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Research Article

Development of Bioanalytical Standardization Parameters of *Alocasia indica* Tuber by High-Performance Thin Layer Chromatography Technique

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ABSTRACT

Alocasia indica is perennial herb growing widely and used as traditional medicine in India, China, and Bangladesh. The divine herb has potent medicinal values for the treatment of different types of illnesses. The High-Performance Thin Layer Chromatography (HPTLC) techniques were used to separate active components from ethanolic extract of tuber part of *A. indica*. This examination was intended to designed a HPTLC fingerprint profile of crude extract of the plant in ethanol. A HPTLC method for the isolation of various active constituents in *A. indica* ethanolic extract has been developed, and solvent system for quercetin the mobile phase used was toluene:ethyl acetate:formic acid (5:2:1) and for analysis of β-sitosterol the mobile phase used was chloroform:ethyl acetate:formic acid (6:4:1). In the present investigation, HPTLC fingerprint of extract of dried tuber part of *A. indica* have been performed and the results demonstrated that important information for standardization. The HPTLC system for routine quality control of present species can be used for ethanolic extract and serve in qualitative, quantitative, and was appropriate for standardization of the plant.

Introduction

Herbal and ethnic medicines having great importance in Chinese, *Ayurvedic*, Kampoian, Korean, and Unani medicinal system as these have been proficiently used in all over the world and blooming into orderly-regulated systems of medicine.[1] Medicinal plants have been playing a significant role in improving human health due to the existence of several active phyto-metabolites.

There are generally two metabolic forms, primary metabolites and secondary metabolites, found in plants, in which generally secondary metabolites showing some important pharmacological activities. Primary metabolites continuously synthesize and utilizing, whereas secondary ones are store up in tissues. Secondary metabolites are accountable for valuable medicinal effects, either alone or with a combination of other metabolites.

In nature, three major secondary metabolites alkaloids (major group) followed by terpenoids and phenolic. Plant phenolic has an ancient history of scientific investigation and stands for the majority plentiful and the widely represented class of plant natural products.[2] The *A. indica* is nutritionally found to contain varying amounts of proteins, ash, crude fiber, carbohydrate, starch, ascorbic acid, oxalates, proteases, nitrate, and tannin. It is cultivated edible aroid in India.

A. indica distributed in Sri Lanka, Bangladesh, India, South Africa, and China. It is generally grown in India and Bangladesh.[3] *A. indica* Schott. is an indigenous herb belonging to family Araceae. Different plant parts are conventionally used in inflammation and in abdomen diseases and spleen diseases. The leaves juice is used as a diuretic, digestive, astringent, laxative, and in management

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of rheumatic arthritis. It also has antifungal and hepatoprotective properties. Chemical constituents of the plant are cyanogenetic glycosides, flavonoids, gallic acid, ascorbic acid, oxalic acid, and lectins.[4]

The great block for promoting herbal drugs is to be short of the scientific evaluation methods and standardization parameters. Major problem in standardization includes, proper identification, their substitutes, adulteration, lack of convincing, and consistent scientific data for their efficacy and safety. This leads to major challenges for standardization of herbal drugs and quality control of the raw materials. The identification of bioactives is a necessary obligation for quality control of plant-based drugs. Any research study could not be proven systematically valid if the sample tested was not authenticated and characterized such that the sample can be reproduced. Herbals may be adulterated, substituted, or contaminated with other substandard species.

Authentication tools may range broadly, depending on plant material and procedure involved, from a straightforward morphological identification of herbals to detailed genetic or chemical approaches.[5] Each recognition method uses special techniques and requires different levels of prior information, infrastructure, and skillsets to achieve proper validation of the herbal product.[6] Some recently promising techniques for ensuring quality are HerboprintTM, thin layer chromatography (TLC), HPTLC, polarography, capillary electrophoresis, and DNA analysis. TLC and HPTLC are consistently used as an important tool for qualitative detection of minute impurities.[7] The present study was aimed to develop chemical fingerprint of tuber of *A. indica* and explore its phytoconstituents with their medicinal uses and its scientific validation by the HPTLC technique.

Materials and Methods

Reagents

β-sitosterol is obtained from Natural Remedies, India, Quercetin from Sigma Aldrich, USA, and solvents of analytical grade were procured from Fischer Scientific, India. TLC aluminum plates pre-coated with silica gel 60 F254 (20 × 20 mm, 0.2 mm thickness) were obtained from E. Merck Ltd., Germany.

