The Impact of Carbon Source on Cell Growth and the Production of Bioactive Compounds in Cell Suspensions of *Hancornia speciosa* Gomes

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Abstract

Belonging to the Brazilian flora, the species Hancornia speciosa (Gomes), known as mangabeira, has bioactive compounds of interest, such as flavonoids, xanthones, and proanthocyanidins. The objective of this study was to determine how the supplementation of sugars in culture medium affects the osmotic potential of the medium, as well as its influence on cell growth and on the concentration of phenolic compounds. For this purpose, after 90 days of subculture, 20 ml aliquots of the cultures were added to flasks containing 20 ml of medium with different sugars (glucose, fructose, sucrose, mannitol, and sorbitol) under a 16-h photoperiod with a spectral range between 400 and 700 nm of photosynthetically active radiation (45-55 µmol m -2 s -1) in a shaker at 110 rpm. After 30 days, the pH, electrical conductivity, osmotic potential, biomass accumulation, and concentrations of phenolic compounds were evaluated. Regardless of their concentration in the medium, the sugars sorbitol and mannitol provided more unfavorable conditions for water absorption at the cellular level, reducing the water potential of the medium. Sucrose favored greater water absorption and biomass accumulation. Among the various sugar concentrations, 3% (30 g/L) sucrose or glucose improved the accumulation of fresh and dry cell weight and the production of polyphenols such as chlorogenic acid, epicatechin, rosmarinic acid, hesperidin, rutin, and quercetin. In addition, they resulted in a higher osmotic potential of the medium and larger cells than other carbon sources. Despite the differences in cell size, no culture conditions compromised cell survival.

Full Text

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Figures

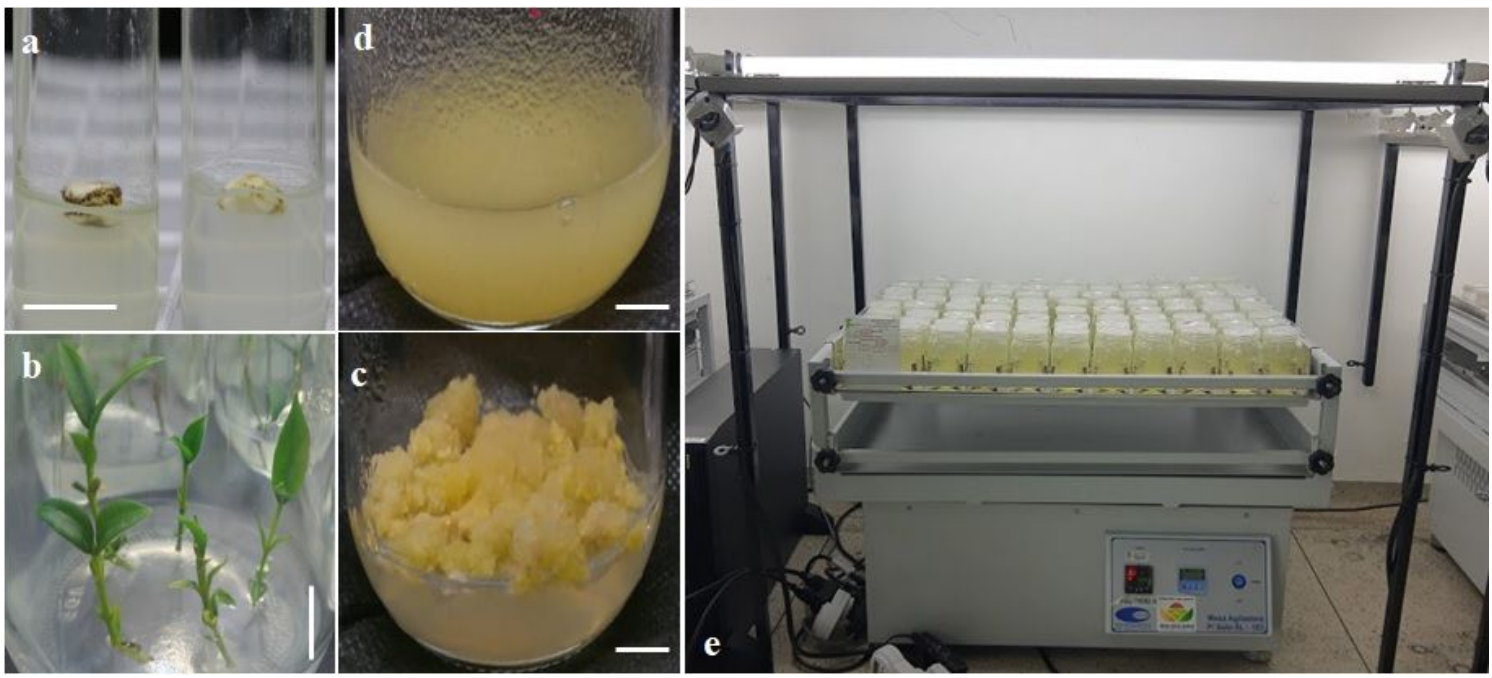

Figure 1

Process of the in vitro establishment and culture of H. speciosa (Gomes) cell culture. Seeds disinfected and inoculated in a tube (A). Explants obtained after 60 days of culture (B). Friable calli 120 days after the beginning of induction (C). Stable cell suspension (D). Orbital shaker used to culture the cell suspension under light support with 50 µmol m-2 s-1 irradiance (E). Bar = 1 cm.

