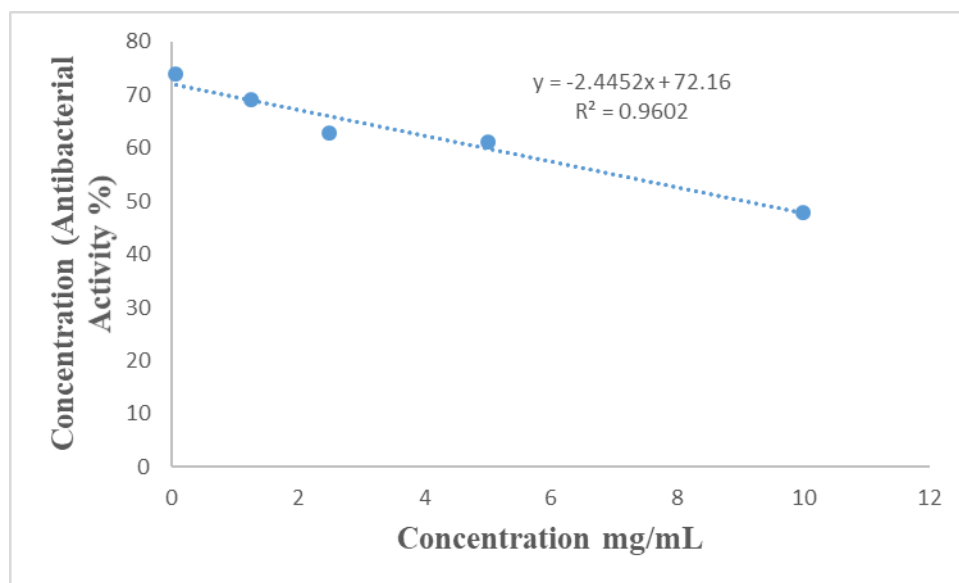
Values are presented as the mean  $\pm$  standard error of 3 replicates (n=3). The same subscripts in the same column within individual solvent show no significant difference at  $P > 0.05$  (Tukey-Kramer's multiple range test)



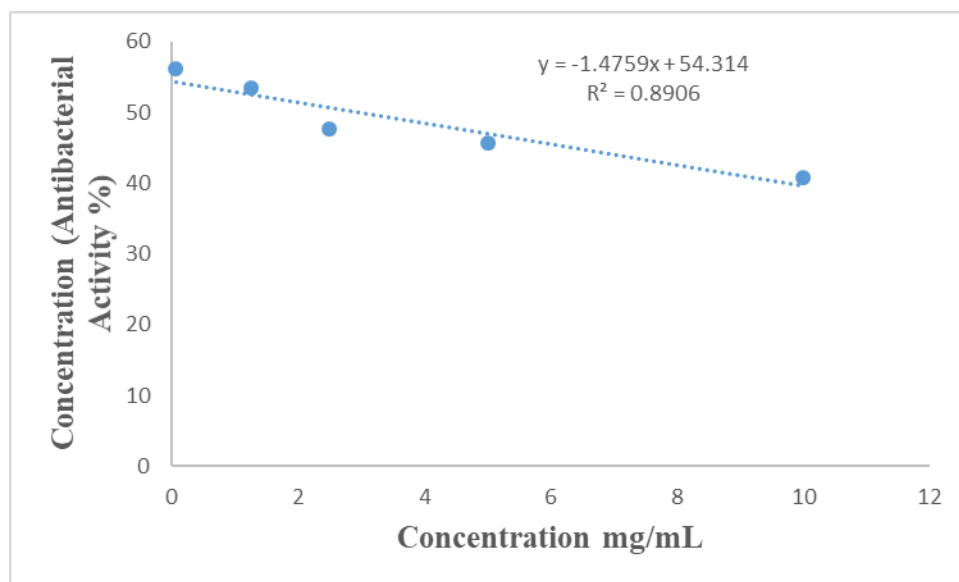
**Auxiliary Figure 20:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *S. aureus* bacteria (Trial #1)



