Comparative Pharmacogonistic Studies of Genuine and Commercial Samples of Trianthema Decandra Linn.

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ABSTRACT: Trianthema decandra Linn. (Fam. ficoidaceae) Commonly known as “Vellai sharunai” in Tamil and Punaranavi in Sanskrit the roots are used in hepatitis, asthma and suppression of menses. Genuine and three commercial samples of R. Decandra in have been compared pharmacognostically for the first time in the present investigation all the pharmacognostic findings reveal adulteration in the commercial samples.

INTRODUCTION

Trianthema decandra Linn. (Fam. Ficoidaceae) commonly known as “Vellai sharunai” in Tamil and ‘Punarnani’ in Sanskrit is commonly used in the siddha and Ayurvedic systems of medicine. It is a prostate weed with branches up to 2 m long distributed in South India and the Deccan, Gujarat, Rajasthan and Uttar Pradesh extending to Haryana. The leaves are eaten during the time of scarcity. The root is used in hepatitis, in asthma and suppression of the menses (Chopra, 1966). The decoction of the root bark is credited with aperient properties. In orchitis, the root is ground with milk and administered. The juice of the leaves is dropped into nostrils to relieve partial head –ache (Anonymous, 1976) the plant has been subjected to the study of abortifacient (Jamwal and Anand, 1962). The zinc and cooper content of the plant has been found out by atukoralia and waidyanatha (1987). The plant as veterinary medicine has also been reported (Reddy and sundarasasam, 1987). Recently the chemistry of medicinal plants of genus Trianthema as been reported (Ashraf and Mohammed, 1993). Even in that report no information, regarding the chemistry of T. decandra has been provided.

Trianthema decandra Linn. Is commonly sold as ‘Sharunai ver’ in the markets of Tirunelveli Kattabomman, V.O. Chidambaranar and kanyakumari Districts. But according to chopra et al. (1966), Trianthema protulacastrum Linn. is also called ‘ Sharunai’ in Tamil Nadu. Both T. decandra and T. Protulacastrum are known as ‘Punarnani’ in Sanskrit. Also T. portulacastrum closely resembles Boeraavia diffusa Linn. (Fam Nyctaginaceae), which is called ‘Punarnava’ in Sanskrit. So there is every possibility of adulteration of one plant with another because of the confusion in names. Hence in the present investigation the genuine and three commercial samples of T. decandra have been compared pharmacognostically for the first time to confirm the authenticity of the genuine sample ant to check up adulteration if any in the commercial samples.
MATERIALS AND METHODS

Fresh authentic specimen of *Trianthema decandara* Linn. Was collected from kilapavoor village, 8 km away from courtallam hills of Western Ghats of south India during January, identified by Prof. L Henry joseph, Department of Botany, St. Xavier’s College, playamlottai, Tirunelveli kattabomman district. Commercial samples were purchases from three leading pharmacies (Nattu Marundhu kadai) in Tirunelveli town (Tirunelvli Kattabomman District), Alwarthirunagari (V.O. Chidambaranar District), Nagercoil (Kanyakumari district) for comparison with genuine sample and were labeled as CS I, CS II and CS III respectively.

Free hand sections were taken, fixed in 70% ethanol, stained with safrannin and tannic green and mounted following the usual plant micro technique (Johansen, 1940). The microscopic photographs were taken by fitting pentax K. 1000 camera (Japan) in trinocular research microscope, LABO TRIUMPH PM -3.Fluorescence analysis of the root powders were carried out according to chase and Pratt (1949). The ash and extractive value were determined according to Pharmacopoeia of India (Anonymous, 1966). The air-dried root powders were successfully extracted with petroleum ether (40-60oC), benzene, chloroform, ethanol and water and the extracts were used for phytochemical analysis according to Brindha et al. (1981). Thin layer and paper chromatographic studies of various extracts were also performed. For TLC studies, Silica Gel G for TLC (Messers BDH Ltd., India) was used and for PC studies, Whatman No.1 filter paper was used. The plates were activated at 110oC for one our and developed in iodine chamber. The fluorescence sports were located using UV-Vis viewing cabinet (Superfit, India) (365 nm).

