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Isolation, characterization of seconadry metabolites from the *Melia azedarach* Linn. Root and to evaluate their *in vitro* antidiabetic activity

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Abstract
Objectives: The present study was to isolate, characterize from the *Melia azedarach* Linn. root and screen them for *in-vitro* antidiabetic activities.
Method: The *M. azedarach* Linn. root coarse powder was subjected to extraction, fractionation and column chromatography to isolate the compounds. The identical eluents were collected and subjected to flash chromatography using Peak track software. The eluents were monitored on TLC and visualized under UV. The isolated compounds were characterized by IR, Mass and ^{1}HNMR and ^{13}CNMR spectra. Later, they were screened for *in-vitro* antidiabetic studies.
Results: Three known compounds 1-methylacrylyl-3-acetyl-11-methoxymeliacapinin, Meliacarpinin B and Quercetin were isolated from *Melia azedarach* root extract. The spectroscopic data confirmed their structures similar to the reported compounds. From the *in-vitro* antidiabatic studies the isolated compounds exhibited α-amylase, sucrase and α-glucosidase inhibitory activities.
Conclusion: We isolated three known compounds from root of *melia azedarach* Linn. and they exhibited significant *in-vitro* antidiabetic activities.

Keywords: *Melia azedarach*, flash chromatography, *in-vitro* antidiabetic drugs

Introduction
Diabetes Mellitus is a group of metabolic disorder characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Type-2 diabetes (T2DM) is caused by combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors (obesity, overeating, lack of exercise, stress) and aging. Several clinical studies have demonstrated that chronic hyperglycaemia is associated with damage, dysfunction, and failure of vital organs and lead to retinopathy, nephropathy, neuropathy, cardiomyopathy, atherosclerosis and stroke. The impaired function of pancreatic β-cells may progress with prolonged T2DM, aging, obesity, insufficient energy consumption, alcohol, smoking, etc are independent risk factors of pathogenesis of T2DM [1, 2]. The epidemiology of DM is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million. The number of people with T2DM is increasing in every country with 80% people with DM living in low- and middle-income countries. Diabetes is fast gaining status of potential epidemic in India. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India [3]. Prolonged use of oral antidiabetic drugs will have drawbacks like resistance, hepatotoxicity, worsen heart disease, weight gain, bloating, flatulence, diarrhoea and abdominal discomfort and pain[4]. Many medicinal preparations contain one or more ingredients of plant origin. According to WHO, about 80% of the population in the developing countries use traditional medicine in the treatment of various diseases [5]. Therefore, plant species used by different ethnic groups should be investigated in order to tap the incredible bio resources for sustainable harvesting of novel bioactive phytopharmaceuticals [6]. The interest in herbal drug research continues with an expectation to novel, safer and more effective compound with all the desired parameters of a drug that could replace the synthetic medicines.
Melia azedarach, (Family Meliaceae) commonly known as Maha neemor (Persian lilac), mainly found in forest of North-West region of Himalaya in India, Pakistan, China and other tropical, subtropical countries. This plant contain a wide range of structurally diverse secondary metabolites have been identified including terpenoids, tetra nortriterpenoids, limonoids, flavonoids, steroids and fatty acids from this plant [7, 8]. Out of them, most predominant are triterpenoids and limonoids which are mainly present in leaves or fruits, and they have been credited for the biological activities including antifeedant,

insecticidal and cytotoxic [9-11]. Previous studies reported that they isolated many compounds from *Melia azedarach* Linn. such as tetracyclic triterpenoids from bark [12], triterpenoids & sterols from leaves & twigs [13], 3-α-tigloyl-melianol and methyl kulonate, limonoids from the fruits [14], Euphane-type triterpenoid from fruits and leaves, and Oxetane-bearing Limonoids from roots[15]. Previous reports had isolated some compounds from fruit and leaf of *M. azedarach* Linn. and reported to possess PTP-1B enzyme inhibitory activity, glucose uptake stimulation in C_2Cl_2 myoblast cells studies [16]. An extensive literature survey from all the scientific sources and revealed that the root of *melia azedarach* Linn. is very effective in the polyurea, polydispisa and beneficial in the management of DM [17]. Hence, the present study is undertaken to isolate the secondary metabolites and investigate the ameliorative effects of plant *M. azedarach* Linn. root against *in vitro* antidiabetic studies.

Materials and Methods

Instrumental analyses: UV and IR were used to measure the absorbance and spectra UV-1800, Shimadzu, Japan and BRUKER ALPHA T – IR Spectrophotometer in our department. ^1H and ^{13}C- NMR spectra were measured with a BRUKER AVANCE II 400 NMR Spectrophotometer and Mass spectra were recorded with WATERS Q-TOF MICROMASS (ESI-MS) from Panjab University, Chandigarh. Chemical shifts were given as δ (ppm) with tetramethylsilane (TMS) as an internal standard.

