QUALITY VARIATIONS IN BLACK MUSLI (CURCULIGO ORCHIOIDES GAERTN.)

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ABSTRACT: Black musli (Curculigo orchioides Gaertn.) one of the ayurvedic dasapushpa and a rejuvenating and aphrodisiac drug. Is on the verge of extinction and needs to be conserved and cultivated. Large variations are also observed in the quality of the crude drug available in the market. Study on the quality of C. orchioides in natural habitat, under cultivation and in trade in south India showed that there was considerable variation with biotypes and habitats. Drugs collected form the natural habitat was superior in quality to that produced by cultivation. Among the market samples collected from the various Zones of kerala, those from the High Ranges were superior in most of the quality parameters, which indicated its superiority for high quality drug formulation. Among the southern states, Tamil Nadu samples ranked next to High Range samples in this respect. There exists large variability in the market samples and there is felt-need for proper standardization of the crude drug for ensuring quality in the drug formulations.

Key Words Curculigo orchioides, black musli, biotype, habitat, crude drug, quality, curculigoside, metabolites.

INTRODUCTION

Golden eye grass or black musli (Curculigo orchioides gaertn.) of the family Amaryllidaceae is a key member of the dasapushpa and a highly useful plant in the indigenous system of medicine. It is a small, geophilous herb, the rhizome of which is used as a rejuvenating and aphrodisiac drug 1, 2. Large variations are observed in the quality of the crude drug available in the market which might affect the efficacy of the drug formulations3. The rhizome of C. orchioides is reported to contain glycosides 4-6. Glycosides are considered to be one of the major pharmacologically active components of the drug. Kubo et al.7 reported the presence of curculigoside (5-hydroxy -2-O-b-D-glucopyranosyl benzoyl-2, 6- dimethoxy benzoate) in C. orchioides and Yamasaki et al.8 extracted it from the rhizome to the tune of 0.2 per cent. The glycosides in C. orchioides which are considered to be pharmacologically important are the glycosides of benzoyl benzoate. This study was undertaken to investigate the biotype variations due to ecology and domestication and to assess the extent of variability in the market samples in the state as well as in the other states of south India.
MATERIALS AND METHODS

Investigations on the quality variations in C. orchioides as influenced by biotypes, habitats and markets were carried out during 2000-'02 at the Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali, Kerala.

Two biotypes of the species C. Orchioides, namely, Panamkuzhi and Vellanikara types were used for the study. The planting material of Panamkuzhi biotype was collected from Panamkuzhi area of Kodanad forest range in the Ernakulam district and the Vellanikara biotype from the rubber plantation of the kerala Agricultural University at vellanikara in Trichur district of Kerala. It is propagated through suckers and leaf tip sprouts during monsoon. The suckers of the two biotypes of C. orchioides were used for raising the crop at the station for evaluating the cultivated situation. Mature plants of both the biotypes were collected from their natural habitat for understanding the natural condition. Crude drugs were purchased directly from two randomly selected markets representing each of the four agroecological zones of kerala and from four southern states of India, namely, Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra to study the market scenario. These samples were evaluated for quality variations in terms of primary and secondary metabolites.

The crude drug samples were processed and analysed for quality parameters such as glucose, sucrose, starch, crude fibre, crude protein, crude fat and ash following standard procedures of Thimmaiah⁹. samples were subjected to extraction of glycosides with ethyl acetate followed by alkali hydrolysis. The hydrolysate was analysed by HPLC and the peaks were identified with the help of authentic standards of benzoic acid derivatives (4-hydroxy 3-methoxybenzoic acid). Curculigoside content was computed from the assay of 2, 6-dimethoxybenzoic acid⁸,¹⁰. The data were analysed following Gomez and Gomez¹¹ and comparison was made on the quality of the crude drugs based on the content of primary and secondary metabolites.

RESULTS AND DISCUSSION

Effort were made to study the difference between biotypes in their chemical composition in terms of primary and secondary metabolites. The proximate analyses (Table 1) showed that the rhizome of Panamkuzhi biotype contained more ash, crude fat, crude fibre, starch, glucose and sucrose while Vellanikara biotype recorded higher crude protein content indicating an inverse relation between crude protein and other primary metabolites. Attempts were also made to identify quality markers taking 4-hydroxy 3-methoxy benzoic acid and 2, 6-dimethoxy benzoic acid as indicator compounds. The content of 4-hydroxy 3-methoxy benzoic acid was higher in the rhizome of Panamkuzhi biotype (Table 2). On the contrary, the reverse was true when the content of 2, 6-dimethoxy benzoic acid and curculigoside were taken into consideration. The relative importance of the two marker compounds in the medicinal effect of the drug is not clearly known. Further C. orchioides possesses a number of therapeutical properties like aphrodisiac, uterine stimulatory, nerve tonic and diuretic and the association of each of the chemical compounds with these widely different properties is also not known, hence it is difficult to establish the superiority of one biotype over the other in this case.
In general, plants in the natural habitat recorded a higher content of ash, crude fat, crude fibre, glucose, sucrose and curculigoside while crude protein and starch were more under cultivated condition. This indicated that C. orchioides growing in natural habitat had better quality compared to those under cultivation as reported by Menon\textsuperscript{12} and menon and Potty\textsuperscript{13, 14} in njavara rice and Kurian et al.\textsuperscript{15} in many medicinal plants.

Among the market samples collected from various zones of kerala, those from High Ranges recorded higher curculigoside content with a comparatively higher values of crude fat, crude fat, crude fibre and ash. The lesser content of the other primary metabolites might be due to their utilization for the production of the secondary metabolite-curculigoside. The data showed that there is a wide variation in the quality of crude drug available in the various zones of the state.

