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Background: Hypericum perforatum L. (family: Hypericaceae), commonly known as St. John's-wort, is a perennial herb and traditionally used for treating anxiety, depression, gastritis, insomnia also menstrual disorders and for healing cuts and burns. In homoeopathy, this remedy is used for the treatment of injuries, tetanus, neuritis, tingling, burning and numbness and constant drowsiness, coccydynia, spasmodic asthmatic attacks with changes of weather, etc.

Objective: The pharmacognostic and fluorescence studies of H. perforatum L. have been conducted to carry out correct identification of plant species for homoeopathic drug preparation and to lay down the standards of the raw drug.

Materials and Methods: The raw drug was supplied by Regional Research Institute of Unani Medicine, Jammu. In the pharmacognostical studies, the macroscopic, microscopic, powder microscopy and fluorescence analysis were performed.

Results: The raw drug was dried, broken and shrivelled pieces of stem, root and leaves. Leaves were pale yellow to brown with prominent blackish-brown dots. The mature stem was circular in shape with two prominent winged projections on both the sides, rays being unibiseriate; pith composed of thin-walled and thick-walled parenchymatous cells with pits. The stomatal index was 22–25 on lower surface, vein-islet 35–43 and palisade ratio 6–10 recorded.

Conclusion: The presented features along with the powder microscopic, organoleptic characters and fluorescence analysis are diagnostic to establish the standards for ensuring correct identity of the raw drug.
Anatomical characterisation and foliar microscopy of *Hypericum perforatum* L.

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**Background:** *Hypericum perforatum* L. (family: Hypericaceae), commonly known as St. John’s-wort, is a perennial herb and traditionally used for treating anxiety, depression, gastritis, insomnia also menstrual disorders and for healing cuts and burns. In homeopathy, this remedy is used for the treatment of injuries, tetanus, neuritis, tingling, burning and numbness and constant drowsiness, coccydynia, spasmodic asthmatic attacks with changes of weather, etc. **Objective:** The pharmacognostic and fluorescence studies of *H. perforatum* L. have been conducted to carry out correct identification of plant species for homoeopathic drug preparation and to lay down the standards of the raw drug. **Materials and Methods:** The raw drug was supplied by Regional Research Institute of Unani Medicine, Jammu. In the pharmacognostical studies, the macroscopic, microscopic, powder microscopy and fluorescence analysis were performed. **Results:** The raw drug was dried, broken and shrivelled pieces of stem, root and leaves. Leaves were pale yellow to brown with prominent blackish-brown dots. The mature stem was circular in shape with two prominent winged projections on both the sides, rays being unibiseriate; pith composed of thin-walled and thick-walled parenchymatous cells with pits. The stomatal index was 22–25 on lower surface, vein-islet 35–43 and palisade ratio 6–10 recorded. **Conclusion:** The presented features along with the powder microscopic, organoleptic characters and fluorescence analysis are diagnostic to establish the standards for ensuring correct identity of the raw drug.

**Keywords:** Drug standardisation, Fluorescence, *Hypericum perforatum*, India, Macroscopy, Microscopy, Powder analysis

**INTRODUCTION**

From the ancient time, plant-based medicines play a key role in healthcare system due to the presence of numerous active chemical constituents. The chemical constituents which are isolated from the plant materials are believed to show better efficacy as compared to the raw plant materials itself but their counter side effects also have been reported in many cases. Therefore, the plant-based drugs are regarded as safe which may be due to the presence of other secondary metabolites acting in protective manner by countering the side effects of the main compounds. The efficacy of any drug is dependent on biotic and abiotic factors. Among these, authenticity of the plant material is one of the important factors which can affect its efficacy.

