IJP (2015), Vol. 2, Issue 4 **(Research Article)**

Received on 13 January, 2015; received in revised form, 19 March, 2015; accepted, 29 March, 2015; published 01 April, 2015

MICROSCOPIC ANALYSIS OF *CURCUMA LONGA* L. USING MULTIVARIATE TEST

Bouzabata Amel

Pharmacognosy laboratory, Faculty of Medicine, Badji-Mokhtar University, Annaba Algeria

Keywords:

Microscopic Evaluation, Statistical Analysis, Crystals

Correspondence to Author:
Dr. Bouzabata Amel

Pharmacognosy laboratory, Faculty of Medicine, Badji-Mokhtar University, Annaba Algeria

E-mail: amelbouz2009@gmail.com

ABSTRACT: Rhizomae Curcumae Longae is the dried rhizome of *Curcuma longa* L. (Zingiberaceae), commonly known as turmeric, has a long history of traditional uses for culinary purposes as a spice and as a food colorant. This study was aimed at establishing the microscopic identification of different commercial samples and developing parameters for discriminating turmeric powders. In this study fifteen samples from different origins were analyzed, and each experiment was performed in triplicate. Statistical techniques were used to analyze the partition of the structure observations. Principal component analysis was applied to the distribution of the forty-five sample observations. In consequence, eleven discriminating structure features were identified. The most diagnostic features are yellow clumps of gelatinized starch, covering trichome, starch granules, vessels, cork, and fibers. The results showed that microscopic observation of rhizoma Curcumae longae powder could be grouped according the presence of non-glandular trichome, and calcium oxalate crystals clusters. These findings revealed that microscopic analysis, coupled with statistical analysis, could provide a simple platform for medicinal plant identification, particularly for the diagnostic authentication of commercial samples.

INTRODUCTION: *Curcuma longa* (*C. domestica* Valeton.) is native to Southeast Asia,[1-3] consumed as a dietary spice and used in the treatment of skin, respiratory, liver and gastrointestinal tract diseases.[4] In India, adulteration of turmeric is a serious problem in local markets.[5] Many reference books still record the microscopic characteristics of turmeric of turmeric (*C. longa*), e.g., the Ayruvedic Pharmacopoeia of India,[6] the Indian Pharmacopoeia,[3] the Chinese Materia Medica,[7] the European Pharmacopoeia,[8] and the American Herbal Pharmacopoeia.[9]

QUICK RESPONSE CODE

DOI:

10.13040/IJPSR.0975-8232.IJP.2(4).173-77

Article can be accessed online on:
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(4).173-77

To our knowledge, there is no data available on the microscopic analysis using multivariate test. Therefore, the present investigation was undertaken to identify the microscopic features of different commercial samples and identify parameters for discriminating and authenticating *C. longa* powder.

MATERIALS AND METHODS:

Sampling:

Fifteen samples of powdered rhizomes of *C. longa* were provided from souks in three locations Annaba [sample 1-6], Souk Ahras [sample 7-11] and El Taref [sample 12-15] district, situated in the North Eastern Algeria during April 2014. The voucher specimens of the rhizomes were deposited in the Department of Pharmacognosy, University of Cairo, Egypt by correlating their morphological and microscopic characters with those described in the literature. The organoleptic characters of the

dried rhizome powder, like colour, odour and taste, and the microscopic characters were evaluated as per standard WHO guidelines. [10]

Mounting:
The powdered material was placed on the slides. Lactic acid (1-3 drops) was added and the materials stirred with a fine pointed needle to distribute the testing agent evenly. The mixture was covered with a glass slip, and any excess liquid exuding from under the cover slip was removed by blotting around its edges gently with filter paper. Lactic acid provided a yellow color for all the lignified elements, and a red orange color for the secretion products, such as the resins and essential oil[11.] Each observation of the individual sample was replicated three times.

Statistical Analysis:
Statistical tests presented in this study were analyzed using SPSS Statistics 17.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA, 2008). Multivariate tests were used to determine the complex relationship among variables. Seven microscopic features were analyzed by correspondence analysis. Analyses examined the relationships between the 45 observations and the associations between variables in two dimensions. Additionally, similar microscopic observations were identified from their positions, with respect to the axes, and the underlying features in the noticed pattern. Finally, the observed patterns were explained based on key features in the microscopic observation.

RESULTS:
Organoleptic characteristics: *C. longa* is a bright, golden-yellow powder with an aromatic and pleasant odour, and a pungent and aromatic taste.

