10.5005/jp-journals-10059-0029

ORIGINAL RESEARCH

Pharmacognostical and Phytochemical Screening of *Allium sativum* Linn. Leaf

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ABSTRACT

Aim: *Allium sativum* L. is a cultivated medicinal plant known as *Rasona* in Ayurveda. In Asian countries, leaves of *Lasuna* are widely used in food recipes and as remedy for cough, asthma, malarial fever, facial paralysis, cardiac disease, etc. It is reported to have high medicinal as well as nutritional value. Therefore, it is felt necessary to study the macroscopy, microscopy, histochemical, physicochemical, and thin-layer chromatographic (TLC) parameters of leaf which are not reported earlier.

Materials and methods: Collected plant specimen was identified, authenticated, and preserved in the herbarium section of the Institute. Shade dried leaves were made into powder. Macroscopic, microscopic, and physicochemical parameters were performed as per the standard procedures described in the Ayurvedic Pharmacopoeia of India (API). Fluorescence analysis, behavior of powdered drug with different chemical reagent, was performed as per the procedures given in the World Health Organization document/guidelines.

Results: Organoleptic analysis showed that leaf powder is light yellowish green in color with pungent odor having acrid taste. Microscopic study revealed the presence of anomocytic stomata on both surfaces. The TLC of methanolic extract corresponds to gallic acid and quercetin standards.

Conclusion: The findings would be useful for identification and standardization of leaf drug and would add the parameters in the API.

Keywords: Camera Lucida, Folk claims, Histochemical tests, *Lasuna*, Microscopy, Pharmacognosy, Thin-layer chromatography.

How to cite this article: Nirawane RB, Rajyadhyaksha SN, Gurav AM, Rao G, Mangal AK, Srikanth N Pharmacognostical and Phytochemical Screening of *Allium sativum* Linn. Leaf. J Drug Res Ayurvedic Sci 2018;3(1):1-8.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

Plants are being used for medicinal and food supplements purpose since time immemorial throughout the world. To avoid side effects of modern medicine, most of the countries are accepting herbal medicine. Nowadays, traditional knowledge extracted from traditional healers, ancient texts, tribals, forest dwellers, and local people is being utilized for betterment of human life. One of the ancient Indian systems of medicine "Ayurveda" also stated that every plant and its part has medicinal value.

In Ayurveda, *Rasona* (*Lasuna*) has corresponded to *A. sativum* L., a perennial bulbous plant belonging to family Alliaceae cultivated as an important condiment crop in the country. Its bulb is being used as ingredient of food spices and also prescribed in the treatment of human ailments like cough, asthma, malarial fever, facial paralysis, constipation, cardiac disease, rheumatism, skin disease, earache, etc.[1] Leaf of *A. sativum* has also been reported in various folk claims to possess medicinal properties and is being used to treat various disease conditions like whooping cough,[2] asthma,[3] rhuematoid-arthritis,[4] and hemorrhoids[5] in single or compound formulations. In India, natives of Arunachal Pradesh state are using leaves, cloves, and roots of *A. sativum* to treat cold, cough, and skin rashes.[6]

Keeping in view the importance of leaf for medicinal purpose as reported in various folk claims, it became necessary to study the detailed pharmacognostical parameters of leaf of *A. sativum*. In this communication, detailed pharmacognostic, physicochemical constituents, and TLC have been investigated and reported.

MATERIALS AND METHODS

Plant Collection

Whole plants of *A. sativum* L. were collected from the botanical garden of Regional Ayurveda Institute for Fundamental Research, Kothrud, Pune, Maharashtra state.

Botanical Identification and Authentication

The plant was identified with the help of flora of Maharashtra state,[7] and authentication data (passport data) were prepared.

Herbarium Preparation

Herbarium specimens were prepared by following standard methods described by Rao and Sharma[8] and deposited in the Institute's herbarium with voucher specimen number 13917/2015.

Processing of Material and Preparation of Wet Sample

Freshly collected leaves were thoroughly washed under running tap water, allowed to drain the water and cut into small pieces. Cut pieces were kept under shade for drying. Some fresh leaves were kept in a glass bottle containing a solution of formalin: glacial acetic acid: 75% ethyl alcohol (10:5:85)[9] for future reference.

Powder Preparation

Shade-dried leaves were made into powder with the help of grinder. Powder was sieved through #60 mesh and stored in airtight bottles for further analysis.