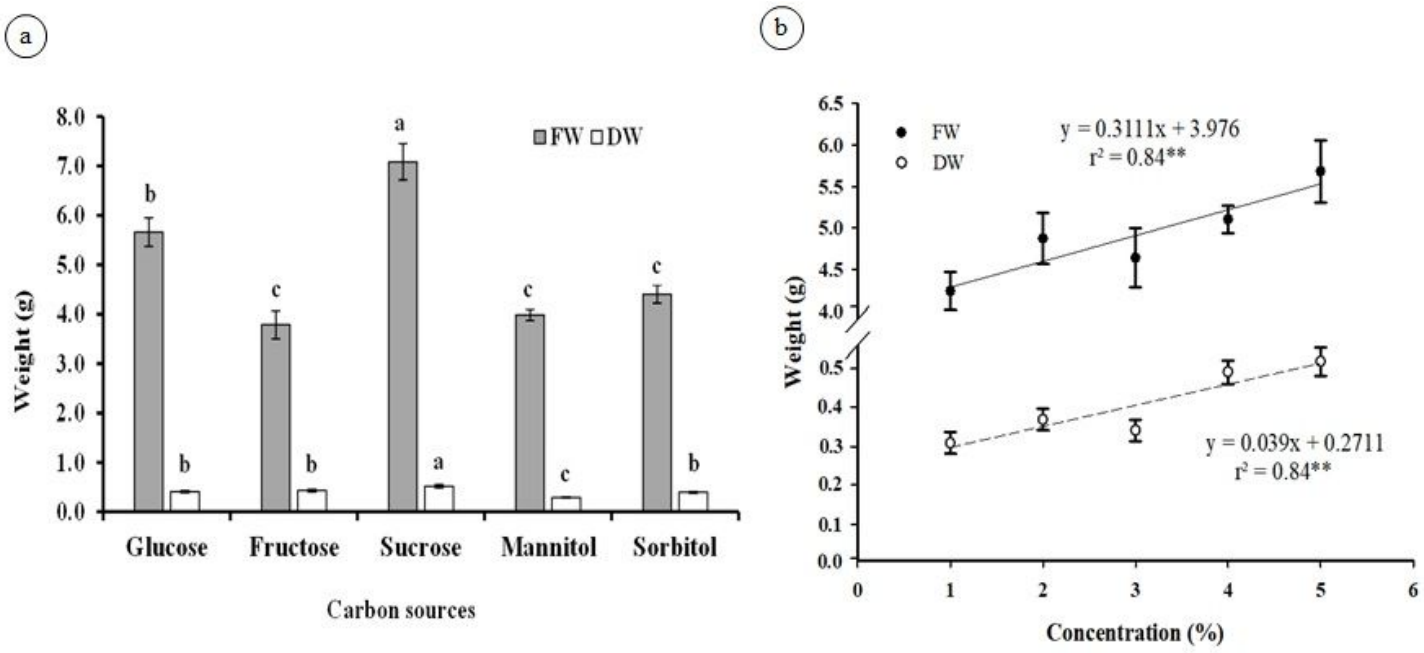

Figure 2

Accumulation of fresh and dry weight of cell-suspension cultures of H. speciosa (Gomes) cultured in different carbon sources (glucose, fructose, sucrose, mannitol, and sorbitol) (A) under different concentrations (1, 2, 3, 4, and 5%) (B). The bars indicate the standard error of the mean; significance: **p<0.01.

