Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 5 (1): 32-40, 2011
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2011.32.40

Structure Confirmation of Rare Conjugate Glycosides from *Glycosmis arborea* (Roxb.) with the Action of β-Glucosidases

[1]D.S. Negi, [1]A. Kumar, [1]R.K. Sharma, [1]N. Shukla, [1]N. Negi, [1]M.L. Tamta, [1]Y. Bansal, [2]Por-gnam Prasert and [2]J.R. Kedutat Cairns

[1]Department of Chemistry, HNB Garhwal University, P.O. Box 75, Srinagar (Garhwal)-246 174, Uttarakhand, India

[2]Institute of Science, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

Corresponding Author: Devendra S. Negi, Department of Chemistry, HNB Garhwal University, P.O. Box 75, Srinagar (Garhwal)-246 174, Uttarakhand, India

ABSTRACT

Two flavanones, 5,5´-dihydroxy-4´-methoxy-7-O-(α-L-rhamnosyl-(1´´>6´´)-β-D-glucopyranosyl) flavanone 1 and (5,5´-dihydroxy-4´-methoxy-5-*O*-β-D-glucopyranosyl) flavanone 2, were isolated by liquid chromatography from ethanolic extracts of *Glycosmis arborea* (Roxb.) leaves and tested for hydrolysis by various β-glucosidases. The β-glucosidases from *Dalbergia cochinchinensis*, *Dalbergia nigrescens*, barley, rice and almond were compared for their ability to hydrolyze the conjugated flavanone glycosides from *G. arborea* by thin layer chromatography of the reaction products. The disaccharide glycoside, compound 1 was only hydrolyzed by the *D. nigrescens* β- glucosidase, which appeared to release the sugar mainly as a disaccharide, while compound 2 was hydrolyzed by all β-glucosidases tested, except Os9BGlu31. These compounds also showed significant antifeedant activity toward the polyphagous crop pest *Spodoptera litura*. This is the first report about the structural elucidation using enzymatic studies of bioactive flavanone glycosides from *G. arborea*.

Key words: β-glucosidases, flavanone glycosides, *Glycosmis arborea*, *Spodoptera litura*, antifeedant activity

INTRODUCTION

Glycosmis arborea (Roxb.) is an evergreen shrub belonging to Rutaceae family and is distributed in warm and temperate regions of India. Roots of this plant are traditionally used to overcome from facial inflammation, rheumatism, anaemia and jaundice. Leaves are useful in treatment of fever, eczema, skin disease, wounds and hepatic disease. Fruits of this plant are used to treat cough and bronchitis (Warrier *et al*., 1995). Previously, carbazole alkaloids and quinoline alkaloids were isolated from the roots of *G. arborea* (Chakravarty *et al*., 1999). A hepatoprotective carotenoid was also isolated from this plant (Kivotani *et al*., 1996).

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a serious polyphagous pest, infesting more than 150 species and is widely distributed in India (Kumar *et al*., 2007). It causes significant losses of crops from 26-100% (Dhir *et al*., 1992), it is also reported to be attacking cotton in North India (Arora, 1993). Pesticides have played a significant role in

increasing agricultural production. However, the continuous use of insecticides, has not only caused death through poisoning and their persistence in environment and food chain, but also caused resistance in pest populations (Wei *et al.*, 2004; Kranthi *et al.*, 2002). Given this circumstance, research needs to be aimed at establishing alternative means of insect control. One promising means is to find phytochemicals from plants and aqueous extracts of the leaves of the medicinal plants *Ipomea carnea* (L.), *Pedalium myrex* (L.) and *Adhatoda vasica* (L.) could have both toxic and antifeedant effects on this pest (Sujatha *et al.*, 2010). An antifeedant is a phytochemical agent that causes a pest to stop eating (Isman *et al.*, 1996). Several plants have been reported to produce a diverse range of secondary metabolites, such as terpenoids, alkaloids polyacetylenes flavonoids, amino acids and sugars that protect the plants from infestation by insects (Patel and Patel, 1996).

