Alexey Arkad'evich Matyushin et al /J. Pharm. Sci. & Res. Vol. 9(8), 2017, 1358-1362

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Acmella oleracea: A Comprehensive Study of Anatomical and Diagnostic Characteristics

Alexey Arkad'evich Matyushin, Olga Vladimirovna Evdokimova

Sechenov First Moscow State Medical University,
119991, Moscow, Russian Federation, Trubetskaya Street, 8-2

Abstract

The article describes anatomical and diagnostic characteristics of a promising source of biologically active substances – *Acmella oleracea. A. oleracea* belongs to *Asteraceae* family and is native to Brazil and Peru, grows in India; it also can be introduced in humid continental climate of Moscow region, but only as an annual plant due to its sensitivity to frost. It is a well-recognized traditional remedy for toothache and gum diseases. Previous studies have demonstrated its antioxidant, antiinflammatory, antibacterial, hepatoprotective, and analgesic activity. The plant is very rich in various chemical substances, namely *N*-alkamides, flavonoids, coumarins, polysaccharides, and terpenoids. Recent publications have introduced several methods of quantification of these components. However, no studies were specifically devoted to comprehensive description of anatomic and diagnostic features of all aerial parts of *A. oleracea,* which can be important for authentification of herbal drug products containing this herb. Therefore, the aim of the study was to describe anatomical and diagnostic characteristics of *A. oleracea* that can be used to distingush the plant from contaminating species and prevent adulteration and falsification of herbal drugs. The study was conducted using dried aerial parts of the herb which were separated into flowers, leaves, and stems. The slides for microscopic examination were prepared and examined according to the Russian State Pharmacopoeia requirements. The authors conclude that the results of the study can be used for identification purposes and in regulatory documents development.

Keywords: acmella olerace, spilanthes, diagnostic features, microscopy

INTRODUCTION

The need for new sources of biologically active substances is driven by the growth of herbal drug products consumption and increasing demand for novel drug products. One of the possible ways to find novel promising drug products is the thorough study of herbs and herbal remedies, that are used in traditional medicine of different countries.

One of such herbs is *Acmella oleracea* (L.) R.K. Jansen, which is native to Brazil and Peru, but also grows in India, China, Taiwan, and can be introduced in humid continental climate, such as Moscow region as an annual plant [1, 2].
A. oleracea is a perennial (annual in some regions due to frost intolerance) herbaceous plant which is also known throughout the world as para cress, toothache plant, Brazilian cress, sechuan button, and eyeball plant. A large number of botanical synonyms, numerous reclassifications, and an ongoing discussion among botanists add to the general confusion about the proper naming of the plant. For example, The Plant List and several other sources list various synonyms for this plant, namely *Anacyclus pyrethraria* (L.) Spreng., *Bidens fusca* Lam., *Spilanthes oleracea* L., *Spilanthes oleracea* Jacq., and *Spilanthes acmella* auct. non (L.) Murr.; however, they all seem to be of the same plant, erroneously attributed to different genera [3, 4, 5, 6].

The plant is about 15-30 cm in height, stems decumbent to usually erect, glabrous to sparse pilose near the top, green to red in color. Petiole is glabrous, narrowly winged. Leaves are opposite, simple, green to reddish; leaf blade is broadly ovate to deltate, glabrous, with dentate margins, truncate bases, and acuminate to acute apexes. Inflorescences are discoid, globe to short conical, yellow with central disk flowers emerging maroon to red, turning yellow-golden as they mature. Water extracts of the plant have specific, pungent taste [2].

A. oleracea shows a wide range of pharmacological properties. For example, its extracts are used in odontalgia and parodontitis [7, 8]; have analgesic [9, 10] and antiinflammatory properties [11]. A DPPH assay confirmed strong antioxidant activity of *A. oleracea* [12], along with sufficient hepatoprotective properties, both in paracetamol-induced hepatic damage [13], and in aflatoxin B1-induced damage [14].

