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Isolation of Phytoconstituents from the leaves of *Anogiessus latifolia* Linn

Shanny Denny[1], Sumit Gupta[3], Usha Gavani[2], Padmaa M Paarakh[2], Ashutosh Mishra[4]

1. *Department of Pharmacognosy, KKECS, Bangalore.*
2. *The Oxford College of Pharmacy, Bangalore*
3. *GIS institute of Professional Studies, Dehradun (U.K).*
4. *A N D College of Pharmacy, Gonda (U. P.)*

Abstract

Chloroform acetone fraction of the methanol extract and crude ethanol extract of the leaves of Anogiessus latifolia Linn (Combretaceae) led to the isolation of 4,18-di methyl, 22-methyl, 15-hydroxy, 11,12-naptha phenanthrene (1) and gallic acid (2). Their structures were elucidated by spectroscopic methods such as UV, IR, NMR, LCMS and Co-TLC pattern. Compound 1 was isolated for the first time from this plant.

***Key words**: Anogiessus latifolia, 4,18-di methyl, 22-methyl, 15-hydroxy, 11,12-naptha phenanthrene, gallic acid, spectroscopic method.*

Corresponding Author's E-mail: - sg4u1983@yahoo.com

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Introduction:

The genus *Anogeissus* (Family. *Combretaceae*) includes four or five different species of tropical trees. Out of these *A. latifolia* is widely distributed in farther India. *Anogeissus latifolia* (Local name- Dhai, Family- Combretaceae) is a small to medium-sized tree up to 36 meters tall, which grows all over Chittagong division in Bangladesh. The bark has been reported to be useful in the treatment of skin diseases, snake and scorpion bite, stomach diseases, colic, cough and diarrhoea. The wound healing and free radical scavenging activities of the plant have also been documented. Updated phytochemical investigations with *A. latifolia* leaves were reported for the presence of (+) leucocyanidin, ellagic acid and glycosides of ellagic and flavellagic acids[1], quercetin, myricetin, and trimethyl ellagic acid[2], quercetin, myricetin and trimethyl ellagic acid[3]. It is reported to posses anti ulcer, antimicrobial[4], antioxidant[5], hepatoprotective[6], wound healing[7], anti-fungal[8], CNS depressant[9], anti-aging[10] and insecticidal[11] activities.

Material and Methods

Plant material

Anogiessus latifolia Linn leaves were collected and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen has been preserved in the Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

General instrument details

UV: Shimadzu UV VIS-1700; IR: JASCO FTIR 5300; LCMS: Agilent 1100 LC-MSD APCI; 1H-NMR (500 MHz).

Extraction and isolation procedure

Coarsely powdered leaves (750 gm) were extracted with petroleum ether followed by benzene, chloroform, acetone, methanol and aqueous by the process of continuous extraction (soxhlation). The crude extracts were evaporated to dryness in a rotary film evaporator. Methanol extract was subjected to column chromatography over silica gel (60-120 mesh) using petroleum ether, taking 100 ml fraction each time. Compound I was isolated from Chloroform : Acetone (7:3) fraction respectively while Compound 2 was isolated from the precipitate obtained from the alcohol extract which was further purified by fractional crystallization

Table 1- Percentage Yield of extracts at Small scale and large scale.

Extracts	Color of the extract	% Yield (w/w)	
		Small Scale	Large Scale
Pet Ether	brownish-green	2.24 %	2.42 %
Benzene	brownish-green	0.9 %	1.4 %
Chloroform	Dark green	0.30 %	0.47%
Acetone	Dark yellow	12.4 %	14.10%
Methanol	Reddish Brown	14.5 %	15.02 %
Aqueous	Dark brown	11.0 %	12.24 %

