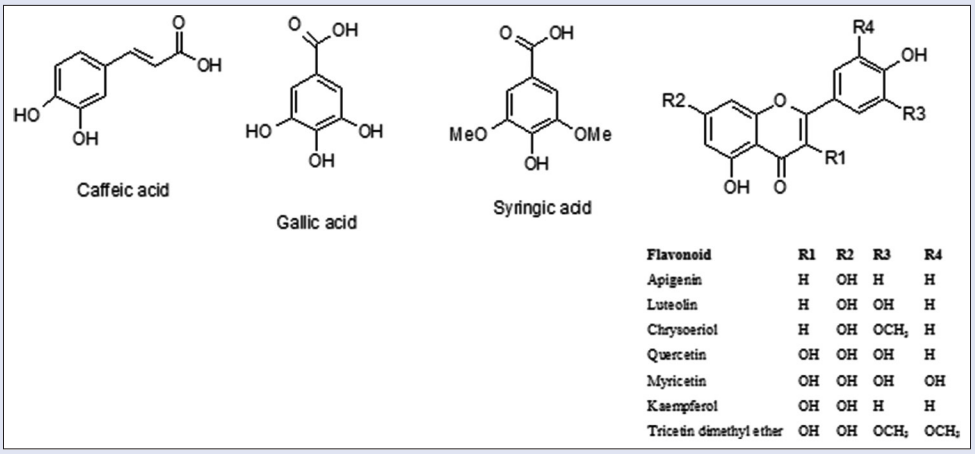


**Figure 1:** Chemical structure of some fragments present in Egyptian carob pods extract

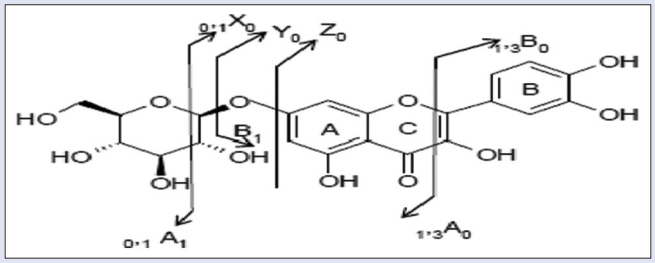

**Figure 2:** Fragmentation of quercetin-*O*-hexoside

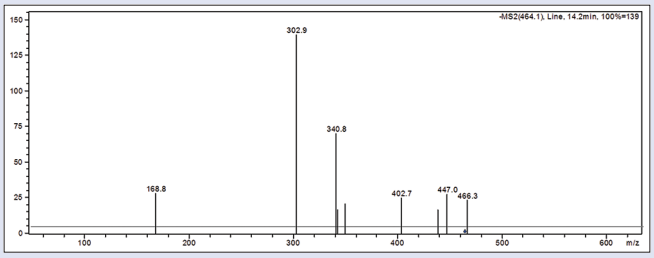


**Figure 3:** Fragmentation of quercetin-*O*-hexoside (peak 14) in negative mode

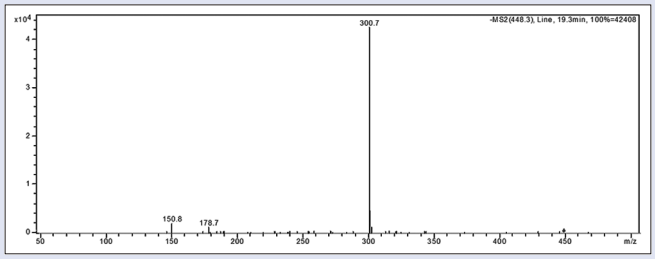


**Figure 4:** Fragmentation of quercetin-*O*-deoxyhexoside (peak 23) in negative mode

nomenclature is proposed by Domon and Castello for glycoconjugates to denote major fragments: $^{k,l}X_j$, $Y_j$, $Z_j$ represent the ions still containing the aglycone, where j is the number of inter glycosidic bond broken (counted from the aglycon), and k and l denote the cleavage within the carbohydrate rings.[7,24-27] Fragmentations of quercetin-*O*-hexoside and quercetin-*O*-deoxyhexoside identifi d in plant extract are shown in Figures 3 and 4, respectively.

