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NOTE

Phytochemical and biological evaluation of *Cassia nodosa* root bark

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Abstract : Phytochemical investigation of the ethanolic extract of the dried root bark of *Cassia nodosa* Buch.-Ham ex Roxb afforded four anthraquinones : 1,8-dihydroxy-3-methylanthraquinone, 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone, 1,5-dihydroxy-3-methoxyanthraquinone and 1,5-dihydroxy-3-methoxy-7-methylanthraquinone, three triterpenoids : lupenone, lupeol and α-amyrin, two sterols : β-sitosterol and its glucoside along with palmitic acid and pinitol. All these have been isolated for the first time from the root bark of this plant. The compounds were characterized by their spectral (IR, Mass, ^{1}H, ^{13}C NMR) data. The extract and various fractions were evaluated for their antibacterial, antifungal and antioxidant activities, where significant effect was observed against the selected test bacteria as also antioxidant activity.

Keywords : *Cassia nodosa*, phytochemistry, antimicrobial, antioxidant.

Introduction

Cassia nodosa Buch.-Ham ex Roxb (Family : Caesalpiniaceae) commonly known as 'Pink Mohur' is a medium sized tree grown in gardens for ornamental purpose. A survey of literature revealed the isolation of anthraquinones and their glycosides, sterols, flavonoids and glycosides from flowers[1-3], leaves[4-6], roots[7], root bark[8], stem[9], stem bark[10] and seeds[11,12]. Since, there is hardly any work on the root bark of this plant, the isolation and identification of constituents and antibacterial, antifungal and antioxidant activities of the extract and various fractions were undertaken.

Results and discussion

1,8-Dihydroxy-3-methylanthraquinone (Chrysophanol) : It was eluted with pet. ether : benzene (1 : 3) and crystallized as dark orange crystals ($CHCl_3$-MeOH), m.p. 187-188 °C[13], $C_{15}H_{10}O_4$, M^+ 254. It gave positive colour reactions with methanolic NaOH and magnesium acetate which indicated its anthraquinone nature.

1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (Physcion) : It was eluted with benzene : ethyl acetate (4 : 1) and crystallized as orange crystals ($CHCl_3$-MeOH), m.p. 198-200 °C[14], $C_{16}H_{12}O_5$, M^+ 284. It gave positive colour reactions for anthraquinones.

Lupenone : It was eluted with pet. ether : benzene (4 : 1) as a white solid and crystallized as white crystals (acetone), m.p. 166-168 °C[15], $C_{30}H_{48}O$, M^+ 424. It gave positive LB and Noller's test for triterpenoids.

Lupeol : Elution with pet. ether : benzene (3 : 2) followed by crystallization furnished white needles (acetone), m.p. 210-212 °C[16], $C_{30}H_{50}O$, M^+ 426. It gave characteristic LB and Noller's test for triterpenoids.

α-Amyrin : It was eluted with pet. ether : benzene (1 : 4) and crystallized as white needles ($CHCl_3$-MeOH), m.p. 183-185 °C[17], $C_{30}H_{50}O$. It gave positive LB and Noller's test for triterpenoids.

Palmitic acid : Elution with pet. ether yielded light yellow waxy solid which on crystallization afforded white amorphous powder (acetone-pet. ether), m.p. 58-60 °C[18], $C_{16}H_{32}O_2$, M^+ 256.

β-Sitosterol : Elution with pet. ether : benzene (1 : 4) followed by crystallization from chloroform-methanol mixture gave white needles, m.p. 135-137 °C. It gave positive LB test for sterols.

β-Sitosterol-β-D-glucoside : Elution with benzene : ethyl acetate (1 : 4) gave white solid which on evapora-

tion crystallized as white granules (MeOH), m.p. 292–293 °C, $C_{35}H_{60}O_6$, FAB mass 599 ($M^+ + Na^+$)[19]. It gave positive LB test for sterols and Molisch's test for sugars and did not reduce Fehling's solution. On hydrolysis it gave β-sitosterol, m.p. 135–137 °C and glucose.