Among the various states, the samples collected from Tamil Nadu recorded higher curculigoside content next to that collected from High Ranges of kerala. Samples from Andhra Pradesh did not contain detectable quantities of curculigoside but possessed higher quantities of crude protein, crude fat, crude fibre and ash which again demonstrated the inverse relationship between the primary and secondary, metabolites. Samples from Karnataka and Maharashtra also recorded very low values for curculigoside. This indicated that there is a wide variation in the quality of crude drug available in the various states of south India as highlighted by Joy et al.\textsuperscript{16} in Alpinia calcarata and curcuma zedoaria, Reynolds \textsuperscript{17} in Panax quinquifolium and Liu et al.\textsuperscript{18} in many herbal drugs. Samples from Tamil Nadu ranked second in quality with respect to curculigoside content next to the samples from the High Ranges of Kerala.

The key attribute of the data is the wide variation in the level of the marker compounds. Sampling locations are situated far apart and hence the source of the material can be expected to be different. This clearly demonstrates that a large variation exists among the C. orchioides plant types sourced from different parts of south India. This wide variation in quality as indicated by these marker compounds proposes a large variation in the therapeutical efficiency as well. Two important aspects that emerge from this study are:

(1) A large variability exists among the natural population of C. orchioides in the subcontinent, which offers the breeder wide scope for improvement of the crop by selection and hybridization.

(2) There is a dire need for developing methods for assessing the quality of crude drugs of C. orchioides and imposing strict quality control to ensure the efficiency of the drug formulations manufactured using the drug.

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Table 1. Proximate analyses of Curculigo orchioides rhizome from various biotypes, habitats and markets (%)

| Source               | Dry matter | Ash | Crude protein | Crude fat | Crude fibre | Starch | Glucose | Sucrose |
|----------------------|------------|-----|---------------|-----------|-------------|--------|---------|---------|
| **Biotype**          |            |     |               |           |             |        |         |         |
| Panamkuzhi           | 93.28      | 7.20| 9.70          | 3.30      | 5.65        | 50.93  | 2.35    | 1.44    |
| Vellanikkara         | 92.59      | 6.25| 12.10         | 2.05      | 4.31        | 46.99  | 0.95    | 0.20    |
| **Habitat**          |            |     |               |           |             |        |         |         |
| Natural              | 92.81      | 8.55| 5.28          | 3.37      | 6.39        | 46.9   | 2.63    | 1.05    |
| Cultivated           | 93.05      | 4.90| 16.52         | 1.71      | 3.56        | 51.02  | 0.67    | 0.58    |
| **Market Samples**   |            |     |               |           |             |        |         |         |
| Kerala               |            |     |               |           |             |        |         |         |
| South zone           | 90.47      | 6.471| 6.121       | 1.500     | 3.326       | 48.53  | 1.436   | 0.745   |
| Central zone         | 89.75      | 8.523| 4.720       | 1.579     | 2.823       | 52.91  | 0.772   | 0.000   |
| North zone           | 91.13      | 6.523| 6.105       | 1.616     | 3.153       | 54.31  | 1.063   | 0.755   |
| High ranges          | 90.75      | 22.206| 5.040      | 1.623     | 3.975       | 52.04  | 0.743   | ND      |
| Tamil Nadu           | 90.40      | 8.026| 6.127       | 1.742     | 3.082       | 52.81  | 1.115   | ND      |
| Karnataka            | 92.05      | 8.607| 6.160       | 1.717     | 3.050       | 53.20  | 1.195   | ND      |
| Andhra Pradesh       | 87.51      | 17.299| 7.304      | 1.855     | 3.675       | 35.16  | ND      | ND      |
| Maharashtra          | 89.40      | 8.545| 6.832       | 1.727     | 2.851       | 52.54  | 0.810   | ND      |
| SEm                  | 2.974      | 0.265| 0.233       | 0.161     | 0.128       | 0.936  | 0.491   | 0.170   |
| CD(0.05)             | NS         | 0.799| 0.703       | 0.485     | 0.385       | 2.823  | 1.479   | 0.513   |
| G.Mean               | 90.94      | 8.818| 7.192       | 1.856     | 3.654       | 50.10  | 1.152   | 0.438   |

ND = Not detected
Table 2. Secondary metabolites in curculigo orchioides rhizome from various biotypes, habitats and markets (ppm)

| Source                | 4-hydroxy-3-methoxy benzoic acid | 2,6-dimethoxy benzoic acid | Curculigoside |
|-----------------------|----------------------------------|----------------------------|---------------|
| **Biotype**           |                                  |                            |               |
| Panamkuzhi            | 115.545                          | 82.035                     | 210.010       |
| Vellanikkara          | 35.295                           | 118.945                    | 304.499       |
| **Habitat**           |                                  |                            |               |
| Natural               | 91.180                           | 135.120                    | 345.907       |
| Cultivated            | 59.660                           | 65.860                     | 168.602       |
| **Market Samples**    |                                  |                            |               |
| Kerala                |                                  |                            |               |
| South zone            | 48.35                            | 183.47                     | 0.007         |
| Central zone          | 1.1                              | 10.01                      | 0.005         |
| North zone            | 68.53                            | 111.28                     | 0.029         |
| High ranges           | 52.42                            | 211.96                     | 0.054         |
| Tamil Nadu            | ND                               | 212.31                     | 0.045         |
| Karnataka             | ND                               | 4.20                       | 0.001         |
| Andhra Pradesh        | 31.40                            | ND                         | ND            |
| Maharashtra           | 38.87                            | 28.25                      | 0.007         |

ND = Not detected