Ultra high performance liquid chromatography-ultraviolet-electrospray ionization-micrOTOF-Q II analysis of flavonoid fractions from *Jatropha tanjorensis*

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ABSTRACT

Background: *Jatropha tanjorensis* (*Euphorbiaceae*) an exotic traditional plant unique to Thanjavur district of Southern India also commonly called as Catholic vegetable. It has been used traditionally in decoctions for treating various ailments and as a health tonic. **Objective:** The objective of the present work is to study a comprehensive characterization of methanolic extract fractions using ultra high performance liquid chromatography (UHPLC) + -electrospray ionization (ESI)-micrOTOF-Q II and correlate their bioactivities. **Materials and Methods:** Phytoconstituents from *J. tanjorensis* leaves were extracted with methanol (MeOH) followed by successive chromatography using linear gradient polar solvents system. All fractions obtained were evaluated for their chemical potential using micrOTOF-Q II techniques and identified key molecules were determined for their anticancer and anti-oxidant potential using *in vitro* methods. **Results:** Successive column chromatography of the MeOH residue yielded six fractions. Compounds such as such as C-glycosylflavones (mono-C-, di-C-), O, C-diglycosylflavones and aglycones were identified for the first time in this plant using UHPLC–ultraviolet–micrOTOF-Q II ESI and a correlation with their anticancer using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay on Ehrlich ascites cells (EAC) and antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl and lipid peroxidation were studied; fraction D extract exhibited the strongest activity against cancer cell. **Conclusions:** LC-mass spectrometry has been successfully applied for a quick separation and identification of the major phytoconstituents. All fractions have shown potent antioxidative activity as compared to standard antioxidant 3,5-di-tert-butyl-4-hydroxytoluene. EAC cell-based cytotoxicity assay also revealed encouraging results. The antioxidant and anticancer activity determined in the present work can be attributed to the presence of flavonoids and flavone glycosides. Present work provides the first scientific report on phytoconstituents of *J. tanjorensis* and its ethnopharmacological significance.

Key words: Antioxidant, Ehrlich ascites cells, *Euphorbiaceae*, flavonoids glycosides, *Jatropha tanjorensis,* liquid chromatography-mass spectrometry

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INTRODUCTION

Jatropha tanjorensis Ellis and Saroja is an exotic plant in Africa, America and Asia (Southern India) and has been used in traditional medicine since long time. *J. tanjorensis* is an herbaceous plant belonging to the family *Euphorbiaceae* and commonly called catholic vegetable, Iyana ipaja, lapalapa. Ethnophamacologically *J. tanjorensis* (Church grass) leaves have been used since ancient.[1]

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Plant leaves were initially and popularly consumed in Nigeria as soup and as a tonic with the claim that it increases blood volume. The leaves are also employed traditionally in the treatment of anemia (as a hematinic agent), diabetes, renal problems, cardiovascular diseases, hypertension and inflammation.[2] *J. tanjorensis* leaves are considered to possess antimicrobial and antiplasmodial properties.[3]

Recent research have proven scientifically its antidiabetic,[4] antiinflammatory, antimicrobial and antioxidant potentials.[5,6] Presences of flavonoids in the selected drug source are of special interest due to their antioxidative properties.[7]

Reactive oxygen species have been implicated in the pathogenesis of many diseases, including liver toxicity, cancer, mutagenesis, etc.[8] Although many synthetic antioxidants are being currently used, there is a growing evidence of consumer's preference for natural antioxidants because of their lower toxicity. *J. tanjorensis* ethanolic extract has already been evaluated scientifically for its free radical scavenging activity.[6]

However, until now, there are no scientific studies on the phytomolecules identified and supporting the traditional use of this plant and thus, the aim of the present work was to develop a method for the identification of major phytoconstituents in the methanolic extract fractions of *J. tanjorensis* and to determine the most active antioxidant and anticancer fraction. The methanolic extract of *J. tanjorensis* was investigated chemically using reverse phase liquid chromatography (LC) coupled to electrospray ionization tandem mass spectrometer (LC/ESI/MSn).

MATERIALS AND METHODS

Chemicals

Following reagents were purchased from Merck (KGaA, Darmstadt, Germany) and used in the present study: HPLC-grade acetonitrile, methanol (MeOH), 3,5-di-tert-butyl-4-hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), silica gel 100-200 Mesh (for column chromatography). 2-thiobarbituric acid (TBA) was obtained from Fluka Chemie GmbH (Buchs, Switzerland). All chemicals were reagent grade, unless otherwise stated.

Plant material

Jatropha tanjorensis (*Euphorbiaceae*) fresh green leaves samples were collected in mid July 2012 from in and around SASTRA University campus, Thanjavur, Tamil Nadu, India.[5] Herbarium voucher specimens were prepared, identified with the deposited specimen at the Raphinet Herbarium (RHT 1291) St. Joseph's College, Tiruchirapalli, Tamil Nadu, India.