Microscopic characteristics: Firstly, four characters were observed in all samples (15), with 100% of total observations:

- Fragments of parenchymateous cells, which are abundant groups; the cells are seen to be rounded to oval in outline with thin, slightly irregular walls.
- Yellow clumps of gelatinized starch, with a bright yellow coloring, the cells are parenchymatous (**Fig. 1, A**) and are also seen to be rounded to oval in outline with thin, slightly irregular walls
- Fibers (**Fig. 1, B).**
- Scattered oil droplets (**Fig. 1, C**). .
- Secondly, seven other characters were also identified, for which the percentage of identification varied:
- Trichomes were identified with 80.0 % of total observations, covering, not very numerous, are quite distinct, scattered, unicellular, elongated, conical and bluntly pointed with moderately thickened walls (**Fig.1, D**).
- Vessels were observed with 97.8% of total observations, fairly abundant, mostly large and reticulate thickened with regularly arranged rectangular pits. A few vessels with spiral or annular thickening also occur (**Fig.1, E**).
- Cork, which is pale brown composed of thin walled cells; appear large and polygonal in surface view, have been observed with 64.4% of total observations (**Fig. 1, F**).
- Oleoresins were observed with 28.9% of total observations.
- Nevertheless, we have identified in some samples crystals of calcium oxalate in two forms; prism with 77.8% and cluster (rarely seen) in 6.7% of total observations.
- Starch grains, were observed with 99.3% of total observations, very abundant, mostly simple, flattened, oblong to oval or irregular in outline with a small point hilum situated at the narrower end, very faint verse striations may be visible on a few of the granules (**Fig. 2**).

Statistical description: The homogeneity or the variability was investigated by multivariate test. The forty-five observations were recorded to the seven microscopic features, using Multiple Correspondence Analysis (MCA).

The two axes explained 48.9% of the observed variation. The X axis and the Y axis accounted for

55.7% and 40.3% of the variations, respectively. The coefficients represent the correlation with the two axes. The oleoresins and covering trichomes were positively linked on the first factor (0.650, 0.591), but weakly related to the second factor (0.020, 0.004). In contrast, it was observed that the calcium oxalate cluster showed a strong link on the second factor and was loaded weakly on the X axis (0.647 *vs.* 0.011) (**Table 1**).

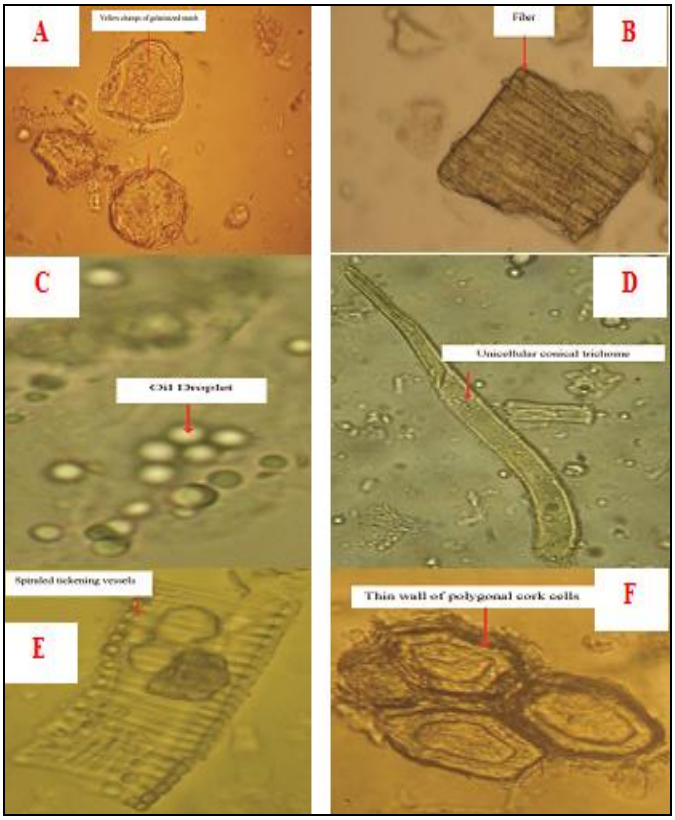

FIG.2: DIFFERENT SHAPE OF STARCK GRAINS OBSERVED IN TURMERIC POWDER.