*Hypericum perforatum* L., commonly known as St. John’s-wort, belongs to the family Hypericaceae. The plant name has been derived from Greek words *hyperikon* that is, hyper + eikon where hyper meaning ‘over’ and eikon meaning ‘apparition;’ as the name suggests that this plant is supposed to have ability to ward off evil spirits.[1] Due to its importance, the ancient people believed that the plant had enigmatic qualities and plants were kept for tutelage from demons and evil spirits.[1]

St. John’s-wort is used in traditional medicine systems, including homoeopathy. Since the ancient Greek period, over 2000 years ago, this plant is has been regarded as a herbal remedy for internal and external ailments such as anxiety, depression, gastritis, insomnia, menstrual disorders, intestinal worms, snakebites and as a wound healer in cuts and burns.[1] In Arab countries, the dried plant is used in the form of vaginal pessary to increase the menstrual flow. In many countries such

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as America, Germany, Russia, England and India, the hot water extracts of dried aerial parts of plants are used for a number of medical health conditions. The vegetable oil prepared from the plant has been used for the treatment of haemorrhoids and inflammation. Red juice extracted from this plant is used in European countries as an expectorant and for wounds and bruises. The leaves of the plant are used for diarrhoea, piles, uterine and rectal prolapse. Recent findings show that nowadays, this plant is used as an antidepressant, sedative, relaxing nerve, anti-inflammatory, anti-cancerous and in the cases of menopausal nervousness, menstrual cramps, neuralgia and rheumatism, cuts and burns. This plant has also shown potent antimicrobial activity against many Gram-positive bacteria and compound hyperforin, sensitivity against many methicillin-resistant and penicillin-resistant Staphylococcus aureus bacterial strains.

In homoeopathy, it is used in for the treatment of nerve injuries due to animals bites, tetanus, neuritis, tingling, burning and numbness, especially in fingers, toes and nails. In addition, it is also used for the treatment of constant drowsiness, post-operative pains, spasms after injury, lockjaw and punctured wounds, coccydynia and spasmodic asthmatic attacks with changes of weather.

**Habit and habitat**

*Hypericum perforatum* is a perennial herb and grows up to 30–70 cm high. The stem is reddish with two longitudinal ridges, when young herbaceous and glabrous, woody at the base. Leaves are glabrous, small, sessile, opposite-decussate, light green, lanceolate to ovate, margin entire. The leaves have few small, dotted, black-coloured glands present throughout the lower leaf surface prominently on margin. The campylodromous type venation pattern seen in leaves where secondary midribs originate from the base of primary midrib, arch upward and reunite toward the apex and each successive secondary midrib is shorter than the previous one. Inflorescence is racemose, flowers are bright yellow, 1–3 cm broad, clustered at the tip of the branch, petals 5, elongated, with small dots along the edges; anthers yellow, style long. The fruit type is a capsule, 5–10 mm long, green, sticky when young; seeds dark brown-black, numerous, 1 × 0.5 mm, cylindrical, having rough surface.

This species is native of Europe and found prominently in pastures, meadows, fields, waste areas, road sides and abandoned mines and quarries of temperate regions of world. This plant has also been reported from temperate locations in Asia, Africa, Australia, New Zealand and North and South America. In India, it is reported from Western Himalayas from Jammu and Kashmir to Himchal Pradesh at an altitude of 2000–3000 m.

This plant is called by various names in different countries that is, Dendhu, Balsana and Bassant in India; Blutkraut, Herrgottsblut and Johaniskraut in Germany; Herba de Millepertuis and Herba de Saint Jean in France and Saint John’s-wort in England. The pharmacognostic and fluorescence studies of *H. perforatum* have been conducted to carry out correct identification of plant species for homoeopathic drug preparation and to lay down the standards of the raw drug.

**Materials and Methods**

The plant material was procured from medicinal plants garden of Regional Research Institute of Unani Medicine, Jammu, with accession number 5614 and collection date 18 August 2018. The whole plant material was preserved in formaldehyde-acetic acid-alcohol (FAA) for the microscopic study and material was air-dried for other macroscopic and powder microscopic studies. The macroscopic, microscopic and powder analysis was carried out in the Pharmacognosy Laboratory, Drug Standardisation Department, Dr. D P Rastogi Central Research Institute (Homoeopathy), Noida.