Extraction

Shade dried and powdered plant material was depigmented using petroleum ether for 48 h and after vacuum-drying, extracted for 72 h with eight-fold amount of MeOH. Both extractions were carried out at room temperature. The process yielded 14% of raw extract on dry weight basis. Depigmented residue was fractionated using silica 100-200 mesh column by stepwise increasing polarity of the eluent to give six fractions, labeled A to F. For LC-mass spectrometry/mass spectrometry (LC-MS/MS) analysis and bioactivity assays, all fractions were dissolved in MeOH.

Liquid chromatography-mass spectrometry analysis

Chemical composition of each fraction was determined by ultra-high performance LC ([UHPLC] + focused) with mass selective detection, using Ultimate 3000 series LC (Dionex, USA) coupled with ESI tandem mass spectrometer (micrOTOF-Q II) (Bruker, Germany). Components were separated using reverse-phase Acclaim 120, reverse phase (RP)-C18 120 Å, 2.1 × 150 mm, 3.0 μm column (Dionex, USA), held at 50°C. Mobile phase consisting of MeOH (A) and 1% aqueous formic acid v/v (B), was used with a discontinuous gradient; 0 min 95% B, to 80% B in 5 min, to 70% B in the next 5 min, at 15th min B reaches 65%, next 5 min to reach 40% B, 2.5 min to reach 0% B, next 2.5 min to reach 95% B until the run ends, with a flow rate of 0.2 ml/min. Chromatographic profiles were acquired in the wavelength at 335 nm. Injection volume was 50 μL. Eluted components were ionized by electrospray ion source (ESI), using N_2 for nebulization (pressure of 34.8 psi) and drying (flow of 7 L/min, temperature of 300°C). Set capillary voltage was 2600 V, end plate offset −500°V, collision cell RF 350.0 Vpp, energy transfer time of 80.0 μs, pre pulse storage of 10.0 μs. Data were acquired in MS/MS (auto) scanning mode. To increase the sensitivity, lower the noise, and simplify the spectra, negative ionization was used. Generated $(M-H)^-$ ions were analyzed using auto-MSn scan mode, in m/z range 50-2500 m/z.

Biological activity

2,2-diphenyl-1-picrylhydrazyl free radical inhibition assay

Selected concentrations (10, 100, 250, 500, and 1000 μg/mL) of *J. tanjorensis* fractions (10 μL) were mixed with 190 μL of MeOH and 100 μL of methanolic solution containing DPPH radicals (67.2 μmol/L).[9] Absorption at 517 nm was measured using NanoDrop 2000 series spectrophotometer (Thermo Scientific, Wilmington, DE, USA) after 60 min of incubation at room temperature. The extract concentration in the reaction mixture, resulting in 50% inhibition of DPPH radicals (IC_{50}), was calculated.

Lipid peroxidation inhibition assay

As a substrate for lipid peroxidation, freshly collected red blood cells (RBCs) were used and were resuspended to make 1% solution in phosphate buffer (37°C, pH 7.2). Selected concentrations (10, 100, 250, 500, 1000 μg/mL) of *J. tanjorensis* fractions (10 μL), each in triplicate, were mixed with 20 μL of 1.875 mmol/L ferrous sulfate and 20 μL of ascorbate (15.4 μg/mL). The control and corrections were also made as previously defined. All probes were incubated at 37°C for 1 h, after which 200 μL of 0.1 mol/L ethylenediaminetetraacetic acid was added to bind the Fe^{2+}, thus stopping the reaction. TBA reagent (2 mL) was added, and the mixture was heated in boiling water bath for 15 min to form a stable, colored

derivative with malondialdehyde (MDA).[10] After that, probes were centrifuged for 15 min at 3700 rpm and the absorbance was measured at 517 nm using NanoDrop 2000 series spectrophotometer (Thermo Scientific, USA). The values of IC_{50} were calculated using GraphPad Software, Inc. CA, USA.

Anticancer assay

Ehrlich ascites cells (EAC) were treated with various concentrations of each fraction for 48 h under 37°C and 5% CO_2 in CO_2 incubator. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was performed in accordance with standard textual method.[11] After treating cells with different concentration of each fraction, absorbance was read out at 590 nm using Epoch microplate spectrophotometer (BioTek Instruments, Inc., VT, USA).

Statistical analysis

Data of the antioxidant and anticancer assays are expressed as the means ± standard deviation of three independent measurements. Statistical analysis of the data was acquired by Graphpad software package.