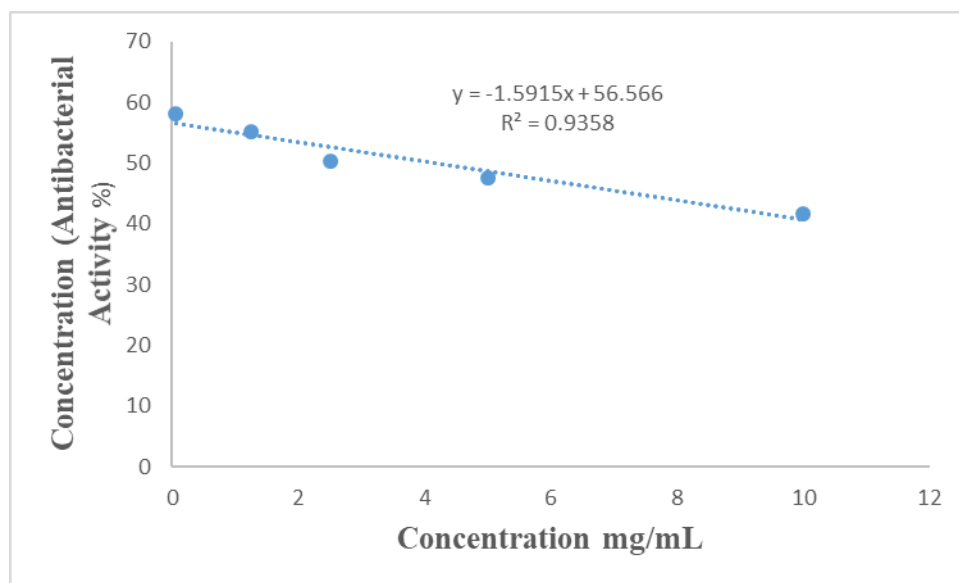
**Auxiliary Figure 21:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *S. aureus* bacteria (Trial #2)



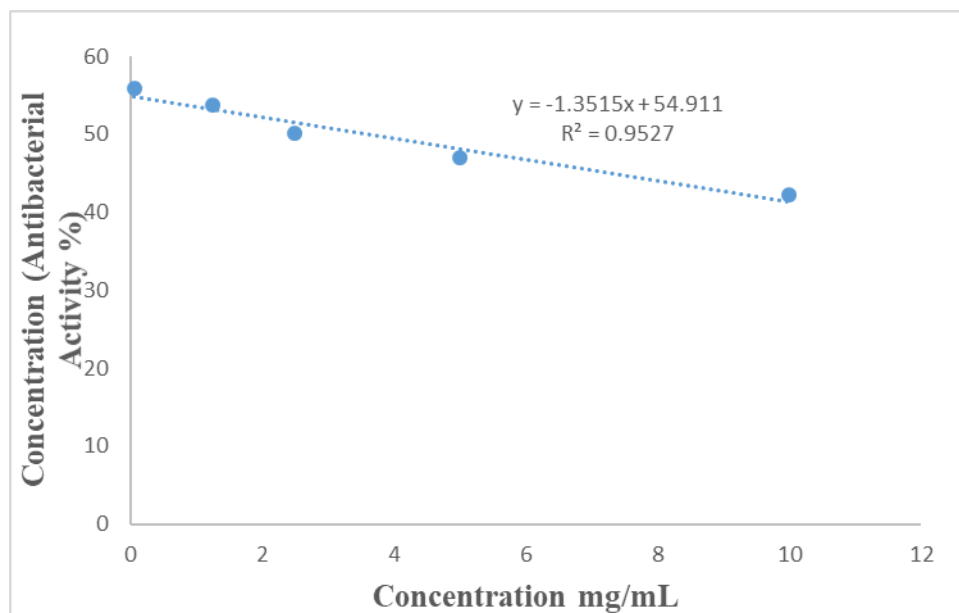
**Auxiliary Figure 22:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *S. aureus* bacteria (Trial #3)



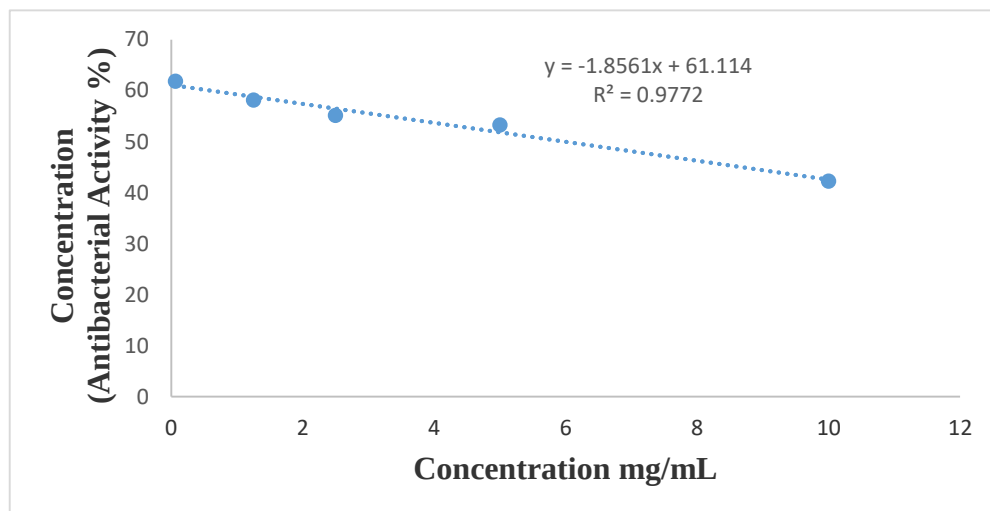
**Auxiliary Figure 23:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *S. aureus* bacteria (Trial #1)



