Microscopic identification of *Echinops spinosus* ssp. *bovei* (Boiss.) Murb. using multivariate tests

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ABSTRACT: The genus *Echinops* (Asteraceae family, Echinopeae class) comprises ca. 120 species and is native to Africa, the Middle East, Europe, and Asia. In Algeria, this genus is represented by the very common species *Echinops spinosus* L., also known as "*Tesskra*", which is used as a diuretic, hypoglycemic, liver disorders, for post-partum care, and for its stomachic effects. The aim was to generate microscopic parameters of the aerial parts which could be used to identify and authenticate *Echinops* powders. The present study reports, for the first time, the detailed pharmacognostic characters of the different parts of *E. spinosus*, through microscopic approaches, coupled with statistical techniques, to analyze the structural observations. Multivariate tests were used to determine the complex relationships among the studied variables. Fifty observations were analyzed from the leaves, flowers, and roots of *E. spinosus*. As a result, fourteen key microscopic features were identified and analyzed by correspondence analysis. The sclereid fibers were positively linked on the first factor (0.600, 0.591), but weakly related to the second factor (0.090, 0.004). It is proposed that microscopic analysis, coupled with statistical analysis, could provide a simple platform for medicinal plant identification. This will be of particular importance for the rapid identification of medicinal plant powders used as a medicinal agent, and may be helpful in developing pharmacopoeial standards for traditional medicines in Africa.

KEYWORDS: Echinops; Algeria; pharmacognostic; parameters; microscopy.

1. INTRODUCTION

The genus Echinops (Asteraceae family, Echinopeae class) comprises ca. 120 species and is native to Africa, the Middle East, Europe, and Asia [1]. *Echinops spinosus* L., locally known as "*Tesskra*", is very common in Algeria, and is used as a diuretic and hypoglycemic agent, for liver disorders, post-partum care, and for its' stomachic effects [2, 3]. Two subspecies are very common: *E. spinosus* ssp. eu. *spinosus* Maire (var. *chaetocephalus* Pomel), and *E. spinous* ssp. *bovei* (Boiss.) Maire (var. *pallens* Maire.) which is also known as *E. bovei* Boiss. [4].

Many diverse research studies have been conducted on *E. spinosus*. However, there is no report on its botanical investigation and only one study is available on *Echinops* which focused on the microscopic identification of *E. albicaulis* Kar. & Kir. [5].

The light microscopic method (LMM) for the identification of medicinal plants has been applied for many years. It is used as a standard procedure in different reference texts for the characterization of plant materials [6], including the Chinese Pharmacopoeia [7, 8], the Chinese Materia Medica [9], the New Compendium of Chinese Materia Medica [10], the British Herbal Pharmacopoeia [11], the American Herbal Pharmacopoeia [12-15], the Japanese Pharmacopoeia [16], the Korea Herbal Pharmacopoeia [17], and the Indian Ayurvedic Pharmacopoeia [18].

The light microscopic method offers several advantages over conventional authentication techniques for medicinal plants including effectiveness, simplicity and low cost, and as noted above, has been widely adopted as an official method in many international herbal pharmacopoeias. However, there are some limitations to this technique, such as usage of the outdated instruments and equipment [19]. Due to the lack of specificity of microscopic features, other methods have been developed to enhance the technique, such as the coupling with multivariate tests. This method was applied for the first time in the authentication of

Curcuma longa L. spices by [20]. Two years later, this method was applied for the identification of similar morphological and microscopic observations of five species in the genus Curcuma: *C. longa*, *C. aromatica*, *C. albiflora*, *C. oligantha* and *C. zedoaria* [21].

Therefore, this study aimed at performing the microscopic analyses focused on identifying key features and characteristics, coupled with multivariate tests of *Echinops spinosus* subsp. *bovei* (Boiss.) Murb., which could serve as markers for the authentication of powdered preparations of this medicinal plant.