RESULTS

This study clearly demonstrated that *J. tanjorensis* extracts contain several classes of plant flavonoids with anticancer and antioxidant potentials. Mostly flavonoid glycosides and aglycones, as well as biflavonoids were identified in the MeOH extract. Although several publications were focusing on this topic,[12] there is still a lack of convincing evidence to determine, which flavonoid class is mostly responsible for the antioxidant and anticancer activity in the plant drug sources. Hence, in the present work attempts were made to detect flavonoids belonging to various class and their anticancer and antioxidant potentials were evaluated.

Crude extract fractionation

Crude methanolic extract (5 g) chromatographed over silica gel 100-200 mesh column and partitioned successively in a linear gradient to give six fractions, A (100% chloroform, 210 mg), B (100% ethyl acetate (EtOAc), 490 mg), C (75% EtOAc: 25% MeOH, 860 mg), D (50% EtOAc: 50% MeOH, 1.35 g), E (25% EtOAc: 75% MeOH, 1.0 g) and F (100% MeOH, 850 mg) as shown in Figure 1. All these fractions were stored at 4°C in dark condition until use.

Liquid chromotography-mass spectrometry analysis

Interesting results were obtained from the LC-MS/MS analysis of *J. tanjorensis* methanolic extract fractions. By UHPLC, a chromatographic method was successfully

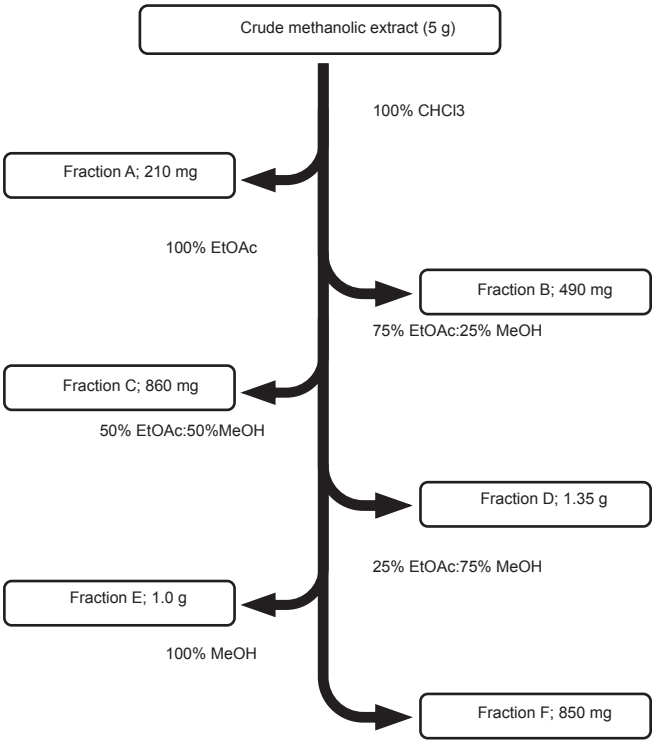

Figure 1: Flow chart of various solvent used and yield of *Jatropha tanjorensis* leaves methanolic extract J fraction

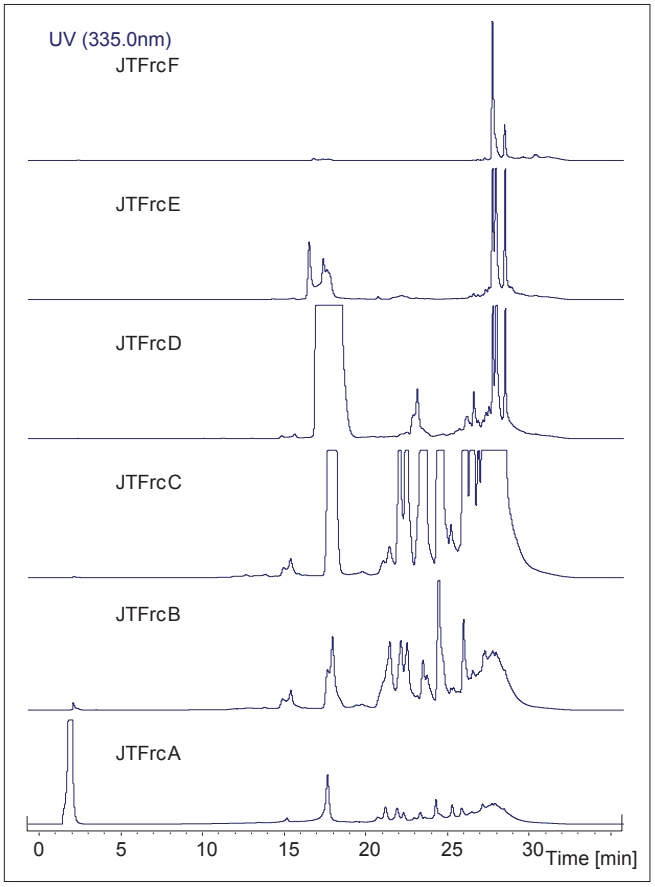

Figure 2: Ultra high performance liquid chromatography + ultraviolet chromatogram of each fraction of *Jatropha tanjorensis* leaves methanolic extract