β-Glucosidases [3.2.1.21] play significant roles in plants, including growth regulation, response to stress, lignifications, cell wall β-glucan degradation, and defense (Cairns and Esen, 2010). These enzymes act on β–glycosidic bonds linking a glucosyl residue to glucose-substituted molecules such as oligosaccharides and aryl and alkyl glucosides, with different specificities. Hessler *et al.* (1997) reported that β-glucosidase from *Saccharopolyspora eryraea* could hydrolyze genistin during fermentation of soy-based media, a β-glucosidase from bifidobacteria in soy milk was capable of converting glucosides to their aglycones 990 m, 590 and 1378 m, respectively (Tsangalis *et al.*, 2002). Pandjaitan *et al.* (2000a, b) treated soy protein isolate β-glucosides with almond β-glucosidase to convert most of its isoflavone glucosides to their aglycone. Suzuki *et al.* (2006) purified, cloned and characterized β-glucosidase from soybean that could hydrolyze isoflavone conjugates, with high activity toward 6"-O-malonyl genistin. Isoflavonoid β-glucosidase have been described from the seeds of *Dalbergia cochinchinensis* and *Dalbergia nigrescens* (Chuankhayan *et al.*, 2005, 2007a,b). These enzymes had high hydrolytic activity on isoflavonoid glycosides from the same seeds and the *D. nigrescens* enzyme can efficiently hydrolyze β-glycosides to remove 6-O-modified glucosyl residues. Most other plant β-glucosidases are not known to be able to hydrolyze these more complex glycosides. In present communication we evaluated the structure and linkage of isolated compounds from *G. arborea* by β-glucosidases with different specificities.

MATERIALS AND METHODS

General experimental procedure: The UV and IR spectra were recorded on Beckman 64 UV spectrophotometer and Perkin-ElmerRX-1 spectrophotometer. NMR spectra were measured on Bruker AVANCE 500 MHz spectrometer. JEOL JMS 600 H was used for MS data. Melting points (uncorrected) were measured on Complab Melting point apparatus. Silica gel 60-120 (Merck) used for isolation of compounds by column chromatography. TLC analyses were carried out using aluminum-backed silica gel 60 F_{254} (0.20 mm thickness) plates (Merck).

Plant material: Leaves of *G. arborea* were collected from Rajaji National Park, Rishikesh, Uttarakhand, India (during flowering season at altitude of 2300-2500 m in October 2005) and identified by Prof R. D. Gaur, a taxonomist of Department of Botany, HNB Garhwal University. The voucher specimen (8977, GUH) have been deposited in the herbarium of the Department of Botany, HNB Garhwal University.

Leaf extraction and isolation: The air dried leaves were grinded and extracted with 90% EtOH. The total ethanolic extract was concentrated under reduced pressure at a temperature below 50°C

to a dark green viscous mass that was partitioned with hexane and n-butanol. The butanol soluble layer was then fractionated into chloroform, ethyl acetate and methanol soluble fractions. Methanol fraction was subjected to column chromatography over 60-120 mesh silica gel (Merck) and eluted with chloroform and MeOH with the increasing polarity. Fractions were collected (100 mL each) and those with similar TLC patterns were mixed together. Compound 1 (46 mg) and 2 (36 mg) were obtained from $CHCl_3$: MeOH (85:15) eluate.

β-Glucosidase enzymes: Almond β-glucosidase was purchased from Sigma, while other β-glucosidase enzymes were produced from recombinant expression systems and seed extracts. Rice β-glucosidases Os3BGlu7, Os4BGlu12 and Os9BGlu31 were produced as thioredoxin fusion proteins in *Escherichia coli* and purified as previously described (Opassiri *et al*., 2003, 2004 2006). rHvBII was similarly produced in the same system as previously described (Kuntothom *et al*., 2009). DnBGlu was purified from *D. nigrescens* seeds (Chuankhayan *et al*., 2005), whereas the recombinant enzyme DnBGlu2 was expressed in *Pichia pastoris* (Chuankhayan *et al*., 2007a). Thai rosewood (*Dalbergia cochinchinensis*) β-glucosidase (dalcochinase, TRW) (Srisomsap *et al*., 1995; Svasti *et al*., 1999) was expressed in *P. pastoris* and purified by hydrophobic interaction chromatography and IMAC (Toonkool *et al*., 2006).

