INT. J. BIOL. BIOTECH., 3 (3): 587-590, 2006.

PHYTOCHEMISTRY AND BIOACTIVITY OF *CASSIA ITALICA*

M. H. Kazmi, Aliha Zehra, Sadia Khan, Farah Siddique and Saira Hameed

Department of Applied Chemistry, University of Karachi, Karachi -75270, Pakistan

ABSTRACT

Phyto chemical studies on *Cassia italica* resulted in the isolation of some known β sistosterol stigmasterol, α myrine, 1,5 dihydroxy-3-methoxy-7-methyl anthraquinone, 1,5 dihydroxy-3 methyl anthraquinone and a new anthraquinone. Structure of new compound was assigned on the basis of spectral studies. The new compound showed anti bacterial activity.

Key words: *Cassia italica*, bioactivity, anthraquinone, phytochemistry.

INTRODUCTION

Cassia (Mill) Lam. ex. F.W Andr is one of the most important genera of Casealpinaceae. It consists of 600 species mostly distributed in tropics and subtropics. In Pakistan 24 species are found. Most of the species are cultivated in Sindh, Balochistan and Punjab (Ali and Nasir, 1977; Baquer, 1889). It is used in indigenous system of medicine for the treatment of constipation, biliousness, gout, and ring worm and other parasites of skin disease (Perry and Metzgar, 1980; Krishna, 969). Saponins, flavonide sterols, triterpenes have been reported (Thomson, 1971) from the hexane insoluble fraction of this plant. We have isolated a new anthraquinone (1) besides five other reported compounds identified as 1,5 dihydroxy-3-methoxy-7-methyl anthraquinone, 1,5 dihydroxy-3-methyl anthraquinone, β-sitosterol, stigmasterol, and α-amyrine. The medicinal importance of *Cassia italica* prompted us to carry out bioactivity of the isolated anthraquinone.

MATERIALS AND METHODS

Cassia italica was collected from Karachi University campus. A voucher specimen has been deposited in the Herbarium of Department of Botany, University of Karachi.

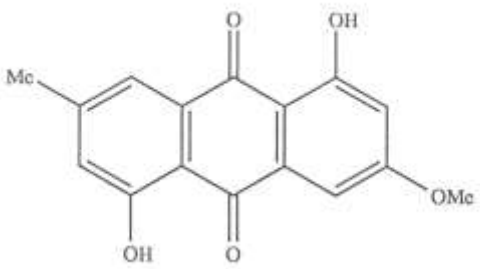
The freshly collected whole plant material (20 Kg) was dried under shade, the material was chopped in small pieces and soaked in methanol for two weeks. The methanol extract was evaporated at reduced pressure to yield dark brown gummy residue (200 gm). It was partitioned between chloroform and water. The chloroform soluble residue was further divided into n-Hexane soluble and insoluble fractions. The hexane insoluble residue was subjected to column chromatography. The elution was carried out with various mixture of n-hexane, chloroform and methanol in order of increasing polarities.

Isolation of sterols and anthraquinone

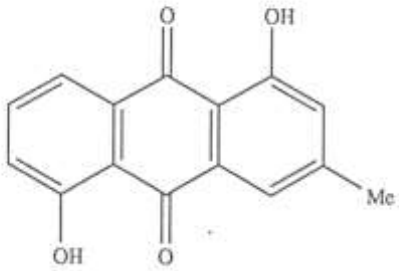
The chloroform soluble fraction was further divided into hexane soluble and insoluble fractions. The hexane soluble fraction was subjected to column chromatography on silica gel. Then elution was carried out with hexane chloroform in increasing order of polarities and obtained β sisterol, sigtrasisterol, α amyrine, Insoluble fraction was also subjected to column chromatography on silica gel, an anthraquinone was eluted at (1:1) n-hexane-chloroform. Finally compound was purified by using different solvent systems on TLC with hexane, chloroform, methanol (3:6.5:0.5). UV light and ceric sulphate spray was used for visualization of sisterols as well as anthraquinone. They were chemically elucidated by E_1 mass, HRMS, ^{1}H NMR and C^{13}NMR spectroscopy.

