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Anticancer, Hepatoprotective, and Antioxidant Activities of Polysaccharides from *Delonix regia* Raf. and *Gleditsia triacanthos* L. Fruits.

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Abstract

Delonix regia Raf. (*DR*) and *Gleditsia triacanthos* L. (*GT*) are deciduous trees of the Fabaceae family. Different parts of the two plant species were used in folk medicine for the treatment of various ailments. This study aims to prepare cold (CPS) and hot (HPS) from the Fabaceae fruits of *DR* and *GT* for the first time, in addition to chemical and biological investigations. CPS and HPS were isolated, filtrated, concentrated, and ethanol precipitated. Quantitative analyses of the two fruit extracts, including their total ash, sugar, protein, and phenolic content, were conducted. Chemically, they were elucidated by gas liquid chromatography (GLC) and Fourier-transform infrared spectroscopy (FTIR). The FTIR and GLC analyses showed that both CPS and HPS have galactomannan-type polysaccharides. Biologically, *in vitro* anticancer activity against four human tumor cell lines, *in vivo* hepatoprotective activity against CCl_4-induced liver injury, and *in vivo* antioxidant activity were studied. The CPS and HPS of the two fruits studied showed very strong selective *in vitro* cytotoxic activity against four human tumor cell lines and very weak activity against normal human lung fibroblasts. They also have significant curative activity for the injured liver and have potent *in vivo* antioxidant activity.

Keywords: Extraction; *Fabaceae* fruits*;* Monosaccharides composition; Quantitative analysis; Bioactivities.

1. Introduction

Many people, both in developing and developed countries, are turning to herbal remedies as a result of the emergence of new diseases, a lack of curative therapy, and the high cost of modern treatments [1-3]. This prompted a slew of scientists to conduct research on a wide range of global botanicals [4-6]. The Fabaceae are a large and commercially significant flowering plant family. The Fabaceae family is the most common in tropical rainforests and dry forests in both Africa and America [7-10]. Furthermore, this family is the third-largest order of seed plants, with approximately 600 genera and 12,000 species. Plants in the Fabacea*e* family are also distinguished by high-molecular-weight galactomannan-derived polysaccharides, and their molecular mass distribution varies depending on the species [11-13]. This type of polysaccharide (PS) is commonly used in various forms for human consumption. They have numerous applications in a variety of industries, including the pharmaceutical, biomedical, textile, food, and cosmetics industries [14, 15].

DR is a member of the Fabaceae family and is widely regarded as one of the world's most beautiful tropical plants. *DR* leaves, barks, seeds, and flowers were traditionally used to treat a variety of disorders, including rheumatism, spasmogenic, cathartic, flatulence, emetic, central nervous system (CNS) depressant, anaemia, fever, insecticidal, gynaecological disorders or dysmenorrhoea, febrifuge, inflammation, diarrhea, bronchitis, and pneumonia in infants, anti-diabetic [16-18], gastric problems, body pain, and rheumatic joint pain [19, 20], as well as root abdominal pain [21, 22]. Fruits of *DR*, on the other hand, were the subject of fewer articles [23, 24].

GT (Cesalpinaceae) is another member of the Fabaceae family that is grown in Egypt, gardens, and

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parks in temperate climates. It contains flavonoid pigments, tanning agents, anthroglycosides, and alkaloids, among other substances [25]. It is used to treat bronchial asthma, stomach ulcers, chronic cholecystitis, and spastic colitis in folk medicine [26].

The liver is an important organ for maintaining homeostasis and for the detoxification of harmful chemicals. Infections, chemicals, alcohol, narcotics, and other exogenous substances are common causes of liver injury, which interferes with the liver's essential metabolic processes and is thus a major health problem. In addition, reactive oxygen species (ROS) damage liver cells [27]. Given this, as well as the fact that chemotherapy may have potentially negative side effects, more research into hepatoprotective drugs derived from natural sources, particularly plant-based polysaccharides (PS), is required.

Plant-based PS have no serious side effects and are nontoxic, which are the main problems with manufactured medicines. Plant PS are thus promising anticancer, wound-healing, and immunomodulatory therapies [28]. PS from the Leguminosae family, such as *Glycyrrhiza uralensis* Fisch, have been shown to have direct anticancer effects on tumor cells as well as immunomodulatory effects via IL-7 cytokine upregulation [29].

Although PS are abundant in *DR* and *GT*, no information on their biological activities has been published. So, the main goals of this study were to (i) isolate and characterize water-soluble PS from these two species using cold and hot water extraction and ethanol precipitation, and (ii) evaluate their *in vitro* anticancer, *in vivo* hepatoprotective, and antioxidant activities.

