INNOVARE JOURNAL OF HEALTH SCIENCES

Vol 1, Issue 3 , 2013 ISSN 2347-5536

ResearchArticle

# QUALITY ASSESSMENT PROFILE OF THE LEAVES OF *Vitis Vinifera* L. (*Vitaceae*) – AN IMPORTANT PHYTOTHERAPY COMPONENT OF TROPICAL DISEASES CONTROL

**K.PERIYANAYAGAM*[1], B.KASIRAJAN[2], V. KARTHIKEYAN[2], R. JANCY GRACELET[2], T. KUMUDA[2]**

[1, 2] **Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, 625020.Tamilnadu, India.**

**Email:**kpn1960@yahoo.com

*Received: 17 September 2013, Revised and Accepted: 30 September 2013*

**ABSTRACT**

Objective: To study in detail the macro and micro morphology including scanning electron microscopy, phyto and physicochemical analysis along with the trace elements determination (EDAX) of the leaves of *Vitisvinifera* L. family Vitaceae, which possesses various bioactive components and many traditional and pharmacologically validated uses in the treatment of many diseases including NTD.

Methods**:** Macroscopy, microscopy including scanning electron microscopy, physicochemical analysis, preliminary phytochemical screening, Energy dispersive X-ray analysis to determine the various trace elements, and other WHO recommended parameters for standardizations were performed.

Results**:** *Vitisvinifera* L. leaves are orbicular-cordate, 5-15cm, more or less deeply, palmately-3-, 5- or 7-lobed, irregularly toothed, glabrescent above, often grey-tomentose beneath, thin membraneous; Microscopic evaluation revealed the presence of ranunculaceous type of stomata in lower epidermis and upper epidermis cell walls are straight polygonal in shape and apostomatic, secretory cells, druses of calcium oxalate crystals, raphide, pearl glands, unicellular trichomes, xylem vessels, phloem, fibres. Vein islet numbers, vein termination numbers, stomatal number, stomatal index and other physicochemical tests like ash values, loss on drying, extractive values were determined. Preliminary phytochemical screening showed the presence of sterols, tannins, proteins and amino acids, flavonoids, terpenoids, volatile oil, saponin, carbohydrates and absence of glycosides, alkaloids, fixed oil.

Conclusion**:** The microscopic analysis using histological identification, microscopic constants and other physicochemical examinations of the leaves of *Vitisvinifera* L. can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf as it was proved to have wide panel of pharmacological and ethno medical use including prevention and treatment of tropical diseases.

**Keywords:***Vitisvinifera*, Vitaceae, Microscopical evaluation, Scanning electron microscope, Energy dispersive X-Ray analysis, neglected tropical diseases.

## INTRODUCTION

Tropical diseases are of great public health problem. The WHO reported that nearly one billion people suffer from one or more neglected tropical diseases [1]. Tropical humid climate facilitates the occurrence of many skin infections and other diseases [2]. Plant materials are used throughout the world as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market. It is therefore important to establish their quality [3]. In this study we selected a widely available plant Common Grape Vine leaf, *Vitisvinifera* L. The Common Grape Vine is popularly known as Angur and dakh, in India and Kodimundiri in Tamilnadu. It belongs to the family Vitaceae [4].Vitaceae family comprises woody climbers, vines, trees, shrubs and succulent's trees which are important food sources. Commercially, Vitaceae families are valuable raw materials for the production of wine, medicine and perfumery. The grape family (Vitaceae) is a relatively small family and comprises about 14 genera and 700 species. Notably, the species of Vitaceae are characterized as woody, vines with leaf opposed tendrils which also includes shrubs and succulents, inflorescence a cyme, corymb or panicle, usually with leaf opposed[8].Leaves and branches extracts investigation by reversed phase HPLC showed the presence of resveratrol, viniferin, balanocarpol[6]. It was reported that the leaves are rich in tannin, flavonoid, procyanidin, organicacids, lipids, enzymes, vitamins[7].The fresh leaves contains procyanidins, anthocyanins, flavanoids, hydroxyl cinnamic acid derivatives, triterpenes, sterols, tannins, polysaccharides, monosaccharides and non-alkaloid nitrogen containing compounds. The stilbene groups, as resveratrol and viniferins, have also been isolated from leaves [8]. The leaves of *V.vinifera* have astringent and haemostatic properties are used in the treatment of leprosy, diarrhea, hemorrhage, varicose veins, hemorrhoids, inflammatory disorder, pain, hepatitis[9,10].Leaves are consumed in some traditional foods (Dolmathes) and used for diarrhoea, vomiting and varicose treatment. As this leaves are useful in the treatment of many diseases including leprosy it is essential to establish standard parameters for identity, quality and purity enabling to obtain newer drug leads for tropical diseases. Antimicrobial, antiviral, antioxidant, antitumor, hypoglycaemic, antinociceptive, wound healing, vasorelaxant, antiasthmatic, analgesic, anti-inflammatory, antipyretic, diuretic, hepato curative effect of leaves of *V.vinifera* have been reported[10-18].

