Introduction

*Juglans* Linn. (Juglandaceae) is a genus of trees distributed in North and South America and from South Europe to East Asia. It is commonly known as Walnuts, some of the species are extensively grown for timber and fruit. *Juglans regia* is only one species, which is found in India (Anonymous, 1997).

Vernacular names

Assam - Kabsing; Bengali - Akhrot, Akrut; English - Common Walnut, Persian Walnut; Hindi - Akhrot; Kannad - Akrodu; Kashmir - Akhor; Malayalam - Akrottu; Marathi - Akroda; Sanskrit - Akschota, Akhoda; Tamil - Akrottu (Kirtikar and Basu, 1994).

Distribution

It is distributed throughout the Himalayas and hills of Assam at altitudes of 1,000-4,000 m and largely cultivated in Afghanistan, Kashmir and Tibet. Other Walnut growing regions are hilly areas of Punjab, Himachal Pradesh and Uttar Pradesh. But the quantity and quality of Walnuts produced from these regions is not usually as good as that from Kashmir (Nadkarni, 1998 and Chatterjee *et al.*, 1994).
Description

It is a large deciduous tree. Leaves and bark aromatic, bark on old stem marked by parallel vertical furrows, grey, young shoots tomentose; leaves 15-38 cm long, imparipinnate, more or less tomentose when quite young; leaflets 5-9 the terminal largest, 7.5-20 by 3.8-10 cm variable, from elliptic to oblong-lanceolate acute or acuminate, usually entire, glabrous or pubescent along the nerves beneath, the lateral leaflets opposite or subopposite, sessile or subsessile; male spikes lateral on the shoots of the previous year, 5-12.5 cm long, often 2 superposed to one leaf-scar, bracts stipitate, stamens 10-20, apiculate; female flowers 1-3, sessile, in a short terminal spike, calyx-tube 6 mm long, ovoid, densely tomentose, limb minute, obscurely 4-toothed; petals green, linear-lanceolate, usually minute; fruit a drupe, 5 cm long, ellipsoid, green, pericarp leathery, aromatic, nut externally distinctly 2-valved corresponding to the 2 carpels of which the ovary is composed, rugose, internally incompletely divided by 2 coriaceous dissepiments one separating the 2 cotyledons the other dividing them into 2 lobes (Kirtikar and Basu, 1994).

Phytoconstituents

The stem bark contains the α-tetralone derivative designated (-) regiolone along with juglone, betulinic acid and β-sitosterol. (-) Regiolone has been identified as 4,8 dihydroxy-1-tetralone (Talapatra et al., 1988). Berberine (0.08%) has been isolated from stem bark (Bhandari and Gupta, 1974). Isolation and identification of cyclo-trisjuglone, β-sitosterol and juglone have also been reported from J. regia root bark (Sidhu et al., 1975). Column chromatography of chloroform extract yielded 3,3'-bisjuglone from root bark of this plant (Pardhasaradhi and Hari Babu, 1978). C_{15}-C_{35} alkanes estimated (0.0036%) in bark by GC, ratio of odd homologous to even homologous alkanes determined as 1: 86 (Rastogi and Mehrotra, 1991).

The unripe fruit husk gave the volatile compounds as 2-methyl-1,4-naphthoquinone, plumbagin(5-hydroxy-2-methyl-1,4-naphthoquinone), β-hydroplumbagin (2,3-dihydro-5-hydroxy-2-methyl-1,4-naphthalenedione), 5-hydroxy-3-methyl-1,4-naphthoquinone and 2,3-dimethyl-5-hydroxy-1,4-naphthoquinone (Anonymous, 2003). Unripe fruit and other parts of the plant yielded a substance, which reduced indophenols dye. This
substance has been identified as α-hydrojuglone glucoside (C_{16}H_{18}O_{9}), which on hydrolysis produced glucose and α-hydrojuglone (1,4,5-trihydroxynaphthalene) (Anonymous, 1997).

Twenty one monoterpenes, two sesquiterpenes, twenty three hydrocarbons along with eugenol and geranic acid have been isolated from leaves (Rastogi and Mehrotra, 1993). The surface waxes from the leaves and fruits of Walnut exhibited a similar chemical composition with primary alcohols (41.6 and 35.7%) as the major lipid class. The other homologous series present in these waxes were hydrocarbons (3.0 and 15.6%), esters (3.5 and 3.4%), aldehyde (5.5 and 10.0%) and fatty acids (8.4 and 4.7%) in smaller amounts. In addition to these common wax lipids, 5-hydroxy-1,4-naphthaquinone (juglone) was identified in the leaf (29.8%) and fruit waxes (28.6%) (Prasad and Gulz, 1990).

Chlorophylls a and b, β-carotene and juglone are detected in lipophilic substances of leaves. The pericarp and leaves have been reported to contains phenolic acids, salicylic, p-hydroxybenzoic, caffeic (predominant in leaves), vanillic, gentisic, syringic (predominant in pericarp), protocatechuic, gallic, p-hydroxyphenyl lactic, p-coumaric, ferulic, sinapic and chlorogenic acids (Anonymous, 2003). Presence of (-)-jasmonic, 6-epi-cucurbic and 6-epi-7-iso-cucurbic acid has been reported in female flowers (Stefania et al., 1989).

The immature fruit and the fresh leaves are rich in ascorbic acid. Leaves may be preserved by exposing them to sulphur dioxide gas and then rapidly drying at 100-110°C. The treated leaves may be preserved or extracted with water to obtain concentrates of ascorbic acid (Anonymous, 1997). Juglone, estradiol and stigmasterol are isolated from pollens (Huanmin and Zongxun, 1987).

The seeds have been reported to furnish phospholipids (438.5-528.9; mg%) with phosphatidylcholines and phosphatidylethanolamines as major components. They contained the biologically active phospholipids, phosphotidyl inositol, 33.3%; phosphotidyl choline, 26.8%; phosphatidyl ethanolamine, 23.1 and cardiohpin, 16.6%.

A globulin, juglansin, has been isolated from the edible kernel. Vitamins of the B group present in the kernel are thiamine 0.33-0.40, riboflavin 0.10-0.16, nicotinic acid 0.58-0.81 and vitamin B_{6} 0.87-1.05 mg/100 g and biotin 2-μg/100 g. The kernel contained also vitamin A and ascorbic acid. The kernel yielded 60-70% of a drying oil, known in the trade as walnut oil. The oil is greenish yellow or almost colourless with a pleasant odour and a nutty flavour. The fatty acids present are palmitic 3-7%, stearic 0.5-3%, oleic 9-30%,

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linoleic 57-76% and linolenic 2-16%. Walnut shell flour contained cellulose, lignin, furfural (5%), pectosans (9%), methylhydroxyamine (6%), sugar and starch (2.5%) (Anonymous, 1997).

Three hydrolyzable tannins, glansrins, together with adenosine, adenine, and 13 known tannins were isolated from the n-BuOH extract of Walnuts. Glansrins was characterized as ellagitannins with a tergalloyl group, or related polyphenolic acyl group based on spectral and chemical evidences (Fukuda and Yoshida, 2003).

The main soluble phenolic compounds of walnut wood extracts were characterized as hydrojuglone glucoside (HJG), quercitrin (QUER) and two ellagic acid derivatives (E₁ and E₂). It was shown that HJG, QUER and E₁ were the dominant phenolic compounds of walnut sapwood (Burtin et al., 1998).

Phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic, sinapic, ellagic, and syringic acid) as well as syringaldehyde and juglone were identified in ripe fruits of ten Walnut cultivars. Analyses were done using a high-performance liquid chromatograph equipped with a diode array detector. It was found that the Walnut pellicle is the most important source of Walnut phenolics. The ratio between the contents in pellicle and kernel varied by at least 14.8-fold for caffeic acid and by up to 752.0-fold for p-coumaric acid (Cholaric et al., 2005).
Chemical structures of phytoconstituents reported from *J. regia* L.

![Chemical structures](image)

- **Juglone**

- **3,3' Bisjuglone**

- **(-) Regiolone (4,8 dihydroxy-1-tetralone)**

- **Tetralones shinanolone, R₁ = H, R₂ = Me**

- **Isoshinanol-one, R₁ = Me, R₂ = H**

- **Sclerone**

- **7- Methyl juglone, R₁ = H, R₂ = Me**

- **Plumbagin, R₁ = Me, R₂ = 0H**

- **Regiolone dibenzoate**

- **Betulinic acid**
**Juglans regia** Linn.

1. **Berberine**
   \[ \text{H}_3\text{CO}^+ \text{OCH}_3 \]
   
2. **β-Sitosterol**
   \[ \text{HO} \]
   
3. **Cyclotrisjuglone, } R=H \]
   \[ \text{RO}_{\text{O}} \text{O}_{\text{O}} \text{OR} \]

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Chapter-2

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Medicinal properties and uses

*Juglans regia* (Walnut) barks are very popular and sold in market under the name Dundasa and is used as tooth cleaning device or for reddening the lips by the people living in all temperate Europe and in the Himalayas, China and Japan (Almas and Al-Lafi, 1995; Anonymous 1997).