Macroscopy

Macroscopic investigations included external morphology, color, texture, odor, and shape of leaf.[10]

Microscopy

Free hand sections of leaf were taken with the help of platinum razor blades and stained with phloroglucinol, followed by HCl, Iodine, and Sudan red-III for identification of various cellular details. Peelings of outer and inner epidermis were separated to study the types of stomata, stomatal index, and stomatal number. Sections were observed under trinocular, Biolux make. Camera Lucida drawings were drawn with the help of prism type Camera Lucida. Microphotographs were taken using Deno Capture 2.0 version 1.4.2.D, the versatile digital eyepiece.[10]

Powder Microscopy

A pinch of powder was taken in a watch glass and stained with phloroglucinol + HCl and Iodine solution. Microslides were prepared and observed under trinocular, Biolux make and microphotographs were snapped with Deno Capture 2.0 version 1.4.2.D, the versatile digital eyepiece.

Histochemical Test

Histochemical tests were performed to confirm the cellular content.[11]

Determination of Physicochemical Parameters

Physicochemical parameters, namely foreign matter, loss on drying, ash values, acid-insoluble ash, extractive values, swelling index, foaming index, pH, behavior of powder drug, and fluorescence analysis of powder were performed as per the standard procedures.[11]

Thin-layer Chromatography

Thin-layer chromatography of methanolic extract of leaf samples was performed using ethyl acetate: toluene: methanol: water: acetic acid (4:3:2:1:0.5) solvent system. Quercetin and gallic acid were loaded as standard on silica-coated aluminum high-performance TLC plate with the help of Camag applicator and run in solvent system by using twin trough chamber, Camag make. Plates were observed in day light, under Ultra Violet light at 254 and 366 nm and derivatized in Iodine vapor, anisaldehyde, and vanillin-sulfuric acid reagents. Rf were calculated, band colors were recorded and photographs were snapped using digital SLR Canon Camera.

RESULTS

Macroscopy

Leaves were 20 to 40 cm long, leaf sheath half of the blade, flat, linear, hollow, dark green, glossy, smooth, having acrid taste with peculiar pungent, and offensive odor (Fig. 1).

Microscopy

Transverse section of leaf is isobilateral showing outer and inner epidermis made up of oval to oblong cells, covered with thick cuticle, followed by single layer of palisade and occasionally two layered. Below this, 9 to 15 layers of spongy parenchyma are made up of circular to oval-shaped cells. Developed and undeveloped vascular bundles are seen toward both epidermis; anomocytic stomata is observed on both epidermis (Fig. 2). Results

Fig. 1: Whole plant of *A. sativum* L.