2. Experimental

2.1. Collection of fruits and extraction of water-soluble PS

DR and *GT* ripe fruits were obtained from the Zoo Garden and El-Orman botanical garden in Giza, Egypt. Plant classification consultant at the Egyptian Ministry of Agriculture in Giza, Mrs. Trease Labib, authenticated the fruits.

To produce cold water extract, 200 g of powdered ripe fruits that had been oven-dried were first extracted in distilled water, and the process took place at room temperature for 3 h. Hot water extract was then obtained by extracting the residual powdered algae remained after cold extraction, a second time at a temperature of 80 °C. In order to obtain CPS and HPS from the fruits of *DR* and *GT*, the extracts were single-filtered and concentrated under decreased pressure at a temperature of no more than 40 °C. The solubilized PS were precipitated by adding a 4-fold of ethanol (95%, v/v)). When the PS that had precipitated were filtered, they were washed twice with 100% ethanol, and they were dried at 40 °C.

2.2. Chemical analysis of PS

Moisture and total ash were calculated using the method given by El-Rafie et al. [30]. Protein content as total nitrogen was calculated using Pearson's MicroKjeldahl method [31]. The crude protein content was determined by multiplying the nitrogen content [expressed as %N] by 6.25. Total sugar content was calculated using phenol-sulfuric acid analysis and glucose as a standard [32]. The Folin-Ciocalteu method was used to determine total phenolic content using a colorimetric technique [33].

2.2.1. Acid hydrolysis of PS

In order to hydrolyze polysaccharides, 10 ml of 1N-H_2SO_4 solution were mixed with 0.1 g of precipitated polysaccharides and heated for 5 h in a water bath at boiling temperature. After adding $BaCO_3$, the mixture was centrifuged, and the precipitate was subjected to two separate washings in water. Evaporation of the solution continued until there was only 2 ml of hydrolysate left. [34].

2.2.2. PS hydrolysate silylation and GLC analyses [34]

A portion of the hydrolysate solution (0.5 ml) was evaporated to dryness in tiny screw-topped septum vials under a stream of nitrogen at 40 °C. When the mixture was almost dry, 0.5 ml of isopropanol was added, and the drying process was repeated under a stream of nitrogen until only a dry, solid residue remained. 0.5 ml of 2.5% hydroxylamine hydrochloride in pyridine was added, stirred, and heated for 30 min at 80 °C before cooling. A milliliter of trimethylchlorosilane:N,O-bis-[trimethylsilyl]-acetamide (1:5) was added and stirred for 30 minutes at 80 °C. The obtained silylated sugar (1 µl) was then analysed using *a* Hewlett-Packard (HP-6890) GLC apparatus under the conditions cited in Reference [34].

2.2.3. FTIR analysis

The dried powder of both CPS and HPS from the fruits of the plants studied was analysed in KBr pellets using a JASCO FT/IR-4100 (JASCO, Tokyo, Japan) instrument in diffuse reflectance mode with a 4 cm^{-1} resolution. Dry CPS and HPS were individually crushed with KBr powder and pressed into pellets for FT-IR spectra in the 400-4000 cm^{-1} frequency range.

2.3. Animals and their diets

2.3.1. Animals: Adult Sprague Dawley albino rats weighing 130-150 g were obtained from the animal house colony of the National Research Center (NRC) in Dokki, Egypt, and kept under the same hygienic conditions and fed a well-balanced diet and water.

2.3.2. Normal diet: It was made up of 54.3% starch, 20% sucrose, 10.5% casein (95% pure), 10% corn oil, 4% mineral mixture, 1% vitamin mixture, and 0.2% cellulose. National Research Council, Agricultural Broad Committee on Animal Nutrition, Nutrient Requirements of Laboratory Animals.

2.3.3. Doses of drugs: The drugs' doses were determined using Paget and Barner's [35] formula and given through a gastric tube at a dosage of 100 mg/kg of body weight (b.wt).

2.4. Cytotoxic activity

At the Egyptian National Cancer Institute, Cairo, Egypt, various plant extracts were evaluated for their in vitro cytotoxic activity, using the Skehan et al. method [36] against four human tumor cell lines, namely, HELA (cervical carcinoma cell line), MCF7 (breast carcinoma cell line), HEPG2 (liver carcinoma cell line), and HCT-116 (colon carcinoma cell line), in addition to a normal human lung fibroblast, WI-38 (ATCC® CCL-75™).

