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A CHEMOTAXONOMIC STUDY OF SOME SPECIES OF ZINGIBER SUBSECTION ZERUMBET

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ABSTRACT

Zingiber zerumbet (L.) J.E. Smith, *Z. amaricans* BL, *Z. aromaticum* Val., and *Z. littorale* Val., which Backer & Bakhuizen v.d. Brink treated as a single species named *Z. zerumbet*, have been found to be chemically and palynologically distinct. This vindicates the species formulation made by Valeton, in which they were distinguished as four separate species.

ABSTRAK

Zingiber zerumbet (L.) J.E. Smith, *Z. amaricans* Bl., *Z. aromaticum* Val., dan *Z. littorale* Val., yang oleh Backer & Bakhuizen v.d. Brink disatukan dalam satu jenis, secara kimia dan palinologi ternyata berbeda. Hal ini mendukung formulasi jenis yang diajukan Valeton.

INTRODUCTION

Ginger and other members of *Zingiber* Boehm. are ultilized in a variety of ways, mainly for spicing, medicine, and culinary purposes. It is mainly cultivated in the tropics, from sea level to 1500 m alt. According to Vavilov (1951) ginger originated in the Indo-Malayan Centre, an area covering India, Sri Lanka, Burma and S.E. Asia. At present its taxonomic problems are baffling for botanists.

In 1916 Valeton concluded that *Z. zerumbet* (L.) J.E. Smith, *Z. amaricans* Bl., *Z. aromaticum* Val., and *Z. littorale* Val., were sufficiently distinct from one another to be treated as different species. Yet 50 years later Backer & Bakhuizen v.d. Brink (1968) considered that these four species were so closely related that they should be treated as one species, *Z. zerumbet* (L.) J.E. Smith.

The purpose of this study is to explore and to clarify the similarities as well as the differences between the four species, in order to evaluate

their taxonomic validity, using a wide range of data such as morphology, chemotaxonomy and palynology. *Z. officinale* Roxb. is also included in this study since this species belongs to the same group *Lampuzia* although placed in a different subsection.

MATERIAL AND METHODS

Living plants as well as herbarium specimens were used in this study. Sixteen accessions of living plants, i.e. four accessions each of *Z. zerumbet, Z. amt&ricans* and *Z. officinale,* three of *Z. aromaticum,* and one of *Z. littorcde,* and ten herbarium specimens were employed (Nasution 1978).

Morphological observations

A total of 47 characters of the rhizomes, stems, leaves and inflorescences, were evaluated. The oldest shoot of each stool was chosen for observation, and maximum values[1] of lengths and breadths were used. Since the living plants did not flower satisfactorily, inflorescence characters were taken from herbarium specimens.

The morphological data were analyzed by numerical taxonomy, using Euclidean distance squared and Ward's method, to produce dendrograms. The chemical data were analyzed using Jaccard's coefficient and the Group Average Clustering Method.

Chertiotaxonomic investigations

Electrophoresis of proteins

Electrophoresis of proteins was conducted in polyacrylamide gel rods following the method of Davis (1964), using a Shandon Universal disc-electrophoresis apparatus. About 10 gm of good fresh rhizomes of more or less similar physiological maturity were sampled from each accessions. After washing with tap water the rhizomes were placed in a mortar into which 5—10 cm^3 tris-glycine buffer of pH 8.3 was added, crushed with a pestle at room temperature, centrifuged for about 5 minutes at 2,000 g and the supernatant decanted and frozen. Samples of 0.01—0.02 cm^3 of protein were analyzed, and after electrophoresis the gels were stained with naphthalene black.

Clfiromatography of phenolic compounds

Chromatographic analyses were conducted by ascending two-dimensional paper chromatography, using a Shandon Universal rack and tank. Fully expanded leaves, generally the fifth and the sixth youngest, were collected from plants. Portions of lamina free of midribs were dried in a forced-air oven at 45°C for about 48 hours. Samples of 0.5 g were ground to a powder and extracted with 4 cm^3 of 0.5% v/v hydrochloric acid in methanoi overnight in darkness.

