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CHEMICAL INVESTIGATION OF *PSIDIUM GUAJAVA* ROOTS

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PSIDIUM GUAJAVA is an economically important plant of medicinal value. Flavonoids, gallic acid and tannins are invariably present in all the parts of the plant *viz.* fruits[1], leaves[2,3], stembark[4], heart-wood[5]. In this investigation roots have been investigated and found to contain β-sitosterol, quercetin, leucocyanidin, gallic acid, 2,3,4-trigalloyl 6-(m-trigalloyl) glucose.

The powdered roots were extracted by the following three different procedures.

i) *Extraction with boiling ethanol*: The extract concentrated and fractionated into light petrol, ether, ethyl acetate and acetone soluble fractions. Petroleum ether fraction gave β-sitosterol, ether fraction gave quercetin, gallic acid and ethyl gallate, while ethyl acetate fraction showed the presence of leucocyanin; from acetone fraction tannin was isolated.

ii) *Extraction with bioling acetone*: The extract concentrated and fractionated into light petrol, benzene, chloroform and acetone soluble fractions respectively. Light petrol gave β-sitosterol, benzene fraction gave quercetin. Chloroform gave leucocyanidin and tannin II was isolated from the acetone soluble fraction of the extract.

Extraction with water was carried out at room temperature and the extract after deionisation over cation and anion exchange resin was concentrated and then fractionated using light petrol ether, ethyl acetate and acetone respectively. Light petrol fraction did not give anything significant, ether and ethyl acetate fractions gave quercetin and leuco-cyanidin respectively and from acetone fraction a tannin was isolated and found to be identical with tannin II.

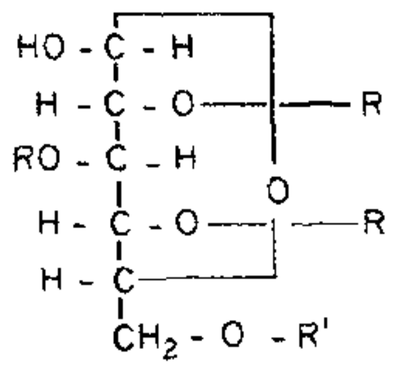
β-sitosterol, quercetin, leucocyanidin, ethyl gallate and gallic acid were identified by comparison with respective authentic samples.

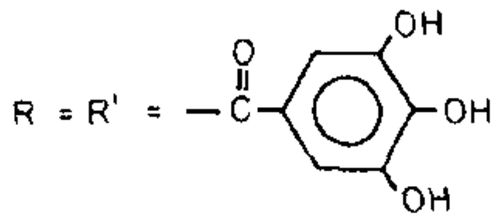
Tannin I – It was isolated as buff-coloured chromatographically homogeneous semicrystalline compound and analysed for $C_{34}H_{28}O_{22}4H_2O$. It gave blue precipitate with ferric chloride[2] and positive Molisch test showing it to be a polyphenol glycoside. However, positive test with AHP reagent indicated the presence of a potential aldehyde group. Acid as well as alkaline hydrolysis of the compound gave gallic acid and glucose. Therefore, it could be a galloyl ester of glucose. Quantitative estimation of glucose and gallic acid in the hydrolysate showed the presence of four units of gallic acid per mole of glucose. Quantitative estimation of glucose and gallic acid in the hydrolysate showed the presence of four units of gallic acid per mole of glucose. As the reducing group of the sugar is free, it could be characterised as tetragalloyl glucose, with four galloyl units attached at 2,3,4 and 6 positions of glucose assuming the sugar to be in pyranose form.

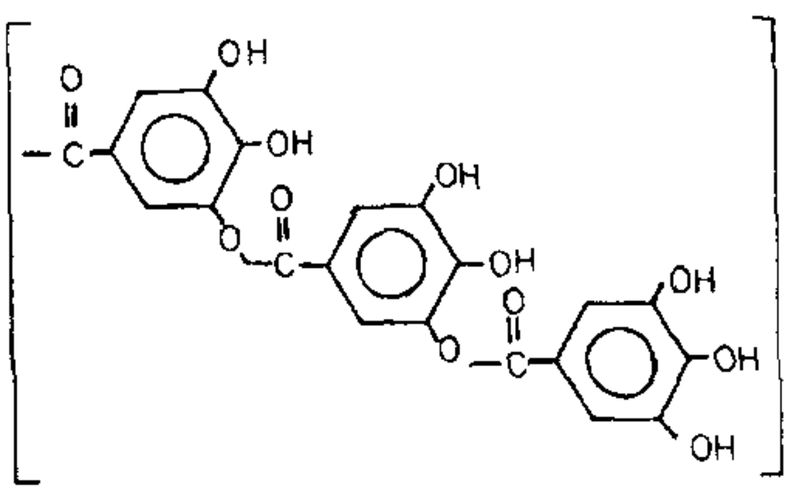
The isolation of ethyl gallate from the ethanolic extract of roots was rather suggestive that depside linkages if present in the genuine tannin might have got ethanolysed during extraction with ethanol, resulting in the formation of ethyl gallate and tetra galloyl glucose. This has been reported in the past by Haworth *et al*[6].

