Research Journal of

Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 8 (3): 133-138, 2014
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2014.133.138

Qualitative and Quantitative Phytochemical Studies of *Acanthus ilicifolius*

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ABSTRACT
The present study was aimed to determine the phytochemicals of *Acanthus ilicifolius by* qualitative and quantitative techniques. Benzene, chloroform, acetone, ethanol and methanolic leave extract of *Acanthus ilicifolius* screened for their preliminary phytochemicals by standard qualitative methods. Based on this, three bioactive compounds viz., gallic acid, quercetin and lupeol were quantified using HPTLC. Results of preliminary screening expressed presence of phenols, flavonoids and triterpenoids. The Rf values of gallic acid, quercetin and lupeol are 0.21, 0.36 and 0.71, respectively. The amount of gallic acid, quercetin and lupeol was 12.26, 58.45 and 71.89 μg mL^{-1}, respectively. In conclusion, quantification of gallic acid, quercetin and lupeol showed good resolution and separation. Further, it will be used to herbal formulation for therapeutic applications.

Key words: HPTLC, lupeol, flavonoid, phytochemical

INTRODUCTION
Phytochemicals are often used to describe the large number of secondary metabolic compounds found in plants and animals with known protective and human consumers functions (Harborne, 1982). *Acanthus ilicifolius* (Family: Acanthaceae) is commonly known as holy mangrove and used as a traditional medicine to treat pain, inflammation and ulcer in Parangipettai coastal village, Southeast coast of India. Furthermore, analgesic, anti-inflammatory, hepatoprotective and antimicrobial activities were validated (Babu *et al*., 2001). Rapid and systematic measurement of specific plant metabolites are a serious challenge for analytical chemists, phytochemists and biochemists because of their inherent structural diversity and dietary impact. In this context, in 2000, the US Food and Drug Administration (FDA) issued a draft of Guidance for Industry Botanical Drug Products (FDA, 2000). Recently, many separation techniques have been proposed to separate, identify and quantify the phytoconstituents in plants. Among that, high performance liquid chromatography is a key technique to quantify the secondary metabolites (Satyavani, 2013). There was insufficiency of scientific data in the phytochemicals of *A. ilicifolius*. Therefore, the present study aimed to evaluate phytochemicals, qualitative and quantitative methods using HPTLC.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves of *A. ilicifolius* were collected from Parangipettai coast, Tamil Nadu, India during January 2008. The vouchered specimen (AUCASMB 01/2008) was deposited in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, India.

Extraction: About 3 kg leaves of *A. ilicifolius* were dried over polythene cover in shade drying method at 21°C and pulverized using a mixer grinder. The coarse powder was used only for the preparation of extract. One kilogram powdered material of *A. ilicifolius* was cold macerated with 3000 mL of benzene, chloroform, acetone, ethyl acetate, methanol and ethanol for three days. After that, the suspension was filtered and the residues were removed. The filtrate of each extract was evaporated and dried at 40°C under reduced pressure in a rota-evaporator to separate the benzene, chloroform, acetone, ethyl acetate, methanol and ethanol extract. The final residual extract was used for further experiments.

Qualitative analysis of phytochemicals: Different extracts viz., benzene, chloroform, acetone, methanol and ethanol in *A. ilicifolius* were used to determine the preliminary phytochemicals followed by the method of Evans (1997).

Preparation of standard solution

Gallic acid: The 10 mg of gallic acid was dissolved in methanol and making upto 10 mL with methanol. Then 1 mL was pipetted out from stock solution and made upto 10 mL with methanol to get the final concentration of 100 $\mu g\ mL^{-1}$.

Quercetin: The 10 mg of quercetin was dissolved in methanol and making upto 10 mL with methanol to get final concentration of 1000 $\mu g\ mL^{-1}$.

Lupeol: The 10 mg of lupeol was dissolved in chloroform and making upto 10 mL with chloroform. Then 1 mL was pipette out from the stock solution and made upto the volume 10 mL with chloroform to get the final concentration of 100 $\mu g\ mL^{-1}$.