Plant materials: The *Melia azedarach* root bark was collected from medicinal garden of SET's College of Pharmacy, Dharwad and authenticated by Dr. GR Hegde, Postgraduate department of studies in Botany, Karnataka University, Dharwad-580 003.

Chemicals: Enzyme: (Type VIB: From porcine pancreas), Sigma, USA and 4-nitrophenyl-α-D-glucopyranoside (NPG), HiMedia were purchased. All other chemicals used were of analytical grade such as starch, Sodium dihydrogen orthophosphate, Disodium hydrogen phosphate, 3, 5-Dinitrosalicylic acid, Potassium sodium tartarate, Sodium hydroxide Maltose monohydrate, Dimethyl sulphoxide, Sucrose. The pure drug meglitol was received as free sample from Dr. Veeresh Veerapur, Professor at Siddaganga College of Pharmacy, Tumkur, Karnataka.

Extraction and Isolation: The roots of *Melia azedarch* Linn. (MA) was collected and washed thoroughly with tap water, shade dried, cut into small pieces and were crushed to moderately coarse powder. The root powder was subjected to methanolic extraction processs by using soxhlet apparatus and which yielded 32.84g of methanolic root extract.

Fractionation of methanol extract: Methanolic extract was subjected to liquid-liquid partition. 30g of extract was dissolved in 300 ml of water and partitioned with ethyl acetate, butanol and water using separating funnel. 13.2, 6.24 and 6.9% w/v yield was obtained with ethyl acetate, butanol and water respectively. Ethyl acetate fraction was further subjected to column chromatography for separation of phytoconstituents.

Column chromatography: 12g of ethyl acetate fraction was packed in a column with silica (60-120) by wet packing method. The column was eluted with different combinations of solvent (1L each). Eluting with petroleum ether and later with petroleum Ether: Chloroform gradient, chloroform, Chloroform: Ethyl acetate gradient, ethyl acetate, ethyl acetate: methanol gradient and methanol. The column eluents were monitored with TLC and identical eluents were mixed, concentrated to dryness. Fraction obtained from Ethyl acetate: Methanol (97:3) showed single spot on TLC [Ethyl acetate: Formic acid: Acetic acid: Methanol (100:11:11:13.5)]. It was designated as compound TG-11 (90mg).

Flash chromatography: Fractions eluted with Chloroform: Ethyl acetate (50:50) obtained from column was further subjected to flash chromatography. 113mg of fraction was packed with silica (240-400) and gradient elution was carried out using chloroform and ethyl acetate. All parameters were set and monitored using Peak track software. The elution with Chloroform: Ethyl acetate (75:25) resulted in a fraction, giving a prominent spot on TLC [Chloroform: Ethyl acetate (60:40)]. The compound was designated as compound TG-03 (33mg).

The ethyl acetate fraction obtained from column was subjected to flash chromatography. 1g of fraction was adsorbed on silica (240-400) and the column was eluted with combination of gradient solvent system (Chloroform and Ethyl acetate). Identical eluates were collected, combined, concentrated and kept aside. Fraction eluted with Chloroform: Ethyl acetate (80:20) has shown prominent spot on TLC [Chloroform: Ethyl acetate (70:30)]. This was concentrated to obtain compound TG-05 (44mg). Later, the isolated compounds TG03, TG05 and TG11 structural elucidation was carried out with help of analytical instruments.

***In-vitro* Antidiabetic studies**

i) **Alpha-amylase inhibition assay** [19, 20]**:** The different concentrations of extract (10 to 400µg) were mixed with 200µL of α-amylase soln were incubated for 10min at 28 ºC. The enzyme reaction was initiated by adding 200µl of 1% starch solution and incubated for 10min at 28 ºC. The enzyme reaction was stopped by adding 400µl 3,5 dinitro salicylic acid solution all the test tubes and transferred to a boiling water bath for 5min. They were made to 5ml with phosphate buffer and the absorbance measured at 540nm. The blank solutions were prepared by replacing the enzyme solution with phosphate buffer. The control incubations, representing 100% enzyme activity were conducted in similar manner, replacing extract with buffer. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing extract with solvent.

The inhibition percentage of α-amylase was assessed by the following formula:

The α-amylase inhibitory activity = (Ac+) – (Ac-) – (As - Ab)/(Ac+) – (Ac-) × 100

Where,

Ac+ that absorbance of 100% enzyme activity (only solvent with enzyme)

Ac- that absorbance of 0% enzyme activity (only solvent without enzyme)

As that absorbance of test sample with enzyme

Ab that absorbance of test sample without enzyme

ii) **Isolation of enzyme** [21]**:** Rats were sacrificed, intestine removed and chilled with ice cold 80mM phosphate buffer. The intestine was then cut open, the mucosa

scraped off with a piece of glass rod and homogenized with cold buffer. Nuclei and large cell debris were removed by cold centrifugation at 2000 to 4000rpm for 10min and supernatant aliquote into 1.5ml vials and stored at -20 °C. The protein content of enzyme preparation was estimated by Lowry method.

