BOTANICAL AND CHEMICAL STUDIES ON SOLANOSTEMA ARGEL

(DEL.) HAYNE GROWN IN KHARTOUM

BY

HATIL, HASHIM AHMED EL-KAMALI

B.Sc. and Ed. (Khartoum)

[1986]

A thesis

submitted to the University of Khartoum

in partial fulfilment of the requirement

for the Degree of Master of Science

University of Khartoum
Faculty of Education
Department of Biology

November 1991
ACKNOWLEDGEMENTS

In presenting this thesis, I wish to express my deepest sense of gratitude to my supervisor, Dr. Abdella El-Khidir Medani, Biology Department, Faculty of Education, for his advice, guidance and encouragement.

I am grateful to my co-supervisor Dr. Mohamed Osman Abdalla El-Sheikh, Medical and Aromatic Herbs Research Institute, for his able guidance through every stage from the initial planning and writing to the final reviewing and editing of this work.

I am also indebted to Dr. Abdul Gabar Nasir, Biology Department, Faculty of Education, for his advice and careful revision of the thesis.

Thanks are also due to the Technical staff of Biology and Chemistry Departments of the Faculty of Education, Chemistry lab of Ahfad College, Geological Research Authority and lastly Medicinal and Aromatic Herbs Research Institute.

I would also like to record my thanks to Mr. Kamal El-Jack, Botany Department, Faculty of Science for his kind help and cooperative. My thanks are also due to Dr. Isam El-Toum, Pathology Department, Faculty of Medicine and Mr. El-Hag Abass, Medical Illustrations unit for the photographs.

Thanks are also due to all those who cooperative with me in the different aspects of this work.

Finally, special thanks are extended to my family whose encouragement and morale were invaluable.
Solmsiaweta argel (Del.) Hayne known as "bargel" in the Sudan was selected for investigation because of its use in popular medicine. Morphological and anatomical studies of the different organs (viz. leaves, stems, roots, flowers & fruits) of argel plants were carried out. Proximate analysis and general phytochemical screening were performed on leaves, stems and roots for the possible presence of certain active constituents with relation to spacing (25 cm, 50 cm, 75 cm & 100 cm) at the plant pre-flowering and flowering stages. Results of phytochemical screening showed the presence of a number of chemical groups, the most important of them are the saponins. In addition, traces of alkaloids were also detected. Distance between plants seems to have no significant effect on the amounts of active ingredients as shown by difference in intensity of colours resulting from the different screening tests. Recommendations were suggested for detailed analysis of saponins and alkaloids as well as raw proteins of argel leaves.
تنامي سولونتيما إرجل هو أحد أجزاء العلاقة الفقارية والتي تضم العديد من النباتات ذات الأوراق الطبية التي لها rôle في بضخامة القلب. ويعتبر هذا النبات من الخضروات التي تنتشر في السودان بأسماع إرجل وقد اكتسبت هيكل هذه النباتات لفائدة شائعة للفائدة في بعض الظروف لعلاج الكبد وضف العروق وتحقيق التوريد وتفعيل. فقد تم تأكيد مرة دراسة النباتات مورفولوجيا وتاريخيا حيث شفت هذه الدراسة الأوراق والخضروات والخضروات والخضروات والعشاق بعده القشرة على أجزاء النبات. ولقد شمل إجراء التجسيد الكيميائي للأوراق وخصائص النباتات لبرقة بعض الخضروات الغليظية والكليمانية بالإضافة إلى سرعة النباتات الكلية للتعضيرات الفعالية الأخرى في هذا الابتكار. وقد ين وبهذه الخضريات مهد مركبة التوريد، كذلك تم إجراء علاج كيميائي عن طريق البحوث والبحث والبحث ونوع الينابيع الطبيعية (كما الأفلاكونيات، الاستروال، الكريكيولات، النانكينيات، السبيرونيات، الفلوسيزوزيما، و/أو إنتركوروبات الكيميودير يد)）、) وعلاقة البحث المحيطة بها ب DataService إصداراً عن النباتات (35 سم. 30 سم. 100 سم) فقد مرحب به في التوريد والتنزير.

دلت الدراسات الكيميائية على وجود عدد من النباتات الكيميائية (الفلافونويدات، الاستروال، الكريكيولات، النانكينيات، السبيرونيات، الفلوسيزوزيما) بينما ظهرت هذه الدراسة على تواجد النباتات الكيميودير يد و
الآثار الحرارية الجليكوسيدية. وهم المجموعات الكبيرة الموصولة بهذا النبات في السلبيات، بالإضافة إلى وجود آثار من العوامل.

لا تظهر النافحة بين النباتات أولاً علامةً بالفعل بالنسبة للجهاز الكبائي لمجموعة المواد المفيدة وذالك من خلال تحليل الألوان المشيدة من مختلف اختبارات المصنع الكيميائي.
CONTENTS

CHAPTER 1

1. Introduction ................................................. 1

CHAPTER 11

2. Literature Review ........................................... 4
   2.1 Family: Asclepiadaceae .................................. 4
      2.1.1 biologically active constituents from the family Asclepiadaceae ...................... 6
   2.2 Genus: Solenostemma ..................................... 6
      2.2.1 Solenostemma argel (Del.) Hayne ................. 7
         2.2.1a Description .................................... 7
         2.2.1b Locality ....................................... 8
      2.2.2 Pharmacological activities of extracts of Solenostemma argel (Del.) Hayne .......... 8
      2.2.3 Biologically active constituents isolated from Solenostemma argel (Del.) Hayne .... 9
      2.2.4 Folkloric uses of Solenostemma argel (Del.) Hayne ........................................ 10
CHAPTER III

3. Materials and Methods .... .... .... .... .... 11

3.1 Materials .... .... .... .... .... .... .... 11

3.1.1 Plant material .... .... .... .... .... .... 11

3.1.2 Apparatus and Reagents .... .... .... .... 12

3.2 Methods .... .... .... .... .... .... .... 12

3.2.1 Land preparation and cultivation procedure 12

3.2.2 Preparation of samples for anatomical study .... .... .... .... .... .... .... 13

3.2.2.1 Microscopic preparations .... .... .... 13

3.2.2.2 Technique of free-hand .... .... .... 13

3.2.2.2a Cutting and fixing .... .... .... 13

3.2.2.2b Sectioning and staining .... .... 13

3.2.2.2c Mounting .... .... .... .... 14

3.2.2.3 Wax-embedded tissue technique .... 14

3.2.2.4 Technique of maceration .... .... 15

(i) Leaves .... .... .... .... .... .... 15

(ii) Woody material(thick sections) .... 15

7.2.2.2 Plant material adopted in biochemical analysis .... 15

3.2.3.1 Determination of moisture content of samples .... .... .... .... .... .... .... 16
3.2.3.2 Determination of total available carbohydrates ...
3.2.3.3 Determination of total nitrogen and crude protein ...
3.2.3.4 Determination of crude fibre ...
3.2.3.5 Determination of ascorbic acid content ...
3.2.3.6 Determination of ash content ...
3.2.3.7 Determination of mineral content of calcium, magnesium, potassium, and sodium ...
   (i) Determination of calcium and magnesium ...
   a. Preparation of digested solution by wet digestion ...
   b. Procedure for calcium assay ...
1/ Normality of versine for Ca ...
2/ Normality of versine for Ca and Mg ...
3/ Determination of milliequivalents of Ca in digested solution ...
4/ Determination of milliequivalents of Ca and Mg in a digested solution ...
c. Determination of Mg concentration 24
   (ii) Determination of K & Na .... 24

a. Ash obtaining from dry ashing 24

b. Calibration of the apparatus and measurement of the samples ..... 25

3.2.4 Method for phytochemical analysis .... 26

3.2.4.1 Preparation of extract .... 26

3.2.4.2 Tests ..... 26

3.2.4.2a Tests for flavonoids .. 26

3.2.4.2b Tests for unsaturated sterols and/or triterpenes 27

3.2.4.2c Tests for cardiac glycosides

Unsaturated steroid nucleus ..... 28

3.2.4.2d Tests for tannins ..... 29

3.2.4.2e Tests for saponins

Proth test ..... 29

3.2.4.2f Tests for alkaloids ... 30

3.2.4.2g Tests for cyanogenic glycosides ..... 30

3.2.4.2h Tests for anthraquinone glycosides ..... 31

3.2.5 Chromatographic method .... 31
(i) Preparation of the extracts for chromatographic investigation of alkaloids 31
(ii) Examination of the extract on thin layer chromatographic plates 32
   a. Preparation of the chromatoplates 32
   b. Spotting, development and visualization 33

CHAPTER IV

4. Results 354

4.1 Morphological and anatomical studies of leaves, stems, roots, flowers & fruits and morphology of seeds of Solenostemma argel (Del.) Hayne grown in Khartoum 35

4.1.1 Morphology and anatomy of the leaf 35
   A. Morphology 35
   B. Anatomy 35
   (i) Lamina 39
      1/ The upper epidermis 39
      2/ Mesophyll 39
      3/ The lower epidermis 41
   (ii) Midrib 41
   (iii) Petiole 41

4.1.2 Morphology and anatomy of the stem 42
   A. Morphology 42
B. Anatomy ......... 42
  1/ The epidermis 42
  2/ Trichomes 45
  3/ Stomata 45
  4/ The cortex 45
  5/ The endodermis 46
  6/ The pericycle 46
  7/ The vascular system 48
  8/ The pith 47

4.1.3 Morphology and anatomy of the root 49

A. Morphology ......... 49
B. Anatomy ......... 49
  1/ The cork ......... 49
  2/ The cortex ......... 49
  3/ The endodermis ......... 51
  4/ The pericycle ......... 51
  5/ The vascular system ......... 51
  6/ The pith ......... 53
  7/ The medullary rays ......... 53

4.1.4 Morphology and anatomy of the flower 55
A. Morphology ......... 55
  (i) Inflorescence ......... 55
  (ii) The flower ......... 55
     1. The pedicel ......... 55

xi
2. The calyx .... ..... .... .. 55
3. The corolla .... ..... .... .. 56
4. The corona .... ..... .... .. 56
5. The androecium .... ..... .... .. 56
6. The pollinium .... ..... .... .. 56
7. The gynoecium .... ..... .... .. 58
8. Anatomy .... ..... ..... ..... .. 58

(i) The calyx .... ..... ..... ..... 58
1/ The epidermis .... ..... .... .. 58
2/ The mesophyll .... ..... .... .. 58
3/ The vascular strands .... ..... 59

(ii) The corolla .... ..... ..... ..... 59
1/ The epidermis .... ..... .... .. 59
2/ The mesophyll .... ..... .... .. 59
3/ The vascular strands .... ..... 59

(iii) The corona .... ..... ..... ..... 61

(iv) The androecium .... ..... ..... 61
1/ The filament tube .... ..... ..... 61
2/ The anther .... ..... ..... ..... 61