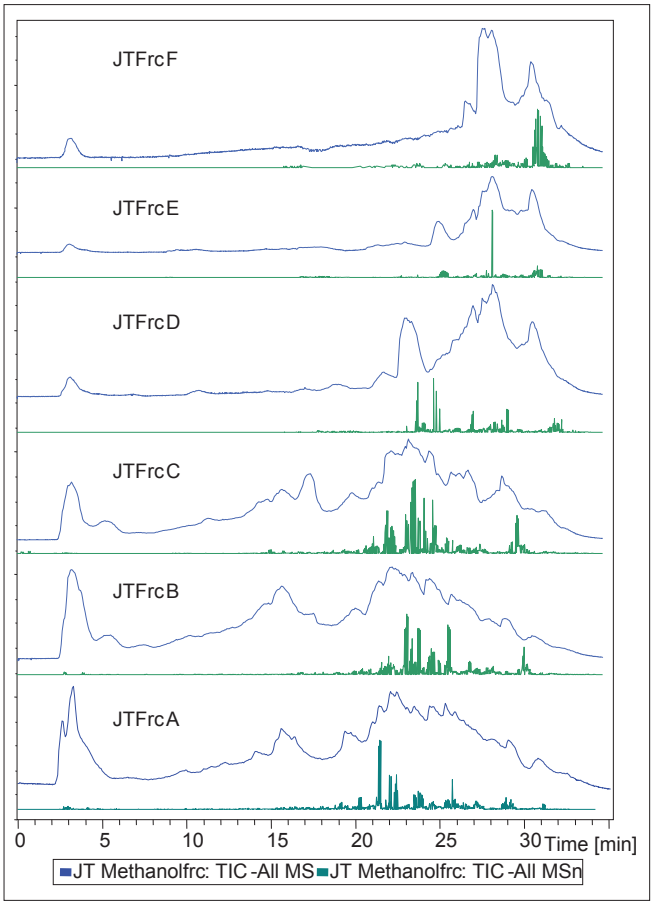

Figure 3: Liquid chromatography-mass spectrometry/mass spectrometry chromatograms (total ion chromatograms [TIC]) of *Jatropha tanjorensis* methanolic extract fractions

developed using Acclaim 120, C18 reverse-phase column from Dionex with ultraviolet detection at 335 nm that could resolve ~ 15 peaks from each fraction [Figure 2]. When this separation was coupled with the ESI/MS/MS mass spectrometry, the Total Ion Chromatograph presented in Figure 3 was obtained. The collected data for the LC/ESI/MS/MS was analyzed by averaging followed by extracted ion analysis using the Hystar DataAnalysis program (Bruker Daltonics, Germany). Averaging and autoMSn options revealed approximately 200 ion signatures in the TIC for each methanolic fraction of *J. tanjorensis* leaves.

Present evaluation of the ESI data yielded about ~100 completely unique ion signatures in the analysis of the *J. tanjorensis*. micrOTOF-Q II analysis was able to identify ~20 constituents by mass spectral matching and several of these assignments were further supported by comparing these data with previously published data of the constituents [Table 1].

Bioactivity assays

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity varied widely from very high to moderate. All samples possessed ability to scavenge DPPH radicals, where most of them had IC_{50} values higher or closer to that with synthetic antioxidant BHT [Figure 4]. Inhibition of lipid peroxidation (LPO) by extract fractions was also demonstrated, with a low IC_{50} as compared to the synthetic antioxidant BHT. All fractions were able to inhibit LPO dose-dependently, as efficient as synthetic antioxidants BHT. Results are presented in Figure 5.

Anticancer activity

Each fraction showed almost similar results. They revealed moderate to high activity. EAC cells when treated with different concentration of each collected methanolic extract fraction showed contrasting results from that of antioxidant findings. Comparatively fraction D and fraction C showed the highest anticancer activity. Results are presented in Table 2.

DISCUSSION

Liquid chromatography-mass spectrometry analysis

The polar constituents are of interest since most of the traditional preparations are only aqueous extractions. RP-UHPLC-/ESI/MS/MS works well for polar molecules and also provide the rapid assessment of plant extracts for the presence of medicinally active compounds with a minimum of prepurification. In LC/ESI/MS/MS the effluent from an UHPLC + is introduced into an ESI source coupled with dual quadrupole and TOF giving typical and well-understood ESI Spectra. The advantage to acquire ESI fragmentation data from a LC separation lies within the subsequent ability to use existing deconvolution and search programs to match results with well-established and commercially available ESI mass spectral databases. Extensive research is needed to develop or upgrade the available MS/MS libraries to prevent the difference in fragmentation patterns among ESI instruments; this would make the identification of the components in a complex mixture very easy.