Sucrase Inhibition assay: The mixture of different concentrations of samples (10 to 400µg) and standard drug and 200µl of enzyme solution were incubated for 10 min at 28ºC. The enzyme reaction was started by adding 100µl of sucrose solution (60 mM) and was incubated for 10min at 28ºC. The reaction was stopped by adding 200µ1of 3,5-dinitrosalicylic acid reagent and transferred into a boiling water bath for 5min and cool under ice-cold water. After attaining to room temperature they were made to 5ml final volume and the absorbance was measured at 540nm by Shimadzu model UV spectrophotometer. An untreated enzyme solution (without samples) was used as control. Control incubations, representing 100% enzyme activity. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing extract with solvent.

The inhibition percentage of α-amylase was assessed by the following formula:
The α-amylase inhibitory activity = (Ac+) – (Ac-) – (As - Ab)/(Ac+) – (Ac-) × 100

Where,
Ac+ that absorbance of 100% enzyme activity (only solvent with enzyme)
Ac- that absorbance of 0% enzyme activity (only solvent without enzyme)
As that absorbance of test sample with enzyme
Ab that absorbance of test sample without enzyme

iii) **Alpha-glucosidase inhibition assay:** Assay of α-glucosidase inhibition was performed according to method described by Li *et al*., 2009 with some modification. A mixture of samples (10 to 400µg) or standard and 200µl of enzyme solution were incubated at 28°C for 10min. After pre-incubation, the enzyme reaction was started by adding 200µl of 2mM p-nitrophenyl-α-D-glucopyranoside solution to each and was incubated at 28 °C for 10min. Then the reaction was stopped by adding 1 ml of 0.1M Sodium carbonate. The absorbance was recorded at 405nm by spectrophotometer. Control incubations, representing 100% enzyme activity were conducted in a similar manner, replacing samples with DMSO. The blanks were prepared without enzymes and meglitol was used as positive control. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing extract with solvent.

The inhibition percentage of α-amylase was assessed by the following formula:
The α-amylase inhibitory activity = (Ac+) – (Ac-) – (As - Ab)/(Ac+) – (Ac-) × 100
Where,
Ac+ that absorbance of 100% enzyme activity (only solvent with enzyme)
Ac- that absorbance of 0% enzyme activity (only solvent without enzyme)
As that absorbance of test sample with enzyme
Ab that absorbance of test sample without enzyme

Results
In the present study, we isolated three compounds TG03, TG05 and TG11 were obtained by subjecting methanolic fraction to column chromatography followed by flash chromatography.

Analysis of compound TG-11
Physical state: Brown powder ; Rf value:0.7 {Ethyl acetate: Formic acid: Acetic acid: Methanol (100:11:11:13.5)} ; Melting point: 190 ºC. The compound TG-11 gave a positive response to Shinoda for flavonoids.

Spectral characteristics: All the spectras of TG11 were shown in Fig 1-4.

IR: 3404.36 cm^{-1}(br, OH), 2918.30 cm^{-1}(CH str. in CH_3), 2850.79 cm^{-1}(C-H str. in CH_2), 1612.49 cm^{-1} (C=C str. of aromatic ring), 1446.61 cm^{-1} (CH deformation in CH_3), 1311.59 cm^{-1} (C-OH), 1024.20 cm^{-1} (CO str.), 609.79 cm^{-1} (phenyl ring substitution bending).

^{1}H NMR: δ 6.0522(d 1H C-6), δ 6.3467(d, 1H C-8), δ 7.6061(d 1HC-2'), δ 6.8937(d 1H C-5'), δ 7.5530(dd 1H C-6'), δ 5.7239(d 1H C1''), δ 4.7400(d 1H C-1'''), δ 1.1289(d 3H C-6''').

^{13}C NMR: δ 158.49(C-2), δ 135.6(C-3), δ 179.29(C-4), δ 166.78(C-5), δ 99.49(C-6), δ 166.78(C-7), δ 94.29(C-8), δ 159.06(C-9), δ 104.02(C-10), δ 123(C-1'), δ 117.29(C-2'), δ 145.33(C-3'), δ 149.78(C-4'), δ 116.33(C-5'), δ 123.06(C-6'), δ 104.02(C-1''), δ 75.29(C-2''), δ 77.02(C-3''), δ 71.33(C-4''), δ 78.0(C-5''), δ 69.78(C-6''), δ 102.72(C-1'''), δ 72.0(C-2'''), δ 72.03(C-3'''), δ 73.06(C-4'''), δ 69.78(C-5'''), δ 16.72(C-6''').