RESULTS

Macroscopic characters

*Trianthema decandara* Linn. Is a prostrate herb, branchlets, glabreanct (without hairs), Leaves: oblong or ovate-elliptic, 1.5-2.5 x0.7-2 cm, subsucculent, papillose, base acute, margin entire, apex obtuse or rounded; petiole to 7 m, dilated amplexcaul. Flowers in submbellate clusters, bracteoles, linear, pedicel 1 to 2 mm not enclosed in petiole, Calyx tube obconical, 2 mm, sepals 5, oblong obovate, subequal green without, pink within, margin scarious, apex mucronate corolla free, pink, stamens 10 unequal, filaments 1.5-2mm pink. Ovary terete, 2 celled, styles 2, filiform. Capsule substerete, beak 2 lobed; seeds:2 in the beak, 2 in the basal part, concentrically ribbed. These macroscopic characters are presented in Fig 1.

MICROSCOPIC CHARACTERS

Root

The transverse section of the root shows the following characteristics: Epidermis is single layered with unicellular root hairs Periderm is 4-5 layered. It consists of cork cambium, cork and secondary cortex. Cortex is parenchymatous. Root trace is seen in the cortex region. Endodermis is distinct and made up of compactly arranged barrel shaped cells wit casparian strips on the radial walls, pericycle is sclerenchmatous. Vascular tissues are polyarch, xylem radial; vascular bundles. Xylem s exarch. Pit is absent. Anomalous secondary growth is seen on the roots.

These microscopic characters of the root are presented in Fig 2a and 2b.
**Stem**

The transverse section of the stem shows the following characters:

Epidermis is single layered and cutinized with epidermal hairs; consist of compactly arranged rectangular cells. Cortex is parenchymatous. Endodermis is single layered and compactly arranged with barrel shaped cell. Casparian strips are seen in the radial walls. Pericycle is 2-3 layered, made up of sclerenchymatous cells. Vascular bundles are arranged in the form of distinct ring embedded in parenchymatous ground tissues. Secondary vascular tissues are seen in the stem. Xylem consist of the cells with bordered pits and simple perforations embedded in ground tissues. Xylem also consists of tracheids and xylem parenchyma. In between secondary xylem and secondary phloem, cambium is seen. Primary phloem usually gets crushed medullary rays are absent. Pith is very large and parenchymatous. Crystals in the form of raphides are seen in the pith region. These microscopic characters of the stem are presented in Fig 3a, 3b and 3c.

**Leaf**

The transverse section of the leaf shows the following characters:

Both upper and lower epidermis consist of parenchymatous cells which are bladder like cells covered by a thin flat continuous layer of cuticle. In the layer epidermis, stomata are frequently seen and they are renunculaceous type. Mesophyll is centric. It consists of large amount of palisade cells and acous tissue. Vascular bundle is located at the centre. Each vascular bundle is located at the centre. Each vascular bundle is surrounded by bundle sheath. The vascular bundles are collateral and endarch. Solitary crystals are seen in the epidermis and mesophyll cells. These microscopic characters of the leaf are presented in Fig 4a, 4b and 4c.

**FLUORESCENCE ANALYSIS**

The genuine and commercial samples of the root powders of *Trianthema decandra* Linn. And the various extracts were examined under UV light (365nm) and also under ordinary light. The root powders were also treated with various reagents and the change in colour as noticed as described case and pratt (1949). These fluorescence characters are recorded in Table-1.

**ASH AND EXTRACTIVE VALUES**

The average value of loss weight on drying, total ash, acid insoluble ash, water soluble ash and the residue on ignition were determined. The extractive values of the root samples in petroleum ethanol (40-60°C), benzene, chloroform, ethanol and water were determined as described in pharmacopoeia of India (Anonymous 1966). The results are presented in Table – 2.

