Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(11)60046-4

Studies on isozymic variation among the South Indian species of *Sphaerostephanos*

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ARTICLE INFO

Article history:
Received 20 February 2011
Received in revised form 28 March 2011
Accepted 13 April 2011
Available online 30 April 2011

Keywords:
Sphaerostephanos
Isoperoxidase
Poly acrylamide gel electrophoresis
Inter-specific variation
Isozymic variation
Isozymic profile
Phylogenetic relationship

ABSTRACT

Objective: To explore the identity and phylogenetic relationships among the three medicinally important species of *Sphaerostephanos* from South India using isozymic profile. **Methods:** The young fronds were homogenized with 3.5 mL of ice-cold homogenizing buffer in a pre-chilled pestle and mortar. The supernatant was subjected to electrophoresis as described by Anbalagan poly acrylamide gel electrophoresis. Staining solutions for isoperoxidase was prepared as per Smila method for the detection of isoenzymes. **Results:** A total of six different bands in five different positions with different molecular weight/Rf values and four active zones have been observed in the isoperoxidase enzyme system of *Sphaerostephanos*. Only one band with MW/Rf 0.399 is common to two different species *i.e. Sphaerostephanos arbuscula* (*S. arbuscula*) and *Sphaerostephanos unitus* (*S. unitus*). Among the remaining four bands, two bands (R_f. 0.23, 0.47) are present in *Sphaerostephanos subtruncatus* (*S. subtruncatus*) and one distinct band has been observed individually in *S. arbuscula* (R_f. 0.507) and *S. unitus* (R_f. 0.56). **Conclusions:** The present preliminary molecular study through isozymic analysis shows the identity of all the three species and the present results confirm distinctness of these three species based on macro-micromorphology, phytochemistry and cytology.

1. Introduction

The primitive vascular plants pteridophytes form the dominant vegetation on the earth next to flowering plants. The megabiodiversity country India is rich in pteridophytes with the presence of about 1 200 species distributed in various taxonomical families. Several species of pteridophytes are medicinally important and they are used by local people to treat various kinds of health problems. The South Indian thelypteroid genus *Sphaerostephanos* is represented by three distinct species *i.e. Sphaerostephanos arbuscula* (Willd.) Holttum (*S. arbuscula*), *Sphaerostephanos subtruncatus* (Bory) Holttum (*S. subtruncatus*) and *Sphaerostephanos unitus* (L.) Holttum (*S. unitus*). In general species of *Sphaerostephanos* are used to treat various health problems. The crushed young leaves are used to rub on scabies. Alternatively, the leaves are boiled and the juice is used to bathe the patient suffering from scabies. For skin conditions associated with measles, the new leaves and shoots are squeezed and the juice is rubbed on the affected area. The plant is also used in treating fever[1]. Yusuf *et al*[2] made the chemotaxonomic survey of flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia. From *S. arbuscula* the medicinally important chemical compound astilbin has been reported[3]. Astilbin prevents concanavalin A-induced liver injury by reducing TNF- α production and T lymphocyte adhesion[4]. The inhibitory effect of astilbin on the arteriosclerosis of murine thoracic aorta transplant has been studied by Zhao *et al*[5]. Astilbin also suppresses acute heart allograft rejection by inhibiting maturation and function of dendritic cells in mice[6]. Song *et al*[7] have also explored the effects of astilbin on the maturation and immunologic function of mouse bone marrow-derived dendritic cells. Antibacterial activity has been proved both in *S. subtruncatus* and *S. unitus*[8,9]. Identity of any medicinally important species is essential in the modern world. They are distinct in macromorphology (frond, rhizome) and micromorphology (morphology and distribution of epidermal glands)[10]. Chemical composition of epidermal glands is also different among these three species[11]. Both *S. unitus* and *S. arbuscula* are diploid

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sexual species with 36 bivalents in Spore Mother Cells[12]. In contrast, *S. subtruncatus* is a tetraploid species (*n*=72). Thus cytologically *S. arbuscula* and *S. unitus* are similar while *S. subtruncatus* is distinct. However, in chemical composition of epidermal glands, *S. arbuscula* and *S. subtruncatus* are more or less similar in having less chemical diversity-eight compounds in each species while *S. unitus* is distinct in having higher degree of chemical diversity (17 different compounds). Based on morphology, cytology and phytochemistry, it is very difficult to identify the medicinal plants in the form of crude drug. Molecular marker is very useful in pharmacognosy. Since the differences in all the morphological and phytochemical characters are ultimately due to the difference in genetical characters, the study on genetical difference may be useful for identity and exploration of phylogenetic relationships among the three medicinally important species of *Sphaerostephanos* from South India. In contrast to DNA marker, isoenzyme analyses are widely used for their relative efficiency and cost-effectiveness, particularly in studies of intra and inter-specific variability[13]. Isozymes are as useful as genetic and biochemical markers and also as good estimators of genetic variability in plant populations. With this background, the differences in the isozymic profile have been analysed in the present study.

