

# Phytochemical and Pharmacognostic Investigation of Antidiabetic *Scoparia dulcis* Linn Scrophulariaceae Whole Plant Grown in Nigeria

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**Abstract:** *Scoparia dulcis* Linn has been widely reported to have pharmacological uses arising from its wide-spread folkloric uses. Some of these pharmacological properties have been evaluated and include antidiabetic, antitumor and antiviral. However, very limited work has been carried out on the Nigerian species toward documenting its ethnomedicinal uses and establishing its phytochemical and pharmacognostic fingerprints. Studies were therefore carried out to determine the phytochemical and pharmacognostic profile of *S. dulcis* L. grown in Nigeria. The phytochemical analysis of the powdered whole plant revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, and terpenes. Successive extraction yielded hexane extract 1.93%, ethyl acetate extract 1.54%, and methanol extract 14.50%. Quantitative pharmacognostic analysis gave moisture content 7.74%, alcohol extractive value 20.00%, water extractive value 20.00%, total ash 6.32%, acid-insoluble ash 0.82% and water soluble ash 0.37%. Leaf and seed coat microscopy is reported here for the first time. Leaf microscopy revealed upper and lower epidermal surfaces made up of wavy-walled somewhat polygonal cells, with abundant stomata, striations, cystoliths and a fair distribution of glandular trichomes with multiseriate heads and particular base cells. The transverse section of the stem indicated a thick layer of cutin preceding the epidermal cells followed by a layer of collenchyma cells, thin layer of pericyclic fibers and a layer of phloem bundle preceding long trachids, proto- and meta- vessels leading to a collateral vascular bundle arrangement. The centre of the section was made up of parenchyma cells with traces of small raphids-type of calcium oxalate crystals. These phytochemical and pharmacognostic fingerprints of *S. dulcis* grown in Nigeria are relevant for developing monograph of this potential drug plant and as quality indices for its development into a phytomedicine. [Researcher. 2010;2(6):7-16]. (ISSN: 1553-9865).

**Key words:** *Scoparia dulcis* L.; phytochemicals; pharmacognosy, microscopy

## 1. Introduction

*Scoparia dulcis* Linn belongs to the family Scrophulariaceae. It is an herbal plant found almost around the globe: in European, African, American and Asian countries. Common names are sweet broomweed, *typycha kuratu* (Japan), *vassourinha* (Brazil) and *escobilla* (Peru). Synonyms are *Scoparia grandiflora*, *Scoparia ternata*, *Capraria dulcis*, *Gratiola micrantha*. *Scoparia dulcis* L. has been used traditionally as a remedy for Diabetes mellitus in India and hypertension in Taiwan. It is used in curing ailments such as fever, diarrhea, ulcer, cancer,

wounds, skin rash, cough and tuberculosis. Usually, the extract is obtained from cold water maceration of the powdered whole plant. The fresh or dried plant has been used for treating stomach aches, inflammation, bronchitis, hemorrhoids and hepatitis and as an analgesic and antipyretic. It is deemed to be a panacea for all ills. In the Gambia, a lotion prepared from the plant is used in curing fever. A hot water infusion or decoction of the leaves or whole plant is used medicinally by indigenous tribes of Nicaragua to treat malaria, stomach disorders, menstrual disorders, insect bites, fevers and heart problems, liver disorders and

venereal diseases. It has been used for blood cleansing, in childbirth and as a general tonic (Burkill, 2000).