As mentioned earlier several reports have been published on the effects of the plant extract and chemical constituents on different biological activities *in vitro* and *in vivo*. An investigation to explore its pharmacognostic examination is inevitable. Hence, in this work we report an attempt on microscopic evaluation including scanning electron microscopy, physicochemical determination and phytochemical screening and energy dispersive X -ray analysis to determine therapeutically important trace elements for the standardization and quality assurance purposes of this cultivar.

## MATERIALS AND METHODS

### Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

### Instruments

- Energy- Dispersive X- Ray spectrometer-Bruker.
- Tescon – VEGA-3 model Scanning electron microscope.
- NIKON Coolpix digital camera.
- Labphot2 microscopic unit.

### Collection and authentication of the leaves of *V. vinifera*

The leaves of the healthy *V.vinifera* selected for our study was collected from Utthupatti, near Kodai Road, DindigulDt, Tamilnadu, India. It was identified, and authenticated by Dr.Stephen, taxonomist, Dept of Botany, The American College, Madurai, Tamilnadu, India. A voucher specimen was deposited at the herbarium of Dept of Pharmacognosy, Madurai Medical College, Madurai, Tamilnadu, India (PCG-277).

### Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted [19].

### Microscopic analysis

Transverse section midrib region of fresh leaf pieces were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol [20] .Sections were taken using microtome. Permanent mount was prepared using safranin fast green double staining technique [21]. In order to supplement the descriptive part the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Coolpix digital camera and Labphot2 microscopic unit.

### 2.5: Microscopical study of leaf using Scanning Electron Microscope:

### SEM sample preparation:

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide and kept in carbon adhesive sheet.Samples were coated with gold to a thickness of 100 AO using hitachi vacuum evaporator. Coated sample were analysed in a TESCON Scanning electron Microscope[22].

### 2.6: Powder microscopy:

Coarse powder of the leaf was used to study the microscopical characters of the leaf [23, 24].

### 2.7: Physicochemical analysis:

Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values and leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index, palisade ratio were determined [23,24].

### 2.8: Preliminary phytochemical screening:

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [25].

### 2.9: Determination of trace elements in leaf of *V.vinifera* by Energy Dispersive X-ray Analysis (EDAX)

Energy- Dispersive X- Ray spectroscopy analysis of *V.vinifera* leaves powder was done to determine quantitatively the various trace elements like potassium, calcium, magnesium, silica etc[26].

## RESULTS

### Macroscopy

*Vitisvinifera* is a a large deciduous climber, climbing by means of intermittent, leaf opposed, long, often bifid tendrils (Fig: 1,2). Stems up to 35m long but in cultivation usually much reduced by pruning; leaves orbicular-cordate, 5-15cm., more or less deeply, palmately-3, 5- or 7-lobed, irregularly toothed, glabrescent above, often grey-tomentose beneath, thin, membranous (Fig: 3) leaf-opposed, rather dense; flowers green, in large panicles (of cymes); the peduncle sometimes bears an unbranched tendril below the flowers; berries very variable in size, ovoid to globose, greenish, purplish or bluish black, edible, generally sweet; seeds 2-4, pear shaped, with a discoidal tubercle at the back.