Finely powdered bark is used as an ingredient for the treatment of bleeding gums as mouth rinse (Lahankar and Rehman, 1990). It can also be used in cosmetics due to the presence of juglone for dentifrices, toothpowders, mouth rinses, deodorants and chewing gums (Anonymous, 2003).

The bark and leaves have alternative, laxative, astringent and detergent properties and are used in the treatment of skin troubles. They are used in herpes, eczema, scrofula and syphilis. Externally the infusion is used as an application to skin eruptions and ulcers (Anonymous, 1997).

Homeopaths prescribe the tincture of its leaves for cutting wisdom teeth. It has also been claimed that a decoction of its leaves makes an effective mouthwash (Francesco, 1977). The bark of *J. regia* is also known to contain 0.5 ppm fluorides and the role of fluorides in inhibiting dental caries has been well documented (Almas and Al-Lafi, 1995). Studies performed on the powdered bark have demonstrated its effectiveness against gum disorders such as bleeding and swelling (Date et al., 1995).

In Turkey, in their traditional system of medicine, the leaf and fruit extracts are used in wound healing and maturation of abscesses (Tabata et al., 1994).

Walnut is described as an anticancer, tonic, blood purifier, and detoxifier agent. The nuts have favorable fatty acids composition and nutrients. Frequent consumption of nuts in the daily diet was associated with a potentially decreased risk of coronary artery disease by decreasing the level of triglyceride and increasing the level of HDL (Ziabeenezhad et al., 2005).

The Walnut plant is most frequently used as anti-parasitics and repellants. Decoction of leaves is efficacious, particularly in stromous sores, fruits are considered to be a tonic and useful in rheumatism. Shells of unripe fruits are vermifugal. Ripe fruit or kernel is palatable and possesses aphrodisiac properties (Guarrera, 1999).
Bioactivities

Antimicrobial activity

Aqueous and alcoholic extracts from *Juglans regia* were tested for their ability to inhibit the growth and some physiological functions of *Streptococcus mutans*. Both aqueous and alcoholic extract strongly inhibited the growth, *in vitro* adherence, acid production and glucan induced aggregation of *S. mutans* (Jagtap and Karkera, 2000).

The antimicrobial activities of *Psidium guajava* and *Juglans regia* leaf extracts, determined by disk diffusion method (zone of inhibition), were compared to tea tree oil (TTO), doxycycline and clindamycin antibiotics. The zones of inhibition were significantly higher than those of TTO and equivalent in case of *Staphylococci* species, but less in case of *P. acnes*, to those obtained from doxycycline or clindamycin. It can be concluded that *Psidium guajava* and *Juglans regia* leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities (Qadan *et al.*, 2005).

The antimicrobial activities of the ethanolic extracts of *Plantago major*, *Ononis spinosa*, *Lythrum salicaria* and *Juglans regia*, all used in the folk-medicine, were tested against Gram negative and Gram positive bacteria as well as yeast like fungi by the agar diffusion method. All plants showed higher antibacterial activity against *Escherichia coli* and *Staphylococcus* species (Citoglu and Altanlar, 2003).

Antifungal activity

The dry extract of the fruit showed antifungal activity against *Aspergillus* species and can be used in herbal antifungal formulations or cream (Binder *et al.*, 1989).

Anti-inflammatory and antinociceptive activities

Ethanolic and aqueous extracts from the leaves of *J. regia* were evaluated for *in vivo* anti-inflammatory and antinociceptive activities. Both the extracts possessed significant antinociceptive activity in varying degrees against p-benzoquinone-induced abdominal contraction in mice and exhibited potent anti-inflammatory activity against carrageenan induced hind paw edema model in mice without inducing any gastric damage (Erdemoglu *et al.*, 2003).
Sedative activity

Sedative activity of *J. regia* L. chloroform extract of leaves and juglone were evaluated in mice. The most important pharmacological activity observed was 6.5 and 0.125 mg/kg doses of chloroform and juglone, respectively. The chloroform extract from Walnut fresh leaves showed an important activity due to juglone (Girzu et al., 1998).

Thyroid hormone enhancing activity

The effect of fruit of *J. regia* on the thyroid hormone levels of mice was investigated using two extracts prepared from the fruits by different methods. The acute toxicities of these two extracts in mice were assessed as well. The extracts prepared from the fruits of *J. regia* enhanced thyroid hormone levels, while they exerted minimal acute toxicity in mice (Aydin et al., 1994).

Hypoglycaemic activity

The extracts of *J. regia* were prepared by boiling the dried leaves with water or macerating it with 80% ethanol. Male Swiss mice were orally loaded with glucose after the extracts had been given by oral gavage. The extract lowered the blood glucose level in mice (Neef et al., 1995).

Antitumor activity

The inhibition of phosphatidylinositol-3-kinase (PtdIns-3-kinase), protein kinase C and c-Src protein tyrosine kinase by a series of halogenated naphthoquinones and quinoline quinones related to the plant-derived naphthoquinones juglone and methyljuglone, which inhibited protein kinase C, has been investigated. Some halogenated quinones showed in vivo antitumor activity without accompanying toxicity, while methyljuglone was without in vivo antitumor activity. Inhibition of PtdIns-3-kinase by the halogenated quinones may provide a lead for the development of more potent and specific inhibitors (Frew et al., 1995).
Succinic dehydrogenase activity

Aqueous extracts of miswak (Salvadora persica; Arak tree) and akhrot (Juglans regia; Walnut tree) were prepared and their effects investigated on growth of Balb/C 3T3 mouse fibroblasts by measuring the mitochondrial succinic dehydrogenase activity. Furthermore, the effects on the viability of various cariogenic bacteria (Streptococcus mutans, Streptococcus salivarius, Lactobacillus casei and Actinomyces viscosus) were also determined. The most sensitive organisms were A. viscosus, followed by S. mutans, S. salivarius, with L. casei being the most resistant. The results showed that aqueous extracts of miswak and akhrot enhance the growth of fibroblasts and inhibit the growth of cariogenic bacteria, with the akhrot extract showe greater activity than miswak (Darmani et al., 2006).
MATERIALS AND METHODS

**Apparatus, equipments and chemicals**

- All the chemicals and reagents were obtained from Qualigen, E. Merck, Himedia and s.d. fine chemicals Ltd.
- Most of the reagents used were of Analar grade.
- Silica gel (60-120 mesh) and silica gel-G, obtained from Qualigen Fine chemicals, Mumbai, were used for the column chromatography and thin layer chromatography (TLC), respectively.
- Anhydrous sodium sulphate was used for drying petroleum ether (60-80°C) and chloroform.
- Melting points were determined on Perfit melting point apparatus and are uncorrected.
- All the weighing was done on single pan Metler Balance.
- Ash values were determined on a Perfit rectangular Muffle furnace (laboratory model) in a silica crucible.
- Ultra violet (UV) spectra were recorded on Beckman DU-6 spectrophotometer in methanol.
- All the Fourier Transform Infra Red (FTIR) spectra were recorded on Jasco FT/IR-5 5000 spectrophotometer using KBr pellets; $\lambda_{\text{max}}$ values are given in cm$^{-1}$.
- $^1$H NMR spectra was screened on Advance Dry 400, Bruker Spectrospin 400-MHz instrument using CDCl$_3$ as a solvent and trimethyl silane (TMS) as an internal standard. Chemical shift are given in $\delta$ (ppm) scale with respect to the TMS as an internal standard and coupling constants (J values) are expressed in Hz. Notation used for spin coupling pattern throughout the manuscript have been mentioned as: s = singlet; d = doublet; dd = double doublet; ddd = doublet of doublet doublet; q = quartet; t = triplet; w = half width; m = multiplet; brs = unresolved broad signal.
- $^{13}$C FT-NMR spectra were recorded on Advance dry 400 Bruker spectrospin 100-MHz with TMS as an internal standard in 5 mm spinning tubes at 27°C.
• Mass spectra (MS) were scanned by effecting FAB ionization JEOL-JMS-DX 303 system, equipped with direct inlet probe system. The m/z values of the more intense peaks are mentioned and the figures in brackets attached to each m/z indicated relative intensity with respect to the base peak.

• Heavy metals analysis were determined by GBC atomic absorption spectrophotometer (AAS), model 932 AA

• Plant materials: The bark of Juglans regia, bark of Mimusops elengi, bark of Symplocos racemosa, flower buds of Syzygium aromaticum and seed of Zanthoxylum alatum were purchased from Khari Baoli, local market of Delhi. The sample were identified on the basis of exomorphic character, review of literature, by Dr. H. B. Singh, taxonomist, NISCAIR, CSIR, New Delhi and by Dr. M. P. Sharma, taxonomist, Dept. of Botany, Faculty of Science, Jamia Hamdard, New Delhi. Voucher specimens are the drugs are preserved in the Phytochemistry laboratory, Faculty of Science, Jamia Hamdard, New Delhi.