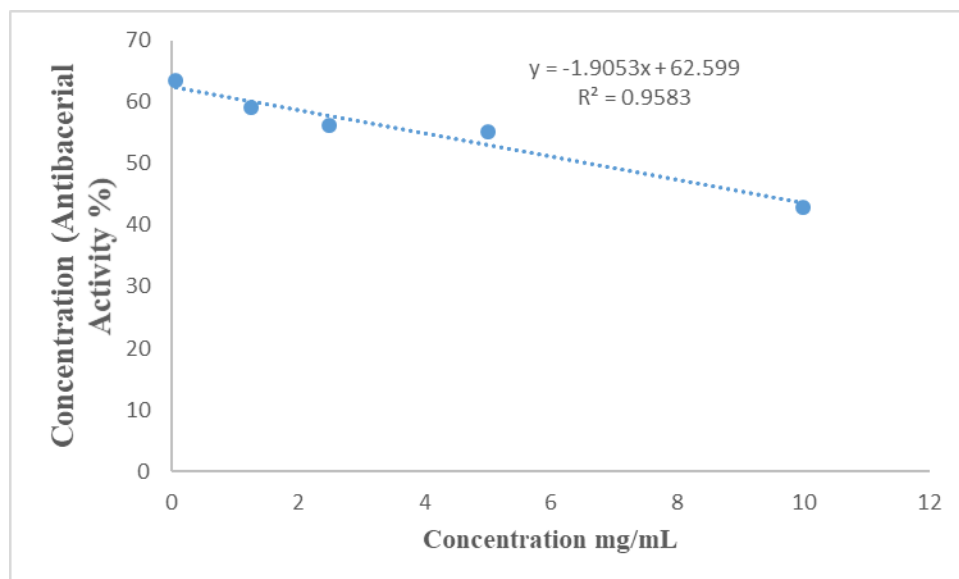
**Auxiliary Figure 24:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *S. aureus* bacteria (Trial #2)



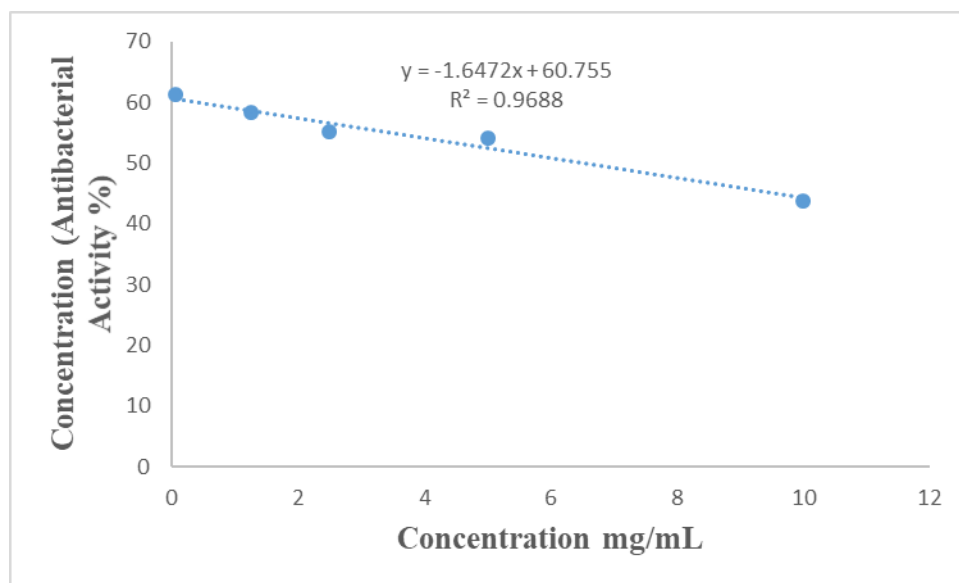
**Auxiliary Figure 25:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *S. aureus* bacteria (Trial #3)