(v) The gynoecium .... ..... ..... ..... 62
1/ The ovary .... ..... ..... ..... 62
2/ The style .... ..... ..... ..... 62
3/ The stigma .... ..... ..... ..... 64

(vi) The pedicel .... ..... ..... ..... 64
1/ The epidermis .... ..... ..... ..... 64
2/ The cortex .... ..... ..... ..... 64

xii
3. The pericycle ........ .... 64
4. The vascular systems ........ 67
5. The pith ........ .... 67
(vii) The bract ........ .... 67

4.1.5 Morphology and anatomy of the fruit ........ 67
A. Morphology ........ .... 67
B. Anatomy ........ .... 69
1. The pericarp ........ .... 69
2. The epidermis ........ .... 69
3. The cortex ........ .... 69
4. The placenta ........ .... 71

4.1.6 Morphology of the seed ........ .... 71

4.2 Chemical composition of Solenostemma argel
(DeJ.) Hayne ........ .... 72
4.2.1 Proximate analysis ........ .... 72
4.2.1.1 Moisture ........ .... 74
4.2.1.2 Crude protein ........ .... 74
4.2.1.3 Crude fibre ........ .... 74
4.2.1.4 Carbohydrates ........ .... 74
4.2.1.5 Ash ........ .... 74
4.2.1.6 Vitamin C (ascorbic acid) ........ .... 75
4.2.1.7 Mineral content ........ .... 75
4.2.2. General phytochemical screening of different morphological organs of Polygoneum angulatum (Bel.) Hayne extract ... 75

4.2.2.3 Test for the presence of flavonoids 82
4.2.2.4 Test for the presence of unsaturated sterols and/or triterpenes ... 88

4.2.2.5 Test for the presence of cardenolides (unsaturated sterol nucleus) ... 91

4.2.2.6 Test for the presence of tannins ... 99
4.2.2.7 Test for the presence of Saponins ... 102
4.2.2.8 Test for the presence of cyanogenic glycosides ... 104
4.2.2.9 Test for the presence of anthraquinone glycosides ... 104

CHAPTER V

5. Discussion ... 105

6. Summary and Conclusions ... 112

7. References ... 114

8. Appendix ... 121

xiv
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Results of proximate analysis of the leaves, stems and roots of <em>Solenostemma argel</em> (Del.) Hayne</td>
<td>73</td>
</tr>
<tr>
<td>2. Results of general phytochemical screening of leaves, stems and roots of <em>Solenostemma argel</em> (Del.) Hayne extract</td>
<td>76</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Solenostemma arget (Del.) Hayne — Vegetative branch ... ... ... ... ... ... 34</td>
</tr>
<tr>
<td>2</td>
<td>The Leaf of Solenostemma arget (Del.) Hayne — (A) Diagrammatic T.S. in the midrib at the middle portion. (B) Detailed T.S. in the midrib at the middle portion ... ... ... ... ... ... 36 (C) Detailed T.S. in part of the lamina at the middle portion. (D) Detailed T.S. at the apex portion ... ... ... ... ... ... ... ... ... ... ... 37 (E) Surface preparation in the upper epidermis. (F) Surface preparation in the lower epidermis. (G) Trichome. (H) Latex tube and latex cell. (I) Calcium oxalate crystals ... ... ... ... ... ... 39</td>
</tr>
<tr>
<td>3</td>
<td>Petiole of Solenostemma arget (Del.) Hayne — Detailed T.S. at portion near base ... ... ... ... ... ... 40</td>
</tr>
<tr>
<td>4</td>
<td>The stem of Solenostemma arget (Del.) Hayne — (A) Diagrammatic T.S. ... ... ... ... ... ... ... ... ... ... ... 43 (B) Detailed T.S. ... ... ... ... ... ... ... ... ... ... ... 44</td>
</tr>
<tr>
<td>5</td>
<td>Root system ... ... ... ... ... ... ... ... ... ... ... 48</td>
</tr>
</tbody>
</table>

xvi
29 Stems and leaves: Reaction for unsaturated sterols and/or triterpenes ... ... ... 92

30 Leaves: Reaction for unsaturated sterols and/or triterpenes ... ... ... ... ... 92

31 and 32 Roots: Reaction for unsaturated sterols and/or triterpenes ... ... ... ... 93

33 and 34 Leaves: Reaction for cardenolides (unsaturated steroid nucleus)... 94

35 and 36 Stems: Reaction for cardenolides (unsaturated steroid nucleus)... 96

37 and 38 Roots: Reaction for cardenolides (unsaturated steroid nucleus)... 97

39 and 40 Leaves: Reaction for tannins - FeCl₃ reagent ... ... ... ... ... 98

41 and 42 Stems: Reaction for tannins - FeCl₃ reagent ... ... ... ... ... ... 100

43 and 44 Roots: Reaction for tannins - FeCl₃ reagent ... ... ... ... ... ... 101

45 T.L.C. of ethanolic extracts of different organs of Solenostrepe angol (Del.) Hayne for their alkaloids ... ... ... ... ... ... ... 103

xix
CHAPTER 1

INTRODUCTION
CHAPTER 1

INTRODUCTION

The plant *Solenostemma argel* (Del.) Hayne\(^1\) is a member of the family Asclepiadaceae, a family which comprises numerous medicinal plants like *Calotropis procera* "lit" R.Br.f., *Marsdenia abyssinica* "Hochst." Schlechter and *Huernia macrocarpa* "A.Rich." Spr.(1) known for their cardiac activity\(^2,3,6\). Argel grows naturally in the northern part of Sudan and it is also widely distributed throughout North Africa (Egypt, Libya & Algeria)\(^4\), and in Saudi Arabia\(^5\). However, among these above-mentioned countries, the Sudan is regarded as the richest source of this plant. The principal production area in the Sudan is the northern region that extends from Berber to Abu Hamed, especially "Rubatab" area. In the Sudan and in other Arabic countries, the plant is known as "Hargel".

The leaves are commonly used as an adulterants of *Aerva*\(^6\), *Bailey*\(^7\), *Hocking*\(^8\) and *Boulos*\(^4\) have mentioned that argel leaves are used as purgative, expectorant, antipyretic, stomachic as well as a remedy in the treatment of gastro-intestinal cramps.
In the literature, only few investigations have been made to study the chemistry of this plant and most of them were made by Sudanese or Egyptian workers.

The present study is divided into two parts. Part one includes a botanical study of *Solenostema argel* (Del.) Hayne; it comprises morphology and anatomy of leaves, stems, roots, flowers and fruits in addition to morphology of seeds. Part two includes a chemical study of argel that comprises: (a) proximate analysis (viz. moisture, crude protein, crude fibre, total available carbohydrates, ash, nitrogen, calcium, magnesium, potassium, sodium and vitamin C) which have been made at only one stage of growth when the plants were in flower; (b) general phytochemical screening for detection of certain plant secondary metabolites (viz. flavonoids, sterols and/or triterpenes, cardenolides [unsaturated steroid nucleus], saponins, alkaloids, cyanogenic glycosides and anthraquinone glycosides) in the leaves, stems and roots. This phytochemical screening was performed at two stages of growth namely pre-flowering and flowering stages for plants grown at four different inter-plant spacings of 25 cm, 50 cm, 75 cm & 100 cm.
The objectives of the present work are:

(a) To determine to some extent the stage for harvest of argel plant which gives the optimum yield of certain chemical compounds;

(b) to clarify that the qualitative determination of these different chemical compounds will add more information about the nature of some chemical groups in this important desert plant;

(c) to add more information about the level of uptake of soil nutrients;

(d) to clarify that the morphological and anatomical studies carried out for this plant can form leading factors as to the sort of growing conditions;

(e) to obtain genuine correct plant free from adulteration for use or for further research through detailed anatomical investigations.
CHAPTER II

LITERATURE REVIEW

2.1 Family: Asclepiadaceae:

The family Asclepiadaceae (Order: Gentiales) consists of about 250 genera and 2000 species. The members of this family are mainly of tropical origin occupying savanna and semi-arid regions and rarely found in rain forests. They are generally twining or erect shrubs but the family also includes perennial herbs. The family Asclepiadaceae is also known as the milkweed family since its members generally have milky latex.

Asclepiadaceae family is divided into two distinguished sub-families: Periplocoideae and Cynanchoideae. The Periplocoideae sub-family includes only 42 genera world-wide and it is characterized by flowers having stalked stamens where pollen is granular and is found in tetrads. Flowers are insect-pollinated.

The Cynanchoideae sub-family has flowers with stamens almost sessile; their pollen is aggregated in two or four wax-like pollinia. This sub-family is divided into four tribes that include 230 genera.
2.2.4 Folkloric uses of *Solenostemma argel* (Bezi)

Haynes:

The most popular use is as a tea (an infusion of the dried leaves and branches are used). An infusion of the leaves is used as a purgative and it also possesses a carminative effect. The water extract of the leaves is used as an antispasmodic, as a tonic tea to ton up the blood and nerves and as a cough remedy for treatment of common colds. It is also used for urinary tract infections and antisyphilitic if used for prolonged periods of 40-80 days. Externally, fine powder of leaves is used for all infected wounds. The smoke of the plant is also considered good for measles. The leaves are considered to be medicinally important in Libya and Chad where a decoction is used to treat neuralgia and sciatica(13,17,18).
3.1 Materials:

3.1.1 Plant Material:

The material used in this work consisted of the separated fresh and dried organs of *Solenostemma argel* (Del.) Hayne obtained from plants cultivated in July 1989 at Khartoum University farm (Hambat, Khartoum North). Fresh materials were used for cutting various sections in the different organs of the plant (viz. leaves, stems, roots, flowers, and fruits). For the general biochemical study, the different organs of the plant (viz. leaves, stems, and roots) were collected at only one stage of growth when the plants were in flower. On the other hand, the extensive phytochemical study for detection of certain plant secondary metabolites was performed at two different stages of growth, namely the pre-flowering and in-flowering stages. All samples except for those required to be fresh were dried in the shade and pulverized to the required size (sieve # 60) prior to analysis.
3.1.2 Apparatus and Reagents:

Apparatus used included different items of instruments, glassware, chemicals and reagents. The different items are shown in the appendix. All reagents and chemicals used were pure and standard.