In Nigeria the plant is known by such names as *roma fada* (Hausa), *aiya* (Igbo), *bibiimbelemo* (Ijo), *mesenmesèn gogoro* (Yoruba), *ndiyang* (Efik), and *ungungbuh* (Gwari). The plant is an erect, shrubby herb that grows up to about 50 cm high (Orhue and others, 2009). It usually has many auxiliary shoots and reproduces from seeds. The stem is more or less woody, ribbed, mainly branched and glabrous. The leaves are opposite or three at a node, oval or narrowly oblanceolate; about 2.5 cm to 5.0 cm long and 1.5 cm wide, widely toothed at the upper part of the leaf and wedge-shaped at the lower part of the leaf. The leaf blade is smooth except that the lower surface has some glandular dots. The inflorescence is a slender raceme with one or two flowers in the upper leaf axils. The fruit is a round capsule (Burkill 2000). The Brazilian folkloric use is to treat bronchitis, gastric disorders, hemorrhoids, insect bites and skin wounds. Asian medicine uses the herb to treat hypertension (Latha and others, 2005). The herb also has antiulcer properties (Melania and others, 2008). In tropical and subtropical regions, the fresh or dried plant of *Scoparia dulcis* has traditionally been used as one of remedies for stomach troubles (Satyanarayana 1969), hypertension (Chow and others, 1974), diabetes (Perry 1980), bronchitis (Freire and others, 1993), and as an analgesic and antipyretic (Gonzales Torres 1986). In Nigeria *S. dulcis* is used for the management of diabetes, as love charm, and chewed to secure favor from people in authorities (Muazzam, Ibrahim Wudil, personal communication, 2009). Scientific literature reveals numerous chemical studies on the herb from various part of the world, except the Nigerian species. Previously isolated biologically active chemical constituents include coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids, catecholamine, noradrenaline and adrenaline which have sympathomimetic effects. Also present are scoparic acid A (**1**), scoparic acid B (**2**), scoparic acid C (Hayashi and others, 1993) (**3**), scopadulcic acid A (**4**) and B (**5**) (Hayashi et al. 1991), scopadulciol or dulcinol (**6**) has been reported as an anti-viral agent, inhibitor of Herpes simplex, and an inhibitor of gastric  $H^+$ ,  $K^+$ -ATPase (Hayashi and others, 1993). Other bioactive constituents are scopadulin (**7**) (Hayashi and others, 1990) which has been identified as contributor to the observed medicinal effect of the plant, hispidulin (**8**) which had been reported to possess bronchodilating and antiasthmatic effect more potent than aminophylline on a molar basis (Kalaya and

others, 1986), scopadiol (**9**), potassium adenosine triphosphatase (ATPase) activator, alpha amyryl, betulinic acid, dulcoic acid, friedlin, glutinol and ifflionic acid (Yamamoto and Gaynor 2005). Betulinic acid is used for the prevention and treatment of cancer. It also has antitumor, antileukemia and antiviral (including HIV) properties. It has cytotoxic activity against malignant brain tumor and bone cancer (Simone, 2008); Scoparic acid B has antitumour activity against human cancer cells. Four scopadulane-type diterpenoids: 4-epi-scopadulcic acid B, scopadulcic acid C (**10**), a new compound, dulcidiol, and iso-dulcinol were isolated in Vietnam. These diterpenoids have antibacterial and antifungal activities (Phan and others, 2006). The chemical structure of scopadulin (an antiviral agent) was confirmed by x-ray crystallography (Hayashi and others, 1990), while scopadulcic acid B had been shown to promote antitumor activities (Hayashi and others, 1991; Nishino 1993).

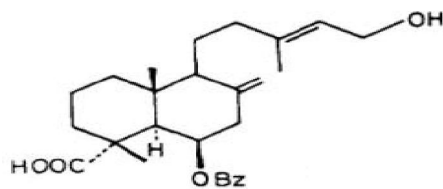
The traditional use of this plant as an abortive or childbirth aid warrants that it should not be taken during pregnancy. An extract of *S. dulcis* recently demonstrated hypoglycemic activity, significantly lowering blood sugar levels in rats. This implies that the plant is probably contraindicated in people with hypoglycemia. The plant however, should not be combined with antidepressants or barbiturates unless under the supervision of a qualified health care practitioner because the plant is associated with sympathomimetic effects (Leslie 2005). The preliminary phytochemical and pharmacognostic fingerprints of this potential drug plant grown in Nigeria toward monograph development and for quality control purposes is reported here for the first time.