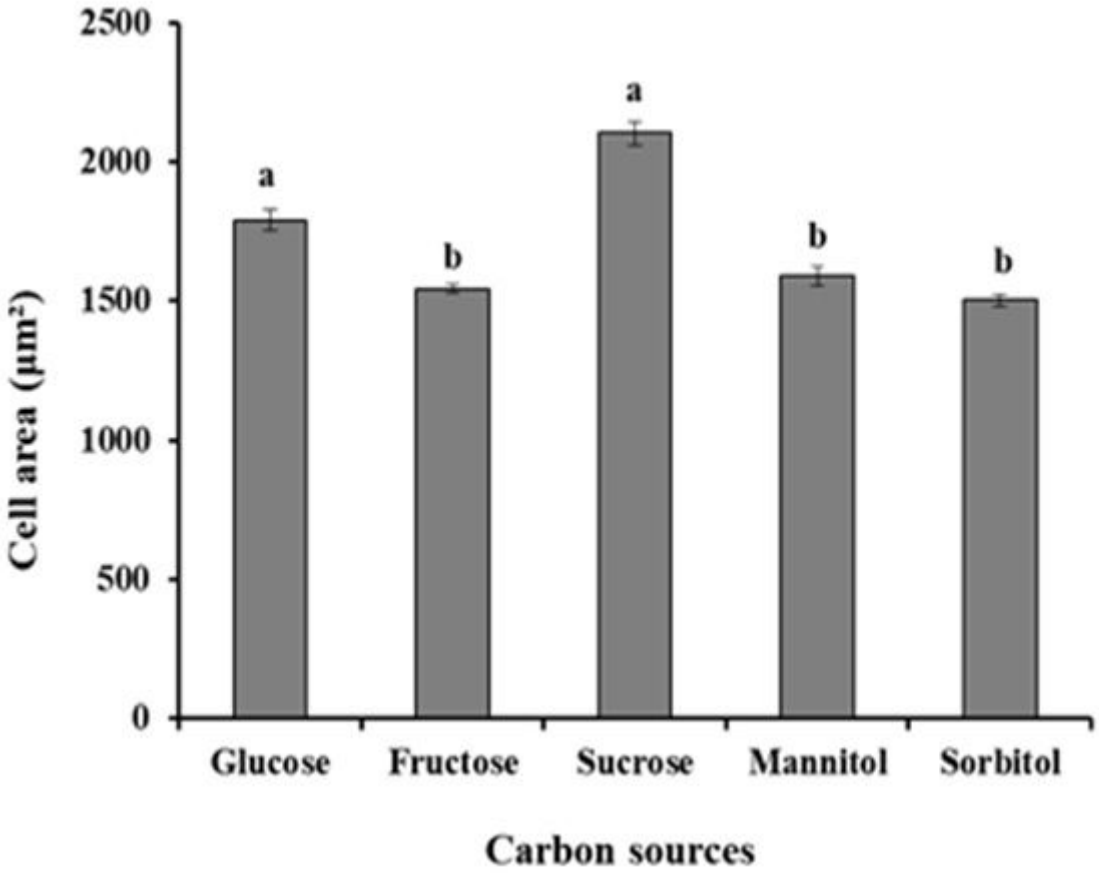

Figure 3

Cell areas (µm-2) observed in cell suspensions of H. speciosa (Gomes) cultured in different carbon sources. The means were compared by Tukey's test. The bars indicate the standard error of the mean; significance: p <0.01.

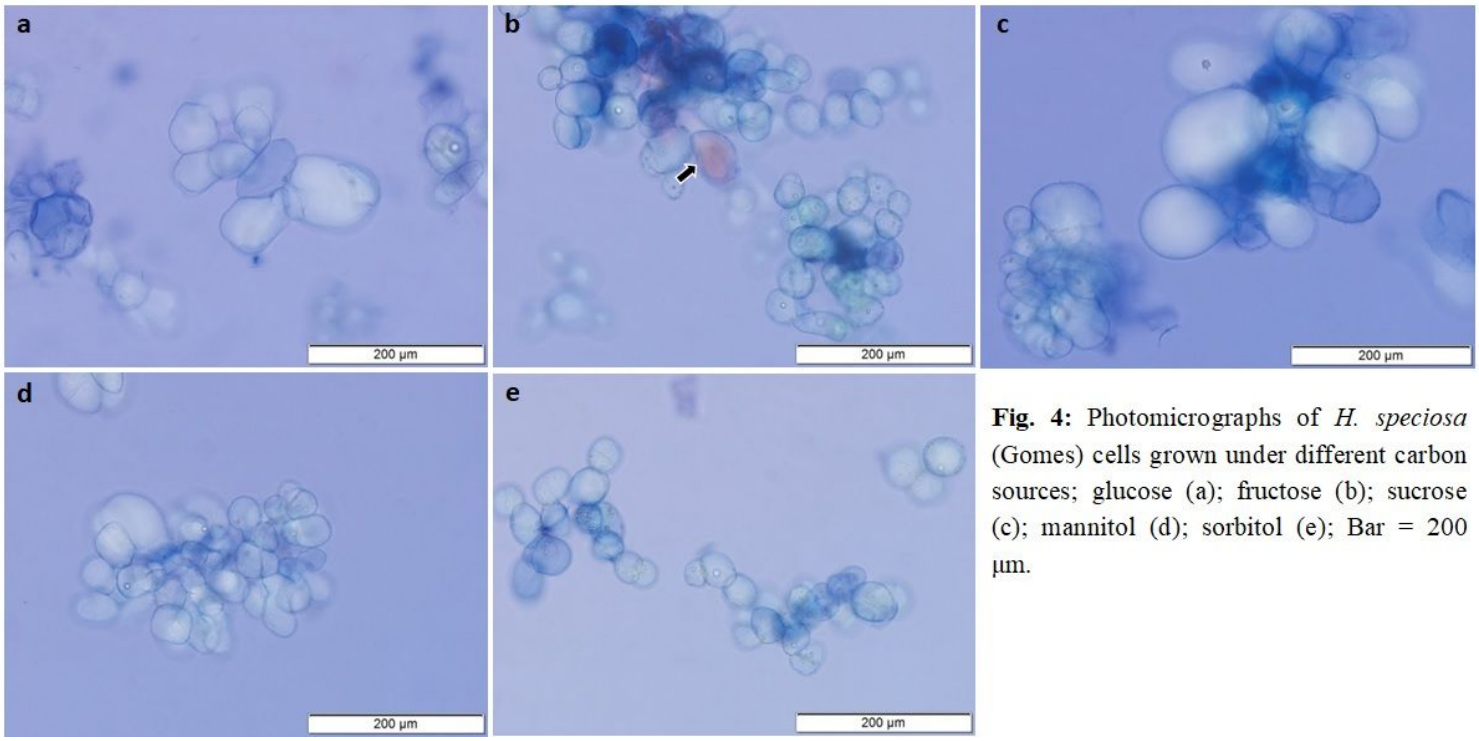

Fig. 4: Photomicrographs of *H. speciosa* (Gomes) cells grown under different carbon sources; glucose (a); fructose (b); sucrose (c); mannitol (d); sorbitol (e); Bar = 200 µm.

Figure 4

Photomicrographs of H. speciosa (Gomes) cells grown under different carbon sources; glucose (A); fructose (B); sucrose (C); mannitol (D); sorbitol (E); Bar = 200 µm. Cell areas (µm-2) observed (F) in cell suspensions. The means were compared by Tukey's test. The bars indicate the standard error of the mean; significance: p <0.01.

