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Full Length Research Paper

Optimization study for the extraction of phenolics-rich silymarin from *Silbum marianum*

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Crude extract of milk thistle (*Silbum marianum*) seeds have been used for centuries as an herbal treatment for liver diseases. The seeds of milk thistle are usually pretreated with organic solvent to defat the seed prior to flavonolignan extraction. The current work aims to obtain maximum yield of phenolics-rich silymarin extract. The tested parameters were seed defatting, temperature, extraction time, solvent type and solvent concentration. The used solvents were water, ethanol, methanol, acetone, acetonitrile and ethylacetate. Comparison of all experiments under the chosen conditions showed that extraction for 3 h of defatted seeds with ethanol (95%) at 50°C proved to have the highest yield of phenolics-rich silymarin extract. With all solvents except water, deffatting leads to higher phenolics-yield. In addition, when using the whole seeds the maximum phenolics yield was obtained at the solvent boiling point.

Key words: Polyphenol, solvent concentration, herbal medicine, ethanol, methanol.

INTRODUCTION

Herbal medicines have provided humans with cures for thousands of years. Nowadays, it is considered one of the main interests of scientific research due to their remarkable therapeutic effects. The majority of them are flowering plants of more than 80 families (Lovkova et al., 2001). The milk thistle is one of those flowering plants of the daisy family [Asteraceae] (Hogan et al., 2007), its seed extract is composed mainly of silymarin [ca.75%] and lipid [ca.25%] (Grenlee et al., 2007; Kroll et al., 2007).

Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent (Kroll, 2007). From a medical point of view, there is a correlation between its significant biological activity and the possible therapy. Several clinical studies have reported its significant effect on the liver function improvement. In addition to its efficacy against liver cirrhosis and chronic hepatitis (liver inflammation), it has been proven to greatly improve, toxin-induced liver damage including severe liver damage from fumonisin B1 and *Amanita phalloides* ('death cap' mushroom poisoning), and gall-bladder disorders (Huseini et al., 2006; Gordon et al., 2006; Greenlee et al., 2007; Gazak et al., 2000; Kroll et al., 2007; Rainone, 2007; Tamayo and Diamond, 2007; El-Adawi et al., 2011).

The extraction of phenolic compounds from plant material is influenced by their chemical structure, the method of extraction, the size of the particles forming the sample, the time and the conditions of storage as well as the presence of interferents (Naczka and Shahidi, 2004). Definitely, the solvent is one of many parameters that could affect the extraction of polyphenols (Troszyńska et al., 2002). The extraction may be carried out by several solvents such as water, methanol, ethanol and acetone. However, aqueous solvents give better yields of extraction than absolute solvents (Spignon et al., 2007).

To evaluate the impact of solvent polarity on the solubility of phenolics from whole or defatted seeds, a

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systematic extraction approach using five commonly used solvents (ethanol, methanol, ethyl acetate, aeceto-nitrile and acetone), three different ratios of solvent to water (95:5, 70:30 and 50:50 v/v), different temperatures and extraction duration time (3 and 5 h) were performed.

MATERIALS AND METHODS

Sampling

Milk thistle was purchased from Mobaco-Co., Egypt. The samples were ground in grinding mill (Retsh Mill) and sieved to produce samples with particle size of less than 150 µm. The raw herb powder of particle size less than 150 µm was sealed and stored at -18°C for further usage.

Chemicals

All the solvents used for extraction were analytical grade (purity > 99%, Sigma Aldrich, Italy).

Pre-extraction sample preparation

In order to get rid of the fats of the sample, 40 g of crushed seeds were soaked in 300 ml of hexane overnight.

Apparatus and instruments

The conventional Soxhlet extraction apparatus was used, consisting of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was an *Advantec* thimble with 22 mm internal diameter and 90 mm external length.

Soxhlet extraction

The time periods were chosen to be 3 and 5 h as for the Soxhlet extraction experiments. Three grams of dried and ground Milk thistle were placed in a Soxhlet apparatus and extracted with 350 ml of an appropriate solvent.