Mass Spectra (EIS-MS)
Molecular formula: $C_{27}H_{30}O_{16}$; Molecular weight: 610.2079 [M+]
GC/MS (m/z): 610 [M+] {$C_{27}H_{30}O_{16}$} other peaks appeared at 610.2079, 595.4074, 360.3364, 301.1535 and 149.0217.
From the melting point, IR and mass spectral data, the compound TG11 was confirmed as Quercetin-3-rutinoside (Rutin).

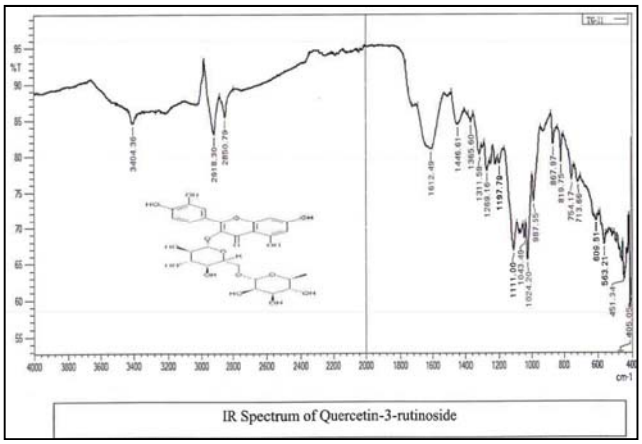

Fig 1: IR Spectrum of TG11

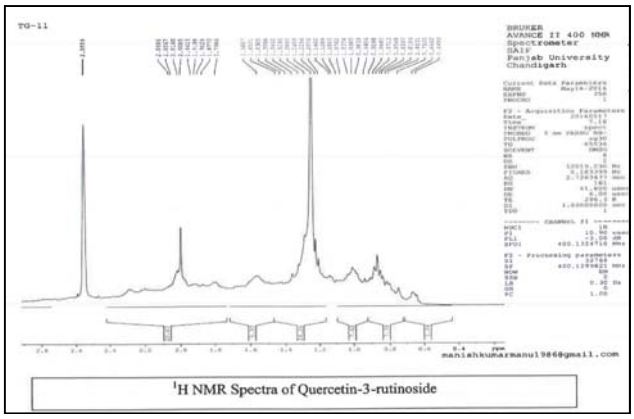

Fig 2: [1]H NMR Spectra of TG11

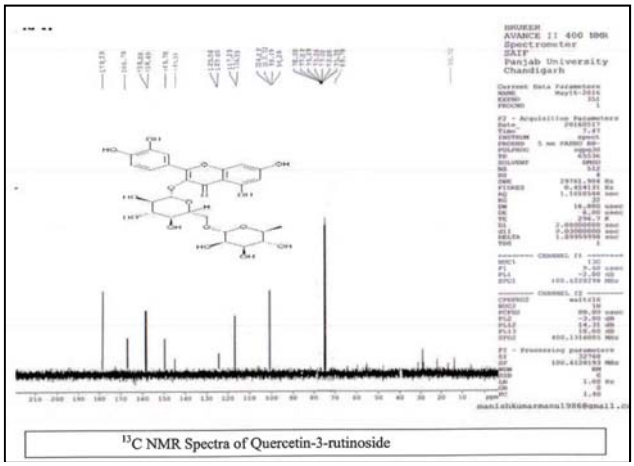

Fig 3: [13]C NMR Spectra of TG11

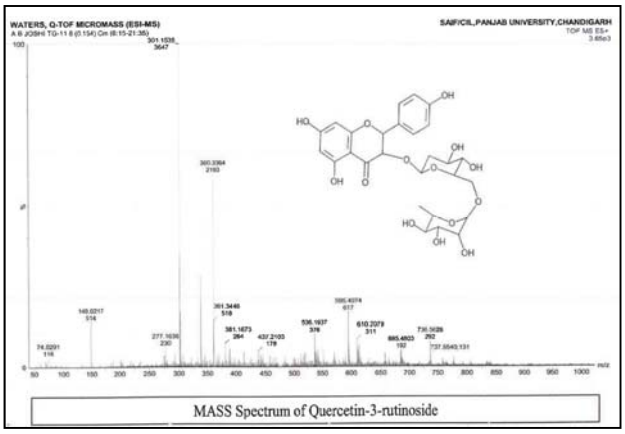

Fig 4: Mass Spectrum of TG11

Analysis of compound TGO3

Physical state: Yellow coloured powder. RF value: 0.5 (Chloroform: Ethyl acetate 60:40)

Spectral characteristics: All the spectras of TG11 were shown in Fig 5-8.