Several studies investigated chemical composition of *A. oleracea,* and it was found, that its main chemical constituents are flavonoids, coumarins, polysaccharides, and terpenoids [15, 16]. Spilanthol, an *N*-alkamide which is also found in several other species of the *Asteracea* family, is recognized as the constituent responsible for the local anesthesia induced by different parts of the plant [17].

One of the main steps in herbal drug standardization is the proper identification of herbal raw materials. The previous studies have investigated the anatomical and diagnostic characteristics of *A. oleracea* to some extent [18, 19]; however, current pharmacopoeial quality control criteria needed for ensuring the identity of herbal raw materials require description of macro- and microscopic characteristics of all aerial parts, i.e. leaves, flowers, and stems of medicinal herbs to distinguish them from related species and possible impurities [20, 21]. Therefore, the aim

of the study was to comprehensively describe anatomic and diagnostical characteristics of *A. oleracea* herb – a promising source of biologically active substances.

MATERIALS AND METHODS

Herbal raw material (*Acmella oleracea* herb) was cultivated and harvested in the Moscow region in the summer 2016, then aerial parts of the plant were shade dried for 10-12 days on drying frames with frequent stirring in orded to provide adequate air circulation.

After the end of the drying period the material was separated into flowers, stems, and leaves, then the slides for microscopic examination were prepared using the following method: about 0.1 g of the material were placed in a 50 ml beaker, then 5-10 ml of 5% sodium hydroxide solution were added, and the content of the beaker was boiled for 10-20 minutes using a hot plate. After that the solution was decantated, and the material was fractionally washed with purified water, waiting for the complete sedimentation of the particles befor each next decantation. After clarification of the washings the material was transferred into a Petri dish where it was separated into different parts according to the General Pharmacopoeial Monograph "A technique of microscopic and microchemical study of herbal medicinal raw materials and herbal medicinal products" [21].

Separated parts of the material were transferred into a drop of inclusive fluid (1:1 glycerol:water solution), covered with a cover glass, and examined using Olympus CX41 microscope (Olympus, Japan) with a 10× eyepiece and several lenses (4×, 20×, and 40×). The photographs were obtained using Canon PowerShot G1X digital camera (Canon, Japan) and the pictures were processed using Adobe Photoshop CS6 software (Adobe, USA).

RESULTS AND DISCUSSION

The description of slides was performed according to the requirement of the general pharmacopoeial monographs on the corresponding morphological groups. All shots are done in surface view.

The microscopic analysis of *A. olearacea* leaves have revealed cells of the lower epidermis with thin sinous walls (Figure 1a), cells with evenly thickened cell walls (Figure 1b), and anomocytic stomata of various sizes, surrounded by 5-7 cells. Upper epidermis is represented by cells with thin, slightly sinous walls.

A large simple multicellular thick-walled scarce hairs (Figure 1c), sometimes with brown content (Figure 1d), can be observed at the edge of the leaf blade. The hair is surrounded by subsidiary cells (Figure 1e).
The mesophyll of the leaf is represented by oval parenchymal cells with round and elongated secretory vesicles (Figure 1f); yellow to yellow-brown uniform and non-uniform content can be observed inside the vesicles (Figure 1g). The vascular system of the leaf is represented by helical, annular, and scalariform vessels (Figure 1h, 1i).
The microscopic analysis of *A. oleracea* flower petals have revealed upper epidermal cells with papillae and drops of volatile oil (Figure 1j), and lower epidermal cells with sinous walls (Figure 1k).

The mesophyll of petals also contains drops of volatile oil. The vascular system is represented by helical vessels marginated by slit-like pores (Figure 1l); vesicles with brown content can be seen along the vessels (Figure 1m). Ground tissue of unripe achenes is composed of thick-walled cells (Figure 2n).