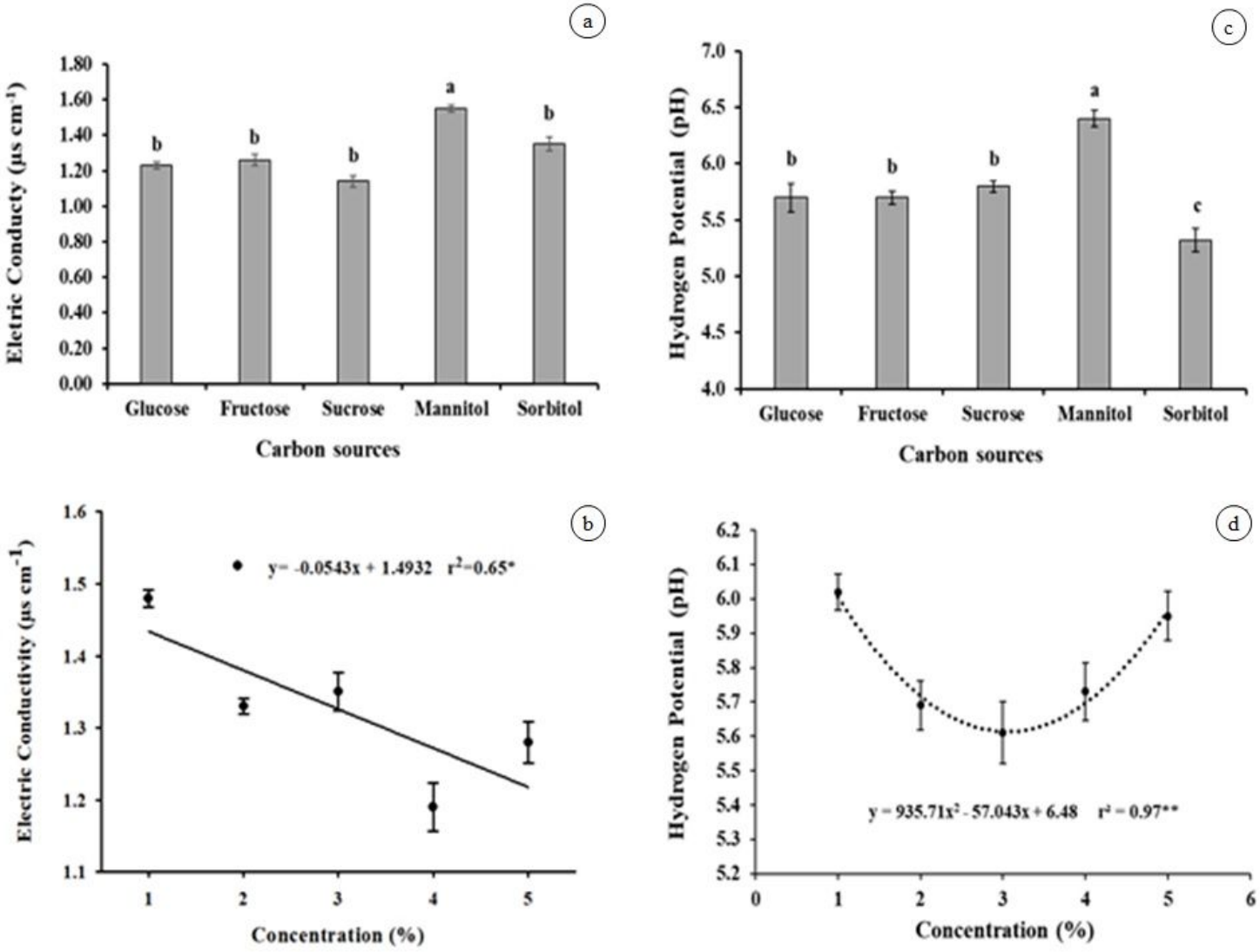

Figure 5

Electrical conductivity (A and B) and pH (C and D) of H. speciosa (Gomes) cell-suspension cultures supplemented with different concentrations of carbon sources. The means were compared by Tukey's test. The bars indicate the standard error of the mean; significance: *p <0.05; **p <0.01.

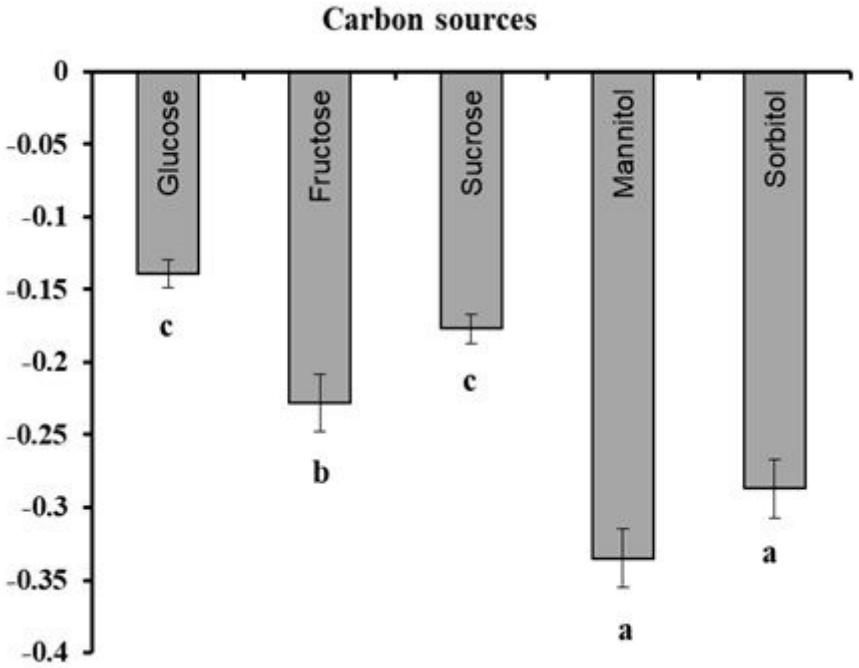

Figure 6

Osmotic potential (MPa) of cell-suspension cultures of H. speciosa (Gomes) supplemented with different carbon sources (glucose, fructose, sucrose, mannitol, and sorbitol). The means were compared by Tukey's test. The bars indicate the standard error of the mean; significance: $p < 0.01$.