• Preparation of slurry: The concentrated extract of drug was taken in a China dish and heated continuously on a water bath by gradually adding methanol in small portions with constant stirring, till desired consistency was obtained. Silica gel (for column, weighed quantity) was then added slowly with continuous mixing with a steel spatula until desired dry mixture was obtained. The larger lumps were broken up and finally passed through a sieve no. 8 to get the slurry of uniform particle size.

• Packing of column: A column of 2.3 m length and 16 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with absorbent cotton wool. The column was clamped and fitted in a vertical position on a stand. The column was then half filled with petroleum ether (b.p.60-80°C). Silica gel (for column) was then poured in small portions and allowed to settle down gently until the necessary length of the column was obtained. The slurry, containing the drug, was loaded on the top of the column and then eluted successively with different solvents in order of increasing polarity of the solvents. The development and elution of the column was carried out with a successive series of different solvents in various combinations such as petroleum ether, petroleum ether: chloroform (9:1, 3:1, 1:1, 1:3, 1: 9 v/v); chloroform,
chloroform: methanol (99.5: 0.5, 99: 1, 98: 2, 97: 3, 95: 5, 90: 10 v/v) and methanol.

- Preparation of the TLC plates: 100 g of silica gel-G was mixed with 400 ml of distilled water by trituration in a glass paste and mortar to form a fine thin paste. The slurry was poured on a clean glass plate and was spread uniformly by means of an applicator to achieve 0.25 mm thickness. The plates were allowed to dry in air at room temperature for 30 minutes.

- Activation of TLC plates: The air dried plates were kept in an electric oven by maintaining the temperature at 110°C for two hours and then stored in a dessicator.

- Equilibration of the chromatographic chamber: About 1 cm height of the solvents was taken in a clean dry chamber. The walls of the chamber were lined with a strip of filter paper impregnated with the solvent system, the chamber was closed and allowed to saturate with vapours of the solvent.

- Application of spots: The base line marked at about 2 cm above from the lower edge. The dissolved fractions were spotted on the plates with fine capillary tubes and allow to dry in air.

- Development of chromatogram: The spotted plates were kept in the chromatographic chamber containing the solvent mixture. The chambers were covered with greased glass plates. The solvent system was allowed to run up to 2 cm below the upper edge of silica gel-G layer. The plates were taken out, solvent fronts marked and dried.

- Homogeneity of the fractions: The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having the same Rf values) were combined together and concentrated. They were then crystallized with suitable solvent system.

- Solvent system for the plates:
  - Petroleum ether (60-80°C)
  - Chloroform
  - Petroleum ether: chloroform (9:1)
  - Petroleum ether: toluene: ethylacetate (10:5:3)
  - Toluene: ethylacetate: formic acid (5:4:1)
Toluene; ethylformate; formic acid (10:10:3)
Chloroform; methanol (9.8:0.2)
Chloroform; methanol; formic acid (9:1:0.5).

- Detection of spots: The air dried plates were viewed in ultra-violet light radiation to look for activation of colour fluorescence and spots were marked, if any. The plates were viewed in iodine vapour chamber and the spots were marked, if any. Freshly prepared spraying reagents, 10% sulphuric acid, Liebermann-Burchard reagent, anisaldehyde-sulphuric acid, vanillin-sulphuric acid, 5% ferric chloride and ceric ammonium sulphate (CAS) were used for detection of spots. The reagents were carefully sprayed on the plates and were subsequently heated in an oven at 110°C for 10-15 minutes. The colours of the visualized spots were noted down and Rf values were calculated.

Preparation of reagents (I. P. 1996)

- Liebermann-Burchard reagent: Concentrated sulphuric acid (2.5 ml) was mixed with 2.5 ml of acetic anhydride and 45 ml of ethanol. This is shaken well and stored.
- Anisaldehyde-sulphuric acid: 0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml MeOH and 5 ml of conc. sulphuric acid.
- Vanillin-sulphuric acid: Vanillin (1g) was dissolved in 95 ml of 95% v/v ethanol. Concentrated sulphuric acid (5 ml) was added to this solution drop by drop with continuous shaking.
- Ceric ammonium sulphate (CAS) solution: Saturated solution of CAS was prepared in 65% sulphuric acid. The solution was kept on steam bath for 15 minutes and left overnight. The clear supernatant solution was decanted and used for spraying. The reagent should be prepared fresh.
- Dragendorff’s reagent stock solution: (Potassium iodobismuthate solution modified) 1.7g of bismuth subnitrate and 20 g of tartaric acid were suspended in 40 ml of distilled water. To the suspension 40 ml of 405 w/v solution of potassium iodide were added, stirred for 1 hour and filtered. This stock solution was kept for several days in light resistant container.
**Dragendorff’s reagent:** The stock solution (5 ml) was mixed with 15 ml of water immediately before use.

**Mayer’s reagent:** Mercuric chloride (1.4 g) was dissolved in 60 ml of distilled water. A solution of 5 g of potassium iodide in 20 ml of distilled water was added to produce 100 ml and kept in a container for use.

**Wagner’s reagent:** Iodine (1.27 g) was dissolved in 5 ml of water and the volume made up to 100 ml. To this solution 100 ml of 2% w/v of potassium iodide solution was added, mixed well and stored in well stopper container for use.

**Million’s reagent:** Mercury (3 g) was dissolved in 27 ml of cold fuming nitric acid and the solution diluted with equal volume of water.

**Ninhydrine reagent:** Ninhydrine (30 g) was dissolved in 10 ml of n-Butanol followed by 0.3 ml of 98% v/v in acetic acid and stored for use.

**Hager’s reagent:** Saturated aqueous picric acid solution was prepared.

**Fehling’s solution-A (Copper sulphate solution):** Carefully selected small crystals of cupric sulphate, (34.66 g) were dissolved showing no trace of effervescence or adhering, moisture in sufficient water to produce 500 ml, and stored in a well closed container.

**Fehling’s solution-B (Alkaline tartrate solution):** Sodium potassium tartrate (176 g) and sodium hydroxide (77 g) were dissolved in sufficient water to produce 500 ml and store in well stopper container. Equal volumes of Fehlings A and B solutions were mixed immediately before use.
EXPERIMENTAL

Plant material

The barks of \textit{J. regia} were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi.

Extraction

The barks of \textit{J. regia} were dried in an oven at 45°C for 2-3 days and coarsely powdered. The ground bark (3 kg) was extracted with ethanol in a Soxhlet apparatus. The ethanol extract was concentrated under reduce pressure to yield a dark red viscous mass (375 g, 12.5%).

Isolation of chemical constituents

The extract (375 g) was dissolved in a minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The air-dried slurry was chromatographed over the silica gel column packed in petroleum ether (60-80°C). The column was eluted with petroleum ether (60-80°C), chloroform and methanol in their various combinations in the order of increasing polarity to isolate the following compounds.

Benzjuglansonic acid (JR-1)

Elution of the column with petroleum ether-chloroform (3:1) gave colourless amorphous powder of JR-1, recrystallized from MeOH.

Yield: 78 mg (0.0026%).

R\text{f}: 0.39 (Chloroform).

m.p. 147-148°C.

UV \lambda_{max} (MeOH): 206, 242, 280 nm (log \varepsilon 2.1, 1.7, 1.7).

IR \nu_{max} (KBr): 3410, 2938, 2862, 1695, 1640, 1535, 1470, 1210, 950, 865 cm\textsuperscript{-1}.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}): \delta 12.22 (1H, brs, COOH), 7.65 (1H, dd, J = 7.2, 2.7 Hz, H-3), 7.60 (1H, dd, t = 7.5, 3.1 Hz, H-6), 7.28 (1H, m, H-5), 6.10 (1H, m, H-4), 3.92 (3H, brs, OMe), 2.17 (2H, brs, H\textsubscript{2}-7), 1.60 (2H, brs, H\textsubscript{2}-7), 1.60 (2H, brs, H\textsubscript{2}-9), 1.26 (2H, brs, H\textsubscript{2}-8).
Naphthjuglanosic acid (JR-2)

Elution of the column with petroleum ether-chloroform (3:1) yielded colourless crystals of JR-2, after recrystallization from MeOH.

Yield: 71 mg (0.0023%).

Rf: 0.61 (Chloroform).

m.p. 176-178°C.

UV λmax (MeOH): 231 nm (log ε 3.8).

IR νmax (KBr): 3469, 2920, 2851, 1695, 1639, 1604, 1550, 1458, 1321, 1242, 1209, 1185, 1068, 1035, 830, 777 cm⁻¹.

1H NMR (CDCl₃): δ 11.74 (1H, brs, H-19), 7.68 (1H, d, J = 7.5 Hz, H-3), 7.64 (1H, dd, J = 8.4, 3.0 Hz, H-8), 7.58 (1H, d, J = 7.5 Hz, H-4), 7.29 (1H, dd, J = 8.4, 3.0 Hz, H-6), 6.15 (1H, m, H-7), 3.92 (3H, brs, OMe), 2.88 (2H, m, H₂-11), 1.57 (4H, brs, 2 x CH₂), 1.26 (8H, brs, 4 x CH₃), 0.88 (3H, t, J = 6.6 Hz, Me-18).