Determination of total phenolic contents (TPC)

The classic technique employed in phenol analysis is the 4-aminoantipyrine colorimetric procedure (Ettinger et al., 1951). The absorbance of the samples was read against blank at 500 nm using spectrophotometer (PerkinElmer Lambda EZ 201, USA). The concentration of the sample was calculated from the standard curve prepared previously.

Statistical analysis

Statistical analysis of data was represented by the mean of triplicate groups± standard deviation.

RESULTS

The TPC of the extract was assumed to be representative of the yield of phenolic-rich silymarin extract.

Ethanol extraction

Defatted seeds

The maximum TPC (77.3%±0.02) was obtained at 95% ethanol concentration, 50°C and 3 h extraction period (Figure 1A). Generally, the TPC went down as the temperature elevated towards the ethanol boiling point (79°C).

Whole seeds

The highest TPC (47.8%±0.02) was recorded at 95% ethanol concentration at the boiling point of ethanol and 5 h extraction period (Figure 1B).

Methanol extraction

Defatted seeds

It can be seen from Figure 2A, that the maximum TPC (55.4%±0.04) was observed at 95% methanol concentration, 50°C, and 3 h extraction duration time (Figure 2A). As long as we elevated the temperature towards the boiling point of methanol (65°C), the lower TPC was gotten.

Whole seeds

The result showed that, yield of TPC was better (40.3%±0.03) when the methanol concentration was 95% at the boiling point and 5 h extraction period (Figure 2B).

Acetonitrile extraction

Defatted seed

As shown in Figure 3A, the maximum TPC (41.1%±0.02) was achieved at 95% solvent concentration and 60°C for 3 h.

Whole seeds

Among all temperatures, the boiling point of acetonitrile (82°C) was the best for getting maximum TPC (29.4%±0.02) at 95% concentration and 5 h extraction period (Figure 3B).

Ethyl acetate extraction

Defatted seeds

Maximum TPC (29.8%±0.01) was obtained at 60°C, 95% solvent concentration and 3 h extraction duration time