## 2. Materials and methods

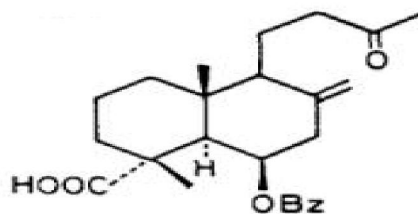
All chemicals used were of analytical reagent grade (supplied by either Merck or Fluka) and used as supplied.

### 2.1 Plant material and processing

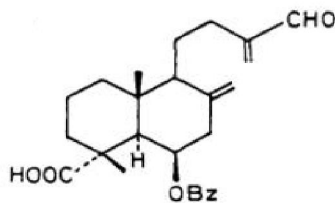
The herb was collected at Chaza in Suleja, Nigeria by Muazzam, Ibrahim Wudil, an ethnobotanist, and was identified and authenticated at the herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, where a voucher specimen was deposited. The whole plant was thoroughly air-dried for two weeks and pulverized using a blender to reduce the particle size.



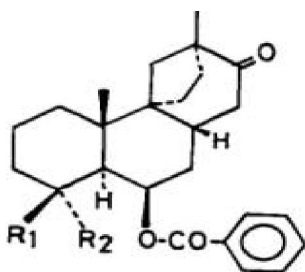
1 Bz=COC<sub>6</sub>H<sub>5</sub>



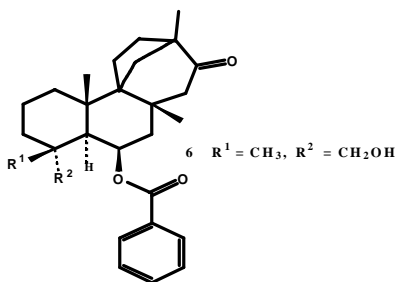
2 Bz=COC<sub>6</sub>H<sub>5</sub>



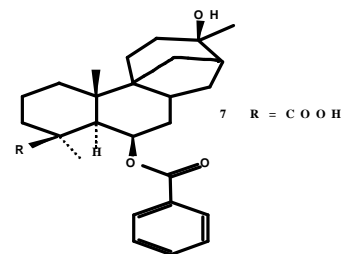
3 Bz=COC<sub>6</sub>H<sub>5</sub>



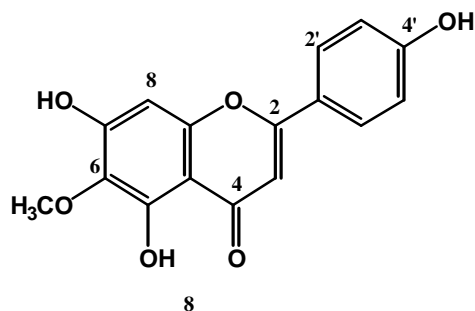
4 R<sub>1</sub>=COOH, R<sub>2</sub>=CH<sub>2</sub>OH  
5 R<sub>1</sub>=Me, R<sub>2</sub>=COOH



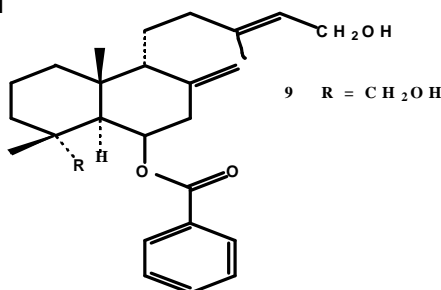
6 R<sup>1</sup> = CH<sub>3</sub>, R<sup>2</sup> = CH<sub>2</sub>OH