13C NMR (CDCl₃): δ 179.11 (COOH), 165.23 (C-1), 142.67 (C-9), 137.17 (C-5), 135.47 (C-10), 125.22 (C-2), 123.85 (C-8), 119.58 (C-3), 118.92 (C-6), 110.47 (C-7), 109.50 (C-4), 56.60 (OMe), 48.16 (C-11), 29.57 (C-12, C-15), 29.51 (C-17), 28.48 (C-13), 26.97 (C-14), 23.11 (C-16), 14.38 (C-18).

+ve FAB-MS m/z (rel. int.): 314 [M]+ (C₂₀H₃₆O₃) (9.3).

Juglansbenzoate (JR-3)

Elution of the column with chloroform afforded light brown amorphous powder of JR-3, recrystallized from MeOH.

Yield: 81 mg (0.0027%).

Rf: 0.72 (Toluene: ethyl formate: formic acid; 10:10:3).

m.p. 208-210°C.

UV λmax (MeOH): 254 nm (log ε 3.9).
Regiaoleate (JR-4)

Elution of the column with chloroform-methanol (49:1) furnished colourless amorphous powder of JR-4, recrystallized from MeOH-CHCl₃ (1:1).

Yield: 135 mg (0.0045%).

Rf: 0.82 (Toluene: ethyl formate: formic acid; 10:10:3).

m.p. 83.85°C.

UV λₓₒₓₓ (MeOH): 249 nm (log ε 2.8).

IR νₓₓₓ (KBr): 2923, 2852, 1740, 1640, 1353, 1263, 1167, 1104, 798 cm⁻¹.

¹H NMR (CDCl₃): δ 7.67 (2H, m, H-2, H-5), 7.19 (2H, m, H-3, H-4), 4.26 (1H, brs, H₂-1'a), 4.06 (1H, brs, H₂-1'b), 3.78 (1H, d, J = 18.0 Hz, H₂-1'"a), 3.66 (1H, d, J = 18.0 Hz, H₂-1'"b), 2.31 (2H, brs, CH₂), 2.03 (2H, brs, CH₂), 1.61 (2H, brs, CH₂), 1.25 (62H, brs, 31 x CH₃), 0.88 (6H, brs, Me-27'a, Me-11'").

¹³C NMR (DMSO-d₆): δ 171.65 (C-7), 161.32 (C-6), 134.96 (C-1), 121.75 (C-5), 118.47 (C-2), 117.26 (C-3, C-4), 66.17 (C-1'), 63.25 (C-1'"), 34.16 (C-2'), 33.89 (C-2'"), 28.97 (32 x CH₂), 14.13 (C-11'"), 13.16 (C-27').

+ve FAB-MS m/z (rel. int.): 670 [M]+ (C₄₅H₇₆O₃) (1.1), 395 (27.8), 155 (67.2), 120 (36.5).

Hydrolysis of JR-4:

Compound JR-4 (15 mg) was refluxed with ethanolic 1N KOH solution (10 ml) for thirty minutes on a steam bath. The reaction mixture was extracted with CHCl₃ (3 x 10 ml).
to remove alcoholic moiety. It was acidified, re-extracted with CHCl₃ (2 x 10 ml) and dried over Na₂SO₄. After evaporation of the solvent, oleic acid was obtained, (TLC comparable).

Regiapalmitate (JR-5)

Elution of the column with chloroform-methanol (9:1) gave colourless amorphous powder of JR-5, recrystallized from MeOH.

Yield: 72 mg (0.0024%).

Rᵣ: 0.65 (Chloroform : methanol; 9:1).

m.p. 86-87°C.

UV λ<sub>max</sub> (MeOH): 206, 247 nm (log ε 1.2, 1.4).

IR ν<sub>max</sub> (KBr): 2919, 2851, 1738, 1612, 1354, 1284, 1237, 1177, 1067, 987, 794, 758 cm⁻¹.

¹H NMR (DMSO-d₆): δ 5.30 (2H, m, H-12', H-13'), 4.08 (2H, brs, H₂-1'), 2.50 (2H, brs, H₂-2), 2.26 (2H, m, H₂-11'), 1.96 (2H, m, H₂-14'), 1.48 (2H, brs, CH₂), 1.23 (86H, brs, 43 x CH₂), 0.83 (6H, brs, Me-16, Me-37').

¹³C NMR (DMSO-d₆): δ 171.23 (C-1), 129.17 (C-12'), 111.45 (C-13'), 62.96 (C-1'), 48.36 (C-2), 33.39 (C-11'), 29.73 (C-14'), 28.97 (42 x CH₂), 26.53 (CH₂), 24.71 (CH₂), 14.13 (Me-16, Me-37').

⁺ve FAB-MS m/z (rel. int.): 772 [M]+ (C₅₃H₇₈O₁₁) (1.1), 533 (22.6), 363 (21.8), 239 (12.9).

Hydrolysis of JR-5:

Compound JR-5 (15 mg) was refluxed with ethanolic 1N KOH solution (10 ml) for thirty minutes on a steam bath. The reaction mixture was extracted with CHCl₃ (3 x 10 ml) to remove alcoholic moiety. It was acidified, re-extracted with CHCl₃ (2 x 10 ml) and dried over Na₂SO₄. After evaporation of the solvent, palmitic acid was obtained, (TLC comparable).

β-Sitosterol (JR-6)

Elution of the column with chloroform-methanol (1:1) furnished colourless amorphous powder of JR-6, recrystallized from MeOH.

Yield: 66 mg (0.002%).

Rᵣ: 0.43 (Chloroform : methanol; 1:1).
m.p. 136-138°C.

UV $\lambda_{\text{max}}$ (MeOH): 205 nm ($\log e$ 4.15).

IR $\nu_{\text{max}}$(KBr): 3430, 2919, 2854, 1620, 1459, 1375, 1121, 1055, 955, 849, 795 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 5.36 (1H, d, $J = 4.64$ Hz, H-6), 3.52 (1H, brs, w $1/2$ 18.5 Hz, H-3 $\alpha$), 1.01 (3H, brs, Me-10), 0.93 (3H, d, $J = 6.50$ Hz, Me-21), 0.84 (3H, t, $J = 6.03$ Hz, Me-29), 0.82 (3H, d, $J = 6.00$ Hz, Me-26), 0.80 (3H, d, $J = 6.0$ Hz, Me-27), 0.68 (3H, brs, Me-18).

$^{13}$C NMR (DMSO-d$_6$): $\delta$ 37.25 (C-1), 31.66 (C-2), 71.82 (C-3), 42.32 (C-4), 140.71 (C-5), 121.73 (C-6), 29.69 (C-7), 31.90 (C-8), 51.23 (C-9), 36.14 (C-10), 21.07 (C-11), 39.77 (C-12), 40.48 (C-13), 56.76 (C-14), 24.29 (C-15), 28.23 (C-16), 56.05 (C-17), 11.97 (C-18), 19.38 (C-19), 36.51 (C-20), 18.77 (C-21), 33.94 (C-22), 26.08 (C-23), 45.83 (C-24), 29.15 (C-25), 19.80 (C-26), 19.02 (C-27), 23.06 (C-28), 11.85 (C-29).

$^{+ve}$ FAB-MS m/z (rel. int.): 414 [M]$^+$ (C$_{29}$H$_{50}$O), (3.9), 400 (3.1), 399 (15.3), 396 (11.5), 381 (6.7), 273 (3.9), 255 (32.5), 240 (3.8), 231 (8.1), 213 (23.2), 198 (11.6), 173 (14.3), 163 (15.9), 161 (24.4), 159 (32.6), 145 (53.3), 133 (41.6), 121 (32.3), 119 (32.5), 107 (53.1), 105 (50.3), 95 (49.2), 93 (39.8), 83 (5.6), 81 (70.1), 71 (31.2), 69 (7.3), 67 (52.5), 54 (85.3), 43 (100).

**Stigmasterol-β-D-glucopyranoside (JR-7)**

Elution of the column with chloroform-methanol (99:1) yielded colourless amorphous powder of JR-7, recrystallized from MeOH.

**Yield:** 90 mg (0.003%).

R$_f$: 0.69 (Toluene: ethyl formate: formic acid; 10:10:3).

m.p. 280-282°C.

UV $\lambda_{\text{max}}$ (MeOH): 247nm ($\log e$ 5.7).

IR $\nu_{\text{max}}$ (KBr): 3471, 2928, 2852, 1638, 1465, 1381, 1260, 1171 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 5.35 (1H, brs, H-5), 5.30 (1H, m, H-22), 5.06 (1H, m, H-23), 4.50 (1H, brs, H-1'), 4.28 (1H, m, H-5'), 4.16 (1H, m, H-2'), 3.92 (1H, m, H-3'), 3.55 (1H, m, w $1/2$ 18.5 Hz, H-3), 3.51 (1H, brs, H-4'), 3.32 (2H, brs, H$_2$-6'), 1.00 (3H, brs, Me-19), 0.91 (3H, d, $J = 6.1$ Hz, Me-21), 0.84 (3H, d, $J = 6.0$ Hz, Me-26), 0.82 (3H, d, $J = 6.0$ Hz, Me-27), 0.80 (3H, d, $J = 6.1$ Hz, Me-29), 0.68 (3H, brs, Me-18).
$^{13}$C NMR (CDCl$_3$): 140.56 (C-5), 130.01 (C-22), 128.28 (C-23), 121.71 (C-6), 107.49 (C-1'), 85.68 (C-5'), 68.23 (C-3'), 65.08 (C-4').

