Research Article

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PHYTOCHEMICAL SCREENING AND HPTLC FINGERPRINTING OF *ANOGEISSUS ACUMINATA* EXTRACTS

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ABSTRACT

Anogeissus acuminata (family Combretaceae) is a plant widely distributed in western parts of India like North Gujarat, Rajasthan, and Madhya Pradesh. Parts of this plant are used in treatment of different conditions in traditional medicine. The plant is also used in other parts of world such as Thailand for treatment of diabetes mellitus. The plant is evaluated for various pharmacological actions. However, systematic phytochemical data for the plant is lacking. Therefore, we evaluated the methanolic extract of plant qualitatively and quantitatively. The methanolic extract of the leaves and bark of plant were found to possess abundant phenolic compounds namely, tannins and flavonoids. Quantitative determination of these extract revealed a presence of 24.57 and 13.63 %w/w tannin and 14.5 and 57% w/w flavonoids in leaf and bark extract respectively. HPTLC fingerprinting analysis of methanolic extract of leaf and bark with mobile phase (toluene: ethyl acetate: formic acid (4.5:3.0:0.2,v/v/v) confirmed the presence of four peaks in both the extracts with different R_f values at 254nm.

Keywords: *Anogeissus acuminata*, quantification of tannins, quantification of flavonoids, Aluminum chloride method, HPTLC fingerptinting.

INTRODUCTION

A herbal drug may encompass several beneficial pharmacological activities, which may improve the therapeutic outcome of the disease. To date, over 400 traditional plant treatments for diabetes have been reported[1], although only a small number of these have received scientific and medical evaluation to assess their efficacy. Major hindrance in amalgamation of herbal medicine in modern medical practices is lack of systematic scientific profiling[2]. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs should be further investigated for discovery of potential anti diabetic medicines[3].

Anogeissus acuminata (Syn *A. pendula*), known as kardhai or dhok, is common in the Northern Gujarat and dry deciduous forests of western India. Leaves of the plant are used in traditional and tribal medicine of Andhra Pradesh to treat painful inflammatory conditions[4]. Aerial parts of the tree are also used for treatment of diabetes mellitus[3]. Lignans isolated from *A. acuminata* have been found to possess HIV1- reverse transcriptase inhibitory activity[6]. Methanolic extract of the leaves has also shown anti- inflammatory and analgesic activity[2]. It has shown promising antidiabetic effect in diabetes mellitus in several studies[5]. However, systematic phytochemical evaluation of methanolic extract of plant is lacking. Therefore, we aimed to perform the phytochemical investigation and HPTLC analysis of the plant.

MATERIALS AND METHODS

Plant material and extraction

Aerial parts and bark of the plant were collected from Khedbrahma, Gujarat in the month of March. Herbarium of the collected sample was submitted for authentication at NISCAIR, Delhi with provided reference no. NISCAIR/RHMD/consult/ 2013/2290/70. Leaves and bark of plant were dried in shade and made into a coarse powder. 50 gm of this powder was extracted in Soxhlet extractor with methanol (200 ml × 3). The filtered extract was dried in vacuum drier and stored at 4°C. The yield for leaves and bark was 18% and 10% w/w respectively in terms of dried starting material.

Phytochemical analysis

The phytochemical investigation of the methanolic extracts of leaf and bark of *Anogeissus acuminata* was carried out using standard protocol[7,8]. The color intensity and appearance of solids in reaction mixture was observed. The results are stated in Table 1.

Quantification of tannins

Estimation of tannin content of the extract was performed by using AOAC Official Method, 1975[9]. 25 ml indigo sulphonic acid was added to conical flask containing 25 ml extract. This mixture was titrated against 0.1 M potassium permanganate solution until golden yellow colour was obtained. Tannin content of extract was calculated by using factor, each ml of 0.1 M potassium permanganate solution is equivalent to 0.004157g of tannin compound calculated as tannic acid.

Quantification of flavonoids

Flavonoid content was determined by Aluminum chloride method using quercetin as standard. Calibration curve of quercetin (6.25, 12.5, 25, 50,100 µg/ml) was prepared. 1 ml of standard or extract solution was taken into 10 ml volumetric flask, containing 4 ml of distill water. 0.3 ml of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added to the mixture. 2 ml of 1M NaOH was added and volume made up to 10 ml with distilled water. The absorbance was recorded at 510 nm using UV-Visible spectrophotometer[10].

HPTLC fingerprinting analysis

Chromatography was performed on Merck Silica gel 60F254 TLC precoated aluminum plates. 20 µl of freshly prepared samples were applied on the plate as a band of 10 mm width with the help of LINOMAT V Automatic Sample Spotter at the distance of 10 mm from the edge of the plate and from each other. Different combinations of mobile phase were used, however, toluene: ethyl acetate: formic acid (4.5:3.0:0.2,*v/v/v*) demonstrated best separation. CAMAG twin trough chamber (10x 10 cm) was used for development of plates. Saturation time of 20 minutes was given. The mobile phase was allowed to run to a distance of 80 mm. The evaluation of developed plates was done at the wavelength of 254 nm with WinCats Software (CAMAG) using TLC scanner 3. .

