TABLE OF CONTENTS

ABSTRACTS ii
ABSTRAK iv
ACKNOWLEDGEMENTS v
TABLE OF CONTENTS vi
LIST OF TABLES xi
LIST OF FIGURES xii
LIST OF ABBREVIATIONS xv

CHAPTER 1: INTRODUCTION
1.0 Introduction 1
1.1 Problem Statement 3
1.2 Objectives of study 4

CHAPTER 2: LITERATURE REVIEW
2.1 *Hibiscus sabdariffa* L. 5
  2.1.1 Botanical Description 5
  2.1.2 Medicinal Value 7
  2.1.3 Antioxidant Activity and Anticholesterol Effects of *H. sabdariffa* 8
  2.1.4 Phytochemical Constituents 9
  2.1.5 UKMR-1 and UKMR-2 variety 10
2.2 Atherosclerosis 12
  2.2.1 Development of Atherosclerosis 12
  2.2.2 High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) 15
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.3</td>
<td>Cholesterol</td>
<td>18</td>
</tr>
<tr>
<td>2.2.4</td>
<td>Triglycerides</td>
<td>20</td>
</tr>
<tr>
<td>2.3</td>
<td>Antioxidant</td>
<td>22</td>
</tr>
</tbody>
</table>

**CHAPTER 3: MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Experimental Material</td>
<td>25</td>
</tr>
<tr>
<td>3.2</td>
<td>Preparation of extracts</td>
<td>25</td>
</tr>
<tr>
<td>3.3</td>
<td>Antioxidant Activity Assay</td>
<td>26</td>
</tr>
<tr>
<td>3.3.1</td>
<td>DPPH Free Radical Scavenging Activity Assay</td>
<td>26</td>
</tr>
<tr>
<td>3.3.1.1</td>
<td>Ascorbic Acid as a Positive References Standard</td>
<td>26</td>
</tr>
<tr>
<td>3.3.1.2</td>
<td>DPPH radical scavenging activity of crude extracts</td>
<td>28</td>
</tr>
<tr>
<td>3.3.1.3</td>
<td>Determination of Percentage of Inhibition</td>
<td>28</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Reducing Power Assay</td>
<td>29</td>
</tr>
<tr>
<td>3.3.2.1</td>
<td>Reducing Power of Standard BHA</td>
<td>29</td>
</tr>
<tr>
<td>3.3.2.2</td>
<td>Reducing Power of Crude Extracts</td>
<td>30</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Metal Chelating Assay</td>
<td>31</td>
</tr>
<tr>
<td>3.3.3.1</td>
<td>Metal Chelating for EDTA Positive Reference Standard</td>
<td>31</td>
</tr>
<tr>
<td>3.3.3.2</td>
<td>Metal Chelating for Crude Extracts</td>
<td>32</td>
</tr>
<tr>
<td>3.3.3.3</td>
<td>Determination of Percentage of Inhibition</td>
<td>32</td>
</tr>
<tr>
<td>3.4</td>
<td>Toxicity Test</td>
<td>33</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Brine Shrimp Lethality Assay</td>
<td>33</td>
</tr>
<tr>
<td>3.4.1.1</td>
<td>Sample Preparation</td>
<td>33</td>
</tr>
<tr>
<td>3.4.1.2</td>
<td>Hatching the Shrimp</td>
<td>34</td>
</tr>
<tr>
<td>3.4.1.3</td>
<td>Test Procedure</td>
<td>34</td>
</tr>
</tbody>
</table>
3.4.2 Oral toxicity Test
  3.4.2.1 Animals and Maintenance 35
  3.4.2.2 Administration of Doses 36
  3.4.2.3 Clinical Observation 37
  3.4.2.4 Clinical Biochemistry Analysis 37
  3.4.2.5 Histopathology 38