Antifeedant activity: The antifeedant activity of the extracts against the polyphagous pest *S. litura* was tested by the leaf dip method. Five percent concentrations of each extract were prepared by dissolving extracts in a small quantity of ethanol and diluting in water containing 0.05% TritonX100. The leaf discs of about 5 cm^2 were prepared out of castor leaf (*Ricinus communis* L.) and were dipped for 30 sec in an extract or compound separately. The leaf discs dipped only in water containing 0.05% TritonX100 were used as controls. The leaf discs were air dried and on each treated leaf disc ten larvae of *S. litura* (one day old) were released. Three replications were maintained for each extract. Larval weight was taken after four days of treatment.

Percent growth reduction was calculated in comparison to control as shown below and the values given in Table 1:

$$\text{Percent growth reduction} = \frac{100 - 100\% \text{ (Treatment wt.)}}{\text{(Control wt.)}}$$

Hydrolysis of compounds: To compare the hydrolytic efficiency of *Dalbergia* rice, barley and almond β-glucosidases towards the isolated compounds, 10 μg aliquots of the compounds were separately hydrolyzed with 0.001 unit of a β-glucosidase in 100 μL of 0.1 M sodium acetate, pH 5.5. The reaction mixtures were incubated at 37°C for 10 min or 16 h, boiled 5 min to stop the reactions, then dried by speed vacuum and resuspended in 100 μL of 10% acetronitrile in 0.1% phosphoric acid/water. A control reaction of water without enzyme was set up in the same manner. The hydrolyzed products were evaluated by Thin-Layer Chromatography (TLC) on analytical silica gel 60 F_{254} aluminum plates (Merck, Darmstadt, Germany) with one of two different solvent systems, which were (A) ethanol: methanol: acetic acid: water (7.5:0.5:1:1) and (B) butanol: methanol: acetic acid: water (7.5:0.5:1:1). Flavonoids were visualized by absorbance under UV light and

Table 1: Digestion of glycosides by plant isoenzymes

	Compound- 1		Compound- 2	
Enzymes	Digestion	Sugar product	Digestion	Sugar product
BGlu1= Os3BGlu7	Not Digested	-----	Digested	Monosaccharide
445-1= Os4BGlu12	Not Digested	-----	Digested	Monosaccharide
BGQ60= recombinant barley β-glucosidase isoenzyme II (rHvBII)	Not Digested	-----	Digested	Monosaccharide
BGlu31= Os9BGlu31	Not Digested	-----	-----	-----
DnBglu= *Dalbergia nigrescens* β-glucosidase	Digested	Disaccharide	Digested	Monosaccharide
DnBglu2= recombinant *Dalbergia nigrescens* β-glucosidase 2	Digested	Disaccharide	Digested	Monosaccharide
TRW= Thai rosewood (*Dalbergia cochinchinensis*) β-glucosidase	Not Digested	-----	Digested	Monosaccharide
Almond β-glucosidase	Not Digested	-----	Digested	Monosaccharide

carbohydrates were visualized by spraying with 10% H_2SO_4 in methanol and incubating at 110°C for 20 min sugars were tentatively identified by comparison with commercial standards.