Plant Material

The *A. indica* was collected from the district of Raipur, Chhattisgarh, in December 2015. Stay away from any type of adulteration during collection. Botanical recognition was performed by Professor N. K. Dubey, Department of Botany, Faculty of science, Varanasi, Uttar Pradesh, where a voucher specimen (accession no. Ara. 2018/1) has been submitted for further reference.

Extraction of Marker Compounds

Shaded dried tubers were powdered and subjected to extraction. The ethanolic extract of *A. indica* (EEAI) was prepared by using soxhlation. The powdered tuber (250 grams) was repeatedly extracted in a round-bottomed flask (1,000 mL) with ethanol (500 mL) 95%. The tuber extract was cooled at room temperature (25–30°C), filtered, and evaporated to dryness under condensed pressure in a rotary evaporator and kept back under refrigeration at 4°C. The yield was found to be 2.35%.

Preparation of Standards and HPTLC Conditions

HPTLC Profile

The HPTLC studies were carried out following Harborne[8] and Wagner *et al.*[9]

Sample Preparation

Stock solutions of all the standard compounds were produced in methanol (1 mg/10 mL). Optimizations of various combinations of solvents for each marker compounds were done for selection of a suitable mobile phase to achieve optimum Rf value, good resolution (in sample extracts), and great sensitivity. The standards were applied as a 6 mm band on 10 × 10 cm HPTLC pre-coated silica gel 60 F254 plates (Merck, Germany) by automatic TLC applicator Linomat-V with nitrogen flow (CAMAG, Switzerland). The plates were seen under UV 254 and 366 nm, and scanned using CAMAG scanner-IV equipped with software winCATS CAMAG 1.3.0 software. For visualization of quercetin and β-sitosterol, HPTLC plates were placed in anisaldehyde-sulphuric acid reagent (5% sulfuric acid in methanol), heated at 105°C for 5 minutes.

Developing Solvent System

The plates were then developed in the respective mobile phases. For quercetin analysis, mobile phase was toluene:ethyl acetate:formic acid (5:2:1), and for β-sitosterol analysis, mobile phase was chloroform:ethyl acetate:formic acid (6:4:1).

Sample Application

Band of EEAI was applied (14 mm in length and 1 µL in concentration) using spray technique. Sample were applied in replica on 3 × 10 cm pre-coated silica gel 60GF-254 aluminum sheets using Linomat 5 applicator attached with CAMAG HPTLC system programmed through winCATS software. For HPTLC analysis, calibration curve of marker compounds were prepared using their respective mobile phases. The stock solution of 1 mg/10 mL concentration of markers diluted to make various concentrations of 100, 200, 300, 400, 500, and 600 ng/mL. The dilutions were marked in triplicate; on 20 × 10 cm HPTLC pre-coated silica gel 60 F254 plates (Merck, Germany) in 6 mm bandwidth.

Development of Chromatogram

The chromatogram was developed after application of bands in twin trough glass chamber (20 × 10 cm) saturated with prepared solvent for 20 minutes.

Detection of Spots

The prepared plate was dried out by hot air to fade away solvents from the plate. For detection plate was sprayed with anisaldehyde sulphuric acid reagent as spray reagent and dried at heated at 105°C for 5 minutes. For better images, the plate was placed in a photo-documentation chamber (CAMAG REPROSTAR 3) and under UV light at 254 and 366 nm, respectively. The Rf values and fingerprint information were recorded by winCATS software.[10]

Phytochemical Test

Phytochemical screening of EEAI was performed for qualitative determination of constituents.[11]

Result

HPTLC fingerprinting of EEAI revealed several peaks. The ethanol extracts showed 2 spots in 5µL, 4 spots in 10 µL, and 6 spots in 20 µL concentrations (Fig. 1). HPTLC profile photo records of the EEAI at 254 nm and 366 nm was shown in Figs 1 and 2 respectively. HPTLC chromatogram of standard and different peaks of phytoconstituents of *A. indica* tuber extract were shown in Figs 3 and 4, respectively. The peak information and Rf values of the EEAI are given in Table 1, with the spots formed at Rf values. 6 spots at the following Rf 0.15 ± 0.008, 0.28 ± 0.021, 0.42 ± 0.035, 0.53 ± 0.026, 0.72 ± 0.016 and 0.84 ± 0.012 (Fig. 1), indicating the presence of 6 different components in EEAI. Preliminary HPTLC analysis of EEAI with relevant standards has shown the presence of β-sitosterol and quercetin at 254 nm and 366 nm at Rf 0.28 ± 0.021 and 0.42 ± 0.035, respectively. Preliminary phytochemical test of EEAI were shown the presence of flavonoids, cynogenetic glycosides, citric acid, ascorbic acid, polyphenolic compounds. HPTLC profile of the ethanolic extract with their height, area, and Rf value are shown in Table 2.