**Figure 1:Habit and habitat of *Vitisvinifera* L.**

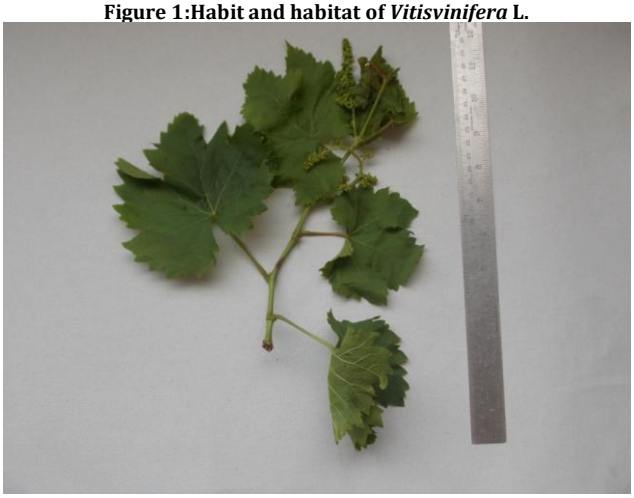

**Figure2: Leaf arrangement of *Vitisvinifera* L.**

**Figure: 3 Dorsal and Ventral view of the *Vitisvinifera*L.**

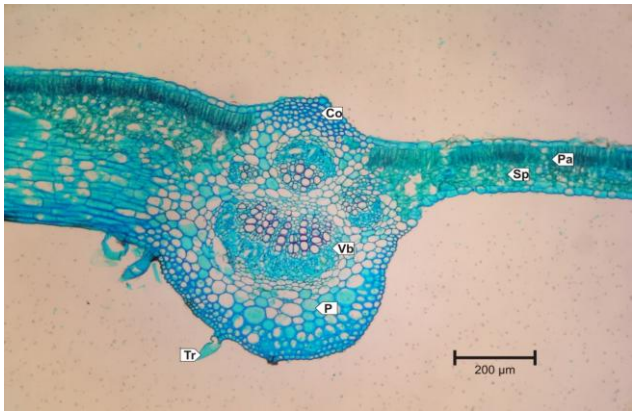


**Figure 4: T.S. of the leaf of *V.vinifera* L. through the Midrib**
Co - Collenchyma, P - Parenchyma, Pa - Palisade tissue, Sp - Spongy tissue, Tr -Trichome, Vb - Vascular bundle

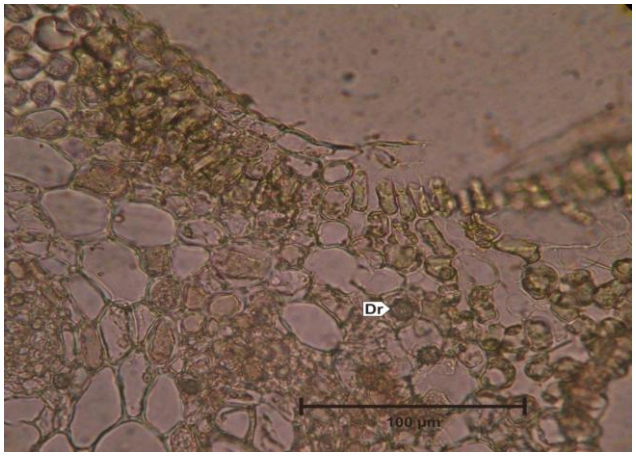


**Figure: 5 CALCIUM OXALATE DRUSES**
Dr - Druses of calcium oxalate crystals

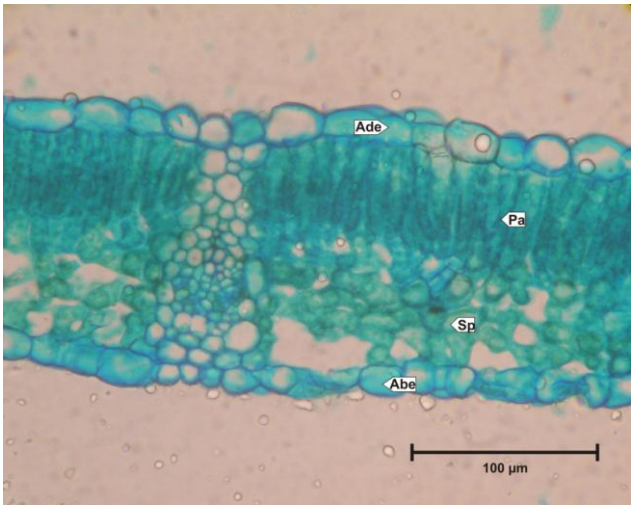


**Figure: 6 T.S. of LAMINA**
Ade - Adaxial epidermis, Abe - Abaxial epidermis, Pa - Palisade tissue, Sp - Spongy tissue

**Microscopy of the leaf**

Transverse section (T.S) of the leaves through the midrib showed the following tissue systems.