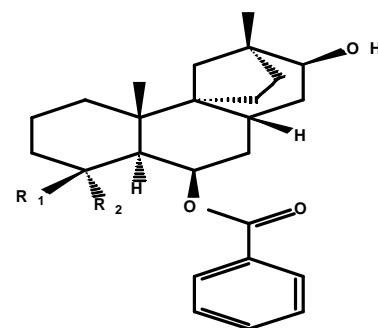
7 R = COOH



8



9 R = CH<sub>2</sub>OH



10 R<sub>1</sub> = COOH R<sub>2</sub> = CH<sub>3</sub>

## 2.2 Extraction and thin layer chromatographic fingerprinting

10g of the air-dried and powdered whole plant was subjected to successive maceration with hexane (3 x 100ml), ethyl acetate (3 x 100ml) and methanol (3 x 100ml) at ambient temperature for 24 hours and vacuum filtered. The filtrates were concentrated to dryness under vacuum at 40°C. Yield

of extracts were determined. The concentrated filtrate was spotted on Silica Gel 60 pre-coated TLC glass plate, previously activated at 105°C for 2 hours, developed with mobile phase solvent systems of hexane-ethyl acetate 4:1; hexane-ethyl acetate 3:2; and ethyl acetate-methanol 1:1 for hexane extract, ethyl acetate extract and methanol extract respectively. Then each plate was sprayed with p-anisaldehyde

sulphuric acid, a specific spray reagent for detection of terpenes and steroids on TLC plates, prepared by mixing 0.50ml p-anisaldehyde, 50.00ml glacial acetic acid and 1.00ml concentrated sulphuric acid. The retardation factors ( $R_f$ ) of all components are reported.

### 2.3 Phytochemical and pharmacognostic analyses

Phytochemical and quantitative pharmacognostic analyses were carried out on the powdered sample while microscopy was carried out on the fresh leaf, stem and dried seed coat. The phytochemical screening and pharmacognostic analyses were carried out using standard methods (Evans 2002, MHFW (1999), and Sofowora 2008).

### 2.4 Microscopy

Fresh sample of the leaves, stem and the dried seed coats were placed in separate petri dishes and sodium hypochlorite solution was added enough to cover the surface and left for about 24 hours when

the various plant parts were completely detached. Microscopic studies were carried out on the upper and lower leaf surfaces, upper and lower seed coats and the transverse sections of the stem.

### 2.5 Determination of water-soluble ash value

Water-soluble ash value was determined as reported in the MHFW (1999) with slight modifications. Briefly, total ash was determined using 2 g of the air-dried powdered sample. The total ash was boiled for 5 minutes with 25 ml of distilled water; the insoluble matter was collected on an ashless filter paper, washed with hot distilled water, and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air-dried powdered plant sample.

## Results

Table 1. Percentage yield of successive extraction of *S. dulcis* powdered whole plant

| Extract       | Hexane extract | Ethyl acetate extract | Methanol extract |
|---------------|----------------|-----------------------|------------------|
| Yield % (w/w) | 1.90           | 1.54                  | 14.50            |

Table 2. Phytochemical analysis of *S. dulcis* powdered whole plant

| Secondary metabolite | Result | TLC* |
|----------------------|--------|------|
| Carbohydrates        | +      |      |
| Saponins             | +      |      |
| Tannins              | +      |      |
| Steroids             | -      | +    |
| Terpenes             | -      | +    |
| Balsams              | -      |      |
| Anthraquinone        | -      |      |
| Flavonoids           | +      |      |
| Alkaloids            | +      |      |

Key: + = Detected, - = Not detected

\* Thin layer chromatograph sprayed with p-anisaldehyde-sulphuric acid reagent.

Table 3. Quantitative pharmacognostic analysis of *S. dulcis* whole plant

| Parameter                        | Value (%) |
|----------------------------------|-----------|
| Moisture content                 | 7.67      |
| Alcohol-soluble extractive value | 20.00     |
| Water-soluble extractive value   | 20.00     |
| Total ash                        | 6.50      |
| Acid-insoluble ash               | 1.00      |
| Water-soluble ash                | 0.50      |