**Phytochemical analysis**

50 gms of air dried root powders of both genuine and commercial samples were separately extracted successively with petroleum ether (40-60°C), benzene, chloroform, ethanol and water and the extracts were tested for various organic compounds such as steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannin, xanthoproteins, anthranquinones and aromatic acids as described by brindha et al and the findings are recorded in Table – 3.
Thin Layer and paper chromatography

Thin layer chromatographic behavior of the petroleum ether (40-60°C), benzene, chloroform and ethanol extracts and paper chromatographic behavior of the water extract of the root powders in the best solvent system are tabulated in Table-4.

DISCUSSION

Fluorescence analysis of both genuine and three commercial samples of the root of Trianthema decandara Linn. Exhibited a characteristic greenish yellow fluorescence when viewed under UV light (365 nm). The fluorescence characters of the extract were almost same in both the genuine and commercial samples. There was not much difference in the total ash values, residue on ignition values and petroleum ether (40 – 60°C), benzene, chloroform extractive values of genuine and CS II and CS III. But CS I has about 1.8% less total ash content and 1.6% less residue on ignition than genuine sample. But variations were observed in acid-insoluble ash, water soluble ash, ethanol and water extractive values between genuine and commercial however among the commercial samples there was a good agreement. Generally the physico-chemical characters of CS III showed the resemblance with those of the genuine sample. Preliminary phytochemical screening of both the genuine and commercial sample indicated the presence of similar type of chemical constituents in both (Table -3). Thin layer and paper chromatographic behaviour of genuine and commercial samples showed almost the same number of spots with similar RF values when viewed in an iodine chamber except one or two additional spots for examples 0.45 obtained in the genuine sample was missing in all the three commercial samples. But a new spot which was absent in the genuine samples was observed at RF values 0.13 and 0.22 in the case of CS II and CS III respectively. In the case of benzene extract, the spot at Rf value 0.75 was missing in CS III. In the case of chloroform extract the sport at Rf value 0.60 present in the genuine sample was missing in all the three commercial sample. In the case of ethanol extract, the spot at Rf value 0.42 in the genuine sample as absent in CS II and CS III. In the case of aqueous extracts, there appeared moderately intense spot at Rf value 0.07 in the genuine samples which was absent in all the three commercial samples however, CS I and CS II showed moderately intense spot at RF value 0.52 which was absent in the genuine and CS III sample. It as been discussed already that there is a confusion in the local names between Trianthema decandara Linn. (Vellai sharunai) and Trianthema portulacastrum (Vatta sharunai). Hence there is every possibility of adulteration of one sample with the other. Moreover the three different localities from which the commercial samples have been collected present great difference in the soil, climatic conditions rainfall and also altitudes. It is therefore expected that the variations result in differences in both physical and chemical properties of these samples. The difference in the physico-chemical characters and chromatographic behavior were more quantitative and such variations are naturally possible as the samples are coming from different sources of collection. Hence it can be concluded that the commercial samples are adulterated.
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LEGENDS FOR THE FIGURES PROVIDED

Fig 1. Macroscopic characters (photographic) of the *Trianthema decandara* Linn

Fig 2a. T.S (Photographic) of the root of *T.decandara* L x100

Fig 2b. portion of the TS (Photographic) of the root sowing cortex, endodermis, pericycle and vascular bundles. x 400

Fig 3a. T.S (Photographic) of the stem of *T.decandara* L x100
Fig 3b. portion of the TS (Photographic) of the stem sowing epidermis, Cortex, vascular bundles. X 100

Fig 3c. portion of the TS (Photographic) of the stem sowing Xylem and phloem. x 400

Fig 4a. T.S (Photographic) of the leaf of T.decandara L x 40

Fig 4b. T.S (Photographic) of the mid rib portion of the leaf of T.decandara L x100

