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Research Article

# PHYSICOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *TAVERNIERA CUNEIFOLIA* (ROTH.) ARN. – A POTENTIAL SUBSTITUTE OF *GLYCYRRIZHA GLABRA* L.

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**ABSTRACT**

The present communication evaluates the physicochemical and preliminary phytochemical properties of *Taverniera cuneifolia* (Roth.) Arn. The roots of *T. cuneifolia* has been considered as a potential substitute of *Glycyrrhiza glabra*, popularly known as Indian Licorice. However, there is no detailed standardized work done so far. The results revealed that the concentration of all the heavy metals were below the WHO/FDA permissible limits. *Escherichia coli, Salmonella Spp.* were found to be absent. Total ash value content was 6.75 %, water soluble ash was 2.61% and sulphated ash was 3.02%. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicated the presence of phenols, alkaloids, steroids, glycosides, flavonoids.

**Keywords:** *Taverniera cuneifolia*, *Glycyrrhiza glabra,* Licorice, Heavy metals, Physicochemical studies, Phytochemical qualitative test.

## INTRODUCTION

Since origin of human's life plants continue to play a curative and therapeutic role in preserving human health against diseases[1]. Today we find a renewed interest in traditional medicines. Traditional ecological knowledge is associated with biodiversity research, bioprospecting, and cultural conservation[1,2,3,4]. In this context, India being a subtropical country is a good repository of plants that are widely used in the preparation of herbal therapies.

In this communication one such plant is selected which is traditionally used by the tribal's of Saurashtra region, in Gujarat. The genus *Taverniera* belongs to the family of Fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries[1]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *G. glabra*[2]. *T. cuneifolia* is locally known as Jethimadh and it is used by the tribal's of Barda Hills of Jamnagar in Western India. It is used as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra*[2]. It is traditionally known to be used as an expectorant, blood purifier, anti-inflammatory, wound healing, antiulcer and in treating spleen tumors[2].

*T. cuneifolia* is a much branched undershrub, 1-2 ft high. Leaves 1-3 foliate, stipules scarious, triangular, acute, deciduous one which is opposite the leaf. Leaflet obovate, thick glaucous, mucronulate. Flowers axillary, 2-6 flowered raceme longer than the leaves. Calyx finely pubescent, teeth triangular, acute. Corolla purplish pink in colour, standard obovate-orbicular, slightly longer than keel, glabrous, veined with dark purple parallel veins, emarginate, pods with 1-2 seeded joints, joints ovoid, echinate[2].

## MATERIALS AND METHODS

### Collection and processing of plant samples:

The plant material was collected from Munjka village, Near Saurashtra University Campus, Rajkot and Rosy port area, Jamnagar, Gujarat, India. The plant material was authenticated at BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Ref.no. BSI/AZC I.12012/ Tech./2011-12 (PL.ID)-55. The shade dried roots were used for the investigation of qualitative phytochemical test, physicochemical tests and microbial contamination.

### Qualitative Phytochemical Tests

### Procedure for HPTLC Analysis

1g of plant sample was accurately weighed, placed in a stoppered tube and 10 ml of methanol (solvent) was added, vortexed for 2 min and left to stand overnight at room temperature (28±2ºC). The contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used as stock solution for further analysis. Same procedure was applied for other solvents like chloroform, ethyl acetate, toluene as well.

### Sample Volume

10 µl of methanol, chloroform, ethyl acetate and toluene extract was spotted as track1 (T1), track 2 (T2), track 3 (T3) and track 4 (T4) respectively.

### Chromatographic Conditions

### Instrument

CAMAG HPTLC system comprising of Linomat IV Spotter, Scanner II, CAMAG CATS 3 software. TLC Plate used was Silica gel 60 F254. Mobile Phase employed was n-butanol: Acetic acid: Water (7: 1: 2). The saturation time was 30 min. Detection wavelength was UV-254 nm and UV-366 nm

### Derivatising agent

Liebermann Burchard Reagent - 5 mL acetic anhydride + 5 mL Conc. $H_2SO_4$ + 50 mL Absolute Ethanol (prepared under cooling ice).

**All the tests were performed according to the following test[2]:**

| Alkaloids | Dragendorff's test |
|---|---|
| Flavonoid | Shinoda test |
| Tannin | Neutral $FeCl_3$ |
| Protein | Biuret test |
| Carbohydrate | Molisch's test |
| Reducing sugar | Fehling's test |
| Saponin | Foam test |
| Resin | Acetic anhydride test |
| Phenol | Neutral $FeCl_3$ |
| Terpenoids | Leibermanburchard test |

**The physiochemical and microbiological tests were done as follows[3,4]:**

| Parameters | Test method |
|---|---|
| **Physicochemical tests** | |
| Water soluble extractive | IP 2007 |
| Alcohol soluble extractive | IP 2007 |
| Moisture content (By KF) | IP 2007 |
| Ash content | IP 2007 |
| Acid insoluble ach | IP 2007 |
| Water soluble ash | IP 2007 |
| Sulphated ash | IP 2007 |
| Swelling index | IP 2007 |
| **Heavy metals** | |
| Lead | API 2008 |
| Cadmium | API 2008 |
| Arsenic | API 2008 |
| Mercury | API 2008 |
| **Microbiological tests** | |
| Total plate count | API 2008 |
| Total fungal count | API 2008 |
| *Escherichia coli* | API 2008 |
| *Pseudomonas aeruginosa* | API 2008 |
| *Staphylococcus aureus* | API 2008 |
| *Salmonella Spp.* | API 2008 |

**(ABR.: IP** – Indian Pharmacopoeia, **API** – Ayurvedic Pharmacopoeia of India.)

**RESULTS AND DISCUSSION**

Roots of *Taverniera cuneifolia* (Roth) Arn. were collected and analysed for the various parameters. Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the drug. The tests were performed using methanol, water and chloroform extracts. Phytochemical test revealed the presence of alkaloid, flavonoid, tannin, protein, reducing sugar and saponin. The results are given in the Table 1.