Table 2- Phytochemical screening

Chemical constituent	Test	Petroleum Ether	Benzene	Chloroform	Acetone	Methanol	Aqeous
Alkaloids	1.Mayer's test	-	-	-	-	-	-
	2.Dragendroff	-	-	-	-	-	-
	3.Wagner's test	-	-	-	-	-	-
	4.Hager's test	-	-	-	-	-	-
Carbohydrates	1.Molisch's test	-	-	+	+	+	+
	2. Benedict's	-	-	+	+	+	+
	3. Fehling test	-	-	+	+	+	+
Anthracene	1.Borntrager's test	-	-	-	-	-	-
Cardenolides	2.Legal test	-	-	-	-	-	-
	3. Baljet test	_	_	_	_	_	_
Saponins	1.Foam test	_	_	_	_	_	_
Phytosterol	1.Salkowaski test	+	+	+	-	-	-
	2. Libermann Burchard test	+	+	+	_	-	-
	3. Libermann test	_	_	_	_	_	_
Fats & oil	Stain test	_	_	_	_	_	_
Resins	1.Acetone water test	_	_	_	_	_	_
Phenols	1. Ferric chloride test	_	_	_	+	+	+
	2.Lead acetate Test	_	_	_	+	+	+
Flavanoids	1.Gelatin test	_	_	_	_	_	_
	2.Shinoda test	_	_	_	+	+	+
	3.Acid Base test	_	_	_	+	+	+
Proteins	1. Ninhydrin test	_	_	_	_	_	_

RESULTS and DISCUSSION

Total two compounds were isolated. Their structures were elucidated by spectroscopic methods such as UV, IR, NMR and LCMS.

In the IR spectra the presence of a broad spectrum at 3431 cm-1 shows the presence of –OH group and the peaks at 2935, 2867 and 1640 cm-1 appeals the presence of aromatic rings which can be further confirmed by the presence of the sharp peak at 7.15 in NMR spectra. In mass spectra the fragmentation peak at 173 and 185 shows the fragmentation of phenanthrene and naphthalene rings respectively.

Compound 2 was identified on the basis of the Co-TLC method in which the Rf value of the sample

compound matched with the Rf value of the standard gallic acid.

After characterization the isolated compound I and II was found to be 4,18-di methyl, 22-methyl, 15-hydroxy, 11,12-naptha phenanthrene and gallic acid.

