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Research Article

Pharmacognostical and Physio-Chemical Evaluation of Indian *Asparagus officinalis* Linn Family Lamiaceae

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ABSTRACT

Asparagus officinalis is an erect, unarmed, branched herbaceous perennial herb. It is considered one of the most important vegetable crops in some Asian, African, European and American countries. *Asparagus* is one of the most nutritionally well balanced vegetables in existence, which is high in folic acid, thiamin, vitamin B6, rutin. Traditionally, the plant is used for the Prevention of kidney and bladder stones, Dropsy, Rheumatic conditions, Liver disease, Bronchial asthma and gout. The pharmacognostic parameters were studied for identification of species through macro and microscopical, physicochemical, phytochemical screening. The plant is characterized by scale-like leaves; scales are very minute, the cladodes fascicled, 0.5 to 1.5 centimeters long and rudimentary, rootstock creeping, thick, tuberously swollen, and short-jointed. Stems (ferns) are with much branched feathery foliage. The anatomy of the root shows presence of covering trichomes and stem showed glandular trichomes. The trichomes which were present on the surface are sessile, quadricellular heads, unicellular stalk with 2 to 4 celler glandular head. The preliminary phytochemical chemical tests showed the presence of, alkaloids, flavonoids, phenolic compounds, steroids, amino acids and proteins. Powdered microscopy shows the presence of large number of vessel elements either entire or fragments.

Keywords: *Asparagus officinalis*, Phytochemical screening, Cladodes, Glandular trichomes, Saponins, Flavanoids.

INTRODUCTION

Asparagus is a large genus with over 160 different species of herbaceous perennials crop of high economic value *Asparagus officinalis* Linn. It is considered one of the most important vegetable crops in some Asian, African, European and American countries whose markets demand a big quantity from fresh green, purple and white spears. *Asparagus* is one of the most nutritionally well balanced vegetables in existence, which is high in folic acid, thiamin, vitamin B6, folacin and a good source of potassium. It represents sources of rutin, a drug which strengthens capillary wall, also, an excellent source of folacin, vitamin B, which helps in the duplication of cells for growth and repair of the body and in blood cell reproduction in the bone marrow. Traditionally, the plant is used for the Prevention of kidney and bladder stones, Dropsy, Rheumatic conditions, Liver disease, Bronchial asthma and gout[1,2,14]. Therefore, it is aimed in this study to bring to light more information about the morphological, anatomical and some important chemical contents of vegetative and reproductive organs of *A. officinalis* L.

MATERIAL AND METHODS

Collection of Plant Material

Asparagus officinalis was commonly known as vegetable herb. It is indigenous to Africa, Asia and European countries. The plant material was collected from the region of Tirupathi in Andhra Pradesh, India in the month of October 2016 and it was authenticated by Dr. Madhava chetty professor of botany department, Andhra university Andhra pradesh. A herbarium is prepared and submitted for future reference in the department of pharmacognosy in Nalanda College of Pharmacy. Under the Vocher no: NCOP/Ph'cog/2016-2017/2550.

Morphological characters

The macroscopic characters such as size, shape, colour, surfaces features, odour and taste of the fresh leaves, stem, and roots were recorded[1,2] and photographed using digital camera. Size measurements were made using vernier caliper.

Anatomical Characters

Plant materials such as leaves, stem and roots were freshly collected from the field and fixed for anatomical studies. Free hand sectioning was performed to obtain a thin transverse section of leaf, stem and root. Microphotographs were taken for identification of cells and tissues[7].

Powder Microscopy

Fresh aerial parts of *Asparagus officinalis* Linn were cleaned and then dried in the shade. It was then powdered separately with the help of electric grinder to a coarse powder. This was subjected to powder microscopy as per the standard procedures mentioned. The powder was stained with different chemical reagents like phluroglucinol : Conc HCL (1:1), iodine and safranin and

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Figure 1: *Asparagus officinalis* Linn.

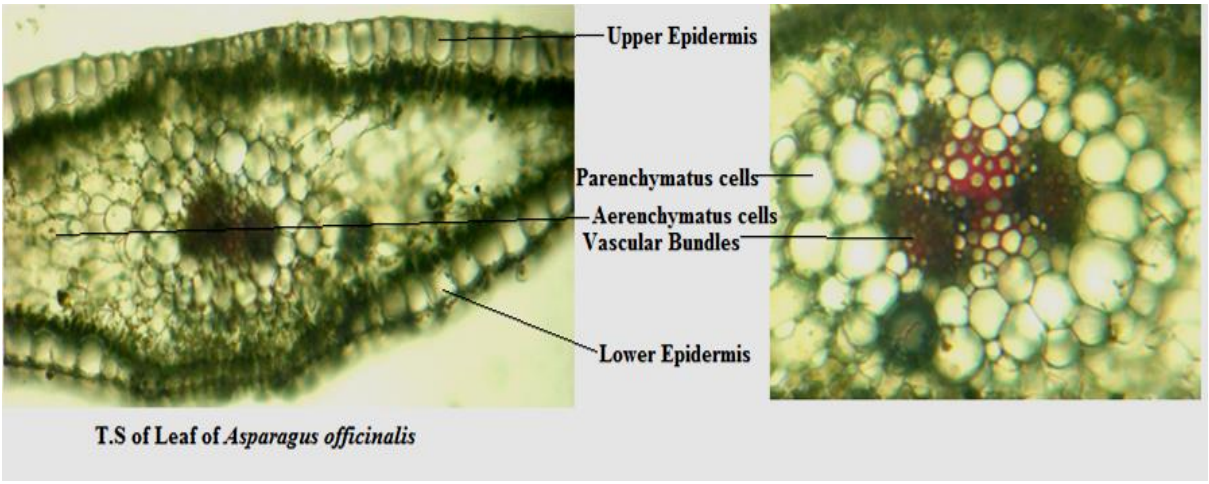

Figure 2: T.S of *Asparagus officinalis* Linn.