2.5. Hepatoprotective activity

2.5.1. Liver injury induction:

The Klassen and Plaa method [37] was used to administer 5 ml/kg of CCl_4 (25%) in liquid paraffin intraperitoneally to rats to induce liver injury

2.5.2. Experimental design

Thirty-six male Albino rats were divided into six groups: The first group, which served as a control, was given a 1 ml oral dose of saline once every day for seven days, both before and after the liver injury. Groups two through five received a daily oral dose of 100 mg per kg of body weight of CPS and HPS for seven days before to and following liver injury. The last group was administered silymarin of 25 mg/kg b.wt (as a standard drug) orally once daily for seven days prior to and following liver injury. After an overnight fast, whole blood was drawn from the retro orbital venous plexus of anaesthetized rats via the eye canthus.

Samples from blood were taken at times zero and seven days before the CCl_4 injection and three and seven days after the injection. Centrifugation was used to separate the serum. Serum alanine aminotransferase (AST), alanine aminotransferase (ALT) [38], and alkaline phosphatase (ALP) [39] were measured using kits from Bio-diagnostic.com, Dokki, Giza, Egypt. The results obtained were statistically analyzed using one-way analysis of variance (ANOVA).

2.6. In vivo antioxidant activity

Seven groups of 42 adult male albino rats were created: One milliliter of saline was administered to and kept with the first (negative control) group. Diabetes was induced in the other animal groups by injecting 150 mg/kg b.wt. alloxan intraperitoneally and fasting overnight, as described by Eliasson & Samet [40]. The second group, which served as a positive control, consisted of diabetic rats that were not given any treatment. The diabetic rats in the third group were treated with a reference medication consisting of vitamin E at a dosage of 7.5 mg/kg. Diabetic rats in the fourth to seventh groups were given 100 mg/kg b.wt. of *DR* and *GT* CPS and HPS soluble extracts, respectively. At the end of the experiment, bio-diagnostic kits were used to measure the amount of glutathione in the blood [41]. Potency was calculated as follows: %Potency = [% change in diabetic rats treated with extract / % change in diabetic rats treated with the reference drug (vitamin E)] x100.

3. Results and Discussion

3.1. Chemical analysis

PS derived from plants are currently gaining popularity as natural medications due to the lack of negative side effects when compared to synthetic chemical therapies. The bioactivities of PS are primarily related to structural and physiochemical properties, such as monosaccharide composition. The objective of this study was, therefore, to isolate CPS and HPS from the fruits of *DR* and *GT*, and the results of their chemical analyses are shown in Tables 1 and 2.

The percent yield was estimated using the dry weight of the plant used for extraction, and the other parameters were provided as a percentage of the extracted PS. DR had the highest PS yield, total ash, total sugar, and protein levels, as shown in Table 1. HPS of *GT* and *DR*, on the other hand, revealed the highest phenolic content, which corresponds to 4.38% and 3.93%, respectively.

The GLC analysis of *DR* and *GT* Fruits fruit monosaccharide composition in PS hydrolysate is shown in Table 2. Based on the type of standard samples injected and computerized peak area

measurements, the results show that galactose, glucose, xylose, and mannose are the most abundant monosaccharides in the CPS and HPS of the studied fruits. Other monosaccharides like arabinose, rhamnose, ribose, fucose, and mannitol are less abundant. These phytochemical constituents are thought to be the primary causes of the diverse bioactivities of *DR* and *GT* fruits. They have distinct pharmacological effects.

Table 1. Percentage and composition of CPS and HPS of *DR* and *GT* Fruits

Plant extract	PS yield [%]	Total ash	Total sugar	Protein	Phenolic contents
CPS of *DR*	4.5	0.73±0.025	86.7±0.32	0.07±0.02	2.60±0.16
HPS of *DR*	8.6	0.85±0.03	93.2±1.30	0.05±0.03	3.93±0.05
CPS of *GT*	3.9	0.75±0.09	85.4±0.21	0.06±0.01	2.43±0.37
HPS of *GT*	7.6	0.72±0.01	92.8±1.04	0.01±0.007	4.38±0.79

Table 2. GLC analysis of the monosaccharide composition and area percentage of PS hydrolysate.

Monosaccharide	Arab.	Xyl.	Rib.	Rham.	Fuc.	Man.	Gala.	Manno.	Gluc.
Retention time [R_t]	8.147	8.383	8.766	9.396	9.475	11.09	13.795	14.23	14.574
Plant extract	Monosaccharide area percentage *								
CPS 1	11.5	11.3	1.3	6.9	4.1	0.8	19.8	1.6	11.8
HPS 2	2.3	1.3	0.2	-	-	1.6	66.6	13.0	4.6
CPS 3	5.1	3.7	-	1.7	5.8	-	29.6	4.2	5.9
HPS 4	3.8	3.4	-	1.2	5.1	-	40.2	5.7	5.8

*****Arab. = Arabinose, Xyl. = Xylose, Rib. = Ribose, Rham.= Rhamnose, Fuc.= Fucose, Man.= Mannitol, Gala.= Galactose, Manno. = Mannose, Gluc. = Glucose.

