Forensic Analysis Of Asparagus *Officinalis* And Identification Of Its Toxin By TLC

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Abstract- India is rich in flora of about 2500 species in which at least 150 medicinal species are used commercially on a very large scale. Asparagus *officinalis* is mainly consumed for its edible shoots. It has also a great value in terms of medicine. It is used as a tonic, antifebrile, antitussive, hair growth stimulator and diuretic agent. It contains several biological activities including anti fungal antiviral, anti tumor, antioxidant, cytotoxic, and molluscicide activities. An attempt has been made to identify the main toxin by chromatographic technique and review of medicinal value. The extract from Leaves and seeds were studied using two different solvents and their Rf values were calculated. For visualization of the spots, the plates were sprayed with sulphuric acid acid to develop coloured spot, then visualized under UV in the range of 254-365 nm. After the microscopic study, phytochemical study the result showed the plant contains main toxin as 1,2-dithiolane-4-carboxylic acid by TLC. In view of Forensic Criminal Investigations, the plant has not been used for Homicidal, or suicidal but mostly may be encountered in accidental cases.

Keywords- Forensic Science, Preliminary screening, Toxin, Asparagus *officinalis*, Criminal Investigations etc

INTRODUCTION

Plants, being the first medicines for human beings, have played a vital role in health care since the ancient times. Traditional plant-based medicines still exist with a great deal of importance to the people living in developing countries and also lead to new drug discovery candidates for a variety of diseases that threaten human health. The Asparagus genus has medicinal importance because of the presence of saponins, steroidal and sapogenins in various parts of the plant. Medicines have been used to cure people and indirectly occupy an important role in the socio-cultural, spiritual and medicinal are native of India and in many countries of Asia and Africa.

Fig. 01 Asparagus *Officinalis* Plant with long stem and needle nobe leaves.

Asparagus *officinalis* is also considered to be an Ayurvedic rejuvenating tonic for overall health and vitality in female. The reputed adaptogenic properties of the plant are attributed to the presence of high concentrations of saponins, known as Shatavarins. Vernacular names of the plant known to be called as Satavari, Shatamuli Toala-gaddalu or Pilli-gaddalu Chatavali Majjigegadde or Aheruballi Narbodh or atmooli Shimaishadavari or Inli-chedi Norkanto or Satawar and Khairva etc in different states of the country by the local people.

Phytochemicals present inAsparagus *officinalis*

The following phytochemicals have been detected in Asparagus *officinalis*

Curcin

Curcin is a toxic albumin belonging to a group of proteins called ribosome-inactivating proteins (RIP), which inhibit prokaryotic and eukaryotic ribosome by specific modification of the larger rRNA,

Tannins

Tannins are the phenolic substances associated with toxic and impaired nutrient absorption and antinutritional effects including reduced food/feed intake, growth retardation. Tannins possess multiple phenolic hydroxyl groups leading to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids, and polysaccharides.

Saponins

These are steroid or triterpene glycoside compounds which are present in a variety of plants. In plants, saponins may serve as anti-feed ants or help in protecting the plant against microbes and fungi. However, saponins are often bitter in taste, and thus, when present in high concentrations would reduce plant palatability in livestock.

Phytates

Phytic acid (known as inositol hexakisphosphate IP6 or phytate when in the salt form) is the principle storage form of phosphorus in most plant seeds. Inositol penta- (IP5), tetra-(IP4), and triphosphate (IP3) are also termed phytates. Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because these animals lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. Phytates also form sparingly digestible phytate–protein complexes, thus reducing the availability of dietary protein.

Lectins

Lectins are carbohydrate-binding (glyco) proteins and are ubiquitous in nature. Plant lectins when consumed by animals survive digestion in the Gastro Intestinal Tract and bind to membrane glycosyl groups of the cells lining.

Materials & Methods

Asparagus *Officinalis* samples were collected from the wild patch of Aravalli range of Bundelkhand region of Jhansi City of Uttar Pradesh (N'25°26'55" and 78°34'11"E) in the month of December. Fresh Leaves and seeds has been collected and stored at 4^0C wrapped in Aluminum foil.

Microscopic Examination

Fresh leaves of Asparagus *Officinalis* were considered for the microscopic examination. For the purpose trinocular microscope was used. Different sectional views were viewed taken from surface view of epidermis and lamina and then measured.

a.(view of leaf after staining with glyscerin)

b. (view of leaf after staining with Saffaerin)

Properties	Observation
Colour	Light Green
Shape	Nodes Linear ,fascicles like pine needles
Size	2.5cm long and 5mm Broad
Venation	Glabrous
Margin	Cordate
Taste	Characteristic, Bitter

Table 1 Microscopic Observations of Asparagus *Officinalis*

Phytomchemical Screening Methods-

Preparation of Extract –

Preparation of the Extract

Asparagus *officinalis* leaves fully dried at room temperature were grinded into powder and for it, ten grams was weighted accurately and extracted with n-hexane in a Soxhlet extraction apparatus. At the end of the extraction process, the flask containing nhexane extract was removed and n-hexane was evaporated by using a rotary evaporator. The weight of the residual extract was measured and precent yield was calculated. The same procedure was used to prepare butanol extract. These extracts were kept in refrigerator for phytochemical study. Extract yield% = W1/W2× 100 Where W1 = net wt. of powder in grams after extraction. W2 = net wt. of powder in grams taken for extraction.