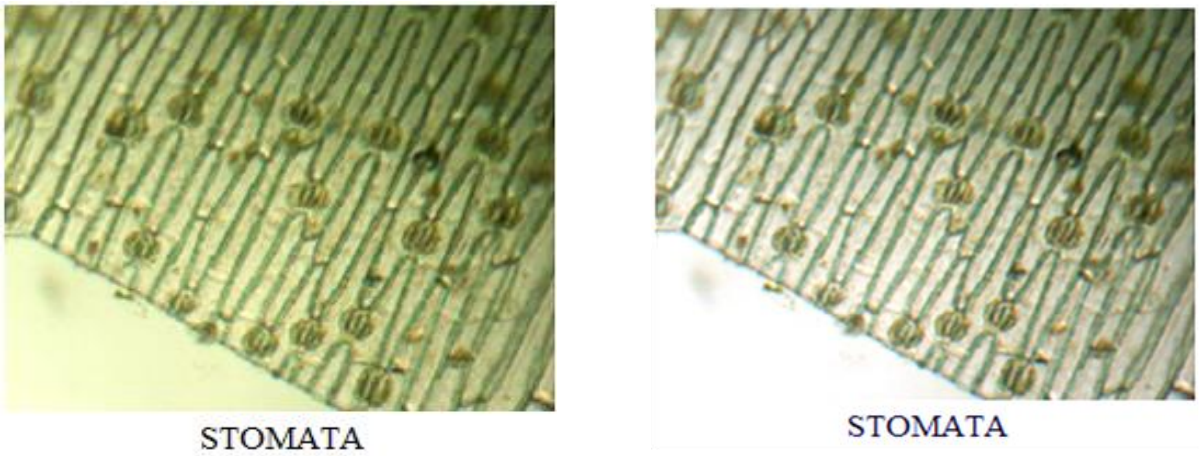

Figure 3: Paracytic stomata.

finally observed under microscope to study the various cell contents[6,8,9,10].

Microscopical measurements

The microscopical measurements such as length and width of the fibres were performed as per the standard reference books[7,10,2].

Chemo-microscopy

The fresh leaves stem, root was taken and thin sections were cut with the help of potato and razor blade. Phluroglucinol and HCl, toludine, and Safranin were used as staining reagents. The slides were neatly prepared and focused under a microscope to study the various cell contents and cell wall thickenings and photographs were taken[10,11].

Fluroscence analysis

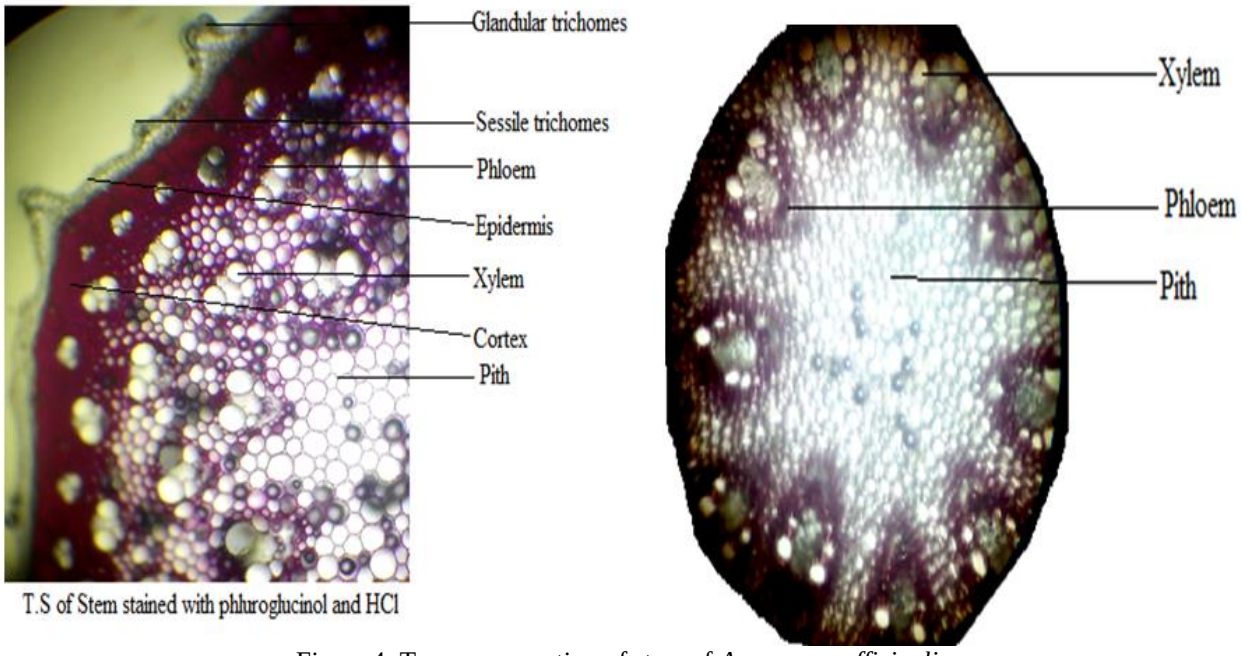

Figure 4: Transverse section of stem of *Asparagus officinalis*.