CPS 1 = Cold water polysaccharides of *DR*, HPS 2 = Hot water polysaccharides of *DR*, CPS 3 = Cold water polysaccharides of *GT*, HPS 4 = Hot water polysaccharides of *GT*.

Figures 1a–d display the typical polysaccharide FTIR spectra [42-44] found in the CPS and HPS of the investigated fruits, in which the band between 3421 and 3434.09 cm^{-1} corresponds to the hydroxyl stretching vibration and the bands between 2921 and 2923 cm^{-1} correspond to a weak C-H stretching vibration. Absorption bands between 1600-1623 cm^{-1} indicate the presence of bound water, whereas bands between 1425-1448 cm^{-1} indicate -CH_3 and -CH_2 bonding vibrations. Stretching vibrations overlap with (C-O-C) glycosidic band vibrations and (C-OH) side group vibrations to form a distinct band in the 1272–1000 cm^{-1} region. The vibrations of the 1-α-D-galactose pyranose ring produce an absorption band between 701 and 777 cm^{-1} [44]. Bands at 875 cm^{-1} have also been found in galactomannan α-linked D-galactopyranosyl units dispersed on the mannopyranosyl main chain [12]. The bending vibrations of the C-O-C, C-O, glycosidic, and C-O-H bonds in the pyranose ring are the cause of the absorption bands that range from 920 to 1137 cm-1 in wavelength. The FTIR and GLC analyses confirmed that the majority of the PS extracted from the fruits of *DR* and *GT* were of the galactomannan type.

3.2. Anticancer activity

It has been demonstrated that the CPS and HPS derived from the two fruits under investigation both have anticancer efficacy against a range of human cancer cell lines, as well as an anti-proliferative effect on cell survival that is dose-dependent. The results of this impact are outlined in Table 3. The CPS and HPS of the fruits of these two plants, as can be seen, showed very potent activity against breast carcinoma (MCF-7) with IC_{50} values of 0.063, 0.028, 4.32 and 0.40; cervix carcinoma (HELA) with IC_{50} values of 0.65, 4.12, 1.44, and 24.18; human colon carcinoma (HCT116) with IC_{50} values of 0.02, 0.01, 14.78, and 0.31 μg/ml; and liver carcinoma (HEPG2) with IC_{50} values of 2.30, 0.02, 3.74, and 1.67μg/ml, respectively. Plant extracts with an $IC_{50} \leq 30$ μg/ml are thought to be anti-cancer agents [45], so the PS isolated from both the CPS and HPS can be used to make anti-cancer drugs. On the other hand, very weak activity was observed against normal human fibroblast WI-38 with IC_{50}= 435.5, 825.4, 703.5, and 2051 μg/ml for CPS and HPS of *DR* and *GT* fruits, respectively.

Because of the structural diversity and variation of these biological macromolecules, PS and attached protein moieties have the potential to modify a large number of immune cells [46-48]. Thus, polysaccharides isolated from both the CPS and HPS of the plant fruits under investigation showed significant cytotoxicity against all cell lines tested, providing a scientific basis for the development of anticancer drugs.