3.2 Methods:

3.2.1 Land preparation and cultivation procedure:

A piece of land within the Khartoum University farm, in Shambat, North Khartoum was selected for the conduction of experiments. The soil was well-prepared, ridged and divided longitudinally into equal plots (each 7 metres x 6 metres). The ripe authenticated seeds of Solenostemma argei (Del.) Hayne collected from plants growing in Shambat were sown in rows 60 cm apart and four different interplant spacings of 25, 50, 75 and 100 cm. The experiments were set in a randomized complete block design with three replicates and the first week of July 1989 was the sowing date. Watering was conducted at intervals of 10–12 days. Thinning, transplanting, weeding and other cultural practices were done when necessary.
3.2.2 Preparation of samples for anatomical study:

3.2.2.1 Microscopic preparations:

Hand sections were cut and stained in safranin and Fast Green. For the study of different organs (viz. leaves, stems, roots, flowers and fruits), microtome sections were used and stained with safranin and Fast Green combination. Much of the present study has been done on microscopical slides, stained and permanently mounted. Temporary mounts of sections and maceration technique of plant material were also performed.

3.2.2.2 Technique of tree-hand:

3.2.2.2a Cutting and fixing:

Woody stems and roots were cut into pieces not larger than 1 cm x 1 cm x 0.5 cm dimensions, transferred to formalin—acetic—alcohol (FAA) for at least 72 hours and then transferred to FAA/Glycerine (1:1) for at least 2 days.

3.2.2.2b Sectioning and staining:

The plant material was placed in the Reichert sliding microtome and some ethanol (50%) was placed on the microtome razor. Sections were cut and transferred by a
camel hair brush to more of the ethanol (50%) in which the tissue has been standing. These were then selected
and transferred to petri dishes containing 50% ethanol. The following runs were performed: safarin (50%) for a
few minutes followed by 50%, 70%, 90%, 95% and absolute alcohol. Then they were transferred to a Fast Green and
absolute alcohol / cedear oil combination (1:1) and finally to xylene.
3.2.2.2c Mounting:
A drop of Canada balsam was placed at the centre of a
clean slide and the plant material was placed in it and
covered with a clean cover-slip. The mounted slides were
put in an oven at 60°C and dried for 3 days.
3.2.2.3 Wax-embedded tissue technique:
The tissues (viz. leaves, flowers and fruits) were cut into pieces not larger than 5 mm thick. Each tissue
was quickly transferred first to FAA, followed consecutively by alcohol 50%, 70%, 90%, 95%,
absolute alcohol and clove oil for at least 6 hours. Sections were then transferred to pure clove oil for 6
hours, followed by clove oil + xylene for 6 hours, pure
xylene for 6 hours, xylene + Wax I paraaffin for 2
hours, and Wax II for 40 minutes, Wax III for 20 minutes
and then to blocking respectively.
3.2.2.4 Technique of maceration:

(i) Leaves:
Leaves are soaked in ethanol (95%) to remove chlorophyll, transferred to aqueous sodium hydroxide solution (8%) and then warmed on a water bath for 45 minutes. They were then washed gently in running water for 2 hours, soaked in hydrochloric acid (10% aq.) for 6 hours and gently washed in running water for 2 hours.

(ii) Woody material (Thick sections):
Sections of stems and roots were boiled in 1% aq. potassium hydroxide solution for one hour then they were washed thoroughly in water. The sections were then placed in chromic acid (25% aq.) for few minutes and finally washed thoroughly in water.\(^{21,22}\)

3.2.3 Methods adopted in biochemical analysis:

Experiments: methods carried out in this work were mainly based on a number of experiments recommended by the “association of official analytical chemists” (A.O.A.C.)\(^{24,25}\), Pearson\(^{26}\) and Osborne\(^{27}\).
3.2.3.1 Determination of moisture content of samples:

Moisture content of the different plant parts were separately determined according to (A.O.A.C.)\(^{(24)}\).

Two-gram samples of each plant part were accurately weighed and placed in separate crucibles. The samples were left in an oven at 105 °C for three hours then transferred to a desiccator for one hour to cool. The samples were finally weighed and moisture percentage was calculated.

3.2.3.2 Determination of total available carbohydrates:

The total available carbohydrate was determined according to Clegg Anthrone Method (1956)\(^{(27)}\).

One-gram sample of each plant part was accurately weighed and placed in separate measuring cylinders. Ten ml of water were added and stirred. Thirteen ml of 52% perchloric acid were added and the mixture was stirred for about 20 minutes and then diluted to 100 ml. The mixture was filtered and then finally diluted with water into a 250 ml graduated flask (sample extract). Ten ml of the sample extract were diluted with water to 100 ml and 1 ml of diluted filtrate was pipetted.
Duplicate blanks and duplicate standards (1 ml of dilute glucose) were alsopipetted out. To all these tubes 5 ml of freshly prepared anthrone reagent were pipetted and stoppered. They were mixed and finally placed in a boiling water bath for exactly 12 minutes. The solutions were transferred to 1 cm glass cuvettes and the absorbance of the samples and standards were read at 630 nm against the reagent blank.

Calculations:

Weight (g) of sample = W
Absorbance of dilute standard = a
Absorbance of dilute sample = b

Then:
Total available carbohydrate (as % glucose) = \( \frac{25 \times b}{a \times W} \)

3.2.3.3 Determination of total nitrogen and crude protein:

Nitrogen content was determined by the macro-Kjeldahl distillation method(27).

Exactly 2 g of the sample was digested in a digestion flask using a catalyst mixture (90% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium
dioxide) and concentrated sulphuric acid (25 ml). The digest was diluted with 50 ml distilled water and made alkaline with concentrated aqueous alkali (100 ml of sodium hydroxide) and then transferred to the steamed generating apparatus. The ammonia was distilled into boric acid solution (50 ml) mixed with indicator solution mixture (methyl red and methylene blue) (4 drops) for 20 minutes. After lowering the receiving flask clear of the condenser, the apparatus was steamed out for further 5 minutes. The distillate was then titrated with 0.1N hydrochloric acid.

Calculations:

Weight (g) of the test portion \( (W) \)

Volume (ml) of hydrochloric acid solution required for the blank test \( (V_1) \)

Volume (ml) of hydrochloric acid solution required for the test portion \( (V_2) \)

Normality of hydrochloric acid \( (0.1N) \)

\[
\text{Total Nitrogen } (\%) = \frac{(V_2 - V_1) \times N}{W} \times 1.4
\]

\[
\text{Crude protein } (\%) = \frac{(V_2 - V_1) \times N}{W} \times 1.4 \times 6.25
\]
3.2.3.5 Determination of crude fibre:

Crude fibre content of the different plant parts were separately determined by the Labconco digestion apparatus according to the A.O.A.C methods (23).

One gram of each sample together with one gram of asbestos were separately placed in a crude fibre estimation beaker (special type) and 200 ml of 0.255N sulphuric acid (hot solution) were added to each beaker. The whole material in each beaker was then transferred to an extraction apparatus equipped with a heater. The mixture was heated to boiling for 30 minutes, left to cool and then filtered through a California modified buchner funnel by suction pressure. The residue was washed with hot water until the washings are free from acid. Then 200 ml of hot 0.313N sodium hydroxide were added followed by boiling the mixture for 30 minutes. The mixture was then left to cool and it was filtered through a California modified buchner funnel. The residue was washed with hot water until the washings were free from alkali. The residue was washed finally with alcohol (25 ml) and then it was transferred to a crucible where it was digested at 132°C for about three hours. The crucible was transferred into the desiccator, cooled to room temperature and weighed ($W_1$). The residue was
ignited in a furnace at 600°C for 30 minutes, left to cool and reweighed (W2). The crude fibre content was calculated as the difference between the dried weight of material and the left ash at 600°C.

\[
\text{Weight of the crude fibre} = (W_1 - W_2) - \text{blank}
\]

\[
\% \text{Crude fibre} = \frac{\text{Weight of the crude fibre}}{\text{Weight of the sample}} \times 100
\]

3.2.3.5 Determination of ascorbic acid (Vitamin C) content:

Thirty grams of green leaves were accurately weighed and blended with 200 ml of 0.4% oxalic acid. Then the mixture was filtered and made up to 250 ml with 0.4% oxalic acid.

An accurate amount of 20 ml of solution was pipetted into a beaker and then it was titrated against 2.6 - Dichlorophenolindophenol to a faint pink colour (end point).

3.2.3.6 Determination of ash content:

Five grams of each dried sample were separately placed in different accurately procelain dishes. The dishes were placed in a muffle furnace at about 550°C until...
light grey ash of constant weight was obtained. Each dish was cooled in the desiccator and weighed and the ash weight was determined and its percentage was calculated.

3.2.3.7 Determination of mineral content of calcium, magnesium, potassium and sodium:

(i) Determination of calcium and magnesium:

Calcium and magnesium contents were determined by the versinate method(29).

a. Preparation of digested solution by wet digestion(30):

Exactly 0.1 g of each dried powdered sample was placed into a 30 ml Kjeldahl flask and 1 ml of concentrated sulphuric acid was then added. Each flask with its content was placed in a quiet flame for five minutes until most of the fumes were removed. One ml of copper sulphate (1%) solution and 4 ml of concentrated sulphuric acid were added and the mixture was heated until gas fumes were removed. Five drops of hydrogen peroxide were added and the mixture was allowed to get digested until a clear light blue colour appeared. Quantitatively, each flask content was transferred to a 100 ml volumetric flask and then the mixture was completed to 100 ml and filtered (digested solution).

21
b. Procedure for calcium assay:

1/ Normality of versine for calcium:

About 5 ml of 0.01N calcium chloride solution was pipetted into a porcelain dish and then it was diluted by 10 ml distilled water.

1.5 ml sodium hydroxide (4.0N) and 0.1 g ammonium purpurate indicator were added, mixed thoroughly and the solution was titrated with 0.01N disodium dihydrogen ethylenediamine tetra-acetate solution (E.B.T.A) until a purple colour was observed.

2/ Normality of versine for calcium and magnesium:

An exact amount of 5 ml of the 0.01N calcium chloride solution was added to 10 ml distilled water and 5 ml of a buffer solution (ammonium chloride + ammonium hydroxide) were pipetted into a porcelain dish and mixed. About 4 drops of Eriochrome Black T indicator (E.B.T) were added. The mixture was then titrated with versinate solution until a blue colour was observed.

3/ Determination of milli-equivalents of calcium in a digested solution:

An exact amount of 5 ml of the digested solution was pipetted into a porcelain dish. 1.5 ml sodium hydroxide (4.0N) was added, then diluted with 10 ml distilled water.
and 0.1 g ammonium purpurate indicator mixture was added. The mixture was titrated with versinate solution until a purple colour was observed.

The calcium amount present in sample was calculated as follows:

\[ V_{\text{versine}} \times N_{(\text{for calcium})} = \text{Milli-equivalents of calcium in sample} \]

(in 5 ml of digested solution)

4/ Determination of milli-equivalents of calcium and magnesium in a digested solution:

An exact amount of 5 ml of the digested solution was pipetted into a procelain dish. 5 ml of the buffer solution (ammonium chloride + ammonium hydroxide) and the 3 drops of Eriochrome Black T indicator (K.B.T) were added. The mixture was titrated with versinate solution until a blue colour was observed.