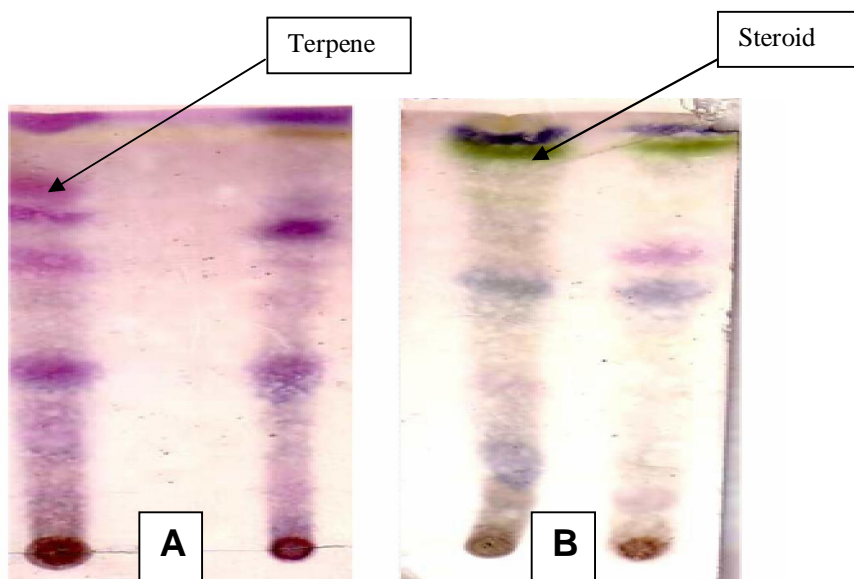
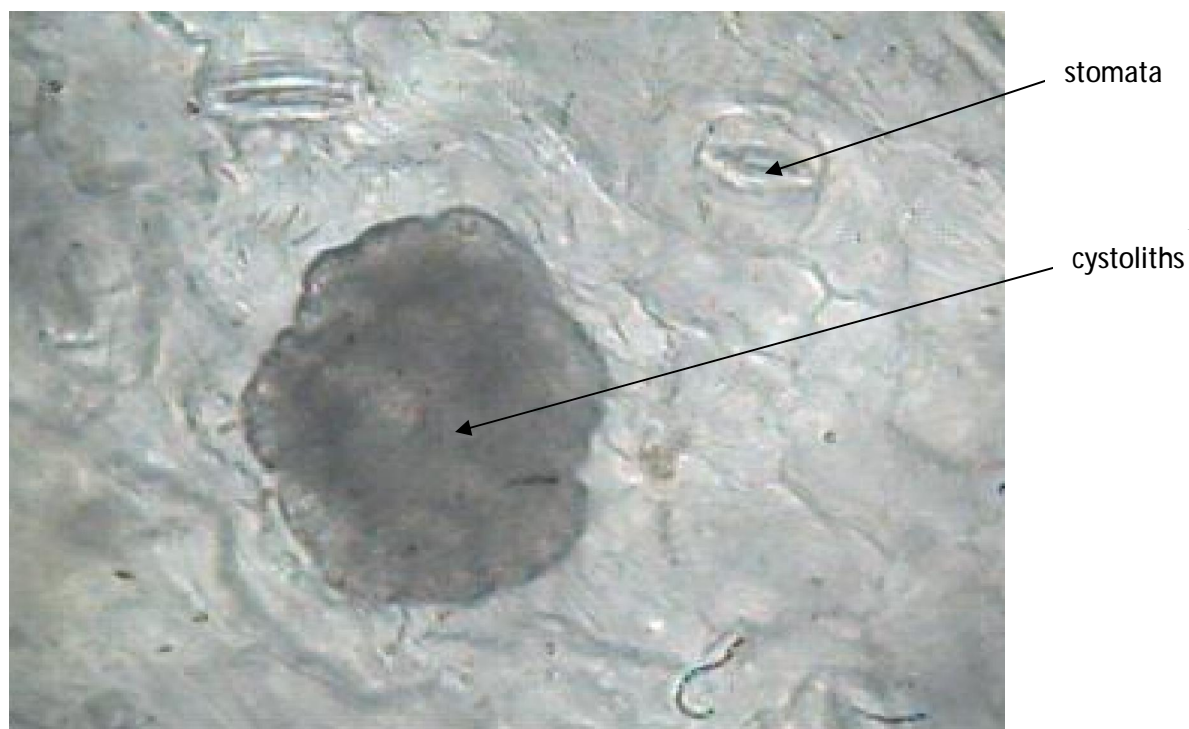


Figure 1. Thin layer chromatoplates of hexane and ethyl acetate extracts sprayed with p-anisaldehyde sulphuric acid. A is hexane extract and B is ethyl acetate extract.