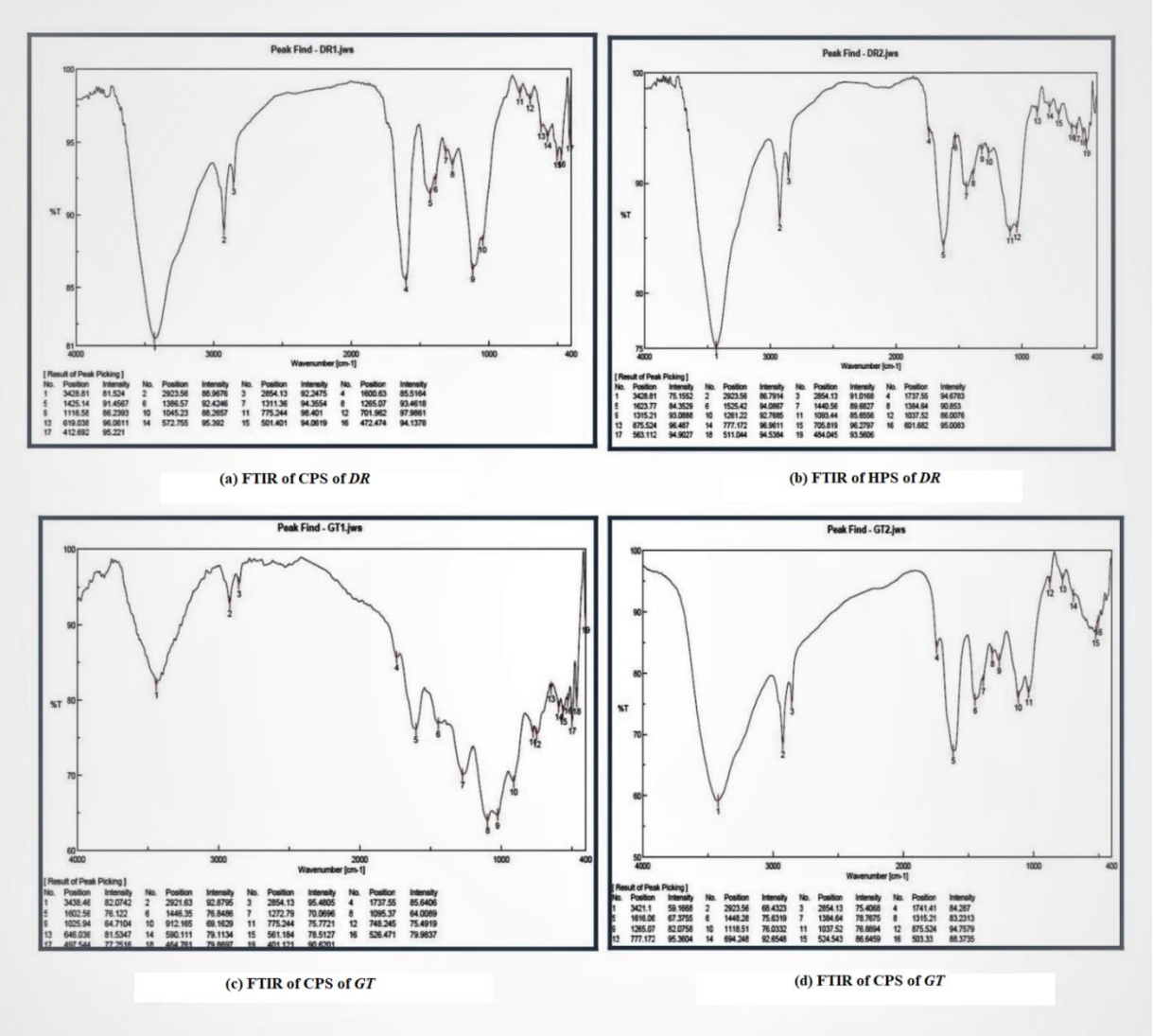

Figure 1a-d. FTIR Spectrum for CPS and HPS of *DR* and *GT*.

Table 3. Cytotoxic activity of the PS of *DR* and *GT* fruits on different cell lines.

Conc. [µg/ml]	Cell Viability [%]				
	MCF-7	**HELA**	**HCT-116**	**HEPG-2**	**WI-38**
			CPS of *DR* fruits		
0	100±2.41	100±1.3	100±0.09	100±0.60	100±3.1
0.01	85.04±3.2	87.47±0.09	69.58±0.52	25.43±1.23	99.6±0.9
0.1	47.04±2.5	62.34±0.46	27.72±0.72	24.55±0.40	99.3±1
1	15.73±0.2	48.46±1.90	25.61±1.06	11.75±1.60	95.6±2.9
10	12.84±0.8	25.69±0.63	19.70±0.41	11.12±0.61	78.8±1.9
100	11.32±0.3	11.72±0.12	10.90±0.60	11.16±031	66.3±2.5
IC_{50}[ug/ml]	0.063	0.65	0.02	2.3	435.5
			HPS of *DR* fruits		
0	100±0.09	100±0.5	100±9.4	100±2.06	100±0.6
0.01	85.94±4.60	95.35±1.41	40.56±0.63	60.20±0.67	95.9±2
0. 1	38.59±0.82	86.80±0.91	40.14±0.78	32.44±2.80	91.2±1.3
1	37.46±0.42	69.74±1.60	33.65±2.01	13.87±1.89	86.9±2.08