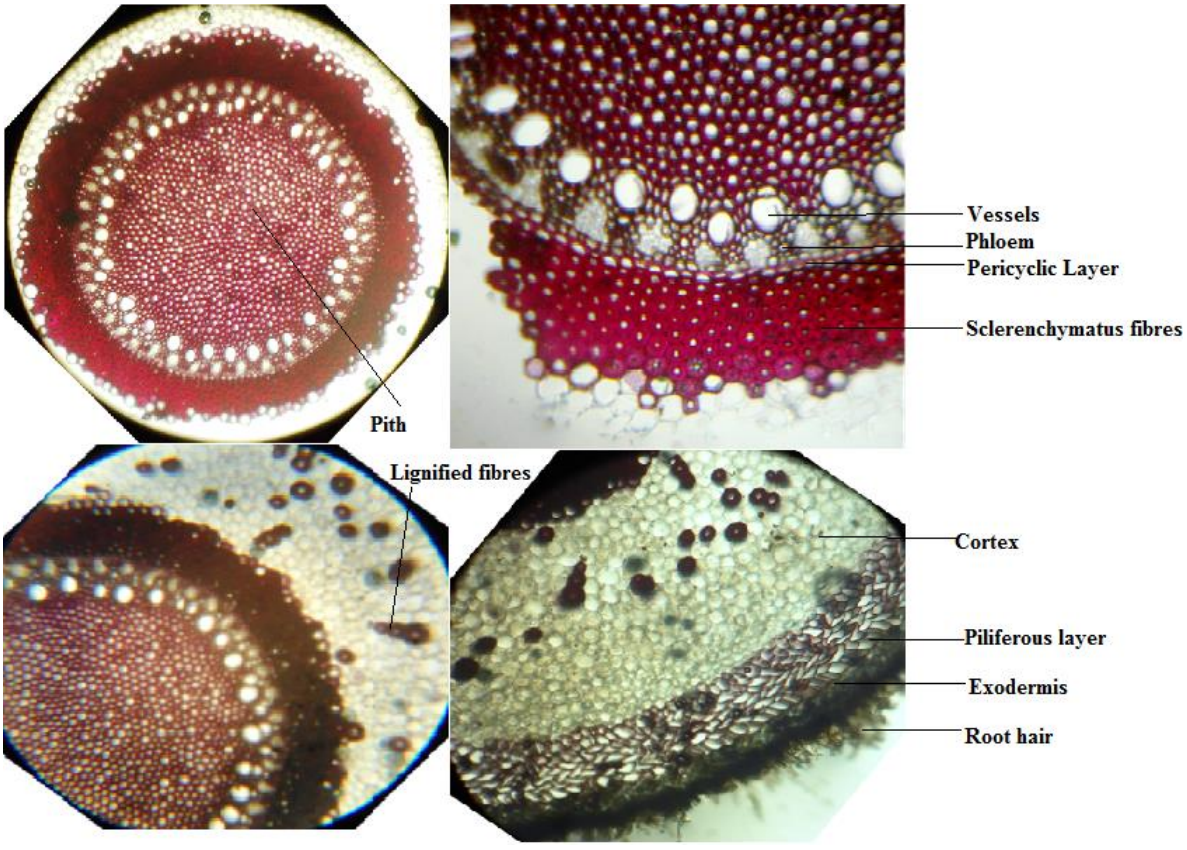

Figure 5: Transverse section of root of *Asparagus officinalis* Linn.

Powdered drug was treated with different chemical reagents and then observed under short wavelength (254nm), long wavelength and (365nm) and day light[6-12,17].

Physico-chemical Parameters[10]

Physicochemical constants such as percentage of total ash, acid-insoluble ash, and water soluble ash. Extractive values were done by using alcohol, petroleum ether, and chloroform water and the percentage yield were calculated as per the methods described in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant material.

Extraction and Phytochemical screening methods[18,19]

About 100g of the whole parts of the plant was packed in thimble then defatted with petroleum ether, extracted with