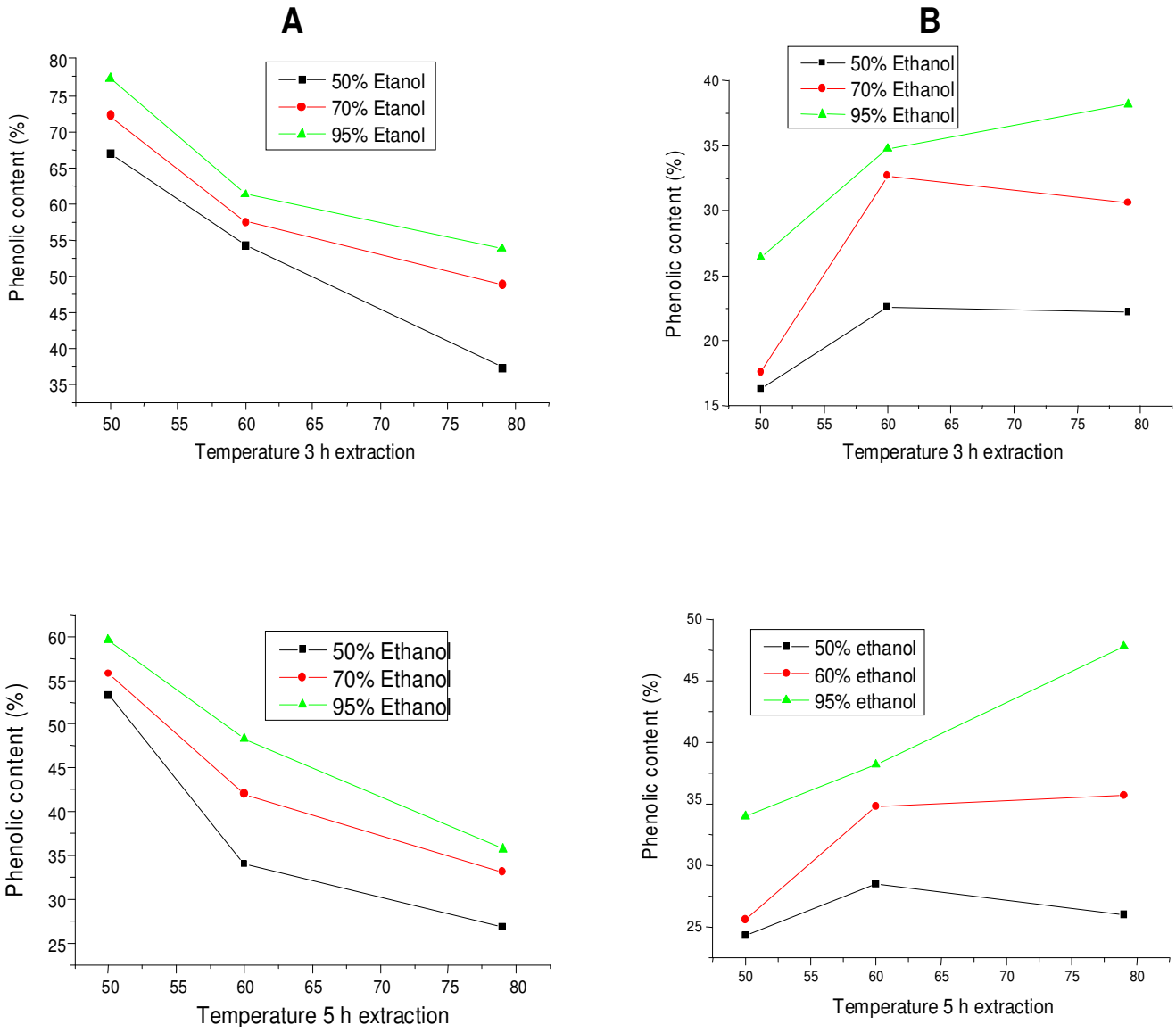

Figure 1. Total phenolic contents% (TPC%), extracted with ethanol at different concentrations. (A) Defatted seeds at different time of extraction period; (B) Whole seeds at different time of extraction period.

(Figure 4A).

Whole seeds

The ethyl acetate has the same trend as the previous solvent, where the maximum TPC (17.2%±0.04) was obtained at the boiling point (77°C), 95% solvent concentration and 5 h extraction duration time (Figure 4B).

Acetone extraction

For both defatted seeds and whole seeds, the optimum conditions for maximum TPC were the boiling point of acetone (56°C) and 95% solvent concentration. The TPC records were 64.7%±0.03 for defatted seeds at 3 h extraction period and 39.0%±0.01 for whole seeds at 5 h extraction period (Figure 5A and B).

Water extraction

As shown in Figure 6A and B, the maximum TPC was 32.2%±0.04 for defatted seeds and 39.9%±0.03 for whole seeds at the boiling point of water and 3 h extraction time.