The microscopic analysis of *A. oleracea* flower sepals have revealed polygonal rectangular thickened cells of upper epidermis located over vein with striated cuticle (Figure 2o) and polygonal cells (Figure 2p). Scarce anomocytic stomata can be seen in the upper epidermis, and cells with very sinous walls and multiple anomocytic stomata – in the lower epidermis (Figure 2q).

The following types of hairs can be observed on the surface of the sepal:

- simple multicellular thick-walled (sometimes with brown content) hairs located along the edges of sepal and bent to the surface of the sepal (Figure 2r);
- simple multicellular thin-walled hairs (subsidiary cells can be seen) (Figure 2s);
- bifarious hairs forming bundles (Figure 2t).

Lower epidermal hairs are simple, without content.
The parenchyma of sepals is represented by rounded thin-walled cells. Vesicles with uniform and non-uniform content (Figure 2u) are located along the veins (after histochemical reaction with Sudan III the content was partially colored orange), drops of volatile oil are also present. The pollen (Figure 2v) is tricolpate, with spiny surface and smooth intina; lypophilic content is found inside the pollen.

The microscopic analysis of *A. oleracea* stem have revealed polygonal rectangular epidermal cells with straight thin walls (Figure 2w) and oblong cells with sinous walls and anomocytic stomata (Figure 2x). Scarce simple thin-walled unicellular hairs with brown content can be observed (Figure 2y).

Vesicles with non-uniform content and drops of volatile oil (Figure 2z) can be seen in in the mesophill, along with separate drops of volatile oil. The vascular system is represented by helical and annular vessels (Figure 2aa).