2. RESULTS

The diagnostic features of the leaf powder are presented in the figure 1. The sample was a greenish powder, with an aromatic odour and a slightly bitter, aromatic taste. The powdered leaves are characterized by an abundance of glandular trichomes with unicellular stalk and bicellular heads. In addition, two different types of glandular trichomes were observed: *i)* with a uniseriate stalk and unicellular glands, *ii)* with a biseriate stalk and multicellular glands. The leaf powder is also characterized by the presence of large convering trichomes. Cells of the epidermis are polygonal, showing cuticular striations and anomocytic stomata. The amount of stomata index underlying ten observations on 1 mm^2 area of the upper epidermis is 29.60±5.64. The pollen grains are fairly small, spherical, with three pores, and the exine is finely warted. Prisms of calcium oxalate, as very small cluster crystals of calcium also occur.
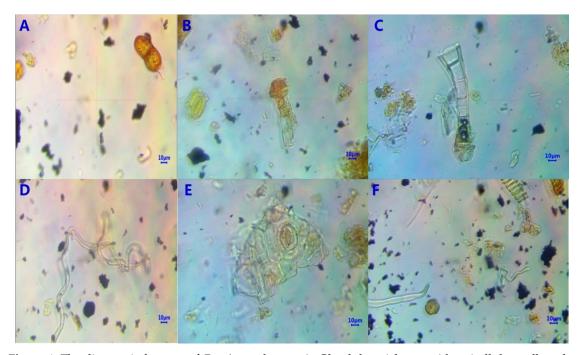

Figure 1. The diagnostic features of *E. spinosus* leaves. A: Glandular trichome with unicellular stalk and a bicellular gland, B: Glandular trichome, C: Glandular trichome, D: Long covering trichome, uniseriate unicellular, E: Fragment of epidermis with cuticular striation and anomocytic stomata, F: Tricoporate pollen grain.

The diagnose features of the powder from the flowers are presented in the figure 2. A light greenish powder, with a characteristic aromatic odour and a slightly bitter and aromatic taste was observed. The powder of the flowers is characterized by an abundance of groups of fibrous cells, cells containing oleoresin and small covering trichomes. Moreover, acicular crystals of calcium oxalate and large covering trichomes, were observed, which are rare. Cells of the epidermis were elongated showing stomata from the margin region. Large trichome is also present rarely in felted mass of thin walled.
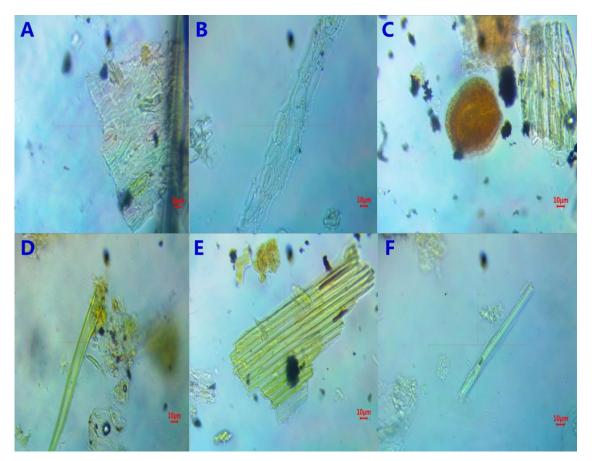
Figure 2. The diagnostic features of *E. spinosus* flowers. A, B: Inner epidermis from near the central region of a bract in surface view showing anomocytic stomata, C: Oil resin cell, D: Covering trichome uniseriate unicellular, E: Part of a group of fibrous cells, F: Acicular crystals of calcium oxalate.