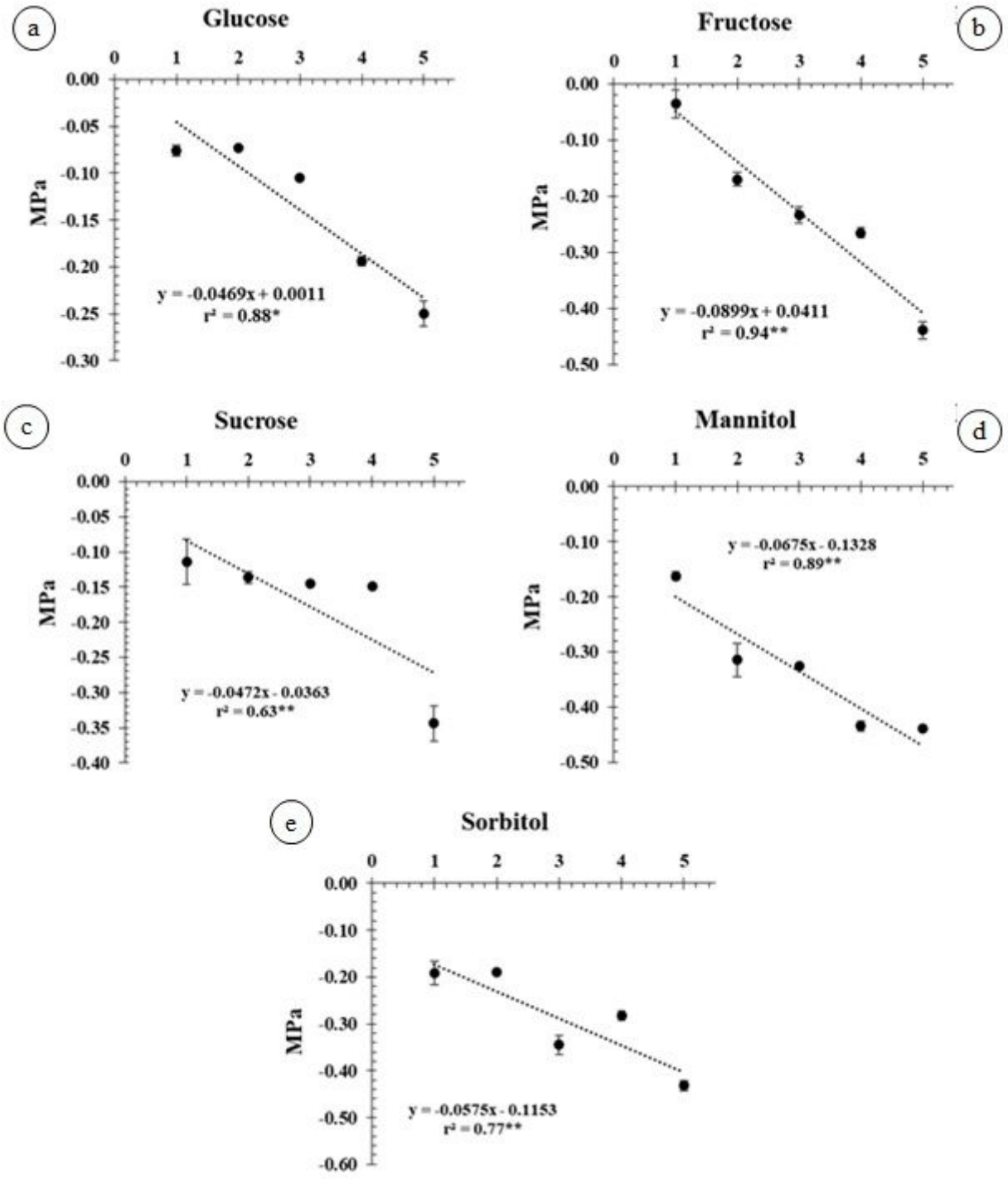

Figure 7

Osmotic potential (MPa) of cell-suspension cultures of H. speciosa (Gomes) supplemented with different carbon sources [glucose (A), fructose (B), sucrose (C), mannitol (D), and sorbitol (E))] andconcentrations (1, 2, 3, 4, and 5%). The bars indicate the standard error of the mean; significance: * p <0.05; ** p <0.01.