Fig 4c. T.S (Photographic) of the Blade portion of the leaf of T.decandara L x100
Table -1 Fluorescene characters of genuine and commercial samples of Trianthema decandra root powder and their extracts in different solvents

| Particular of treatment | Under ordinary light | Under UV light (365 nm) |
|-------------------------|----------------------|------------------------|
|                         | Genuine              | (Commercial) Palayamkottai | (Commercial) Alwarthirunagari | (Commercial) Nagercoil | Genuine              | (Commercial) Palayamkottai | (Commercial) Alwarthirunagari | (Commercial) Nagercoil |
| Powder as such          | Pale yellow          | Pale yellow             | Pale yellow                  | Pale yellow           | Greenish yellow     | Greenish yellow         | Greenish yellow             | Greenish yellow           |
| Powder + IN NaOH (aqueous) | Pale brown          | Dark brown              | Reddish brown                | Reddish brown        | Pale green          | Greenish yellow         | Greenish yellow             | Greenish yellow           |
| Powder + IN NaOH (ethanolic) | Yellow at edge pale brown at centre | Brownish yellow         | Pale brown                   | Yellow at edge green at centre | Yellowish green     | Yellowish green         | Yellowish green             | Yellowish green           |
| Powder + IN HCl         | Yellow               | Pale reddish brown      | Pale reddish brown           | Pale reddish brown   | Pale reddish brown | Yellow at edge brown at centre | Brown                   | Dark brown               |
| Powder + 1:1 H2 SO4     | Pale brown           | Pale reddish brown      | Pale reddish brown           | Pale reddish brown   | Dark green          | Brown                   | Dark brown               | Brown                   |
| Powder + 1:1 HNO3       | Reddish brown        | Red orange              | Red orange                   | Red orange           | Brown               | Brown                   | Dark brown               | Brown                   |
| Extracts                |                      |                        |                            |                    |                    |                        |                        |                        |
| a) petroleum ether (40o-60oC) | Yellowish green      | Yellowish green         | Pale brown                  | Pale brown           | Yellow              | Yellow                  | Yellow                  | Yellow                  |
| b) Benzene              | Yellowish brown      | Yellowish brown         | Pale brown                  | Pale brown           | Pale brown         | Yellow at centre pale pink at edge | Yellow                | Greenish yellow         |
| c) Chloroform           | Yellowish brown      | Yellowish brown         | Pale brown                  | Pale brown           | Yellowish green     | Yellow                  | Greenish yellow         | Greenish yellow         |
| d) Ethanol              | Yellowish brown      | Yellowish brown         | Pale brown                  | Pale brown           | Lemon yellow        | Yellow                  | Greenish yellow         | Greenish yellow         |
| e) Water                | Yellowish brown      | Yellowish brown         | Pale brown                  | Yellowish brown      | Pale yellow         | Greenish yellow         | Greenish yellow         | Greenish yellow         |
Table 2: Physicochemical characters of the root of genuine and commercial samples of Trianthema decendra

| Particulars                   | Genuine sample (%) | Commercial sample          |
|-------------------------------|--------------------|-----------------------------|
|                               | Palayamkottai      | Alwarthirunagari | Nagercoil |
| Total ash                     | 8.45               | 6.60                       | 8.02      | 8.60     |
| Acid insoluble ash            | 3.72               | 1.11                       | 1.33      | 1.28      |
| Water soluble ash             | 2.73               | 1.95                       | 1.25      | 1.80      |
| Residue on ignition           | 7.30               | 5.70                       | 7.70      | 7.42      |