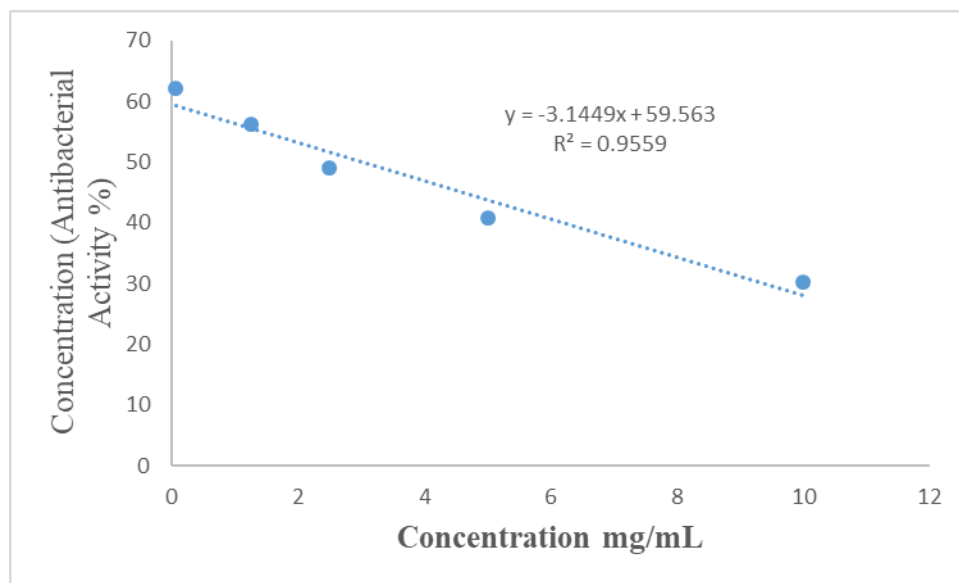
**Auxiliary Figure 26:** Auxillary Figure Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia americana* L for *S. aureus* bacteria (Trial #1)



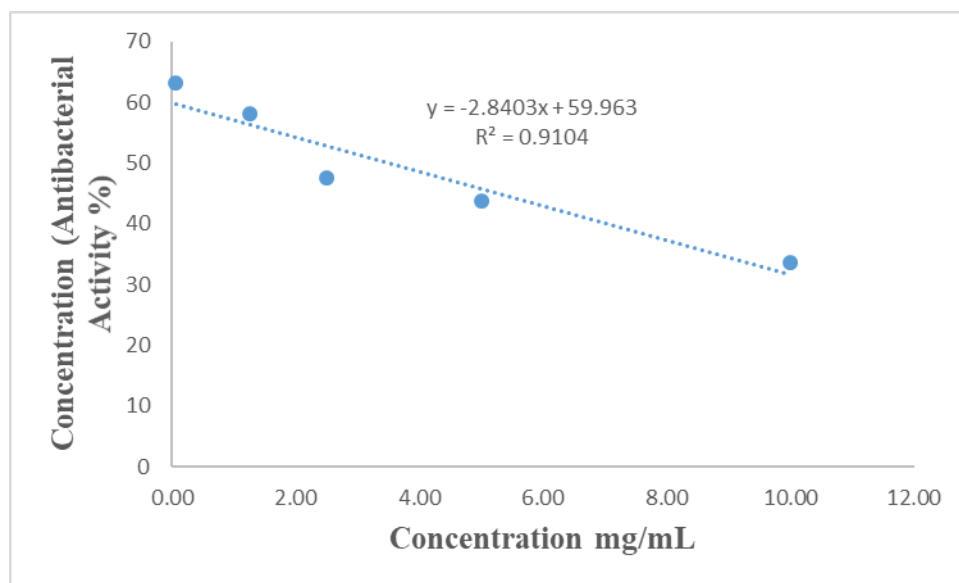
**Auxiliary Figure 27:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia americana L* for *S. aureus* bacteria (Trial #2)