Table 4. Retardation factors ( $R_f$  values) of hexane, ethyl acetate and methanol extracts of *S. dulcis* whole plant.

| Spots | Hexane extract | Ethyl acetate extract | Methanol extract |
|-------|----------------|-----------------------|------------------|
| 1     | 0.28           | 0.55                  | 0.71             |
| 2     | 0.68           | 0.69                  | 0.93             |
| 3     | 0.80           | 0.83                  |                  |
| 4     | 0.91           | 0.87                  |                  |
| 5     | 0.94           | 0.93                  |                  |
| 6     | 0.99           | 0.97                  |                  |

Mobile phase solvent system for Thin Layer Chromatography: Hexane extract (hexane-ethyl acetate 4:1); ethyl acetate extract (hexane-ethyl acetate 3:2); and methanol extract (Ethyl acetate-Methanol 1:1).

Figure 2. Cystoliths containing calcium carbonate in the upper leaf surface of *S. dulcis* (Magnification: x400)

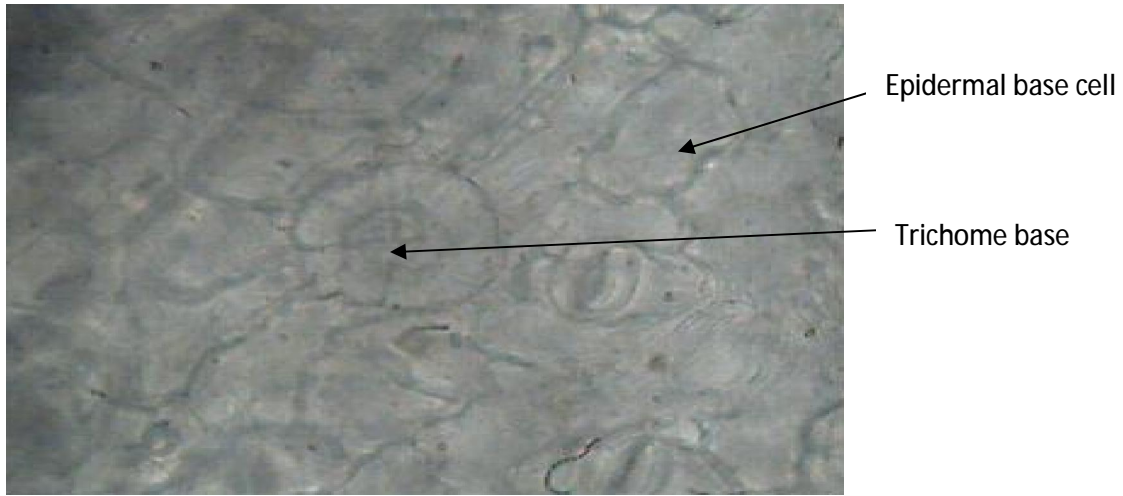


Figure 3. The upper surface of *S. dulcis* leaf showing the glandular trichome (Magnification: x400)