RESULTS AND DISCUSSION

The leaves extract of *G. arborea* was fractionated using a liquid–liquid partition procedure with solvents of increasing polarity. The methanol fraction was then purified using open column chromatography over silica gel 60-120 mesh to yield two flavanone glycosides 1 and 2. Compound 1 has molecular formula $C_{28}H_{34}O_{15}$ it was identified as 5,5´-dihydroxy-4´-methoxy-7-*O*-(α-L-rhamnosyl-(1´´´>6´´)-β-D-glucopyranosyl flavanone, whereas compound 2 with molecular formula $C_{22}H_{24}O_{11}$, was characterized as 7,5´-dihydroxy-4´-methoxy-5-O-β-D-glucopyranosyl flavanone (Fig. 1.) both are known compounds and their structures were confirmed on the basis of comparison of their spectroscopic data with reported literature (Wei *et al*., 2007; Jovanoic *et al*., 1994).

Both compounds are firstly reported from this plant. For the confirmation of glycoside linkage we digested 1 and 2 by commercial almond β-glucosidase or rice Os3BGlu7, Os4BGlu12 and Os9BGlu31 or barley rHvBII β-glycosides on incubation at overnight 30°C the compound 1 was not digested by these enzymes, whereas 2 was digested by Os3BGlu7, Os4BGlu12 and rHvBII, to further confirm the glycoside linkage, we digested the compounds with DnBGlu, DnBGlu2 and TRW (Thai rosewood). TLC showed that the recombinant enzyme DnBGlu2 could completely digest compound 1 (spot three, Fig. 2a), while DnBGlu from seed digested it slightly (Fig. 2b, spot nine). DnBGlu is an isoflavone 7-O-β-D-glucosidase and acuminosidase (3.2.1.161) (Chuankhayan *et al*., 2005).

Recombinant DnBGlu2 and DnBGlu can cut glucose, 6-acetyl-glucose, 6-malonyl-glucose and 6- β-dα-apiosyl-β-dα-glucose off from the 7 hydroxyl of isoflavonoids (Chuankhayan *et al*., 2005, 2007a). The digestion of compound 1 by DnBGlu2 suggested that compound may contain a 6-O-modified glucose for the sugar, since DnBGlu2 hydrolyzes flavonoid 6-β-D-apiosyl-β-D glucosides and 6-malonyl glucosides much better than Thai rosewood (*Dalbergia cochinchinensis*) β-glucosidase (TRW, dalcochinase) (Srisomsap *et al*., 1995) and the other enzymes could not hydrolyze these types of sugars. β-Glucosidases from almond, *D. nigrescens* (DnBglu and DnBglu2) and *D. cochinchinensis* (TRW) were also able to hydrolyze compound 2. Digestion of both compounds is shown in Table 1. This inferred that enzyme DnBGlu2 efficiently hydrolyzed the α-lα-rhamnosyl-1,6-β-dα-glucosyl moiety from the 7-O of the flavanone. To our knowledge, this is the first time that DnBGlu, DnBGlu2 or any other β-glucosidase has been reported to hydrolyze an α-lα-rhamnosyl-1,6-β-dα-glucoside.

