The bark of maritime pine (Pinus pinaster Aiton, also called Pinus maritima) has been widely used as an herbal remedy for various degenerative diseases, and is predominantly used for its antioxidant activity. A standardized extract that complies with the U.S. Pharmacopeia (USP) monograph is derived from the outer bark of P. pinaster (Pycnogenol®). The Pycnogenol extract (PE) consists of approximately 65–75% procyanidins made up of catechin and epicatechin subunits of varying chain lengths. Other constituents of the PE include polyphenolic monomers, phenolic acids, or cinnamic acids, and their glycosides. Research shows that the components of PE are highly bioavailable. Furthermore, one of the important characteristics of PE that has emerged in studies is that the complete mixture of the extract demonstrates greater biological effects than a similar amount of any of its individually purified components [1].

This study aims to find out the optimum conditions for the determination of pycnogenol and some components of proanthocyanidins. It has been used five different types of pine barks which are growing in Turkey. Pinus brutia, Pinus halapensi, Pinus nigra, Pinus pine, Pinus sylvestris Pine Barks were assured from Bursa Orman Müdürlüğü, Adana Orman Müdürlüğü and Manisa. Pine bark extract was obtained using the method developed by Masquelier (1987). A quantity of 10.0 g of pine bark was further ground for 1 min, at a speed set 100.0 mL of boiling water and cooled down to 20°C. Liquid was collected after filtration and then sodium chloride (Merck) was added up to saturation and the precipitate formed was removed by vacuum filtration. Subsequently, the filtrate was extracted three times with ethylacetate (Merck) (10.0 mL filtrate/1.0 mL ethylacetate (v/v)). The ethyl acetate phase was collected and dried using anhydrous sodium sulphate (Panreac) and reduced to 1/5 of its volume [2]. The extract was enpoured into three volumes of chloroform (Merck), under mechanical stirring. The proanthocyanidins were precipitated and collected by vacuum filtration. The light-beige-color powder extract obtained was dissolved in water and methanol and injected to HPLC.

Perkin Elmer 200 HPLC and Pekin Elmer UV-VIS detector was used for determination of Pycnogenol. 50 µL sample injected for each analysis.

Standard addition calibration method was applied for the determination of pycnogenol content in samples. Analyses were performed at 285 nm with a flow rate of 1.0 mL/min. Separation was achieved on a ODS-3 column (5 µm 4.6x250 mm) applying gradient elution starting with water and acetonitrile. Catechin, epicatechin, taxifoline, gallic acid, caffeic acid, ferulic acid, vanillic acid, epigallocatechin gallate were separated successfully from each other.

KEYWORDS: pine bark, pycnogenol, extraction, HPLC

REFERENCES