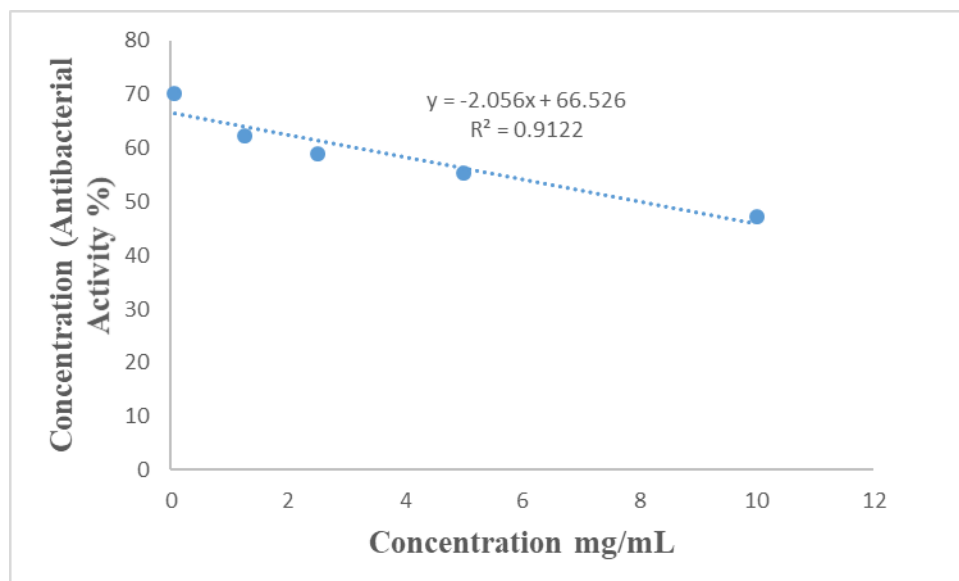
**Auxiliary Figure 28:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia Americana L* for *S. aureus* bacteria (Trial #3)



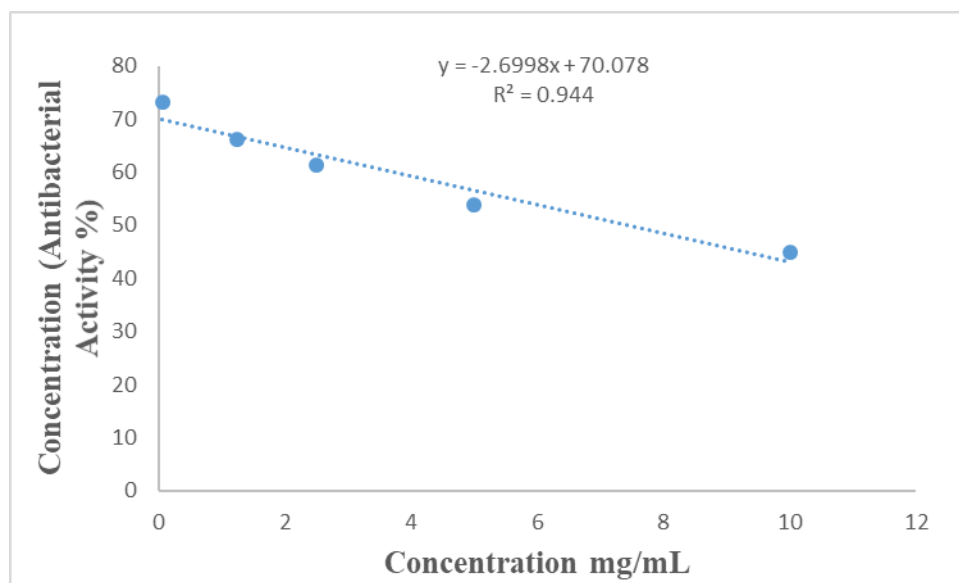
**Antimicrobial Figure 29:** activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *E. coli* bacteria (Trial #1)



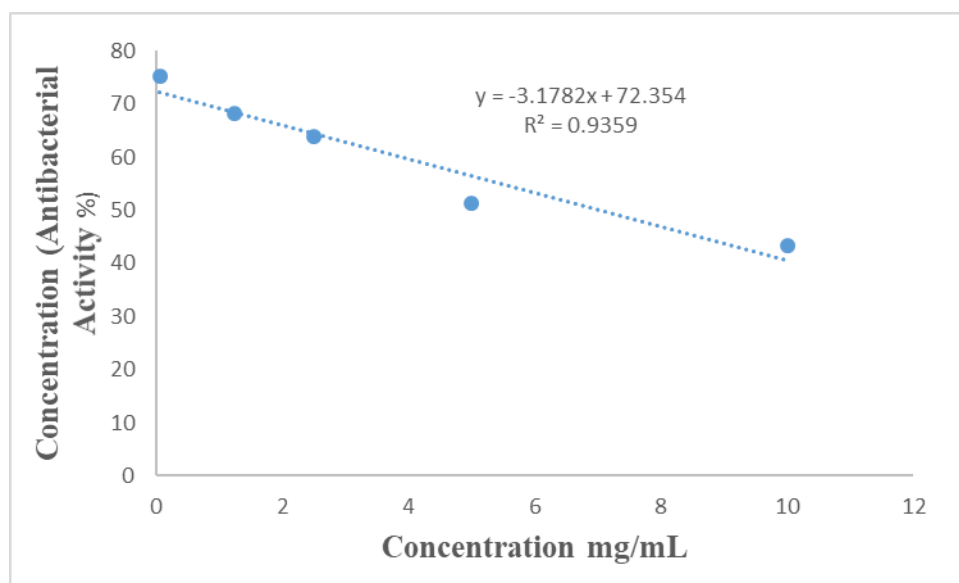
**Auxiliary Figure 30:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *E. coli* bacteria (Trial #2)