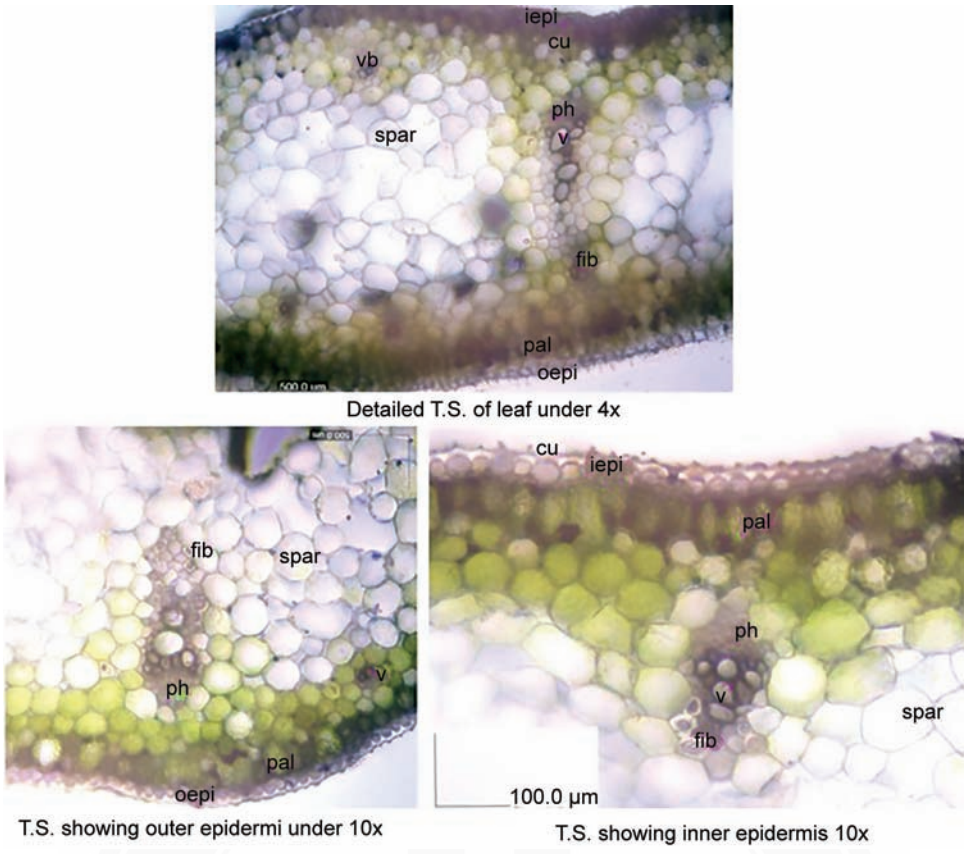

Fig. 2: Transverse section of leaf of *A. sativum* L. with microscopic view

Note: (cu: cuticle, fib: fibres; iepi: inner epidermis; pal: palisade cells; ph: phoem; spar: spongy parenchyma; oepi: outer epidermis; v: vessels, vb: vascular bundle)

of quantitative microscopic parameters, i.e., stomatic number and stomatic index are given in Table 1.

Table 1: Quantitative microscopic parameters of *A. sativum* L. leaf

Parameter	*Result per sq. mm*
Stomatal number	
a) Inner epidermis	17.75
b) Outer epidermis	30.66
Stomatal index	
a) Inner epidermis	14.55
b) Outer epidermis	46.60

Powder Microscopy

Leaf powder is light yellowish green in color, fine, peculiar pungent and offensive odor with acrid taste. Powder microscopy showed fragments of outer and inner epidermis in surface view showing anomocytic stomata and underlying palisade cells, and transversally cut fragment of leaf showed spongy parenchyma, vascular bundle, isolated cells of palisade, annular vessels, spiral vessels, and fragment of fiber (Figs 3 and 4).

Histochemical Test

Histochemical test revealed the presence of lignified cell wall only. Details of results are given in Table 2.

Physicochemical Parameters

While observing the result of physicochemical parameters, foreign matter was 0.11%, the loss on drying was not more than 5.64% w/w, total ash value was not more than 12.44% w/w, acid-insoluble ash value was not more than 0.67% w/w, water-soluble extractive was not more than 42.71% w/w, and the alcohol-soluble extractive was not more than 5.18% w/w. Details of the results of physicochemical parameters are depicted in Table 3. Behavior of powder drug and fluorescence analysis of powder are given in Tables 4 and 5.

Thin-layer Chromatography

Thin-layer chromatography of the methanolic extract shows seven spots under 254 nm and five spots under 366 nm. On exposure to iodine vapor, 10 spots appeared, whereas after spraying with anisaldehyde and vanillin-sulfuric acid reagents, totally 12 and 8 spots were observed respectively. Rf 0.53 and 0.74 matched with Rf of standard quercetin gallic acid respectively. Details of results are given in Tables 6 and 7 and Figures 5 and 6.