The macroscopic characteristics, namely shape, size, colour, texture, odour and taste were recorded and photographs of raw drug were taken. The plant material preserved in FAA was taken for morphoanatomical studies in which the qualitative and quantitative microscopy of drug was performed. In qualitative analysis, preserved samples were dehydrated through alcohol-TBA-xylene series followed by embedding in paraffin wax. The sectioning of embedded specimen blocks was done at 15–25 μm thickness with the help of rotary microtome model WESWOX Model-MT 1090A and the sections were stained as per the standard method. The prepared slides were studied under the microscope, observations were recorded and photomicrography of different parts in different magnifications was done by Radical RTC S-7 Trinocular microscope.

For the organoleptic evaluation, the raw drug and its powder form were analysed on the basis of sensory characters such as taste, odour and colour. In powder microscopy, powder was treated with different chemicals, stained with safranin and mounted with glycerine and observed under microscope.

For quantitative microscopy, the preserved leaves were treated with saturated chloral hydrate solution for clearing. The peelings from both surfaces were taken for the study of stomatal number and index, where peelings were stained with safranin and mounted on slides with glycerine and examined under the microscope. For vein-islet, vein termination number and palisade ratio determination whole leaf were taken in place of peelings and after staining with safranin and the sample was mounted on slide. The vein-islet, vein termination and stomatal number were calculated by drawing area of 1 mm² as per standard method.

The fluorescence analysis of powder was done to check the presence of fluorescence active compounds through fluorescence activity as per standard method. Under this method, the powder was taken and treated with a few drops of the reagents solution. The mixture were kept in the ultraviolet (UV) fluorescence analysis cabinet and observed under the visible light, short UV light (254 nm) and long UV light (365 nm). The changes in the colour of powder were observed and recorded.
Chemicals and reagents
All the chemicals used in the study were of analytical grade and were procured from Fisher Scientific, Mumbai, India, and E. Merck India Limited.

RESULTS
Macroscopic description
The plant material was dried, broken and shrivelled pieces of stem, root and leaves were obtained. Leaves were pale yellow to brown with prominent blackish-brown dots along the entire lower surface; stem was pale yellow; odour was balsamic and the taste was found bitter and somewhat astringent [Figure 1a].

Microscopic description
Root
In young root, the transection showed wavy circular outline. The epidermis was single layered with elliptical cells. The cortical cells were parenchymatous and not differentiated followed by undifferentiated endodermis, pericycle, narrow secondary phloem and a broad secondary xylem. The pith was composed of thin-walled, anisodiametric, parenchymatous cells without any intercellular spaces.

Figure 1: Hypericum perforatum L. a) Raw drug; b) Transverse section of young root showing undifferentiated cortical region; c) Transverse section of mature root showing periderm, secondary cortex and broad vascular region; d) Enlarged view of transverse section of root showing periderm, secondary cortex and secondary phloem; e) Enlarged view of transverse section of root showing secondary xylem region and rays; f) Enlarged view of transverse section of root showing parenchymatous pith disintegrating towards centre; g) Longitudinal section of root showing bark region, stone cells and patches of thick walled parenchyma; h) Longitudinal section of root showing uniseriate, biseriate and multiseriate rays.
In mature root, the transection showed circular outline. The periderm was 2–3 layered, cells were flattened and thickened. A zone of 2–8 layered stone cells present below the periderm and secondary cortex was multilayered with parenchymatous, anisodiametric cells without intercellular space. A few patches of thick-walled parenchymatous cells also present in this region. The secondary phloem region was present below the cortical region, which included sieve elements, companion cells and phloem parenchyma. The secondary xylem was composed of tracheids, vessels and xylem parenchyma. In longitudinal view, the tracheary elements were with scalariform thickening and bordered pits. The bordered pits were alternate type, present throughout the surface of wall. The tracheary elements were with narrow lumen and were found with spiral and annular thickenings. The rays were both uniseriate and multiseriate and cells were parenchymatous. The pith was composed of thin-walled, anisodiametric, parenchymatous cells, without any intercellular spaces. At maturity, pith started...
degenerating thus creating a large cavity at the centre. Any type of inclusion bodies were not observed throughout this region [Figure 1b-h].