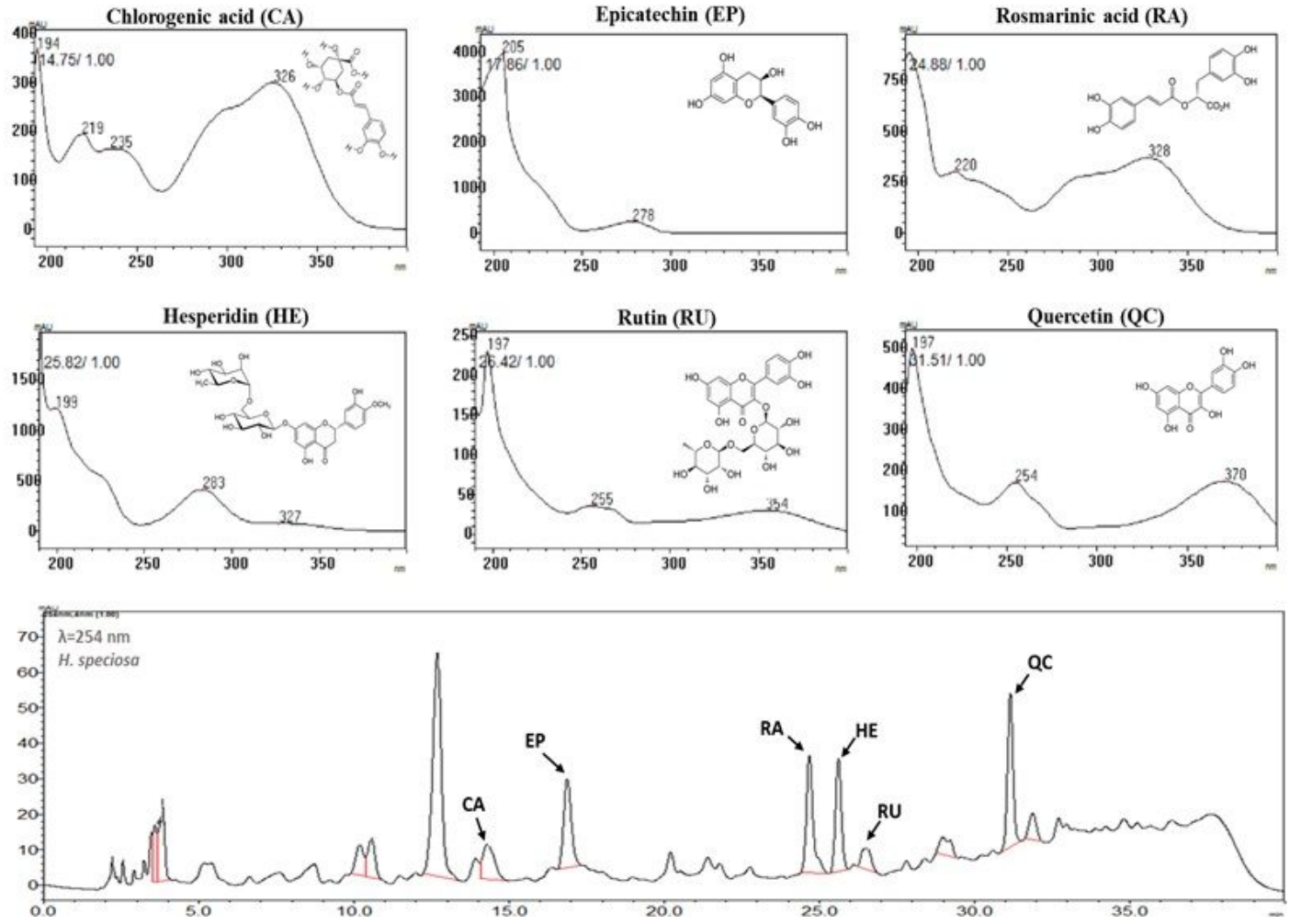

Figure 8

Spectra and chromatographic separation of phenolic compounds obtained by HPLC-DAD from H. speciosa (Gomes) cell suspensions grown with different carbon sources.