DISCUSSION

Results of the present study shows that, in the case of

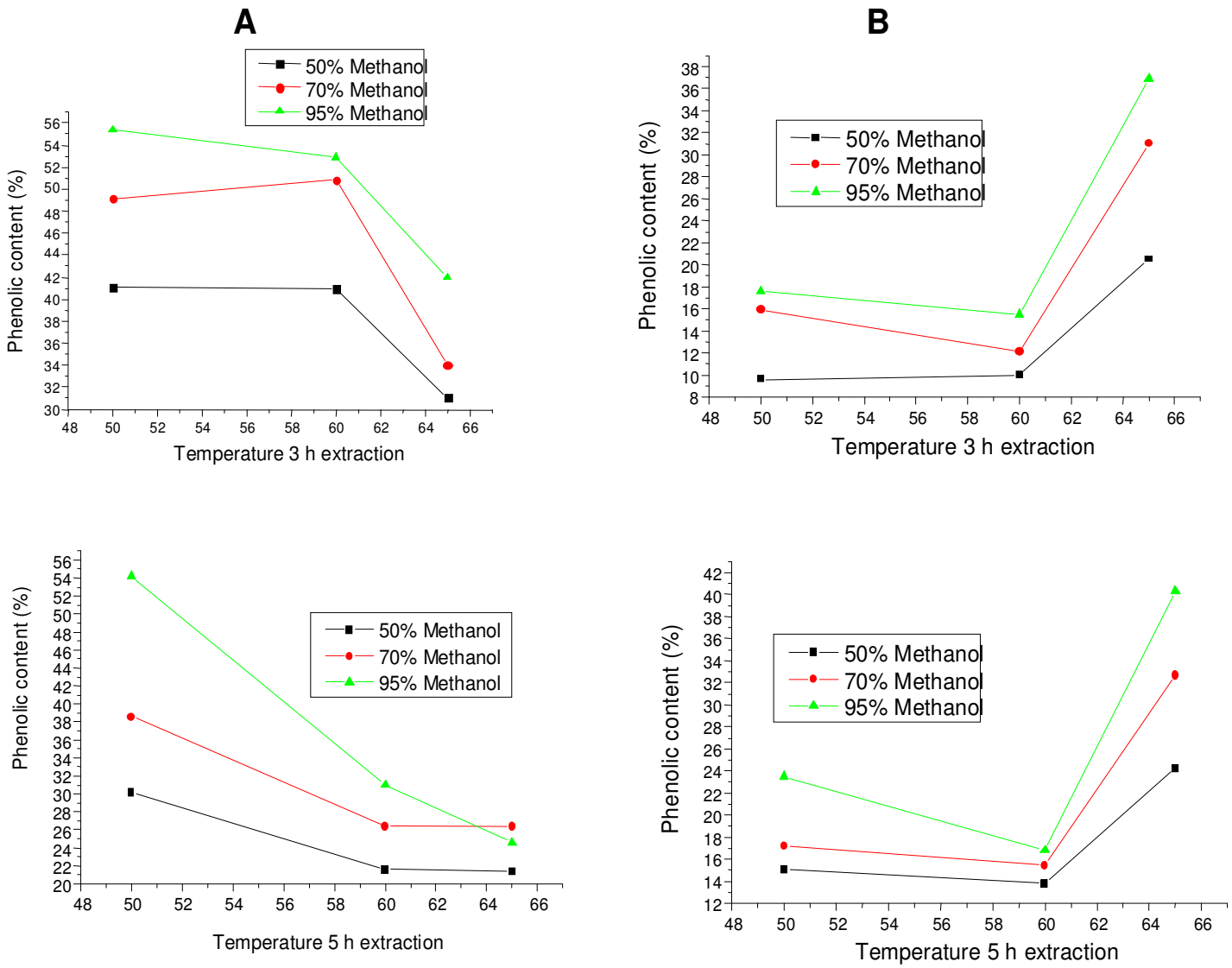

Figure 2. Total phenolic contents% (TPC%), extracted with methanol at different concentrations. (A) Defatted seeds at different time of extraction period; (B) Whole seeds at different time of extraction period.

defatted seeds, among all solvent extracts, aqueous (95%) ethanol extract offered the highest TPC (77.3%±0.02 at 50°C for 3 h), followed by aqueous acetone extract (64.7%±0.03 TPC at 50°C for 3 h) while moderate TPC were recorded for methanol (55.4%±0.04 at 50°C for 3 h) and acetonitrile (41.1%±0.02 at 60°C for 3 h). However, only 29.8%±0.01 of TPC was recovered when ethyl acetate was used at the same concentration (95% at 60°C at 3 h). Our results agreed with Wallace et al. (2003), where the extraction with ethanol resulted in the highest TPC in comparison with methanol, acetonitrile, and acetone as the solvents.