Test for Alkaloids

In 5ml stock extract 2 ml HCl was added. It made acidic medium and then 1ml of dragendroff's reagent was added, which gave orange or red color precipitate. It indicated the presence of alkaloids.

Test for Amino Acid

In 2 ml stock extract 40% NaOH solution was added in a test tube. After that one drop of 1% CuSO4 solution was added in it. A blue color appeared which showed the presence of amino acids in the extract.

Test for Anthraquinones

2M HCl was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then it was cooled and filtered. The filtrate was extracted with chloroform layer which was later on separated and shaken with 10% KOH and it became pink-red which showed the presence of anthraquinones in the extract.

Test for Flavonoids

Dilute NaOH (1N) was added to one ml extract, then yellow color in plant extract appeared, and soon it became colorless, when few drops of acid (10%H2SO4) were added to it. It indicated the presence of flavonoids in the extract.

Test for Glycosides

On water bath, 1ml of extract hydrolysed with HCl for few hours and cooled at room temperature. Then to it 1 ml pyridine was added with a few drops of sodium nitropruside solution, which further made alkaline with NaOH solution. Pink to red color appeared, which indicated the presence of glycosides in the extract.

Test for Phytosterol

The extract was refluxed with solution of alcoholic KOH till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for presence of phytosterol. The residue was dissolved in few drops of dilute acidic acid; 3ml acetic anhydride was added followed by few drops of conc. H2SO4 Bluish colour
indicated the presence of phytosterol in the extract.

Test for Saponins

Extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes continuously. A formation of layer (1cm) of foam showed the presence of saponins in the extract.

Phyto-chemical analysis

Serial No	Chemical Tests	N-hexane Extract	Butanol Extract
1	Alkaloids	+	+
2.	Amino acid	+	+
3.	Anthraquinones	+	-
4.	Flavonoids	+	+
5.	Glycosides	+	+
6.	Phytosterol	-	-

Note- (+)Present and (-) Absent

Table2: Phyto-Chemical Analysis

Indentification Of Asparagusic Acid By TLC

Asparagusic acid is an organosulfur compound with the molecular formula $C_4H_6O_2S_2$ and is systematically named 1,2-dithiolane-4-carboxylic acid. The molecule consists of a cyclic disulfide functional group (a 1,2-dithiolane) with a carboxylic acid side chain. It is found in asparagus and is believed to be the metabolic precursor to odorous sulfur compounds.

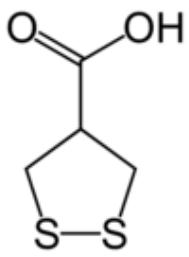

Fig.01 Asparagusic Acid (1,2-dithiolane-4-carboxylic acid)

Chromatographic Methods -Thin Layer Chromatography (TLC)

For identification of the Asparagus *officinalis* plant main toxin, TLC was prepared. Two suitable solvent system prepared for the computation of the Rf value with the help of a measuring scale. Here the extracted leaves of Asparagus *officinalis* was used as solute to identify the 1,2-dithiolane-4-carboxylic acid and for study and identification two different solvents were used.

TLC Data

Sr. No.	Compound	Solvent System	Std. Rf. Value	Rf.Value Extract
1	1,2-dithiolane-4-carboxylic acid	v/v ratio ethanol (96%) — aqueous ammonia (25% NH3) — water	0.75	0.74
2	1,2-dithiolane-4-carboxylic acid	v/v ratio ethanol (96%) — aqueous ammonia (25% NH3) — water	0.80	0.79

RESULTS AND DISCUSSION

In the present study an attempt has been made to study Asparagus *officinalis* plant by Macro and Microscopic, Phytochemical and TLC examination. These examinations are found to be very useful tools for the identification and characterization of Asparagus *officinalis* leaves. A simple, accurate and precise analytical method is used for the analysis of leaves and seeds of Asparagus *officinalis* which could be useful in future forensic identification of unknown plant material.

Phytochemical studies were carried out for the identification of Asparagus *officinalis* leaves with standard plant leaves. Thin layer chromatography studies showed the presence of active principles of Asparagus *officinalis*. This is further suggested that the proposed methods are simple, sensitive, reproducible, and economical and requires very less equipment. These can be employed for qualitative evaluation of Asparagus *officinalis* leaves and also for the routine forensic analysis Asparagus *officinalis*. Therefore, this could be a method of choice for official
monographs in Forensic Toxicology.

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