1,5 dihydroxy-2-hydroxy methyl-antraquinone

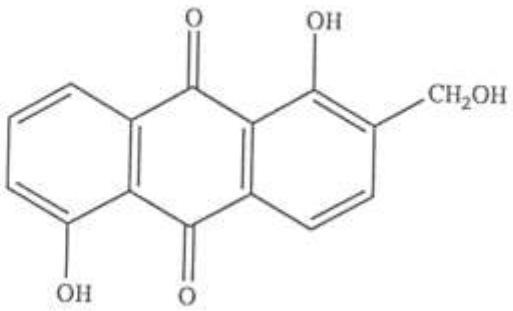
MP 204-206^o, $[\alpha]^{25}$ D^{126} ($CHCl_3$).UVλ_{max} EtoH nm 289 (log ε4.46) , 350 (log ε4.54), 450(log ε4.65), 475 (log ε4.67); IR γmax (KBr) cm^{-1} 2800 – 3400, 1625, 1325; MS m/z (rel int); 270 [$C_{15}H_{10}O_5$] (80) 242 [M-CO]$^+$ (50) 214 [M-CO]$^+$ (25), 183 [M-CH_2OH]$^+$ (20) ^{1}H NMR ($CDCl_3$ 500 MHZ) δ:12.26[1H,S,OH,1],12.16[1H,S,OH-5],4.63[2H, d, CH_2OH-2], C^{13}-NMR ($CDCl_3$, 500 MHZ) δ:162.64 (C-1),107.53 (C-2) 120.16 (C-6), 117.82 (C-7), 137.20 (C-8), 13460 (C-8a), 163.62 (C-9), 113.32 (C-9a), 192.73(C-10) 135.36 (C-10a) and 64.15(CH_2OH-2). The physical and spectral data identified compound 1 as 1, 5 dihydroxy 2 hydroxy methyl anthraquonone reported earlier from assignment were made through comparison with related compounds (Aktar *et al*., 1992).



1



2



3

Anthraquinone Compounds

Bioactivity

The antibacterial activity of compounds was determined by Agar well diffusion method proposed by Jaffer *et al*. (1988). One loop full of 24 hr – old culture of selected bacteria spread on the surface of Mueller Hinton Agar plates wells were dug in the medium with the help of sterile borer. A stock solution of new test compounds (2 mg / ml) was prepared in DMSO and dilutions of the stock solution containing hundred microlitre of each dilution was added to their respective wells and after 24 hr growth inhibition of bacteria was observed.

Table. I Antibacterial Activity of a new and some known compounds of *Cassia italica.*

a) 1,5 dihydroxy-3-methoxy-7-methyl anthraquinone

Name of Organisms	Concentration of 1,5 dihydroxy -3-methoxy-7 methyl anthraquinone ($\mu g/100\mu l$) used with zone of inhibition (mm)				Control (DMSO) 100 μl
	50	100	150	200	
Bacillus anthracis	+	18 mm	22 mm	25 mm	+
Corynebacterium pseudodiphthericum	+	10 mm	12 mm	16 mm	+
Pseudomonas aeruginosa	+	10 mm	12 mm	15 mm	+
Pseudomonas pseudomalliae	+	12 mm	15 mm	22 mm	+
Staphylococcus aureus	+	2.8 mm	3.8 mm	10 mm	+
Vibrio cholerae	+	2 mm	4 mm	6 mm	+
Salmonella typhi	+	2 mm	4 mm	5 mm	+
Shigella dysenteriae	+	2 mm	3 mm	6 mm	+
Shigella sonnei	-	-	-	-	-

b) 1,5 dihydroxy–3–methyl anthraquinone

Name of Organisms	Concentration of 1,5 dihydroxy 3-methyl anthraquinone ($\mu g/100\mu l$) used with zone of inhibition (mm)				Control (DMSO) 100 μl
	50	100	150	200	
Bacillus subtilis	+	2 mm	3 mm	5 mm	+
Vibrio cholerae	+	+ mm	+ mm	2 mm	+
E. coli 76	+	+ mm	+ mm	2 mm	+
Salmonella typhimurium	+	2 mm	3 mm	4.5 mm	+
Streptococcus lactis	-	-	-	-	-
Salmonella typhi	+	+ mm	2 mm	4 mm	+
Shigella dysenteriae	+	+ mm	2 mm	3 mm	+
Shigella sonnei	+	+ mm	3 mm	4 mm	+
Proteus vulgaris	-	-	-	-	-

c) 1,5 dihydroxy–2–hydroxy– methyl anthraquinone

Name of Organisms	Concentration of 1,5 dihydroxy -2-hydroxy-methyl anthraquinone ($\mu g/100\mu l$) used with zone of inhibition (mm)				Control (DMSO) 100 μl
	50	100	150	200	
Bacillus subtilis	+	2 mm	3 mm	5 mm	+
Vibrio cholerae	+	3 mm	5 mm	6 mm	+
E. coli 76	-	-	-	-	-
Salmonella typhimurium	+	2 mm	4 mm	4 mm	+
Streptococcus lactis	-	-	-	-	-
Salmonella typhi	+	2 mm	4 mm	5 mm	+
Shigella dysenteriae	+	+	+	3.5 mm	+
Shigella sonnei	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-

RESULTS AND DISCUSSION

Compound 1 gave the characteristic colour reaction of 1,5-dihydroxy anthraquinone derivative (Imres et al. 1974). The HRMS gave the $[M]^+$ peak at m/z 270.0459 corresponding to the molecular formula $C_{15}H_{10}O_5$ (Calcd. $C_{15}H_{10}O_5$, 270.241). The UV spectrum showed absorption maxima at 289 (log ε4.46), 350 (log ε4.54), 450 (log ε4.65), and 475 (log ε4.67) nm. The last maximum was characteristic of 1,5 dihydroxy anthraquinone system (Thomson *et al*., 1971). The IR spectrum showed bonds at 3400 (OH group) and at 1600 cm^{-1} for chelated carbonyl group.