In the case of whole seeds, the highest TPC (47.8%±0.02) was recorded for aqueous ethanol extract followed by methanol extract (40.3%±0.03), acetone (39.9%±0.01), acetonitrile (29.4%±0.02) and finally the ethyl acetate extract (17.2%±0.04). This yield of TPC was obtained at the same concentration of all solvents (95%) and at the boiling point of each solvent for 5 h extraction time. These results confirm the previous work by Wallace et al. (2005) as it reflects the importance of lipid removal (defatting) step since the solubility of phenolics in different solvents is hindered by the presence of fat.

Duan et al. (2004) were the first group that used the hot water as an extraction solvent for milk thistle. In the present study, the yield of TPC increased with increasing water temperature. However, the boiling point of water proved to be an efficient extraction temperature for both whole and defatted seeds. Extraction of whole seeds with boiling water returned maximum yields of 39.9%±0.03.

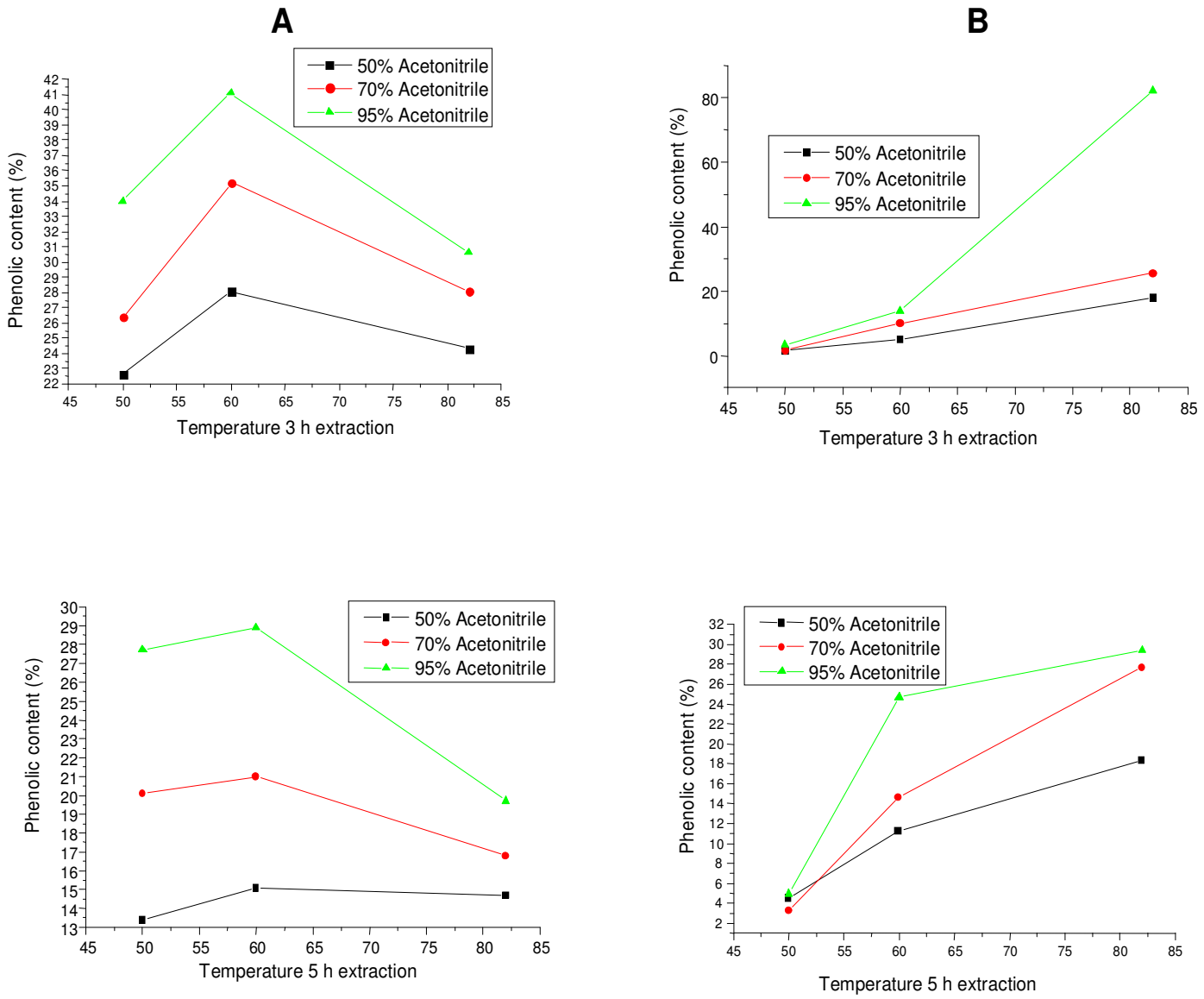

Figure 3. Total phenolic contents% (TPC%), extracted with acetonitrile at different concentrations. (A) Defatted seeds at different time of extraction period. (B) Whole seeds at different time of extraction period.

Conclusion

The structural diversity of phenolic compounds presents a significant challenge for developing a uniform methodology that is suitable for extraction of all phenolics or a specific class of phenolic compounds. The issue of developing a satisfactory extraction procedure is further complicated as phenolics can be found in free, conjugated and polymeric forms or may coexist as complexes with carbohydrate, protein or other plant components. All of the aforementioned factors directly impact the solubility of phenolics in different solvents.

In the case of defatted seeds, for all solvents, moderate extraction temperature (50 to 60°C) resulted in a significant increase in TPC yield except for the acetone solvent, which gave the highest yield at boiling point. On the other hand, water gave the highest yield at boiling point with the whole seed. In addition, when using the whole seeds, the maximum TPC was obtained at the solvent boiling point. The current study shows that the yield of extracted phenol is influenced by the concentration of solvent in water, where 95% concentration was the optimum concentration for all solvents. Extraction period of 3 h resulted in the best TPC for both seeds (whole and defatted seeds). Longer extraction time decreased the TPC extracted, possibly because of some loss of phenolic compounds via oxidation and these products might polymerize into insoluble compounds.