The calculation of milli-equivalents of calcium and magnesium was as follows:

\[ V_{\text{versine}} \times N_{(\text{for Ca A Mg})} = \text{Milli-equivalents of calcium and magnesium in 5 ml of digested solution} \]
c. **Determination of magnesium concentration:**

Mg concentration in each sample was calculated, subtracting milli-equivalents of calcium value from the milli-equivalents of calcium and magnesium.

(ii) **Determination of potassium and sodium:**

Potassium and sodium contents were determined by the atomic absorption method.

a. **Ash obtaining from dry ashing:**

The ash obtained from the assay for ash content was treated with 5 ml of 6.0N hydrochloric acid and evaporated to dryness on a hot plate at a low temperature. 15 ml of 3.0N hydrochloric acid were added and the dish was heated on a hot plate until the solution just boiled. The dish was then cooled and the mixture was filtered in a graduated flask. 10 ml of 3.0N hydrochloric acid were added to the residue and then the solution was heated until it just boiled. The dish was cooled and filtered into the graduated flask and then it was washed three times with water. The content of the flask was cooled and diluted to a specific mark with water. A blank without sample was prepared by the same amount of reagent.
b. Calibration of the apparatus and measurement of the samples:

Stock standard solutions were prepared as follows:
- 10 ml standard solution (1000 ppm)
- 100 ml Distilled water

Standard solutions were prepared as follows:
- 5, 10 or 15 ml Required volume
- 10 ml Cesium chloride solution
- 10 ml Concentrated hydrochloric acid
- 100 ml Distilled water

Calculations:

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Volume of stock solution</th>
<th>Volume of solution taken for reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample weight</td>
<td>Volume taken for sample</td>
<td></td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{Dilution Factor} & = \frac{\text{Volume of stock solution}}{\text{Sample weight}} \times \frac{\text{Volume of solution taken for reading}}{\text{Volume taken for sample}} \\
\text{Metal content} & = \text{Dilution factor} \times \text{apparatus reading}
\end{align*}
\]

\[
\begin{align*}
50 & \times 100 = 200 \text{ (0.32 ppm)} \\
5 & \times 5
\end{align*}
\]

25
3.2.4 Method for phytochemical analysis:

3.2.4.1 Preparation of extract:

The dried powder of the different morphological parts of *Solanostemma argel* (Del.) Hayne (viz. leaves, stems and roots) were extracted separately. A sample of 100 g of each of these plant parts was accurately weighed and separately extracted in a Soxhlet apparatus. Each sample was continuously extracted for about four hours with 80% ethanol. The cooled solution was filtered and enough 80% ethanol was passed through the marc to adjust the volume of the filtrate to 500 ml. This prepared extract was used for the various following tests.

3.2.4.2 Tests:

3.2.4.2a Tests for flavonoids:

A 75 ml aliquot of the prepared extract was concentrated by removing all solvent by distillation under reduced pressure. The concentrated extract was dried on a water bath to remove any residual solvent. cooled and the residue was defatted by several extractions with petroleum ether (b.r. 60 - 80°C). The
defatted residue was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for the following tests:

1. To 3 ml of the filtrate in a test tube, 1 ml of 1% aluminium chloride solution in methanol was added. The tube was observed for the formation of a yellow colour that indicated the presence of flavonols, flavones and/or chalcones.

2. To 2 ml of the filtrate, 0.5 ml of concentrated hydrochloric acid and a few magnesium turnings were added. Production of a definite colour change to pink or red was taken as a presumptive evidence that flavonols or flavones were present in the test sample.

3.2.4.2b Tests for unsaturated sterols and/or triterpenes:

A 40 ml aliquot of the prepared extract was concentrated by removing all solvent by distillation under reduced pressure. The concentrated extract was dried on a water bath to remove any residual solvent. The residue was cooled and stirred several times with petroleum ether (b.p. 60–80°C) to remove most of the
colouring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphate and then it was filtered. Five ml portion of the chloroform solution was mixed with 0.5 ml of acetic anhydride, followed by two drops of concentrated sulphuric acid. The gradual appearance of green – blue, pink – purple colours was taken as an indication of the presence of sterols (green – blue) and/or triterpenes (pink – purple) in the plant sample.

3.2.4.2c Tests for cardenolides:

Unsaturated steroid nucleus:

Twenty ml of the prepared extract was concentrated by removing all solvent by distillation under reduced pressure. The concentrated extract was dried on a water bath to remove any residual solvent. The residue was cooled and it was then extracted several times with petroleum ether (b.r. 60 – 80 °C) to remove most of the fats and pigments. The defatted residue was extracted with chloroform and the chloroform extract was dried over anhydrous sodium sulphate and then it was filtered. To 5 ml of the dried chloroform extract 0.5 ml of acetic anhydride followed by two drops of concentrated sulphuric
acid were added. A change of colour to green or blue indicated the presence of sterols.

3.2.4.2d Tests for tannins:

Twenty five ml of the prepared extract was concentrated by removing all solvent by distillation under reduced pressure. The concentrated extract was dried on a water bath to remove any residual solvent. The residue was extracted several times with n-hexane and then filtered. The insoluble residue was then stirred with 10 ml of hot saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. To 5 ml of this solution, few drops of ferric chloride test reagent were added. The formation of (blue-black) or green colour was taken as an evidence for the presence of tannins.

3.2.4.2e Tests for saponins:

Froth test:

About 0.5 g of the dried powder of the different organs under investigation were separately placed in a clean test tube with 10 ml of distilled water. The tube
was stoppered and shaken vigorously. The formation of a "honey comb" froth that persists for at least half an hour was taken as an evidence for the presence of saponins.

3.2.4.2f Tests for alkaloids:

A 30 ml aliquot of the prepared extract was evaporated to dryness in an evaporating dish on a water bath. About 5 ml of 2N HCl acid were added and stirred while heating on the water bath for 10 minutes, cooled, filtered and divided equally into three test tubes. To one portion of the filtrate, few drops of Mayer's reagent were added. To the second portion few drops of Wagner's reagent were added while to the third portion few drops of Dragesdorff's were added. A slight turbidity or a heavy precipitate in either of the three test tubes was taken as a presumptive evidence for the presence of alkaloids.

3.2.4.2g Tests for cyanogenic glycosides:

About 3 g of the dried powdered plant was placed in 125 ml Erlenmeyer flask and sufficient distilled water was added to moisten the sample, followed by 1 ml of chloroform. A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which
was used to stopper the flask. A change in the colour of sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycosides.

3.2.4.2h Tests for anthraquinone glycosides:

To 1 g of the powdered plant was added 10 ml N/2 potassium hydroxide containing 1 ml of 3 % hydrogen peroxide solution. The suspension was boiled for 3 - 5 minutes then cooled, filtered and 5 ml of the filtrate was acidified with 10 drops of glacial acetic acid. This acidified mixture was extracted by shaking with 10 ml of benzene. A 5 ml aliquot of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. A pink to red colouration of the alkaline layer indicated the presence of anthraquinone.

3.2.5 Chromatographic method (32,33):

(i) Preparation of the extracts for chromatographic investigation of alkaloids:

20 g of each of the air dried powdered leaves, stems and roots were separately extracted with ethanol
(80%) in a Soxhlet apparatus. The ethanolic extract in each case was separately concentrated under reduced pressure.

(ii) Examination of the extract in thin layer chromatographic plates:

Extracts of different morphological parts of Solenostoma argel (Del.) Hayne were examined on thin layer chromatographic plates.

a. Preparation of the chromatoplates:

The glass plates were cleaned thoroughly before use, for this they were washed with water and detergent, drained and dried. A final wash with a piece of cotton soaked into acetone was done. The adsorbent (stationary phase) was made into a slurry with water, usually in proportion of one gram of adsorbent (silica gel) and 2 ml of water (30 g silica gel and 60 ml distilled water). The slurry was then thoroughly stirred and spread on the glass plates (20 cm x 20 cm size) using an adjustable spreading device (apparatus made by Desaga) giving layers of 0.25 mm thick. The plates
were allowed to dry in air and then activated in hot air oven at 110°C for half an hour and the plates were allowed to cool.

b. Spotting, development and visualization:

The activated plate was then placed flat on the laboratory bench and samples spotted carefully at the starting points 1.5 cm apart and 2 cm from the lower edge of the plate. After the solvent was evaporated, the plates were placed vertically in a glass tank which contained a suitable solvent to a depth of about 1.5 cm and the chromatogram developed. The developer was allowed to ascend a suitable distance (12 cm). The plates were removed from the jar, allowed to dry spontaneously and the spots were located by means of daylight, UV light (long & short) and finally sprayed by Dragendorff's reagent.
CHAPTER IV

RESULTS
Fig. (1): Dolostemma urgel (Del.) Hayne -
Vegetative branch
CHAPTER IV

RESULTS

4.1 Morphological and anatomical studies of leaves, stems, roots, flowers and fruits and morphology of seeds of Solanostemma argel (Del.) Hayne grown in Khartoum:

Morphology and detailed anatomy descriptions of Solanostemma argel (Del.) Hayne were mainly based on some references (23, 34, 35).

4.1.1 Morphology and anatomy of the leaf:

A. Morphology:

Leaves (Fig. 1) are simple, opposite, 3.5 - 4.5 x 1 - 1.5 cm, linear-lanceolate, extipulate, entire, coriaceous when dry, fleshy when alive, cuneate at the base, dark green above, prominently veined beneath, pubescent. The petiole is 4 mm long.

B. Anatomy:

A transverse section of a leaf is shown in Fig. 2A. The leaves show an isobilateral structure. Both surfaces of leaves are covered with a smooth cuticle and possess both stomata and trichomes.

35
Fig. (2):

The leaf of *Soleostemma argel* (Del.) Hayne

A. Diagrammatic T.S. in the midrib at the middle portion

B. Detailed T.S. in the midrib at the middle portion.

( Col., collenchyma; cr., crystal of calcium oxalate; cut., cuticle; ep., epidermis; l.ep., lower epidermis; pol., palisade; ph., phloem; p.ph., perimedullary phloem; s.p.n., spongy mesophyll; tri., trichome; u.ep., upper epidermis; v., vein; v.b., vascular bundle; xy.v., xylem vessels.)
C. Detailed T.S. in part of the lamina at the middle portion.

D. Detailed T.S. at the apex portion.

( Cr., crystal of calcium oxalate; cut., cuticle; l.t., latex tube; l.ep., lower epidermis; pal., palisade; sp.m., spongy mesophyll; tri., trichome; u.ep., upper epidermis; v., vein; v.b., vascular bundle ).
E. Surface preparation in the upper epidermis.

