ORIGINAL ARTICLE

Development of quality control standards for two *Epaltes* species

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Abstract

Background: Whole plant of *Epaltes* species, namely, *Epaltes divaricata* L. (Cass) and *Epaltes pygmaea* DC. traditionally used as medicine in Sri Lanka to cure various ailments, both plants have potent hepatoprotective and diuretic activities. **Materials and Methods:** In this study, whole plant of *E. divaricata* and *E. pygmaea* were collected, identified and subjected for its quality control parameters such as physicochemical, heavy metal, nutraceutical analysis, microbial contamination, aflatoxins, and pesticide residue as per standard methods. **Results:** The results revealed that both plants showed sufficient mineral elements, within the limit of heavy metals, free from pesticide, aflatoxins and microbial contamination and its use is safe. **Conclusion:** The study ensures that these quality control parameters do help in the proper standardization of the crude drugs in drug development process.

Key words: *Epaltes divaricata*, *Epaltes pygmaea*, quality control parameters

INTRODUCTION

Standardization is an important factor to ensure the quality and safety of herbal medicine. Unfortunately, most of the herbal products do not have drug regulatory approval to demonstrate their safety and efficacy.[1] According to the World Health Organization (WHO) guidelines, the plant material needs to be standardized with respect of safety and quality before use. Several analytical parameters such as physicochemical constants, estimation of elements, vitamins, heavy metals, microbial contamination, aflatoxins and pesticide residue are to be carried out as a measure of quality check.

The genus *Epaltes* is used in traditional Ayurvedic medicine in Sri Lanka to alleviate jaundice, urethral discharges, acute dyspepsia, diaphoretic, diuretic, and a stimulating expectorant.[2] Two weeds belonging to this genus have been found to be available in South India in minor quantities, *viz*., *Epaltes divaricata* L. (Cass) and *Epaltes pygmaea* DC. Roots of *E. divaricata* are mostly used as astringent and a health tonic.[3] The literature review revealed that the pharmacognostic standardization, preliminary phytochemical studies[4] and isolation of five closely related eudesmane derivatives from the acetone extract[5] and hepatoprotective[2] diuretic activity of the plant were reported earlier.[6] Eleven chemical constituents have been identified from n-hexane extract of the plant of *E. divaricata* by gas chromatography-mass spectrometry (GC-MS) analysis.[7]

Major active chemical constituents of *E. pygmaea* are lupeol acetate, stigmasterol, stigmasterol acetate, apigenin, luteolin, apigenin-7-O-glucoside and luteolin-7-O-glucoside[8] hepatoprotective and diuretic activity of aqueous and alcoholic extracts of *E. pygmaea* has been reported.[9] However, detailed study of quality control parameters has not been done. In the present study, whole plants of *E. divaricata* and *E. pygmaea* were subjected to physicochemical, heavy

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metals analysis, pesticide residues, aflatoxins, microbial contamination, and nutraceutical analysis.

MATERIALS AND METHODS

Plant Material

Plant materials were collected from Thirunelveli district, Tamil Nadu; the species were identified and authenticated by Dr. S. Amerjothy, Retired HOD, Department of Plant Biology and Plant Biotechnology, Presidency College Chennai – 600 005, Tamil Nadu, India and Dr. V. Chelladurai, Retired Officer, Survey of Medicinal Plant Unit-Siddha, Palayamkottai, Tamil Nadu, India. Voucher specimens of *E. divaricata* (00629) and *E. pygmaea* (00630) have been deposited in the herbarium of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai, Tamil Nadu, India.

Sample Preparation

The plants were thoroughly washed with deionized water, shade dried at room temperature for about 15 days, ground into powder with a mechanical grinder and finally stored in airtight bottles before analysis.

Chemicals and Solvents

All chemicals and solvents used in these studies were of analytical grade obtained from E.Merck, Mumbai, Maharashtra, India.

Analytical Parameters

The procedures recommended as per WHO guidelines[10,11] were followed to determine the parameters such as moisture content, total ash, water-soluble ash, and acid-insoluble ash. The percentage of alcohol-soluble and water-soluble extractive was also determined.

