Algerian Journal of Natural Products 7:1 **(2019)** 645-650

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Extraction and Thermogravimetric Characterization of Lignin Phenolic Polymers from Date Seeds by Mild Alkaline Solutions

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Received: 27/02/2019 /Accepted: 27/07/2019

Abstract: Date seeds are abundant lignocellulosic waste materials contain lignin phenolic polymers that act as antioxidants in food, cosmetic and pharmaceutical industries. Lignin have been extracted by several methods including alkaline medium. In the present work, optimum alkaline conditions for extraction of and de-polymerization of lignin from date seeds into low molecular weight phenolic compounds were studied. Date seeds surface were characterized before and after extraction by FTIR and thermal analysis (TGA and DTG).Total phenolic content, TPC and antioxidant capacity of extracts were evaluated spectrophotometrically. Maximum TPC was 124±8.78 µg GAE/mL extract and optimum antioxidant capacity was determined as DPPH free radical inhibition (I= 76.56%). The optimum values of TPC and DPPH inhibition were attained at 0.005- 0.010M Na_2CO_3.

Keywords: *extraction, antioxidant, DPPH, total phenolic, date seeds, TGA*

I. Introduction

Date seed, DS of fruit of Phoenix dactylifera L. is an abundant by-product material (about 7 million tones) and it is popular in the Middle East. DS are lignocellulosic compounds that composed of 23%, 20 and 55% of lignin, α cellulose and hemicellulose, respectively [1] and it also contains antioxidants and phenolic compounds [2]. Phenolic compounds exist as free or bind with cell wall component so their extraction depends on matrix components in plant [3].

Several workers have reported antioxidant capacity and total Phenolic content of several varieties of DS from different countries after extractions by methanol, ethanol, water, acetone, formic and dimethyl sulfoxide [4-8]. Alkaline treatment of biomass cause of solubility of hemicellulose and lignin parts [9].

Lignin is a biopolymer of polyphenolic contain p-coumaryl, coniferyl, and sinapyl alcohol, which linked together through carbon-carbon or ether bonds. Lignin have been characterized by Fourier Transform infrared, FTIR and thermogravimetric, TGA analysis [10-11]. Lignin's phenolic compounds were known as natural antioxidant compounds [12] to scavenge free radicals and protect humans from oxidation damage. They have been used in food, cosmetic and pharmacetical industries [13].The antioxidant potential of lignin depends on the type of lignocellulose material and extraction solvents [14]. Organic, aqueous acidic or alkaline solvents have accomplished for extraction of lignin. Acidic extraction was reported as less effective to bound phenolic compounds and alkaline extraction was recommended [15,16]. Alkaline extraction of lignin from cell wall (delignification) is as a result of hydrolysis of both ester and ether bonds of lignin and hemicellulose and converting into high amount of lower molecular mass of phenolic compound [17, 18]. Delignification of lignocellulose in alkaline medium have been reported by several investigators [19-23] Alkaline extraction were carried out by hydroxide ion or carbonate ion however strongly alkaline solution may lead to decrease of lignin extraction [24] and lignin extracted in carbonate more than in hydroxide ion [17]. Some researchers reported that phenolic compounds might be converted into non-oxidative quinones in strong alkaline medium [24-25]. The ability of extracted phenolic compounds as free radical scavenger has been characterized using diphenyl picrylhydrazyl (DPPH) method

This work was aimed to study the effect of mild alkaline carbonate concentration on extraction of antioxidant and total phenolic content of extracted date stone as well as characterization of DS surface after extraction by analyzing residual solid DS by FTIR and TGA methods. Antioxidant capacity was deduced by diphenylpicrylhydrazyl (DPPH) radical scavenging method however, total phenolic content, TPC was determined by Folin–Ciocalteu reagent.

II. **Experimental Section**

II.1. Materials

Date stone, DS was separated from date fruits (Sokary type),washed with distilled water, grounded and sieved to 814micron.Sodium carbonate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin–Ciocalteu reagent and methanol were obtained from Sigma-Aldrich or chemical pure company.

II.2. Extraction and characterization method

Six different concentrations of Na_2CO_3 solutions ($1x10^{-4}$-0.05M) were prepared and shaken with 5 gram of DS for four hours in water bath shaker (JSSB-30T) at 150 rpm then filtered. The modified stones were characterized by Fourier Transforms infrared (FTIR-8400S SHIMADZU) and thermogravimetric (SDT 600). Filtrates were analyzed for antioxidant potential by DPPH radical scavenging and total phenolic content (TPC) methods.