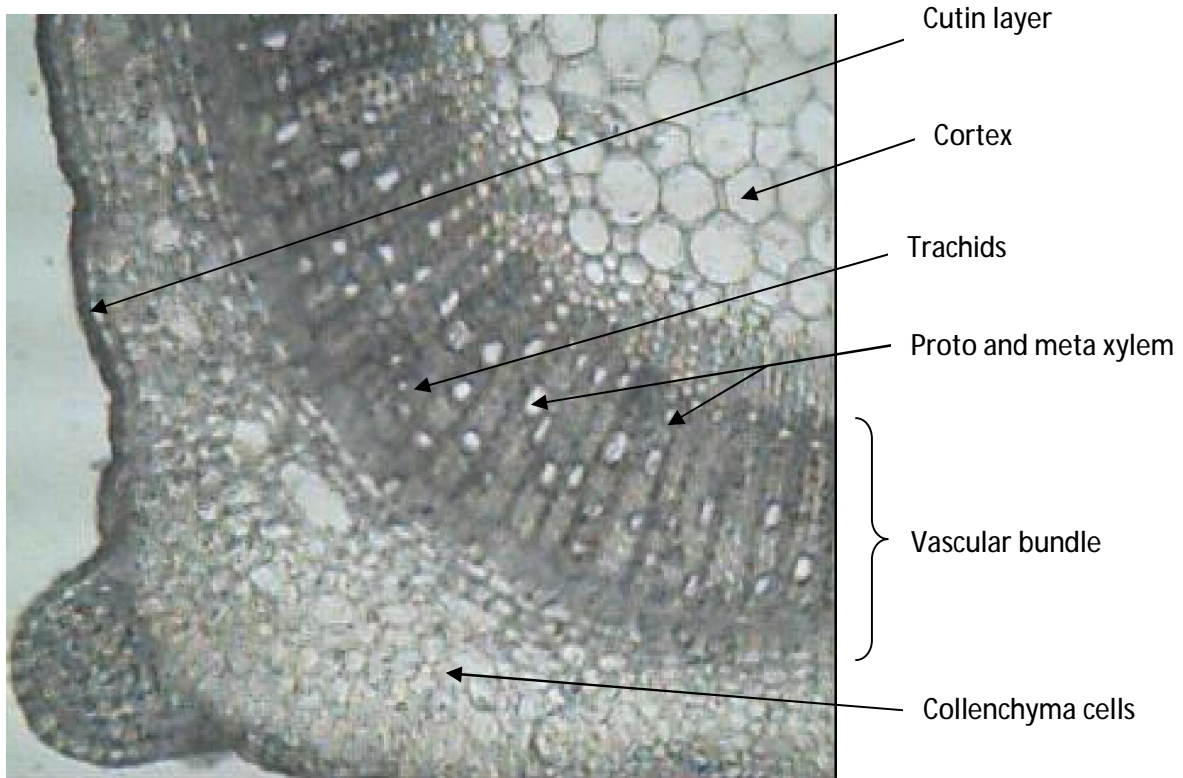


Figure 4. Transverse section of *S. dulcis* stem (Magnification: x400)

