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Glycoside from the Seed Powder of *Syzigium cumini* (L)

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Abstract, The seeds of *Syzigium cumini* (L) were shade dried, powdered and subjected for soxhlet extraction for twenty four hours through ethanol solvent. The extractive was concentrated. And it was then partitioned using petrol, benzene, ether, ethyl acetate, acetone and methanol. The soluble fractions were separated. The acetone soluble fraction was subjected for Si-gel CC and gradient eluted with ethyl acetate, acetone mixture in various concentrations. The elutes collected from ethyl acetate, acetone (9, 2) were combined and crystallized from ether as a light yellow needles. This compound was dissolved in aqueous ethyl alcohol (1, 1 v/v) and then treated with Tokadiastase. Liberation of free rhamnose was detected, suggesting the presence of alpha linkage between sugar moiety and aglycone. The resultant compound provided enough evidence regarding "5, 7-dihydroxy-6, 2-dimethoxyisoflavone-7-O-alpha-L-rhamnoside.

Keywords, *Syzigium cumini;* Glycoside; Seed

Introduction

Interplay of the Bio-compounds of plant origin serves the progression of development and growth of animals, which lead to establish the concept of "Use of plant products for the sustainable human health". *Syzigium cumini* (L), a poly-embryonic species (Family, Myrtaceae) (Chase and Reveal, 2009), is a tropical tree of great economic and medicinal importance. It is a large evergreen widely distributed forest tree of India, Sri Lanka, Malaysia and Australia. Topical application of ten microliters of various concentrations of acetone extractives of tender stem pieces of *Syzigium cumini* (L) to the fifth instar larvae of silkworm, *Bombyx mori* (L) was found to inhibit the chitin deposition in the integument. The percent inhibition of integument – chitin seems to be titre dependent. The ID -50 values for *Syzigium cumini* (L) was found measured 3.60 micrograms (Vitthalrao et al, 2011). The edible fruits made it to introduce from India and tropical Asia to Southern Africa. It has been successfully introduced to many tropical countries like West Indies, East and West Africa and some sub tropical regions like Florida, California, Algeria and Israel for its medicinal importance. The ripe fruits are purplish black in color, juicy, almost odorless, with pleasant, slightly bitter, astringent taste. Different solvent (methanol, water, chloroform, n-hexane and ethyl acetate) extraction methods were used for getting the highest amount of nutrients and have and have been applied for medicinal use (Ruan et al, 2008). The methanol and acidified methanol were found suitable for the extraction of total anthocyanin content and antioxidant capacity of the fruits of *Syzigium cumini* (L) (Kheaw-on, et al, 2009; Faria, et al, 2011). Two types of tannins present in the fruits have been identified by NMR, MALDI – TOF MS and HPLC in *Syzigium cumini* (L). Hydrolysable tannin ellagi tannins and condensed tannin epiafzelechins have been identified and structure has been predicted through NMR studies (Zhang and Lin, 2009). The extracts have been reported for a good DPPH radical scavenging and FRAP activity, which is the supportive proof of presence of potential antioxidants in the fruits of *Syzigium cumini* (L). This plant has some of the important compounds (like , mallic acid, oxalic acid, gallic acid, betulic acid, tannins, flavonoids and essential oils (Table – 1), which are present at the different parts of the tree and can either act in combination or individually to cure some disease and health problems. Glucose and fructose are the main source of sweeteners in the fruits of *Syzigium cumini* (L). The fruits of this plant are with absolutely no trace of sucrose and therefore, the only fruit with minimum calories. Many more studies have been carried out in the last few decades to confirm the activity of fruits, seeds and stem bark against diabetes mellitus (Chaudhary and Mukhopadhyay, 2012). Phytochemical significance of seeds of *Syzigium cumini* (L) lies in presence of rich contents of proteins; calcium and the Jambolin (the glycogen), which helps in controlling the blood sugar level by switching off the mechanism of starch converting to sugar Leelavinothan et al, 2006). The Glycosides was the major component in the phytochemical analysis of *Syzigium cumini* (L) (Kumar et al, 2009). The structural detailed studies on Glycosides are lacking. Therefore, the attempt on Isolation of Glycoside has been planned.

Material and Methods

The ripen fruits of *Syzigium cumuni* (L) were collected from the Malegaon farm they were identified through the experts and confirmed. The individual seed from each fruit was separated. All the seeds were shade dried and powdered through

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the use of domestic mixture. The known quantity (100gm) of seed powder was subjected for soxhlet extraction, for 24 hours, through the use of ethyl alcohol (95%). The extract allowed for cooling and concentration. The concentrated extract was partitioned using petrol, ether, ethyl acetate, acetone and methanol. And soluble fractions were separated. The acetone soluble fraction was concentrated and subjected to Si-gel CC and gradient eluted with ethyl acetate, acetone mixture in various concentrations. The elutes collected from ethyl acetate, acetone (9,2) were combined on the basis of Rf value (TLC monitored) and crystallized from ether as a light yellow colored needles (Compound I), weight of which found measured 367mg. The compound was subjected for screening test for glycosides (Kumar, et al, 2009). Then the compound was analyzed for molecular formula, melting point and spectral analysis.