10	26.57±1.30	45.79±2.80	31.90±1.30	10.01±0.23	84.3±0.4
100	22.62±0.23	26.37±1.03	14.30±0.45	05.01±0.01	63.2±3.9
IC_{50}[µg/ml]	0.028	4.12	0.01	0.02	852.4
			CPS of *GT* fruits		
0	100±1.05	100±0.060	100±1.44	100±1.52	100±1.3
0.01	73.25±0.43	85.57±0.42	91.59±0.77	90.14±0.79	91.974±0.3
0. 1	72.23±1.60	67.42±1.30	87.74±0.43	87.91±1.27	79.5±0.84
1	68.60±0.63	60.98±0.48	82.81±1.31	75.34±0.53	78.5±2.73
10	37.51±1.30	30.56±±0.73	62.39±0.68	33.50±0.44	73.4±0.87
100	33.12±0.61	15.62±0.23	18.37±0.93	04.86±1.52	66.32±4.57
IC_{50}[µg/ml]	4.32	1.44	14.78	33.74	703.5
			HPS of *GT* fruits		
0	100±0.40	100±0.32	100±1.3	100±1.05	100±0.25
0.01	94.56±1.90	85.11±1.90	80.40±1.90	90.93±1.24	97.60±0.63
0.1	71.98±0.31	76.22±0.74	73.50±0.71	84.49±0.65	96.00±0.72
1	60.49±2.80	73.38±1.60	54.84±0.09	61.54±1.30	89.80±1.20
10	46.05±1.01	51.89±0.34	44.26±1.60	21.60±0.46	73.80±3.24
100	35.60±0.23	42.15±1.30	36.92±2.80	06.59±0.23	65.02±4.57
IC_{50}[µg/ml]	0.40	4.18	0.31	1.67	2051

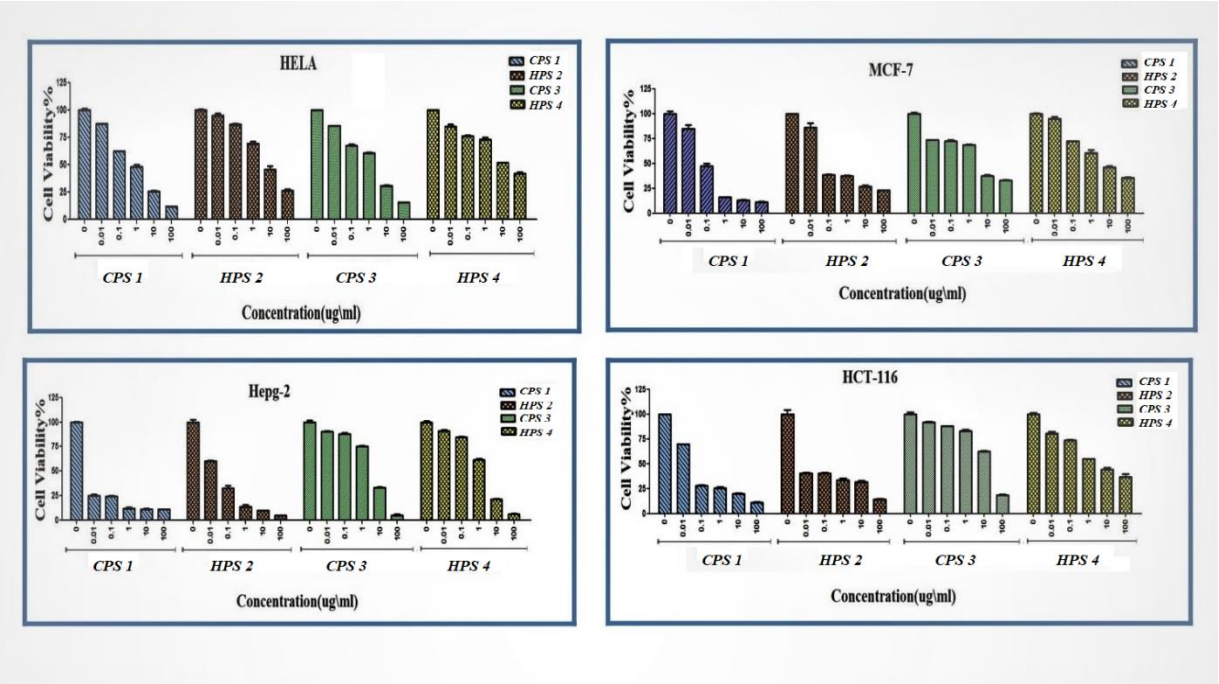

Figure 2. Effect of PS of *DR* and *GT* fruits on cell viability % of different cell lines