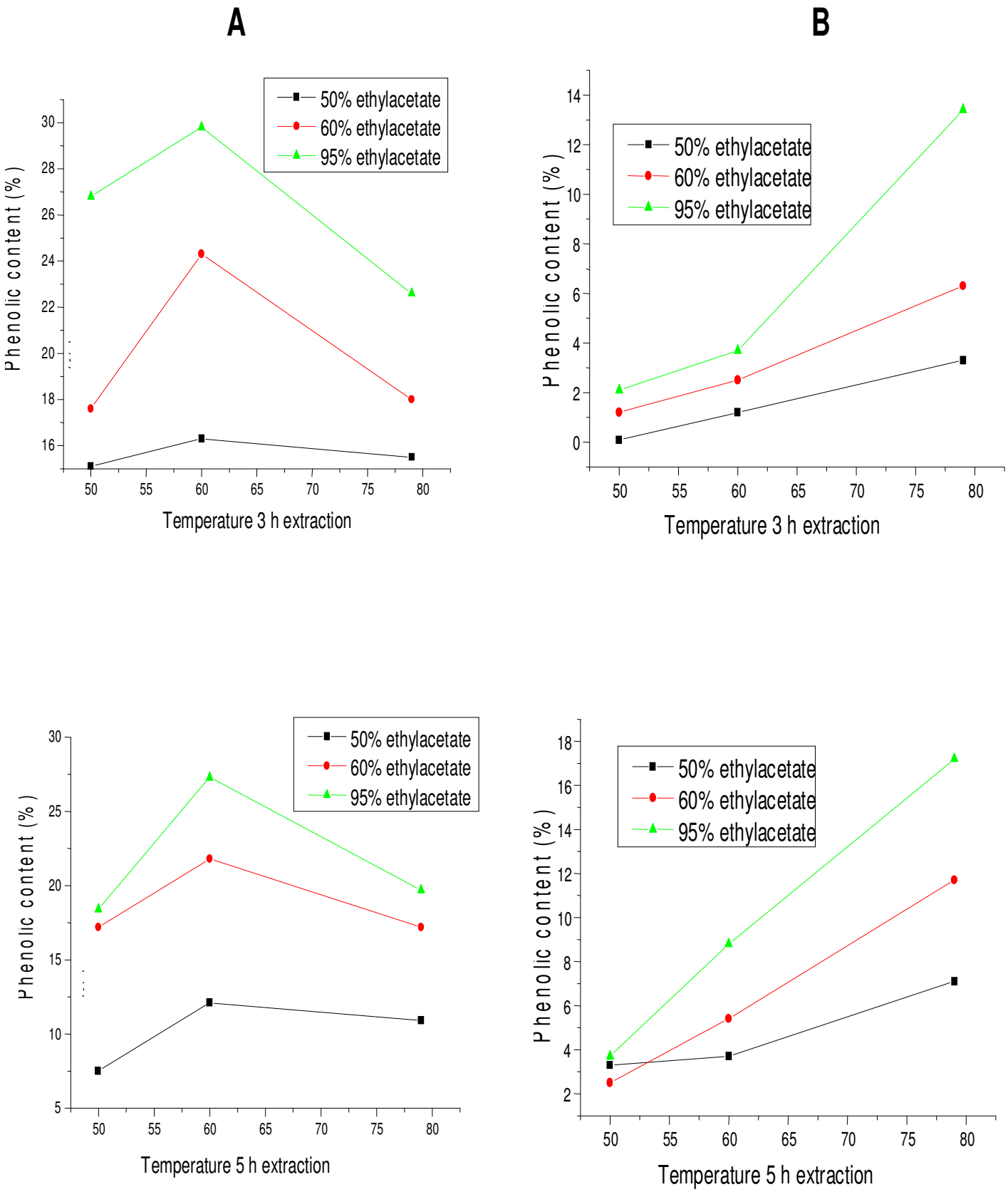

Figure 4. Total phenolic contents% (TPC%), extracted with ethyl acetate at different concentrations. (A) Defatted seeds at different time of extraction period; (B) Whole seeds at different time of extraction period.