IR: 3419.79 cm^{-1}(br. O-H str), 1708.93 cm^{-1} (ester), 1619.15 cm^{-1}(C=O str. ketone).

[1]H NMR (DMSO) : δ 4.9440 (dd 1H C-1), δ 2.0763(dt 1H C-2α), δ 2.5556(dt 1H C-2β), δ 4.5912(dd,1H C-3), δ 2.556(d 1HC-5), δ 3.9046(dd 1H C-6), δ 4.7456(d 1H C-7), δ 3.7263(s 3H, C-9), δ 4.0501(d 1H C-15), δ 2.0763(ddd 1H C-16β), δ 1.8779(dd 1H C16α), δ 2.07(dd 1H C-17), δ 1.6077(s 3H C-18), δ 3.8775(d 1H C-19α), δ 4.0677(d 1H C-19β), δ 5.9695(s 1H C-21), δ 4.7456(d 1H C-22), δ 6.3426(d 1H C-23), δ 3.3849(d 3H C-28α), δ 3.7263(d 1H C-28 β), δ 1.0060(s 3H C-29), δ 1.5757(s 1H C-30), δ 6.3426(s 1H 20-OH), δ 3.7263(s 3H 11-OMe), δ 3.8775(s 3H 12-OMe), δ 2.0096(s 3H OAc), δ 6.3426 (m1H 3'), δ 1.9177(s 3H 4').

[13]C NMR : δ 70.13(C-1), δ 22.23(C-2α), δ 72.24(C-3), δ 40.18(C4), δ 36.16(C-5), δ 70.13(C-6), δ 39.97(C-7), δ 36.16(C-8), δ 73.93(C-9), δ 83.66(C-10), δ 51.22(C-11), δ 169.0(C-12), δ 95.13(C-13), δ 93.22(C-14), δ 83.66(C-15), δ 29.67(C16α), δ 51.22(C-17), δ 22.13(C-18), δ 70.13(C-19α), δ 86.93(C20), δ 109.34(C-21), δ 107.13(C-22), δ 144.93(C-23), δ 78.73(C28α), δ 19.00(C-29), δ 16.93(C-30), δ 51.22(12-O-Me), δ 170.193(3-O-Ac), δ 163.55 (1'), δ 137.66(2'), δ 128.86(3'), δ 11.44(4').

Mass spectra (EIS-MS)

Molecular formula: $C_{34}H_{44}O_{14}$; Molecular weight: 676

EI-MS (m/z): 699{(M+Na)+ $C_{34}H_{44}O_{14}$} other peaks appeared at 676.12(M+), 663, 598, 597, 555, 443, 393, 317, 301, 289, 235, 177, 149.

From the IR, [1]HNMR, [13]C NMR and Mass spectral data, the compound TG03 was confirmed as 1-methylacrylyl-3-acetyl-11-methoxymeliacapinin.