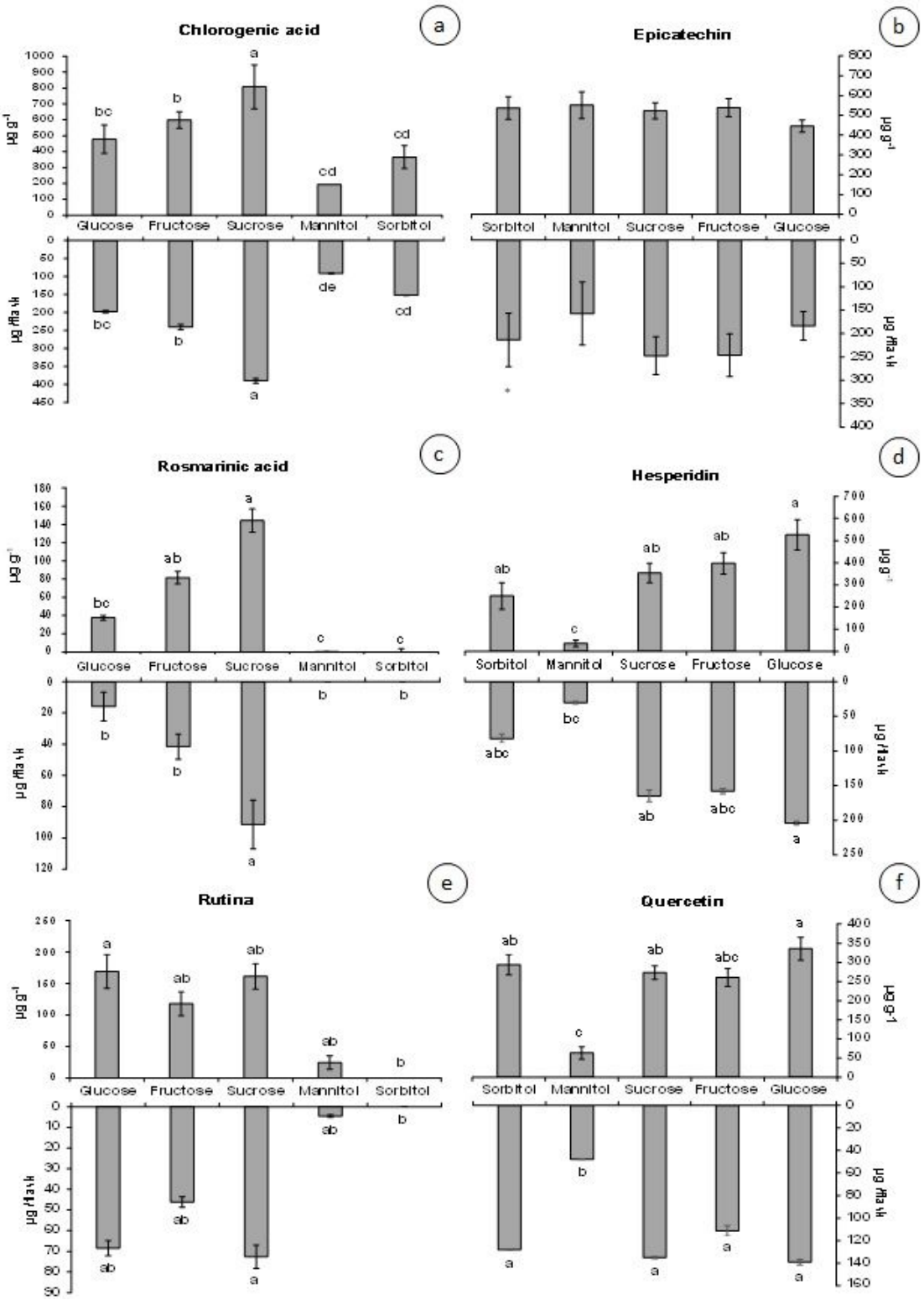

Figure 9

Concentration and yield of chlorogenic acid (A), epicatechin (B), rosmarinic acid (C), hesperidin (D), rutin (E), and quercetin (F) in cell-suspension cultures of H. speciosa (Gomes) supplemented with different carbon sources. The means were compared by Tukey's test. The bars indicate the standard error of the mean; * not significant.