Screening Test for Glycosides,

The compound (in the form of extract) was hydrolyzed with HCl for an hour in water bath. Few drops of sodium nitroprusside solution were added. The content was then made alkaline. Pink color appeared, which the indication for presence of Glycosides is.

Acid Hydrolysis of Compound (I),

The Compound (I) was refluxed with aqueous seven percent sulphuric acid for six hours and then extracted with ethyl acetate (EtOAC). The EtOAC layer when worked up gave 5, 7-dihydroxy-6, 2-dimethoxyisoflavone m. p. 234 – 235 degree celcius. The aqueous layer was found to contain L-rhamnose (PC, n-BuOH-HOAc-H2O 4, 2, 1, RF ~ 0.38). The glycoside (I) (20 mg) was dissolved in aqueous EtOH (20ml) 1,1 v/v, and was then treated with Tokadiastase (10ml) at 35 to 45 degree celcious for three hours and then at room temperature for 48 hours. The liberation of free rhamnose was detected by PC, Rf-0.38 and suggested the presence of alpha linkage between sugar moiety and aglycone. The present experimental out puts provided enough evidence that the Compound (I) should be 5, 7-dihydroxy-6, 2-dimethoxyisoflavone-7-alpha-L-rhamnoside.

Result and Discussion

The light yellow colored needle form compound (I), with molecular formula C23H24O10, with melting point 234-235^{0}C responded to all the characteristic tests of isoflavone (V. Narayan and T. Sheshadri, 1971; D. Adinarayan and J. R. Rao, 1972). Further, it results a positive Molisch Test. This is the clear indication of having "Isoflavone-Glycoside". The absorption maximum at 264nm in methyl alcohol was a typical of isoflavone. The IRpeaks were at 3380 chelated (OH) 2860 (OMe), 1665 (alpha, beta unsaturated C=O), 1070-1030 (Glycoside C-O). The bathochromic shift of 11 nm in band I on addition of AlCl3 / HCl (relative to that with MeOH) indicated the presence of free hydroxyl group at C-5. Acid hydrolysis of the Compound (I) yielded L- rhamnose (Co-PC, CO-TLC) and compound (II), which was characterized and identified as 5,7-dihydroxy-6,2'-dimethoxyisoflavone with molecular formula C17 H14 O6; with melting point 194 -195^{0}C [M] m/z 314, by direct comparison of its mp, UV, IR, H-NMR and C- NMR spectral data with those reported in literature (Abdul, et al, 1984). Acetylation of I (Ac2O/ pyridine) gave a penta acetate derivative with molecular formula C31H32O14, m. p. 156 – 157 degree Celcious [M+] m / z 628, which confirmed the presence of two free hydroxyl groups in the aglycon part of (I).In the H – NMR spectrum a peak at delta 8.20 (1H, s , H – 2) and a peak at delta 155.14 (C- 2) in the C-NMR spectrum of acetylated derivative (III) further confirmed its Isoflavone Skeleton character (Wenkert and Gottlieb,1977; Petter, et al, 1976). The substitution pattern in ring B was inferred from H-NMR coupling pattern H-3 proton appeared at delta 7.10 (1H, dd, J=8.5, 1.5 Hz), which was coupled to an ortho proton at C-4 and also further coupled to a meta proton at C-5. Appearance of a double doublet of a doublet at delta 7.41 (1H, J=8.5, 1.5 Hz), which was coupled two ortho protons at C-3 an C-5 and also coupled to a meta proton at C-6. The H-5 proton also appeared as double doublet at delta 7.01 (1H, ddd, J=8.5, 1.5 Hz) indicating its coupling with two ortho protons at C-4 and C-6. A double doublet at delta 7.29 (1H, dd, J=7.5, 1.5Hz) was assigned to H-6 coupled to an ortho proton at C-5 and Meta proton at C-4. The anomeric portion of L-rhamnose appeared as a doublet at delta5.10. In the EIMS spectrum the molecular ion peak at 314 and another ion peak at 182 correspond to glycone and aglycone respectively. A strong peak at m/z 299 corresponding to a loss of methyl group strongly suggested the presence of methoxy group in ring A of Compound (I) at C-6 position (Rogriguez, et al, 1972). C-NMR spectrum showed twenty three carbon atoms in compound (I). The oxygenation pattern was confirmed by comparison of its C-NMR spectrum with compound having similar oxygenation pattern (Abdul et al, 1984). The specific rhamnoside from the *Syzigium cumini* (L) seed powder has been isolated; Characterized and named through the standard nomenclature. This rhamnoside may open a new avenue in the field of "Medicinal Use of *Syzigium cumini* (L)".

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