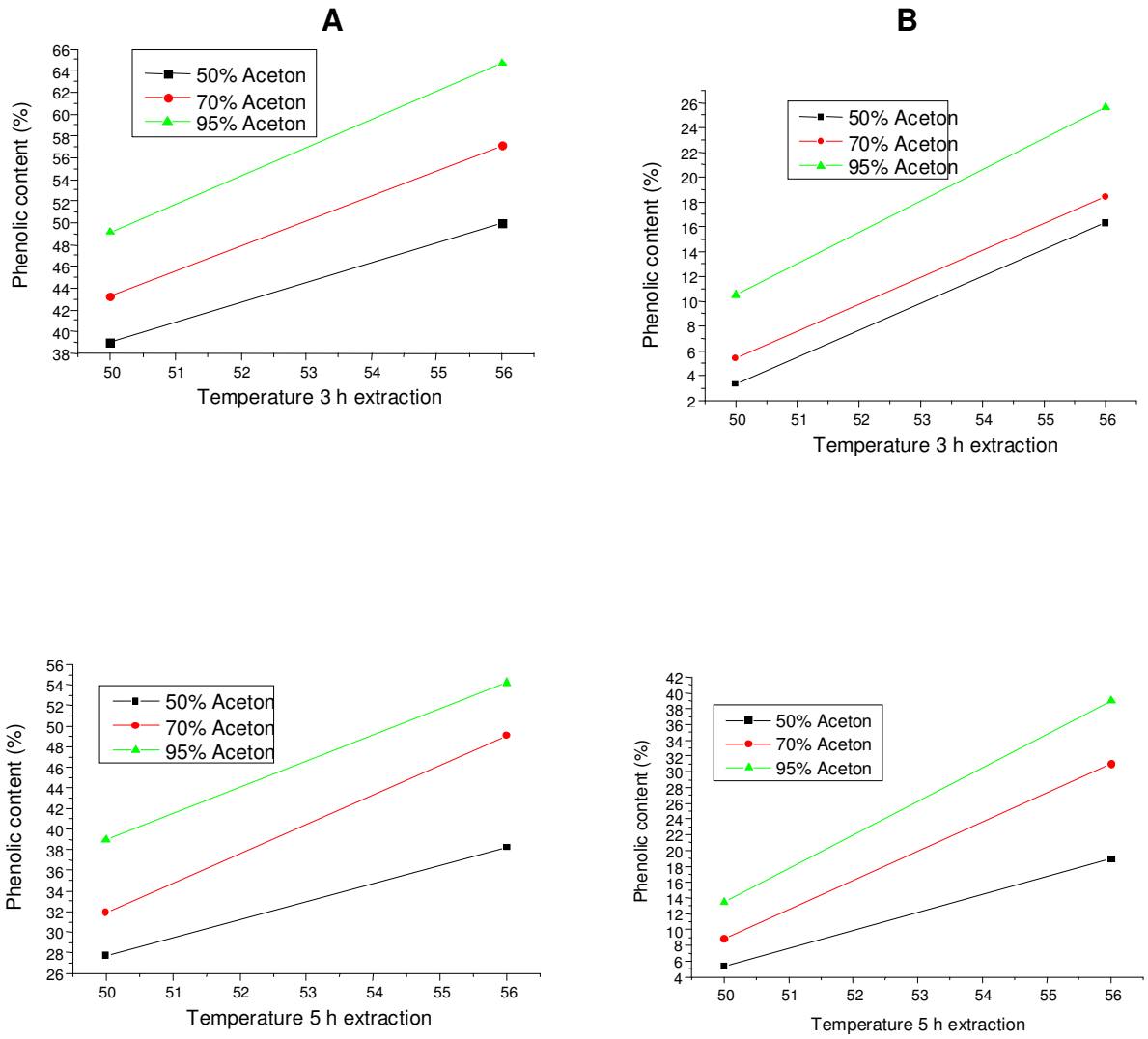

Figure 5. Total phenolic contents% (TPC%), extracted with acetone at different concentrations. (A) Defatted seeds at different time of extraction period. (B) Whole seeds at different time of extraction period.

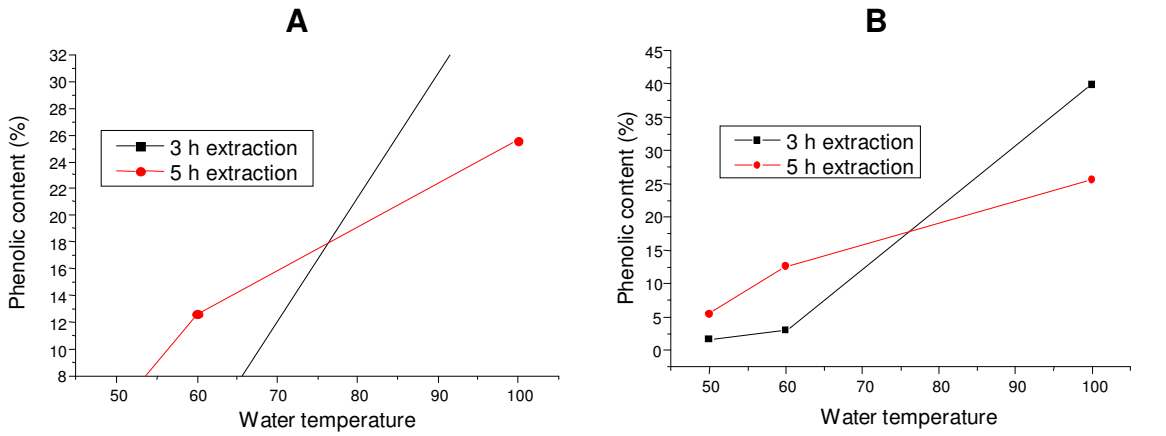

Figure 6. Total phenolic contents% (TPC%), extracted with water. (A) Defatted seeds at different time of extraction period; (B) Whole seeds at different time of extraction period.

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