**Stem**

Transection of young stem showed lenticular shape. The outer epidermal cells were elliptical in developing stage, cortical region was with anisodiametric parenchymatous cells, without intercellular space. A few tanniniferous cells were present in this region. In the inner region, single-layered endodermis and pericycle were present, followed by a thin zone of secondary phloem. A few pericyclic fibres were present in this region, however, these fibres were not observed in mature stem. The cambium cells in a single layer and developing secondary xylem with parenchymatous pith were present in this region. A few outer cells of pith had started differentiating and started developing pitted parenchymatous cells.

Transection of the mature stem showed circular outline with two-winged projections opposite to each other. The epidermis was single layered, cells were rectangular, flattened and covered with a thin cuticle, often interrupted by stomata. The wing of the mature stem had a large lysogenous cavity probably developed due to degeneration of chlorenchymatous cells.

![Figure 3: Hypericum perforatum L. a) TLS of stem showing uniseriate rays, xylem with bordered pits and xylem parenchyma; b) RLS of stem showing xylem with bordered pits and ray parenchyma; c) Longitudinal section of stem showing xylem with bordered pits and scalariform perforation plate; d) Vertical section of leaf through midrib region showing vascular bundle and collenchyma tissue; e) Vertical section of lamina region showing vascular bundles; f) Leaf section showing palisade cells and spongy parenchyma cells with large inter-cellular space; g) Leaf section showing mesophyll gland; h) Leaf peel of adaxial surface showing epidermal cells with granulated margin.](image_url)
Below the epidermis, 2–3 layers of crushed chlorenchymatous cells were present. The cortical region was very narrow with 1–2 layers of anisodiometric, thick-walled parenchymatous cells without intercellular spaces. The secondary phloem was composed of sieve elements, companion cells and phloem parenchyma. The schizogenous secretory gland also present in this region. Secondary xylem ring was broad and rays were mainly uniseriate but rarely multiseriate rays were also present. The tracheids were with bordered pits. The pith composed of two types of cells: Thin-walled parenchymatous cells, toward the outer region and thick-walled pitted parenchymatous cells filled with dense content, toward the centre. Both types of cells were without intercellular spaces and were degenerating toward the centre and in mature stem, the pith was hollow [Figure 2a-h and 3a-c].

**Leaf**

The leaves were hypostomatous with anisocytic stomata measuring 20–26 × 16–23 micrometre (μm). The epidermal cells were polygonal in shape and dotted glands were present throughout the margin on lower surface. The trichomes were absent throughout the leaf surface. The vertical section of leaf showed dorsiventral structure with winged margins curved at the edges. The upper and lower epidermis was single layered with large, rectangular cells, about 0.2–0.4 μm long and covered by thick cuticle. The mesophyll was differentiated into a single-
layered palisade cell and 2–3 layered spongy parenchyma. The spongy parenchyma was with a large intercellular space. A few schizolysogenous type secretory glands were present throughout the lamina. The midrib region of abaxial side was convexed, epidermis was single layered covered with thick cuticle, followed by a zone collenchymatous cells on both sides, which was 2–4 layered on the lower side and was single layered on the upper side, followed by 2–3 layers of ground parenchymatous cells. The vascular region had large, crescent-shaped meristele at the centre having collateral, conjoint and open vascular bundles surrounded by parenchymatous sheath [Figure 3d-g].

**Micrometric analysis**
Leaves of drug were hypostomatous, having anisocytic stomata, present on the lower surface. The stomata were unevenly distributed throughout the surface of leaves. Stomata varied from 184–218 per millimetre (mm) square. The stomatal index was 22–25 on lower side, vein-islet 35–43, palisade ratio 6–10 and vein termination observed 85–110 per mm square [Figure 3h and 4a-c].

**Powder analysis**

**Organoleptic evaluation**
Powdered drug was brown in colour, rough in texture, odour was balsamic and the taste was bitter and somewhat astringent.