0.02 cms of leaf extracts were applied to Whatman No. 1 filter paper. After chromatography in the first solvent (butan-1-01 : ethanoic acid : water = 3:1:1) for about 2—3 hours, the papers were dried then run in the second solvent (ethanoic acid : water = 15% v/v) for 3 hours. After drying, the papers were examined under U.V. light and U.V. light with ammonia fumes.

Chromatography of essential oils

Chromatography of essential oils was conducted using thin-layer chromatography, with a Shandon TLC apparatus. Samples of 20 to 25 gm of good fresh rhizomes of more or less similar physiological maturity were used from each species. They were grated and subjected to steam distillation to obtain 100 cms distillates. 10 cm^3 of diethyl ether was added to the distillate, shaken, and the supernatant collected.

The silica gel (Kieselgel G type 60, Merck) was spread on glass plates. Samples of about 0.02 cm^3 were separated by chromatography in benzene and ethyl ethanoate (95 : 5) for almost 3 hours, then sprayed with vanillin-sulphate (0.17 g vanillin, 33 cm^3 ethyl alcohol, 1.0 cm^3 concentrated sulphuric acid), heated in an oven at 100°C for about 12 mins, and then examined immediately.

Pollen observations

Out of five species studied, only four species had pollen, namely *Z. officinale, Z. zerumbet, Z. amaricans* and *Z. aromaticum.* Examination was conducted under a scanning electron microscope at magnifications between x 800 and x 1600.

RESULTS AND DISCUSSION

Morphological studies

Evidence from morphological characters recorded from living plants comprising rhizomes, stems and leaves indicated two main groups among the species studied; the first group consisting of *Z. zerumbet* and *Z. aromaticum* and the second comprising *Z. amaricans* and *Z. littorale*

However, the dendrogram constructed from morphological characters of the inflorescence was slightly different from the dendrogram constructed from data from the vegetative parts. It showed that the inflorescence of *Z. littorale* is completely different from the other species,

while that of *Z. zerumbet* is almost identical to *Z. amaricans* and *Z. aromaticum.* This is in agreement with Valeton's observation when he reported that the spike of *Z. aromaticum* resembled that of *Z. amaricans,* whereas the shape of its labellum and its staminodes showed strong resemblance to *Z. zerumbet.*

Morphological characters of *Z. officinale* recorded both from living and herbarium specimens proved it to be distinct from the other species mentioned above.

Chemotaxonomic studies

Proteins of rhizomes

The results obtained indicate that eight bands are the maximum number recorded from any one species. Bands Nos. 5, 7, 10, 12 and 15 can be considered characteristic for *Z. zerumbet,* band No. 17 for *Z. amaricans* and band No. 16 for *Z. aromaticum.* It was noticed that band No. 9 reflects the close affinities between Z. *amaricans, Z. aromaticum* and *Z. littorale* (Fig. 1).

From the dendrogram constructed it appeared that *Z. zerumbet* and *Z. littorale* can be distinguished, but the close similarity between *Z. aromaticum* and *Z. amaricans* agrees with the similarities of inflorescence morphology of these two species.

The protein extracts of *Z. officinale* produced 11 bands in a different pattern.

Phenolic compounds of leaves

Data obtained indicate that five spots, namely Nos. 2, 3, 4, 6 and 9 were found in all species. However, Nos. 1, 7, 10 and 12 were only found in *Z. zerumbet* and hence may be considered characteristic for that species. The same is true for spots No. 17 and 20 for *Z. amaricans,* spots Nos. 23, 24, 25 and 26 for *Z. aromaticum,* and spot No. 27 for *Z. littorale.*

The presence of spots Nos. 28, 29 and 30, which appeared purple under U.V. light and then turned into purplish yellow under U.V. light with ammonia fumes, may perhaps characterize *Z. officinale* (Fig. 2).

Furthermore., the dendrogram constructed indicated that the low level of relationship between *Z. officinale* and *Z. zerumbet* suggested by protein constituents was also borne out by the phenolic compounds.