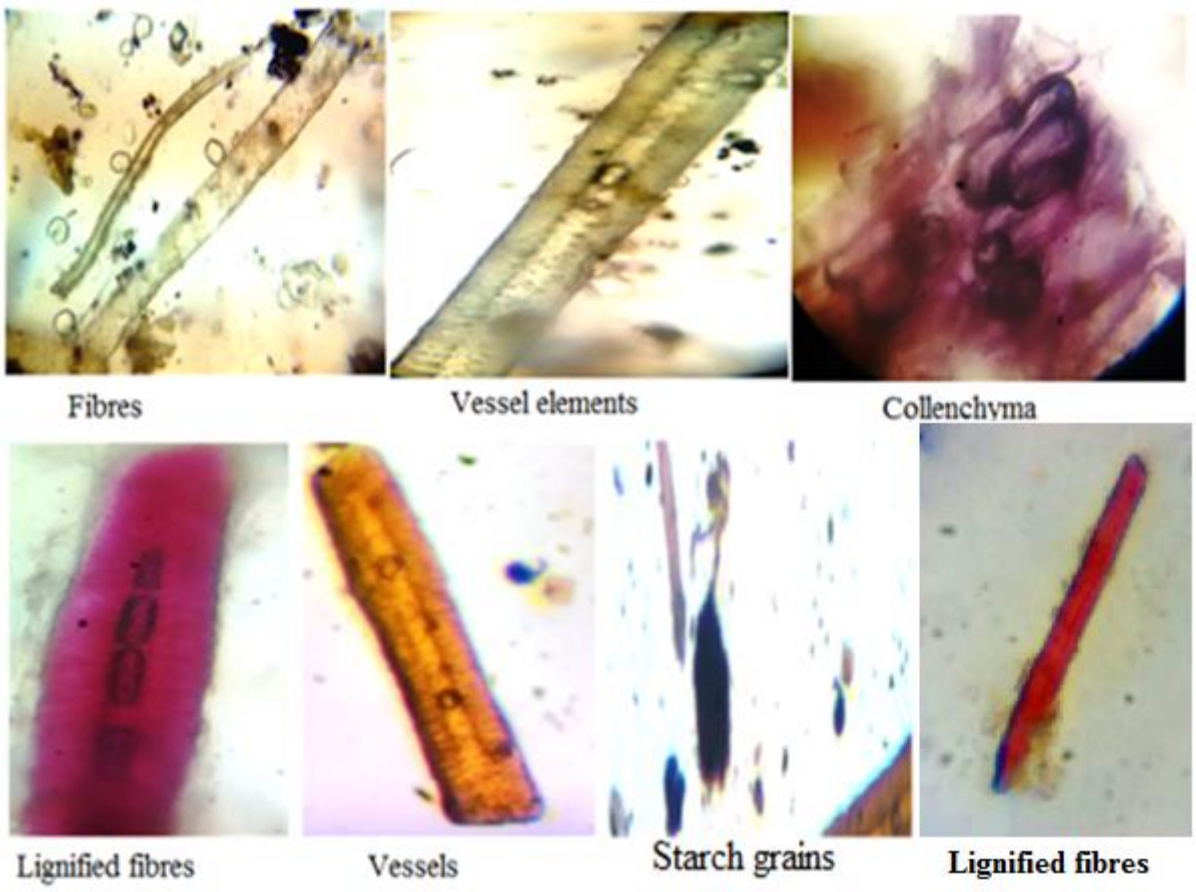

Figure 6: Powder microscopy of *Asparagus officinalis*.

Table 1: Fluorescence analysis of crude powder of *Asparagus officinalis.*

Reagents used	*Day light*	*Long wavelength*	*Short wavelength*
Phluroglucinol & HCl	*Pink*	*Black*	*Dark Pink*
Safranin	*Pink*	*Black*	*Greenish black*
Conc HCl	*Yellow*	*Black*	*Light green*
Conc. HNO_3	*Reddish brown*	*Black*	*Brown*
Conc. H2SO4	*Dark brown*	*Black*	*Dark brown*
Chloroform	*Light brown*	*Reddish black*	*Pale brown*
Glacial acetic acid	*Yellowish brown*	*Black*	*Yellowish brown*
Wagner's reagent	*Yellowish brown*	*Black*	*Pale green*
Sudan red –III	*Reddish brown*	*Black*	*Greenish yellow*
Dragondroff's reagent	*Yellowish red*	*Dark red*	*Greenish yellow*
Benedict's reagent	*Bluish green*	*Blackish purple*	*Light green*
Methanol	*Light green*	*Red*	*Greenish yellow*
Conc.HNO_3+NAOH	*Pale brown*	*Dark purple*	*Green*
Glacial acetic acid + Conc.HNO_3	*Light yellow*	*Black*	*Light green*

Table 2: Physicochemical properties of crude powder of *Asparagus officinalis*.

Parameters	Content
Ash Values	
Total ash values	27.5%
Acid insoluble ash values	26.5%
Water soluble ash values	21%
Extactive values	
Water soluble extractive values	0.32
Alcohol soluble extractive values	0.18

different solvents such as hexane, chloroform, methanol, alcohol and water and then extracted with alcohol as per the polarity of the solvent, for 34 hrs at 30ºC. The thick mass was evaporated with the help of rotary vacuum evaporator and percentage yield was calculated. The extracted crude drug was subjected to the phytochemical screening, the presence of different compounds viz. alkaloid (Dragendorff's test), anthraquinone (Borntrager's test), flavonoid (Shinoda test), steroidal glycosides (LB test), proteins (Biuret test), reducing sugar (Fehling solution test) and saponin (foam test) etc. were detected by usual methods prescribed in standard reference books[18,19].