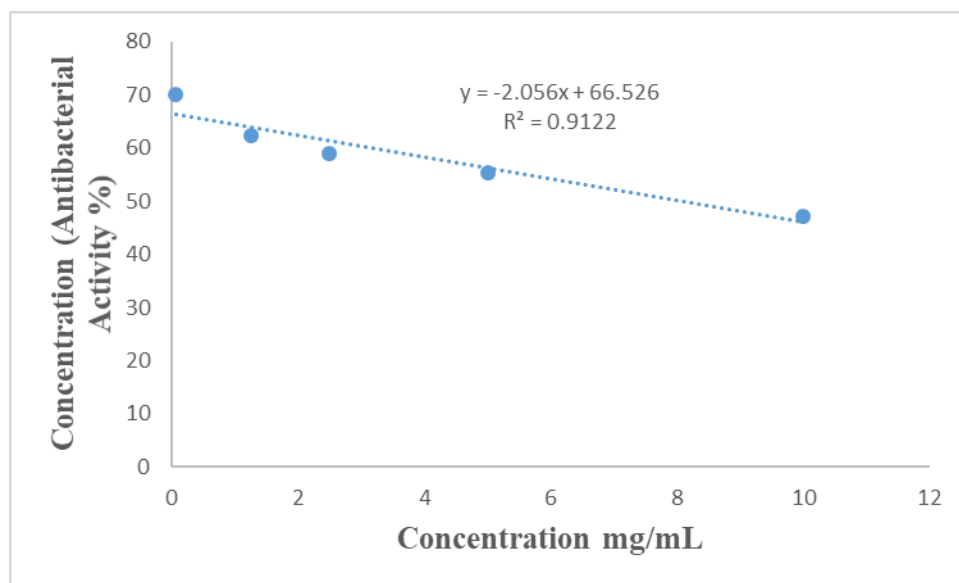
**Auxiliary Figure 31:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia caffra* S for *E. coli* bacteria (Trial #3)



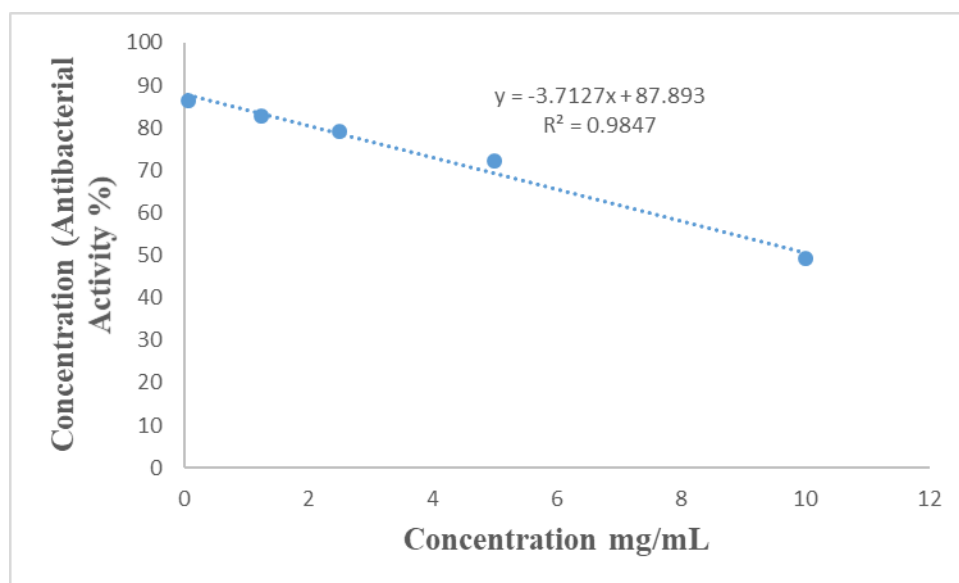
**Auxiliary Figure 32:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia americana* L for *E. coli* bacteria (Trial #1)