All compounds that were identified are novel components of the selected plant leaves [Figure S1]. Major identified constituents are flavonoids and its derivatives with different degree of glycosides [Table 1].

Bioactivity assays

In the present work, fractions containing flavonoids were detected in the MeOH extract of *J. tanjorensis* along with their structural pattern. These structures provided supporting evidences for their free radical scavenging, antioxidant, and anticancer properties.

Table 1: ESI-MS and ESI-MS/MS product ions of fractions from *Jatropha tanjorensis* leaves methanolic extract

Fraction	Identified compound	*R*t (min)	Mass	MS (parent ion)	MS/MS (product ion)
A	Vitexin	20.3-20.6	432	431	283, 268, 311, 323, 341, 239, 254, 295
	Homoorientin	17.3-17.5	448	447	298, 299, 327, 285, 269, 240, 205, 225, 259, 310, 339, 357
	6-c-pentosyl-8-c-hexosyl apigenin	17.5-18.0	564	563	353, 383, 325, 297, 365, 395, 413, 425, 283, 443, 455, 473, 207, 503, 455
	Saponarin	18.9-19.1	594	593	353, 311, 383, 325, 297, 365, 395, 413, 431, 283, 271, 473, 207, 341, 473
	Cosmosiin	25.9	432	431	268, 240, 211
	Rhoifolin	25.4-25.8	578	577	269, 324, 353, 296, 383, 444
	Syringetin-3-O-glucoside	23.0-23.3	508	507	327, 283, 301, 344, 271, 217, 243, 357, 311, 253
	Luteolin-7-O-glucoside	26.4-26.5	448	447	284, 256, 297, 199, 241, 327
	Petunidin-3-O-beta-glucopyranoside	24.8-24.9	479	478	285, 299, 271, 323, 313, 256, 230, 447, 462
	Isorhamnetin-3-glucoside-4'-glucoside	24.9-25.0	640	639	285, 300, 315, 225, 271, 339, 369, 431, 459, 624
	Quercetin-3-O-glucoside	25.1-25.2	464	463	271, 300, 256, 243, 227, 283, 311, 323, 339
	Peonidin-3-O-beta-galactopyranoside	25.9-26.1	463	462	285, 315, 314, 299, 297, 271, 255, 243, 227, 323, 311, 230, 447, 462
	Vitexin-2"-O-rhamnoside	25.4-25.8	578	577	353, 383, 269, 339, 323, 297, 369, 395, 311, 283, 459, 473, 504, 413, 531, 207, 237
B	Vitexin	23.5-23.7	432	431	283, 311, 293, 268, 269, 323, 239
	Homoorientin	26.5	448	447	327, 299, 272, 284, 311, 339, 357, 256, 225, 434, 241, 205
	6-c-pentosyl-8-c-hexosyl apigenin	18.8-19.3	564	563	353, 383, 365, 325, 297, 337, 395, 425, 443, 473, 503, 207, 236
	6-c-pentosyl-8-c-hexosyl apigenin dimer	21.3-21.4	1159	1158	563, 353, 383, 365, 325, 297, 337, 395, 425, 443, 473, 503, 207, 236
	Prunin	23.4	434	433	271, 283, 227, 311, 253, 323, 293, 241, 211, 199
	Rhoifolin	24.0-24.1	578	577	353, 383, 325, 297, 269, 324, 413, 444, 457, 473, 233
	Kaempferol-3-o-rutinoside	24.7-24.9	594	593	353, 383, 325, 297, 283, 311, 365, 395, 413, 455, 473, 503, 207, 192
	Kaempferol-3-o-rutinoside dimer	21.0-21.1	1188	1187	593, 353, 383, 325, 297, 283, 311, 365, 395, 413, 455, 473, 503, 207, 192
	Syringetin-3-O-galactoside	24.9-25.1	508	507	463, 285, 217, 301, 243, 327, 447, 489
	Luteolin-7-O-glucoside	24.6-24.7	448	447	284, 256, 227
	Petunidin-3-O-beta-glucopyranoside	25.0-25.1	479	478	285, 299, 271, 313, 327, 447, 462, 227, 255, 203
	Cyanidin-3,5-di-O-glucoside	18.2-18.3	610	609	327, 357, 298, 313, 339, 369, 399, 429, 447, 207, 285, 298, 399, 192
	Delphinidin-3-O-[2''-O-beta-xylopyranosyl-beta-glucopyranoside]	15.5-15.8	597	596	271, 313, 227, 293, 295
	Isorhamnetin-3-glucoside-4'-glucoside	25.2	640	639	300, 285, 315, 339, 447, 459, 433, 624, 271, 353, 207, 225, 192,
	Keracyanin	25.4-25.5	595	594	323, 285, 271, 395, 353, 443, 311, 339
	Quercetin-3-O-glucoside	25.2-25.4	464	463	271, 300, 255, 243, 283, 311, 227
	2",3",4',5,6",7-hexa-O-methylisovitexin	27.2-27.4	516	515	394, 364, 300, 323, 282, 351, 422, 333, 311, 271, 234
C	Vitexin	23.4-23.5	432	431	283, 311, 293, 271, 323, 239, 270, 269
	Homoorientin	20.9-21.0	448	447	299, 327, 294, 253, 225, 207, 357, 238, 269
	Cyanidin-3,5-di-O-glucoside	18.4-18.8	611	610	327, 357, 298, 313, 339, 369, 447, 207, 285, 298, 399, 192, 223, 259
	6-c-pentosyl-8-c-hexosyl apigenin	19.2-19.3	564	563	353, 383, 325, 297, 365, 337, 311, 395, 413, 425, 443, 283, 271, 473
	Prunin	20.0-20.3	434	433	271, 227
	Delphinidin-3-O-[2''-O-beta-xylopyranosyl-beta-glucopyranoside]	15.5-15.6	596	595	271, 313, 297, 295, 227
	Keracyanin	20.9-21.0	594	593	311, 297, 271, 283, 341, 354, 431, 473, 209
	6-c-hexosyl-8-c-pentosyl apigenin	19.9-20.0	580	579	369, 399, 339, 429, 381, 411, 312, 441, 459, 205, 353
	Reynoutrin	16.0-16.5	434	433	342, 357, 243, 217, 327, 299, 228, 271, 205
	Rhoifolin	25.8-25.9	578	577	269, 324, 353, 296, 383, 444