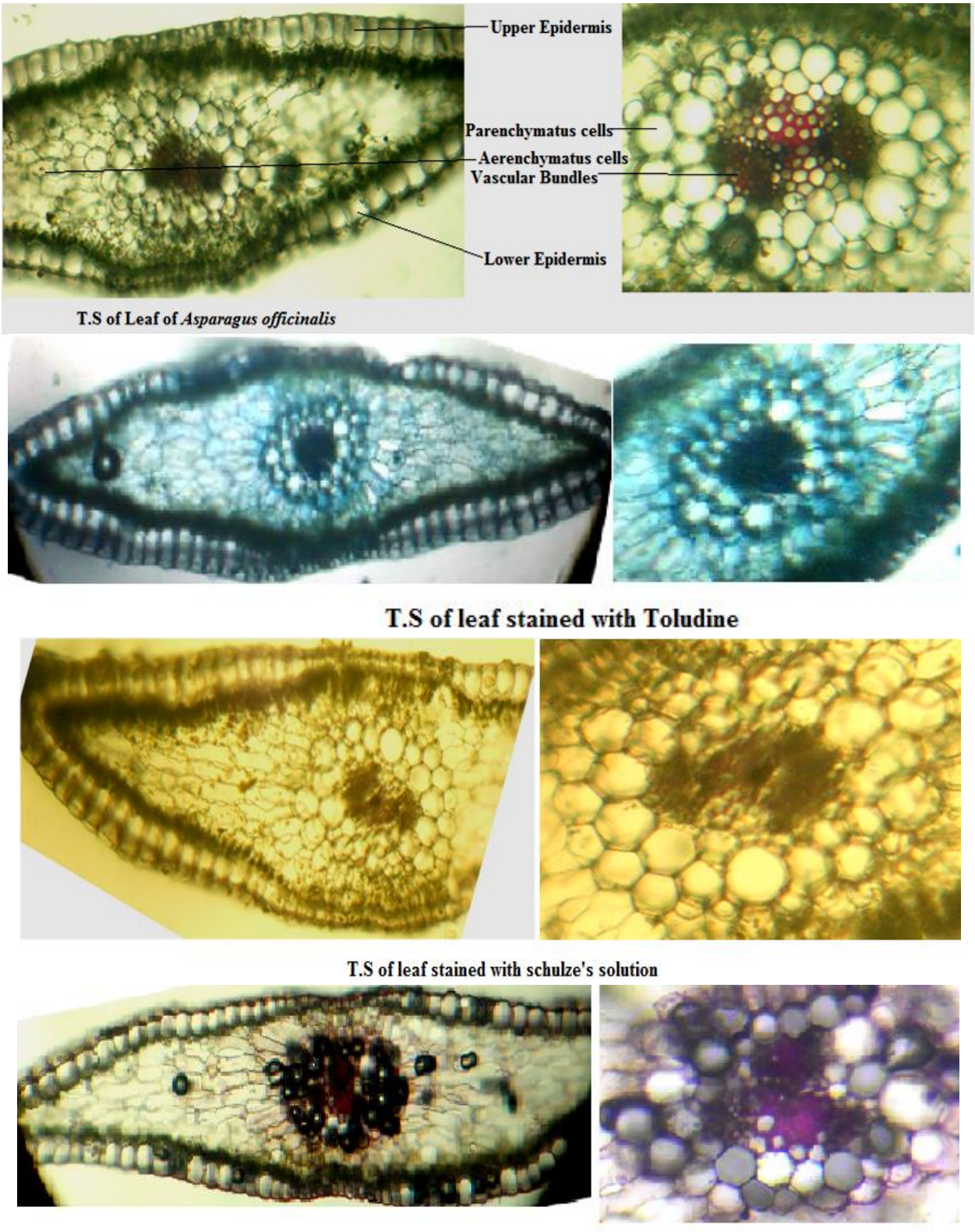

Figure 7: Chemo-microscopy of Leaves of *Asparagus officinalis* Linn.

RESULTS

Morphological characters of the plan

Leaves

Fernlike (actually branches functioning as leaves). They appear somewhat like pine needles. Alternate and reduced to scales on main stem, glabrous. Leaves of upper branches are linear. To 2.5cm long, 5mm broad in group of 1.5 per

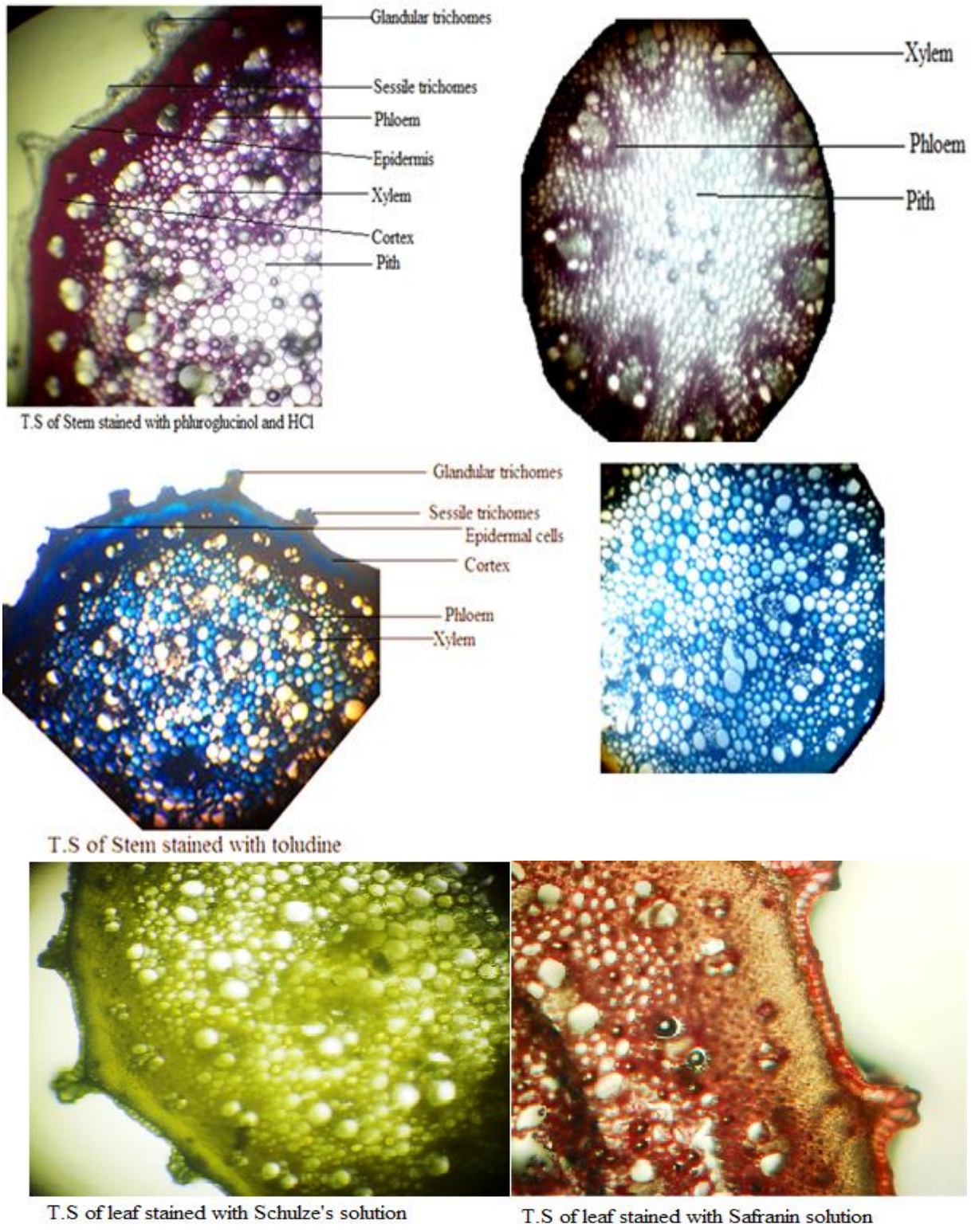

Figure 8: Chemo-microscopy of stem of *Asparagus officinalis*.