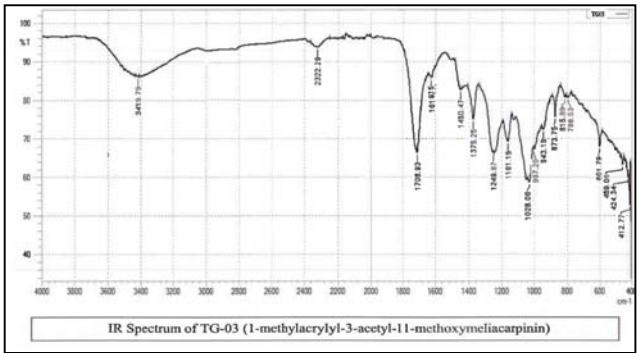

Fig 5: IR spectrum of TG03

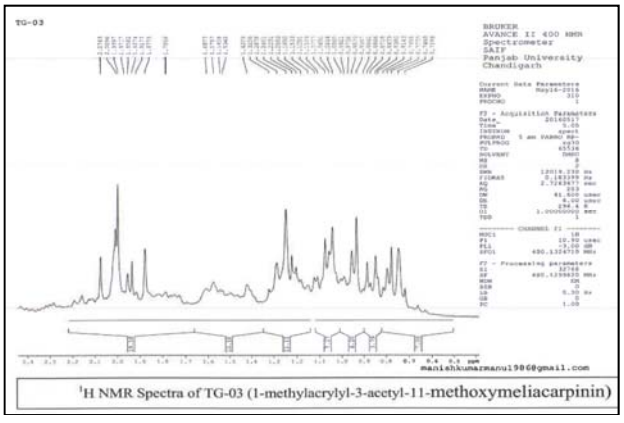

Fig 6: [1]H NMR Spectra of TG03

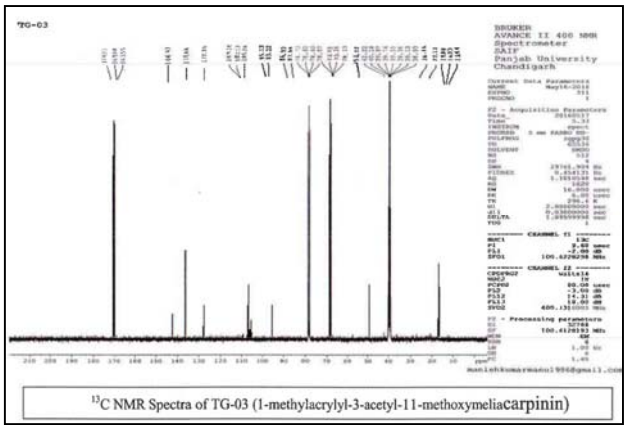

Fig 3: ^{13}C NMR Spectra of TG03

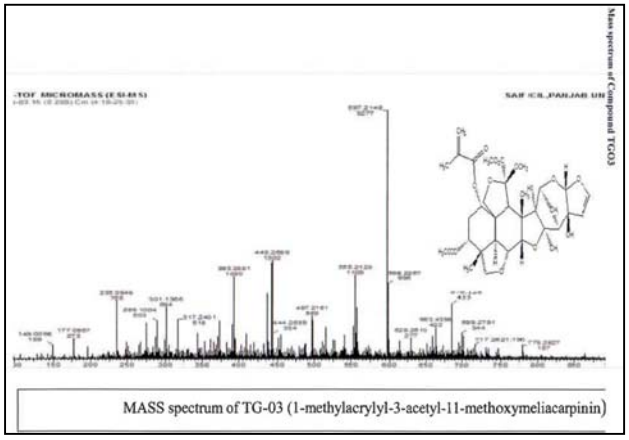

Fig 8: Mass Spectrum of TG03

Analysis of compound TGO5
Physical state: light brown colour powder
RF value: 0.7 (Chloroform: Ethyl acetate 70:30)

Spectral characteristics: All the spectras of TG11 were shown in Fig 9-12.

IR:3404.36 cm^{-1}(br. OH str), 1743.49 cm^{-1} (ester), 1708.93 cm^{-1}(C=O str. carboxylic ester)

^{1}H NMR (DMSO) :δ 1.3879(s 1H C-1), δ 2.1828(m 2H C-2), δ 4.9868(brt 1HC-3), δ 4.9868(brt 1H C-3), δ 2.7259(d 1H C-5), δ 3.9188(d 1HC-6), δ 4.5142(d 1H C-7), δ 3.1570(S 1H C-9), δ 4.1275(brs 1H C-15), δ 2.2162(m 2H C-16), δ 2.1591(m 1H C-17), δ 1.5133(s 3H C-18 CH3), δ 4.9723(d 1H C-19), δ 5.7002(s 1H C21), δ 4.8346(d 1H C-22), δ 6.3395(d 1H C-23), δ 3.5503 (d 1H C-28), δ 0.9797 (s 3H C-29), □ 1.5667(s 3H C-30), δ 4.2265(S 1H C-14), δ 3.4633(s 3H C-11), δ 3.8241(s 3H C-12), δ 6.7864(qq 1H C-3'), δ 1.8140(dq 3H C-4'), δ 1.8140(dq 3H C-5').

^{13}C NMR: δ 24.34(C-1), δ 29.05 (C-2), δ 78.06 (C-3), δ 39.80(C-4), δ 39.17 (C-5), δ 39.38 (C-6), δ 77.92 (C-7), δ 46.69 (C8), δ 46.69 (C-9), δ 40.22(C-10), δ 111.03(C-11), δ 178.97(C-12), δ 86.34(C-13), δ 86.29(C-14), δ 85.23(C-15), δ 29.05(C-16), δ 64.94(C-17), δ 29.05(C-18), δ 64.94(C-19), δ 86.34(C-20), δ 111.03(C-21), δ 110.02(C-22), δ 146.0(C-23), δ 76.06(C-28), δ 18.4(C-29), δ 17.2(C-30), δ 164.94(C-1'), δ 129.0(C-2'), δ 138.97(C-3'), δ 14.5(C-4'), δ 12.1(C-5').

Mass spectra (EIS-MS)
Molecular formula: $C_{33}H_{44}O_{12}$; Molecular weight: 633 [M+1]+
EI-MS(m/z): 633 [M+1]+ {$C_{33}H_{44}O_{12}$} other peaks appeared at 633, 511, 445, 371, 302, 301, 297, 274, 223,149.
From the IR, 1HNMR, 13C NMR and mass spectral data, the compound TG05 was confirmed as Meliacarpinin B.