II.3. Free Radical Scavenging Method

DPPH radical scavenging assay was utilized for determination of antioxidant potential by following the inhibition of strong absorption band at 517 nm by UV-Visible spectrophotometer (Thermo Scientific™ GENESYS™ 10S)[26]. 2mL of DPPH (0.2mM in methanol) was added to 0.1 ml of each DS extract solution and incubated in dark for 15 min. The absorbance was recorded at 517nm and the percentage of inhibition of DPPH radical activity, % I was calculated according to the following equation:

$$\%I = \frac{(A_o - A_1)x100}{A_o} \quad (1)$$

Where A_o is absorbance of control, A_1 is the absorbance of control plus extract.

II.4. Measurement of the total phenolic contents

The amount of total phenolic compounds, TPC of CDS extracts were determined using Folin-Ciocalteu reagent, FC with respect to gallic acid standard [27]. FC reagent react with TPC in alkaline medium to give blue color that proportional with the amount of TPC. In 10 mL volumetric flask 2mLof extract, 2.5 mL of diluted FC reagent (10%) and 2mL of Na_2CO_3 were mixed were kept in water bath at 50ºC for 5minutes. The mixture was analyzed spectrophotometrically at 760nm and TFC was calculated as gallic acid equivalent, GAE by comparing with standard curve of gallic acid.

III. Results and Discussion

DS was extracted with variable concentration of Na_2CO_3 solutions and the remaining solid DS were characterized with and TGA.

III.1. FTIR characterization

FTIR spectrum of raw DS and CDS (Fig.1) show characteristic bands of lignin aromatic skeletal 1600, 1491, 1525, and 1446 cm^{-1} [28-31] and ether bonds 1246 and 1033 cm^{-1}. In CDS, bands have lower intensity which indicates the extraction of lignin.

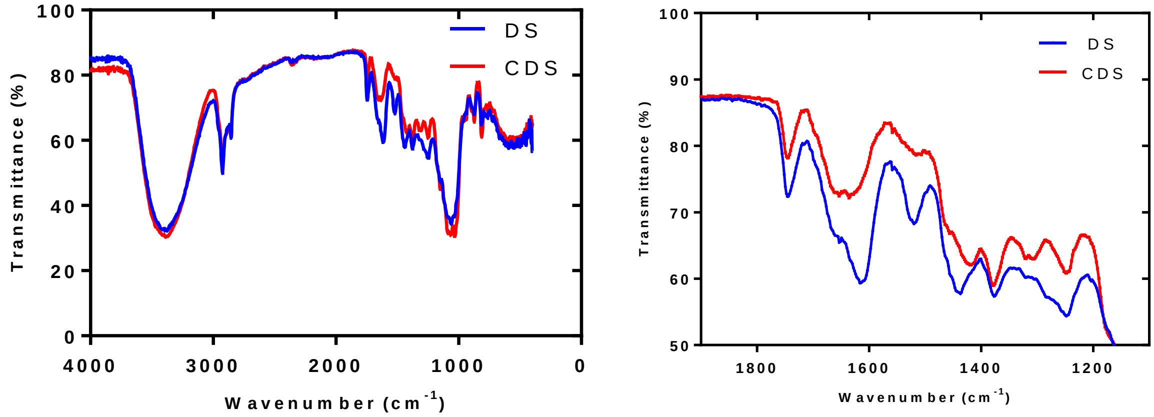

Figure 1. *IR characterization of DS and CDS*

III.2. TGA characterization

Thermogravimetric analysis for raw and modified of DS or carbonate modified DS (CDS) were performed at a heating rate of 10 °C per minute. The dose of around 2.5- 4.70 mg of each were heated at 25 up to 900 °C