Table 1: Phytochemical screening of methanolic extracts of leaf and bark oF *Anogeissus acuminata*

Chemical constituent	Test	Bark extract	Leaf extract
Alkaloids	Dragendorff test	+	+
	Hager's test	+	+
	Mayer's test	-	-
	Wagner's test	-	-
Glycosides	Borntrager's test	-	-
	Legal's test	-	-
Carbohydrates	Molisch's test	-	-
	Fehling's test	-	-
Tannin	$Fecl_3$ test	+++	+++
Triterpenoids	Liberman Buchard test	-	-
Flavonoids	Shinoda's test	++	++
	Alkali test	++	++
	Acid test	++	++
Phenols	$Fecl_3$ test	+	+
	Lead acetate test	+	+
Saponins	Foam test	+	+
Steroids	Salkowskis test	-	-
	Liberman Buchard test	-	-
Fixed oils and fats	Spot test	-	-
Proteins and free amino acid	Biuret test	+	+

+ = presence, - = absence

Table 2: Tannin and flavonoid content of leaf and bark extracts

	Leaf	Bark
% of tannin in extract (w/w)	24.57%	13.63%
% of flavonoids in extract (w/w)	14.5%	57%

Table 3: Peak list and R_f values of chromatograms of *Anogeissus acuminata* extracts at 254nm

Peak No	Maximum R_f	Peak Area	% Area
Leaf extract			
1	0.12	3081.7	39.02
2	0.26	1445.4	18.30
3	0.38	2349.7	29.75
4	0.51	1020.8	12.93
Bark extract			
1	0.31	5804.2	13.42
2	0.38	18932.1	43.77
3	0.44	9525.1	22.02
4	0.50	8996.4	20.80

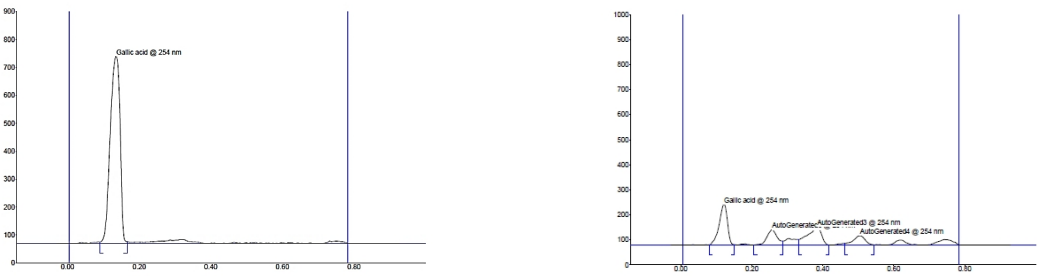

Figure 1: HPTLC chromatogram of gallic acid and leaf ectract

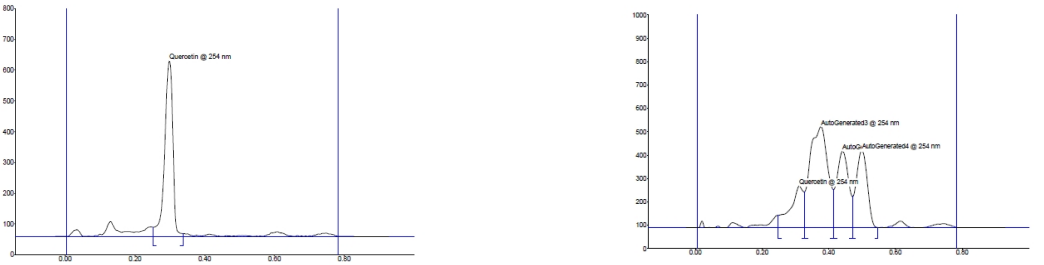

Figure 2: HPTLC chromatogram of quercetin and bark ectract

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical tests of the extracts revealed the presence of several chemical constituents as shown in Table 1**.** Major constituents noted were tannins, flavonoids, phenols and saponins**.**

Quantification of tannins and flavonoids

By preliminary screening it was observed that both extracts have abundant tannins and flavonoids. Therefore, tannin and flavonoid contents of leaf and bark extracts were determined. (Table 2)

HPTLC fingerprinting analysis

In the current study HPTLC analysis of *A. acuminata* methanolic extract was performed using gallic acid and quercetin as reference standards, as plant was found to be rich in tannins and flavonoids. For optimization of method, different combinations of mobile phases were tried with varying results. Among the different systems tried toluene**:** ethyl acetate**:** formic acid **(**4.5**:**3.0**:**0.2,*v/v/v***)** showed best separation of chemical constituents**.** The fingerprinting analysis of both methanolic extracts by HPTLC chromatograms revealed the presence of four peaks at R_f values as presented in Table 3. Peak 1 in leaf extract (R_f value= 0.12) was found comparable with that of standard gallic acid at wavelength 254 nm. Peak 1 in bark extract (R_f value = 0.31) was found to be corresponding with that of quercetin (Figure 1, 2) at 254 nm.

CONCLUSION

Methanolic extract of *Anogeissus acuminata* was found to have notable amount of tannins and flavonoids. HPTLC fingerptinting analysis of the extracts revealed the presence of gallic acid in leaf and quercetin in bark extract. These are potent active principles known to have multiple biological actions. This implicates and justifies the varied pharmacological actions possessed by the plant.

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