Contd...

Table 1: Continued

Fraction	Identified compound	Rt (min)	Mass	MS (parent ion)	MS/MS (product ion)
D	Vitexin	23.4-23.5	432	431	283, 311, 293, 270, 239, 207
	Homoorientin	21.8-22.2	448	447	327, 298, 312, 284, 218, 206, 233, 339, 355
	6-c-pentosyl-8-c-hexosyl apigenin	21.1-21.2	564	563	353, 383, 325, 297, 206, 311, 365, 221, 413, 473
	Kaempferol	27.8-27.9	286	285	199, 211, 227, 257, 266, 239,
	3',7-dimethoxy-3-hydroxyflavone	29.0-29.1	298	297	256, 227, 284, 269, 199, 211, 183
	Peonidin	28.0-28.4	301	300	256, 227, 284, 256
	Chrysoeriol	28.5-28.5	300	299	256, 227, 284, 199, 211, 239
	Baicalein	28.4-28.7	270	269	225, 241
E	Vitexin	23.5-23.5	432	431	283, 268, 269, 311, 206, 229, 256
	Naringin	22.4-22.5	580	579	447, 463, 323, 419, 295, 435, 492, 347, 391, 295
	Kaempferol	27.8-27.9	286	285	199, 211, 256, 239, 227, 268, 269
	Chrysoeriol	28.4-28.5	300	299	256, 227, 284, 199, 211, 239
	Kaempferol-3-o-rutinoside	25.2-25.4	593	592	339, 383, 463, 363, 321, 283, 293, 271, 215, 295, 407, 425, 477, 505
	3',7-Dimethoxy-3-hydroxyflavone	25.8-25.9	298	297	211, 255, 254
	7,8-Dihydroxy 6-methoxycoumarin 8-β-D-glucopyranoside	27.5	370	369	311, 267, 339, 227, 255, 211, 239, 283, 297
	Gossypetin 3-methylether	29.8-29.9	332	331	253, 269, 281, 241, 205, 223
	Iristectorin A	28.1-28.4	492	491	285, 255, 271, 192, 242
	6a, 12a-Didehydroamorphigenin	28.8-28.9	408	407	283, 271, 347, 348, 227, 243, 201, 297, 216, 313
	Dihydroquercetin	29.5-29.7	304	303	241, 254, 257, 226, 284
F	Vitexin	24.1-24.5	432	431	283, 311, 293, 295, 269, 323, 341
	Isoorientin	24.4-24.7	448	447	284
	Prunin	23.0-23.2	434	433	271, 283
	Chrysoeriol	28.4-28.5	300	299	256, 227, 284, 211, 199, 239, 269
	Cosmosiin	25.9-26.2	432	431	268, 283, 240
	3',7-dimethoxy-3-hydroxyflavone	28.9-29.0	298	297	256, 227, 284, 199, 211, 239, 269
	Isorhamnetin	27.9-28.0	316	315	271, 243, 227, 203, 300, 255, 215
	Delphinidin	29.0	303	302	256, 227, 284, 199, 211, 238, 269, 246
	Embinin	31.1-31.3	606	605	355, 337, 564, 531, 242, 207, 225, 290, 295, 310, 423, 499, 513, 220, 373, 387, 401, 445