**Midrib:**

Transverse section of leaf shows a small protuberance on the adaxial surface and convexity on the abaxial side. Epidermis in single layered and some of the epidermal cells elongate to form simple unicellular, trichome and characteristic pearl glands also occur. Hypodermal region of adaxial side contain 3 or 4 rows of collenchyma cells and 1or 2 rows in abaxial side. Ground tissue is made up of parenchymatous cells (Fig: 4).Some cells contain druses of calcium oxalate crystals. (Fig: 5).

**Lamina:**

Leaf is dorsiventral in structure. Epidermis is single layered and made up of small transversely elongated cells. Palisade tissue is compactly arranged & composed of a single layer of closely arranged cells. Spongy tissue is composed of small, loosely arranged oval to round parenchyma cells (Fig: 6).The raphide sacs are varying in length. Besides the raphide needles, they contain a varying amount of mucilage. In some places only mucilage cells are seen without raphides. Side by side with the raphides, clustered crystals also frequently occur in the mesophyll. The individual raphides pointed at one end but bidentate at the other end.

Vasculature is represented by four bundles, of which, an arc shaped large vascular bundle in the centre and small bundle opposite to the larger one and two smaller ones on either side of these bundles.

**Vein-islets & vein termination:**

The secondary and tertiary veins forming distinct vein islets.Raphides are clearly seen. A few vein terminations are forked.

**Trichome:**

Trichome is non-glandular, simple and unicellular with blunt end. Pearl glands are usually spherical structures each with a short stalk, the interior consisting of large polygonal cells, surrounded by an epidermis perforated by a stoma, the latter usually but not invariably situated opposite the stalk of the gland (Fig 7). Cells of the glands are rich in protein, oil and sugar.

**Epidermis in surface view:**

The stomata are restricted to the abaxial side of the leaf. The adaxial epidermal cell walls are straight, polygonal in shape and devoid of stomata (Fig 8). The Abaxial epidermal cells are small in size and perforated by ranunculaceous type of stomata (Fig 9).

**T.S. of Petiole:**

Transverse section of petiole is nearly circular in outline. Epidermis is single layered and covered by thin cuticle. The cortex is differentiated into 2 zones. The outer region shows patches of collenchyma cells. In between these patches of parenchyma cells are seen. Inner cortical region is composed of 2 to 4 layers of oval – round parenchyma cells. Vasculature is represented by a ring of isolated bundles, accompanied by two cortical bundles on either side of the groove in the adaxial surface of the petiole. Vessels are round-oval in shape and arranged in radial rows. Phloem is made up of sieve tubes, companion cells &parenchyma cells. Vascular bundles are covered on the adaxial side by pericyclicfibres. They are isolated bundles of fibres or a composite continuous ring of sclerenchyma. Secretory cells with amorphous contents are widely distributed in the prenchymatous tissues. Large mucilage cells are also seen. The central pith region is composed of penta – heptagonal closely arranged parenchyma cell (Fig: 10).

**Microscopical study of leaf using Scanning Electron Microscope**

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of microstructures not previously recognized and apparently simple structure which may be extremely complex was observed (Fig 11).

**Powder microscopy**

The analysis of the dried powder of the leaf showed Green in colour, coarse powder, no characteristic odour and taste.Fragments of leaf with ranunculaceous stomata.Simple non glandular unicellular trichomes.Clustered crystals embedded in parenchyma cells.Individual raphides and Parenchyma cells.Xylem and phloem vessels with companion cells (Fig 12).