F. Surface preparation in the lower epidermis.

G. Trichomes.

H. Latex tube and latex cell.

I. Calcium oxalate crystals.

( Cr., crystals of calcium oxalate; i.e., latex cell; l.t., latex tube; st., stomata; tri., trichome.)
Fig. (3):

Petiole of *Solenostemma argai* (Del.) Hayne -
Detailed T.S. of portion near base.

(Col., collenchyma; cr., crystal of calcium oxalate; ep., epidermis; par., parenchyma; ph., phloem; p.ph., perimedullary phloem; v.b., vascular bundle).
3. The lower epidermis:

It is covered with smooth, thin cuticle and consists of a single layer of cells much smaller in size than those of the upper epidermis; these cells appear rounded, oval, or tabular in transverse section. Stomata are present, and they are few in number; abundant non-glandular trichomes are also present.

(ii) Midrib: (Fig. 2A)

Below the epidermis there is cortex which is composed of two parts: a collenchyma tissue the cells of which are very heavily thickened in their angles and cortex parenchyma. The cortex contains numerous crystals of calcium oxalate. Endodermis is not distinguishable. Vascular tissues are in a crescent with end recurved and numerous bundles. Phloem occurs on both sides of the xylem and hence the vascular bundle is bicolateral.

(iii) Petiole: (Fig. 3)

The epidermis is highly cuticularised and is composed of round to rectangular cells. This enclosed a ground tissue of parenchyma except on either side of the median
bundle where the peripheral 3 - 4 layers are collenchymatous. The bundles are numerous. There are 7 - 10 separated groups of vascular bundles in the petiole, of which the median is the largest and 1 or 2 bundles occurring in the wing are the smallest. Phloem occurs on both sides of the xylem. Vascular tissues are present in a crescent with ends recurved. Latex tubes occur associated usually with the vascular bundles.

4.1.2 Morphology and anatomy of the stem:

A. Morphology:

The stem is erect herbaceous, woody at the base, 60 - 100 cm high, velvety pubescent. (Fig. 1).

B. Anatomy: (Fig. 4A & B)

1. The epidermis:

The epidermis consists of tabular - polygonal, nearly isodiametric cells with straight anticlinal walls, covered with a thick, striated cuticle.
Fig. (4):

The stem of *Solenostemma argol* (Del.) Hayne -

A. Diagrammatic T.S.

(Col., collenchyma; cr., crystal of calcium oxalate; cut., cuticle; en., endodermis; ep., epidermis; fas., fascicular cambium; l.t., latex tube; m.r., medullary ray; par., parenchyma; p.f., pericyclic fibres; p.ph., perimedullary phloem; ph., phloem; pi., pith; tri., trichome; ve., vessel; xy.f., xylem fibres; xy.par., xylem parenchyma.)
3. Detailed T.S.

(col., collenchyma; cr., crystal of calcium oxalate; cut., cuticle; en., endodermis; ep., epidermis; l.t., latex tube; m.r., medullary ray; par., parenchyma; p.f., pericyclic fibres; p.ph., perimedullary phloem; ph., phloem; pi., pith; tri., trichome; ve., vessel; xy.f., xylem fibre; xy.par., xylem parenchyma).
2. Trichomes:

These are multicellular, uniseriate, non-glandular, slightly curved hairs with thick warty walls and pointed apices.

3. Stomata:

The stomata are of the usual anomocytic type. The guard cells are oval each being surrounded by 5–7 subsidiary cells.

4. The cortex:

This is comparatively narrow, consisting of an outer zone of 6–7 rows of collenchyma cells and 6–8 rows of thin-walled parenchyma cells. The latter are mostly rounded with chloroplasts and starch granules. Numerous rosettes of calcium oxalate and few clusters and prisms of calcium oxalate are also present. Few latex cells and latex tubes (round or oval in transverse section) are observed.
5. **The endodermis**:

It is formed of one row of thin-walled cells, without intercellular spaces and containing starch granules and calcium oxalate crystals.

6. **The pericycle**:

It is formed of an interrupted ring of fibers separated by parenchyma cells. The pericyclic fibers are usually in groups of commonly 20 - 24 cells. They are polygonal or rounded in transverse section. The pericyclic parenchyma are most or less rounded having thin cellulosic walls and contain numerous starch granules.

7. **The vascular system**:

Secondary xylem and secondary phloem tissues are produced both by the fascicular and the interfascicular cambium.

**The phloem**:

The secondary phloem appears as isolated groups of cells between the cambium and the pericycle. The tissue is rapidly thin, so that the total amount of such phloem
is small. It is rich in laticiferous cells containing latex, as well as latex tubes.

**The cambium:**

It forms a distinct zone of 3–4 rows of cambiform cells.

**The xylem:**

The secondary xylem forms a wide zone of lignified thick-walled radially arranged elements traversed by narrow medullary rays. The xylem parenchyma are polygonal, axially elongated with lignified walls. The medullary rays are uniseriate or bisetaceous. The xylem consists of vessels, xylem fibers and xylem parenchyma.

8. **The pith:**

It is large and composed of rounded parenchyma cells with wide intercellular spaces and starch granules. Calcium oxalate crystals and numerous laticiferous and ramifying branched latex tubes are observed. Supernumerary strands of perimedullary phloem are scattered in the periphery of the pith.
Fig. (5):

Salicostemma argel (Del.) Hayne – Root system
4.1.3 Morphology and anatomy of the root:

A. Morphology: (Fig. 5)

The main root is tap, pale yellow about 8 cm x 1.5 m. It bears numerous lateral roots which measure up to 50 x 3.5 cm.

B. Anatomy:

A transverse section in the root (Fig. 6 A & B) reveals the following structure:

1. The cork:

Cork cells are formed by the cork cambium of the pericycle. This is stratified into about 3-6 thin radial zones. Secondary growth is followed by the rupture of the outer tissues.

2. The cortex:

The cortex is comparatively wide, of secondary origin (phelloderm) and is formed of thin-walled parenchymatous cells. The cells contain numerous starch
Fig. (b): The Root of *Soilonostoma argii* (Del.) Hayne

A. Diagrammatic T.S.

(C, c, cork; tok.com., cork cambium; cor., cortex; cr., crystal of calcium oxalate; fas.com., fascicular cambium; in.fas.com., interfascicular cambium; m.r., medullary ray; starch granule; ve., vessel; xy.f, xylem fibres; xy.par., xylem parenchyma).
granules, calcium oxalate crystals and isolated latex cells.

3. **The endodermis**:

   It is destroyed soon after the incidence of secondary growth.

4. **Pericycle**:

   This is a relatively narrow zone of tissue compared with the cortex. Its component cells are largely parenchymatous.

5. **The vascular system**:

   **The phloem**:

   The phloem is narrow and consists mainly of parenchyma with scattered sieve tubes.

   **The cambium**:

   The cambium zone is formed of a row of thin-walled, tangentially elongated subrectangular parenchymatous cells.
B. Detailed T.S.

(Cork; cork cam., cork cambium; cr., crystal of calcium oxalate; fas.cam., fascicular cambium; in.fas.cam., interfascicular cambium; m.r., medullary ray; ph., phloem; s.gr., starch granules; ve., vessel; x.f., xylem fibre; x.par., xylem parenchyma).
The xylem:

The xylem constitutes about one half of the diameter of the root, and is formed of compact, radiating, lignified elements. It is difficult to trace the primary xylem arches in the old root due to the vigorous growth of the secondary xylem. The secondary xylem contains vessels of various diameters accompanied by fibres and parenchyma cells. The wood fibres are the main constituent of the wood, usually with straight, regular or very slightly irregular outline, wide lumens and moderately thin lignified walls. The wood parenchyma cells are usually consisting of rectangular to subrectangular, axillary elongated cells, with lignified walls.

b. The pith:

No pith is present.

7. The medullary rays:

The primary and secondary medullary rays are numerous, being uniseriate traversing the phloem and xylem regions. The cells in the phloem region are parenchymatous, cellulosic and mostly rectangular, but in the xylem region the cells are mostly rectangular, radially elongated, and with lignified walls.
Fig. (7) :

Flower of *Solanostemma argei* (Del.) Hayne

A. Inflorescence
B. Entire flower
C. Floral diagram
D. Floral formula

( Ant., anther; cap., corpusculum; coru., corona; ov., ovary; ped., pedicel; pet., petal; sep., sepal; tri., trichome )
4.1.4 Morphology and anatomy of the flower:

A. Morphology:

(i) Inflorescence: (Fig. 7A)

It consists of bracteate axillary cymes with about 30 mm long carried on a short peduncle 5 – 10 mm long. The whole inflorescence carries 35 – 46 flowers.

Bracts are linear – lanceolate, 6 – 7 x 2 – 2.5 mm, entire, acute at the apex, greenish to whitish, hairy on both surfaces.

(ii) The flower: (Fig. 7B &C)

Flowers hermaphrodite, regular, 1.0 – 1.4 cm long, shortly pedicellate, with characteristic odour and with characteristic intense bitter taste.

1. The pedicel:
It is erect cylindrical, hairy, light green in colour, 5 x 2.5 mm.

2. The calyx:
It consists of 5 fused oblong sepals, 3 – 5 x 1.5 – 2 mm, greenish, entire, acute at the apex, with prominent
midrib which is glandular and hairy.

3. The corolla:

It consists of 5 petals which are fused, oblong - linear.

5 = (2.5 - 3) mm white.

4. The corona:

It is cup-shaped white, with five short rounded
induplicate lobes. 3 x 2.5 mm, glabrous.

5. The androecium:

It is composed of five epipetalous, monadelphous stamens.
The anthers are oblong appendaged and alternating with 5
gland-like pollen-carriers. Pollen grains are
present at one anther lobe forming club-shaped, flattened and pendulous pollen masses. Each two adjacent
stamens are fused together by translators, tied with
corpusculum. Pollen masses, translators and corpusculum,
form the pollinium.

The pollinium:

It is ovate in shape rounded at the base gradually narrowing
and tapering at the apex. Translators are differentiated
Fig. (a):

A. Sepal of *Solenostemma argel* (Del.) Hayne.
   (i) Diagrammatic T.S.
   (ii) Detailed T.S.

B. Petal of *Solenostemma argel* (Del.) Hayne.
   Detailed T.S.

C. Corona of *Solenostemma argel* (Del.) Hayne
   Detailed T.S.

(Cut., cuticle; ep., epidermis; l.ep., lower epidermis; l.t., latex tube; par., parenchyma; ph., phloem; r.cr., rosette crystal of calcium oxalate; tri., trichome; u.ep., upper epidermis; v.e., vessel; v.b., vascular bundle).
6. The **gynoecium**:
   It is syncarpous composed of 2 united carpels; ovary superior; ovules numerous with axile placentation; style single with 5 stigmatic lobe.

B. **Anatomy**:

(i) The **calyx**; (Fig 8A)

1. The **epidermis**:
   Both epidermis are formed of polygonal cells having straight anticlinal walls and covered by thick striated cuticle. Stomata are present on both surfaces, mostly of anomocytic type. Trichomes are multicellular and covered by warty cuticle.

