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Determination of bioanalytical parameters for the standardization of *Abroma augusta*

Dinesh K. Patel[1,2], S. P. Dhanabal[2*]

[1]*Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi-221005, India*
[2]*J.S.S. College of Pharmacy, Ooty- 643 001, India*

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ABSTRACT

Objective: To set the analytical parameters for simultaneous determination of different phytochemicals present in the *Abroma augusta* (*A. augusta*). **Methods:** Different phytochemical test including total phenol and total flavonoid content were carried out in the present investigation. Further, a rapid and simple high-performance thin-layer chromatographic (HPTLC) method was developed for the qualitative and quantitative analysis. Moreover antimicrobial study was also performed in the present investigation to know the presence of biological contamination. **Results:** Preliminary phytochemical analysis revealed the presence of glycoside, alkaloid, carbohydrate and steroid in the *A. augusta*. Total phenol and total flavonoid content were found to be 0.29% and 0.50% w/w respectively. All tested microorganism in the *A. augusta* extract were found to be under the limit. Chloroform: methanol: acetic acid: H_2O (170:25:25:4) was used as a solvent system for the qualitative and quantitative analysis through HPTLC Method. HPTLC analysis revealed 1.87 % w/w of linoleic acid in the *A. augusta* extract. **Conclusions:** Results showed that the selected parameters in the present investigation would set the new standards for the qualitative and quantitative analysis of *A. augusta*.

1. Introduction

Plants offer important source of food and drugs for human being science very early age and many of the available drugs have been derived from plant and other natural sources. Several plants have been used for the treatment of various types of disorders even without knowing its side effect, proper function, and phytoconstituents. About two to three decade ago, most of the drugs were obtained from natural sources[1-3]. The plants, parts of plants and other material from plants have been used in the practice from time immemorial for the prevention and treatment of various health aspects. More than 25% of the drugs prescribed worldwide are mainly derived from plant source. A large number of drugs in the WHO's essential medicine list is exclusively derived from plant origin. In India, about 80% of the rural population uses medicinal herbs for their primary health concern[4-6]. In the recent years, plant derived products gain much more importance in the world in the form of medicinal products, nutraceuticals and cosmetics. Herbal formulations have reached widespread acceptability as therapeutic agents for the treatment of various disorders. Standardization of herbal formulations is essential in order to assess the quality of drugs. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market[7]. Standardization is a process that ensures a predefined amount of quantity, quality, and therapeutic effect of ingredients in each dose. For quality control of herbal products, high performance thin layer chromatography (HPTLC) is a popular method for the analysis of herbal medicines. HPTLC fingerprint profile is best choice for standardization followed by determination of specific active phytoconstituents.

Ahroma augusta (Family: Sterculiaceae) (*A. augusta*), commonly known as Devils's cotton is a popular plant, mainly used for the treatment of various types of disorder

*Corresponding author: Dr. S. P. Dhanabal, Professor & Head, Department of Phytochemistry & Phytomedicine, JSS college of Pharmacy, Ooty-643 001, India.
Tel: +919443095697
E-mail: dhanskanaksahil@rediffmail.com

in the traditional system of medicine. *A. augusta* is one of the widely found plant all over in India and Australia[8,9]. The whole plant contain several alkaloids and secondary metabolites including steroids, triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosidesand phenylethanoid glycosides[10]. The leaves of *A. augusta* contain octacosanol, taraxerol, β-sitosterol acetate, lupeol, an aliphatic alcohol ($C_{32}H_{66}O$) and mixture of long chain fatty diols. Abromine, the active constituent of the *A. augusta* identified as betaine is mainly responsible for antihyperglycemic activity[11]. Different parts of *A. augusta* are useful in treating diabetes, stomachache, dermatitis, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, nerve stimulant, weakness, hypertension, uterine disorders, dermatitis, inflammation, rheumatic pain of joints and headache with sinusitis[10]. The plant is reported to have hypolipidemic effect, however root bark is reported to contain antifertility agent[8,9].