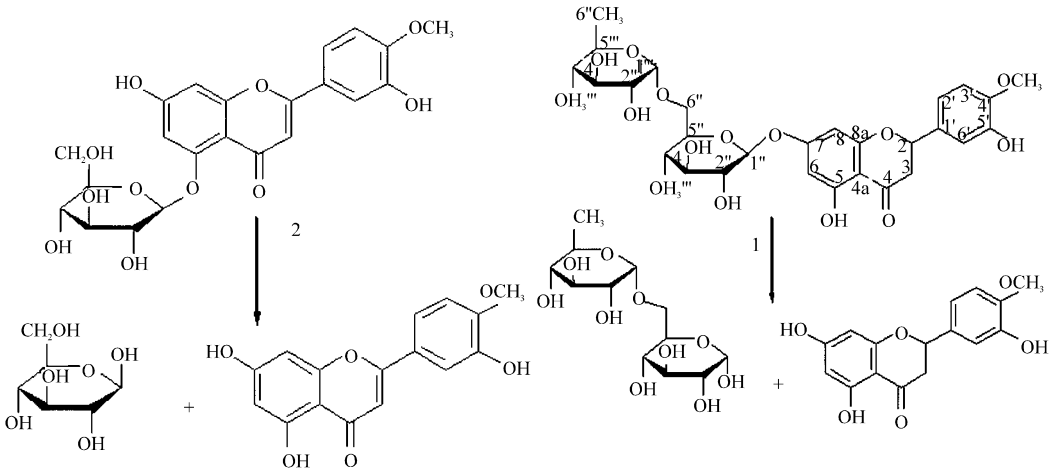

Fig. 1: Structure and hydrolysis of compound 1 and 2

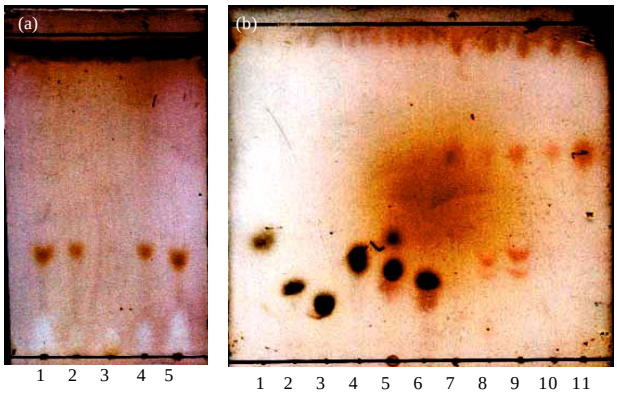

Fig. 2: The hydrolysis of Compound 1 by *Dalbergia* β-glucosidases. Solvent system A [ethanol methanol: acetic acid: water (7.5:0.5:1:1)] was used to separate the glycosides and aglycones in TLC 1a, while solvent system B [butanol: methanol: acetic acid: water (7.5:0.5:1:1)] was used in TLC 1b. Compound 1 was digested overnight with DnBGlu (1a spot 2 and 1b spot 8), recombinant DnBGlu2 (1a spot 3 and 1b spot 9), Thai rosewood β-glucosidase (1a spot 4 and 1b spot 10), and almond β-glucosidase (1a spot 5 and 1b spot 11). The control digest (Compound 1) is shown in Fig. 3a spot 1 and 3b spot 7, while sugar standards are shown in Fig. 2b as follows: 1, glucose; 2, cellobiose; 3, lactose; 4, galactose; 5, sucrose; and 6, maltose

It is not completely unexpected, since the enzymes natural substrates appear to include α-lα-apiosyl-1,6-β-D-glucosides (Chuankhayan *et al*., 2005, 2007b), but it has not been previously demonstrated. This suggests that the active site can accommodate the binding of a range of sugars, in addition to malonyl and acetyl groups bound to the 6-O of the glucosyl residue. This makes it useful for determining whether a given flavonoid 7-O-glycoside has a (1→6)-linked disaccharide or

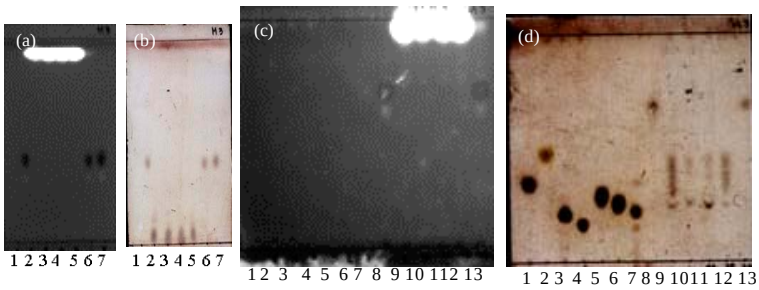

Fig. 3: (a-d) Hydrolysis of Compound 2 by β-glucosidases, as analyzed by TLC

Table 2: Antifeedant activity of leaf extracts of *G. arborea* and its compounds against *S. litura*