2. The **mesophyll**:
   It is formed of 10-15 rows of rounded parenchymatous cells with wide intercellular spaces. Numerous calcium oxalate crystals and latex tubes were observed.
3. The vascular strands:

Vascular bundles are collateral and the phloem elements are accompanied by latex tubes.

(ii) The corolla: (Fig 8B)

1. The epidermis:

Epidermal cells are polygonal or isodiamic with straightened cuticle. The stomata are rare, of the anomocytic type and present on the lower surface only. Trichomes are absent.

2. The mesophyll:

This is undifferentiated into palisade layer and the spongy tissue. It is formed of 8–15 rows of rounded parenchymatous cells with wide intercellular spaces. Numerous rosettes of calcium oxalate and latex tubes were observed.

3. The vascular strands:

Vascular bundles are collateral.
Fig. (9) :

A. The Androecium of the flower of *Solenostephan argel* (Del.) Hayne :
   (i) T.S. in the Anther.
   (ii) T.S. in the filament tube.
   (iii) Pollinium.

B. The Gynoecium of the flower of *Solenostephan argel* (Del.) Hayne:
   Diagrammatic T.S. in the ovary.

( Ant., anther; ant.l., anther lobe; con., connective; cop., corpusculum; f.c., fibrous cells; f.t., filament tube; ov., ovary; ov.l., ovule; pl., placent; po.s., pollen sac; pol., pollinium; sty., style; t.s.c., tapetal cells; ts., translator; v.b., vascular bundle; w., wing.)
(iii) **The corona**: (Fig 8C)

This is composed of upper and lower epidermis and of parenchyma ground tissue with wide intercellular spaces. The cells of the corona are free of contents.

(iv) **The androecium**: (Fig.9A)

1. **The filament tube**:

T.S. in the filament tube shows that it is formed of an outer and inner epidermis enclosing in between them a parenchymatous cells with comparatively wide intercellular spaces. Latex tubes and narrow vascular strands are scattered in the ground tissue.

(ii) **The anther**:

The outer wall is composed of an epidermis of polygonal, slightly elongated cells.

The anther wall is limited in the inner side by disorganised tapetal cells surrounding the pollen sac.

The fibrous layer is restricted to the ventral side of each pollen sac where it forms a band that run into the wing-like edge. The fibrous layer is formed of 4-6
rows of thick walled lignified cells having no intercellular spaces and showing bar-like reticulate thickening. This layer is gradually decreased to 1 - 2 rows of cells in the wing like - part of the anther. The rest of the anther wall is formed of thin - walled parenchymatous cells having narrow intercellular spaces.

(v) The gynoecium:

1. The ovary: (Fig 98)

The epidermis of the ovary is formed of polygonal isodiametric cells which enclose a ground tissue of parenchyma with narrow intercellular spaces. Some of the cells contain crystals of calcium oxalate.

2. The style:

The epidermis is composed of small polygonal elongated thin - walled cells and covered by thin cuticle. The ground tissue is composed of 8 - 10 rows of rounded parenchymatous cells and traversed by small vascular bundle.

52
Fig. (10):

Pedicel of *Solenostemma argel* (Del.) Hayne

Detailed T.S.

( Cor., cortex; cut., cuticle; ep., epidermis; i.t., latex tube; ph., phloem; pi., pith; p.m.ph., perimedullary phloem; r.cr., rosette crystal of calcium oxalate; tr., trichome; xy., xylem).
3. The stigma:

The internal tissue of the stigma is formed of polygonal isodiametric cells with thin walls and small intercellular spaces. Crystals of calcium oxalate and laticiferous cells are commonly present.

(vi) The pedicel: (Fig 10)

1. The epidermis:

The epidermis consists of tabular - polygonal, nearly isodiametric cells covered by thick striated cuticle. Stomata are mostly of apomocytic type with 5 subsidiary cells. Trichomes are multicellular unicellular uniseriate.

2. The cortex:

The cortex consists of 2 - 3 rows of thick-walled collenchyma cells followed by 6 - 8 rows of thin-walled rounded parenchyma cells with wide intercellular spaces. The cells contain starch granules, crystals of calcium oxalate, latex tubes and latex cells.

3. The pericycle:

Is parenchymatous.
Fig. (11):

The Brock of Solanum mammosum argel (Del.) Hayne

Diagrammatic T.S.

( l.ep., lower epidermis ; l.t., latex tube ; r.ep.,
rosette crystal of calcium oxalate ; tri., trichome ;
u.ep., upper epidermis ; v.b., vascular bundle )
Fig. (12):

Fruit of *Solenostemma argol* (Del.) Hayne

A. Ripe and unripe fruits

3. Opened fruit showing the seeds
4. The vascular system:

It is formed of an interrupted ring of collateral bundles.

5. The pith:

The pith is comparatively wide with peripheral groups of perimedullary phloem. Few starch granules are also present. The pith is traversed by laticiferous tubes.

(vii) The bract: (Fig. 11)

Both epidermis are formed of polygonal cells and are covered by striated cuticle. Stomata and trichomes are present on both surfaces. The bract is structurally similar to the sepals with the exception of the epidermal cells which are axially elongated.

4.1.5 Morphology and anatomy of the fruit:

A. Morphology:

The fruit (Fig 12) is an aggregate of follicles which are oblong – ovate, smooth, greenish – brown with
Fig. (13):

Fruit of *Solenostoma argel* (Del.) Hayne

A. Diagrammatic T.S.

B. Detailed T.S.

(Col., collenchyma; cut., cuticle; end., endosarp; ep., epidermis; t., fibres; l.c., latex cell; l.k., latex tube; per., parenchyma; s.gr., starch granule; v.b., vascular bundle).
numerous dark brown areas and rough corrugated surface. It measures 4 – 5 cm in length and up to 3 cm in the width with a pendulous, cylindrical, yellowish - green stalk.

B. Anatomy:

1. The pericarp:

The outline of the pericarp of the mature fruit, in transverse section (Fig. 13), appears rounded to ovoid.

2. The epidermis:

The epidermis consists of a single row of polygonal, isodiametric cells covered with a thick striated cuticle. Stomata are of the anomocytic type surrounded by 5 – 6 subsidiary cells. They carry non-glandular, multicellular, uniseriate trichomes.

3. The cortex:

This is formed of 4 – 5 rows of collenchyma cells followed by a zone of groups of lignified fibres, and

69
Fig. (14):

Seed of *Solenostemma argel* (Del.) Hayne
Mature seeds
separated by thin-walled parenchyma cells. The rest of
the cortex is a ground tissue of thin-walled parenchymatous
cells with numerous starch granules, latex cells and latex tubes.

4. The placenta:

This forms a considerable part of the fruit. It
consists of masses of soft, thin-walled parenchyma and
vascular bundles.

4.1.6 Morphology of the seed: (Fig. 14)

The average number of seeds per fruit was 68 seeds.
The seed had an average weight of 6.0 mg. The length
varied from 0.4 to 0.5 mm and the breadth from 0.1 to
0.5 mm. The seeds are dark brown in colour, turrid,
ovoid, channelled down one face, minutely tuberculate.
At the narrow end, long plumes of silky tufts of hairs
present and measure about 2 cm in length and these are
useful in dispersal by wind.

71
4.2 Chemical composition of *Solenostemma argel* (Del.) Hayne:

4.2.1 Proximate analysis:

The results of proximate analysis of the leaves, stems and roots of *Solenostemma argel* (Del.) Hayne are shown in Table (1). Plants were analyzed at only one stage of growth when the plants were in flower. The results are expressed on dry matter basis for 100 g of material.
Table 1: Results of proximate analysis of the leaves, stems and roots of *Solenostemma orgel* (Del.) Hayne:

<table>
<thead>
<tr>
<th>Items</th>
<th>Leaves (%)</th>
<th>Stems (%)</th>
<th>Roots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>6.61</td>
<td>5.94</td>
<td>6.56</td>
</tr>
<tr>
<td>Crude protein content</td>
<td>14.25</td>
<td>3.50</td>
<td>1.75</td>
</tr>
<tr>
<td>Crude fibre content</td>
<td>15.20</td>
<td>30.80</td>
<td>39.10</td>
</tr>
<tr>
<td>Carbohydrate content</td>
<td>24.87</td>
<td>24.37</td>
<td>12.56</td>
</tr>
<tr>
<td>Ash content</td>
<td>16.76</td>
<td>8.60</td>
<td>6.65</td>
</tr>
<tr>
<td>Vitamin C content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.70 mg/100g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b. Macro-elements (expressed in %):

<table>
<thead>
<tr>
<th>Elements</th>
<th>Leaves (%)</th>
<th>Stems (%)</th>
<th>Roots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>2.28</td>
<td>0.56</td>
<td>0.28</td>
</tr>
<tr>
<td>Calcium + magnesium</td>
<td>3.09</td>
<td>3.57</td>
<td>4.42</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.60</td>
<td>0.50</td>
<td>0.40</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.48</td>
<td>3.05</td>
<td>4.02</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.06</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.40</td>
<td>4.34</td>
<td>3.20</td>
</tr>
</tbody>
</table>

* N.B.: Each percentage is a mean of three determinations.
4.2.1.1 **Moisture:**

The average moisture content was 6.61% in leaves, 5.94% in stems, and 6.56% in roots.

4.2.1.2 **Crude protein:**

Crude protein content was 14.25% in leaves, 3.50% in stems, and 1.25% in roots.

4.2.1.3 **Crude fibre:**

Crude fibre content was 15.2% in leaves, 30.80% in stems, and 39.10% in roots.

4.2.1.4 **Carbohydrates:**

Total available carbohydrate content was 24.87% in leaves, 24.37% in stems, and 12.56% in roots.

4.2.1.5 **Ash:**

The ash content was 16.76% in leaves, 8.60% in stems, and 6.65% in roots.
4.2.1.6 Vitamin C (Ascorbic acid):

Argel leaves have 27.7 mg / 100 g plant tissue ascorbic acid.

4.2.1.7 Mineral content:

The nitrogen content was assayed as 2.28 % in leaves, 0.56 % in stems and 0.28 % in roots. The calcium content was 0.60 % in leaves, 0.50 % in stems and 0.40 % in roots whereas the magnesium content was 2.48 % in leaves, 3.05 % in stems and 4.02 % in roots. The potassium content was 6.40 % in leaves, 4.34 % in stems and 3.20 % in roots whereas the sodium content was the same in leaves and roots (0.06 %) but it was 0.04 % in stems.

4.2.2 General phytochemical screening of different morphological organs of Solenostemma argel (Del.) Hayne extract:

The results of the general phytochemical screening of ethanolic extracts of leaves, stems and roots of Solenostemma argel (Del.) Hayne are collectively shown in Table (2).

