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QUALITATIVE ASSAY OF AMINO ACIDS AND AMIDES IN SEEDS AND SEEDLINGS OF *TEPHROSIA PURPUREA* IN VINDHYAN EGION

Rashmi Arnold*

Department of Botany, Govt. Model Science College, Rewa, M.P.

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***Correspondence for Author**
Rashmi Arnold
Department of Botany, Govt. Model Science College, Rewa, M.P.

ABSTRACT

Tephrosia purpurea is a plant found in open spaces in rocky and barren grounds a weed. The plant *Tephrosia purpurea* (L) Pers is an important source of medicinal drugs and has a well recognized place in the indigenous system of medicine. It has been mentioned in both the Ayurvedic and Unani systems of medicine. Tephrosia L. or Sarpunkha is a wild legume belonging to family Leguminosae (Sub family-Papilionaceae) and grows throughout India and Western Himalaya. The seeds of *Tephrosia purpurea* showed strong dormancy. The reason of the dormancy in the seeds is due to the yellowish pigment with a strong odour given out by the seed coat when the seed comes in contact with moisture. Seed germination initiated by hydration and activation of enzymes, is accompanied by breakdown of reserve food material, its transportation resynthesis and utilization before new substances are synthesized on account of Photosynthesis. Amino acids, as constituents of proteins play the most important role in these changes. Chromatographic methods are generally followed to assess such changes.

KEY WORDS: *Tephrosia purpurea*, seeds and seedling, chromatographic methods, etc.

INTRODUCTION

The genus Tephrosia L. or Sarpunkha is a wild legume belonging to family Leguminosae (Sub family-Papilionaceae) and grows throughout India and Western Himalaya. The plant also relieves dental pain, asthma, leprosy, arrests bleeding. According to Ayurveda, this plant is digestible, anthelmintic, alexiteric, and antipyretic. It is alternative cure for the diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma, poisoning etc. Leaves are tonic to intestines and a promising appetizer.[1,2,3]

The constituents of Tephrosia include alkaloids, saponins, glycosides, Tannins, flavonoids etc. Some of the constituents may have direct activity and the other inert substances may increase bioavailability, reduce toxicity, or stimulate the action via a synergistic activity. Sometimes the isolated constituent- containing preparation may be stronger than the whole plant.[4,5]

Tephrosia purpurea containing formulations are prescribed in ayurveda mainly as liver correctives and restoratives. They contain aqueous or alcoholic extracts of *Tephrosia purpurea*. They are found to be effective in treating various disorders like, alcoholic liver, cirrhosis, viral hepatitis, pre-cirrhotic condition, protein energy malnutrition and radiation etc.[6,7,8]

According to Ayurveda literature this plant has also given the name of "Sarwa wranvishapaka" which means that it has the property of healing all types of wounds. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders. In Ayurvedic system of medicine various parts of this plant are used as remedy for impotency, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders. The plant has been claimed to cure diseases of kidney, liver spleen, heart and blood. The dried herb is effective as tonic laxative, diuretics and deobstruents. It is also used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles.[9,10]
R Zafar *et al* were successfully developed and maintained the root, stem and leaf calli of *Tephrosia purpurea* L. pers on Murashinge and Skoog's medium Supplemented with various plant growth regulators. The content of rotenoids and rutin in the callus cultures were estimated by spectrophotometric method.[11] Sinha *et al* isolate an isoflavone, 7,4,,-dihydroxy-3,,,5,,-dimethoxyisoflavone, and a chalcone, (+)-tephropurpurin, both novel compounds, as well as six constituents of known structure, (+)-purpurin, pongamol, lanceolatin B, (−)-maackiain, (−)-3-hydroxy-4-methoxy-8,9-methylenedioxypterocarpan, and (−)-medicarpin, were obtained as active compounds from *Tephrosia purpurea*, using a bioassay based on the induction of quinone reductase (QR) activity with cultured Hepa 1c1c7 mouse hepatoma cells. Additionally, three inactive compounds of known structure, 3,,-methoxydaidzein, desmoxyphyllin B, and 3, 9-dihydroxy-8-methoxycoumestan, were isolated and identified. The structure elucidation of compounds 1 and 2 was carried out by spectral data interpretation.[12]

The area of the present work extends through various startigraphic terrains belonging to a wide span of geological events. The geological age of the various rocks that occur within the periphery of the region some of these rocks are ranging in age from 2500 m.yr. or more while the rocks of volcanic origin formed nearly 60 m.yr. ago are also found. Thus, almost all the important stratigraphic columns of the Indian shield are represented here. Rocks of the Vindhyans super group occur in all the four districts of the area of investigation. It is interesting to note that the first survey made to identify the black formations (Vijayagarh shale) west of the Sone river valley which were until then supposed to be coal seams. Oldham (1856) carried out a systematic study and grouped the rocks in "Vindhyan system". A lot of work has been done on the various aspects of these rocks over the past fourteen decades.

The geology of the region which encompasses four districts is as follows.