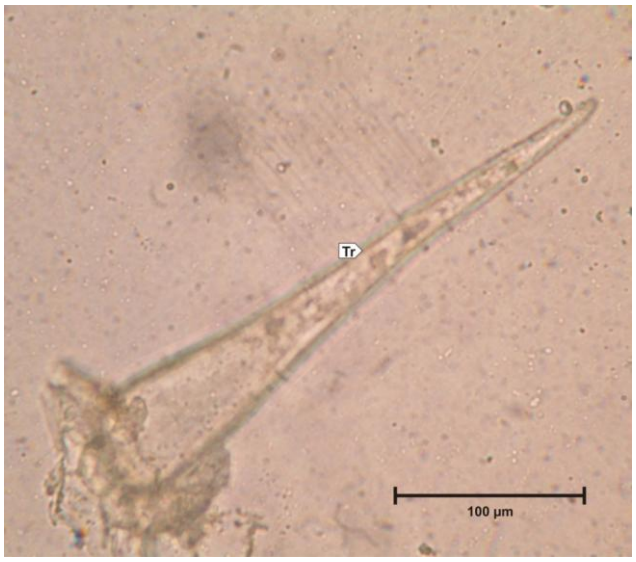


**Figure:7Unicellular trichome**
Tr-Trichome

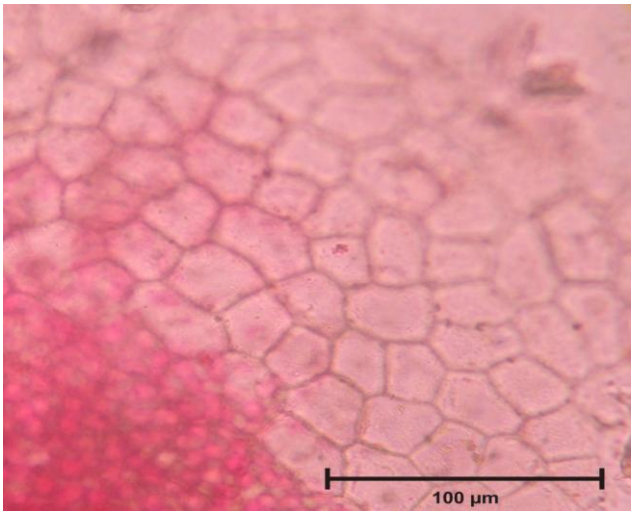


**Figure 8: APOSTOMATIC UPPER EPIDERMIS**

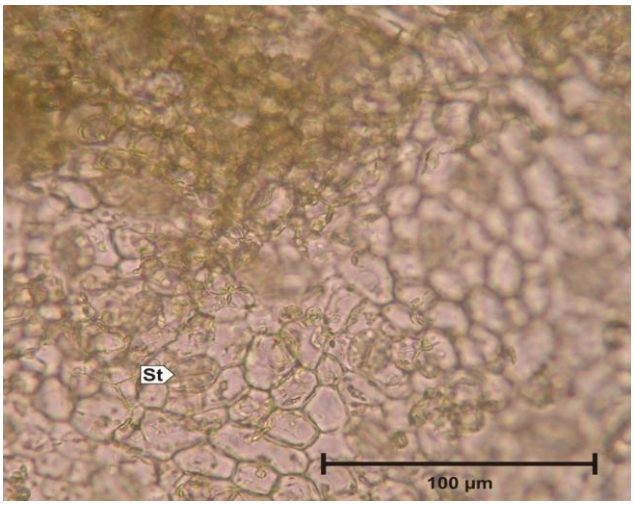


**Figure: 9 Lower epidermis showing stomata**
St -Stomata

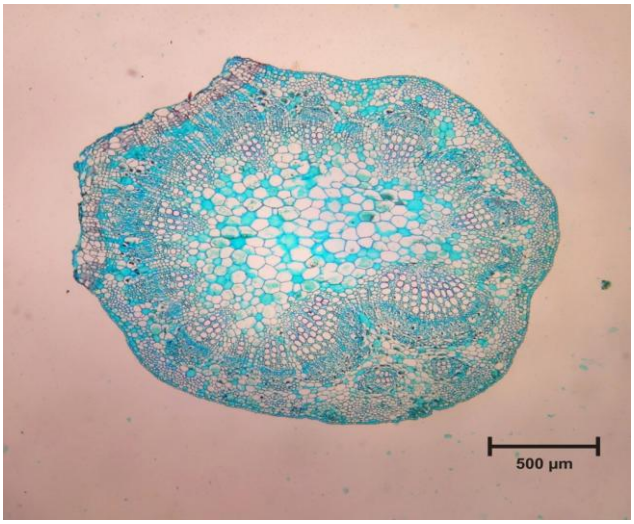


**Figure:10 T.S. of Petiole**

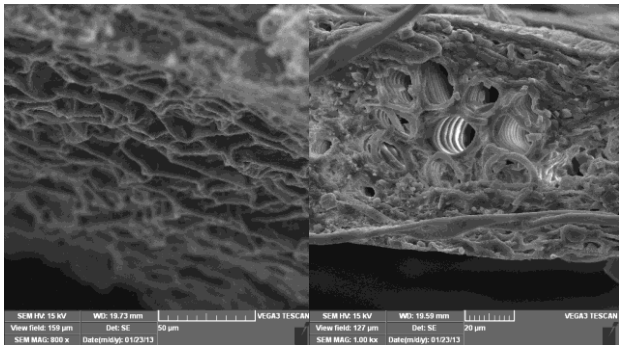

**Figure 11: Scanning electron microscopy of Vitisvinifera L.**

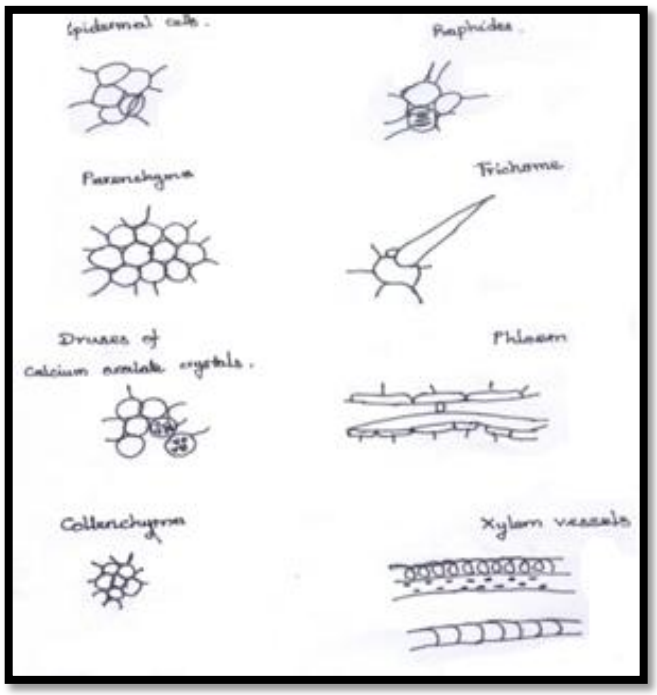


**Figure 12: Powder microscopy of *Vitisvinifera*L.**

**Physicochemical analysis**

Physicochemical parameters were found as follows: Total ash 8.96%w/w, acid insoluble ash 2.78 % w/w, water soluble ash 6.61%w/w, ethanol soluble extractive value 1.72%w/w, water soluble extractive value 14.58%w/w, loss on drying 2.8%w/w and foreign organic matter was nil. Leaf constants were as follows vein islet number 14, Vein termination number 24, stomatal number (lower epidermis) 23, stomatal index 6.5, and palisade ratio 5.9.

**Preliminary phytochemical screening**

Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, sterols, volatile oil, proteins and amino acids, tannin, saponins, carbohydrates, and absence of alkaloids, cyanogenetic glycosides, anthroquinone glycosides, cardiac glycosides, fixed oils.

**Determination of trace elements in leaf of *V.vinifera* by Energy Dispersive X-ray Analysis (EDAX)**

Energy- Dispersive X- Ray spectroscopy analysis of *V.vinifera* leaves powder showed the presence of calcium (1.95%), potassium (1.26%), silica (0.99%), and magnesium (0.13%).