**Powder microscopy**
Powder was coarse, brownish, with characteristic balsamic odour, taste bitter somewhat astringent. Powder microscopy revealed the presence of fragments of parenchymatous cells, a few palisade cells, fragments of tracheary elements with annular and spiral thickening, pitted parenchymatous cells, a few collenchymatous cells and sclereid fibres [Figure 4d-h].

**Fluorescence analysis**
The fluorescence analysis of raw drug was performed after treating it with different chemicals. The result of analysis is given in Table 1.

Fluorescence analysis is mainly used for the screening of chemical constituents present in the raw material at primary level. This study suggests further possibility of the presence of relative constituents in the plant material. This may be a good tool for characterisation of chemical constituents present in the plant material in a very short time.

**DISCUSSION AND CONCLUSION**
Morphological and anatomical identification of any plant material is the first step toward its characterisation. The present study has been conducted to reveal the detailed macroscopic, microscopic and powder analysis, namely root, stem, leaf and petiole of the plant sample. This study will be helpful for identification, authentication and standardisation of raw plant material. Extensive work has been performed on this plant by several authors,[18,19] still some characters which are diagnostic for plant identification were not reported in their studies. However, in this study, these (characters) have been reported for the first time. The tracheids and vessels present in root, while stem was with scalariform perforation plate [Figure 1h, 3c]. In leaves, walls of epidermal cells ornamented with granulated margin [Figure 3h]. The young stem outline shaped as convex lens [Figure 2a]. A few pericyclic fibres

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<td>20.</td>
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Conc.: Concentrated, dil.: Dilute
were present in stelar region at young stage but not observed in mature stem. The secondary xylem consisting of tracheary elements with bordered pits.

Previously, Barbara and Ewa\textsuperscript{[18]} reported hypostomatous leaves, absence of stomata on stem and pitted parenchymatous pith. However, in the present study, stomata were observed on the stem. Perrone \textit{et al.}\textsuperscript{[19]} also reported hypostomatous type leaves but they also did not report the presence of stomata on the stem. They also reported two wings in the stem with ring porous wood, which is also seen in this study. Erkara and Tokur\textsuperscript{[20]} reported amphistomatous leaves, but we have found that the stomata were confined only to the lower surface.

The described macroscopic, microscopic and fluorescence microscopy along with powder studies are unique diagnostic characteristics of \textit{H. perforatum} in addition to available information in Homoeopathic Pharmacopoeia of India that will help in identification and authentication of the raw drug materials to ensure the quality. Thus, this evidence-based research will be helpful for the correct and complete standardisation of the raw drug and to differentiate the authentic plant material from its adulterants.

\section*{Acknowledgment}

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\section*{Conflict of Interest}

Authors declare no conflict of interest.

\section*{References}


Caractérisation anatomique et microscopie foliaire de Hypericum perforatum L. : un médicament homéopathique

Contexte: Hypericum perforatum L., communément appelé millepertuis, est un arbuste vivace appartenant à la famille des Hypericaceae. Cette plante est utilisée en homéopathie pour traiter l'anxiété, la dépression, la gastrite, l'insomnie, les troubles menstruels, etc. et pour guérir les coupures et les brûlures. En homéopathie, ce remède est utilisé pour les symptômes de blessures, le tétanos, la névrite, les picotements, les brûlures, l'engourdissement, la somnolence constante, la coccydynie, les crises d'asthme spasmodiques avec les changements de temps, etc. Objectif: Les études pharmacognostiques et de fluorescence de Hypericum perforatum L. ont été menées pour identifier les espèces végétales correctes pour la préparation de médicaments homéopathiques et pour établir les normes de la drogue brute. Matériaux et méthodes: Le médicament brut a été fourni par le Regional Research Institute of Unani Medicine, Jammu. Les études pharmacognostiques, y compris l'analyse macroscopique, microscopique, microscopique des poudres et fluorescente, ont été réalisées. Résultat: La drogue brute a été trouvée sous forme de morceaux séchés, brisés et ratatinés de tige, de racine et de feuilles. Les feuilles étaient de couleur jaune pâle à brune avec des points marron noirâtre proéminents. La tige mature était de forme circulaire avec deux projections ailées proéminentes sur les deux côtés, les rayons étant unibisériés ; la moelle était composée de cellules parenchymateuses à paroi mince et épaisse avec des piqûres ; l'indice stomatique était de 22 à 25, l'îlot de la veine de 35 à 43, le rapport de palissade de 6 à 10. Conclusions: Les caractéristiques présentées, ainsi que les caractères microscopiques et organolectiques de la poudre et l'analyse par fluorescence, permettent d'établir des normes pour garantir l'identité correcte du médicament brut.
Anatomical and foliar microscopy of Hypericum perforatum L.: A homeopathic medicine