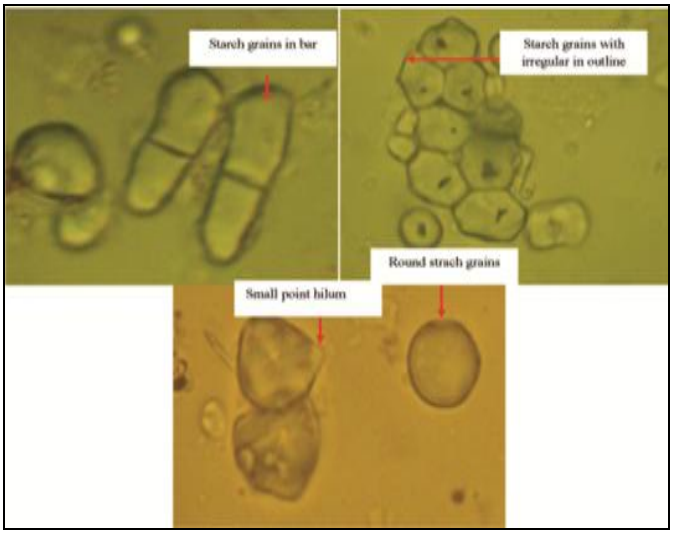

FIG.2: DIFFERENT SHAPE OF STARCK GRAINS OBSERVED IN TURMERIC POWDER.

TABLE 1: MEASURE OF DISCRIMINATION.

Microscopic Feature	Dimension		Average
	1	2	
vessels	0.026	0.077	0.052
oleoresins	0.650	0.020	0.335
cork	0.303	0.383	0.343
covering trichomes	0.591	0.004	0.297
starch grains	0.222	0.003	0.112
calcium oxalate prisms	0.111	0.393	0.252
calcium oxalate cluster	0.011	0.647	0.329
Total	1.914	1.527	1.721
Percentage of variance	27.342	21.818	24.580

The projection of matrix is plotted in **Fig. 3** in two dimensions, and the position of observation depicted underlying the analyzed parameters. Two groups have been distinguished on the basis of the presence of covering trichome. Group I is characterized by the lack of covering trichome and included similar observations [11,16,12,10,18,17]. Group II is characterized by the presence of a covering trichome. However, two subgroups were distinguished in Group II, related to the cluster crystals of calcium oxalate. In this way, the clusters were identified in all observations of the subgroup I [4, 5, 6], while they were absent in the subgroup II.

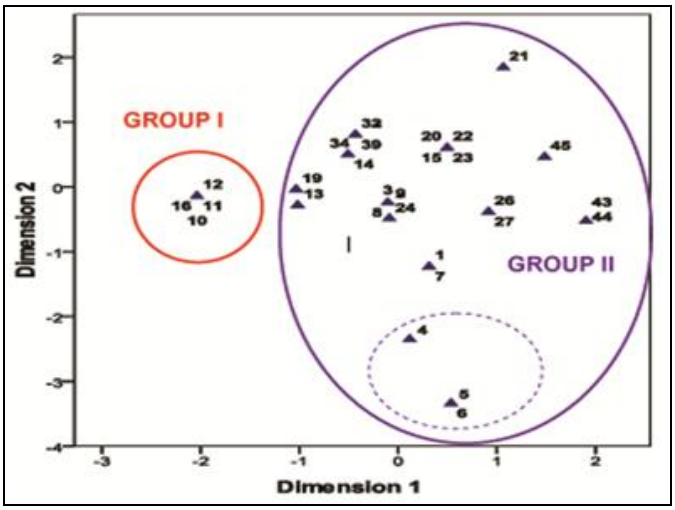

FIG.3: PROJECTION OF MATRIX OF THE POSITION OF OBSERVATION UNDERLYING THE ANALYZED PARAMETERS.

DISCUSSION: Microscopic identification is the oldest, simplest and cheapest method for plant characterization, thus is to be preferred when its use is feasible. In a previous study, the anatomical description of *C. longa* rhizome was reported, as well as microscopic characteristics of the powder. It includes cork, starch, fibers and vessels.[13] In different Pharmacopoeias, included the Indian [3] Chinese [3] European [8] and American [9] the

microscopic identification of turmeric powder was documented.

However, the adulteration of this spice is exceedingly prevalent in India, and probably the most subject to admixture is turmeric. The use of a microscope is important for the detection of adulterating and contaminating materials. Nevertheless, to identify adulteration by another species of the same genus *Curcuma* (*C. xanthorrhiza*, *C. aromatica*, and *C. zedoaria*) is often difficult, particularly in the starch grains, and the oleoresin cells which are destroyed by boiling.[5] Although the adulteration of *C. longa* by *C. aromatica* and *C. zedoaria* can be detected chemically from the presence of camphor and camphene, which occur as minor components in the essential oil of the latter two species.[14]

Therefore, the combination between microscopic data and statistical methods was built in this study to distinguish discriminating features in the authentication of *C. longa*. In a first step, eleven microscopic features were described. In second step, the forty-five observations were submitted to the statistical analysis recorded to seven microscopic features included trichomes, starch grains, vessels, cork, oleoresins, prism and cluster of calcium oxalate.