**DISCUSSION**

Utilization and exploration of the available sources of medicinal plants which are enriched with bioactive compounds and have been tested traditionally to reduce illness, social exclusion and mortalities may be cost effective way. Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin .The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost [27]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials. In our present work we selected the leaves of *V.vinifera*L. (Vitaceae). The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters. Leaf shows a small protuberance on the adaxial surface and convexity on the abaxial side. Simple unicellular trichome, pearl gland, and druses of calcium oxalate crystals, presence of ranunculaceous type of stomata in the lower epidermis were observed. The secondary and tertiary veins forming distinct vein islets, raphideswere clearly seen. The scanning electron microscopy study showed the above structures in 3D view. No diagnostic feature and new kind of microstructures not previously recognized and apparently simple structure which may be extremely complex were observed. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash value is particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adulterated drug. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Preliminary phytochemical screening showed the presence of volatile oil, steroids, flavonoids, terpenoids, saponins, reducing sugars, carbohydrates, protein and amino acids and absence of alkaloids, fixed oils and glycosides. Identification of inorganic minerals of the powdered leaves of *V.vinifera* by Energy Dispersive X ray Analysis (EDAX) showed the presence of calcium, potassium, silica, and magnesium.

**CONCLUSION**

The present work was undertaken with a view to lay down standards of *Vitisvinifera* leaves which could be useful to detect the authenticity of this medicinally useful plant to control some of the neglected tropical diseases. Microscopical evaluation and physicochemical standards and preliminary phytochemical reports can be useful to substantiate and authenticate this drug [28].

**Conflict of interest statement:**

We declare that we have no conflict of interest.

**ACKNOWLEDGEMENT**

The authors thank all helping hands particularly Dr.Stephen, taxonomist, Dept of Botany,The American College, Madurai, Tamilnadu, India and R.Ravishankar, senior technical officer, central instrumentation fascility, CSIR-CECRI, Karaikudi, Tamilnadu, India.

**REFERENCES**

1. Medium-term strategic plan 2008–2013[amended draft] and Proposed programme budget 2010–2011.Geneva, World Health Organization, 2009 (available at http://apps.who.int/gb/e/e_amtsp3.html; accessed on Jan 2013).