Tannin II – It was a microcrystalline compound, chromatographically homogeneous and analysed for $C_{48}H_{36}O_{33}.4H_2O$. It gave all the characteristic tests of tannins[6] similar to tannin I. On acid as well as alkaline hydrolysis it gave glucose and gallic acid. Quantitative hydrolysis showed the presence of six moles of gallic acid per mole of glucose.

In order to get more information about the structure, it was methylated with diazomethane and subsequently hydrolysed when 3,4,5-tri-O-methyl and 3,4-di-O-methyl gallic acids were obtained which were identified by mmp and co-chromatography with authentic samples. The detection of 3,4-di-O-methyl gallic acid in the hydrolysate was indication of the presence of depside links in the molecule. Intensity of the two spots of 3,4,5-tri-O-methyl gallic acid and 3,4-di-O-methyl gallic acid was compared with the intensity of the spots obtained by running the artificially prepared mixtures of these acids in different molecular proportions on descending strip chromatogram. The 2:1 proportion of 3,4,5-tri-O-methyl and 3,4-di-O-methyl gallic acid respectively, agreed with that of the hydrolysate, which supported the conclusion that four ester and two depside links are present in the molecule.



Tannin I R = R' = 

Tannin II - R' = 

The presence of four ester linkages in the molecule of tannin would mean that one hydroxyl of glucose moiety is free. Positive test with AHP reagent indicated the presence of free aldehyde group. Therefore, the ester linkages must be present at 2,3,4 and 6-positions provided glucose moiety has pyranose ring structure.

The position of the two depside linkages was decided by methanolysis of the tannin which gave traces of gallic acid, methyl-*m*-digallate and another tannin, which compared on paper chromatography and TLC with one obtained from the ethanolic extract of the roots (tannin I). This tannin on hydrolysis gave gallic acid and glucose. Gallic acid estimation (86.28%) and glucose (22.29%) suggested that there might be four gallic acid units per mole of glucose. This suggested that the remaining two gallic acid units must be linked with depside links.

From the above discussion it could be concluded that in tannin II four galloyl units are linked through ester linkages at 2,3,4, and 6 positions of glucose molecule and at any one of these positions a trigalloyl chain is present having two gallic acid units linked through depside linkages. Although the exact position of the trigalloyl chain could not be decided, the stereochemistry of the molecules as well as biogenesis of this class of compounds favour position-6. In all the naturally occurring gallatannins so far reported, the longest chain of gallic acid residues is always attached at position-6.

The low optical rotation $[\alpha]_D^{20} + 9.8$ (in Me_2CO) of the gallotannin II, suggested that probably glucose was present here in β-form.

From the above discussion, it could be concluded that tannin II is the genuine tannin and tannin I is its artefact obtained by alcoholysis of the depside linkages during extraction with ethanol.

7 March 1984

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OCCURRENCE OF BAGH BED (UPPER CRETACEOUS) IN HARDASPUR-UMRI AREA, JHABUA DISTRICT, MADHYA PRADESH.

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THE present note records the occurrence of richly fossiliferous strata of sandstone and limestone belonging to Bagh Beds in the area near the village of Hardaspur (74° 38′: 22° 25′) Umri (74° 37′: 22° 23′), in Jobat (74° 34′: 22° 25′), Jhabua district, M.P. These have earlier been reported as Lameta[1] (figure 1).

Near the villages of Hardaspur and Umri, ridges of limestones underlying the sandstone are exposed. The sandstone is nearly horizontal and unconformably rests over the Archean basement (granite gneiss). It is hard and friable at places, showing various shades of yellowish brown, reddish brown, and grey colours. This sandstone unit is identical with the Nimar