The results showed that two groups have been differentiated recorded to the presence of non-glandular trichome and the calcium oxalate crystals. Moreover, it appeared that the presence of calcium oxalate cluster is a discriminated parameter of the group II and differentiated between subgroups. Indeed, this criterion is rarely observed and is present only in three observations of the subgroup II-1.

Taking into account the literature data, no calcium oxalate crystals have been identified in *C. longa*.[3, 6, 7, 8, 9, 10] In contrast, only one study has reported on the prism-shaped calcium oxalate crystals which were identified in *C. pseudomontana*.[15]

According this study, it may be suggested that the presence of crystals of calcium oxalate in *C. longa* samples support the adulteration. However, the chemical methods as tool for authentication are necessary in quality control of *C. longa*.

CONCLUSION: Fifteen commercial samples of turmeric were observed. Eleven key microscopic features were identified. All the samples contained yellow clumps of gelatinized starch granules, fibers, and oil droplets. However, the percentage of observation of other features varied. The oleoresin was identified in 28.9% and cork in 64.4% of the total observations. Therefore, the forty-five observations were submitted to multivariate analysis that suggested the existence of two groups of samples distinguished on the basis of the presence or the lack of the non-glandular trichome. In addition, calcium oxalate crystal clusters were the feature key to identify subgroups. It was reported that *C. longa* rhizome was characterized by the lack of calcium oxalate crystals, and this point appeared useful to analyze this group of samples. This finding revealed that microscopic analysis, coupled with statistical analysis, could provide a platform for plant identification, particularly in the authentication of commercial samples.

ACKNOWLEDGEMENT: The Author would like to thank Professor Emeritus Geoffrey A. Cordell, Natural Product Inc., Evanston, IL, USA, for his review of the manuscript.

REFERENCES:

1. Kubitzki K, Huber H: Flowering Plants, Monocotyledons: Alismatanae and Commelinanae (except Gramineae). New York: Springer-Verlag 1998.
2. Mabberley DJ: Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classification and Uses. 3rd ed., Cambridge: Cambridge University Press 2008.
3. Indian Pharmacopoeia Commission, Indian Pharmacopoeia. 6th ed. vol. 3, Ghaziabad: Indian Pharmacopoeia Commission 2010.
4. Bouzabata A, Boukhari A: Variation in the traditional knowledge of *Curcuma longa* L. in North-Eastern Algeri. International Journal of Biological, Veterinary, Agricultural and Food Egineering 2014; 8(11): 1141-1145.
5. Jansen PCM: Dye and Tannins. Netherlands: Plant Resources of Tropical Africa 3 PROTA Foundation, Backhuys Publishers CTA, Wageningen 2005.
6. The Ayruvedic Pharmacopoeia of India, unspecied date. Part I, Vol I; pp: 60-61.
7. A coloured Atlas of the Chinese Materia Medica Specified in Pharmacopoeia of the People's Republic of China, Pharmacopoeia Commission of the Ministry of Public Health, P.R. China Guangdong: Science and Technology Press 1995.

8. European Pharmacopoeia. 8 th ed. Strasbourg, France: Council of Europe 2013.
9. American Herbal Pharmacopoeia AHP, Botanical Pharmacognosy-Microscopic Characterization of Botanical Medicines. Boca Raton: Taylor and Francis Group 2011.
10. World Health Organization (WHO),. Monographs on Selected Medicinal Plants. Volume 1. Geneva 1999.
11. Wichtl M, Anton R: Plantes Thérapeutiques: Tradition, Pratique Officinale, Science et Thérapeutique. 3ième ed. allemande. Strasbourg, France: Lavoisier 1999.
12. Zhao Z: Application of microscopic techniques for the authentication of herbal medicines. Microscopy: Science, Technology, Applications and Education 2010; 4(2): 803-812.
13. Kadam PV, Yadav KN, Patel FA, Karjikar FA, Patidar MK, Patil MJ: Pharmacognostic, Phytochemical and Physico-chemical studies of *Curcuma longa* Linn. rhizome. International Journal of Pharmacy 2013; 3(3): 514-520.
14. Sen BAR, Sen Gupta P, Ghose Dastidar N: Detection of *Curcuma zedoaria* and *Curcuma aromatica* in *C. longa* (Turmeric) by Thin Layer Chromatography. Analyst 1974; 99: 153-155.
15. Hiremath GB, Kaliwal BB: Pharmacognostic Evaluation of Rhizome of *Curcuma pseudomontana* J. Graham. IJPBS 2014; 5(2): 242-250.

How to cite this article:
Amel B: Microscopic Analysis of *Curcuma Longa* L. Using Multivariate Test. *Int J Pharmacognosy* 2015; 2(4): 173-77:.doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(4).173-77.