Heavy Metal Analysis

Heavy metals such as lead, cadmium, mercury, and arsenic were analyzed using inductively coupled plasma-MS (ICP-MS).[12] Accurately weighed coarsely powdered air-dried plant materials (5 g) was taken in a 250 ml conical flask and added 20 ml of conc. nitric acid, 16 N and 5 ml of perchloric acid (70%). The flask was heated at 60-80°C on a heating mantle until complete digestion occurred resulting in a clear solution. The solution was filtered through Whatman No. 41 filter paper into a 100 ml standard volumetric flask, and the volume was made up to 100 ml with deionized water. The sample was used for the analysis of lead, cadmium, mercury, arsenic, chromium, and nickel by ICP-MS.

Operating Parameters

Name of the instruments	Agilent 7500 CX
Detector	Quarter pole mass detector
Carrier gas	Argon
Flow rate	0.94/min
Plasma temperature	7000 K
Collision gas for collision cell	Helium
Library software	Chemstation

Pesticide Residue and Aflatoxins

Pesticide residues were analyzed using GC-MS Shimadzu instrument equipped with electron capture detector as per the method of AOAC.[13] Aflatoxins were determined by Kobra cell technique using Shimadzu high performance liquid chromatography (HPLC) instrument.[14]

Determination of Microbial Contamination

Microbial contamination present in the sample, *viz*., *Enterobacteriaceae*, *Salmonella*, and *Staphylococcus aureus* were estimated as per the method WHO.[10] For determination of microbial load, 1 g of each sample was weighed accurately in separate flasks and 99 ml of sterile distilled water was added. The samples in the flask were kept in a mechanical shaker for few minutes to obtain uniform suspension of microorganisms. The dilution is 1:100 or 10^{-2} from, which 1 ml was transferred to 9 ml of sterilized distilled water to make a 1: 1000 dilution and this procedure was repeated up to 10^{-6} dilution. Each 0.1 ml of sterile diluted sample was inoculated to the sterile plates containing nutrient agar, SS agar and potato dextrose agar (PDA) by spread plate method. Nutrient agar and SS agar plates were inoculated at 37° for 24 h and PDA plates were inoculated at room temperature for 3-5 days. Bacterial and fungal colonies were counted using a colony counter.

Nutraceutical Analysis

Determination of carbohydrate,[15] fat,[16] and protein[17] were estimated by standard methodology. Calorific value was determined by the combination of four times of carbohydrate, nine times of fat and four times of protein (carbohydrate × 4 + fat × 9 + protein × 4), multiplied with formula and after adding all the values, finally get the calorific value.[15] Estimation of elements was determined as per standard textual procedures.[18] Estimation of vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), and niacinamide by HPLC method.[19]

RESULTS

The physicochemical parameters of both plants were investigated and reported in Table 1. The heavy metals analysis

of two species is shown in Table 2. The report of analysis for aflatoxins in the two species is given in Table 3. The result of various pesticidal residues is shown in Table 4. The microbial contamination of the plants is shown in Table 5. The nutraceutical composition of the plants is shown in Table 6.

DISCUSSION

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. Loss on drying which infers the presence of excess moisture is conducive to the promotion of mold and bacterial growth, and subsequently, to deterioration and spoilage of the drug. The moisture content was calculated to be 9.42% in *E. divaricata* and 13.85% in *E. pygmaea* (*E. divaricata* < *E. pygmaea*). The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The total ash content of *E. divaricata* and *E. pygmaea* was found to be 9.01% and 11.31%, respectively, which is due to the presence of inorganic matter present in these *Epaltes* species. Inorganic matter was found to be higher in *E. pygmaea* than *E. divaricata*. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. The acid-insoluble ash indicates the presence of siliceous matter in the drug. It was found to be 1.64% in *E. divaricata* and 2.27% in *E. pygmaea*, respectively. More siliceous matter was found in *E. pygmaea* than in *E. divaricata*. The water-soluble ash is used to detect the presence of inorganic salts exhausted by water and it was found to be 4.24% in *E. divaricata* and 5.91% in *E. pygmaea*. The alcohol soluble extractive value revealed the presence of polar compounds such as glycosides of flavonoids, steroids and triterpenoids present in the plants. It was found to be 14.5% in *E. divaricata* and 11.0% in *E. pygmaea*. The water-soluble extractive value revealed the presence of water-soluble matters such as sugars, carboxylic acids, vitamins, and amino acids and it was found to be 15.7% in *E. divaricata* and 19.9% in *E. pygmaea*.