**Extractive values**

|                   | Palayamkottai | Alwarthirunagari | Nagercoil |
|-------------------|---------------|------------------|-----------|
| Petroleum ether   | 4.04          | 4.12             | 4.40      | 4.35      |
| (40°C-60°C)       |               |                  |           |           |
| Benzene           | 2.48          | 2.88             | 2.74      | 2.78      |
| Chloroform        | 8.36          | 8.48             | 8.88      | 8.60      |
| Ethanol           | 14.52         | 18.32            | 16.84     | 17.40     |
| Water             | 16.88         | 21.28            | 23.00     | 22.20     |
| Extracts                      | Samples | Colour | Steroids | Triterpenoids | Reducing sugars | Aromatic acids | Alkaloids | Phenolic compounds | Anthraquinones | Saponins | Xanthoproteins | Tannins | Flavonoids |
|------------------------------|---------|--------|----------|---------------|----------------|---------------|-----------|-------------------|----------------|----------|----------------|---------|-----------|
| petroleum ether (40°-60°C)    | G       | -      | +        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS1     | -      | +        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS2     | -      | +        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS3     | -      | +        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
| Benzene                      | G       | -      | -        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS1     | -      | -        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS2     | -      | -        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS3     | -      | -        | -             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
| Chloroform                   | G       | -      | +        | +             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS1     | -      | +        | +             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS2     | -      | +        | +             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
|                              | CS3     | -      | +        | +             | +              | +             | +         | -                 | -              | -        | -              | -       | -         |
| Ethanol                      | G       | -      | -        | +             | +              | +             | -         | -                 | -              | +        | +              | +       | -         |
|                              | CS1     | -      | -        | +             | +              | +             | +         | +                 | +              | -        | +              | +       | -         |
|                              | CS2     | -      | -        | +             | +              | +             | +         | +                 | +              | -        | +              | +       | -         |
|                              | CS3     | -      | -        | +             | +              | +             | +         | +                 | +              | -        | +              | +       | -         |
| Water                        | CS1     | -      | -        | +             | +              | +             | -         | -                 | +              | +        | +              | +       | -         |
|                              | CS2     | -      | -        | +             | +              | +             | -         | -                 | +              | +        | +              | +       | -         |
|                              | CS3     | -      | -        | +             | +              | +             | -         | -                 | +              | +        | +              | +       | -         |

G= Genuine; CS1 = Palayamkotta; CS2 = Alwarthirunagari; CS3 = Nagercoil
# Table - 4 Thinlayer and paper chromatographic behavior of the root extract of genuine and commercial samples of Trianthema decandra

| Name of the extract | Name of the solvent system used | Genuine sample | Commercial sample |
|---------------------|---------------------------------|----------------|-------------------|
|                     |                                 | Rf value in iodine camber | Rf value under UV light (365 nm) | Rf value in iodine camber | Rf value under UV light (365 nm) |
|                     |                                 | CS1 | CS2 | CS3 | CS1 | CS2 | CS3 | CS1 | CS2 | CS3 | CS1 | CS2 | CS3 |
| petroleum ether (40o-60oC) | Chloroform: Benzene (9:1)       | 0.42 | 0.54 | -   | 0.42 | 0.13 | 0.22 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.95 | -   | -   | 0.95 | 0.42 | 0.42 | -   | -   | -   | -   | -   | -   |
| Benzene             | Chloroform: Benzene (4:1)       | 0.09 | 0.20 | -   | 0.09 | 0.20 | 0.20 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.75 | 0.98 | -   | 0.75 | 0.98 | 0.98 | -   | -   | -   | -   | -   | -   |
| Chloroform (100%)   | Chloroform (100%)               | 0.09 | 0.27 | -   | 0.09 | 0.40 | 0.98 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.60 | -   | -   | 0.72 | 0.74 | 0.98 | -   | -   | -   | -   | -   | -   |
| Ethanol (100%)      | Benzene (100%)                  | 0.11 | 0.42 | -   | 0.11 | 0.68 | 0.72 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.17 | 0.75 | -   | 0.17 | 0.94 | 0.72 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.27 | 0.94 | -   | 0.27 | 0.94 | 0.72 | -   | -   | -   | -   | -   | -   |
| Water               | n-Butanol Aceticacid Water (4:2:1) | 0.09 | 0.70 | -   | 0.09 | 0.52 | 0.94 | -   | -   | -   | -   | -   | -   |
|                     |                                 | 0.94 | -   | -   | 0.94 | 0.94 | 0.94 | -   | -   | -   | -   | -   | -   |

For water extract paper chromatographic studies have been performed o= Less intense o= More intense = Moderately intense CS1 = Palayamkottai; CS2 = Alwarthirunagar; CS3 = Nagercoil.