Essential oils of rhizomes

In general the distributions of spots among the species are nearly identical. However, spot No. 5 appeared only in *Z. zerumbet* and *Z. aromaticum,* while spot No. 6 was only found in *Z. amaricans* and *Z. littorale.* From this, it seems that, as far as essential oils are concerned, *Z. zerumbet* is closer to *Z. aromaticum,* and *Z. amaricans* to *Z. littorale.*

In *Z. officinale* 13 spots have been recorded (Fig. 3).

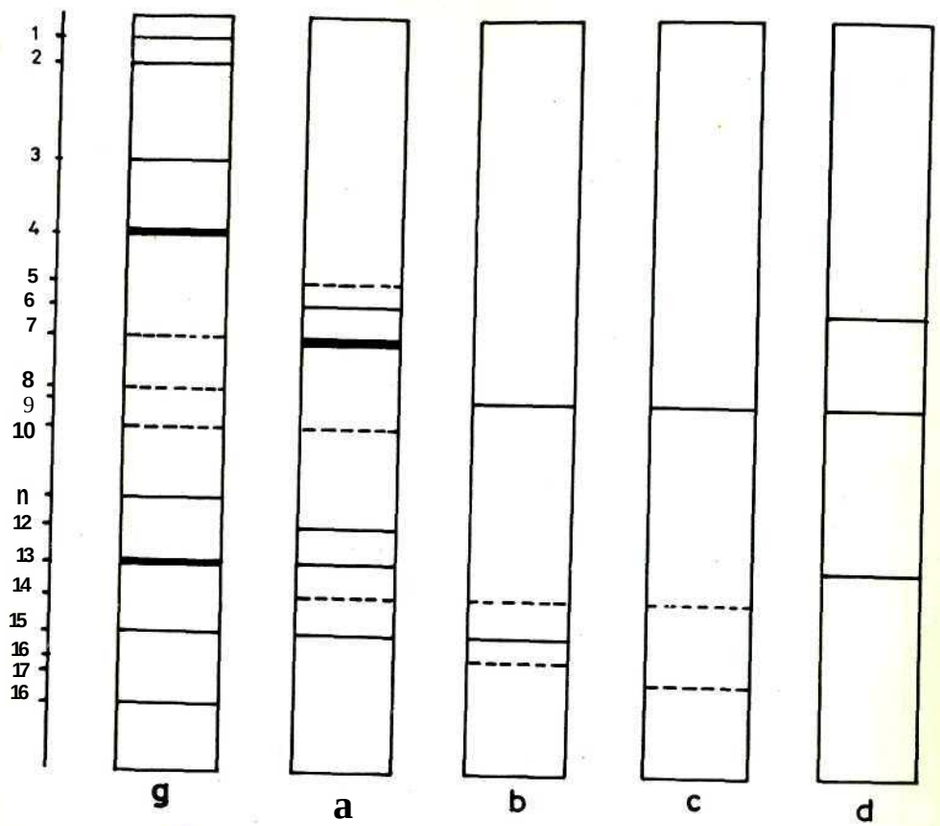


FIG. 1. Diagram representing protein bands after disc-electrophoresis. g = *Z. officinale,* a = *Z. zerumbet,* b = *Z. amaricans,* c = *Z. aromaticum,* d = *Z. littorale.*

Pollen observations

Examination of pollen indicates that there are distinguishing features which offer some additional evidence in classifying these species. In general, the size and shape of the pollen grains are similar, but their surface structures are distinctive. Two main points can be noticed, viz. the presence of branching supra-tegillar processes on *Z. aromaticum* which differentiates it from the other species, and the absence of pores on tegillar processes of *Z. officinale,* which is characteristic for this species (Fig. 4-5).