Estimation of gallic acid and quercetin: The 100 mg of ethanolic extract was dissolved in ethanol and making upto 10 mL to get the concentration of 10 $mg\ mL^{-1}$ (Test GA and Test Q). The solution was filtered and used for chromatographic analysis.

Estimation of lupeol: The 100 mg of the ethanolic extract was dissolved in chloroform and making upto 10 mL to get the concentration of 10 $mg\ mL^{-1}$ (Test L). The solution was filtered and used for chromatographic analysis.

HPTLC analysis: The samples were spotted in the form of bands with CAMAG microlitre syringe on a precoated silica gel GF254 plates (20×20 cm with 0.2 mm thickness, E. Merck) using camag linomat V. Automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in CAMAG glass twin trough chamber previously saturated with the solvent for 30 min. The distance was 8 cm subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC scanner in absorbance at 254, 280 and 538 nm operated by Wincats software 4.03 (Table 1).

Table 1: Quantification of gallic acid, quercetin and lupeol in *A. ilicifolius*

Parameters	Gallic acid	Quercetin	Lupeol
Stationary phase	Silica gel GF254 plates	Silica gel GF254 plates	Silica gel GF254 plates
Mobile phase	Toluene: Ethyl acetate: Formic acid (6:4:0.8)	Toluene: Ethyl acetate: Formic acid (6:4:0.8)	Toluene: Ethyl acetate: Formic acid (6:4:0.8)
Standard	Gallic acid (100 μg mL^{-1})	Quercetin (1000 μg mL^{-1})	Lupeol (100 μg mL^{-1})
Sample	Test GA (10 mg mL^{-1})	Test Q (10 mg mL^{-1})	Test L (10 mg mL^{-1})
Migration distance	80 mm	80 mm	80 mm
Scanning wavelength	254 nm	280 nm	538 nm
Mode of scanning	Absorption (deuterium)	Absorption (deuterium)	Absorption (deuterium)

Table 2: Phytochemical screening of *A. ilicifolius* extracts

Phytochemical test	Reagents used	Benzene	Chloroform	Acetone	Ethanol	Methanol
Alkaloids	Mayer's reagent	N	N	N	P	P
	Wagner's reagent	N	N	N	P	P
	Hager's reagent	N	N	N	P	P
Carbohydrates	Benedict's reagent	P	P	P	P	P
	Fehling's reagent	P	P	P	P	P
Saponin	Foam test	N	N	N	N	N
Glycosides	Modified brontrager's	P	P	P	P	P
Phytosterol	Salkowski's test	P	P	P	P	P
Resins	Acetone water test	N	N	N	N	N
Phenols	Ferric chloride test	N	P	N	P	P
Tannins	Gelatin test	N	P	N	P	P
Terpenoids	Copper acetate test	N	P	N	P	P
Flavonoids	Alkaline reagent test	N	P	N	P	P
	Lead acetate test	N	P	N	P	P
	Shinoda test	N	P	N	P	P
Proteins and amino acids	Xanthoproteic test	P	P	P	P	P

P: Positive, N: Negative

RESULTS AND DISCUSSION

Phytochemical studies: Qualitative results of *A. ilicifolius* extracts indicated the presence of alkaloids, flavonoids, phenols, carbohydrate, tannins, terpenoids, glycosides and proteins significantly high amount in ethanolic extract of *A. ilicifolius*. The chloroform and acetone extracts having alkaloids, flavonoids, phenols, carbohydrate, tannins in moderate amount. Ethanolic extract of *A. ilicifolius* showed the presence of higher levels of terpenoids and phenolics as compared to other extracts (Table 2). Hence, the quantification was carried out in the ethanolic extract.