Era Recent	Alluminium Laterite	Shahdol, Sidhi Rewa , Satna
Mesozoic/ Early Tertiary/ Palaeozoic	Deccan Traps Lametas Upper Gondwana	Shadol Shadol Shadol
Early/ Palaezoic	Upper vindhyans Lower Vindhyans	Rewa, Satna Shahdol Sidhi, Shadol
Precambrian/ Purana Archaean	Bijawar Crystalline complex Greisses & Schists	Sidhi, Shahdol Sidhi Shahdol

MATERIALS AND METHODS

Biochemical Studies of Seed and Seedling

Treated seeds and seedlings were subjected to biochemical analysis. The analysis of amino acids was carefully done in the present study.

1. Separation and Identification of Amino Acids (Extraction of soluble fractions)

Method outlined by Steward *et al*., 1954 was followed.[13] Presoaked seeds and germinated seedlings were separately boiled in 80% ethyl alcohol for 10-15 minutes. Prolonged heating was avoided lest some nitrogenous compounds should be destroyed. Alcohol was decanted and materials were finally grounded with the help of a glass mortar and pestle. The grounded material was quantitatively transferred to the original alcoholic solution with alcohol washings. This was again boiled for five minutes with occasional stirring and filtered. The alcohol extract was kept in evaporating dishes. The residue was re-extracted following the same process. The two filtrates which contained amino acids and amides were evaporated to

dryness on a water bath and the residue was dissolved in 20% ethanol to make the final solution of one gm. fresh weight of the material per ml.

The solution was centrifuged at 2000 rpm for 30 minutes to remove precipitated solution protein in 20% alcohol. The clean liquid was decanted and kept in small sterilized bottles for use of chromatographic analysis of amino acids and amides.

2. Chromatographic Separation of Amino Acids and Amides

The method outlined by Cousden *et al.*, 1944 was followed to obtain a complete resolution of different amino acids and amides.[14] Whatmann No.1 special grade chromatographic filter papers of 28×28 cm were used and the marker point was spotted at a distance of 3 cm from the adjacent edges of the filter papers. A 0.025 ml of 80% alcoholic solution fraction was applied at the sport with the help of a micro pipette using an infra-red lamp for quick drying. The spotted filter papers were hung in the chromatographic chamber with its lower edge just dipping in the solvent mixture. Stainless Steel clips were used to hold the filter paper on the glass rod. Care was taken not to allow the edge of the filter paper to touch either the base or the sides of the solvent dish. The first running solvent mixture was used as a Partridge's solvent (1948) modified by fowden (1954), consisting of phenol (80%) saturated with 0.5% (Wt/vol.) ammonia and water in proportion 80: 1.5: 20. When the solvent had traversed up to the edges of chromatographic paper after 12 to 14 hrs run, the chromatographic paper was removed from the chamber and hung at room temperature (25^0C-1^0C) for two days to remove excess of phenol.[15, 16]

The dried chromatograms were rotated at right angles towards the side of marker spot and were developed in the second solvent system of N-Butenol: acetic acid: water 4: 1: 5. The required running time was 12-14 hours. The chromatograms were removed from the chamber after the second run and hung at room temperature for 12 hours to remove the acetic acid vapors from the chromatograms. The filter papers, dry to touch, were now ready for development of amino acid and amide spots.

The location reagent used for detection of amino acids and amides was 0.1% ninhydrin in N-Butenol. Spraying was done with an all glass Shandon automizer. The chromatograms were first allowed to dry at room temperature and then warmed in a labotherm at 85^0C for 30 minutes. The spots which appeared on the chromatograms into room condition to absorb

moisture. A map already prepared using reference amino acids was used for comparison and identification of separated amino acids on the chromatograms.

RESULTS AND DISCUSSIONS

Qualitative Assay of Amino Acids and Amides in *Tephrosia Purpurea* Seeds and Seedlings

Metabolic changes at the time of germination of seed have fascinated a good number of workers in the past[17, 18, 19] Seed germination initiated by hydration and activation of enzymes, is accompanied by breakdown of reserve food material, its transportation resynthesis and utilization before new substances are synthesized and utilization before new substances are synthesized on account of Photosynthesis. Amino acids, as constituents of proteins play the most important role in these changes. Chromatographic methods are generally followed to assess such changes.[20] In present study the qualitative estimation of Amino acids was done by method of paper chromatography.

Chromatographic Separation of Amino Acid and Amides

Alcoholic extract of presoaked seeds and seedlings were prepared, the chromatograms were developed and shown in fig and table. Table-1 shows the presence of various amino acids in seed and seedlings.

Table-1 Showing presence of Amino Acids and Amides in soaked seeds and seedlings (Untreated) of *Tephrosia purpurea.*

S. No.	Name of Amino Acid and Amides	Seed (Soaked)	Seedling (untreated)
1.	Leucines	+	+
2.	Phenylalanine	+	+
3.	Valine	+	-
4.	Y aminobutyric acid	+	-
5.	Tyrosine	-	-
6.	Protine	+	-
7.	B Alanine	-	+
8.	Alanine	-	-
9.	Glutamic acid	+	-
10.	Threonine	-	-
11.	Arginine	-	-
12.	Aspartic acid	+	-
13.	Serine	-	-
14.	Glycine	-	-
15.	Asparagines	+	+
16.	Glutamine	-	-

17.	Histidine + Lysine	+	-
18.	Cysteric acids	+	+

+ = Present = Absent

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