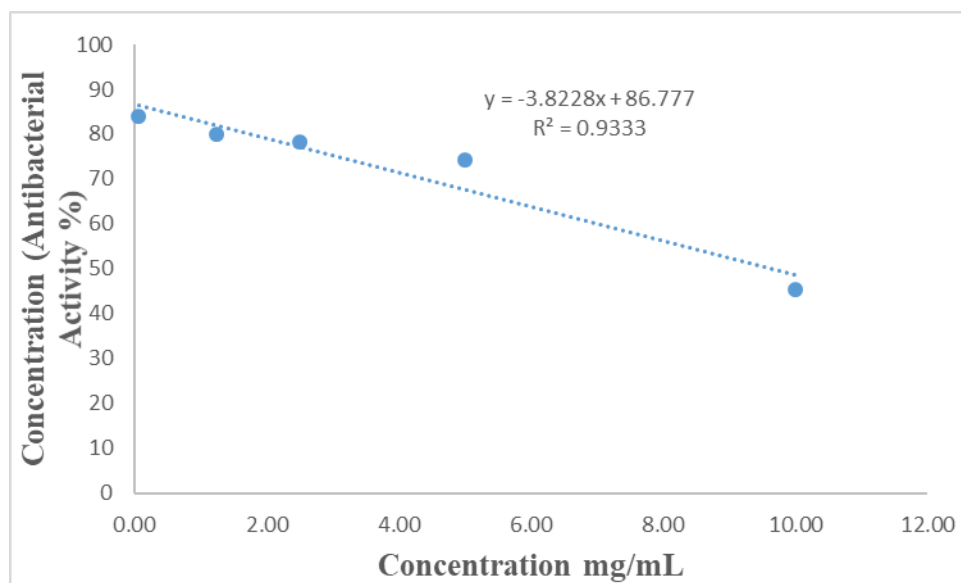
**Auxiliary Figure 33:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia americana L* for *E. coli* bacteria (Trial #2)



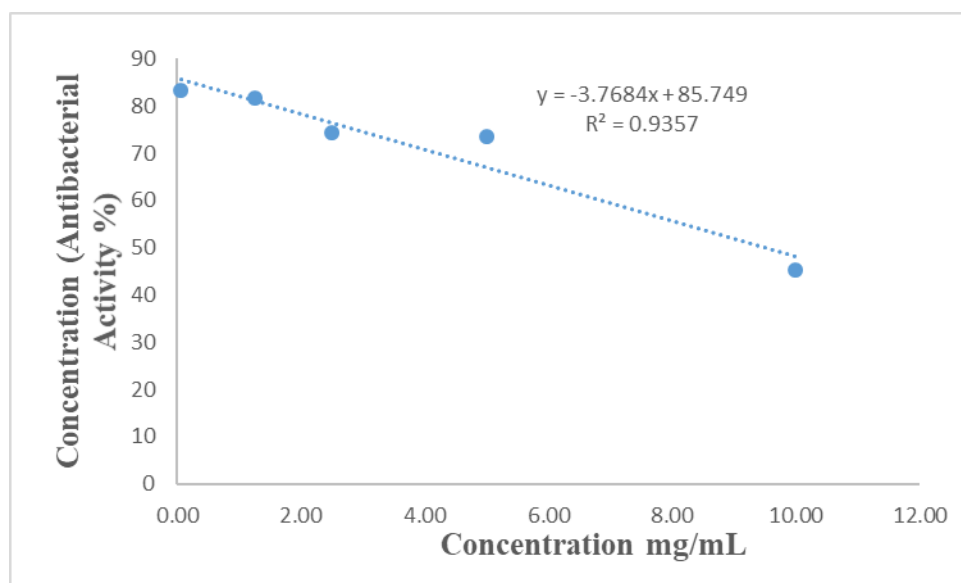
**Auxiliary Figure 34:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Ximenia americana L* for *E. coli* bacteria (Trial #3)