Particulate	Average larval wt. mg±SD	Mortality (%)	Percent growth reduction(±)
$CHCl_3$ extract	12.8±2.1	50	93 (1.12±0.075)
Hexane extract	13.02±0.56	30	90 (1.72±0.23)
Butanol extract	13.5±0.85	35	80 (1.65±0.78)
1	10.5±2.15	NS	24 (1.37±0.09)
2	11.2±1.85	NS	22 (1.32±0.36)
Control (0.005% Triton X100)	17.3+1.45	00	0.00

Experiment done in triplicate, ±SD value

glucosyl group, by comparing the hydrolysis by DnBGlu2 to that by one of the many β-glucosidases that can only hydrolyze flavonoid glucosides.

Os3BGlu7 is best at hydrolyzing long celloligosaccharides, but can hydrolyze some glycosides such as pyridoxine glycoside (Opassiri *et al*., 2004). It has β-D-glucosidase, β-D-fucosidase, β-D-mannosidase and β-D-galactosidase activities. Os4BGlu12, another rice isoenzyme expressed in germinating shoots, also hydrolyzes oligosaccharides and has β-fucosidase, β-glucosidase, β-galactosidase, β-xylosidase and α-L-arabinosidase activities (Opassiri *et al*., 2006). Barley rHvBII is a recombinant enzyme nearly the same as barley BGQ60 (Leah *et al*., 1995) and barley β-glucosidase isoenzyme II (Hrmova *et al*., 1996, 1998). It is a β-D-mannosidase with β-D-glucosidase, β-D-fucosidase and β-D-galactosidase activities as well.

In Fig. 3a and b, the TLC was run in solvent system A, while 3c and d show the TLC run in solvent system B. 3a and c are the images of the TLC taken under UV light, which show the dark absorbing spots for the glycosides and the bright fluorescent spots for the aglycone, while 3b and d show the TLCs after development with 10% sulfuric acid and heating. Sugar standards are shown in TLC 3c and d as follows: 1, glucose; 2, apiose; 3, cellobiose; 4, lactose; 5, galactose; 6, sucrose and 7, maltose. The control reaction and unreacted substrate are shown in Lanes 1 and 7 in 3a and b and the control reaction in lane 8 (Compound 2) in Fig. 3c and d. The enzymes used to digest were Os4BGlu12 (Fig. 3a and b lanes 2 and 5, Fig. 3c and d lanes 9 and 12); Os3BGlu7 (Fig. 3a and 3b lane 3 and C and D lane 10), barley rHvBII (A and B lane 4 and C and D lane 11) and Os9BGlu31 (A and B lane 6 and Fig. 3c and d lane 13).

An evaluation of the antifeedant activities of extract, fractions and isolated compounds from *G. arborea* on *S. litura* larvae was also carried out and the results are shown in Table 2. The chloroform extract had significant activity and showed the maximum mortality of 50% and a growth reduction of 93% for *G. arborea*. The hexane and butanol extracts caused lower mortalities and reductions in growth rates, while in the pure compounds did not cause mortality but cause

growth reduction in the larva. It should be noted that compounds in water extracts from other Indian medicinal plants have also been shown to have toxic and antifeedant activities toward *S. litura* larvae (Sujatha *et al.*, 2010). However, the addition of more antifeedant compounds to combat this pest should be useful.

ACKNOWLEDGMENTS

Authors are thankful to Director, Uttarakhand Council for Science and Technology (UCOST), Uttarakhand, India and University Grants Commission (UGC) New Delhi for financial assistance. Phimonphan Chuankhayan, Pornphimol Metheenukul, Teerachai Kuntothom, Sukanya Luang, Salila Pengthaisong, Thipwarin Rimlumduan, and Sompong Sansenya are thanked for production of enzymes. Support for enzyme work that was provided by the Thailand Research Fund and Suranaree University of Technology, Thailand.