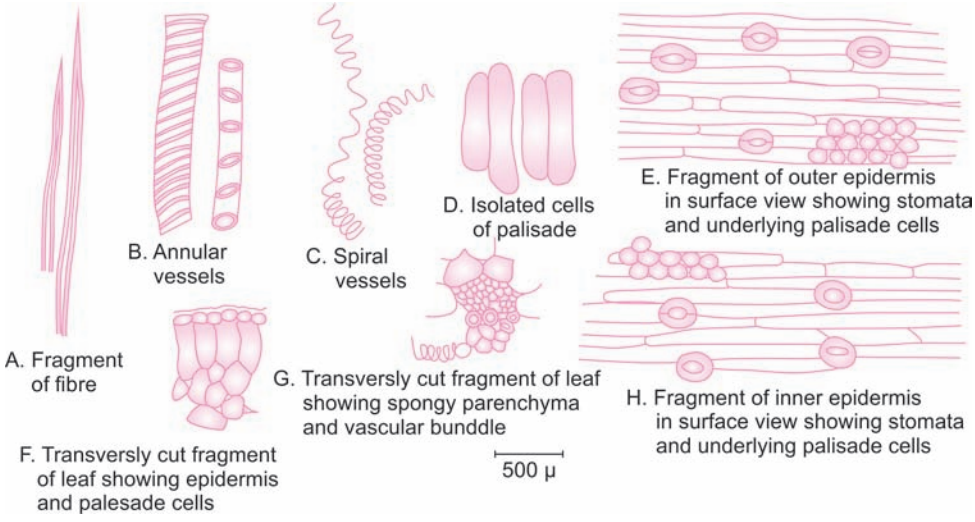

Fig. 3: Camera Lucida drawings of powder characters of *A. sativum* L. leaf

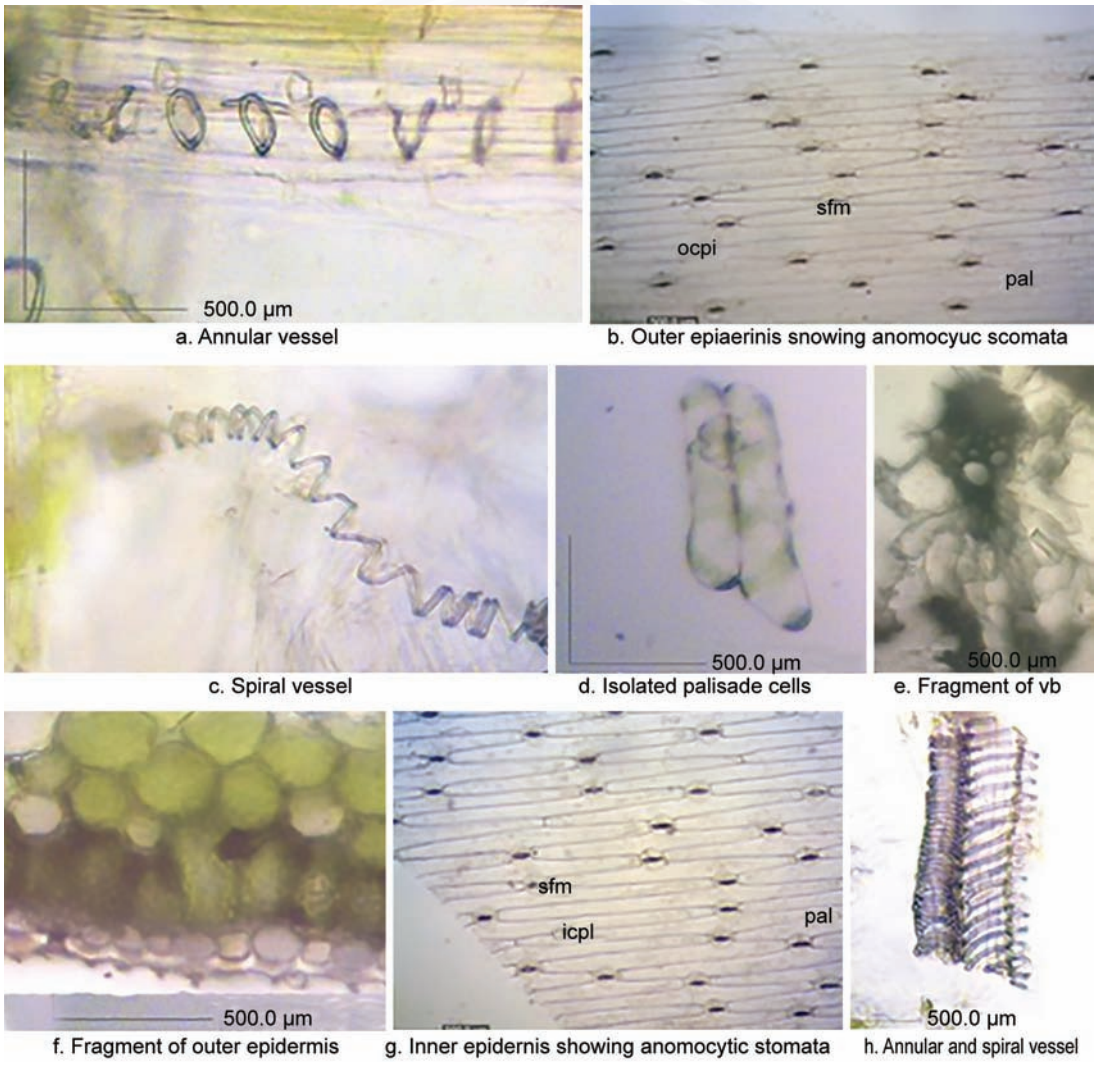

Fig. 4: Powder characters of *A. sativum* L. leaf
Note: (iepi, inner epidermis; pal, palisade cells; oepi, outer epidermis; stm, stomata, vb, vascular bundle)