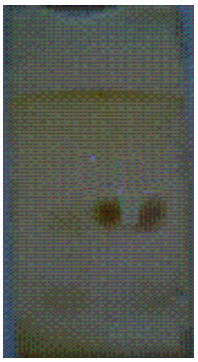


Fig. 1 **CO- TLC for Compound II**

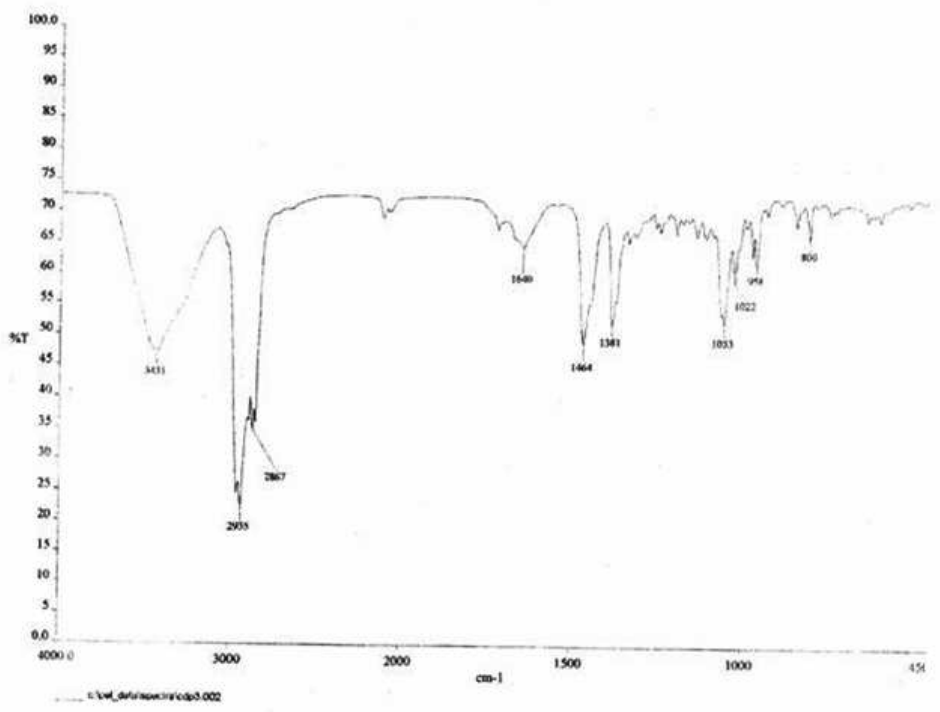


Fig. 2 IR spectra of Compound I

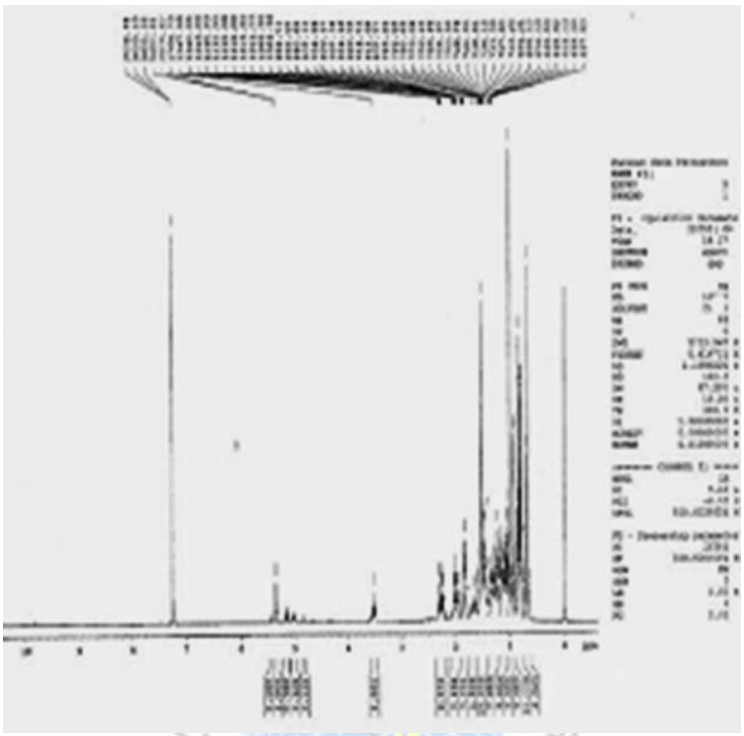


Fig. 3 NMR spectra of Compound I

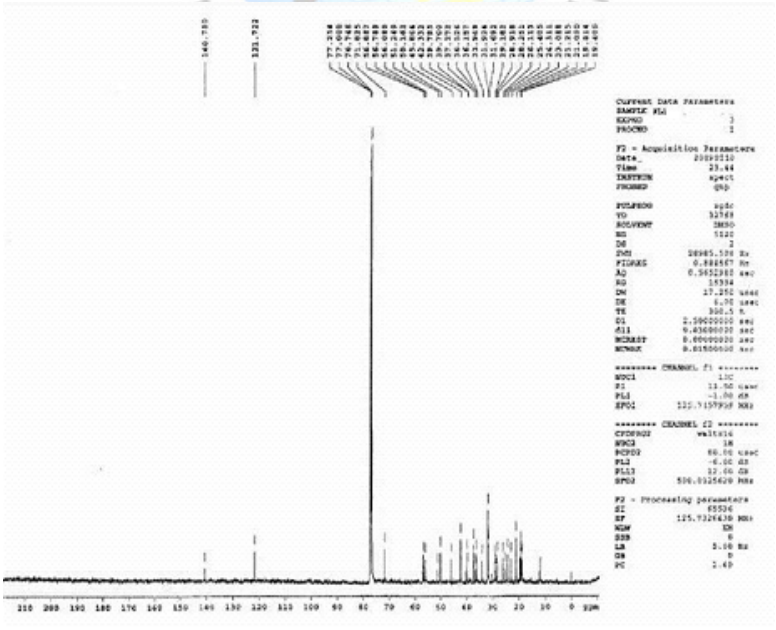


Fig.4 NMR Spectra of Compound I

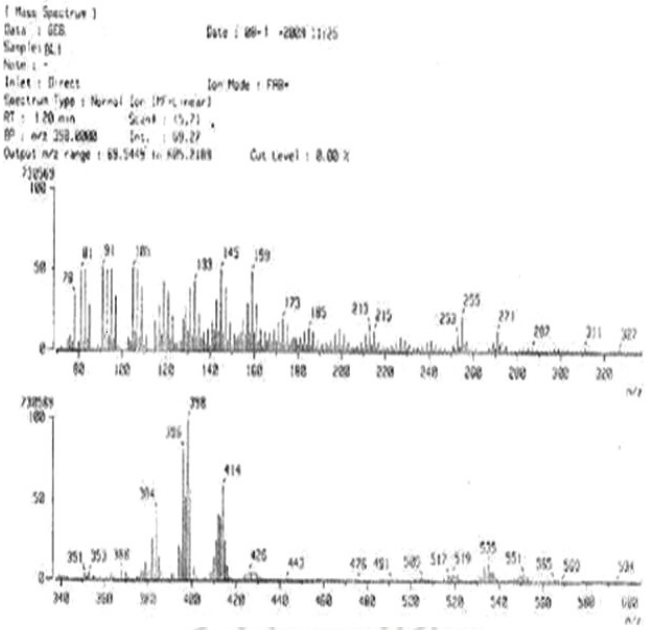