1,5-Dihydroxy-3-methoxyanthraquinone : It was eluted with benzene : ethyl acetate (3 : 2) and crystallized as orange powder (C_6H_5-MeOH), m.p. 189–190 °C[20], $C_{15}H_{10}O_5$, M^+ 270. The colour reactions with methanolic NaOH and magnesium acetate indicated its anthraquinone nature.

1,5-Dihydroxy-3-methoxy-7-methylanthraquinone : It was eluted with benzene : ethyl acetate (1 : 1) and crystallized as yellow needles (C_6H_5-MeOH), m.p. 190–192 °C[21], $C_{16}H_{12}O_5$, M^+ 284. It gave positive colour reactions with methanolic NaOH and magnesium acetate which indicated its anthraquinone nature.

D-Pinitol : It was eluted with ethyl acetate and crystallized as white powder (MeOH), m.p. 185–187 °C[22], $C_7H_{14}O_6$, M^+ 194.

Antibacterial and antifungal activity :

The ethanolic extract and various fractions of the root bark of *C. nodosa* were also screened for antibacterial and antifungal activity against the bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeroginosa* and fungi *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Penicillium chrysogenum* by disc diffusion method[23]. Gentamycin and ketoconozole were used as reference compound for evaluating antibacterial and antifungal activities respectively. It was observed that the ethanolic extract exhibited significant activity against *E. aerogenes*, *P. aeroginosa* and *S. aureus*, with efficacy against *E. aerogenes* (AI = 1.32) even better than that of the standard (Table 1). Amongst the various fractions, the dichloromethane was most effective against *E. aerogenes* (AI = 1.60) and *P. aeroginosa* (AI = 0.96). In case of antifungal activity (Table 2), the ethanolic extract and various fractions exhibited only mild activity against all fungi except *P. chrysogenum* where no activity was observed.

Antioxidant activity :

The antioxidant activity of ethanolic extract of the root bark of *C. nodosa* was studied by carrying out the free radical scavenging activity employing DPPH (1,1-diphenyl-2-picrylhydrazyl) radical[24] and total reduction capability by Fe^{3+}-Fe^{2+} transformation[25]. In both cases, ascorbic acid was used as reference marker. The extract exhibited significant free radical scavenging activity using DPPH. The low absorption value at 10 μg/ml indicated its effectivity even at low concentration. Also, IC_{50} was found to be nearly equivalent to that of the reference compound, ascorbic acid (5.2 μg/ml) thus, indicating high antioxidant potency of the root bark of *C. nodosa* (IC_{50}

Table 1. Antibacterial activity of *C. nodosa*

Sl. no.	Test microbes		Nature of extract/fraction			
			Ethanol	Pet. ether	Dichloro-methane	Ethyl-acetate
1.	*B. subtilis*	IZ[a]	13.00	16.60	15.80	12.20
		AI[b]	0.60	0.78	0.76	0.54
2.	*E. aerogenes*	IZ	22.00	10.00	27.00	14.00
		AI	1.32	0.54	1.60	0.78
3.	*E. coli*	IZ	13.00	11.00	13.80	10.60
		AI	0.70	0.58	0.82	0.52
4.	*P. aeruginosa*	IZ	16.20	15.40	19.30	17.70
		AI	0.88	0.76	0.96	0.84
5.	*S. aureus*	IZ	17.20	10.80	14.70	11.40
		AI	0.86	0.54	0.76	0.62

[a]IZ = Inhibition zone (in mm) including the diameter of disc (6 mm).

[b]AI = Activity index = Inhibition zone of sample/Inhibition zone of standard; Standard : Gentamycin.

Table 2. Antifungal activity of *C. nodosa*

Sl. no.	Test microbes		Nature of extract/fraction			
			Ethanol	Pet. ether	Dichloro-methane	Ethyl-acetate
1.	*A. flavus*	IZ[a]	12.80	11.60	15.20	11.80
		AI[b]	0.54	0.52	0.60	0.44
2.	*A. niger*	IZ	18.00	11.00	13.40	16.20
		AI	0.68	0.40	0.52	0.58
3.	*C. albicans*	IZ	12.00	11.80	10.70	10.20
		AI	0.60	0.58	0.62	0.44
4.	*P. chrysogenum*	IZ	-	-	-	-
		AI	-	-	-	-

[a]IZ = Inhibition zone (in mm) including the diameter of disc (6 mm).