+ve FAB-MS m/z (rel. int.): 574 [M]$^+$ (C$_3$H$_3$O$_6$) (6.8), 412 (55.3), 397 (100), 381 (20.1), 379 (17.3), 273 (11.1), 255 (26.3), 253 (12.6), 213 (28.3), 198 (26.7).

Hydrolysis of JR-7:

Compound JR-7 (15 mg) was refluxed with 2N alcoholic KOH solution (10 ml) for 3 hours. The reaction mixture was acidified to Congo red and extracted with CHCl$_3$ (3 x 15 ml). The organic phase was washed with water (2 x 10 ml), dried over Na$_2$SO$_4$ and evaporated to get $\beta$-sitosterol, m.p. mmp and Co-TLC comparable. The aqueous phase was concentrated and subjected to paper chromatography using butanol: glacial acetic acid: water (4:1:5) (top layer) as a solvent system for standard samples of monosaccharide. The paper was sprayed with aniline hydrogen phthalate, comparable with glucose.

$\beta$-Sitosterol-$\beta$-D-glucoside (JR-8)

Elution of the column with chloroform afforded colourless amorphous powder of JR-8, recrystallized from MeOH.

Yield: 500 mg (0.01667).

R, : 0.75 (Chloroform: ethyl acetate 9.2:0.8).

m.p. 315-318°C.

UV $\lambda_{max}$ (MeOH): 207 nm (log $\varepsilon$ 4.15).

IR $\nu_{max}$ (KBr): 3440, 3300, 3250, 2965, 2870, 1620, 1460, 1375, 1121, 1055, 955, 850, 785 cm$^{-1}$.

$^1$H NMR: $\delta$ 5.32 (1H, d, J = 4.64 Hz, H-6), 4.88 (1H, brs, H-1), 4.86 (1H, brs, w 1/2 18 Hz, H-3), 4.25 (1H, d, J = 5.02 Hz, H-5), 3.69 (1H, m, H-2), 3.65 (2H, m, H-3, H-4), 3.09 (2H, m, H-6), 1.24 (3H, brs, Me-19), 0.96 (3H, d, J = 6.5 Hz, Me-21), 0.91 (3H, d, J = 6.03 Hz, Me-26), 0.89 (3H, d, J = 6.01 Hz, Me-27), 0.83 (3H, t, J = 6.5 Hz, Me-29), 0.66 (3H, brs, Me-18).

$^{13}$C NMR: 36.80 (C-1), 30.54 (C-2), 73.37 (C-3), 40.32 (C-4), 148.30 (C-5), 121.09 (C-6), 29.23 (C-7), 31.37 (C-8), 49.58 (C-9), 35.45 (C-10), 20.54 (C-11), 40.05 (C-12), 45.14 (C-13), 56.14 (C-14), 23.81 (C-15), 26.98 (C-16), 55.38 (C-17), 11.57 (C-18), 19.59 (C-19), 36.17 (C-20), 18.82 (C-21), 33.34 (C-22), 27.73 (C-23), 45.14 (C-24), 28.55 (C-25), 19.59
(+ve FAB-MS m/z (rel. int.): 576 [M]+ (C_{35}H_{58}O_{7}), 400 (3.1), 399 (15.3), 396 (11.5), 381 (6.7), 367 (3.6), 273 (3.0), 255 (32.5), 240 (3.8), 231 (8.1), 213 (23.2), 198 (11.6), 173 (14.3), 163 (15.9), 161 (24.4), 159 (32.6), 145 (53.3), 133 (41.6), 121 (32.3), 119 (32.5), 107 (50.3), 95 (49.2), 93 (39.8), 83 (5.6), 81 (70.1), 71 (31.2), 69 (57.3), 67 (52.5), 55 (85.3), 43 (100).

Hydrolysis of JR-8:

Compound JR-8 (15 mg) was refluxed with 2N alcoholic KOH solution (10 ml) for 3 hours. The reaction mixture was acidified to Congo red and extracted with CHCl₃ (3 x 15 ml). The organic phase was washed with water (2 x 10 ml), dried over Na₂SO₄ and evaporated to get β-sitosterol, m.p. mmp and Co-TLC comparable. The aqueous phase was concentrated and subjected to paper chromatography using butanol: glacial acetic acid: water (4:1:5) (top layer) as a solvent system for standard samples of monosaccharide. The paper was sprayed with aniline hydrogen phthalate, comparable with glucose.

β-Sitosterol-β-D-glucuronopyranoside (JR-9)

Elution of the column with chloroform-methanol (49:1) provided colourless amorphous powder of JR-9, recrystallized from MeOH.

Yield: 85 mg (0.004%).

Rₜ: 0.72 (Toluene: ethyl formate: formic acid: 10:10:3).

m.p. 278-280°C.

UV λₘₐₓ (MeOH): 240 nm (log ε 2.7).

IR νₘₐₓ (KBr): 3421, 3390, 2917, 2844, 1710, 1472, 1372, 1261, 1170, 1096, 1023, 804 cm⁻¹.

¹H NMR (DMSO-d₆): δ 5.32 (1H, brs, H-6), 4.90 (1H, brs, H-1'), 4.41 (1H, m, H-5'), 4.22 (1H, d, J = 7.2 Hz, H-2'), 3.62 (1H, m, H-3'), 3.59 (1H, m, H-4'), 3.06 (2H, brs, H₂-6'), 3.39 (1H, brm, w ½ = 16.5 Hz, H-3a), 1.04 (3H, brs, Me-19), 0.97 (3H, d, J = 6.2 Hz, Me-21), 0.87 (6H, brs, Me-26, Me-27), 0.85 (3H, m, Me-29), 0.68 (3H, brs, Me-18).

+ve FAB-MS m/z (rel. int.): 590 [M]+ (C_{35}H_{58}O_{7}) (21.6), 396 [M-C₆H₅O₃-Me]+ (26.3), 394 [M-C₆H₁₀O₆]+ (20.5).
Hydrolysis of JR-9:

Compound JR-9 (15 mg) was refluxed with ethanolic 1N KOH solution (10 ml) for thirty minutes on a steam bath. The reaction mixture was extracted with CHCl₃ (3 x 10 ml) to remove alcoholic moiety. It was acidified, re-extracted with CHCl₃ (2 x 10 ml) and dried over Na₂SO₄. After evaporation of the solvent, glucuronic acid was obtained, (TLC comparable).
RESULTS AND DISCUSSION

Compound JR-1, designated as benzjuglansonic acid, was obtained as colourless amorphous powder from petroleum ether-chloroform (3:1) eluants. It gave effervescences with sodium bicarbonate solution indicating the presence of a carboxylic group in the molecule. Its IR spectrum exhibited distinctive absorption bands for carboxylic group (3410, 1695 cm\(^{-1}\)) and aromatic ring (1535, 950 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectrum its molecular weight was established as 194 consistent to a molecular formula of a disubstituted aromatic acid, C\(_{11}\)H\(_{14}\)O\(_3\).

The mass spectrum of JR-1 showed important ion fragments appeared at m/z 179 [M-Me]\(^+\) and 149 [M-COOH]\(^+\) suggesting attachment of the carboxylic group.

The \(^1\)H NMR spectrum of JR-1 displayed a broad signal of one-proton at \(\delta\) 12.22 (H-10) attributed to carboxylic proton. Two ortho-, meta-coupled doublets of one-proton at \(\delta\) 7.65 (J = 7.2, 2.7 Hz), 7.60 (J = 7.5, 3.1 Hz) and two multiplets of one-proton at \(\delta\) 7.28 and 6.10 were assigned to aromatic protons, H-3, H-6, H-5 and H-4. A three-proton broad signal at \(\delta\) 3.92 was ascribed to methoxy proton and the signals resonating between \(\delta\) 2.17-1.26 were associated with the methylene protons.

A deshielded carbon signal in the \(^{13}\)C NMR spectrum at \(\delta\) 188.90 and an upfielded carbon signal at \(\delta\) 56.61 were attributed to carboxylic carbon (C-10) and methoxy carbon respectively. Six carbon signals appearing between \(\delta\) 161.05-109.45 were attributed to aromatic carbons and the signals between \(\delta\) 49.13-29.61 were assigned to methylene carbons.

These spectral data lead to establish the structure of JR-1 as 2-methoxy-benzyl-\(n\)-benzoic acid. This is a new aromatic acid isolated from a natural source for the first time.
Compound JR-2, designated as naphthjuglanosoic acid, was obtained as colourless crystals from petroleum ether-chloroform (3:1) eluants. It gave effervescences with sodium bicarbonate solution indicating the presence of a carboxylic group in the molecule. Its IR spectrum exhibited characteristic absorption bands for carboxylic group (3469, 1695 cm\(^{-1}\)) and aromatic ring (1604, 1550, 830 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectrum its molecular weight was established as 314 consistent with a molecular formula of C\(_{20}\)H\(_{16}\)O\(_4\).