2. SharmaKK, Kotoky J, Kalita JC, Sarma GC.Traditional use of medicinal plants for anti-ringworm therapy in some parts of Kamrup District of Assam, a North Eastern State of India.*Asian Pac J Trop Diseases* 2012; 2(2): S316-S319.
3. Periyanayagam K, Jagadeesan M, Kavimani S, Vetriselvan T. Pharmacognostical and Phyto-physicochemical profile of the leaves of *Piper betle* L. varPachaikodi (Piperaceae) - Valuable assessment of its Quality. *Asian Pacific Journal of Tropical Biomedicine* 2012:S506-S510.
4. Anonymous. *The Wealth of India Raw materials, Sp-W, Vol X,* New Delhi, National Institute of Science communication and information resources (NISCAIR), CSIR; 2005.p. 527-58.
5. Najmaddin C, Hussin K, Maideen H. Comparative study on the anatomy and palynology of the three variety of *Vitisvinifera* variety (family Vitaceae). *Afr J Biotechnol* 2001;10(74):1686-74.
6. Felicio JD, Santos RS, Goncalez E. Chemical constituents from *Vitisvinifera*L. (Vitaceae). *Arq. Inst. Biol* 2001; 68(1**)**:47-50.
7. Sendogdu N, Aslan M, Orhan DD, Ergun F, Yesilada E. Antidiabetic and antioxidant effect of *Vitisvinifera L* leaves in streptozotocin – Diabetic rats. *Turkish. J. Pharm. Sci* 2005; 3(1):7-18.
8. Singh J, Singh AK, Singh A. Analgesic and anti-inflammatory activity of methanolic extract of *Vitisvinifera L* leaves. *Pharmacologyonline*2009;3:496-504.
9. Kumar KA, Vijayalakshmi K. GC-MS analysis of phytochemical constituents in ethanolic extract of *Punicagranatum* peel and *Vitisvinifera* seeds. *Int J Pharma and Bio sci* 2011; 2(4):B461-B468.
10. Orhan DD, Orhan N, Ozcelik B, Ergun F. Biological activities of *Vitisvinifera*L leaves. *Turk. J. Biol* 2009;33:341-48.
11. Hawary SE, Fouly KE, Gohary HME, Meselhy KM, Slem A, Talaat Z. Phytochemical and Biological Investigation of *Vitisvinifera* L. (Flame cultivar), Family Vitaceae Cultivated in Egypt. *Nat Sci* 2012; 10(10):48-59.
12. Katalinic V, Mozina SS, Generalic I, Skroza D, Ljubenkov I, Klancnik A. Phenolic Profile, Antioxidant capacity, and Antimicrobial Activity of Leaf Extracts from Six *Vitisvinifera* L. Varieties. *Int .J. Food Properties* 2013; 16:45-60.
13. Singh J, Singh AK, George M, Joseph L. Antiinflammatory and antipyretic activity of *Vitisvinifera* Leaves extract. *T. Ph. Res* 2010; 3:172-82.
14. Abramovic H, Terpinc P, Generalic I, Skroza D, Klancnik A, Katalinic V, Mozina SS. Antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosmarinusofficinalis*) and vine (*Vitisvinifera*) leaves. *Croat. J. Food Sci. Technol* 2012; 4(1):1-8.
15. Askari GA, Kahouadji A, Mousaddak M, Ouaffak L, Charof R, Menname Z. Evaluation of Antimicrobial activity of aqueous and ethanolic extracts of Leaves of *Vitisvinifera*collected from different regions in Morocco. *American-Eurasian J. Agric. & Environ. Sci* 2012; 12(1):85-90.
16. Patil PB, Patil SB, Naikwade NS. Evaluation of antitumor and antioxidant activity of *Vitisvinifera* L. against Ehrlich Ascites Carcinoma induced mice. *IJPRD* 2011; 3(3):65-72.
17. Sadaiah B, Kumar KTS, Kavitha CHN, Babu SM, Reddy VP. Antinociceptive activity of methanolic extract of leaves of *Vitisvinifera*. *Der. Pharmacia Sinica* 2011; 2(2):190-97.
18. Kumar CH, Ramesh A, Kumar JNS, Ishaq BM. A Review on hepatoprotective activity of medicinal plants. *IJPSR* 2011; 2(3):501-15.
**19.** Mukherjee PK. *Quality control of Herbal drugs- An approach to evaluation of botanicals* 1st edn New Delhi, Business Horizon; 2012.
**20.** Kunle, FolashadeO,Egharevba, Omoregie H, Ahamadu, Ochogu P. Standardization of herbal medicines-A Review. *Int J Biodiver Conser*2012; 4(3):101-12.
**21.** Asokan J. *Botanical microtechnique principles and practice*.1st edn. Chennai, Plant anatomy research centre; 2006.
22. Heywood VH. The Systemics Associations, Scanning Electron Microscopy, Systemics and evolutionary appplications, Proceedings of an International Symposium held at the Department of Botany, 1971. Spl vol.4, University of Reading, Academic Press, London: 1-16.
**23.** WHO. *Quality control methods for medicinal plant materials*. Geneva: World Health Organisation, 1998.
**24.** Evan WC. *Trease and Evans Pharmacognosy* 16th edn, London Saunders Elsevier; 2009, p563-70.
**25.** Gokhale SB, Kokate CK. *Practical Pharmacognosy*. 12th edn, NiraliPrakashan; 2008: p13-19.
**26.** Pirzada AJ, Iqbal P, Shaik W, Kazi TG, Ghani KU. Studies on the elemental Composition and antifungal activity of medicinal plant LippianodifloraL. Against skin fungi. J. Pak Assoc. Dermat2005;15: 113-18.
**27.** Serrano R, Silva G.da, Silva O. Application of light and scanning electron microscopy in the identification of herbal medicines. In: Mendez vilas A, Diaz J. (eds). *Microscopy: Science, Technology, Application and Education.* Vol.1, Sapin, Formatex Research Centre; 2010: p182-90.
**28.** Kumar S, Kumar V, Om Prakash. Microscopic evaluation and physiochemical analysis of *Dilleniaindica* leaf. *Asian Pac J Trop Biomed* 2011; 1(5): 337-40.