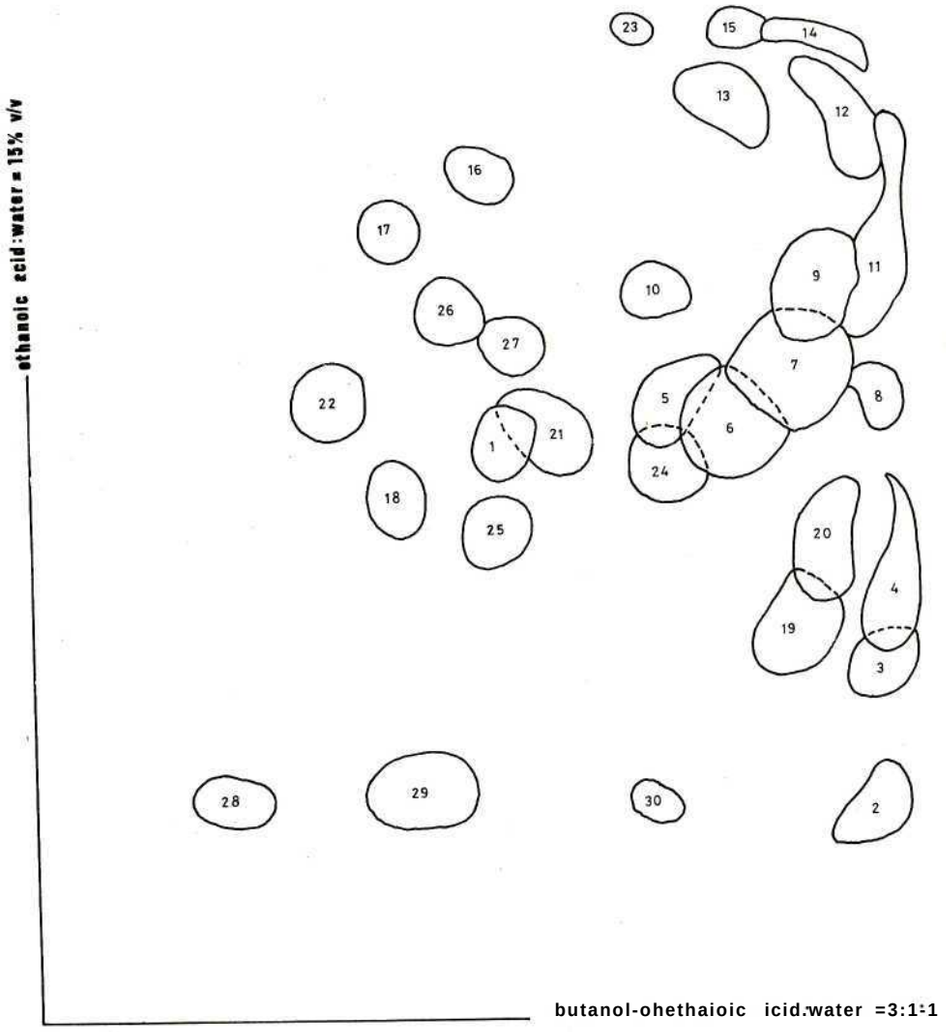
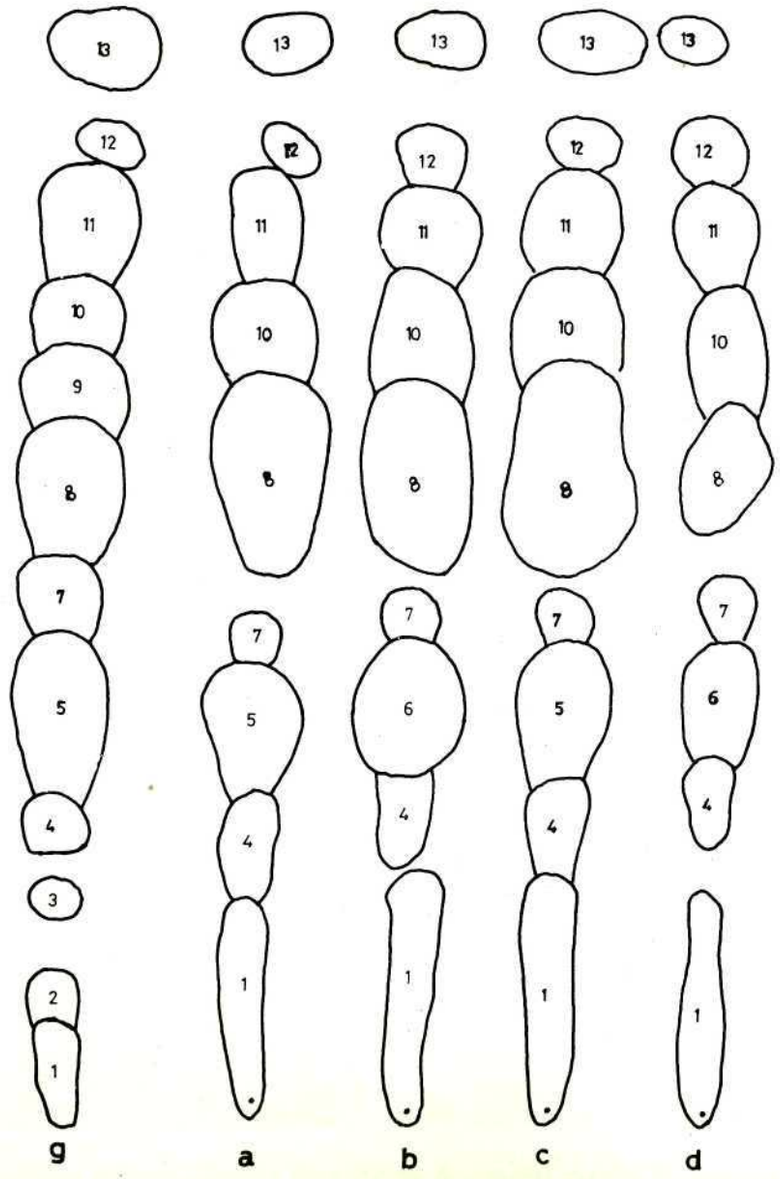
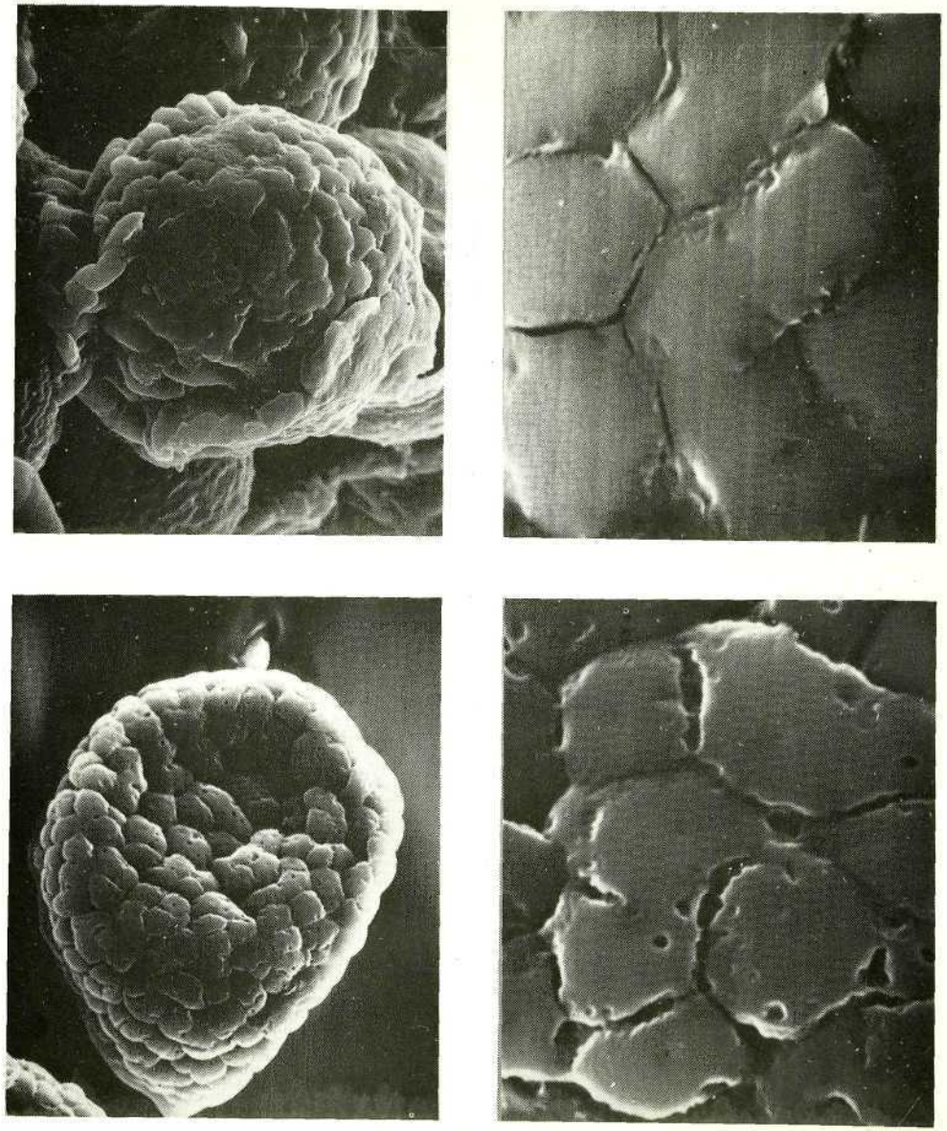