The \(^1\)H NMR spectrum of JR-2 displayed a broad signal of one-proton at \(\delta 11.74\) attributed to carboxylic proton H-19. Two ortho-coupled doublets of one-proton each at \(\delta 7.68\) (\(J = 7.5\) Hz), 7.58 (\(J = 7.5\) Hz) and two ortho-, meta-coupled double doublets of one-proton each at \(\delta 7.64\) (\(J = 8.4, 3.0\) Hz), 7.29 (\(J = 8.4, 3.0\) Hz) were assigned to aromatic protons H-3, H-4 and H-8, H-6, respectively. A one-proton deshielded multiplet at \(\delta 6.15\) was accounted for aromatic H-7. The methylene protons appeared between at \(\delta 2.88 - 1.26\) and a triplet of three-protons resonated at \(\delta 0.88\) (\(J = 6.6\) Hz) was assigned to methyl C-18 primary protons.

The \(^{13}\)C NMR spectrum exhibited a deshielded carbon signal at \(\delta 179.11\) and an upfielded carbon signal at \(\delta 56.60\) attributed to carboxylic and methoxy carbons, respectively. The signal at \(\delta 165.23\) was assigned to oxygenated carbon (C-1) and signal at \(\delta 142.67\) was attributed to C-9. The remaining aromatic carbons appeared between at \(\delta 137.17-109.50\), methylene carbons resonated between \(\delta 48.16-23.11\) and methyl carbons at \(\delta 14.38\) (C-18).

On the basis of these spectral data analysis the structure of JR-2 has been elucidated as 1-methoxy-2-n-octanylnaphthyl-9-oic acid. This is a new aromatic acid isolated from a natural source for the first time.
Compound JR-3, designated as juglansbenzoate, was obtained as a light brown amorphous powder from chloroform eluents. Its IR spectrum exhibited characteristic absorption bands for ester group (1738 cm\(^{-1}\)) and unsaturation (1599 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectrum its molecular weight was established as 670 consistent with a molecular formula of an aromatic ester, \(\text{C}_{25}\text{H}_{58}\text{O}_{3}\).

The mass spectrum of JR-3 displayed important ion peaks at \(m/z\) 120 \([\text{C}_{6}\text{H}_{10}\text{COO}]^+\), 155 \([\text{M-515}]^+\) and 395 \([\text{M-275}]^+\) suggesting attachment of the ester group in aromatic ring. The generation of prominent ion peaks at \(m/z\) 395 \([\text{O(CH}_2)_6\text{CH}_3]^+\), 155 \([\text{C}_{11}\text{H}_{23}]^+\) and 120 \([\text{M-395-C}_{11}\text{H}_{23}]^+\) indicated that \(n\)-undecanyl salicylic acid was esterified with a \(\text{C}_{27}\) aliphatic alcohol.

The \(^1\)H NMR spectrum of JR-3 showed two deshielded two-proton multiplets at \(\delta\) 7.67 and 7.19 attributed to aromatic protons H-2, H-5 and H-3, H-4, respectively. Two one-proton broad signals at \(\delta\) 4.26, 4.06 and two one-proton doublets at \(\delta\) 3.78 \((J = 18.0\ Hz)\) and 3.66 \((J = 10.0\ Hz)\) were associated with oxygenated methylene protons H-2-1' and H-2-1'', respectively. Three two-proton broad signals at \(\delta\) 2.31 (\(\text{CH}_2\)), 2.03 (\(\text{CH}_2\)), 1.61 (\(\text{CH}_2\)) and a 62-proton broad signal at \(\delta\) 1.25 were associated with the methylene proton of the aliphatic chains. A broad signal of six-proton at \(\delta\) 0.88 was attributed to terminal methyl protons (Me-27'a, Me-11').

The \(^{13}\)C NMR spectrum of JR-3 exhibited important signals for ester carbon at \(\delta\) 171.65 (C-7), the aromatic carbons between \(\delta\) 161.32-117.26, oxygenated methylene carbon signals at \(\delta\) 66.17 (C-1') and 63.25 (C-1''). The methylene carbons at \(\delta\) 34.16 (C-2'), 33.89 (C-2''), 28.97 (32 x \(\text{CH}_2\)) and methyl carbons at \(\delta\) 14.13 (C-11') and 13.16 (C-27').
On the basis of above spectral data analysis the structure of JR-3 has been elucidated as o-undecanyl-n-heptacanyl-2-hydroxy-benzoate. This is a new phytoconstituents isolated from a natural source for the first time.

![Chemical Structure of JR-3]

Juglansbenzoate (JR-3)
Scheme 2.1: Mass fragmentation pattern of juglansbenzoate (JR-3)
Compound JR-4, designated as regiooleate, was obtained as colourless amorphous powder from chloroform-methanol (49:1) eluants. Its IR spectrum exhibited characteristic absorption bands for ester group (1740 cm⁻¹), unsaturation (1640 cm⁻¹) and long aliphatic chain (798 cm⁻¹). On the basis of mass spectrum its molecular weight was established as 576 corresponding to a molecular formula of a fatty acid ester, C₃₀H₆₂O₂. It indicated two double bond equivalents, one each of them was adjusted to the ester linkage and vinylic bond, respectively.

The mass spectrum of JR-4 showed important ion peaks at m/z 311 [M-265]⁺ and 295 [M-281]⁺ suggesting that oleic acid was esterified with C₂₁ aliphatic alcohol.

The ¹H NMR spectrum of JR-4 displayed a two-proton multiplet at δ 5.28 attributed to the vinylic protons H-9 and H-10. Two doublets of one-proton at δ 4.0 (J = 9.6 Hz) and 3.96 (J = 9.6 Hz) were ascribed to oxygenated methylene protons H₂-1'a and H₂-1'b. A broad signal of two-proton at δ 2.24 accounted for the methylene protons adjacent to the ester group. The remaining methylene protons resonated between δ 1.96-1.18 and a broad signal of six-proton at δ 0.81 accounted for terminal primary methyl protons Me-18 and Me-21'.

The ¹³C NMR spectrum of JR-4 showed important signals at δ 178.10 (C-1) for ester carbon, δ 129.12 (C-9) and 128.17 (C-10) for vinylic carbons, δ 60.98 (C-1') for oxygenated methylene carbon, signals between at δ 33.38-22.12 for methylene carbons and at δ 14.37 for C-18 and C-21' for methyl carbons. Acid hydrolysis of JR-4 yielded oleic acid (TLC comparable).

On the basis of the spectral data analysis the structure of JR-4 has been established as n-heneicosanyl-9-octadeconoate. This phytoconstituent has been isolated first time from this plant.

\[
\text{H}_3\text{C}(-\text{CH}_2)_7\text{CH}=-\text{CH}(-\text{CH}_2)_7\text{CO}-\text{O}-(\text{CH}_2)_{20}\text{CH}_3
\]

Regiooleate (JR-4)
Scheme 2.1: Mass fragmentation pattern of regiaoleate (JR -4)
Compound JR-5, designated as regiapalmitate, was obtained as a colourless amorphous powder from chloroform-methanol (19:1) eluants. Its IR spectrum exhibited characteristic absorption bands for ester group (1738 cm\(^{-1}\)) and unsaturation (1612 cm\(^{-1}\)) and long aliphatic chain (740 cm\(^{-1}\)). On the basis of mass spectrum its molecular weight was established as 772 corresponding to a molecular formula of a fatty acid ester, C\(_{53}\)H\(_{104}\)O\(_2\). It indicates two double bond equivalents, one each of them were adjusted in the ester group and vinylic linkage.

The mass spectrum of JR-5 showed important ion peak at m/z 239 suggesting that a palmitic acid was esterified with a C-37 alcohol.

The \(^1\)H NMR spectrum of JR-5 displayed a two-proton multiplet at \(\delta\) 5.30 attributed to vinylic proton H-12' and H-13'. Two broad signals integrating for two protons each at \(\delta\) 4.08 and 2.50 were attributed to oxygenated methylene protons H-2-1' and the methylene protons H-2-2 adjacent to the ester group. Two multiplets at \(\delta\) 2.26 and 1.96 integrating for two-proton each were assigned to allylic protons H-11' and H-14'. A broad signals of eighty six-protons at \(\delta\) 1.23 was accounted to the remaining methylene protons and a broad signal of six-protons at \(\delta\) 0.83 was associated with the terminal primary methyl protons, Me-16 and Me-37'.

The \(^{13}\)C NMR spectrum of JR-5 showed important signals for ester carbon at \(\delta\) 171.23 (C-1), vinylic carbons at \(\delta\) 129.17 (C-12') and 111.45 (C-13'), oxygenated methylene carbon at \(\delta\) 62.96 (C-1') and the remaining methylene carbons resonated between at \(\delta\) 48.36-24.71. The signals resonating at \(\delta\) 14.13 were assigned to terminal methyl carbons Me-16 and Me-37'. Acid hydrolysis of JR-5 yielded palmitic acid (TLC comparable).