3.3. Hepatoprotective activity

Serum levels of AST, ALT, and ALP were utilized individually as biochemical markers for acute liver injury to examine the hepatoprotective effects of CPS and HPS. Figure 3a-c shows the results of the recorded liver function enzyme concentrations as well as the calculated percentages of change observed after 72 hours and 7 days of CCl_4 administration. The prophylactic effect of both CPS and HPS extracts was investigated by administering each extract for 7 consecutive days prior to CCl_4-induced liver injury. To assess the potential curative effect of the tested PS, rats with induced liver injury were treated with the extracts for another 7 consecutive days, after which their liver enzyme levels were measured and compared to those recorded after 72 hours of CCl_4 administration. The following conclusions can be drawn from Figures 3a-c:

a) In the case of AST (Fig.3a), the HPS of *GT* fruit extract had the greatest protective effect on the liver, as measured by the percentage of change prophylactic at 72 hours after liver injury (58.61%), followed by the HPS of *DR* fruits (65.74%), when compared to silymarin (35.03%). The HPS of *DR* fruits, on the other hand, had the highest curative activity for injured livers (25.68%), which was nearly equal to silymarin (27.44%), and was followed by the CPS of *GT* fruits (23.33%).

b) In the case of ALT (Figure 3b), in comparison with silymarin (37.52%), the HPS of *DR* fruits exhibited the strongest therapeutic activity for the injured liver, as measured by the percent drop in the ALT enzyme level and the highest percentage of change curative (27.63%).

c) Figure 3c also shows that the CPS of *GT* fruits had the highest percentage of change curative activity (38.96%) when compared to the HPS of *DR* fruits (35.96%), while the silymarin protective activity was 56.91%.

In general, the results obtained (Fig. 3a-c) showed that the activities of AST, ALT, and ALP were significantly reduced ($p< 0.01$) in rats treated with both CPS and HPS, indicating that they attenuated CCl_4-induced liver injury, had hepatocyte regeneration effects, and were able to stabilize membrane structures in injured livers [49].