Table 2: Histochemical tests of *A. sativum* L. leaf sections and powder

Purpose of test	*Chemicals to be used*	*Observation*	*Result*
Lignified cell walls	Phloroglucinol HCl	Pink to cherry red color	+
Cuticular cell walls	Sudan red-III	Orange red or red	−
Aleurone grains	Iodine	Yellowish brown to brown	−
Fats, fatty oils, volatile oils and resins	Sudan red−III	Orange red to red	−
Mucilage	Ruthenium red	Pink	−
Starch	Iodine	Blue or reddish blue	−
Calcium oxalate crystals	Hydrochloric acid	Dissolves	−
Calcium carbonate crystals	Hydrochloric acid	Dissolves with effervescence	−

Table 3: Physicochemical parameters of *A. sativum* L. leaf

Parameter	*Result*
Foreign matter	0.11% w/w
Loss on drying	5.64 % w/w
Total Ash value	12.44 % w/w
Acid insoluble ash value	0.67 % w/w
Water soluble extractive value	42.71 % w/w
Alcohol soluble extractive value	5.18 % w/w
Swelling index	0.5
Foaming index	<1cm
pH of aqueous extract	4.56
pH of Alcoholic extract	5.54

DISCUSSION

Results obtained from the study clearly showed that the leaf of *A. sativum* L. has peculiar macroscopic, microscopic, and physicochemical characters. Macroscopic study revealed peculiar pungent and offensive odor of leaf, whereas microscopically, developed and undeveloped vascular bundles on outer and inner epidermis, as well as presence of anomocytic stomata on both the epidermis, were observe. Presence of fiber, annular and spiral vessels, single- or double-layered palisade layer and offensive odor with acrid taste are the diagnostic characters of leaf. In physicochemical parameters, water-soluble extractive value was much higher as compared with alcohol-soluble extractive. The high solubility in water indicates the best suitability of drug for extraction with water or water-based preparations. Thin-layer chromatography of methanolic extract of leaf confirmed the presence of quercetin and gallic acid at 0.74 and 0.53 Rf respectively which would be proved as reference for identification and standardization of leaf.

CONCLUSION

The macroscopic, microscopic, physicochemical data, and TLC of this study would be useful for identification and authentication of leaf drug of *A. sativum* L.

Table 4: Behavior of *A. sativum* L. leaf powder with different chemical reagents

Name of test	*Result*
Conc. H_2SO_4	a) Powder floats on surface
	b) On shaking, few particles move down up to 2 cm and remain suspended
Conc. HNO_3	a) Powder floats on surface; became pinkish
	b) On shaking, 0.2 cm foam ring forms, few particles move downward and remain suspended up to 3 to 4 cm
Conc. HCl	a) Powder floats on surface no change in color
	b) Few particles slowly moving downward c) On shaking, particles remain suspended and few settle down
Glacial acetic acid	a) Few particles settle down slowly
	b) On shaking, particles remain suspended
5% I_2 water	a) Powder floats on surface
	b) On shaking, few particles settle down, few remain suspended, and few float on surface
5% $FeCl_3$	a) Powder floats on surface
	b) On shaking, particles remain suspended; few settle down at the bottom and few float on surface
5% NaOH	a) Powder floats on surface
	b) On shaking, particles move down slowly; remain suspended and float on surface
5% KOH	a) Powder particles float on surface
	b) Few particles settle down, few particles move down slowly and few are suspended