Heavy metals such as arsenic, mercury, and cadmium were not present in both species; the presence of lead was found only to be 1.22 ppm in *E. divaricata* and 0.89 ppm in *E. pygmaea*, respectively, which are within the permissible limit (10 ppm) indicating that these plants are safe to utilize as drugs. The aflatoxins B_1, B_2, G_1, and G_2 were below the detecting level revealing that they are free from toxins and are safe for internal use. Further the studies indicated that the absence of these aflatoxins would help to increase in shelf life of the raw drug. Pesticides are not detected (DL, 0.005 mg/kg) for the two *Epaltes* species indicating that they are safe for their usage. Determination of microbial contamination, *viz*., Bacteria, fungi count, *Enterobacteriaceae, Salmonella* sp. and *Staphylococcus* sp. are found to be within the limit for the two *Epaltes* species as per WHO guidelines[10] revealing that they are free from pathogen and can be used as drugs.

Table 1: Physicochemical values of two *Epaltes* species

Parameters	*E.divaricata* (% w/w)	*E.pygmaea* (% w/w)
Moisture content	9.42	13.85
Total ash	9.01	11.31
Water-soluble ash	4.24	5.91
Acid-in soluble ash	1.64	2.27
Alcoho1-soluble Extractive	14.5	11.0
Water-soluble extractive	15.7	19.9

Values are mean of 3 readings, *E. divaricata: Epaltes divaricata, E. pygmaea: Epaltes pygmaea*

Table 2: Heavy metal analysis of two *Epaltes* species

Heavy metals	*E. divaricata*	*E. pygmaea*	*Permissible* limit (API,2008)
Lead	1.22 ppm	0.89 ppm	10 ppm
Cadmium	ND	ND	0.3 ppm
Mercury	ND	ND	1 ppm
Arsenic	ND	ND	3 ppm

API: Ayurvedic Pharmacopoeia of India, ND: Not detected, *E. divaricata: Epaltes divaricata, E. pygmaea: Epaltes pygmaea*

Table 3: Pesticidal residue of two *Epaltes* species

Pesticides	*E. divaricata*	*E. pygmaea*
o, p´-DDD	ND	ND
p, p´-DDD	ND	ND
o, p-DDE	ND	ND
p, p´-DDE	ND	ND
o, p´-DDT	ND	ND
p, p´-DDT	ND	ND
α-Endosulfan	ND	ND
β-Endosulfan	ND	ND
α-HCH	ND	ND
β-HCH	ND	ND
γ-HCH	ND	ND
δ-HCH	ND	ND

ND: Not detectable; Detection limit for the instrument: DL-0.005 mg/kg. *E. divaricata: Epaltes divaricata, E. pygmaea: Epaltes pygmaea*

Table 4: Aflatoxins in two *Epaltes* species

Aflatoxins	*E. divaricata*	*E. pygmaea*
B_1	BDL	BDL
B_2	BDL	BDL
G_1	BDL	BDL
G_2	BDL	BDL

Detection limit for the instrument: DL-0.03 mg/kg.
BDL: Below detecting limit, *E. divaricata: Epaltes divaricata, E. pygmaea: Epaltes pygmaea*

Table 5: Determination of microbial load for two *Epaltes* species

Name of the sample	TBC	TFC	Parameters CFU/g		
			Enterobacteriaceae	***Salmonella* sp.**	***Staphylococcus aureus***
E. divaricata	$<10^5$	$<10^3$	$<10^2$	Absent	Absent
E. pygmaea	$<10^4$	$<10^3$	$<10^1$	Absent	Absent
WHO limits for external	$<10^7$/g	$<10^4$/g	$<10^3$/g	Absent	Absent

TBC: Total bacterial count, TFC: Total fungal count, CFU: Colony forming units, *E. divaricata: Epaltes divaricata, E. pygmaea: Epaltes pygmaea*

Table 6: Nutraceutical composition of two *Epaltes* species

Parameters	***E. divaricata*** **(100 g)**	***E. pygrmaea*** **(100 g)**
Carbohydrate (%)	5.786	6.89
Fat (%)	1.245	0.024
Protein (%)	4.764	4.87
Calcium (mg)	14.5	23.7
Magnesium (mg)	12.2	12.1
Sodium (mg)	15.8	6.78
Potassium (mg)	5.67	0.90
Phosphorous (mg)	1.33	0.55
Zmc (mg)	6.77	0.21
Calorific values (Kcal)	55 (approximately)	46 (approximately)
Chromium	Nil	Nil
Nickel	Nil	Nil
Vitamin B1 (mg)	0.786	0.878
Vitamin B2 (mg)	0.989	0.967
Vitamin B6 (mg)	0.567	0.222
Niacin amide (mg)	0.089	0.3099