2. Material and methods

2.1. Procurement of extract and preliminary phytochemical analysis

In the present investigation, crude extract of *A. augusta* was purchased from Garlico Herbal Concentrate (M.P.), India. In order to know the different phytoconstituents in A. angusts extract, preliminary phytochemical analysis was performed as per the standard methods[12]. Moreover the presence of different phytoconstituents in the *A. augusta* extract was also confirmed through thin layer chromatography (TLC) analysis[13]. Further the crude extract of *A. augusta* was also subjected to the total phenol and total flavonoid content determination[14,15]. Microbiological assay were also performed in the present investigation as per the standard official procedure[16,17]. The quantification of linoleic acid in the *A. augusta* was determined through high performance thin layer chromatography (HPTLC) techniques.

2.2. Determination of total phenol and flavonoid content

2.2.1. Preparation of standard solution

10 mg of standard chemical (Gallic acid, rutin) was dissolved in 10 mL distilled methanol to get 1 000 μg/mL solution. Further it was serially diluted with methanol to obtained desired concentration of the solution.

2.2.2. Preparation of test solution

100 mg of extract was dissolved in 10 mL of methanol to get 10 000 μg/mL solution. These solutions were diluted with methanol to obtain lower dilution.

2.2.3. Total phenol determination

Total phenol content was determined in the *A. augusta* extracts by Folin-Ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation a green blue complex is measurable at 750 nm. 0.4 mL of extract separately mixed with 2 mL of folin reagent and 1.6 mL of Na_2CO_3. After shaking, it was kept for 2 h (Reaction time) and then absorbance was measured at 750 nm using gallic acid standard curve. The total phenol content was expressed as gallic acid equivalent in % w/w of extract.

2.2.4. Total flavonoid determination

Aluminum chloride colorimetric method was used for the total flavonoid content determination. Plant extract (0.5 mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 μg/mL in methanol.

2.3. Development of analytical parameters for HPTLC standardization

2.3.1. Preparation of standard

For the preparation of the calibration curve in the quantitative analyasis different concentration of the standard stock solution were prepared in the HPLC grade methanol.

2.3.2. Preparation of samples

For the preparation of the samples solution weight exactly 1 000 mg of the extract and dissolve in methanol and then sonicated for 10 min and the final volume of the solutions was made up to 5 mL to get stock solutions. All the needed concentration of the samples was prepared from the stock solution by suitable dilution.

2.3.4. Chromatographic conditions

For the chromatography analysis pre-coated silica gel 60 F_{254} HPTLC plates were used. All the samples and standard were dissolved in the HPLC grade methanol and filter through microne filter before application. Samples and standard compound were applied in a 6 mm band for qualitative analysis and 8mm bands for quantitative analyasis, 15 mm from the bottom of the plate, using an automated TLC applicator Linomat V (Camag, Switzerland) with nitrogen flow. These critical parameters for the HPTLC analysis were maintained for all analyses performed. The respective analytical parameters for the fingerprint and quantitative analysis were presented in the Table 1 and Table 2.

2.3.5. HPTLC plate development

For HPTLC analysis plate were developed in AMD2 development chamber using chloroform: methanol: acetic acid: H_2O (170:25:25:4) as a solvent system. The compositions

of the mobile phase were selected based on testing of different solvent compositions of varying polarities. HPTLC analyses were performed at (20±5) ℃ and 50% relative humidity. After development of the plate of the separated constituents, compounds were quantified using a TLC scanner (Camag, model 3) equipped with CATS software (Camag). Slit width 8 mm × 0.4 mm; wavelength 254 nm; absorption reflection scan mode were used in the present analysis.

Table 1
Analytical and chromatographic conditions for HPTLC fingerprint analysis of *A. augusta*.

Parameters	Analytical conditions
Analysis	HPTLC fingerprint analysis of *A. augusta*
Plate material	HPTLC precoated plates silica gel Merck $60F_{254}$
Solvent system	Chlroform:methanol:acetic acid:H_2O (170:25:25:4)
Application mode	CAMAG automatic TLC Sampler III
Development mode	Ascending

Table 2
Chromatographic parameters for HPTLC quantification of linoleic acid in *A. augusta*.