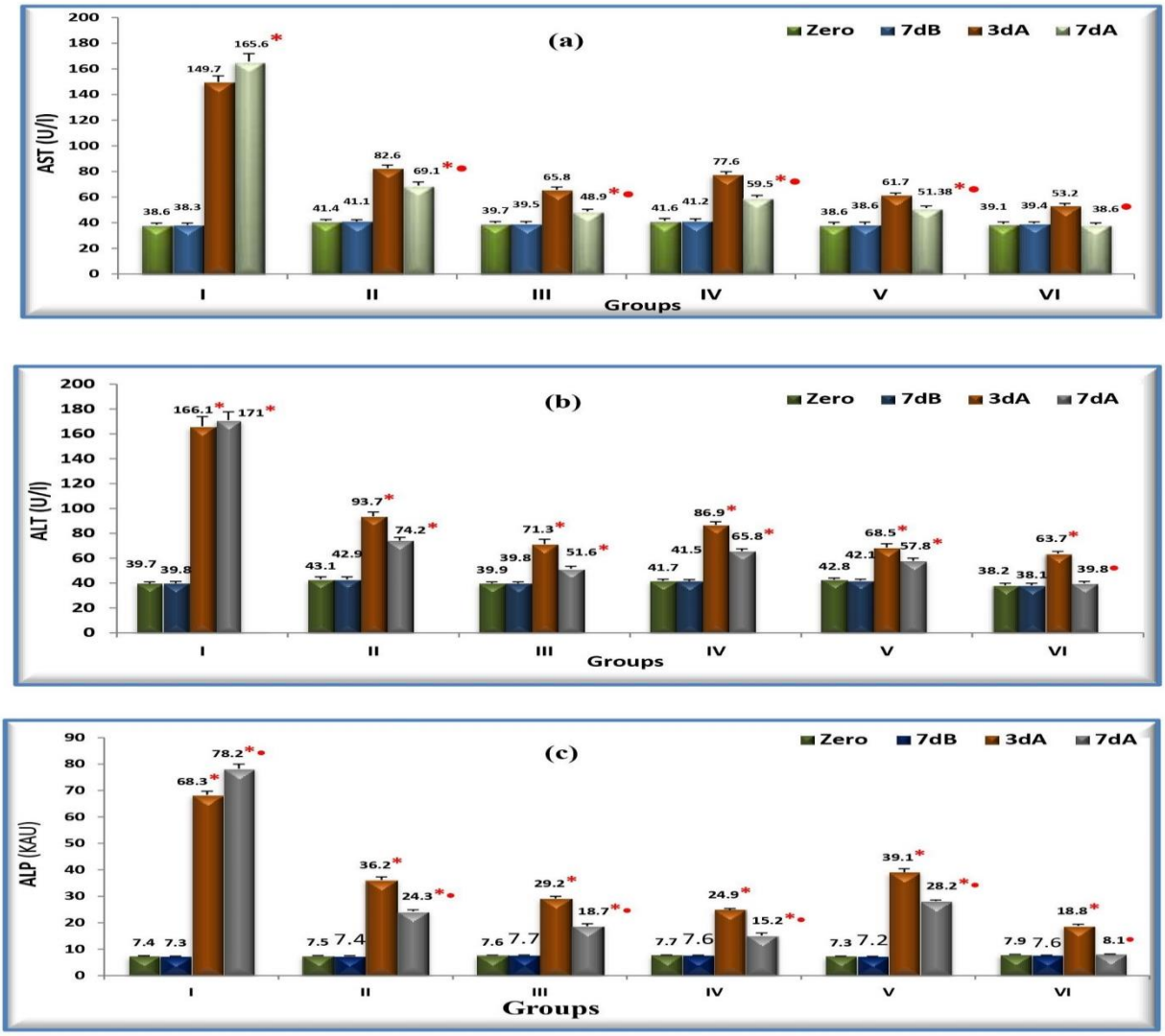

Figure 3a-c. Effect of the PS extracts of *DR* and *GT* fruits and Silymarin on serum enzyme levels [AST, ALT, and ALP] on CCl_4 liver injury rats: [a] **I**= a control group before and after liver injury; **II and III =** treated with CPS and HPS of *DR* (100 mg/kg); **IV and V =** treated with CPS and HPS of *GT* (100mg/kg); **VI =** treated with silymarin (25 mg/kg); **7dB** = seven days before CCl_4 liver injury; **3dA** = three days after CCl_4 liver injury; **7dA** = seven days after CCl_4 liver injury; *Statistically significant from zero and 7d groups after CCl_4 liver injury at p. <0.01; •Statistically significant from 3dA group after CCl_4 at $p<0.01$.

3.4. Antioxidant activity

Glutathione (GSH) is one of the endogenous enzymes that is used as a marker for the detection of ROS. The low level of GSH observed in the diabetic control group in the current study (22.7 ± 0.6 mg %) in Table 4 could be explained by the accumulation of superoxide anion and hydrogen peroxide (ROS), which would have been effectively scavenged by this enzyme [50]. These complications were caused by the rats' lowered antioxidant defense system and could be compensated for by external antioxidants, such as vitamin E in diabetic rats (VEDC) 36.2 ± 1.3 mg %. As a result, administration of CPS and HPS of *DR and GT* fruits significantly increased tissue GSH levels (34.6 ± 1.1, 34.1 ± 0.9, 35.8 ± 1.2, and 35.5± 0.8 mg % respectively) when compared to the diabetic control group, implying their compensatory response to oxidative stress as they decrease the endogenous hydrogen peroxide produced, thus diminishing toxic effects due to this radical or other free radicals derived from secondary reactions [50]. As a result, as indicated by earlier investigations on the antioxidant activity of PS isolated from different plant extracts, both CPS and HPS obtained from *DR* and *GT* fruits could be considered good sources of natural antioxidants [51-53].

Table 4. Antioxidant activity of *DR* and *GT* PS extracts and vitamin E drug on diabetic male albino rats (n = 6)

Group	Blood glutathione [mg%]	% of change	potency
Control	36.9± 1.4	--	--
Diabetic [DC]	22.7 ± 0.6*	--	--
Diabitic + vit E[VEDC]	36.2 ± 1.3	59.47	100
Diabitic + 1	34.6 ± 1.1	52.42	88.15
Diabitic + 2	35.8 ± 1.2	57.70	97.00
Diabitic + 3	34.1 ± 0.9	50.22	84.44
Diabitic + 4	35.5± 0.8	56.39	94.82

* Statistically significant from the control group at $P < 0.01$.
1 = CPS of *DR*, 2 = HPS of *DR*, 3 = CPS of *GT*, 4 = HPS of *GT*.

4. Conclusions

Natural resources are crucial in the development of medications. Medicinal plants, in particular, are an abundant source of bioactive phytoconstituents with no known adverse effects and potent pharmacological effects. Taking these considerations into account, the cytotoxic, hepatoprotective, and antioxidant properties of CPS and HPS extracts of *DR* and *GT* fruits were investigated. The bioactivities of CPS and HPS from both *DR and GT* fruits were found to have very powerful *in vitro* cytotoxic activity against human tumor cell lines: breast carcinoma (MCF-7), human colon carcinoma (HCT-116), liver carcinoma (HEPG2), and cervix carcinoma (HELA). Furthermore, when compared to silymarin as a positive reference drug, HPS from *DR* and CPS from *GT* fruits had the best therapeutic efficacy for the injured liver as measured by the percent decrease in AST, ALP, and ALT enzyme levels. Besides that, the HPS from both fruits demonstrated significant *in vivo* antioxidant activity. These studies on the PS fruits of these species were conducted for the first time.

Conflicts of interest

There are no conflicts to declare.

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