HPTLC: High performance liquid chromatography method is a very desirable method for phenolic constituent's estimation from *A. ilicifolius*. Toluene:Ethyl acetate:Formic acid (6:4:0.8 v/v/v) as mobile phase gave the best resolution of gallic acid and quercetin (Rf-0.22, 0.36), respectively. Toluene:Ethyl acetate (7:3 v/v) as mobile phase for lupeol with Rf values 0.70 of the other components of the methanolic extract of *A. ilicifolius*. The identity of band of gallic acid, quercetin and lupeol in *A. ilicifolius* extract was confirmed by overlay in UV absorption spectra with those of the standards gallic acid and quercetin, while identity of bands of lupeol in *A. ilicifolius* extract was confirmed by overlay in visible spectra with those of the standard lupeol using CAMAG TLC scanner 3. The peaks of gallic acid, quercetin and lupeol in *A. ilicifolius* extract were confirmed by overlaying the absorption spectra (Fig. 1). The amount of gallic acid, quercetin and lupeol was 12.26, 58.45, 71.89 μg mL^{-1}, respectively.

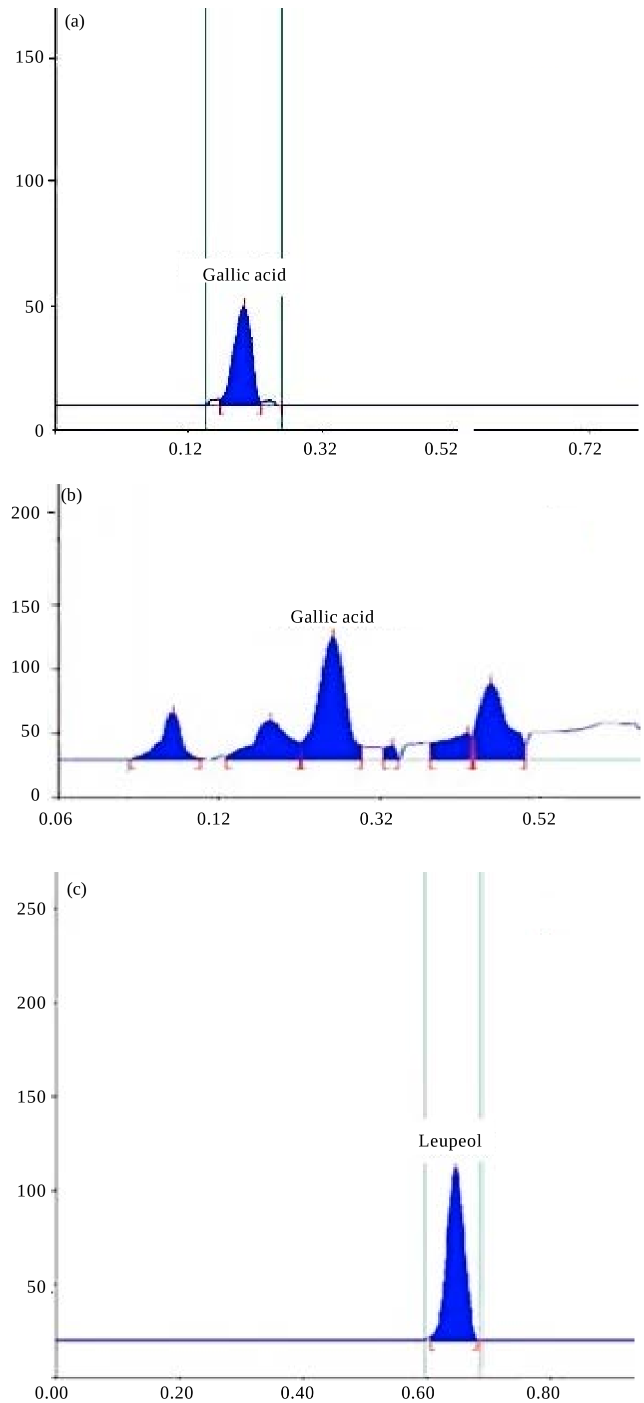

Fig. 1(a-f): Continue

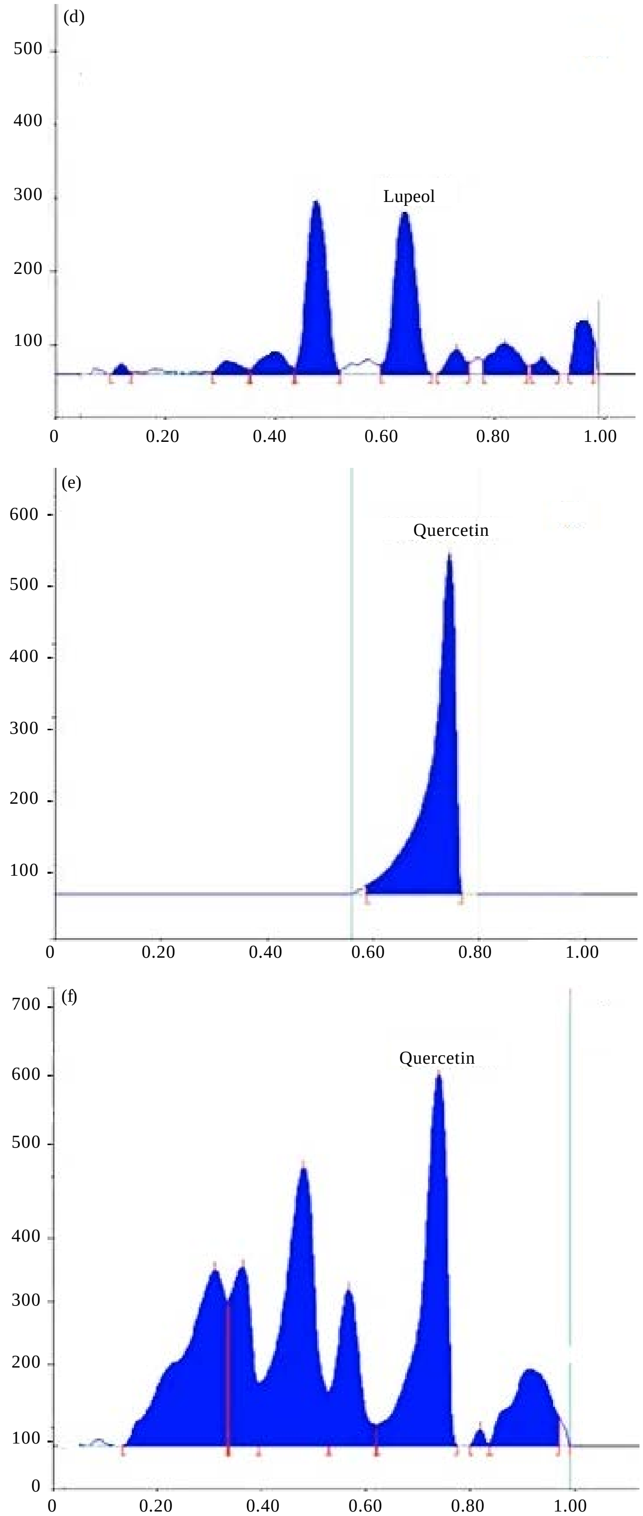

Fig. 1(a-f): HPTLC chromatogram of ethanolic extract of *Acanthus ilicifolius*, (a) Standard gallic acid, (b) Gallic acid of AIEEt, (c) Standard lupeol, (d) Lupeol of AIEEt, (e) Standard quercetin and (f) Quercetin of AIEEt

CONCLUSION

In conclusion this the first report of gallic acid, quercetin and lupeol was identified and quantified from *A. ilicifolius*. Quantification of gallic acid, quercetin and lupeol showed good resolution and separation. Further, it will be used to herbal formulation for therapeutic applications.

ACKNOWLEDGMENT

The authors are grateful to the authorities of Annamalai University and BioMed Research Management Services, Portonovo, Tamil Nadu, India for providing the necessary support.

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