node, glabrous, appearing as if in fascicles like pine needles.

Stems

Stems are up to 6 feet tall, herbaceous, erect, much branched, glabrous, from rhizomes. Braches are thin drooping. The cladodes (Modified stems) arise in the axis of scale leaves. The arial shoots carry cladodes (cladophlls), which were found in fascicles (3-6) on each node.

Roots

The tuberous roots are 30 – 100 cm long, 0.7 – 1.5 cm diameter (Fig 1d), odourless, cylindrical with slightly tapering ends and sweetish in taste. Older tubers were dark brown whereas young tubers pale yellow in colour. Scars

Table 3: Phytochemical screening of crude powder of *Asparagus officinalis*.

Phyto-constituents	Petrolium ether	n-hexane	Toluene	Chloroform	Alcohol	Methanol	Water
Carbohydrates	-	-	-	-	+ve	+ve	+ve
Proteins	-	-	-	-	+ve	+ve	+ve
Amino acids	-	-	-	-	+ve	+ve	-
Fats	+ve	+ve	-	+ve	+ve	-	-
Alkaloids	-	-	-	-	+ve	+ve	+ve
Glycosides	-	-	-	-	+ve	+ve	+ve
Flavonoids	+ve	+ve	-	-	+ve	+ve	+ve
Tannins & Phenolic	-	-	-	-	+ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	-	-	+ve	+ve	+ve
Vitamin-C	-	-	-	-	+ve	-	-

and protuberances of lateral rootlets were seen all over the outer surface with longitudinal wrinkles. Texture was hard and roots breaks with uneven fibrous fracture when dried. Irregular longitudinal furrows developed when root was peeled and dried. (Figure no-1)

Anatomical characters of the plant

Microscopic characters of leaf

Transverse section of young leaves showed the presence of single layer of epidermal cells. It has upper epidermis and lower epidermis. The type of stomata was found to be paracytic stomata, the number of stomata in the abaxial side (Lower) more than the adaxial one. Mesophyll consists of irregular parenchyma. There are poor developed collateral vascular bundles found in continuous line. There are two rows of prolonged radial cholenchymatous cells are present. The vascular bundles are surrounded by one two rows of compactly arranged parenchymatous cells. Vascular bundle feature of two alternative arms of xylem with two strands of phloem is expressed. (Figure no-2)

Microscopic characters of stem

T.S. of young stem showed presence of ridges and furrows along with most of the typical monocot characters such as large pith and irregularly arranged vascular bundles. Epidermis consisted of single layer of cells possessing thick layer of cuticle followed by 2-3 layers of parenchyma. It consists of numerous numbers of trichomes. The trichomes which were present on the surface are sessile, quadricellular heads, unicellular stalk with 2 to 4 celler glandular head. Cortex consists of 2-3 layers of collenchymatous cells and 4-5 layers of lacunar collenchyma. Numerous vascular bundles were embedded in ground tissue. Pith is relatively narrow. The larger vascular bundles placed towards the inner side and smaller ones developing in peripheral region. Vascular bundles were of collateral and closed type with metaxylem present on its outer side. This type of central cylinder is known as atactostele. In addition centrally placed pith composed of polygonal parenchymatus cells having triangular intercellular spaces. (Figure- no4)

Transverse section of root

In cross sections, the adventitious root has a round form. The first layer, called rhizoderma was represented by small cells, some of them became absorbing hairs by elongation, followed by a cortex of 3 subsectors, exodermis (4-5 layers of suberificated cells), cortical parenchyma (15-18 ranks of oval cells with intercellular spaces and deposited the reticence substances). The last layer of cortex is endodermis. A sheath of stone cells surrounding, the endodermis, includes cells with casparian strips interrupted by passage cells. The central cylinder includes the pericycle xylem and phloem bundles, medullary rays and centrally pith region. (Figure no-5)

Powder Microscopy

The powdered crude drug of leaf, stem and root revealed the presence of fibres, vessels; vessel elements are both fractured and entire. Some amount of starch grains was observed throughout the powder crude drug. (Figure no-6)

Microscopical measurements

Measurements of length and width of fibres was performed. The minimum value was found to be 152.9µ, average value is 2022.45µ and maximum length of fibres was found to be 3892µ respectively, the minimum value was found to be 38.92µ, average value is 436.46µ and maximum width of fibres was found to be 834µ respectively.

Chemo-microscopy

The transverse section of the leaf was stained with various chemicals and reagents to study the cell wall thickenings and cell contents present in the leaf of *Asparagus officinalis*. When the leaf section was treated with Phluroglucinol and concentrated HCL, the lignified cells were stained pink in colour (Figure). When a leaf section was stained with safranin solution the lignified cells stained dark pink colour. Section stained with Schulze's solution exhibited lignin in yellow colour, cutin and suberin as yellow to brown colour. The T.S of the leaf when stained with toludine blue which is a polychromatic stain imparted lignin containing cells blue to bluish-green colour which indicates the presence of lignin, cutin, suberin and cellulose in the leaf section.