On the basis of the spectral data analysis and chemical reactions, the structure of JR-5 has been established as \(n\)-heptyl triacont-12-enyl hexadecanoate. This constituent first report of a fatty acid ester isolated from either a natural or synthetic source.
Scheme 2.3: Mass fragmentation pattern of regiapalmitate (JR-5)
Compound JR-6, designated as β-sitosterol was obtained as colourless amorphous powder from chloroform eluants. It responded positively to Liebermann-Burchard test for steroids. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3430 cm\(^{-1}\)) and unsaturation (1620 cm\(^{-1}\)). Its mass spectrum had a molecular ion peak at \(m/z\) 414 corresponding to a steroidal formula, \(\text{C}_{29}\text{H}_{59}\text{O}\). It indicated five double bond equivalents, four of which adjusted to the steroidal carbon skeleton and one in the olefinic linkage. The other diagnostic important peaks were observed at \(m/z\) 399 [M-Me]\(^+\), 396 [M-H\(_2\)O]\(^+\), 273 [M-side chain]\(^+\), 255 [273-H\(_2\)O]\(^+\), 213 [255-ring D fission]+ and 198 [213-Me]\(^+\). These fragments suggested that it was a \(\text{C}_{29}\) sterol possessing one double bond and a \(\text{C}_{10}\) saturated side chain. The ion fragments at \(m/z\) 55 [\(\text{C}_{11,0}\text{-C}_{15}\) fission-H\(_2\)O]\(^+\), 69 [\(\text{C}_{2,3}\text{-C}_{5,10}\text{-C}_{6,7}\) fission]\(^+\), 83 [\(\text{C}_{2,3}\text{-C}_{5,10}\text{-C}_{7,8}\) fission]\(^+\) indicated that hydroxyl group was located at ring A, which was placed at C-3 on the basis of biogenetic grounds. The mass spectrum indicated the presence of an ethyl group in the side chain which was placed at C-24.

The \(^1\)H NMR spectrum of JR-6 showed a one-proton doublet at δ 5.36 (\(J = 4.64\) Hz) assigned to H-6 proton. A broad multiplet at δ 3.52 with \(W_{1/2}\) 18.50 Hz showed the presence of 3α methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 equatorial and axial protons. Three doublets, integrating for three protons each, at δ 0.93 (\(J = 0.65\) Hz) and 0.84 (\(J = 6.0\) Hz), and 0.82 (\(J = 6.0\) Hz), were attributed to C-21, C-26 and C-27 secondary methyls and a three-proton triplet at δ 0.83 (\(J = 6.03\) Hz) was ascribed to C-29 primary methyl protons. The remaining two C-18 and C-19 tertiary methyl signals appeared as singlet at δ 0.68 and 1.01 respectively. The presence of all the methyls in the region δ 0.68-1.01 suggested that these functionalities were attached to saturated carbons. The remaining methylene and methine protons resonated in the region δ 2.28-1.03.

Further evidence for the structure JR-6 was provided by its \(^{13}\)C NMR spectral data which showed the presence of 29 carbon atom in the molecule. Signals at δ 40.71, 121.73 and 71.82 were assigned to C-5, C-6 unsaturated carbons and C-3 carbinol carbon. The β-configuration of the ethyl group was confirmed by comparison of chemical shifts of
carbons and protons of the side chain in the $^1$H NMR and $^{13}$C NMR spectra of JR-6 with $\beta$-sitosterol and other related sterol, e.g. stigmaster-4-en-3-one, stigmaster-4-en-6-\beta-O-3-one (Greca et al., 1990) and lawsaritol (Gupta et al., 1992). The $H_{3-29}$ resonance of 24-R-configuration ($\delta$ 0.83) was more upshifted as compared to the 24-S resonance ($\delta$ 0.86) (Rubinstein et al., 1976).

Based on these evidences the structure of JR-6 has been formulated as stigmasta-5-en-3-\beta-ol.

\[ \text{\beta-Sitosterol (JR-6)} \]

**Compound JR-7,** designated as stigmasterol-\beta-D-glucopyranoside, was obtained as colourless amorphous powder from chloroform-methanol (99:1) eluants. It gave positive test for sterol glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3471 cm$^{-1}$) and unsaturation (1638 cm$^{-1}$). On the basis of mass and $^{13}$C NMR spectrum its molecular weight was established as 574, consistent with a molecular formula C$_{35}$H$_{58}$O$_6$.

The mass spectrum of JR-7 showed important ion fragments appearing at $m/z$ 412 [M-C$_6$H$_{11}$O$_5$, glycoside]$^+$, 273 [412-C$_{10}$H$_{19}$, side chain]$^+$, 397 [M-177]$^+$, 381 [M-193]$^+$, 379 [M-195]$^+$, 255 [273-H$_2$O]$^+$, 253 [M-321]$^+$, 213 [M-361]$^+$, 198 [213-Me]$^+$ which suggested that it was a C$_{29}$ sterol possessing one double bond in the carbocyclic ring system and one double bond in the side chain. The mass spectrum indicated the presence of an ethyl group in the side chain which was placed at C-24 on the basis of biological analogy as well as similarities in chemical shifts of protons and carbons of the side chain with related compounds.
The $^1$H NMR spectrum exhibited a one-proton broad signal at $\delta$ 5.35 and two multiplets of one-proton each, at $\delta$ 5.30 and 5.06 assignable to vinylic H-5, H-22 and H-23, respectively. A one-proton broad signal at $\delta$ 4.50 and a one-proton broad multiplet at $\delta$ 3.55 with half width of 18.5 Hz were assigned to anomeric H-1' and carbinol H-3 protons, respectively. The remaining hydroxymethine protons of sugar moiety appeared between $\delta$ 4.28 - 3.55. A two-proton broad signal at $\delta$ 3.32 was accounted hydroxymethylene H$_2$-6' protons. Two three-proton broad signals at $\delta$ 0.68 and 1.00 were assigned to tertiary C-18 and C-19 tertiary methyl protons. Four doublets at $\delta$ 0.91 ($J = 6.1$ Hz), 0.84 ($J = 6.0$ Hz), 0.82 ($J = 6.0$ Hz) and 0.80 ($J = 6.1$ Hz), all integrating for three protons each, were associated with C-21, C-26, C-27 and primary C-29 methyl protons, respectively. The presence of all the methyl signals in the range $\delta$ 1.00-0.68 suggested the location of these functionalities on the saturated carbons.

The $^{13}$C NMR spectrum of JR-7, showed important signals for vinylic carbons at $\delta$ 140.56 (C-5), 121.71 (C-6), 130.01 (C-22) and 128.28 (C-23), anomeric carbon at $\delta$ 107.49 (C-1') and sugar carbons between $\delta$ 85.68 - 65.08, respectively. Acid hydrolysis of JR-7 yielded stigmasterol and D-glucose (TLC comparable).

On the basis of these evidences, the structure of JR-7 has been established as stigmasta-5,22-dien-3β-ol-3β-D-glucopyranosyl.

![Stigmasterol-3-D-glucopyranoside (JR-7)](49)
Compound JR-8, designated as β-sitosterol-β-D glucoside, was obtained as colourless amorphous powder from the chloroform eluents. It responded positively to steroidal glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3430, 3300, 3250 cm⁻¹) and unsaturation (1620 cm⁻¹). Its mass spectrum displayed a molecular ion peak at m/z 576 corresponding to a molecular formula of glycoside of a phytosterol, C₃₅H₆₆O₆. It indicated six double bond equivalents, four of which adjusted to a tetracyclic carbon framework of a sterol and the remaining two in the vinyl linkage and glycoside unit. Its mass spectrum showed important ion peaks at m/z 414 [M-C₆H₁₁O₆]^+, 399 [414-Me]^+, 396 [M-C₆H₁₂O₆]^+, 273 [414- side chain]^+, 255 [273-H₂O]^+, 213 [255-ring D fission]^+ and 198 [213-Me]^+. These fragments suggested that it was a C-29 sterol possessing one double bond and a C-10 saturated side chain. The ion fragments generated at m/z 55 [C₁₁H₁₀-C₄₅ fission-H₂O]^+, 69 [C₂₃-C₃₄-C₆₇ fission]^+, 83 [C₂₃-C₄₆-C₇₈ fission]^+ indicated that hydroxyl group was located in ring A which was placed at C-3 on biogenetic grounds. The mass spectrum indicated the presence of an ethyl group in the side chain, which was placed at C-24 on the basis of biological analogy, as well as similarities in chemical shifts of proton and carbons of the side chain with related compounds (Ali, 2001).