REFERENCES

Arora, R., 1993. Growth and Sporodoptera litura (Fab.) On Hirsutum cotton in Panjab. Indian. J. Ecol., 20: 37-42.

Cairns, J.R.K. and A. Esen, 2010. β-Glucosidases. Cell. Mol. Life Sci., 67: 3389-3405.

Chakravarty, A.K., T. Sarkar, K. Masuda and K. Shiojima, 1999. Carbazole alkaloids from roots of glycosmis arborea. Phytochemistry, 50: 1263-1266.

Chuankhayan, P., T. Rimlumduan, J. Svasti and J.R.K. Cairns, 2007a. Hydrolysis of soybean isoflavonoid glycosides by Dalbergia β-glucosidases. J. Agric. Food Chem., 55: 2407-2412.

Chuankhayan, P., T. Rimlumduan, W. Tantanuch, N. Mothong and P.T. Kongsaeree *et al.*, 2007b. Functional and structural differences between isoflavonoid β-glycosidases from *Dalbergia* sp., Arch. Biochem. Biophys., 468: 205-216.

Chuankhayan, P., Y. Hua, J. Svasti, S. Sakdarat, P.A. Sullivan and J.R.K. Cairns, 2005. Purification of an isoflavonoid 7-O-beta-apiosyl-glucoside beta-glycosidase and its substrates from *Dalbergia nigrescens* Kurz. Phytochemistry, 66: 1880-1889.

Dhir, B.C., H.K. Mohapatra and B. Senapati, 1992. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F). Ind. J. Plant Protect., 20: 215-217.

Hessler, P.E., P.E. Larsen, A.I. Constantinou, K.H. Schram and J.M. Weber, 1997. Isolation of isoflavones from soy-based fermentation of the erythromycin-producing bacterium *Sacharopolyspora erthraea*. Applied Microbol. Biotechnol., 47: 398-404.

Hrmova, M., A.J. Harvey, J. Wang, N.J. Shirley and G.P. Jones *et al.*, 1996. Barley β-D-glucan exohydrolases with β-D-glucosidase activity: Purification, characterization and determination of primary structure from a cDNA clone. J. Biol. Chem., 271: 5277-5286.

Hrmova, M., E.A. MacGregor, P. Biely, R.J. Stewart and G.B. Fincher, 1998. Substrate binding and catalytic mechanism of a barley β-D-glucosidase/(1,4)-β-D-glucan exohydrolase. J. Biol. Chem., 273: 11134-11143.

Isman, M.B., H. Matsuura, S. MacKinnon, T. Durst, G.H.N. Towers and J.T. Arnason, 1996. Phytochemistry of the meliaceae: So many terpenoids, so few insecticides. Recent Adv. Phytochem., 30: 155-178.

Jovanoic, S.V., S. Steenken, M. Tosic, B. Marjanovic and M.G. Simic, 1994. Flavonoids as antioxidants. J. Am. Chem. Soc., 116: 4846-4851.

Kivotani, T., K. Masuda, H. Ageta, A.K. Chakravarty and B. Das, 1996. Glycoric acid, a new degraded carotenoid from *Glycosmis arborea*. Acta Cryst., 52: 3216-3217.

Kranthi, K.R., D.R. Jadhav, S. Kranthi, R.R. Wanjari, S.S. Ali and D.A. Russell, 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot., 21: 449-460.

Kumar, J.P., R.K. Tanwar, M.C. Jat, A.D. Handapani, O.M. Bambawale and D. Monga, 2007. Spodoptera litura an emering pest on BT Cotton (Cry1AC) under North Indian conditions. Pesticide Res. J., 19: 197-200.

Kuntothom, T., S. Luang, A.J. Harvey, G.B. Fincher, R. Opassiri, M. Hrmova and J.R.K. Cairns, 2009. Rice family GH1 glycosyl hydrolases with β-D-glucosidase and β-D-mannosidase activities. Arch. Biochem. Biophys., 491: 85-95.