The diagnostic features of the powder from the roots powder are presented in the figure 3. A brownish powder, with a characteristic aromatic odour and an astringent taste was observed. The diagnostic characters are an abundance of sclereids which vary considerably in size, and shape. Two types can be readily distinguished: those which are rectangular to ovale in outline and are heavily thickened with a small lumen. The most abundant ones are more elongated with moderately thickened and striated walls with numerous pits and were developed as *fibrous sclereids*. Numerous covering trichomes also occurred, which are unicellular, thin walled shortly conical, and with a swollen base. Long covering uniseriate and unicellular trichomes were frequently observed. Fairly abundant crystals of calcium oxalate, in acicular form were also found scattered. Very occasionally fragments of cork appeared in the surface view, and the cells were thin-walled and polygonal form. Very occasionally groups of small, lignified vessels with spiral or annular thickening. The scattered brown globular masses (lignin bodies) were also observed.

On the other hand, for multivariate analysis, the two axes showed 22.3% of the observed variation. The X and Y axes accounted for 25.3% and 19.2% of the variations, respectively. The coefficients represent the correlation between the two axes. The sclereid fibers were positively linked on the first factor (0.600, 0.591), and very weakly related to the second factor (0.090, 0.004). In contrast, it was observed that the fiber and stomata showed a positive link on the second factor and was loaded weakly on the X axis (0.488/0.425 vs. 0.001/0.025) (Figure 4). In addition, the long covered trichomes showed a positive link on both the X and Y axes (0.227/0.373).
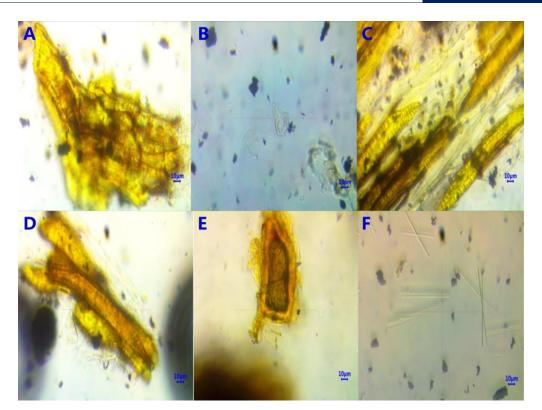
Figure 3. The diagnostic features of *E. spinosus* roots. A: Fragment of cork, B: Conic covering trichome, C: Part of a group of sclereids fibres, D: An isolated fibrous sclereid, E: An isolated sclereid, F: Acicular calcium oxalate crystals.
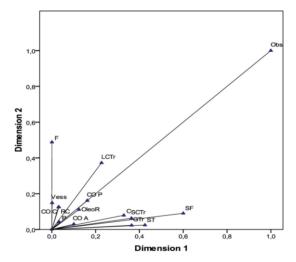

Figure 4. Projections of the fourteen analyzed parameters in the X and Y axes. *CO: cork, **Vess**: vessels, **Oleo R**: oleo resin, **LCTr**: long covering trichomes, **SCTr**: short covering trichomes, **GTr**: glandular trichomes, **PO**: pollen, **F**: fibers, **CO P**: prisms of calcium oxalate, **CO C**: clusters of calcium oxalate, **CO A**: acicular of calcium oxalate, **St**: stomates, **SF**: sclereids fibers, **Rcell**: cells of resin.*

The projection of matrix is plotted in Figure 5 in two dimensions, and the position of observation depicted underlying the analyzed parameters. Two groups were distinguished based on the presence of sclereid fibers. Group I is characterized by the lack of sclereids and included forty observations [1L, 40R, 3L, 37R, 39R, 41R, 6L, 4L, 2L, 13L, 8L, 12L, 10L, 5L, 14L, 11L, 9L, 16L, 15L, 32F, 25F, 33F, 50R, 31F, 22F, 7L, 17F, 34F, 20F, 21F, 23F, 26F,24F, 18F, 27F, 35F, 19F, 29F, 30F and 28F]. Group II is characterized by the presence of sclereids including ten observations 46R, 44R, 43R, 42R, 36R, 38R, 45R, 47R, 48R, and 49R. Two subgroups were distinguished in Group II, relating to the presence or the absence of fibers. In this way, the fibers were absent in all observations of the subgroup I-A [,1L, 40R, 3L, 37R, 39R, 41R, 6L, 4L, 2L, 13L, 8L, 12L 11L, and 10L].
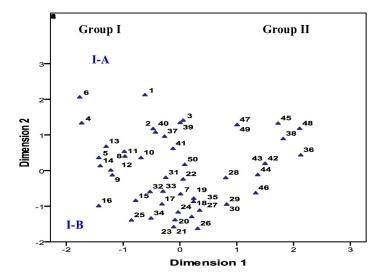
Figure 5. Projection of matrix of the position of observation.