ESI: Electrospray ionization; MS: Mass spectrometry

Table 2: Cytotoxicity assay performed on each fraction from *Jatropha tanjorensis* leaves methanolic extract

Fraction detail	IC_{50} in μg/ml
A	162.4
B	20.13
C	11.38
D	8.03
E	20.84
F	54.53

Antioxidant activity

All fractions were subjected to the antioxidant activity assays. Since, it is now recognized that there is no single confirmatory test to evaluate antioxidant activity of the compounds with wide spectra of structures, modes of action, and physical and chemical properties,[13] two assays were employed as a part of the present investigation.

2,2-diphenyl-1-picrylhydrazyl is a stable radical and is often used in assessing antioxidant activity. The free radical DPPH possesses a characteristic absorption at 517 nm (purple in color), which decreases significantly when exposed to radical-scavengers (due to hydrogen atom transfer from antioxidant to DPPH). A lower absorbance at 517 nm indicates a higher radical-scavenging activity of fraction.[14] In this assay, the ability of the methanolic extract fractions of *J. tanjorensis* leaves acted as donors of hydrogen atoms or electrons in the transformation of DPPH radical into its reduced form DPPH-H was investigated.

The activity observed is in a very good correlation with its chemical composition, where the most active fractions contain flavonoid glycosides and anthocyanins (Frc A, B, C, and F) and comparatively lesser in aglycones containing fractions (Frc D and Frc E). It is notable that mostly flavonoid glycosides (with apigenin, luteolin, delphinidin, petunidin and isorhamnetin sugars) seem to contribute significantly to radical-scavenging activity (showed a low IC_{50}) [Figure 4].

One of the main detrimental effects of reactive radical species (especially OH) is LPO that is, oxidative

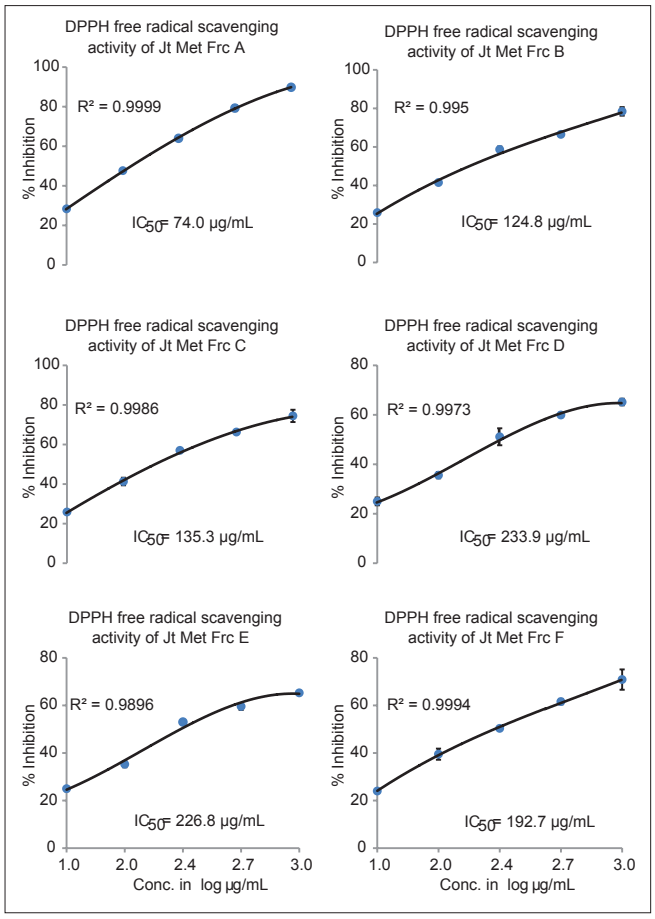

Figure 4: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay result of each fraction from *Jatropha tanjorensis* leaves methanolic extract

degradation of lipids, leading to biological membrane damage and possibly to cell death or the formation of mutagenic/carcinogenic products. The best known LP product is MDA and it has been used most widely as a biomarker in various studies associated with lipid peroxidation. The determination of MDA may be problematic because of its high reactivity and water solubility, and it is therefore necessary to generate stable derivatives. One of the most commonly used is TBA adduct, which can be determined spectrophotometrically. In the present work, freshly collected 1% RBCs solution was used as a substrate for LP due to its high content of polyunsaturated fatty acids. LP of polyunsaturated fatty acids was triggered by Fe^2+ and ascorbate (which, through fenton reaction, generate OH radicals).[15]

All the fractions were in correlation with the test results with chemical composition of fractions. It is known that flavonoids, either that with catechol-like substitution on B ring or with 4-oxo-3-hydroxy or 4-oxo-5-xydroxy substitution, are efficient in inhibiting LP, both through radical scavenging and through chelation of iron ions.

