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

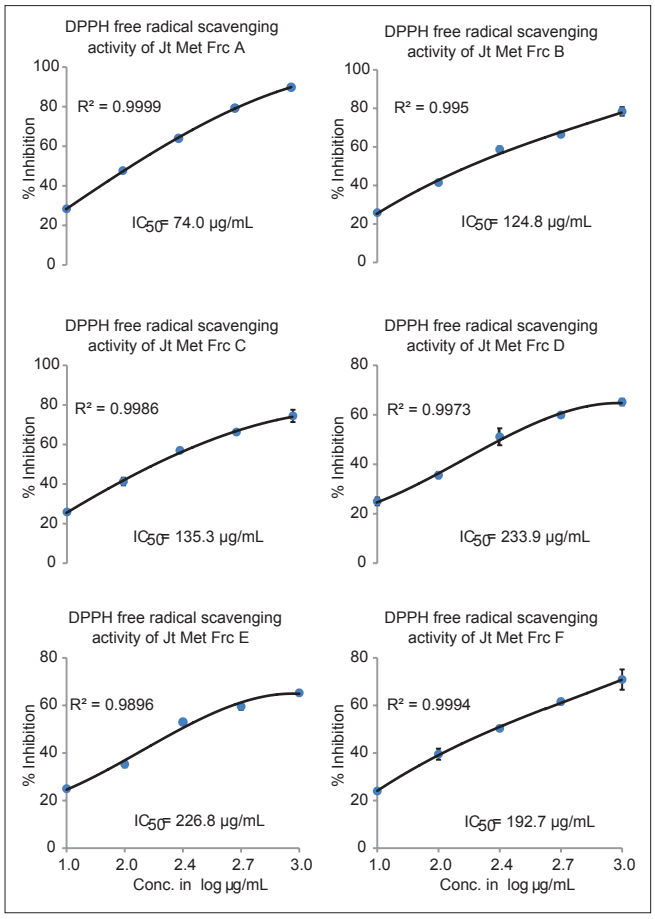

Figure 4: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay result of each fraction from *Jatropha tanjorensis* leaves methanolic extract

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

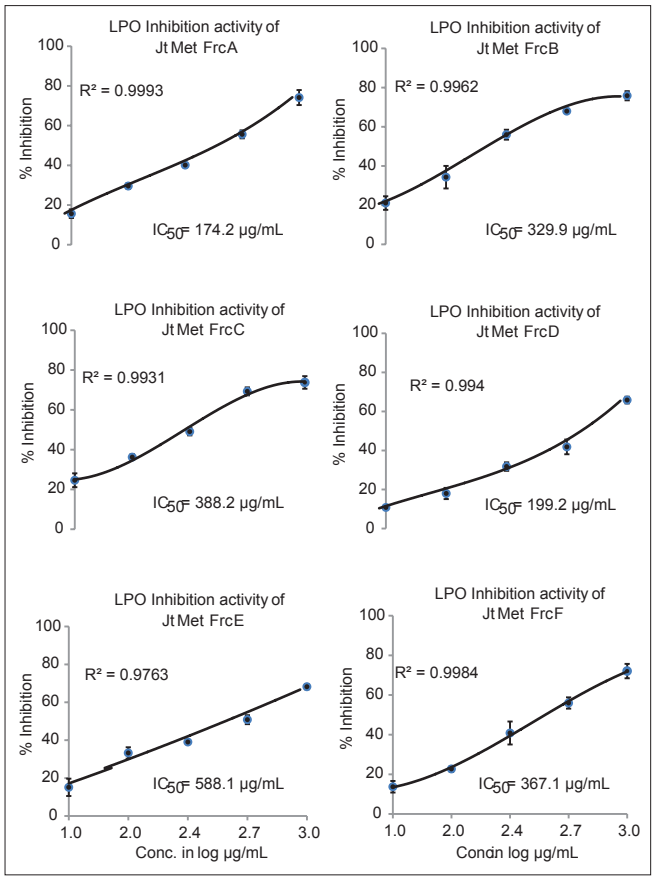

Figure 5: Lipid peroxidation inhibition assay result of each fraction from *Jatropha tanjorensis* leaves methanolic extract

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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