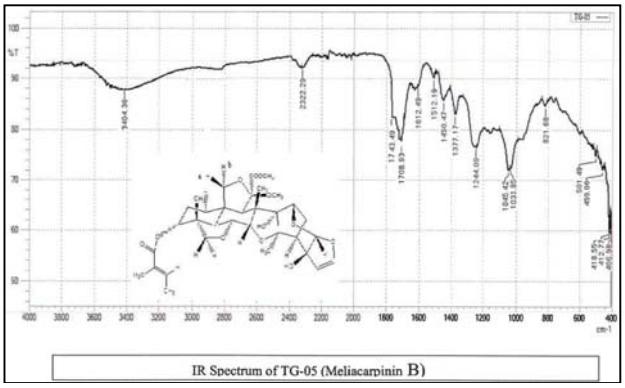

Fig 9: IR Spectrum of TG05

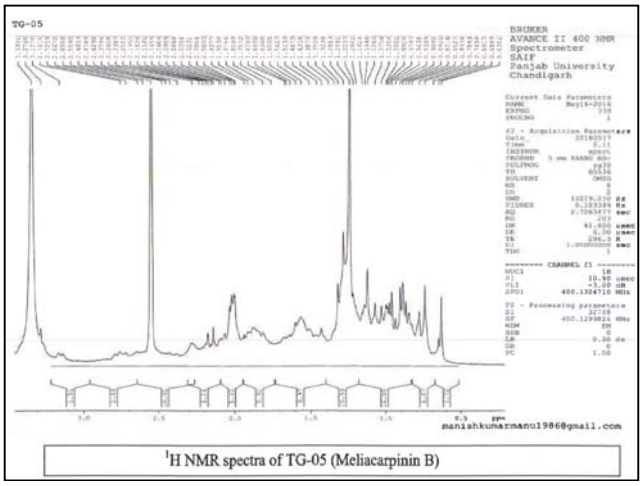

Fig 10: ^{1}H NMR Spectra of TG05

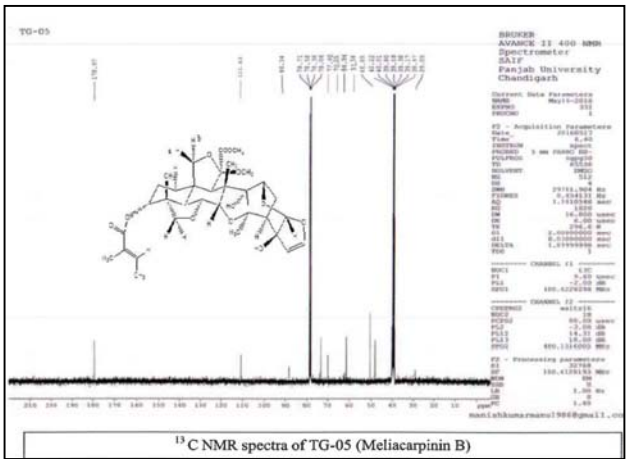

Fig 11: ^{13}C NMR Spectra of TG05

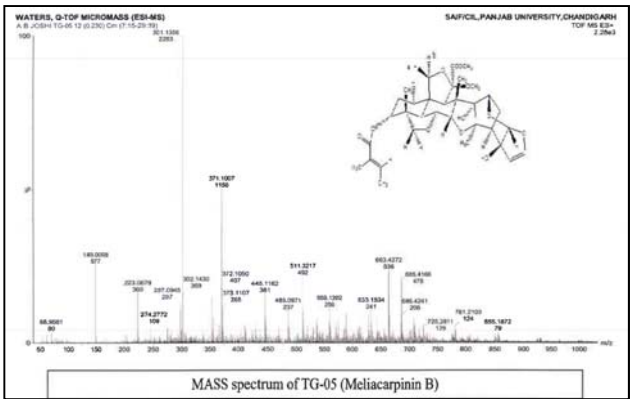

Fig 12: Mass Spectrum of TG05

In-vitro antidiabetic studies

The isolated compounds were evaluated for *in-vitro* antidiabetic activities such as- α-amylase, sucrase and α-glucosidase inhibitory assays. The results were shown in Table 1.

Table 1: the IC_{50} values of α-amylase, sucrase and α-glucosidase inhibitory concentration of isolated compounds of *Melia azedarach* Linn. root.