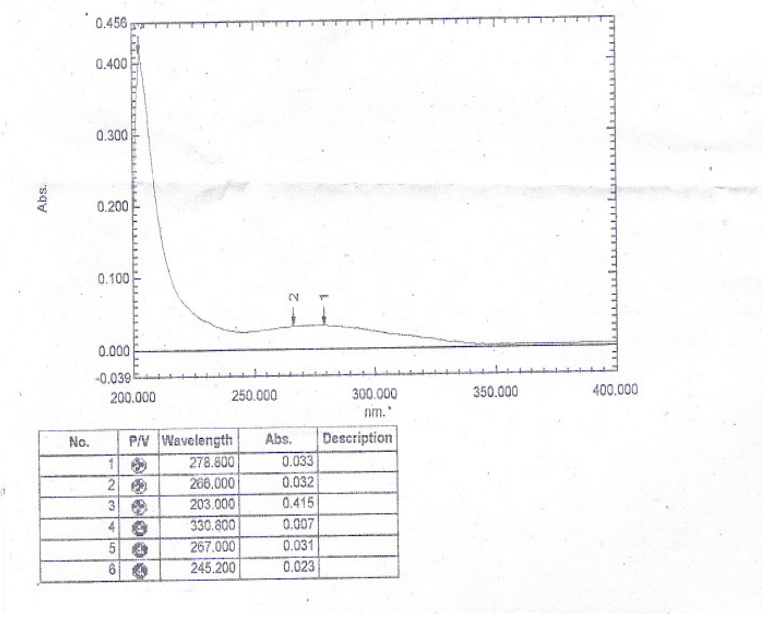


Fig.5 Mass spectra of Compound I



No.	P/V	Wavelength	Abs.	Description
1		278.800	0.033	
2		266.000	0.032	
3		203.000	0.415	
4		330.800	0.007	
5		267.000	0.031	
6		245.200	0.023	

Fig.6 UV spectra of Compound I

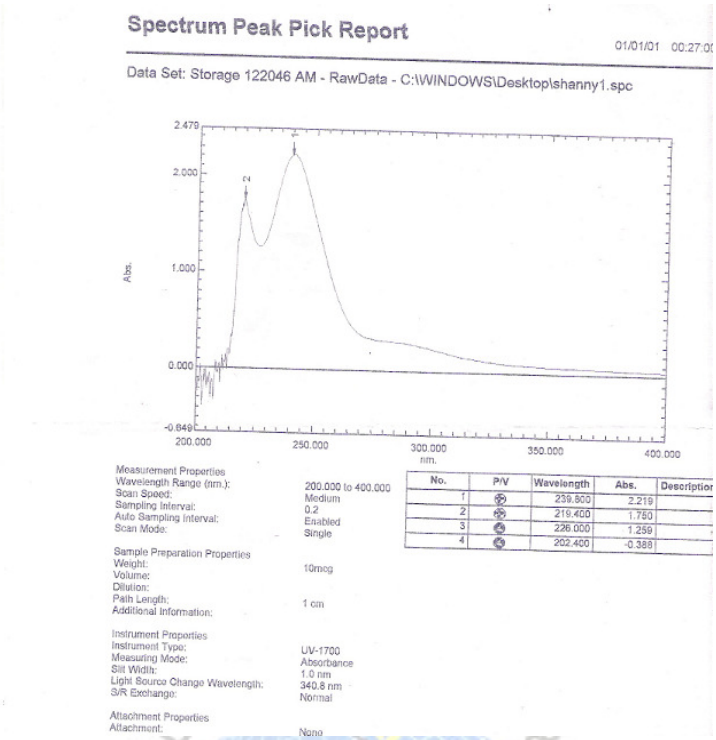


Fig. 7 UV spectra of Compound II

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