75
### 4. Tannins

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>++</th>
<th>++</th>
<th>++</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### 5. Saponins

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>++</th>
<th>++</th>
<th>++</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

---

78
7. Cyanogenic glycosides

<table>
<thead>
<tr>
<th></th>
<th>l</th>
<th>s</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>l</th>
<th>s</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Anthraquinone glycosides

<table>
<thead>
<tr>
<th></th>
<th>l</th>
<th>s</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>l</th>
<th>s</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Key: l = leaves; s = stems; r = roots; (+) = relatively higher amounts; (••) = low amounts; (−) = not detectable; (±) = traces; 1 = pre-flowering stage of plant growth; 11 = flowering stage of plant growth.
4.2.2.1 Test for the presence of flavonoids:

The presence of flavonoids in the different morphological organs (viz. leaves, stems and roots) of *Solanostemma argel* (Del.) Hayne were detected separately by aluminium chloride and magnesium - in - conc. HCl reagents.

The results of using aluminium chloride reagent for flavonoids detection are shown in Figures 15, 16, 17, 18, 19 and 20 and it is described in table (2). Figures 15 and 16 showed no detection of flavonoids in leaves of plants spaced at 25 cm during the pre-flowering and flowering stages or at 100 cm in the pre-flowering stage according to colours which appear as dark green. Traces of flavonoids are suspected to be present in leaves of plants spaced at 50 cm and 75 cm during pre-flowering and flowering stages (yellow - green) and these substances are most probably present at 100 cm during the flowering stage due to appearance of yellow colours.
Figures 17 and 18 and the explanatory table (2) showed that flavonoids are most probably present in stems of plants at pre-flowering and flowering stages due to intensity of yellow colours shown and their presence seems to be unaffected by the factor of spacing.

Figures 19 and 20 and the table (2) showed that flavonoids are suspected to be present in trace amounts in root extracts of plants in pre-flowering and flowering stages (light yellow = light green or yellow) and their presence is unaffected by the factor of spacing.

The results of using magnesium in concentrated HCl as a comparative reagent are shown in Figures 21, 22, 23, 24, 25 and 26 and the explanatory table (2).

Figures 21 and 22 showed that flavonoids are suspected to be present in leaves of plants spaced at 75 cm in pre-flowering and flowering stages or at 100 cm in the flowering stage (dark = red). As indicated from the test, no flavonoids have been detected in leaves of plants spaced at 25 cm and 50 cm during pre-flowering and flowering stages or at 100 cm during pre-flowering stage (blue = green).
Figures 23 and 24 showed that flavonoids are most probably present in stems of plants at the flowering stage in low amounts and their presence seems to be unaffected by the factor of spacing (red). No flavonoids have been detected in stems at the pre-flowering stage (yellow = brown) but traces are suspected to be present in plants spaced at 100 cm (dark = red).

Figures 25 and 26 showed that no flavonoids have been detected in root extracts of plant (light yellow, yellow = green or yellow).

4.2.2.2 Test for the presence of unsaturated sterols and/or triterpenes:

The results of phytochemical screening for the presence of unsaturated sterols and/or triterpenes in the alcoholic extracts of leaves, stems and roots are shown in figures 27, 28, 29, 30, 31 and 32 and it is described in table (2).
Figures 27, 28 and 29 showed that unsaturated sterols and/or triterpenes are most probably present in leaves of plants at pre-flowering and flowering stages in comparatively higher amounts and their presence seems to be unaffected by the factor of spacing (blue - green).

Figures 27, 29 and 30 showed that unsaturated sterols and/or triterpenes are most probably present in stems of plants at the pre-flowering and flowering stages in comparatively higher amounts and their presence also seems to be unaffected by the factor of spacing (blue - green) except at 50 cm during pre-flowering stage (pink - purple).

Figures 31 and 32 showed that unsaturated sterols and/or triterpenes are most probably present in roots of plants at the pre-flowering and flowering stages and their presence is also unaffected by the factor of spacing (blue - green and/or pink - purple).

4.2.2.3 Test for the presence of cardenolides (Unsaturated steroid nucleus):

The results of phytochemical screening for the presence of cardenolides (unsaturated steroid nucleus) in
the alcoholic extracts of leaves, stems and roots are listed in table 2 and also are shown in figures 33, 34, 35, 36, 37 and 38.

Figures 33 and 34 showed that cardenolides are most probably present in leaves of plants spaced at 25 cm, 50 cm and 100 cm during the pre-flowering and flowering stages in comparatively higher amounts (blue - green). Traces are suspected to be present in leaves of plants spaced at 75 cm at pre-flowering and flowering stages (blue - green and/or dark red).

Figures 35 and 36 showed that cardenolides are most probably present in stems of plants at pre-flowering and flowering stages in higher amounts and their presence is unaffected by the factor of spacing (blue - green).

Figures 37 and 38 showed that cardenolides are most probably present in roots of plants spaced at 100 cm in pre-flowering stage, 75 cm in flowering stage or also at 50 cm in pre-flowering and flowering stages (blue - green). No cardenolides have been detected in roots of plants spaced at 100 cm during flowering stage, at 75 cm during pre-flowering stage or also at 25 cm during pre-flowering and flowering stages (pink - purple).
4.2.2.4 Test for the presence of tannins:

The results of phytochemical screening for the presence of tannins in the alcoholic extracts of leaves, stems and roots are listed in Table (2) and also are shown in figures 39, 40, 41, 42, 43 and 44.

Figures 39 and 40 showed that tannins are most probably present in leaves extracts during pre-flowering and flowering stages in higher amounts and their presence seems to be unaffected by the factor of spacing (black).

Figures 41 and 42 showed that tannins appear to be most probably present during the pre-flowering and flowering stages in low amounts and their presence is unaffected by the factor of spacing (light black).

Figures 43 and 44 showed that no tannins have been detected in roots extracts of plants during the pre-flowering and flowering stages (yellow = brown or dark brown).
4.2.2.5 Test for the presence of saponins:

The formation of persistent foams during plant extraction or during the concentration of plant extracts is reliable evidence that saponins are present. Referring to table (2), it is shown that saponins are most probably present in the three different morphological organs (viz. leaves, stems and roots) of argel plants. Plants at the pre-flowering and the flowering stages show comparatively higher amounts of saponins and the presence of these compounds seems to be unaffected by the factor of spacing.

4.2.2.6 Tests for the presence of alkaloids:

The results of phytochemical screening for the presence of alkaloids in the alcoholic extracts of leaves, stems, and roots are listed in table (2) using Wagner's, Mayer's and Dragendorff's reagents. All these reagents gave positive results in table (2) using Wagner's, Mayer's, and Dragendorff's reagents. In case of the Dragendorff's reagent, thin layer chromatography was used and the developed chromatograms showed clear orange spots with Rf values of 0.33 and

102
T.L.C. of ethanolic extracts of different organs of *Gelonostemma argel* (Kel.) Hayne for their alkaloids

1 = Alcoholic extract of leaves.
2 = Alcoholic extract of stems.
3 = Alcoholic extract of roots.
Solvent system
Toluene:acetone:acetic acid
(1 : 2 : 0.02)

1  2  3
0.29 with toluene/aceton/acetic acid (1:2:0.02) as the solvent system. The spots were not resolved satisfactorily.

4.2.2.7 Test for the presence of cyanogenic glycosides:

Table (2) also shows the results of the phytochemical screening for surveying the presence or absence of cyanogenic glycosides. The results show the absence of the cyanogenic glycosides in the three different morphological organs at the pre-flowering and flowering stages.

4.2.2.8 Test for the presence of anthraquinone glycosides:

The results of the phytochemical screening for the presence or absence of anthraquinone glycosides were shown in Table (2). No anthraquinone glycosides have been detected in plants.
CHAPTER V

DISCUSSION
CHAPTER V
DISCUSSION

The results of proximate analysis of Solanostemma argel (Del.) Hayne as manifested in Table (1) show that there is no significant difference in the average moisture content of leaves (6.61 %) and roots (6.56 %). The average moisture content is (5.94 %) in stems. This result reflects that the root system of argel as a semi-desert plant keeps more moisture than stem.

Crude protein content is higher in leaves (14.25 %) than that in stems (3.5 %) and roots (1.75 %) which is normal. Nevertheless, more work has to be performed to evaluate argel leaves as a source of proteins. In addition, amino acid content of these proteins need to be analyzed.

Crude fibre content is found to be higher in roots (93.1 %) than that in stems (30.8 %) and leaves (15.2 %). Also there is no significant difference in total available carbohydrate in leaves (24.37 %) and stems (24.37 %) but the content was shown to be lower in roots (12.56 %) indicating that the root system of argel may not be considered as a storage organ for carbohydrates or

105
that its respiration activity may be higher than that of stem or leaf.

The ash content was 16.76% in leaves which is higher when compared to that in stems (8.50%) or in roots (6.65%). Also, argel leaves were shown to have a fair amount of plant tissue ascorbic acid with an average of 27.7 mg/100 g.

It is to be stressed that many factors contribute to the amounts of the different chemical components of plant parts. These factors may include climatic conditions, season of the year, age of the plant when collected in addition to the type of soil supporting the plants growth and the nature of the root system.

Table (1) also indicates the results of the chemical analyses of some of the elements important for plant nutrition. Elemental analysis generally reflects the amounts of different macro and micro elements required by a plant species to support its growth and metabolism. Results show that the nitrogen content was high in leaves (2.28%) in comparison to that in stems (0.56%) and roots (0.28%). This comparatively high nitrogen content in leaves may be attributed to the presence of the
different nitrogen-containing metabolites like amino acids and enzymes in the leaf, the most important plant organ for active metabolism. Proteins approximately contain 18% nitrogen on a dry weight basis. In addition, the total amount of leaf nitrogen was found to be directly proportional to the rate of photosynthesis. This is because the bulk of leaf nitrogen is directly involved in photosynthesis as a component of photosynthetic enzymes and chlorophyll.

The highest percentage of calcium is found in the leaves (0.60%) followed by the stems (0.50%) and lastly the roots (0.40%), whereas the highest content of magnesium is found to be in the roots (4.02%) followed by the stems (3.05%) and lastly the leaves (2.48%). Crystals of calcium oxalate are of common occurrence in the leaves (Fig. 2a) and this may justify the presence of calcium in relatively high levels in this plant part. The investigated species also shows high levels of potassium uptake in comparison with the rest of the elements. This is because high potassium concentration is needed for the purpose of activation of numerous enzymes. Highest percentage of potassium was found in leaves (6.40%) than in stems (4.34%) while the roots contain 3.20%. There is no difference in sodium content of leaves and roots (0.06%) while in stems the content is relatively lower (0.04%).
Alkaloids, tannins, saponins, cyanogenic glycosides, flavonoids and anthraquinones are the active ingredients generally screened in a medicinal plant. Table (2) summarizes the results of the general phytochemical screening for the possible presence of certain active constituents with relation to spacing (25 cm, 50 cm, 75 cm and 100 cm) at two stages of growth. The pre-flowering and flowering stages are the two stages of growth when argel leaves are generally picked by collectors.