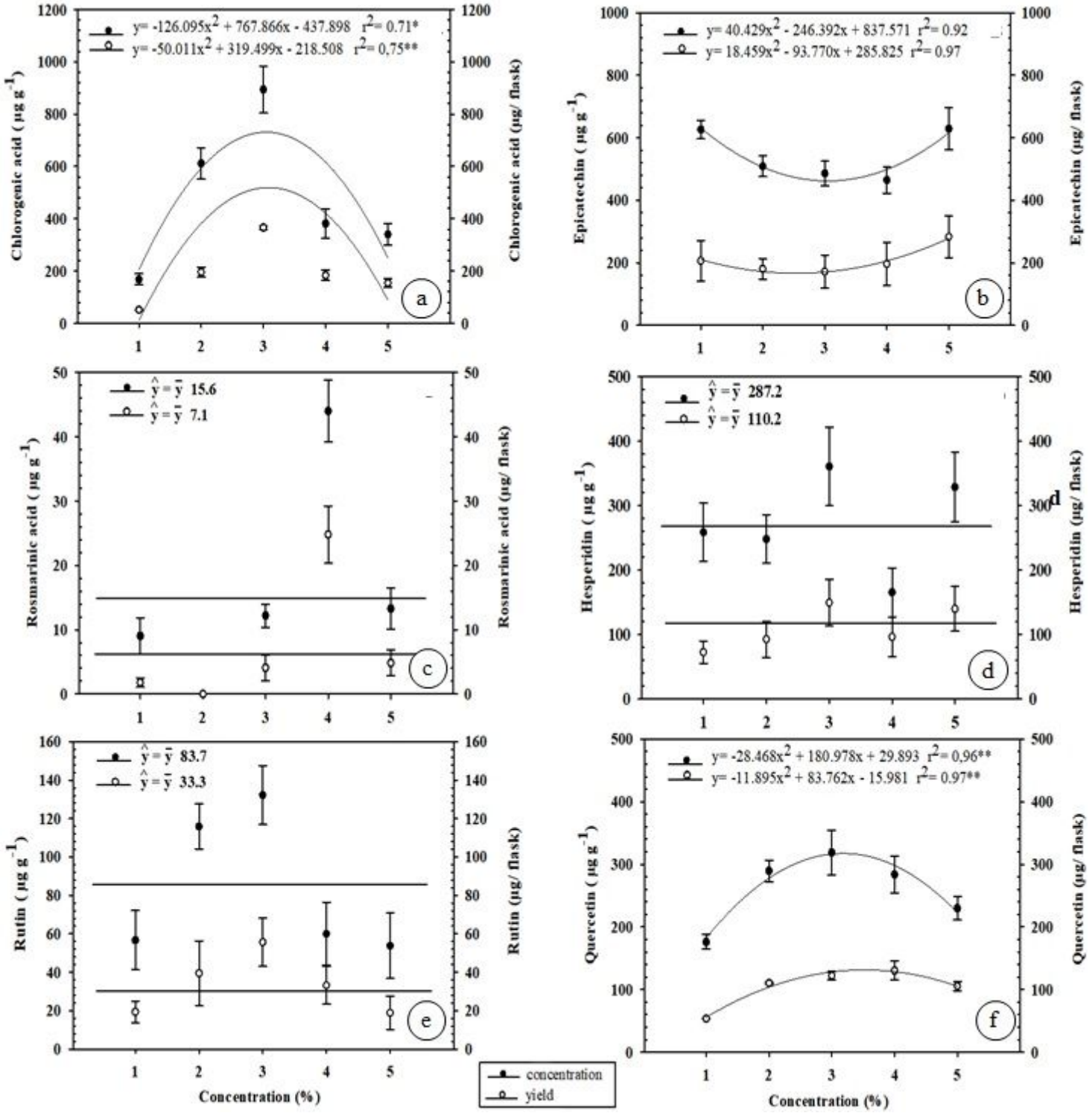

Figure 10

Concentration and yield of chlorogenic acid (A), epicatechin (B), rosmarinic acid (C), hesperidin (D), rutin (E) and quercetin (F) of the cell-suspension cultures of H. speciosa (Gomes) supplemented with different concentrations. The bars indicate the standard error of the mean.

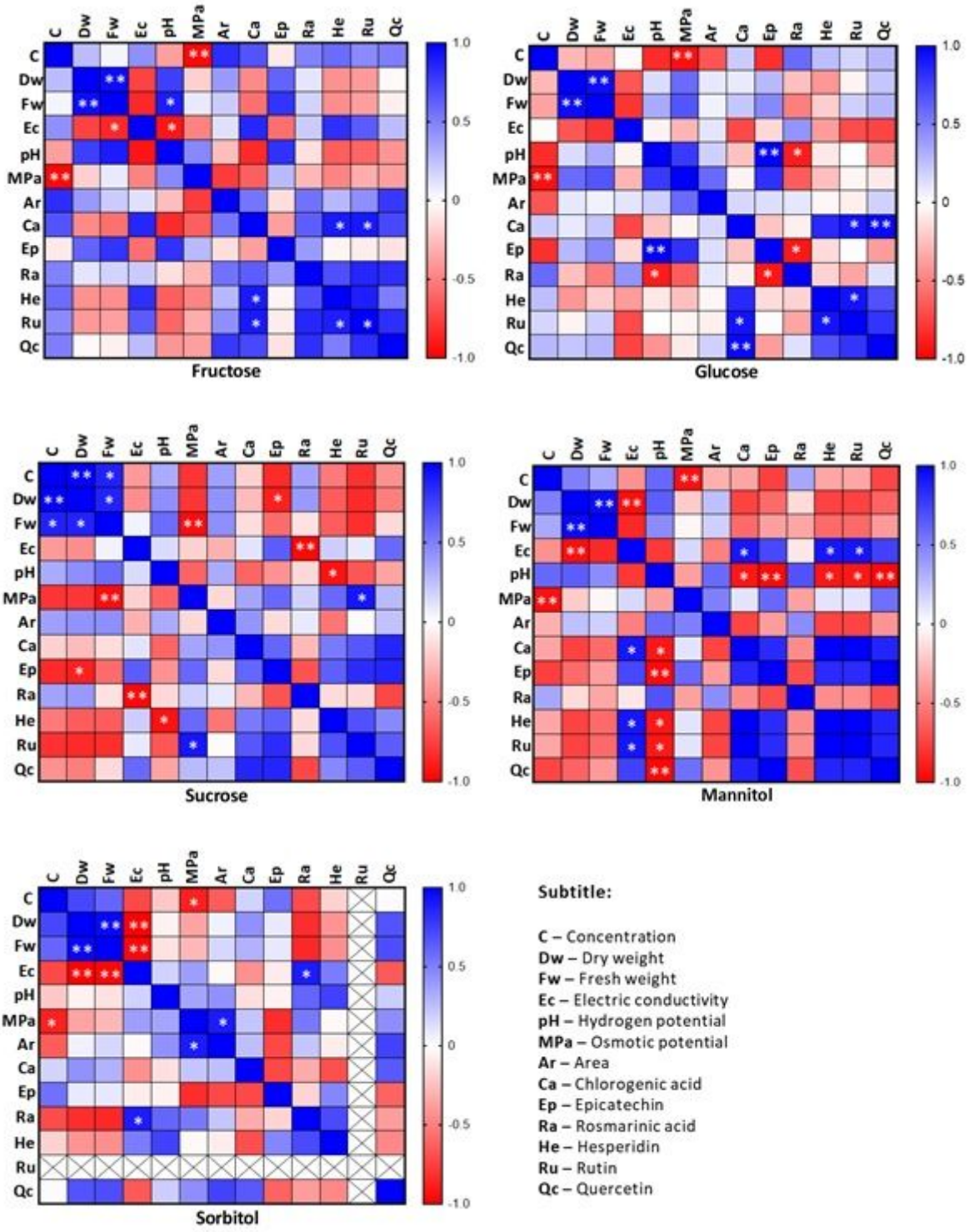

Figure 11

Estimates of heatmap correlations according to Pearson's coefficient for multiple variables (dry weight; fresh weight; pH; electrical conductivity; osmotic potential; cell area; chlorogenic acid, epicatechin, rosmarinic acid, hesperidin, rutin, and quercetin concentrations) after 30 days of H. speciosa (Gomes) cell culture in different carbon sources. ** and * are significant at 1 and 5% by the t-test, respectively. Significance: * ($p \leq 0.05$), ** ($p \leq 0.01$).