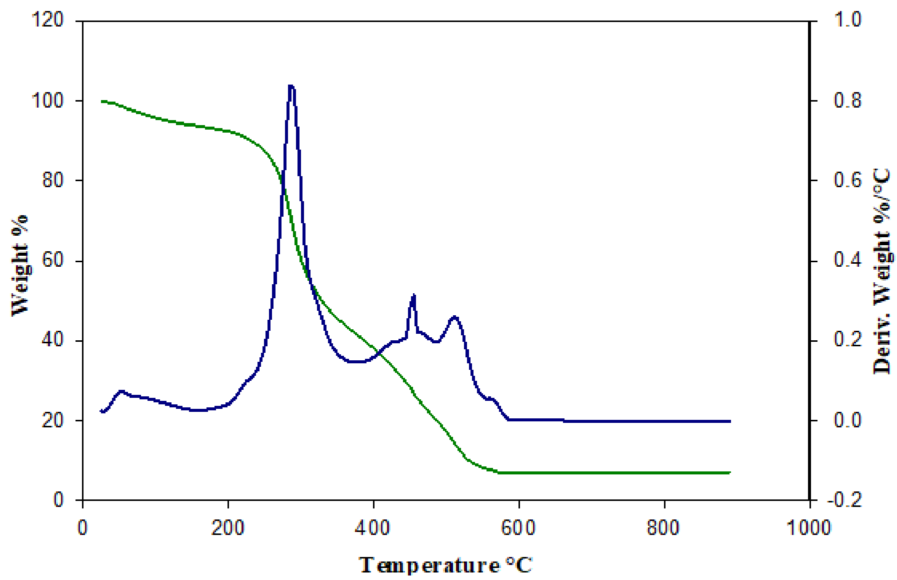

Fig.2. *TGA and DTG analysis of raw DS*

In Fig,2 there are three characteristic TGA&DTG peaks of untreated DS at 275,465 and 505ºC representing hemicellulose, cellulose and lignin peaks, respectively. Delaying of degradation of lignin is due to due to cleavage of interunit linkage of polymer structure producing phenolic monomers then decomposing of aromatic ring of lignin above 500 ºC [32]. The initial mass loss which started at 50ºC correspond to water and volatile components and the second mass loss was started at 200ºC to300ºC corresponding to hemicellulose degradation*. The third mass loss was due to lignin degradation at 490-550 ºC.

In case of CDS samples the position of DTG peaks of lignin (Fig.3) were varied from DS according to stabilization of lignin and re-dissociation again on varying Na_2CO_3 concentration [33].