Nutrition involves the relationship of food and nutrients to health. The nutritional science also includes many aspects of related disciplines such as physiology, food chemistry, toxicology, pediatrics, and public health.[20] Plants are the outsourcing of many nutritive components; it is mandatory to identify the methodology for estimating and extracting the nutritive phytoconsituents. All human beings require a number of complex organic compounds as added caloric requirements to meet the need for their muscular activities.[21]

Carbohydrates, fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively a smaller part.[22] The carbohydrates are main source and store of energy. They are the starting substances for biological synthesis of many compounds.[23] The percentage of carbohydrates is found to be more in *E. pygmaea* than in *E. divaricata*, while the protein amounts are equal in both plants. Fat content is found to be more in *E. divaricata* when compared to *E. pygmaea*. *E. divaricata* illustrates more calorific value (55 kcal) when compared to *E. pygmaea* (46 kcal); the higher calorific value is due to the higher quantum of carbohydrates and fats.

Elements such as calcium, magnesium, potassium, sodium, phosphorous, zinc, vitamins such as vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B6 (pyridoxin), and niacinamide are identified. Calcium is found to be high in *E. pygmaea* than in *E. divaricata*. Calcium is an important element in the treatment of bone loss,[24] nerve-impulse transmission and in the mechanism of neuromuscular system.[21] Magnesium plays an important role in enzyme activity; deficiency interfere with transmission of nerve and muscle, impulses, causing irritability, and nervousness; prevent heart diseases.[25] Magnesium content is found to be almost equal in both plants. Both sodium and potassium take part in ionic balance of the human body and maintain tissue excitability.[26] The content of sodium and potassium is found to be higher in *E. divaricata* than in *E. pygmaea*.

Zinc is a component of many metalloenzymes and also a membrane stabilizer and a stimulator of the immune response.[27,28] Zinc deficiency causes hair loss, diarrhea, and delayed sexual maturation.[29,30] Phosphorus is important in maintaining bone structure and modulating plasma and bone formation[25] and blood sugar level; normal heart contraction dependent on phosphorus.[31] The zinc and phosphorus content are found to be high in *E. divaricata* than in *E. pygmaea*. Chromium and nickel are not found in both plants.

Thiamine (vitamin B1) is needed for functioning of nervous system and it helps in releasing energy from carbohydrates.[32] Thiamine deficiency, demonstrated by its decrease in urine as well as the increase of transketolase activity coefficient, leads to beriberi disease.[33] *E. divaricata* and *E. pygmaea* was found to contain 0.786 mg/g and 0.878 mg/g of thiamine, respectively. Riboflavin (vitamin B2) helps release energy from foods and is essential for healthy eyes, skin, nails, and hair.[34] The symptoms of vitamin B2 deficiency are local inflammation of mucosal membranes and disturbances in the functioning of the nervous system.[35] *E. divaricata* and *E. pygmaea* contains 0.989 and 0.967 mg/g of riboflavin. *E. divaricata* and *E. pygmaea* was found to contain 0.567 and 0.222 mg/g of pyridoxine (vitamin B6). It helps to form red blood cells and is needed for metabolism, normal reproductive process, and healthy pregnancies.[36] Niacinamide is active in preventing the disease pellagra,[37] niacinamide deficiency

may cause neurological and skin problems.[24] *E. divaricata* and *E. pygmaea* was found to contain 0.089 mg/g and 0.309 mg/g of niacinamide. It is lesser (0.089%) in *E. divaricata* than when compared to *E. pygmaea* (0.3%).

CONCLUSION

It can be concluded that the data obtained in the present work revealed that the physicochemical standards were generally used for deciding the identity, purity and strength of the drug. The heavy metal content and microbial load were found to be well within the limit; the absence of pesticide and aflatoxins indicates both plants found to be free from such contamination and safe for use. Both plants were scientifically validated for nutraceutical composition. They contain fat, protein, carbohydrates, and vitamins such as thiamine, riboflavin, pyridoxine and niacinamide, elements such as calcium, magnesium, potassium, sodium, phosphorus, and zinc. These plants have a good source of nutrients. The data obtained from all the above study would be useful in the identification of these *Epaltes* species and serve as standards.

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