Compounds	IC_{50} in µg		
	α-amylase	Sucrase	α-Glucosidase
Meglitol	< 50	<50	<50
MEF	205.5±3.9	114.5±8.3	109.7±5.1
Quercetin-3-rutinoside (Rutin)	135.2±8.1	98.7±8.4	51.9±5.1
1-methylacrylyl-3-acetyl-11-methoxymeliacapinin	87.7±2.1	66.8±8.1	60.6 ± 6.7
Meliacarpinin B	148.5±3.6	69.9±3.3	92.1 ± 1.5

MEF – Ethyl acetate fraction of *Melia azedarach* root. Meglitol was used as positive control. Data presented is the mean ± SD of samples runs in triplicate.

Discussion

Hyperglycaemia is an abnormal postprandial increase of blood glucose level, has been linked to the onset of type 2 insulin-dependent diabetes mellitus (NIDDM, Type 2 diabetes) and associated with cardiovascular complications. α-amylase and α-glucosidase (sucrase, maltase, isomaltase) are key enzymes involved in starch breakdown and intestinal glucose absorption, respectively. The inhibition of these enzymes can slow down the passage of carbohydrates into the bloodstream and significantly decreases the postprandial increase of blood glucose level after a mixed carbohydrate diet which has an important strategy in the management of type 2 diabetes [16]. A main drawback of currently used therapeutic α-glucosidase inhibitor (Acarbose) has strong α-amylase inhibitory activity and side effects as abdominal distension, flatulence, meteorism and diarrhoea. Therefore, natural compounds are beneficial because of their lower inhibitory activity against α-amylase, stronger inhibitory activity against α-glucosidase and can be used in the management of postprandial hyperglycaemia with minimal side effects.

Recent article reported that they isolated many compounds from *Melia azedarach* Linn. such as tetracyclic triterpenoids from bark [15], triterpenoids & sterols from leaves & twigs [16], 3-α-tigloyl-melianol & methyl kulonate, limonoids from the fruits [17], euphane-type triterpenoid from fruits and leaves, and oxetane-bearing limonoids from roots [18]. Therefore, the present study was conducted to isolate the secondary metabolites from root of *Melia azedarach* Linn. and they were screened for *in-vitro* antidiabetic activities.

The phytochemical study led to the isolation of one flavonoid – quercetin-3-rutinoside (Rutin) and two limonoids –1-methylacrylyl-3-acetyl-11-methoxymeliacapinin and meliacarpinin B. All the compounds were identified by comparing their chemical and spectral data with that of published literatures [22]. Recent paper reported the isolation of Quercetin-3-rutinoside (Rutin) from the leaves but here we isolated the same compound in root of *Melia azedarach* [23].

In this study, we screened the isolated compounds for *in-vitro* antidiabetic activities such as α-amylase, α-glucosidase and sucrase activity. These enzymes have main role in the digestion of starches present in diet. The potency of the inhibition was determined experimentally, and the data were expressed using IC_{50} values. The results of enzyme inhibitory activity of ethyl acetate fraction of methanolic root extract of *melia azedarach* (MEF), 1-methylacrylyl-3-acetyl-11-methoxymeliacapinin and meliacarpinin B and quercetin-3-rutinoside (Rutin) were shown in Table 1. The IC_{50} values of ethyl acetate fraction of *melia azedarach* root were 205µg/ml, 114µg/ml, 109. 7µg/ml for α-amylase, sucrase and α-glucosidase inhibitory activity respectively. The IC_{50} quercetin-3-rutinoside (Rutin) were 87.7µg/ml, 66.8µg/ml, 60. 6 µg/ml for α-amylase, sucrase and α-glucosidase inhibitory activity respectively. The IC_{50} values of values of 1-methylacrylyl-3-acetyl-11-methoxymeliacapinin were 135.2µg/ml, 98.7µg/ml, 51.9µg/ml for α-amylase, sucrase and α-glucosidase inhibitory activity respectively. The IC_{50} values of meliacapinin B were 148.5µg/ml, 69.9µg/ml, 92.1µg/ml for α-amylase, sucrase and α-glucosidase inhibitory activity respectively. The present study indicates that isolated compound quercetin-3-rutinoside (Rutin) shown significant enzyme inhibitory activity and could be useful in managing the postprandial hyperglycaemia in diabetes mellitus.

Conclusion

From the study we isolated three known compounds from *M. azedarach* root and they were confirmed as from IR, NMR, MASS spectra as quercetin -3-rutinoside, 1-methylacrylyl-3-acetyl-11-methoxymeliacapinin and meliacarpinin B. They were identified by comparing their chemical and spectral data with that of published literatures and screened them for *in-vitro* antidiabetic activities. The compound quercetin -3-rutinoside had shown significant enzyme inhibitory activity would be helpful to manage uptake glucose-induced increased levels in hyperglycaemia. and in *in-vitro* antidiabetic studies. Further, the in compounds must be analyzed in animal studies. Further research work may be extended *in-vivo* in experimental animals to elucidate its mechanism.

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