It is clear from table (2) and figures from (fig. 15) to (fig. 44) that some chemical ingredients in various concentrations were detected in the three morphological plant parts while other ingredients seemed to be absent. In general, the factor of spacing was shown to be insignificant. This conclusion may be attributed partly to the fact that the general phytochemical screening is more qualitative than it is quantitative. The other factor is that argel is a desert plant and it is drought resistant. This characteristic property is related to its deep, profusely branched root system (fig. 5) which absorbs water and nutrients from a large mass of soil. Such property may hold the factor of spacing relatively ineffective.
All the tests were more sensitive to show the presence of flavonoids in stems with traces in leaves and roots both in the pre-flowering and flowering stages.

Unsatuated sterols, triketones and carotenoids were found to be almost equally present in the three plant parts at both pre-flowering and flowering stages.

Flavonoids were shown to be present most in leaves with little in stems but roots were devoid of them. This was evident in both stages of growth.

Saponins were satisfactorily detected in the three plant parts at both pre-flowering and flowering stages while alkaloids were found to be present as traces in the three morphological parts at both stages of growth. The three morphological parts at both stages of growth were shown to be devoid of cyanogenic or anthraquinone glycosides.

Flavonoids class, particularly flavonols, flavones or/and chalcones make a significant distribution to yellow colouration (Figures 17, 18, 19 and 20). These results confirm with the findings of Knaedel and co-workers (1974) who indicated the presence of kaempferol, a flavonol, in leaves of ergot. Flavonoids class, particularly flavonols and/or flavones are suspected to be most probably present in plants after treatment with Mg conc. 0.1%. These results agreed with the findings of Habermann (1973) who stated that:

"only flavonones among the flavonoids give intense cherry red color".
All morphological organs tested gave strong positive tests for sterols and/or triterpenes. These results agreed with the findings of Sandermann (1962) who stated that: "triterpenes should hardly be expected to be suited for chemotaxonomic purposes, since they are distributed among the entire plant kingdom". However, no cyanogenic glycosides or anthraquinone glycosides have been detected in leaves, stems or roots of argel plant.

On the other hand, leaves and stems of argel plant gave positive tests for tannins but roots were found to be devoid of them. (Figures 39, 40, 41, 42, 43 & 44) Also, all morphological organs tested gave strong positive tests for saponins. From these results, further research work on this species for its saponin content could be promising.

One of the species belonging to this family, namely Calotropis procera, has been investigated by Mohammed (2) who reported that it was devoid of anthraquinone glycosides and saponins in flower, stem, root bark and stem bark. Flavonoids were detected in the flower while tannins are found in traces in flower and stem bark. Alkaloids were detected in flower, root bark and stem bark but they were devoid in stem wood.
Although detection of alkaloids in argel plants has not been reported before, tests for alkaloids in the three morphological parts showed positive results which were confirmed by the three reagents: Mayer's, Wagner's and Dragendorff's. Thin layer chromatography showed at least two spots which gave a characteristic orange colour when sprayed with Dragendorff's reagent. Therefore, it is recommended that more work has to be carried out concerning alkaloids in argel.
SUMMARY AND CONCLUSIONS
The present study is divided into two parts. Part one includes a botanical study of *Selenostemma argel* (Del.) Hayne; it comprises morphology and anatomy of leaves, stems, roots, flowers & fruits in addition to morphology of seeds. Part two includes a chemical study of argel that comprises: (a) proximate analysis (viz. moisture, crude protein, crude fibre, total available carbohydrate, ash, nitrogen, calcium, magnesium, potassium, sodium & vitamin C) which have been made at only one stage of growth when the plants were in flower; (b) general phytochemical screening for detection of certain plant secondary metabolites (viz. flavonoids, sterols, tannins, alkaloids, saponins, cyanogenic glycosides & anthraquinone glycosides) in leaves, stems & roots. This phytochemical screening was performed at two stages for plant growth namely pre-flowering and flowering stages for plants grown at four different inter-plant spacings of 25 cm, 50 cm, 75 cm and 100 cm.

The most prominent findings of the morphological study are the gamopetalous corolla, staminal column and corona and styylar head with 5 gland-like pollen carriers alternating with and adhering to anther.
follicular fruits, flattened seeds with silky coma and opposite extipulate leaves. The laticiferous tubes, intraaxillary phloem, superficial development of the cork, a broad pericycle containing isolated groups of fibres, narrow medullary rays in the wood, anomocytic stomata, bicolateral vascular bundles and non-glandular hairs are the most important anatomical characteristics of argal plant.

The phytochemical screening revealed that some chemical ingredients (viz. flavonoids, sterols and/or triterpenes, cardiacenolides "unsaturated steroid nucleus", tannins, saponins, alkaloids) were detected in the three morphological plant parts while other ingredients (viz. cyanogenic glycosides & anthraquinone glycosides) seems to be absent.

In general, the factor of spacing was shown to be insignificant. The most important secondary constituents are the saponins in addition to traces of alkaloids which presence was reported for the first time.

There is no significant difference between the pre-flowering and flowering stages as to the presence or absence of these different chemical groups.

113
REFERENCES


116


119


APPENDIX
Apparatus and reagents:

Instruments:

Reichert sliding microtome, Rotary microtome - Baird and Tatlock microtome - sargent, microscope, mettler H3D balance, oven, hot plates, heater, heating device, water bath, muffle furnace, fume-hood, desiccator, vacuum pump, Labconco digestion apparatus, Soxhlet extracting apparatus (capacity 250, 500 ml), distillation apparatus, blender, spectrophotometer, atomic absorption spectrophotometer - Perkin ELMER 2380 (for Na: 330.7 nm, for K: 766.5 nm), rotary evaporator.

An adjustable spreading device (apparatus) - made by Desaga, scalpels, razor blades, forceps, mounted needle, L-moulds - brass, camel hair brush, filter papers - Schleicher and Schuell No. 150, trays, cotton.

Glassware:

Petri - dishes, glass slides and cover-slips, Coplin jar, graduated measuring cylinder (100 ml), glass rod, pipettes (capacity 5, 10 and 20 ml), burettes, test
tubes, volumetric flasks (capacity 50, 100, 250, 500 and 1000 ml), Kjeldahl flask (capacity 30 and 500 ml), separatory funnel, glass plates (20 cm x 20 cm), porcelain crucible, California modified Buchner funnel (Labconco cat. # 55100).

**Chemicals:**

**Acids:**

Hydrochloric acid (concentrated, 0.1N, 2N and 10% aq.); perchloric acid (52%) [270 ml perchloric acid (sp. gr. 1.70) + 100 ml distilled water]; sulphuric acid (concentrated, 0.255N, 77%: [1 part of conc. H₂SO₄ + 1 part of H₂O], (760 ml sulphuric acid sp. gr. 1.84 + 330 ml distilled water); boric acid; [40 g boric acid (H₃BO₃) + 1000 ml distilled water]; oxalic acid (40%): [4 g oxalic acid + 1 litre distilled water]; oxalic acid (10%) for measuring the dye strength [50 g oxalic acid + 500 ml distilled water]; ascorbic acid for measuring dye strength [0.05 g ascorbic acid + 250 ml 10% oxalic acid]; acetic acid anhydride; acetic acid glacial; chromic acid (20 - 30% aq.).
Reagents:

Buffer solution of ammonium chloride and ammonium hydroxide: [67.5 g NH₄Cl + 570 ml conc. NH₄OH + 1000 ml distilled water]; calcium chloride standard solution (0.01N): [0.5 g calcium carbonate + 10 ml hydrochloric acid; "1 part hydrochloric acid + 3 parts H₂O" + 1000 ml distilled water]; copper sulphate solution (1%); hydrogen peroxide solution (3%, 30% volume); aluminium chloride (1% w/v in methanol); normal saline solution (0.9% w/v solution sodium chloride in distilled water); glucose standard solution: [100 mg glucose + 100 ml distilled water]; glucose dilute standard solution: [10 ml of strong standard + 100 ml distilled water; "1 ml = 0.1 mg glucose"]; sodium nitrate standard
solution for atomic absorption spectroscopy: potassium nitrate standard solution for atomic absorption spectroscopy: cesium chloride solution: [50 g cesium chloride + 200 g Al(NO₃)₃·9H₂O + 1000 ml distilled water]
- Formalin - Acetic - Alcohol (FAA): [850 ml 70% ethanol + 100 ml 40% formaldehyde + 50 ml acetic acid glacial; anthrone reagent (freshly prepared): [0.1% anthrone + sulphuric acid "760 ml H₂SO₄ + 330 ml distilled water"], versine (0.01%): [2 g E.D.T.A "Disodium dihydrogen ethylene diamine tetr-acetate solution" + 0.5 g magnesium chloride + 1000 ml distilled water]
- Ferric chloride reagent: [5 g ferric chloride + 100 ml distilled water]
- Moyer's reagent: [1.35 g mercuric chloride + 5.0 g potassium iodide + 100 ml distilled water]
- Wagner's reagent: [5 g iodine + 10 g potassium iodide + 100 ml distilled water]
- Modified Dragendorff's reagent: [A) 10 ml acetic acid + 0.85 g bismuth nitrate + 40 ml distilled water (B) 5 g potassium iodide + 20 ml distilled water, equal volumes of A and B are mixed. 1 ml stock solution was mixed with 2 ml of acetic acid and complete to 10 ml distilled water]
- Sodium picrate paper: [A piece of filter paper was dipped in 1% picric acid, left to dry then dipped in 10% solution of sodium carbonate and air dried].
Dyes:

Mixed indicator solution: [2 g methyl red + 1 g methylene blue + 1000 ml ethanol (96% v/v); dye solution for determination of ascorbic acid: [0.2 g 2,6-Dichlorophenolindophenol + 500 ml distilled water]; dye strength: [5 ml ascorbic acid + 5 ml 10% oxalic acid]. Then the solution was titrated with dye to faint pink colour. (dye strength = 1 / titre); ammonium purpureate indicator: [0.5 g indicator + potassium sulphate powder]; Eriochrome Black T. indicator (E.B.T): [0.5 g indicator + 4.5 g hydroxylamine – hydrochloric acid + 100 ml ethanol (95%)]; safronin stain (1 g was dissolved in 100 ml 50% ethanol); Fast Green stain: [0.5 g was dissolved in 100 ml ethanol].

Mounting material:

Cedar oil, clove oil, Canada balsam, paraffin wax – congealing point about 50°C, xylene, glycerine.