[b]AI = Activity index = Inhibition zone of sample/Inhibition zone of standard; Standard : Ketoconazole.

(-) = No activity.

Table 3. Antioxidant activity

Test material	Free radical scavenging effect						Total reduction capability				
	Absorbance (nm)[a]						Absorbance (nm)				
	Concentration (μg/ml)						Concentration (μg/ml)				
	10	20	40	60	80	IC_{50}	62.5	125	250	500	1000
C. nodosa	0.158	0.152	0.151	0.149	0.147	5.1	0.524	0.688	0.792	0.876	1.362
Ascorbic acid	0.121	0.101	0.064	0.061	0.051	5.2	0.381	0.504	0.544	1.241	2.162
[a]Blank.	3.913										

= 5.1 μg/ml). A study of total reducing power indicated that at low concentration, the root bark extract of *C. nodosa* exhibited higher absorbance, i.e. better reducing power than the ascorbic acid (standard). Thus the above studies indicated that the root bark extract possesses significant antioxidant effect (Table 3).

Experimental

General : Melting points were determined in open end capillaries using Gallenkamp melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on Jeol AL 300 MHz and 75 MHz instrument using $CDCl_3$ and DMSO-d_6 as solvents and TMS as the internal reference respectively. EIMS spectra were recorded on a Hitachi model RMU 6E and Jeol D-300 mass spectrometer. The IR spectra were recorded as KBr pellets on an 8400S Shimadzu FT-IR spectrometer. Column chromatography was run using silica gel (60–120 mesh) and TLC on silica gel G in different solvent systems. In general, ceric ammonium sulphate and UV light were used for visualization of TLC spots. However, for anthraquinones 10% methanolic NaOH was used.

Plant material : The root bark of *C. nodosa* Buch.-Ham ex Roxb was collected from University of Rajasthan campus, Jaipur during the rainy season and identified in Department of Botany, University of Rajasthan, Jaipur (Herbarium Sheet No. RUBL 20159).

Extraction and isolation : Air-dried and coarsely powdered root bark of (2.8 kg) was exhaustively extracted with ethanol (95%) on a steam bath for 3 × 8 h. The pooled extract was concentrated under reduced pressure to give a reddish brown semi-solid mass (122.0 g). This ethanolic extract was re-extracted with pet. ether, dichloromethane and ethyl acetate which on concentration afforded 2.8 g, 0.7 g and 82.0 g dark reddish brown solids respectively. Since pet. ether and dichloromethane fractions exhibited a similar TLC profile (benzene : ethyl acetate, 1 : 1), they were mixed together and chromatographed over silica gel which on elution with solvent of increasing polarity afforded eight compounds which included two anthraquinones, 1,8-dihydroxy-3-methylanthraquinone and 1,8-dihydroxy-6-methoxy-3-methylanthraquinone; three triterpenoids, lupenone, lupeol and α-amyrin; one long chain hydrocarbon, palmitic acid and two sterols, β-sitosterol and β-sitosterol-β-D-glucoside. Further, ethyl acetate soluble fraction when applied over silica gel column with solvents of increasing polarity afforded three compounds including two anthraquinones, 1,5-dihydroxy-3-methoxyanthraquinone and 1,5-dihydroxy-3-methoxy-7-methylanthraquinone and a cyclitol, pinitol. All these compounds have been isolated for the first time from the root bark of this plant.

Conclusion

Phytochemical investigation of the ethanolic extract of the dried root bark of *Cassia nodosa* Buch.-Ham ex Roxb afforded eleven compounds including four anthraquinones, three triterpenoids, two sterols along with palmitic acid and pinitol. All these have been isolated for the first time from the root bark of this plant. The extract and some fractions were found to exhibit significant activity against the selected test bacteria as well as antioxidant effect.

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