The broad band ^{13}C-NMR showed fifteen carbon atoms, their multiplicities were determined through DEPT experiment to keeping the last polarization phase angle θ=45°, 90° and 135°. It showed the presence of nine quaternary one methylene and five methane carbon atoms. The ^{1}H-NMR spectrum showed the characteristic downfield signals for the chelated hydroxyl groups at δ12.26 and δ12.16 and a hydroxyl methyl group which gave 2H doublet at δ4.63. This was confirmed by the down field methylene signal in the C13 NMR spectrum at δ64.12 ppm and an M-29 peak in the mass spectrum. The proton at C-3 and C-4 formed an AB system showing an ortho coupling of 8HZ between them, allowing us to assign position 2 to primary alcoholic group.

The compound (1) was tested against number of organisms. It was found to be active against *B. anthracis*, *Corynebacterium pseudodiphthericum*, *Pseudomonas aergenosa* and *P. paseudomalliae*. Zone of inhibition also increased with the conc. of the compound. In the case of *B. anthracis* the maximum zone of inhibition (25 mm) was observed against 200 µl /100 µl.

The compound (2) was tested against a number of organisms. It was found to be active against *B. subtitles.* Zone of inhibition was 5 mm at 200µg / 100µl. While *S. typhimurium, S. dysenteriae; S. sonnei* showed 5 mm zone

of inhibition. The compound was low resin. The compound was not active against *S. lactis, Proteus vulgaris* which showed no zone of inhibition.

The new compound (3) was found to be active against *Vibro cholerae*. The zone of inhibition was 6 mm at 200 µl /100 µl. While *B. subtilis, Salmonella typhi* showed 5 mm zone of inhibition. The compound was not active against *E. coli* 76, *S. sonnei, S. lactis* and *P. vulgaris* which showed no zone of inhibition.

The compound 1 was conformed as 1,5 dihydroxy anthraquinone derivative by (Imres *et al*., 1974) and further structure was conformed by (Thomson *et al*., 1971)

The data obtained for antibacterial activity of the known as well as unknown confirms the results obtained by Dharelal *et al*. (1974) and Baquir *et al*. (1985)

This preliminary study in compounds were established the occurrence of antibacterial component. Structure elucidation of the active compounds and further work will be reported in due courses the present antibacterial studies are in accordance with the finding of Fransworth (1961).

REFERENCES

Ali, S. I. and E. Nasir (1977). *Flora of west Pakistan* No. 54, Department of Botany, University of Karachi p.16.

Baquar, S. R. (1989). *Medicinal and Poisonous plant of Pakistan*. Printas Karachi, Pakistan p. 88.

Perry, L. M. and J. Metzgar (1980). *Medicinal plants of East and South Asia*. The MIT press Cambridge. England p.210.

Krishna, A. Murhi, eds. (1969). *The wealth of India*. Council of Scientific and Industrial Research, New Dehli Vol 2 p. 94

Thomson, R. H. (1971). *Naturally occurring Quniones*. 2nd Edn. Academic Press p.367.

Imres S., Oztunc, A. and Büyüktimkin, Ziganein und Ziganein – 1 – Methylather: (1974). *Zsei Neue Anthrachinone Aus Digitals Schischkinii Phytochemistry*, 13: 681.

Kitanaka, S., M. Igarashi and M Takido (1985). Formation of Pigments by the Tissue culture of *Cassia accidentalis J. Chem. Pharm. Bull*., 33, p. 971

Akhtar, N., A. Malik and S.U. Kazmi (1992). Procergenin, and Antibacterial Cardenolide from *Calotropis procera*. *Phytochemistry*, 31: 2821.

Dhar, M.L., B.N. Dhawan, C.R. Persad, R.R. Rastogi, K.K. Singh and T.S. Tandon (1974). Serreming of Indian Plants for biological activity part V. *Ind. Jour, Exp. Biol.*, 12: p.512.

Baqir, S.N., S. Dilnawaz and M.S. Rafi (1985). Screening of Pakistani plants for antibacterial activity. *Pak. J. Sci. Ind. Res.*, 28: 4.

Farnsworth, N. R. (1961). The pharmacognosy of the per-winkles vinca and catharanthus. *Lloydia* 24: 105.

(*Accepted for publication May 2006*)