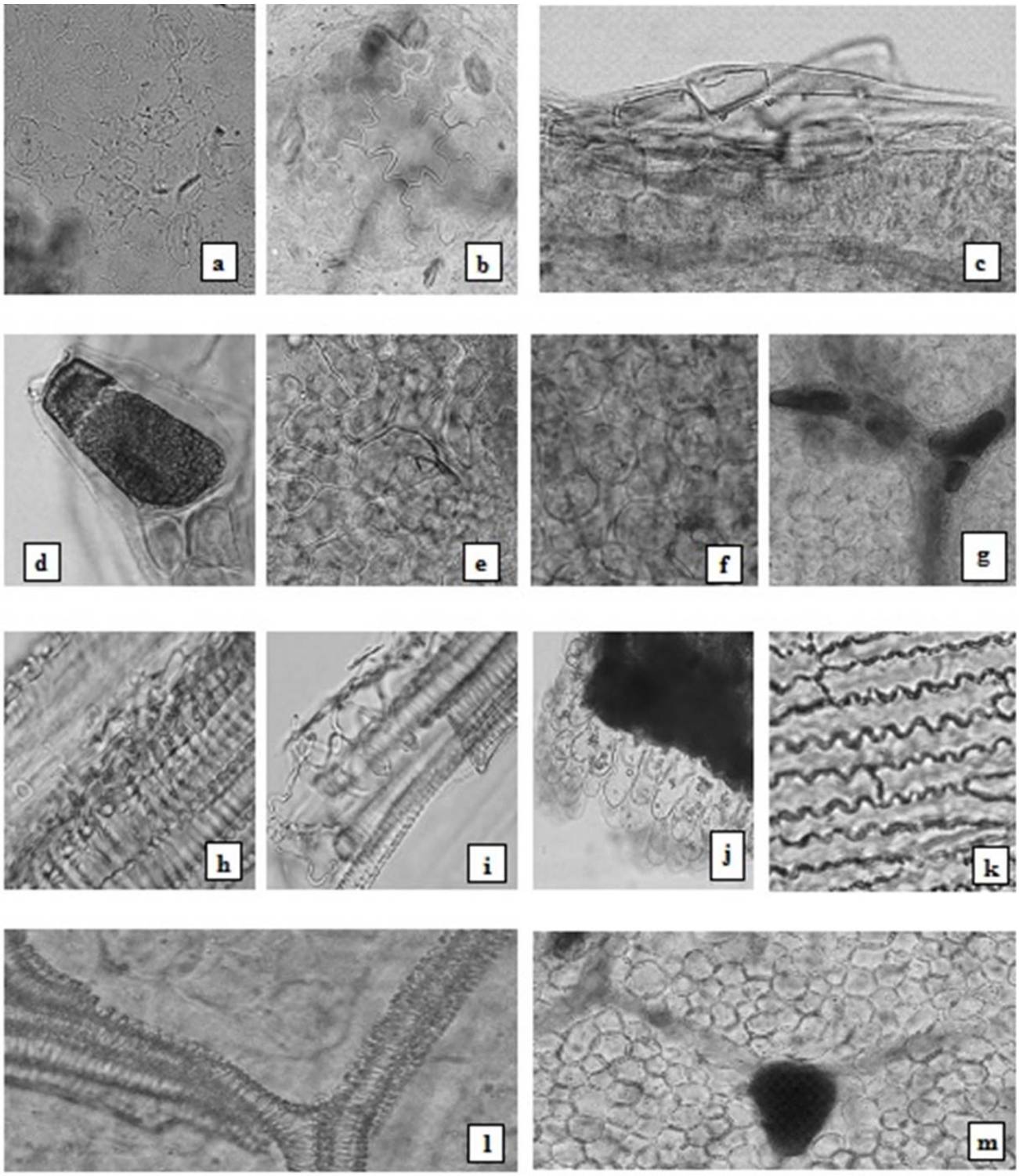

Figure 1. Leaves (a – i):

a – lower epidermis cells, anomocytic stomata of various sizes (200×);
b – cells with thickened walls (200×); c – simple hair (200×); d – fragment of a simple thick-walled hair with brown content (200×); e – fragment of an epidermis with subsidiary cells (200×); f – parenchymal cells (200×); g – secretory vesicles along the vessels (200×); h – annular and scalariform vessels (400×); i - helical and scalariform vessels (400×). **Petals (j – m):** j – upper epidermal cells with pappilae (200×); k – lower epidermis cells (200×); l – helical vessels (200×); m – vesicles along the vessels (200×).