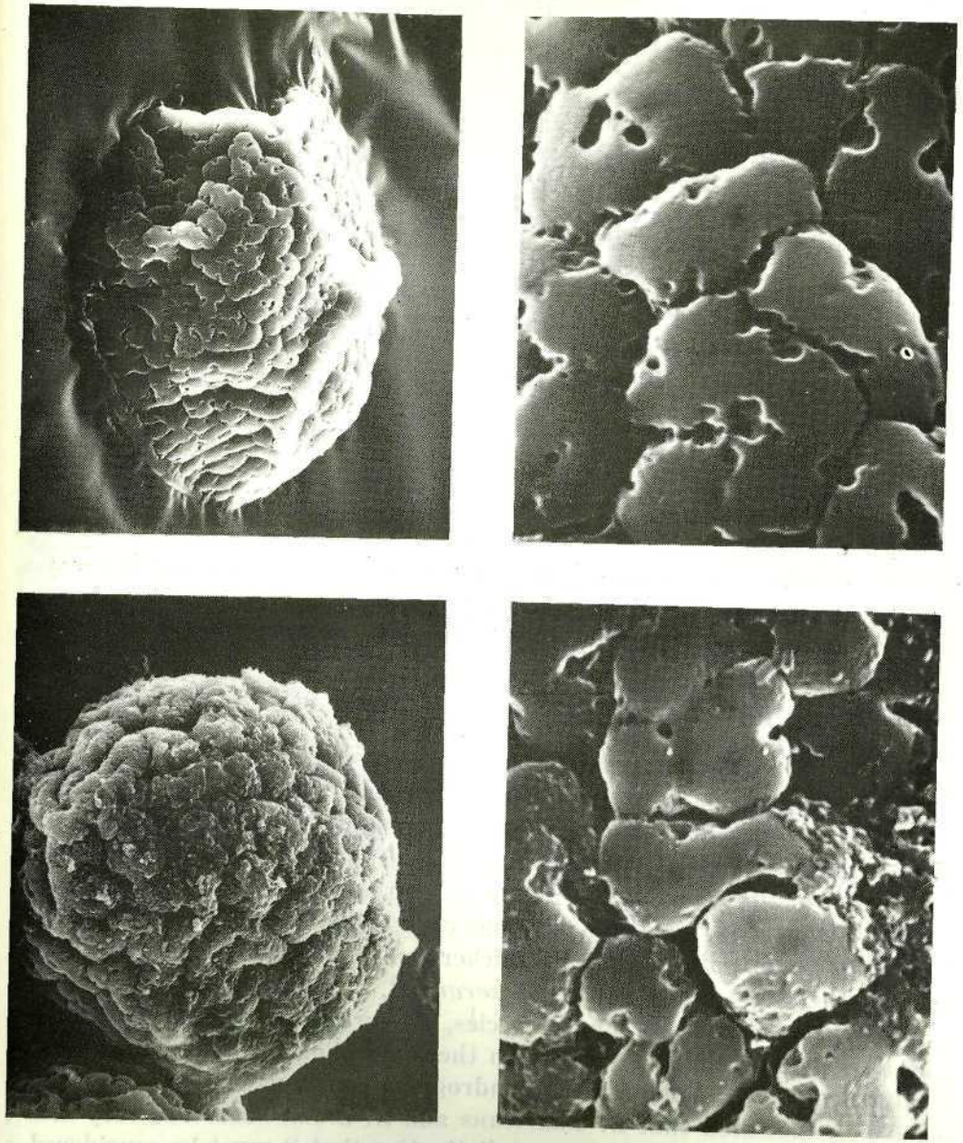
FIG. 2. Master chromatogram of phenolic spots after paper chromatography.