Antecedentes: Hypericum perforatum L., comúnmente conocido como hierba de San Juan, es un arbusto perenne perteneciente a la familia Hypericaceae. Esta planta se utiliza en homeopatía para tratar la ansiedad, depresión, gastritis, insomnio, trastornos menstruales, etc., y también para curar cortes y quemaduras. En homeopatía este remedio se usa para los síntomas de lesiones, tétanos, neuritis, hormigueo, ardor, entumecimiento, somnolencia constante, coccidinia, ataques asmáticos espasmódicos con cambios de clima, etc.

Objetivo: Los estudios farmacognósticos y de fluorescencia de Hypericum perforatum L se han realizado para identificar las especies de plantas correctas para la preparación de fármacos homeopáticos y establecer los estándares de fármaco crudo.

Materiales y métodos: La droga cruda fue suministrada desde / por el Instituto Regional de Investigación de Medicina Unani, Jammu. Se realizaron los estudios farmacognósticos que incluyeron análisis macroscópico, microscópico, microscopía de polvo y fluorescente.

Resultado: La droga cruda se encontró en trozos secos, rotos y arrugados de tallo, raíz y hojas. Las hojas eran de color amarillo pálido a marrón con puntos prominentes de color marrón negruzco. El tallo maduro era de forma circular con 2 salientes alados prominentes en ambos lados, los radios eran uniberiados; médula compuesta por células parenquimatosas de paredes delgadas y paredes gruesas con hoyos; índice estomático 22 - 25, islote venoso 35 - 43, relación de empalizada 6 - 10 registrada.

Conclusiones: Las características presentadas junto con el microscopio en polvo, los caracteres organolépticos y el análisis fluorescente son diagnósticos para establecer los estándares para garantizar la identidad correcta del fármaco crudo.

金丝桃（金丝桃 金丝桃 L.）的解剖学特征和叶面显微镜检查。一种顺势疗法的药物 背景介绍: 金丝桃 金丝桃 L 俗称圣约翰草,属于金丝桃科多年生灌木。这种植物在顺势疗法中用于治疗焦虑、抑郁、胃炎、失眠、月经紊乱等,还可以治疗割伤和烧伤。在顺势疗法中,这种药用于治疗受伤、破伤风、神经炎、刺痛、燃烧、麻木、持续昏睡、尾椎病、天气变化时痉挛性喘息发作等症状。

目标: 金丝桃的药理认知和荧光研究。已经进行了正确的植物品种鉴定，以备同治药物的制备，并制定了原料药的标准。

材料和方法: 原药由查谟的乌纳尼医学区域研究所提供。药理研究包括宏观、微观、粉末显微镜和荧光分析。

结果: 原药被发现为干枯、破碎和干瘪的茎、根和叶块。叶子呈淡黄色至褐色，有突出的黑褐色小点。成熟的茎是圆形的，两边有两个突出的翼状突起，射线是单双列的；髓部由薄壁和厚壁的实质细胞组成，有孔；气孔指数22-25，静脉小管35-43，宫腔比值6-10。

结论: 所呈现的性状及粉末的显微镜，感官特征和荧光分析是诊断性的，以建立标准，确保原料药的正确身份。