Leah, R., J. Kigel, I. Svendsen and J. Mundy, 1995. Biochemical and molecular characterization of barley seed β-glucosidase. J. Biol. Chem., 270: 15789-15797.

Opassiri, R., J.R.K. Cairns, T. Akiyama, O. Wara-Aswapati, J. Svasti and A. Esen, 2003. Characterization of a rice β-glucosidase genes highly expressed in flower and germinating shoot. Plant Sci., 165: 627-638.

Opassiri, R., Y. Hua, O. Wara-Aswapati, T. Akiyama, J. Svasti, A. Esen and J.R.K. Cairns, 2004. β-glucosidase, exo-β-glucanase and pyridoxine transglucosylase activities of rice BGlu1. Biochem. J., 379: 125-131.

Opassiri, R., B. Pomthong, T. Okoksoong, T. Akiyama, A. Esen and J.R.K. Cairns, 2006. Analysis of rice glycosyl hydrolase family 1 and expression of Os4bglu12 β-glucosidase. BMC Plant Biol., 6: 33-33.

Pandjaitan, N., N. Hettiarachchy and Z.Y. Ju, 2000a. Enrichment of genistein in soy protein concentrate with beet β-glucosidase. J. Food Sci., 65: 403-407.

Pandjaitan, N., N. Hettiarachchy, Z.Y. Ju, P. Crandall, C. Seneller and D. Ombek, 2000b. Enrichment of genistein in soy protein concentrate with hydrocolloids and β-glucosidase. J. Food Sci., 65: 591-595.

Patel, V.B. and J.R. Patel, 1996. Ovicidal property of some indigenous plant extracts against *Spodoptera litura*. Gujarat Agric. Univ. Res. J., 21: 137-140.

Srisomsap, C., J. Svasti, R. Surarit, V. Champattanachai and K. Boonpuan *et al*., 1995. Isolation and characterization of β-D-Glucosidase/β-D-Fucosidase from i. Pierre J. Biochem., 119: 585-585.

Sujatha, S., B. Joseph and P.S. Sumi, 2010. Medicinal plants and its impact of ecology, nutritional effluents and incentive of digestive enzymes on *Spodoptera litura* (fabricious). Asian J. Agric. Res., 4: 204-211.

Suzuki, H., S. Takahashi, R. Watanabe, Y. Fukushima and N. Fujita *et al*., 2006. An isoflavone conjugate-hydrolyzing β-glucosidase from the roots of soy bean (*Glycine max*) seedlings. J. Biol. Chem., 281: 30251-30259.

Svasti, J., C. Srisomsap, S. Techansakul and R. Surarit, 1999. Dalcochinin-8'-O-β-D-Glucoside and its β-glucosidase enzyme from *Dalbergia cochinchinensis*. Phytochemistry, 50: 739-743.

Toonkool, P., P. Metheenukul, P. Sujiwattanarat, P. Paiboon and N. Tongtubtim *et al*., 2006. Expression and purification of dalcochinase, a β-glucosidase from *Dalbergia cochinchinensis* Pierre, in yeast and bacterial hosts. Protein Express. Purific., 48: 195-204.

Tsangalis, D., J.F. Ashton, J. McGill and N.P. Shah, 2002. Enzymatic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase producing bifidobacteria. J. Food Sci., 67: 3104-3113.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty, 1995. Indian Medicinal Plants a Compendium of 500 Species. Orient Longman Pvt. Ltd., New Delhi, pp: 82-83.

Wei, H.Y., Y.P. Huang and J.W. Du, 2004. Sex pheromones and reproductive behavior of *Spodoptera litura* (Fabricius) moths reared from larvae treated with four insecticides. J. Chem. Ecol., 30: 1457-1466.

Wei, H., P. Wu, X. Ge, M. Liu and X. Wei, 2007. Chemical constituents of the seeds of *Ammopiptanthus* (L.) and their systematic and ecological significance. Biochem. Syst. Ecol., 35: 274-280.