2. Materials and methods

Plants *S. arbuscula* (Willd.) Holttum, *S. subtruncatus* (Bory) Holttum and *S. unitus* (L.) Holttum were collected from the natural habitats and established in the green house attached to the Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India. From the mother plants, croziers were collected and homogenized with 3.5 mL of ice-cold homogenizing buffer in a pre-chilled pestle and mortar. The supernatant was subjected to electrophoresis as described by Anbalagan[14] on poly acrylamide gel electrophoresis (PAGE). Staining solutions for isoperoxidase was prepared as per Smila[15] for the detection of iso-enzymes. After the electrophoresis, the gels were incubated in the staining solution for a few minutes under dark condition till the clear bands appeared. The gels were fixed with 7% acetic acid solution for 30 min, washed with distilled water and photographed using the gel documentation system manufactured by Biotech, Yercaud, India. Based on the isoenzyme banding profile the zymogram was constructed.

3. Results

A total of six different bands in five different positions with different molecular weight/Rf values and four active zones have been observed in the isoperoxidase enzyme system of *Sphaerostephanos* (Figure 1). Only one band was observed in zone three PRX3[1] (0.230) and it was restricted to *S. subtruncatus*. Zone four expressed with two bands in one position PRX4[1] (0.399) and jointly expressed in *S. unitus* and *S. arbuscula*. Zone five also showed only one band PRX5[1] (0.470); and it is present only in *S. subtruncatus*. The other two species failed to express any band in the zone five. Two bands were observed in zone six. PRX6[1] (0.507) was expressed only in *S. arbuscula* and PRX6[2] (0.560) was present only in *S. unitus* (Table 1).

Table 1
Isoperoxidase profile of *Sphaerostephanos* spp.

MW/R_f Value	*S. arbuscula*	*S. subtruncatus*	*S. unitus*
0.230		+	
0.399	+		+
0.470		+	
0.507	+		
0.560			+

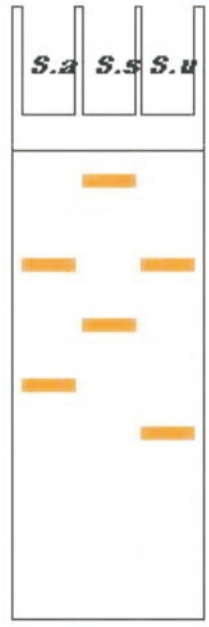

Figure 1. Zymogram of the isoperoxidase profile of *Sphaerostephanos* spp.
S. a-S. arbuscula; *S. s-S. subtruncatus; S. u-S. unitus.*

4. Discussion

All the three south Indian species are similar in having two different bands in each species. But they differ in having bands of different molecular weight and Rf values. Only one band with MW/Rf 0.399 is common to two different species, *i.e. S. arbuscula* and *S. unitus*. Among the remaining four bands, two bands (R_f. 0.230, 0.470) are present in *S. subtruncatus* and one distinct band has been observed individually in *S. arbuscula* (R_f. 0.507) and *S. unitus* (R_f. 0.560). Thus the present preliminary molecular study through isozymic analysis shows the identity of all three species and the present results confirm distinctness of these three species based on macro-micromorphology, phytochemistry and cytology. The two diploid species share the band with R_f value of 0.399 which is absent in the tetraploid species *S. subtruncatus*. *S. arbuscula* is both morphologically and cytologically primitive in having erect rhizome and with

diploidal chromosome number (n=36)[16-19]. *S. subtruncatus* is morphologically primitive and cytologically advanced in having erect rhizome and tetraploidal chromosome number (n=72) and *S. unitus* is morphologically advanced and cytologically prmitive in having long creeping rhizome and diploidal chromosome number (n=36). The same kind of trend is also observed in the present molecular study in having different bands with different molecular weight and different Rf values. Thus the distinct banding patterns of the presently studied medicinal plants will be useful for the pharmacognostical identification of the crude drugs of the same species in the near future. The present study results were directly coincided with previous observations[20-28]. Further studies on molecular taxonomy on these species will give more knowledge about the molecular identity and phylogenetic relationship among these three South Indian species of *Sphaerostephanos*.

Conflict of interest statement

We declare that we have no conflict of interest.

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