Anticancer activity

All fractions were subjected to the cytotoxicity activity against EAC using MTT reagent. Results obtained clearly proves that aglycones and anthocyanidins (kaempferol, chrysoeriol, baicalein, 3',7-dimethoxy-3-hydroxyflavone and peonidin) has potent anticancer property than its related flavonoid sugars with various degree of C and O linkages.

It was also noted that fractions containing mono-glycoside flavonoids (Frc D, E and F) and with more di-C-glycosides (Frc C) has shown better cytotoxicity effect than the fractions containing di-glycosides (C-O and O-O linkages). Although Fraction B do contain di-glycosides, but the linkage is through C-O and O-O di-glycosidic, which may be the reason of decreasing the cytotoxic potency. IC_{50} values of each fraction were calculated using GraphPad Software, Inc. CA, USA.

CONCLUSION

Present results demonstrated that methanolic fractions of *J. tanjorensis* leaves obtained by successive solid-liquid extractions with solvents of different polarities possess antioxidant and anticancer activities. In the present work, six fractions of *J. tanjorensis* obtained and main constituents of each fraction were identified and correlated with the bioactivity obtained. UHPLC-ESI-Q-TOF technique has been successfully applied for a quick separation and identification of the major phytoconstituents. The present work provides the first report on the mentioned phytomolecules from *J. tanjorensis*. The antioxidant activity of flavonoid fractions has been assessed by scavenging DPPH free radical and LPO using freshly collected RBC as a substrate, where possible, synthetic antioxidants BHT was also used as a standard. An attempt was made to correlate the chemical composition of the extracts with its antioxidant and anticancer activity and to determine, which groups of biomolecules possess most potent activity. All fractions have shown a very high antioxidative activity as compared to standard antioxidant (BHT). Significant antioxidant activity was determined for most of the fractions by the DPPH assay (lowest IC_{50} of 74.04 µg/ml) and LPO (174.2 µg/ml). EAC cell based cytotoxicity assay also revealed encouraging results. Methanolic extract fractions of *J. tanjorensis* have shown potent anticancer property as proved by MTT bioassay (highest cytotoxicity with IC_{50} of 8.03 µg/ml). The antioxidant and anticancer activity determined in the present work can be attributed to the presence of flavonoids and flavones glycosides. For the pharmaceutical products production, the preparation of the enriched extracts may be of interest. In this paper, it has been demonstrated that it is possible to obtain extracts

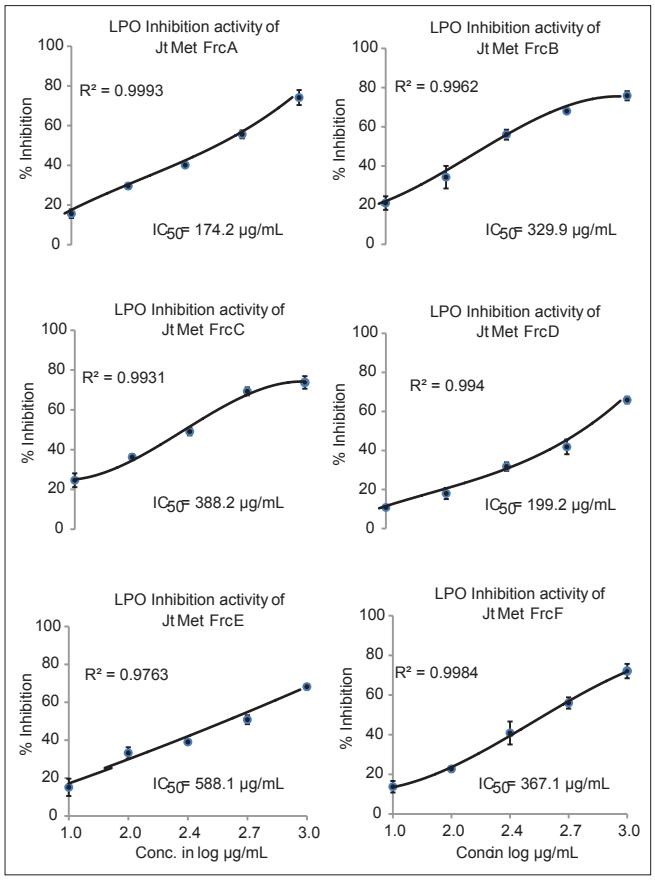

Figure 5: Lipid peroxidation inhibition assay result of each fraction from *Jatropha tanjorensis* leaves methanolic extract

with high levels of flavonoids by using a relatively simple procedure, which appears to be a suitable candidate to develop a new therapeutic agent against cancer.

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