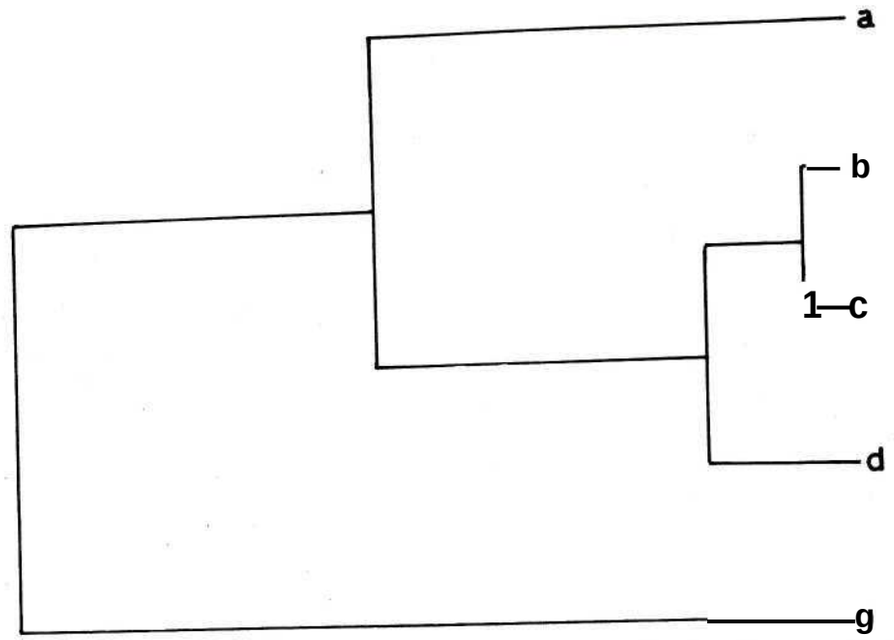
PIG. 3. Chromatogram representing the distribution of spots of essential oils after TLC. g = *Z. officinale,* a = *Z, zerumbet,* b = *Z. amaricans,* c = *Z. aromaticum,* d = *Z. littorale.*