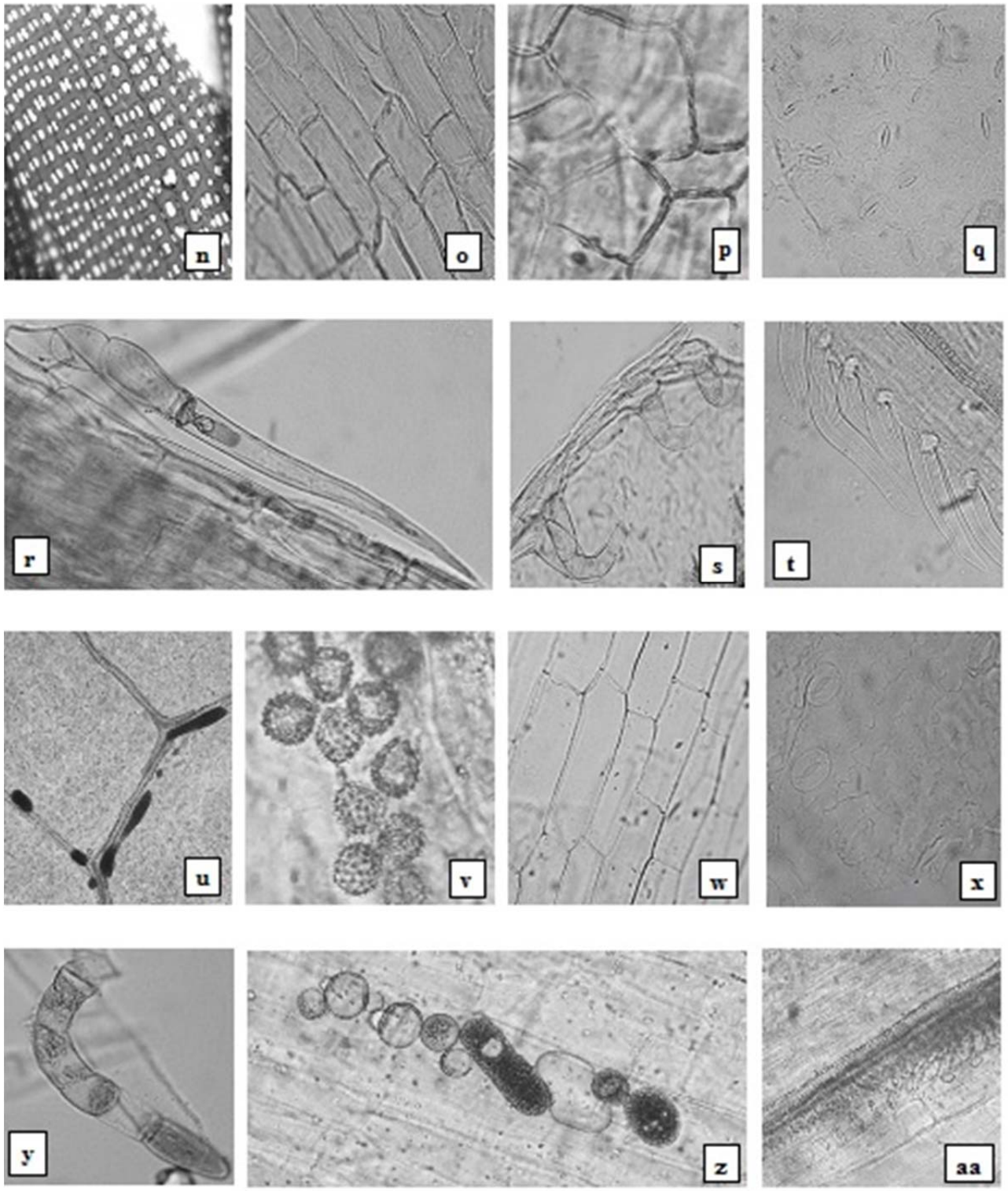

Figure 2. Petals (cont., n): n – achene ground tissue (200×).

Sepals (o – v): o – polygonal rectangular cells of upper epidermis over vein with striated cuticle (200×); p – upper epidermis polygonal cells (200×); q – lower epidermis cells with very sinous walls and anomocytic stomata (200×); r – simple multicellular hair with brown content (200×); s - simple multicellular thin-walled hairs (200×); t – bifarious hairs (200×); u – vesicles along vessels (200×); v – tricolpate pollen (400×). **Stem (w – aa):** w – polygonal rectangular epidermal cells (200×); x – oblong epidermal cells (200×); y – thin-walled hairs (200×); z – vesicles with non-uniform content (200×); aa – vascular system (200×).

CONCLUSION

Anatomical and diagnostic characteristics of *A. oleracea* leaves, flowers, and stems which allow proper identification of this herbal raw material are described. The results of the study can be used for standardization and quality control of *A. oleracea* herbal raw material and herbal drug products containing this plant.