For flavonoid aglycone and its glycosides in the negative mode, the spectra showed both the deprotonated molecule $[M - H]^-$ of the glycoside and the ion corresponding to the deprotonated aglycone $[A - H]^-$. The latter ion was formed by loss of the pentose (132 units), hexose (164 units), or deoxyhexose (146 units) moieties from the glycosides. The presence of deprotonated aglycone $[A - H]^-$ at m/z 301 demonstrated the presence of the quercetin, while m/z 269 represented apigenin, m/z 317 indicated myricetin, and m/z 285 demonstrated luteolin and kaempferol. For flavonoid aglycone and its glycosides in the positive mode, the spectra showed both the protonated molecule $[M + H]^+$ of the glycoside and the ion corresponding to the protonated aglycone $[A + H]^+$. The presence of protonated aglycone $[A + H]^-$ at m/z 273 demonstrated the presence of the naringenin, m/z 301 represented chrysoeriol, and m/z 331 indicated tricetin dimethyl ether.[3-5,13-19]

### *Structure characterization of phenolic acids by tandem mass spectrometry*

In hydrolysable tannins, hydroxyphenolic acids such as gallic acid can be esterifi d by D-glucose, yielding gallotannins. Thus, anion of gallic acid (m/z 169) was often found in MS analyses of tannins. Loss of (m/z 152) indicated the presence of galloyl moiety. Moreover, the presence of syringic acid derivatives was indicated by the presence of (m/z 198).[3,5,15,11,12]

## Standard-free relative quantification by peak area of liquid chromatography and mass spectrometry

The use of LC/MS allowed the identifi ation and quantifi ation of 36 compounds in the Egyptian carob pods methanolic extract. Twenty-six compounds were identifi d in the negative mode corresponding to 85.4% of plant dry weight [Table 1], while ten compounds were identifi d in the positive mode representing 16.1% of plant dry weight [Table 2], with the prevalence of flavonoids (75.4% of plant dry weight) predominantly represented by two methylapigenin-*O*-pentoside isomers (20.9 and 13.7% of plant dry weight) and luteolin-*O*-hexoside (10.6% of plant dry weight). On the other hand, phenolic acids (17.3% of plant dry weight) were present in various forms mainly syringic acid derivative

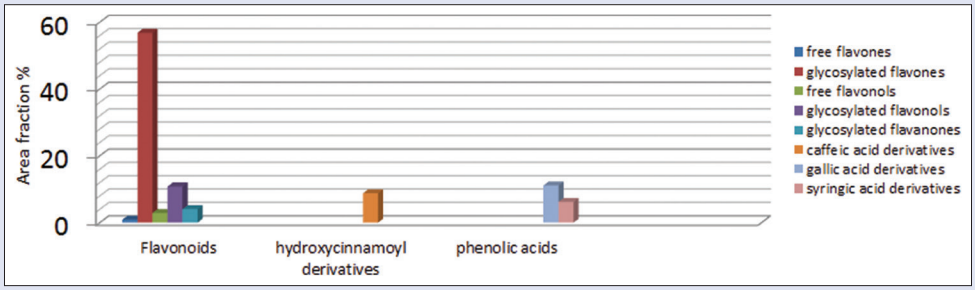


**Figure 5:** Graphical representation of major constituents in carob pods methanolic extract

and trigalloyl hexose (representing 6.2 and 3.3% of plant dry weight, respectively). Two hydroxycinnamoyl derivatives were detected in relatively appreciable amount (8.8% of plant dry weight) and identifi d as hexosylcaffeic acid derivatives (7.9 and 0.9% of plant dry weight). Figure 5 illustrates a graphical representation of major constituents in carob pods methanolic extract.

## CONCLUSION

The present data shows that LC/ESI-MS/MS$^n$ is a useful tool for characterization and quantifi ation of different phenolic compounds present in Egyptian carob pods, which is performed for the fi st time. The results of this study clarifies that carob pods not only has a high content of phenolic antioxidants, comparable to other Mediterranean foods such as olives[3] but also contains a rich variety of individual components from several classes: flavonoids including; free flavones (0.9% of plant dry weight), glycosylated flavones (56.8% of plant dry weight), free flavonols (2.9% of plant dry weight), glycosylated flavonols (10.8% of plant dry weight), and glycosylated flavanones (4.0% of plant dry weight); in addition to the presence of hydroxycinnamoyl derivatives (caffeic acid derivatives; 8.8% of plant dry weight) and phenolic acid derivatives (gallic acid derivatives; 11.1% of plant dry weight and syringic acid derivatives; 6.2% of plant dry weight).

To date, carob consumption has been based on the pleasant taste of the drink, especially in the Mediterranean region. The identifi ation of various compounds present in carob pods opens a new door to an increased understanding of the different health benefits brought about by the consumption of popular carob and its products.

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### Conflicts of interest

There are no confli ts of interest.

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