PIG. 4. Pollen grain of *Zingiber officinale*. A: general outlook, B: detailed. Pollen grain of *Zingiber zerumbet*. C: general outlook, D: detailed.



PlG. 5. Pollen grain of *Zingiber aromabicum. A:* general mitlnnt n. j frain of *Zingiber amaricans.* C: generfl otuLk,^ D^'d^tafled?



PIG. 6. Dendrogram representing- relationships between species based on combined Morphological characters, protein contents, phenolic compounds and essential oils, g = *Z. officinale,* a = *Z. zerumbet, h — Z. amaricans,* c. = *aromaticum,* d = *Z. littorale.*

CONCLUSIONS

The total range of results obtained from various sources comprising morphology, proteins, phenolic compounds and essential oils, produced a combination of 108 characters (excluding pollen) which were employed to produce an overall dendrogram (Fig. 6).

The results accumulated in the present work indicate that, although such data as anatomy, cytology and crossability should not be ignored, the suggestion put forward by Backer and Bakhuizen v.d. Brink Jr. to merge the four species, namely *Z. zerumbet, Z. amaricans, Z. aromaticum* and *Z. littorale*, into a single species, *Z. zerumbet*, cannot be defended because it is possible to distinguish them morphologically, chemically and palynologically. Although the dendrogram constructed from the overall evidence indicated that *Z. amaricans* and *Z. aromaticum* are very close, the pollen of *Z. aromaticum* is so distinctive that it must be considered a distinct species.

Furthermore, it is also clearly noticed that *Z. officinale* is not closely related to the other four species. It is morphologically, chemically **and** palynologically distinct.

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