Table 5: Fluorescence analysis of *A. sativum* L. leaf powder

Name of test	*Daylight*	*254 nm*	*366 nm*
Powder as such	Green	–	–
Powder + H_2O	Umber	Light herbage green	Herbage green
Powder + HCl	Dark umber	Dark green	Dark green
Powder + HNO_3	Pale orange	Pale herbage green	Dark herbage green
Powder + H_2SO_4	Brown umber	Dark green	Black
Powder + G. acetic acid	Amber	Citrine green	Dark citrine green
Powder + 50% HCl	Sienna	Greenish glaucous	Dull green
Powder + 50% HNO_3	Pale orange	Herbage green	Dark herbage green
Powder + 50% H_2SO_4	Umber	Greenish glaucous	Olivaceous
Powder + 50% G. acetic acid	Amber	Herbage green	Dark herbage green
Powder + 1 N NaOH	Pale amber	Herbage green	Dark herbage green
Powder + 1 N NaOH	Pale amber	Herbage green	Dark herbage green
Powder + 5% iodine	Dark amber	Herbage green	Dark herbage green
Powder + 5% $FeCl_3$	Brown	Dark green	Black
Powder + Liq NH_3	Greenish amber	Herbage green	Dark herbage green

Table 6: Rf and corresponding band color observed in daylight and UV light

Rf values of daylight	*Color of band*	*Rf values of 254 nm*	*Color of band*	*Rf values of 366 nm*	*Color of band*
0.06	Pale brown	0.05	Gray	0.06	Gray
0.19	Pale brown	0.27	Gray	0.24	Black
0.25	Pale brown	0.36	Faint purple	0.74*	Faint red
0.73	Pale brown	0.53#	Faint purple (gallic acid)	0.89	Faint red
0.74*	Greenish yellow (quercetin)	0.74*	Yellow (quercetin)	0.90	Yellow
0.94	Green	0.78	Faint purple	–	–
–	–	0.94	Faint purple	–	–

*Gallic acid; #Quercetin as a standard marker

Table 7: Rf and corresponding band color observed in iodine, anisaldehyde, vanillin-sulfuric acid

Rf values of iodine	*Color of band*	*Rf values of anisaldehyde*	*Color of band*	*Rf values of vanillin*	*Color of band*
0.09	Pink	0.12	Purple	0.08	Bluish purple
0.14	Slightly yellow	0.18	Gray	0.13	Bluish purple
0.18	Slightly yellow	0.19	Bluish green	0.18	Bluish purple
0.29	Slightly yellow	0.25	Bluish green	0.44	Bluish gray
0.4	Slightly yellow	0.29	Gray	0.53#	Bluish gray
0.49	Slightly yellow	0.39	Yellow	0.74*	Brownish black
0.53#	Greenish yellow	0.53#	Gray	0.77	Light brownish black
0.71	Slightly green	0.56	Greenish blue	0.86	Bluish purple
0.74*	Dark green	0.61	Gray	–	–
0.87	Fluorescent green	0.74*	Brownish black	–	–
–	–	0.81	Gray	–	–
–	–	0.87	Bluish gray	–	–

*Gallic acid; #Quercetin as a standard marker

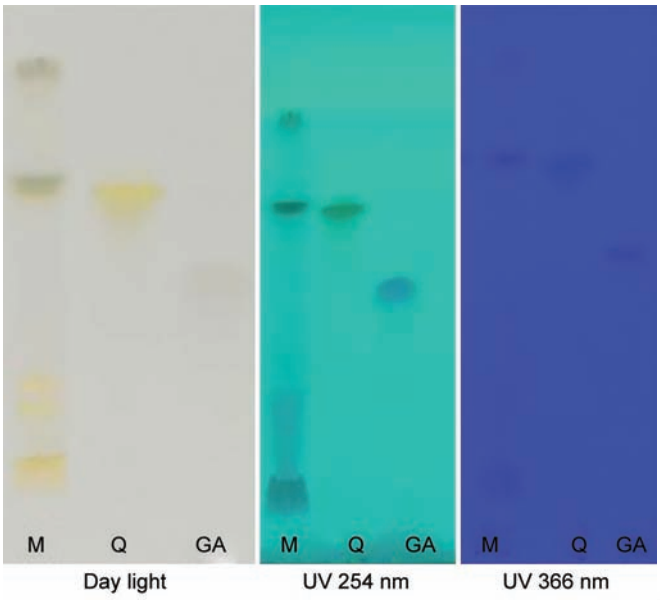

Fig. 5: The TLC of *A. sativum* L. extracts (M: Methanolic extract; Q: Quercetin; GA: Gallic acid) in daylight, ultraviolet light