The transverse section of the stem when treated with Phluroglucinol and HCL stained lignified cells pink in colour. TS of stem stained with toludine blue lignified cells bluish green colour and whereas the cutin, and suberin containing cells were stained blue colour. When stem section was stained with safranin solution the lignified cells stained dark pink colour. Stem section stained with

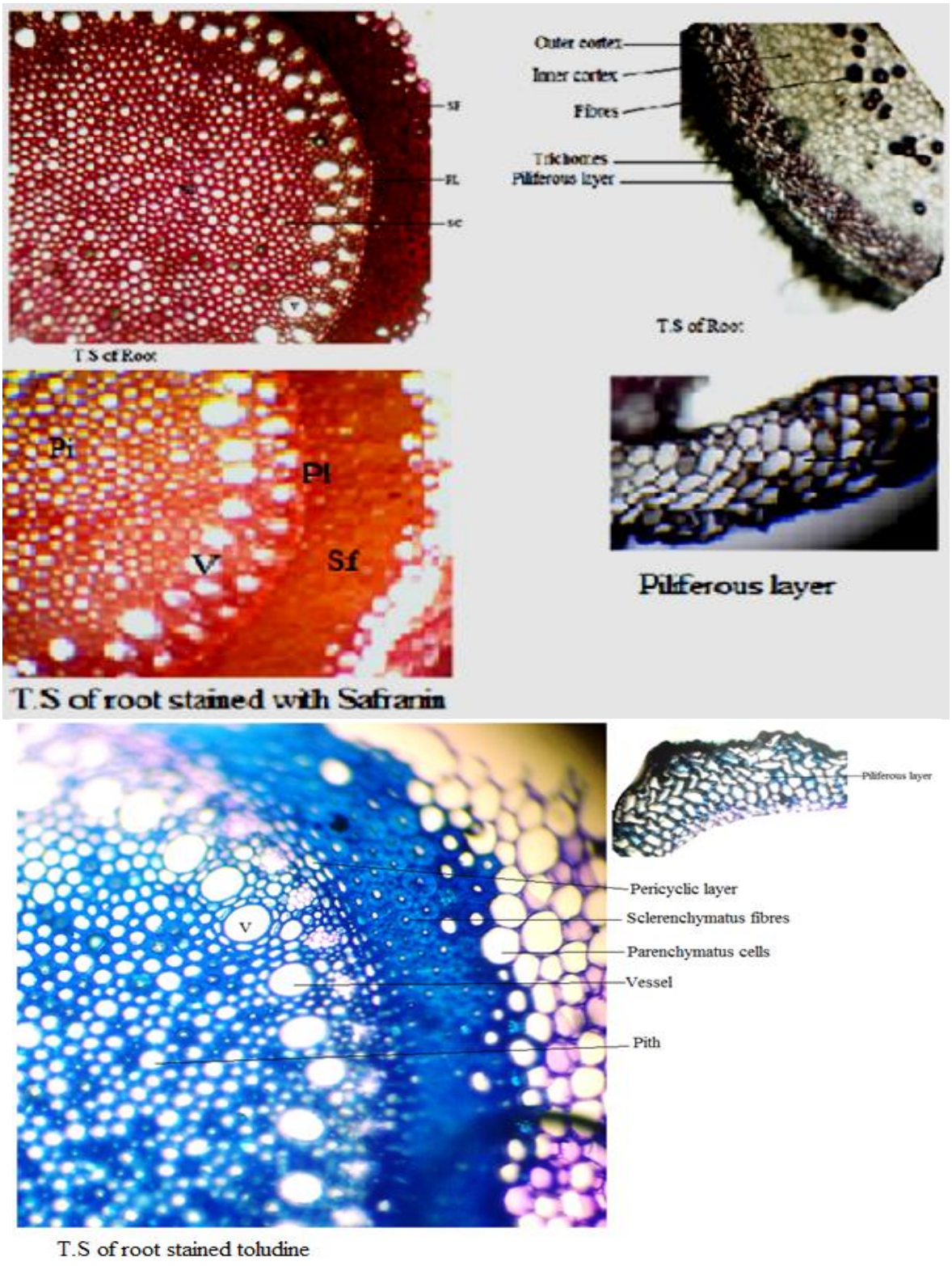

Figure 9: Chemo-microscopy of roots of *Asparagus officinalis* Linn.

Schulze's solution exhibited lignin in yellow colour, cutin and suberin as yellow to brown colour.

The transverse section of the root when treated with Phluroglucinol and Conc HCL stained piliferous layer, sclerenchymatus and pith region containing lignified cells dark pink in colour. When root section was stained with safranin solution the lignified cells stained dark orange to red in colour. TS of root stained with toludine blue lignified cells blue in colour and whereas the cutin, and suberin containing cells were stained blue colour. Parenchymatus cells present in cortex region stained in violet colour.

Fluroscence analysis

The fluorescence analysis of the powdered plant drug showed different fluorescent colours. Powdered crude drug showed different shades in day light, long wavelength and short wavelength. In day light it showed green to yellow colour, under long wavelength it shows black colour and in short wavelength it shows green to brown colour and the results were tabulated as follows (Table 1).

Physico-chemical Parameters

The percentage of total ash, acid insoluble ash and water soluble ash gives an idea about the purity and quality of the drug. Water soluble extractive, alcohol soluble extractives were determined by standard method and the results were tabulated as follows (Table-2).