The ¹H NMR spectrum of JR-8 exhibited a one-proton doublet at δ 5.32 (J = 4.64 Hz) assigned to vinylic H-6 proton. A broad multiplet at δ 4.86 with W₁/₂ of 18.0 Hz was ascribed to 3 α methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 equatorial, C-4 axial protons. Three doublets, integrating for three protons each, at δ 0.96 (J = 6.5 Hz), 0.91 (J = 6.03 Hz) and 0.89 (J = 6.0 Hz) were accounted to C-21, C-26 and C-27 secondary methyl protons, respectively. The remaining two tertiary C-18 and C-19 methyl signals appeared as three-proton each broad signal, at δ 0.66 and 1.24, respectively. A three-proton triplet at δ 0.83 (J = 6.5 Hz) was attributed to C-29 primary proton. The presence of all the methyl in the region δ 0.68-1.01 suggested that these functionalities were attached to saturated carbons. C-1 anomeric proton and other protons of the glucose moiety were observed at δ 4.88 (H-1'), 4.25 (H-5'), 3.65 (H-3' and H-4') and 3.09 (H₂-6'). The remaining methylene and methine protons resonated in the region δ 2.28-1.03.
Further evidence for the structure JR-8 was provided by its $^{13}$C NMR spectral data which showed the presence of 35 carbon atom in the molecule. Signals at $\delta$ 140.30, 121.09 and 73.37 were assigned to C-5, C-6 unsaturated carbons and C-3 carbinol carbon. The anomeric carbon appeared at $\delta$ 100.97 (C-1'). The remaining sugar protons appeared between at $\delta$ 79.20-61.06. The $\beta$-configuration of the ethyl group was confirmed by comparison of chemical shifts of carbons and protons of the side chain in the $^1$H NMR and $^{13}$C NMR spectra of JR-8 with $\beta$-sitosterol and other related sterol, e.g. stigmast-4-en-3-one, stigmast-4en-6-$\beta$-O-3-one (Greca et al., 1990) and lawsaritol (Gupta et al., 1992). Alkaline hydrolysis of JR-8 furnished $\beta$-sitosterol and $\alpha$-D glucose (TLC comparable).

Based on these evidences the structure of JR-8 has been formulated as stigmasta-5-en-3$\beta$-D-glucopyranoside.

\[ \text{\beta-Sitosterol-D-glucopyranoside (JR-8)} \]

Compound JR-9, a phytosterol glycoside, was obtained as colourless amorphous powder from chloroform-methanol (49:1) eluants. Its IR spectrum exhibited distinctive absorption bands for hydroxyl groups (3421, 3390 cm$^{-1}$), carboxylic group (1710 cm$^{-1}$) and unsaturation (1653 cm$^{-1}$).
Its +ve FAB mass spectrum displayed a molecular ion peak at m/z 590 consistent with the molecular formula, C_{35}H_{58}O_{7}. Fragments ion peaks generated at m/z 396 [M-C_{6}H_{9}O_{3}]^{+} and [M-C_{6}H_{11}O_{5}]^{+} supported the compound to be a glycoside of C_{29}H_{50}O.

The $^1$H NMR spectrum of JR-9 showed a one-proton downfield signal at δ 5.33 attributed to H-6 vinylic proton. A one-proton broad signal at δ 4.90 was ascribed to H-1 anomeric proton. Other sugar protons resonated between δ 4.41-3.06 as multiplets. A one-proton broad multiplets at δ 3.39 ($\Delta v_2 = 16.5$ Hz) was ascribed to H-3α -carbinol proton.

Two three-proton broad signals at δ 1.04 and 0.68 were associated with C-19 and C-18 tertiary methyl signals, respectively. A three-proton doublet at δ 0.97 (J = 6.2 Hz) accounted for C-21 secondary methyl protons. A six-proton broad signal was ascribed to C-26 and C-27 secondary methyl protons. A three-proton multiplet at δ 0.85 was due to C-29 primary methyl protons. Acid hydrolysis of JR-9 yielded β-sitosterol and D-glucuronic acid (TLC comparable).

On the basis of these findings the structure of JR-9 has been established as stigmast-5-en-3-O-β-D-glucuronopyranoside.

β-Sitosterol-β-D-glucuronopyranoside (JR-9)
Table 2.1: Details of phytoconstituents isolated from the bark of *Juglans regia*

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Column eluants</th>
<th>Compound name</th>
<th>Mol. for. Mol. wt.</th>
<th>Melting point °C</th>
<th>Yield (%)</th>
<th>R&lt;sub&gt;r&lt;/sub&gt;</th>
<th>Nomenclature (Remarks)</th>
</tr>
</thead>
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<tr>
<td>JR-1</td>
<td>Pet. ether : CHCl&lt;sub&gt;3&lt;/sub&gt; (3:1)</td>
<td>Benzjuglansoic acid</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; [194]</td>
<td>147-148</td>
<td>0.002</td>
<td>0.39 (a)</td>
<td>2-Methoxy-benzyl-n-benzoic acid (New)</td>
</tr>
<tr>
<td>JR-2</td>
<td>Pet. ether : CHCl&lt;sub&gt;3&lt;/sub&gt; (3:1)</td>
<td>Naphthjuglansoic acid</td>
<td>C&lt;sub&gt;26&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; [314]</td>
<td>176-178</td>
<td>0.002</td>
<td>0.61 (a)</td>
<td>1-Methoxy-2-n-octanyl naphth-9-0ic acid (New)</td>
</tr>
<tr>
<td>JR-3</td>
<td>Chloroform</td>
<td>Juglansbenzoate</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;22&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; [670]</td>
<td>208-210</td>
<td>0.002</td>
<td>0.72 (b)</td>
<td>o-Undecanoyl-n-heptanoyl-9-hydroxy benzoate (New)</td>
</tr>
<tr>
<td>JR-4</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH (49:1)</td>
<td>Regiaoleate</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; [576]</td>
<td>83-85</td>
<td>0.004</td>
<td>0.82 (b)</td>
<td>n-Hentioconyl-9-octadecanoate</td>
</tr>
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<td>JR-5</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH (9:1)</td>
<td>Regiapalmitate</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; [772]</td>
<td>86-87</td>
<td>0.002</td>
<td>0.65 (c)</td>
<td>n-Heptyltrimethyl-12'-ethyl hexadecanoate (New)</td>
</tr>
<tr>
<td>JR-6</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH (1:1)</td>
<td>β-Sitosterol</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O [414]</td>
<td>136-138</td>
<td>0.002</td>
<td>0.43 (d)</td>
<td>Stigmasta-5-en-3-β-ol (Known)</td>
</tr>
<tr>
<td>JR-7</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH (99:1)</td>
<td>Stigmasterol-β-D-glucopyranoside</td>
<td>C&lt;sub&gt;35&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt; [574]</td>
<td>280-282</td>
<td>0.003</td>
<td>0.69 (b)</td>
<td>Stigmasta-5,22-dien-3 β-ol-3 β-D-glucopyranosyl (Known)</td>
</tr>
<tr>
<td>JR-8</td>
<td>Chloroform</td>
<td>β-Sitosterol-β-D-glucoside</td>
<td>C&lt;sub&gt;33&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt; [576]</td>
<td>315-318</td>
<td>0.016</td>
<td>0.75 (c)</td>
<td>Stigmasta-5-en-3 β-β-D-glucopyranoside (Known)</td>
</tr>
<tr>
<td>JR-9</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH (49:1)</td>
<td>β-Sitosterol-β-D-glucuronopyranoside</td>
<td>C&lt;sub&gt;37&lt;/sub&gt;H&lt;sub&gt;38&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt; [590]</td>
<td>278-280</td>
<td>0.004</td>
<td>0.72 (b)</td>
<td>Stigmasta-5-en-3 β-β-D-glucopyranoside (Known)</td>
</tr>
</tbody>
</table>

Solvent systems: [a] Pure chloroform; [b] Toluene: ethyl formate: formic acid (10:10:3); [c] Chloroform-methanol (9:1); [d] Petroleum ether: Chloroform (1:3); [e] Chloroform: ethyl acetate (9:2:0.8)
Spectrum 1: $^1$H NMR of benzjuglansoic acid (JR-1)

Spectrum 2: $^{13}$C NMR of benzjuglansoic acid (JR-1)
Spectrum 3: $^1$H NMR of naphthjuglansoic acid (JR-2)

Spectrum 4: $^{13}$C NMR of naphthjuglansoic acid (JR-2)
Spectrum 5: $^1$H NMR of juglansbenzoate (JR-3)
$H_3C(CH_2)_7CH=CH(CH_2)_7CO-O-(CH_2)_20CH_3$

Spectrum 6: $^1H$ NMR of regiooleate (JR-4)
Regiapalmitate (JR-5)

\[ \text{H}_{16}\text{C(CH}_2\text{)}_{18}\text{CO}=\text{O}=\text{O}=\text{O}(\text{CH}_2)_{11}\text{CH}^=\text{CH}-(\text{CH}_2)_{15}\text{CH}_3 \]

Spectrum 7: \textsuperscript{1}H NMR of regiapalmitate (JR-5)
Spectrum 8: $^1$H NMR of stigmasterol-$\beta$-D-glucopyranoside (JR-7)

Spectrum 9: $^{13}$C NMR of stigmasterol-$\beta$-D-glucopyranoside (JR-7)
Spectrum 10: $^1$H NMR of $\beta$-sitosterol- $\beta$-D-glucuronopyranoside (JR-9)
REFERENCES