3. DISCUSSION

According to the World Health Organization [22], the macroscopic and microscopic description of a plant is the first step to establish the identity and the degree of purity of such materials and should be carried out before any tests are undertaken [23]. This study is an attempt to establish, the diagnostic characteristics of *E. spinosus L.* The present microscopic observations of different parts of the plant, coupled with statistical analysis provides useful information for developing initial quality control parameters for the crude drug. In this first step for *E. spinosus*, the microscopic studies of the leaf powder showed the presence of covering trichome in different shape and size; and glandular trichomes.

These results are in agreement with those of [5] who identified long covering trichomes in the lower epidermis of *E. albicaulis* Kar. et Kir leaf material. The stoma type was also identified in the lower epidermis as an anomocytic type. In addition, needle-shaped crystals were also found in the form of rafids, which was also identified in the leaf of *E. spinosus* L. However, no glandular trichomes have been identified in the leaf material of *E. spinosus* L., which may be a microscopic marker of identification.

In the second step of this study, we identified microscopic features of the flowers and roots were identified. In this regard, specific characteristics were found, namely special cells with oleoresin in the flowers, and sclereids in the roots with different shape and size.

The results showed that the two groups of samples have been differentiated according to the presence of fibers and sclereids. Moreover, it appears that the presence of fibers is a discriminating parameter of the group II and serves to differentiate between the subgroups. Indeed, this criterion was absent present the subgroup II-1.

4. CONCLUSION

Fifty observations were analyzed from the leaves, flowers, and roots of *E. spinosus* (leaves, flowers and roots). Fourteen key microscopic features were identified. Multivariate analysis suggested the existence of two groups of samples distinguished by the presence or absence of sclereids. In addition, the fibers were a key feature key to identify the subgroups. This finding revealed that microscopic analysis, coupled with statistical analysis, could provide a platform for positive plant identification, particularly in the authentication of commercial samples [20].

5. MATERIALS AND METHODS

5.1. Sampling

Samples of *Echinops spinosus* subsp. *bovei* (Boiss.) Murb. were provided in 2017 from the El Tarf district, situated in the North Eastern Algeria. Voucher specimens of the plant samples were deposited in the herbarium of the Conservatory and Botanical Garden, Geneva, Switzerland under reference number G00403753. The identification of the species was carried out by Dr G. Debelaire, by correlating the

morphological characters with those described in the literature. The different plant parts were dried in the sun and crushed into a powder as traditionally used. The dried cured organ was powdered and the powder was suspended in water and subjected to microscopic evaluation. The organoleptic characters of the dried powder (e.g. colour, odour and taste), and the microscopic characters were evaluated according to standard WHO Guidelines [22].

5.2. Microscopic preparation and mounting

The individual powdered plant materials were placed on microscope 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 protected with a cover slip, and any excess liquid exuding from under the cover slip was removed by gentle blotting 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 [24]. Each sample was observed with an Amscope microscope B120b-9M equipped with Amscope 3.7. 7303 software for a digital camera (2003-2016). Sixteen observations were made for the leaf samples [1L-16L], nineteen observations for flowers samples [17F-35F] and fifteen observations for the roots samples [36R-50R].

5.3. 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 relationships among variables. Fourteen microscopic features were analyzed by correspondence analysis. The analyses examined the relationships between the fifty observations and the associations between the 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 patterns. Finally, the observed patterns were explained based on key features in the microscopic observations.

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