Antibacterial Activity of Polyphenols of *Garcinia Indica*

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The aim of present work is to study the antibacterial activity of polyphenols isolated from the ethyl acetate soluble of methanol extract of stem bark of *Garcinia indica* against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* by paper disc method. The results showed good antibacterial activity against *S. aureus* at higher concentrations, moderate at lower concentrations, against *S. typhi* moderate at higher concentrations but no activity against *E. coli* even at higher concentration for flavononylflavone. With proauthocyanin *S. Aureus, S. Typhi* and *E. coli* showed good antibacterial activity at higher concentration only.

Key words: Antibacterial activity, biflavonoid, flavononylflavone, *Garcinia indica*, proauthocyanidin

Garcinia has more than 200 listed polygamodioecious trees and shrubs distributed widely in nature, of which 30 are identified in India. *Garcinia indica* (choiss) belongs to Clusiaceae (earlier Guttiferae) family is a slowly growing polygamodioecious tree[1]. It is distributed through out topical Asia, Africa and Polynesia[2]. In India it is found in the topical humid evergreen rain forest of Western Ghats of South India as well as in the North Eastern states of India[3]. It is popularly known as *kokum* in Hindi, *amsol* in Marati and *punarpulli* in Malayalam in India[1]. It is now included under the list of endangered species of medicinal plants of South India[4].

The root is astringent[5]. Fruit fat is demulcent and emollient[6]. It is a remedy for dysentery and diarehia, tumors, heart complaints, stomach acidity and liver disorders[7]. Fruit rind extracts have been shown antifungal and antioxidant properties[5]. Garcinol, the compound isolated from fruit rind exerts antiinflammatory effects and is a neuroprotectant[8].

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(-) Hydroxycitric acid from leaves and fruit rind is antiobesity and anti cholesterol drug[9]. Seed oil rich of long chain fatty acid methyl esters can be used as environmental friendly non toxic biodiesel[10] a substitution for conventional diesel. The fruits are also used to prepare red beverage which has bilious action[11]. The fruit rind is widely used traditionally in Srilanka and southern parts of India because of pleasant flavour and sour taste for culinary purposes.

D-leucine from leaves[12], (-) hydroxyl citric acid from leaves and fruit rind[13], fatty acids and glycerides from seeds[14], anthocyanin glycosides from fruits[15], garcinol, isogarcinol and cambaginol from fruit rinds[16], phenolic compounds like xanthones, biflavanoids from heartwood[17] and stem bark[18] and fatty acids from seed oil[19] were so far isolated from this plant.

In this communication we report isolation, characterisation and antibacterial activity of two poly phenols – a proauthocyanidin (I) and a biflavanoid (III) from the ethyl acetate soluble fraction of methanol extract of stem bark of *G. indica* and preparation of their derivatives.

Stem bark of *G. indica* (500 g) was procured from south canara of Karnataka state, India during summer. The plant was identified and voucher specimen was deposited with Department of Botany, Osmania University, Hyderabad, India. The collected plant material subjected to shade drying and reduced to shavings. These shavings were coarsely powdered and first defatted with petroleum ether and then extracted with methanol under cold percolation method. The methanol extract on concentration provided a dark coloured semisolid (9 g). This semisolid mass was successively extracted with chloroform and ethyl acetate. The ethyl acetate soluble were washed with distilled water to remove water soluble compounds and dried over sodium sulphate. After solvent removal a brown coloured solid (5 g) was obtained. It was dissolved in ethyl acetate (50 ml) and fractionally precipitated with petroleum ether (15 ml) for 25 times. The solid fractions 5 to 15 were combined and the process of fractional precipitation was repeated. The solid obtained from the last 10 fractions were mixed and purified on safodox LH 20 using ethanol-dichloromethane (1:1 v/v) to get proanthocyanidin I, mp is 230° (300 mg).

The alkali solubles of the solid fractions 18 to 25 was chromatographed over silica gel (200 mesh) and eluted with benzene, benzene - ethyl acetate 8:2, 7;3, 1:1 (v/v) and ethyl acetate successively. Benzene - ethyl acetate (7:3) fractions gave biflavonoid III, 340° (100 mg).

The proauthocyanidin (I) (fig. 1a) gave positive ferric chloride test suggests the presence of phenolic –OH. On treatment with dimethyl sulphate and potassium carbonate in acetone yielded octamethyl ether (Ia) suggesting eight phenolic –OH were present in the molecule. This ether further on treatment with acetic anhydride in pyridine yielded triacetate of ether (Ib). This suggests that three more –OH's in the form of alcoholic nature were also present in the molecule. The high resolution mass spectra of I, Ia and Ib suggested that I is dimeric proauthocyanidin. Based on biogenetic considerations the linkage of dimer has intramolecular flavonyl linkage between C-4 and C-8. On hydrolysis of this proauthocyanidin (I) with methanolic hydrochloric acid cyanidin chloride (II)