Parameters	Analytical conditions
Analysis	Estimation of linoleic acid in *A. augusta*
Plate material	HPTLC precoated plates silica gel Merck $60F_{254}$
Solvent system	Chlroform:methanol:acetic acid:H_2O (170:25:25:4)
Application mode	CAMAG automatic TLC Sampler III
Development mode	Ascending

3. Results

For the development of the analytical parameters, different phytochemical analysis were performed in the present investigation. Glycoside, alkaloid, carbohydrate and steroid were found to be present whereas other phytoconstituents such as tannin, triterpenoid, triterpenoid, saponin, amino acid and protein were found to be absent in the *A. augusta* extract. TLC analysis were also performed for *A. augusta* extract, which showed two spots in chloroform: methanol: H_2O (64:50:100) solvent system with 0.81 and 0.89 R_f value and three spot in tolune: acetone: glacial acetic acid (3:2:2) solvent system with 0.83, 0.70, 0.93 R_f value respectively. HPTLC fingerprint analysis revealed the presence of five prominent spots in chloroform:methanol:acetic acid:H_2O (170:25:25:4) solvent system (Table 3). Total phenol and flavonoid content were determined as per the standard official methods and were found to be 0.29% and 0.50 % w/w in the *A. augusta* extract. Microbiological assay were also performed in the present investigation to know the biological contaminant in the samples and found that E. coli and salmonella was found to be absent whereas total bacterial count and yeast & moulds contents were found to be in the limit. HPTLC techniques were used in the present investigation to quantifiy the linoleic acid content in the *A. augusta*. Chloroform:methanol:acetic acid:H_2O (170:25:25:4) solvent system was found to be most suitable solvent system for the quantification of linoleic acid in the *A. augusta* and for fingerprint analysis. The content of linoleic acid in *A. augusta* was found to be 1.87% w/w.

Table 3
HPTLC fingerprint analysis of *A. augusta*.

No of spot	Solvent system	R_f value	Maximum peak height	Peak area (%)
5	Chlroform: methanol: acetic acid: H_2O (170:25:25:4)	0.31	28.4	4.58
		0.42	19.3	1.71
		0.68	34.9	7.84
		0.88	214.3	47.97
		1.00	246.7	37.90

4. Discussion

For the optimization of the suitable solvent system for HPTLC analysis, different compositions of the mobile phase were used in the TLC analysis before quantitative analysis. Once the solvent system optimized, all the samples and standard were developed in the HPTLC plates in order to know the suitability of the chosen solvent system. In the present study chloroform:methanol:acetic acid:H_2O (170:25:25:4) was found to be the most suitable solvent system for simultaneous determination of linoleic acid in the *A. augusta* extract and fingerprint analysis. Data related to the HPTLC fingerprint analysis was presented in the Table 3. HPTLC analysis was performed as per the method optimized above and the respective quantities of the linoleic acid were calculated as per standard method. From the data analysis it was found that *A. augusta* extract contains measurable amounts of linoleic acid. The specificity of the developed method was investigated by analyzing the standard marker compound and the extract at different concentrations. The presence of the respective linoleic acid was confirmed by comparing the Rf values and spectra of the sample spot with that of the standard. The peak purity of the standard compound and samples was determined by comparing the spectra at three different concentration using peak area, peak height and R_f values of the spot.

Due to the unmatched availability of chemical diversity, natural product plays an important role in the drug development and discovery program. Phytochemical analysis revealed the presence of various phytoconstituents in the *A. augusta* such as glycoside, alkaloid, carbohydrate, etc. These phytoconstituents have been reported to have multiple biological effects such as anti-inflammatory, antiallergic, antioxidant, antidiabetic, aldose reductase inhibitory potential, anti-viral and anti-cancer activities[18]. Several medicinal plants have been used as dietary supplements and for the treatment of various disorders without proper knowledge of their mode of action. Therefore, there is a need of more well documented standard i.e. clinical trials and more laboratory work to justify their pharmacological actions and toxicity for safe and effective treatment[19]. Aalkaloids, flavonoids, tannins, phenols, saponins, and

several other compounds play an important role in the defense mechanism against microorganisms, insects and other herbivores in the plants[20]. Phytochemical standards are generally used for determining the identity, purity and strength of the drug source. These parameters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration[7,21,22]. Phytochemical standardization includes preliminary phytochemical analysis and quantification of different phytoconstituents (marker compounds) present in the extract[23]. For the standardization of the plant extract/material, HPTLC technique plays an important role due to analysis of several samples simultaneously with less time. TLC and HPTLC techniques solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis[24].

Therefore, from the above-mentioned analytical parameters, it was found that *A. augusta* contain significant amount of phenol, flavonoid and linoleic acid. The above mention analytical parameters could be useful to the researcher to quantify the phytochemical present in the *A. augusta* and these parameters will also be helpful for the standardization of the *A. augusta* in the future.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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