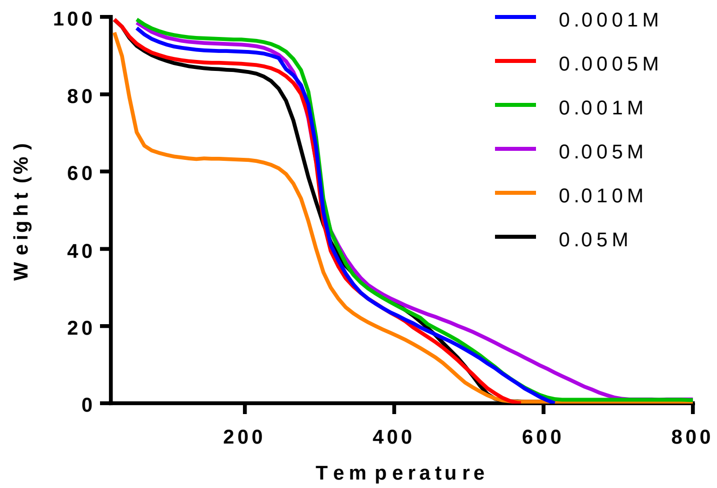

Fig.3 *TGA analysis of CDS at variable concentrations of Na_2CO_3*

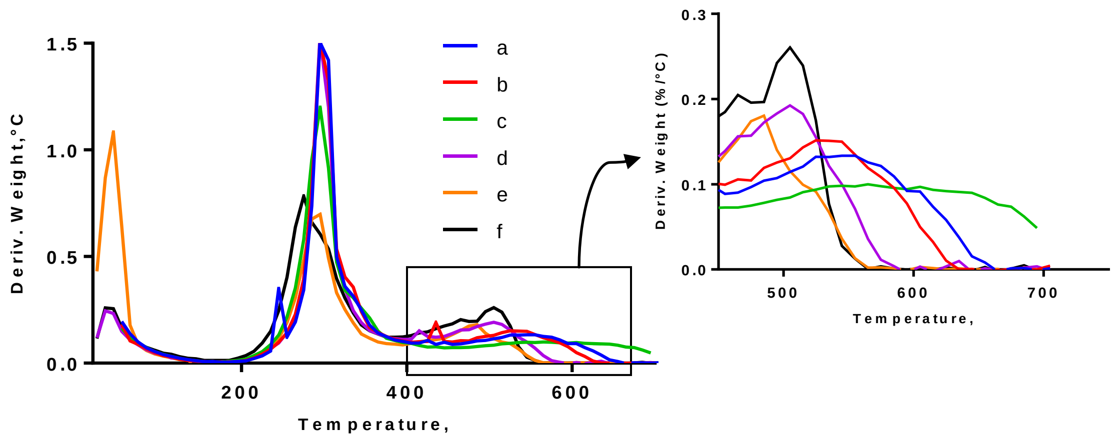

Fig4.*Comparison of DTG of CDS samples at variable Na_2CO_3 concentration*

Increasing alkaline concentration shift DTG band to lower temperature except of Na_2CO_3 0.001M concentration, which indicates the increasing solubility of lignin, by increasing Na_2CO_3 concentrations. Maximum extraction of lignin may be at 0.01M of Na_2CO_3 because it show DTG peak at lowest temperature and lower mass loss in TGA. Utilizing of 0.001M Na_2CO_3 for DS extraction may increase stability of DS's lignin and retard its extraction. This may be due to formation of larger macromolecule [31]

III.3.Antioxidant capacity

Antioxidant capacity of CDS extracts were estimated by DPPH free radical scavenging capacity (table 1)

Table 1. Effect of [Na_2CO_3] on total antioxidant capacities, % I of CDS extracts

[Na_2CO_3],M	**0.0001**	**0.0005**	**0.0010**	**0.0050**	**0.0100**	**0.0500**
%I	5.47	10.45	8.24	76.56	72.91	8.13
± Standard Deviation	1.11	0.64	0.83	0.63	1.03	1.43

Generally, at higher concentration of Na_2CO_3 the antioxidant capacity of CDS extract increase by increasing carbonate concentration lead to hydrolysis of lignocellulosic ester bond, which enhance lignin extraction. Lignin extract are contain phenolic hydroxyl and ortho- methoxy substituted aromatic ring that responsible of lignin antioxidants. In case of utilizing 0.001M of Na_2CO_3 the antioxidant capacity of CDS, extracts slightly decrease. This may be due to formation of other stable lignin type which concordant with DTG results. Largest antioxidant capacities (I≅77-73%) were at 0.005and 0.01M carbonate extracts, which correspond to pH 9.3 and 9.8. However, utilizing 0.05M concentration of Na_2CO_3 lead to diminishing antioxidant capacity from 77% to 9% due to the increase of extraction of hemicellulose, which suppress antioxidant capacity. Hemicellulose are carbohydrates which have ability to linked to phenolic groups by hydrogen bonding and interfere in antioxidant properties [34, 35]

III.4.Total phenolic content

In alkaline medium Lignin are hydrolyzed to monomer lower molecular weight phenolic compounds. Phenolic compounds have been considered as antioxidants by free radical scavenging and metal chelating [36-37]. TPC was determined as µg gallic acid equivalent (GAE) per mL of extract from calibration curve of standard gallic acid which have an equation $y = 0.0091x + 0.0135$ and correlation factor R^2=0.998. Total phenolic content of CDS extract increase linearly with increasing carbonate concentration and reach maximum value at 0.01M thereafter it decrease (table2). Increasing of TPC are due to de-poymarization of lignin and formation of monomer phenolic compounds however, decreasing of TPC at 0.05M Na_2CO_3 are due to re-polymerization of highly reactive phenolic compounds [38, 23].

Table 2. *Total phenolic content of CDS extract at variable concentration of Na_2CO_3*

[Na_2CO_3], M	**0.0001**	**0.0005**	**0.0010**	**0.0050**	**0.0100**	**0.0500**
µg GAE/mL	63.556	50.185	72.222	106.370	124.040	83.889
± Standard Deviation	1.56	1.50	4.12	1.74	8.78	0.44

From above results, it is noticed that the optimum alkaline condition for lignin total antioxidant extraction of DS are Na_2CO_3 concentration are 0.05-0.01M which give lowest temperature of lignin DTG band and highest antioxidant capacity, %I and TPC.

IV. Conclusion

Mild alkaline carbonate are optimum condition for extraction of maximum amount of lignin antioxidants of abundantly by-product date seeds which is important in cosmetic and pharmaceutical industrials.

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