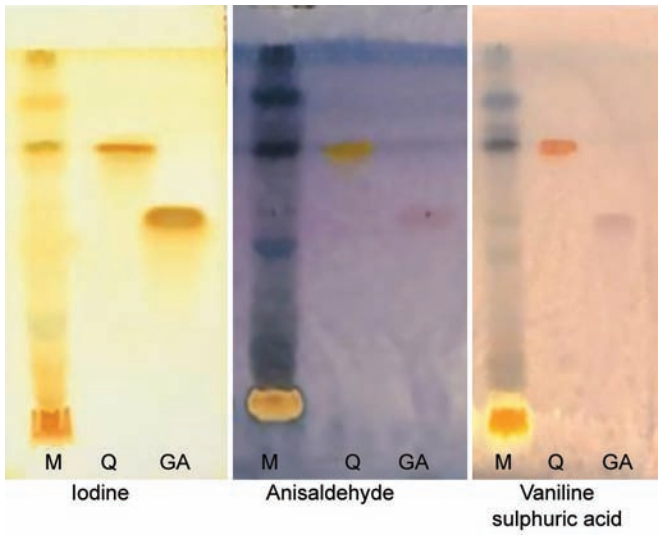

Fig. 6: The TLC of *A. sativum* L. extracts (M, methanolic extract; Q, quercetin; GA, gallic acid) in iodine, anisaldehyde, vanillin-sulfuric acid

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हिन्दी सारंश

एलियम सेटाईवम एल. की पत्ती का भैषजअभिज्ञानीय तथा पादप रासायनिक जांच

उद्देश्यः एलियम सेटाईवम लिन. कृशियोग्य औषधीय पादप है, जिसे आयुर्वेद में लहसुन के नाम से जाना जाता है। एशियाई महाद्वीप के देशो में लहसुन की पत्तियों को भोज्य व्यंजनों में तथा अनेक रोगों जैसे खांसी, श्वास, मलेरिया ज्वर, अद्रित तथा हृदय सम्बन्धी रोगो इत्यादि में, औषधि के रूप में अधिकतर उपयोग किया जाता है। लहसुन में पोषक व विटामिन के रूप में उच्च औषधीय गुण होते है तथा एशिया महाद्वीप के लोक दावों में प्रतिवेदित किया गया है। लहसुन के पूर्व में अप्रतिवेदित मैक्रोस्कोपी, माइक्रोस्कोपी, हिस्टोकेमिकल, फिजीकोकेमिकल तथा थिन लेयर क्रोमेटोग्राफी (टी. एल. सी.) मापदण्डों का अध्ययन करना आवश्यक समझा गया।

सामग्री और विधिः संगृहीत पादप नमूने को पहचाना गया, प्रमाणित किया गया तथा संस्थान के पादपालय अनुभाग में संरक्षित किया गया। छाया में सुखाई हुयी पत्तियों का चूर्ण बनाया गया। ए. पी. आई. में वर्णित मानक प्रक्रिया के अनुसार वृहत, सूक्ष्म तथा फिजीकोकेमिकल मापदंडो का निष्पादन किया गया। विश्व स्वास्थ्य संगठन में उल्लिखित प्रक्रियाओं के अनुसार फ्लुओरोसेंस विश्लेषण तथा विभिन्न रासायनिक अभिकर्मकों के साथ चूर्ण की हुई औषधी के व्यवहार का निष्पादन किया गया।

परिणामः ऑर्गेनोलेप्टिक विश्लेषण दर्शाता है कि हल्के पीले हरे रंग का तथा तीक्ष्ण गंध युक्त पत्ती चूर्ण स्वाद में कसैला है। दोनों सतहों पर ऐनोमोसाइटिक स्टोमेटा पाया जाता है। एक्वियस तथा मिथेनोलिक एक्सट्रेक्ट्स की टी.एल.सी., मानक जैसे गेलिक अम्ल तथा क्वेरसिटिन की टी.एल.सी. के संगत आर.एफ. वेल्यू तथा कलर बैंड विकसित हुई।

निष्कर्षः प्राप्त तथ्य, पत्ती के रूप में संगृहीत औषधी की पहचान तथा मानकीकरण में बहुत उपयोगी है तथा भारतीय फार्माकोपिया में ये मापदंड शामिल होंगे।

मुख्य शब्दः एलियम सटाईवम, केमेरा लूसिडा, फ्लुओरोसेंस, लोकदावे, हिस्टोकेमिकल टेस्ट, लहसुन, माइक्रोस्कोपी, फार्माकोग्नॉसी।