The presence of four heavy metals namely Lead, Cadmium, Arsenic, Mercury and microbial contamination were analysed in the sample and the results are shown in the Table 2. The concentrations of all the heavy metals were below the WHO/FDA[2,3] permissible limits. In microbial contamination *Escherichia coli, Salmonella Spp.* were absent and *Pseudomonas aeruginosa, Staphylococcus aureus* were found to be present. Physicochemical parameters of the root of *T. cuneifolia* are depicted in Table 2. The moisture content of the root was found to be 4.48%. Total ash value of the plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value content was 6.75%. The negligible amount of acid insoluble siliceous matter present in the root was 1.23%. The water soluble ash was 2.61% and sulphated ash was 3.02%. The water soluble extractive value indicated the presence of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The results are given in Table 2.

**Table 1: Preliminary phytochemical qualitative test for roots of *T. cuneifolia*.**

| Natural Product | Test Performed | Result |
|---|---|---|
| Alkaloids | Dragendorff's test | + |
| Flavonoid | Shinoda test | + |
| Tannin | Neutral $FeCl_3$ | + |
| Protein | Biuret test | + |
| Carbohydrate | Molisch's test | - |
| Reducing sugar | Fehling's test | + |
| Saponin | Foam test | + |
| Resin | Acetic anhydride test | - |
| Phenol | Neutral $FeCl_3$ | - |
| Terpenoids | Leibermanburchard test | - |

**Table 2: Physicochemical parameters of roots of *T. cuneifolia***

| Parameters | Result |
|---|---|
| **Physicochemical Tests** | |
| Water soluble extractive | 17.19 % |
| Alcohol soluble extractive | 14.98 % |
| Moisture content (By KF) | 4.48 % |
| Ash content | 6.75 % |
| Acid insoluble ach | 1.23 % |
| Water soluble ash | 2.61 % |
| Sulphated ash | 3.02 % |
| Swelling index | Absent |
| **Heavy Metals** | |
| Lead | Not Detected |
| Cadmium | 0.1021 ppm |
| Arsenic | Not Detected |
| Mercury | Not Detected |

| | |
|---|---|
| **Microbiological Tests** | |
| Total plate count | 178 x 102 cfu/g |
| Total fungal count | 17 x 102 cfu/g |
| *Escherichia coli* | Absent |
| *Pseudomonas aeruginosa* | Present |
| *Staphylococcus aureus* | Present |
| *Salmonella Spp.* | Absent |

**ABR.: IP** – Indian Pharmacopoeia, **API** – Ayurvedic Pharmacopoeia of India, **ppm** – parts per million, cfu – colony forming unit.

Thin layer chromatographic technique was used to separate the chemical compounds present in the drug. Various solvent systems were used to separate the maximum number of chemical compounds in the drug.

TLC of the methanol extract developed in the mobile phase of Butanol: Acetic acid: Water (7: 1: 2) (Figure.1) and observed 9 spots at Rf value 0.18, 0.21, 0.26, 0.28, 0.32, 0.42, 0.53, 0.57, 0.76.

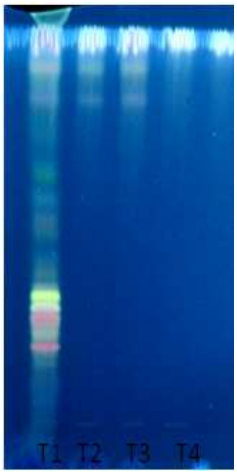


**Fig. 1: TLC profile of the root of *T. cuneifolia* taken in different extracts:**

T1- Methanolic extract, T2- Chloroform extract, T3- Ethyl acetate extract,

T4- Toluene extract (Under UV 366nm).

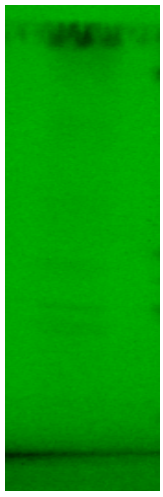

**Fig. 2: TLC profile of the roots of *T.cuneifolia* (Under UV 254nm)**

## CONCLUSION

Phytochemical, physicochemical, toxic heavy metal analysis, microbial contaminants of the root sample along with TLC studies was done for the authentication of quality control of raw drugs. Roots of *Taverniera cuneifolia* exhibits a set of characters, which would help to identify the drug in dried condition.

It has been concluded from this study that estimation of heavy metals and microbial contamination is highly essential for raw drugs or plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safer use of herbal drugs.

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