DISCUSSIONS

The quantitative determination of pharmacognostical parameters is useful for setting standards for crude drugs. Identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Macroscopic studies play an important role for primary identification of drugs[15]. The leaves of *Asparagus officinalis* were pine needle shaped green in colour, the cladodes (Modified stems) arise in the axis of scale leaves. The young stems were green in colour, stems are erect, much branched, glabrous, from rhizomes. Braches are thin drooping. the roots are in yellow to dark brown coloured.

Microscopic studies or a structural detail helps the secondary identification of drugs. The microscopical studies of the leaf showed the presence of epidermal cells, cortex region consists of loosely arrainger parenchymatus cells and Vascular bundle feature of two alternative arms of xylem with two strands of phloem is expressed. Microscopy studies of stem showed the presence of numerous number of glandular trichomes such as sessile, quadricellular heads, unicellular stalk with 2 to 4 celler glandular head. Vascular bundles were of collateral and closed type with metaxylem present on its outer side. This type of central cylinder is known as atactostele. The chemomicroscopical study is application of chemical tests to small quantities of its histological section which helps for the study of different constituents of drugs. This study gives chemical composition of the cell walls of the tissue in the plant. When leaf and stem stained with phluroglucinol and HCl, it shows the presence of lignin. When stained with safranin all lignified and cutinized tissues turned redish orange colour and Schulze's solution (Chlorzinc- iodide) stained lignin yellow, cutin and suberin yellow or brown. Polychromatic stain toluidine blue O stained lignin containing cells bluish in colour. The microscopical measurements set a limit and range for identification of authenticity that can be present in the plant specimen.

Measurements of the length and width of fibres was performed. Test for ash values was performed to determine quality and purity of a crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate content of crude drug affects 'Total ash value'. Such variables were removed by treating with acid and then acid insoluble ash value was determined. Extractive values useful for the evaluation of a crude drug which gives an idea about the nature of the chemical constituents present in a crude drug and also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. In this plant water extractive values were more when compared with the alcoholic extractive values. Fluorescence studies of the leaf, stem and root give a wide range of colour changes at day light, UV-chamber (256nm and 365nm). These colour changes reflect the nature of the chemical components present in the pant parts when exposed to the respective chemical reagent[16]. Hence, this parameter is very important technique for the proper identification of the plant species. The powder of the different parts of the plant was subjected to the preliminary chemical tests showed the presence of various constituents like alkaloids, flavonoids, glycosides, tannins, phenolic compounds, resin and saponins. The information obtained from preliminary phytochemical screening will be useful in finding out the genunity of the drug.

CONCLUSION

Standardization is the prime need of time.These help in the establishment of quality and identity profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. The results obtained in the present investigation are encouraging and can be used as an effective reference data for the standardization of *Asparagus officinalis* Linn.

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REFERENCES

1. Evans WC. Trease and Evans pharmacognosy. 14th Ed, WB Saunders Ltd. London, UK, 1996, 541-578.
2. Wallis T E., *Text book of Parmacognosy*, 5th edition, CBS Publishers, New Delhi, pp 111 &566, (2005).
3. Asparagus Root is a highly regarded herb worldwide retrived on April17th 2014.
4. Al-Snafi AE. Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants. Thi qar University, 2013.
5. Germplasm Resources Information Network, United States Department of Agriculture, Agricultural Research service. Beltsville Area, http://www.ars-grin.gov/cg-bin/npgs/html/taxon.pl?300050[12 Sep 2012].2.
6. Iyengar M A., *Anatomy of crude drugs*, 9th edn, Manipal Press Ltd, Pg.No:42, (2004).
7. Iyengar M.A., *Pharmacognosy of powdered crude drug*, 7th edition, Manipal press Ltd, Manipal, pp 8, (2005).

8. Khandelwal K R., *Practical pharmacognosy*, 19th edn, Nirali Prakashan, Pune, pp 146-165, (2009).
9. Kokate C K, *Practical Pharmacognosy*. 4th edn, Vallabh Prakashan, New Delhi, pp.10-27, (2008).
10. Kokate C K., Purohit A P., Gokhale S B., *Pharmacognosy*, 44th edn, Nirali Prakashan, pp11.59 – 11.60, (2004).
11. Kulkarni Y A., Gokhale S B., Yele S U., Surana S J., Tatiya A U.,Pharmacognostical and preliminary Phytochemical investigations on the bark of *Persea macrantha* (Nees) Kosterm (Lauraceae), *Indian Journal of Natural Products and Resources*, 2(2),211-217,(2011).
12. Bindu Gopalakrishnan., (Late)Sharadha N S., Pharmacognsotical studies on stem bark of *Madhuca longifolia* (Koen.) Macbr. Var. *Latifolia* (Roxb.) A. Cheval, *Indian Journal of Natural Products and Resources*, 3(2), 232-236, (2012).
13. Asparagus Root is a highly regardes herb world wide retrieved on 17th April 2014 at14:30.
14. Nature Gate, Asparagus officinalis,www.luontoportti.com/suomi/en/kukkakasvit/asparagus [22 jul 2013].
15. WHO. the International pharmacopeia, test methods and general requirements, 3rd edition, volume 4, world health organization, Geneva, (1998).
16. Sandhya S., Venkata Ramana K., Vinod K R., Pharmacognostical standardization of *Tephrosia purpurea* Pers root, *Ancient Science of life*, 30(1),1-6, (2010).
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-phenol reagent. J Biol Chem 1951; 193:265-275.
18. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Amer Pharm Assoc 1949; 38:324-331.
19. Houghton PJ, Raman A. Laboratory Handbook for the Fractional of Natural Extracts. Chapman and Hall, London, UK, 1998, 154-162.