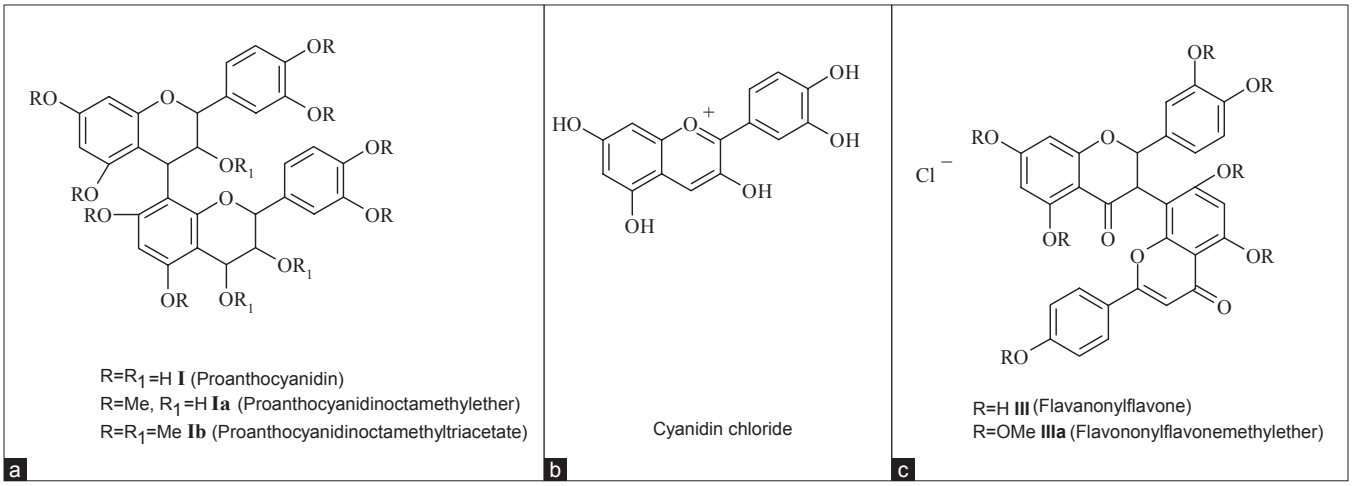

Fig. 1: Structures of polyphenols
(a) Proantocyanidin and derivatives, (b) Cyaniding chloride, (c) Flavanonylflavone and derivative

(fig. 1b) only obtained. No stereo chemical studies were carried out for these compounds.

The biflavanoid (III) (fig. 1c) gave positive phenolic and positive flavonoid tests with ferric chloride and Mg+HCl reagents respectively. This implies that the flavonoid is a phenolic hydroxy compound. On treatment with dimethyl sulphate and potassium carbonate in acetone a hepta methyl ether (IIIa) was formed. This methyl ether further treatment with acetic anhydride in pyridine did not yielded any acetyl derivative. This suggests that no alcoholic hydroxys were present in the molecule. based on ^{1}H, ^{13}C NMR and high resolution mass spectra the compound has been confirmed as I-5,II-5,I-7,II-7,I-3/,I-4/,II-4/-heptahydroxy[I-3,II-8]flavononylflavone (III), a compound earlier reported from *Garcinia nervosa* by Babu *et al.*[20]. This is the first report from this plant and second report front *Garcinia* species.

The antibacterial studies were carried out by paper disc method which is easy, better and comparatively fast when compared with tube dilution method or phenol coefficient method or slide cell technique[21]. Ciprofloxacin was used as standard anatibacterial agent (1 mg/ml). The activity was evaluated by using 24 h cultures of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* and the nutrient broth Labelmco was prepared from beef extract as culture medium. The bacteria strains used in this experiment were cultured in the Microbiology Department, Osmania University, Hyderabad.

Culture medium prepared by taking Labelmco, sodium chloride and peptene in double distilled water. The solution was filtered and the pH was adjusted to 6.8-7.0. The medium was sterilised in autoclave at 121° at 15 Lbs pressure for 15 min. The test samples of 10 mg were dissolved in 10 ml of acetone to get 1000 µg/ml dilution. Different dilutions like 400 µg/ml, 200 µg/ml, 100 µg/ml and 50 µg/ml were prepared from this.

Paper disc of 4 mm dia were dipped in test solutions of different dilutions and standard solution. After drying the disc it was placed on culture medium in petridishes and seeded with 1 ml of experimental bacteria culture of *S. aureus, S. typhi* and *E. coli* and incubated at 37±1° for 24 h. The petridishes were checked for growth inhibition zone after 24 h. The crude and flavononylflavone showed good activity against *S.aureus* even at lower concentrations while partial with proauthocyanidin. *E. coli* showed partial activity with crude and proauthocyanidin at higher concentrations no activity with flavononylflavone even at higher concentrations. Crude, flavononylflavone and proauthocyanidin showed medium activity only at higher concentrations with *S. typhi*. All the derivatives showed no activity with bacteria even at higher concentrations. Table 1 showed the anti bacterial activity of the compounds.

TABLE 1: ANTI BACTERIAL ACTIVITY OF CRUDE EXTRACT AND POLY PHENOLS OF *GARCINIA INDICA*

Bacteria	Crude				Comp I				Comp III				CF_{10}
	A	B	C	D	A	B	C	D	A	B	C	D	E
Staphylococcus aureus	10	13	19	30	12	19	-	-	10	13	18	-	9
Salmonella typhi	10	18	-	-	18	-	-	-	19	-	-	-	6
Escherichia coli	17	19	-	-	17	-	-	-	-	-	-	-	8

Values are mean inhibition zone in mm, A=400 µg, B=200 µg, C=100 µg, D=50 µg, E=10 µg; CF_{10}=Ciflofloxacin 10 µg

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