**Auxiliary Figure 35:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *E. coli* bacteria (Trial #1)

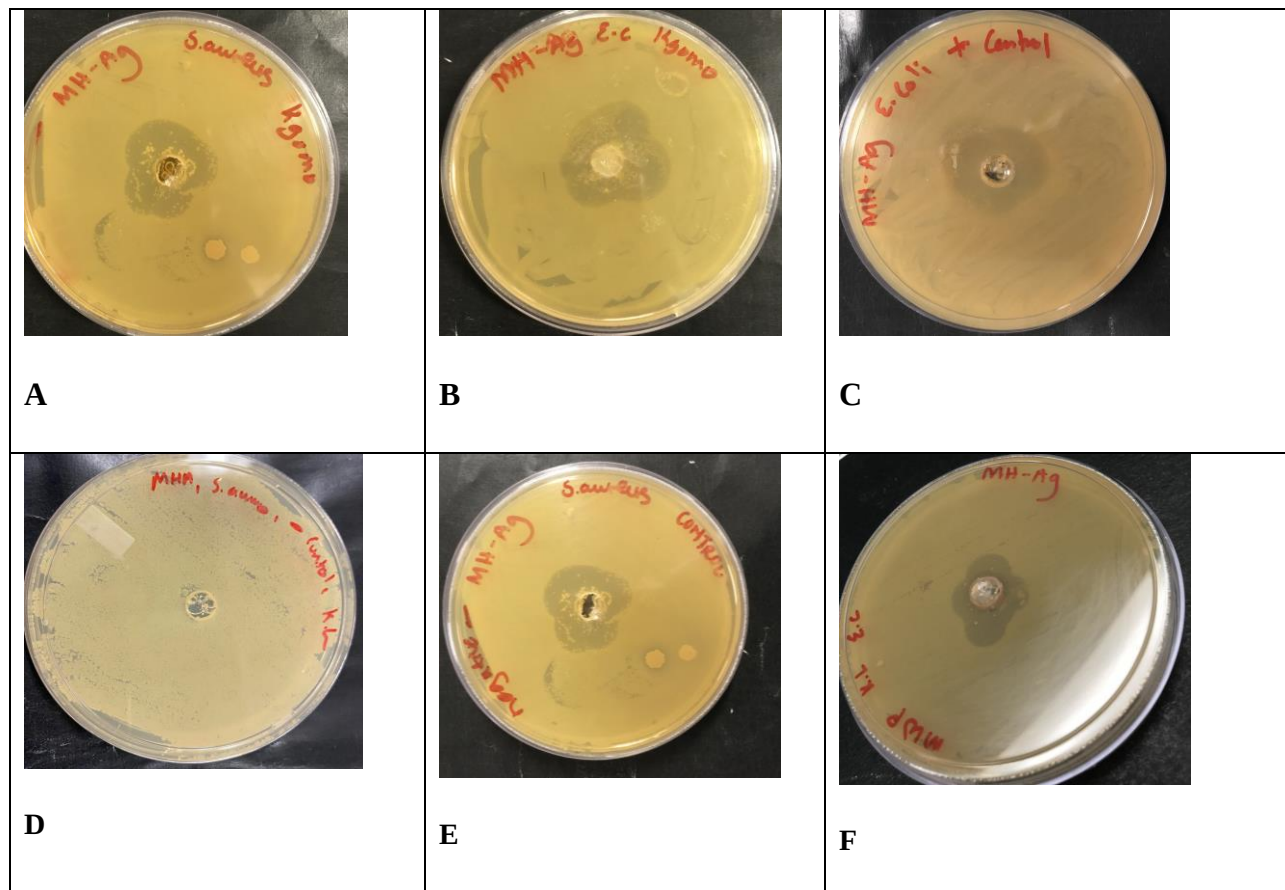


**Auxiliary Figure 36:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *E. coli* bacteria (Trial #2)



**Auxiliary Figure 37:** Antimicrobial activity (Minimum Inhibitory Concentration) of methanolic extract of *Mimusops zeyheri* for *E. coli* bacteria (Trial #3)

**Auxiliary Table 18:** below shows the zone of inhibition from the selected plate of Bacterial strains (*S. aureus* and *E. coli*). A= (*X. caffra* S for *S. aureus* bacteria), B= (*X. caffra* S for *E. coli*), C= (Gentamici positive control for *E. coli*), D= (Negative control for *S. aureus*), E= (*X. americana* L for *E. coli* bacterial strain), F= (*Mimusops zeyheri* for *S. aureus* bacterial strain), G= (Gentamicin positive control for *E. coli*), H= (*Ximenia americana* L for *E. coli* bacterial strain), I= (*X. americana* L for *S. aureus* bacterial strain), J= (*X. caffra* S for *S. aureus* bacteria), J= (*Mimusops zeyheri* *E. coli* bacteria), K= (Negative Control for *S. aureus* bacteria)

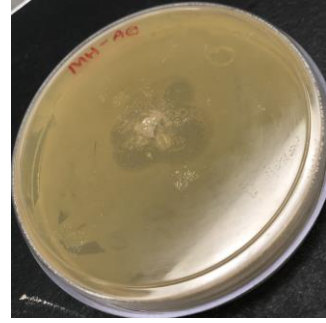




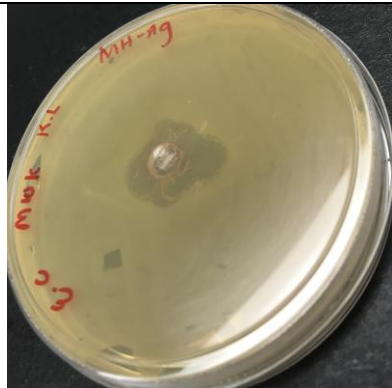
G



H



I



J



K