Table 1: Rf value of different phytoconstituents peak with color and intensity

Sample name	*No.*	*Colour*	*Intensity*	*Rf value*
Alocasia indica	1.	blue	dark	0.15 ± 0.008
	2.	purpule	medium	0.28 ± 0.021
	3.	pink	low	0.42 ± 0.035
	4.	pink	low	0.53 ± 0.026
	5.	pink	low	0.72 ± 0.016
	6.	purpule	medium	0.84 ± 0.012

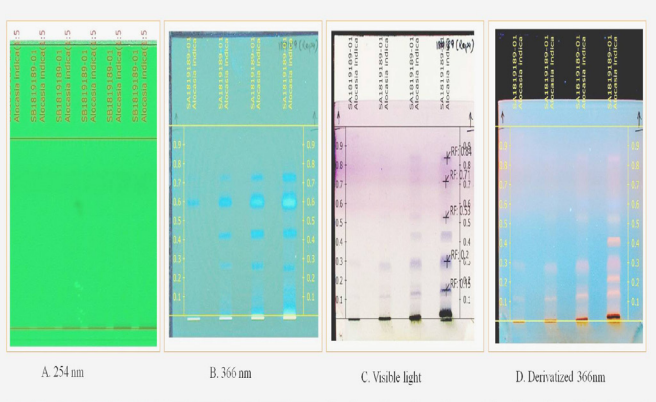

Fig. 1: HPTLC profile of ethanol extract of *A. indica* (tuber)

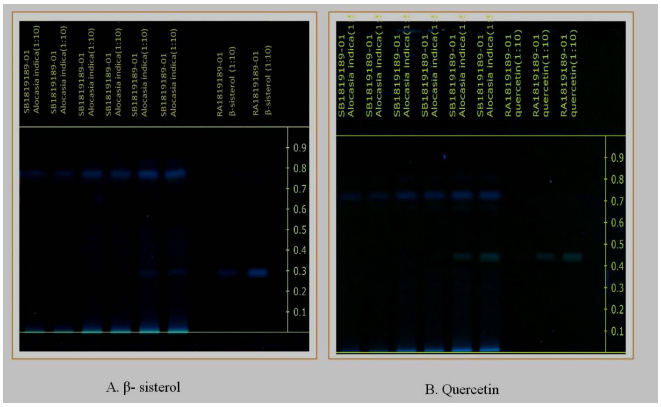

Fig. 2: HPTLC profile of standard (**A:** β-sitosterol; **B:** Quercetin)

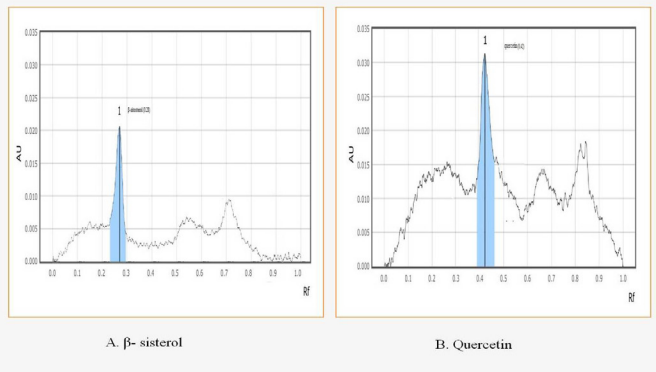

Fig. 3: HPTLC chromatogram of standard phytoconstituents peaks

Table 2: HPTLC profile of the ethanolic extract of *A. indica* (tuber)