3.5 Hypercholesterolemic Assay 39
  3.5.1 Total Cholesterol Measurement 41
  3.5.2 Triglycerides Measurement 42
  3.5.3 High Density Lipoprotein (HDL) Cholesterol Measurement 43
  3.5.4 Atherosclerotic Plagues Analysis 44
    3.5.4.1 Sample Abstraction 44
    3.5.4.2 Fixation and Staining of Organ Specimens 45

3.6 Phytochemical Analysis 46
  3.6.1 Thin-layer Chromatography (TLC) 46
  3.6.2 LC-MS/MS Analysis 47
    3.6.2.1 Sample Preparation 47
    3.6.2.2 LCMS/MS conditions 47

3.7 Statistical analysis 48

CHAPTER 4: RESULTS
4.1 Extraction of Samples 49
  4.1.1 Water Content in Plant Samples 49
  4.1.2 Average Yield of Crude Extract 50
4.2 Antioxidant Activity Assay
   4.2.1 DPPH Free Radical Scavenging Activity Assay
   4.2.2 Reducing Power Assay
   4.2.3 Metal Chelating Assay

4.3 Toxicity Test
   4.3.1 Brine Shrimp Lethality Assay
   4.3.2 Oral toxicity Test
      4.3.2.1 Clinical Observation
      4.3.2.2 Clinical Biochemistry Analysis
      4.3.2.3 Histopathology Analysis

4.4 Hypercholesterolemic Analysis
   4.4.1 Body weight
   4.4.2 Triglycerides Measurement
   4.4.3 Total Cholesterol Measurement
   4.4.4 High Density Lipoprotein (HDL) Cholesterol Measurement
   4.4.5 Low Density Lipoprotein (LDL) Cholesterol Measurement
   4.4.6 Atherosclerotic Plagues Analysis
      4.4.6.1 Atherosclerotic Plagues Analysis for Normal Group
      4.4.6.2 Atherosclerotic Plagues Analysis for Cholesterol Group
      4.4.6.3 Atherosclerotic Plagues Analysis for Arab Group
      4.4.6.4 Atherosclerotic Plagues Analysis for UKMR-1 Group
      4.4.6.5 Atherosclerotic Plagues Analysis for UKMR-2 Group

4.5 Phytochemical Analysis
   4.5.1 Thin layer Chromatography (TLC)
      4.5.1.1 Thin layer Chromatography (TLC) of Arab Variety
      4.5.1.2 Thin layer Chromatography (TLC) of UKMR-1 Variety
      4.5.1.3 Thin layer Chromatography (TLC) of UKMR-2 Variety
4.5.2 LC-MS/MS Analysis
  4.5.2.1 LC-MS/MS Analysis of Arab Variety 93
  4.5.2.2 LC-MS/MS Analysis of UKMR-1 Variety 108
  4.5.2.3 LC-MS/MS Analysis of UKMR-2 Variety 123

CHAPTER 5: DISCUSSION

5.1 Preparation of Plant extracts 134

5.2 Analysis of Antioxidant Activity 136
  5.2.1 DPPH Free Radical Scavenging Activity Assay 137
  5.2.2 Reducing Power Assay 138
  5.2.3 Metal Chelating Assay 139

5.3 Toxicity Test 141
  5.3.1 Brine Shrimp Lethality Assay 141
  5.3.2 Oral toxicity Test 142
    5.3.2.1 Clinical Observation 143
    5.3.2.2 Clinical Biochemistry Analysis 144
      i. Liver function test 144
      ii. Renal function test 149
  5.3.2.3 Histopathology Analysis 152

5.4 Hypercholesterolemic Analysis 153
  5.4.1 Body weight 153
  5.4.2 Triglycerides Measurement 154
  5.4.3 Total Cholesterol Measurement 157
  5.4.4 High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) Cholesterol Measurement 159
  5.4.5 Atherosclerotic Plagues Analysis 161
5.5 Phytochemical Analysis  
5.5.1 Thin layer Chromatography (TLC) Analysis  
5.5.2 LC-MS/MS Analysis  

CHAPTER 6: CONCLUSION  

APENDIX  

References  
Publication