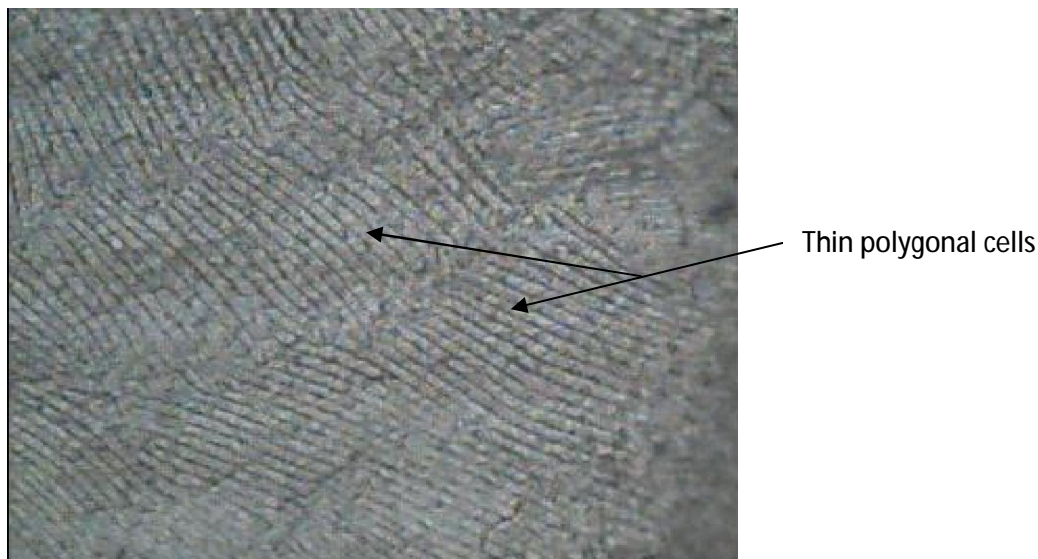


Figure 5. Upper surface of *S. dulcis* seed coat showing polygonal cells (Magnification: x400)

## Discussion

The phytochemical screening of the plant shows the presence of saponins, tannins, steroids, flavonoids, alkaloids and terpenes. Some secondary metabolites have been reported in the literature to have pharmacological activities. For instance, some alkaloids had been reported to have anticancer and antiviral activity, saponins have been reported to be cardiotonics, while flavonoids have anti-inflammatory activity (Evans 2002).

The presence of tannins may be responsible for ability of *S. dulcis* to cure diseases such as diabetes, diarrhea, sore throat, skin ulcer and dysentery. The presence of flavonoids in *S. dulcis* may be responsible for its uses to cure cancer, inflammations and allergies (Cushine and others, 2005). The alkaloids may be responsible for the anticancer, antidiabetics, antiaging and antiviral activities of this herbal plant (Evans 2002).

The herbal plant *S. dulcis* in its dried form is expected to have a long shelf-life with reduced chance of microbial growth due to its relatively low moisture content of 7.67%. Total ash value of 6.50% indicates low inorganic components in the herbal plant. Acid-insoluble ash value of 1.00% indicates high digestibility when the plant is consumed. Water-soluble ash value of 0.50% is indicative of negligible level of water-soluble minerals absorption from the plant when it is consumed.

The alcohol-soluble extractive value of 20.00% and water-soluble extractive value of 20.00% shows that both solvents would be good for extraction of this potential drug-plant. Extraction with methanol gave the highest yield in the successive extraction, showing it is the best solvent for extraction among the three solvents used. The thin layer chromatography shows that at least six components are present in the hexane extract, at least six in ethyl acetate extract and at least two in methanol extract

The TLC plates sprayed with p-anisaldehyde-sulphuric acid showed the presence of terpenes and steroids which were not detected during the phytochemical screening. Based on this observation, we recommend that when in doubt, spray reagents be used to confirm phytochemical test results.

The epidermal surfaces (upper and lower) of the leaves (figures 4 and 5) are characterized by wavy walled somewhat polygonal cells, with abundant stomata, striations, cystoliths and a fair distribution of glandular trichomes with multiseriate heads and particular base cells. The transverse section across the stem is characterized by a thick layer of cutin preceding the epidermal cells (1 or 2 layers), followed by a layer of collenchyma cells, thin layer of pericycle fibers, a layer of phloem bundle preceding long trachids, and proto- and meta- vessels leading to a collateral vascular bundle arrangement. The centre of the section was made up of general cortex



(parenchyma cells) with traces of small raphids type of calcium oxalate crystals.

The upper and lower surfaces of the seed coat exhibited long thin polygonal cells towards the tip and then thick walled pitted sclerenchyma cells at the tip of the seed coat. Occasional strands of spiral xylem vessels transverse the length of the seed coat, neither trichomes nor stomata were detected.

### Conclusions

*Scoparia dulcis* grown in Nigeria is rich in secondary metabolites and has numerous uses in traditional medicine to treat several ailments, ethnomedicinally reputable as antidiabetics. It has potential for development into a phytomedicine. More work is ongoing in our laboratories to isolate and characterize the chemical constituents of the Nigerian plant and to ascertain its antidiabetic properties. The phytochemical and pharmacognostic fingerprints reported here for this potential drug plant could be useful for monograph development and for quality control purposes.

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