	Start		*Max*			*End*		*Area*	
Peak	*Rf*	*Height*	*Rf*	*Height*	*%*	*Rf*	*Height*	*Area*	*%*
1.	0.114	0.212	0.129	0.223	9.42	0.161	0.194	0.009	6.9
2.	0.272	0.238	0.34	0.270	11.04	0.340	0.212	0.012	7.17
3.	0.41	0.471	0.49	0.499	20.38	0.4923	0.378	0.024	14.31
4.	0.532	0.115	0.573	0.334	32.14	0.587	0.138	0.027	34.55
5.	0.71	0.23	0.741	0.339	36.93	0.762	0.193	0.012	6.67
6.	0.81	0.113	0.831	0.122	42.1	0.840	0.19	0.01	6.1

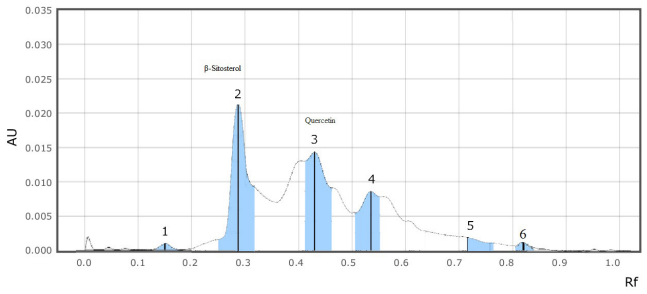

Fig. 4: HPTLC chromatogram of *A. indica* tuber extract showing different peaks of phytoconstituents

Discussion

Plants are being rich in bioactive molecules used as source material for a variety of modern drugs. From ancient times, a number of populations depended on plants as part of different medical systems and also on plant originated drugs via the complementary and alternative medicinal system.[1] Quality and scientific validation are one of the major issues to be discussed. Quality assessment and equivalence of herbal preparation is an essential prerequisite of the herbal industry. As per WHO guidelines, an herbal product required to be standardized with respect to safety before releasing it into the market. Phytochemical analysis, with fingerprinting and marker investigation for quality, ensure of the herbal drugs, is the favored approach. The importance of the marker analysis has been discussed in the various reports.[12,13] The present study presented a comprehensive approach which assimilates extraction and HPTLC fingerprinting for detection and quantization of chemical compounds in *A. indica* and further, their evaluation, to present active compound as the optimum chemical marker to ensure the quality of *Alocasia* derived drugs. Active compounds were accomplished on account of their abundance, stable existence, and noticeable on HPTLC and also by classifying their implication using multivariate statistical analysis. Principal chemical components determined in *A. indica* were β-sitosterol and quercetin. These were considered for selecting a marker compound for *A. indica*.

Quercetin is a natural flavonoid widely present in fruits, vegetables, and nuts. Quercetin having an assemblage biological actions as reported in recent years by different research groups. So, attention in open-handed benefits to human health by administering quercetin as a food supplement or dietary component has rapidly grown. Quercetin having various properties, like antimicrobial, antioxidant, anti-inflammatory, anti-tumoral, anti-bacterial, anti-viral, anti-aging, antithrombotic, anti-aggregatory, and vasodilator effects,[14-16] whereas β-sitosterol (24-ethylcholesterol) is natural phytosterol found in a lot of nuts, beans, and seeds. It is a major constituent of saw palmetto, grapple plant, *Urtica dioica*, and quite a lot of other natural remedies. β-sitosterol used in the management of cholesterol levels by preventing its intestinal absorption. It also has anti-inflammatory, analgesic properties, and reducing the symptoms of benign prostatic hyperplasia in various animal models.[17]

The HPTLC, as the advanced analytical technique, has the benefit of quick analysis as numerous samples can be run concurrently and ensuing quick comparison of phytochemical profiles.[18,19] This analytical technique used for the concurrent analysis of different constituents present in the *A. indica* plant material. HPTLC analysis of EEAI with relevant standards has shown the presence of β-sitosterol and quercetin.

Phytochemical analysis is used to confirm the authentic nature of the crude drug, therefore, it plays an imperative part in prevention of adulteration. Phytoconstituents obtained from natural resources have been increasing importance in day by day due to the health-promoting activity. So, it is necessary to make sure the quality, safety, and efficacy of herbal drugs before its consumption.[20,21] Phytochemical tests of EEAI were shown the presence of flavonoids, cyanogenetic glycosides, citric acid, ascorbic acid, and polyphenolic compounds. These phytoconstituents having multiple biological activities like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, and analgesic.[22] The data of the present study may be helpful as a standard to recognize to distinguish from its adulterants and other related species.

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