REFERENCES

1. Kuo-Fang Chung, Yoshiko Kono, Chiu-Mei Wang, Ching-Peng, Notes on Acmella (Asteraceae: Heliantheae) in Taiwan. *Botanical studies,* 2008; 49, 73-82
2. Matyushin A.A. and Evdokimova O.V., *A study of microscopic and diagnostic features of Acmella Oleracea (L.)* R.K. Jansen flowers. Modern aspects of herbal and natural raw materials use in medicine. Moscow: Sechenov First Moscow State Medical University, 2017, pp. 145-147.
3. *The Plant List.* Retrieved Jule 30, 2017 from http://www.theplantlist.org
4. Duke, A. J., *Duke's Handbook of Medicinal Plants of Latin America.* CRC Press, 2008, pp. 832.
5. Grubben, G.J.H., Vegetables. Backhuys Publishers, 2005, pp. 667
6. Hind N. and Biggs N., Plate 460. Acmella oleracea *Compositae. Curtis's botanical magazine,* 2003; 20(1): 31-39.
7. Matyushin A.A., Nesterova O.V., Malanova O.A. and Popkov V.A., The prospects for pharmaceutical use of herbal raw material containing alkylamides. *The journal of scientific articles "Health and Education Millenium*", 2017; 1: 123-128.
8. Tiwari K.L., Jadhav S.K. and Joshi V., An updated review on medicinal herb genus *Spilanthes. Chin. J. Integr. Med.,* 2011; 9: 1170-1178.
9. Peiris K.P.R, Silva G.K.J., Ratnasooriya W.D., Analgesic Activity of Water Extract of *Spilanthes acmella* Flowers on Rats. *J. Trop. Med. Plants*., 2001; 2(2): 120-126
10. Rios M.R. and Olivo H.F., Natural and synthetic alkylamides: applications in pain therapy. *Studies in Natural Products Chemistry*, 2014; 43: 79–118
11. Dubey S., Maity S., Singh M., Saraf S.A. and Saha S. P., Phytochemistry, pharmacology and toxicology of Spilanthes acmella: a review. *Advances in pharmacological sciences. 2013*; 2013. Retrieved Jule 30, 2017 from https://www.hindawi.com/journals/aps/2013/423750/
12. Mallikarjuna Rao T., Ganga Rao B. and Venkateswara Rao Y., Antioxidant activity of Spilanthes Acmella extracts. *International Journal of Phytopharmacology,* 2012; 3(2): 216-220
13. Suja S. R., Latha P. G., Rajasekharan S., and Pushpangadan P.,. Antihepatotoxic activity of *Spilanthes ciliate*. *Pharmaceutical Biology*, 2003; 41(7): 536–541.
14. Shyamal L., Latha P.G. and Suja S.R. et al., Hepatoprotectiveeffect of three herbal extracts on aflatoxin B1-intoxicated rat liver. *Singapore Medical Journal*, 2010; 51(4): 326–331.
15. Prachayasittikul S., Suphapong S., Worachartcheewan A., Lawung R., Ruchirawat S., and Prachayasittikul V., Bioactive Metabolites from *Spilanthes acmella* Murr. *Molecules,* 2009; 14(2): 850-867.
16. Abeysiri G.R.P.I., Dharmadasa R.M., Abeysinghe D.C. and Samarasinghe K., Screening of phytochemical, physico-chemical and bioactivity of different parts of *Acmella oleraceae* Murr. (Asteraceae), a natural remedy for toothache. Industrial crops and products, 2013; 50: 852-856
17. Barbosa A. F., Carvalho M. G.de, Smith R. E. and Sabaa-Srur A. U.O., Spilanthol: occurrence, extraction, chemistry and biological activities. Revista Brasileira de Farmacognosia, 2016; 26(1): 128-133.
18. Kavya M. D. and Pramod V. P., Phytochemical and pharmacognostic investigations of *Spilanthes acmella* Murr. World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(11): 979-988.
19. Alcantara, B. N., Kobayashi Y. T., Barroso K. F., Ribeiro S.I. D., Almeida M. B. and Barbosa W. L. R., Pharmacognostic analyses and evaluation of the *in vitro* antimicrobial activity of *Acmella oleracea* (L.) RK Jansen (Jambu) floral extract and fractions. Journal of Medicinal Plants Research, 2014; 9(4): 91-96
20. Potanina O.G. and Samylina I.A., Pharmacopoeial requirements for microscopic analysis of raw medicinal plant materials. Pharmacia, 2015; 4: 47-48
21. The State Pharmacopoeia of the Russian Federation, XIII ed., Vol. III. Retrieved Jule 30, 2017 from http://193.232.7.120/feml/clinical_ref/pharmacopoeia_3/HTML/