Research Article

Hot Water and Ultrasound-Assisted Extraction of Polysaccharides from *Vernonia amygdalina*

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ABSTRACT

Polysaccharides are one of the most essential biological macromolecules and are widely distributed in nature. Polysaccharides of medicinal plants have been extensively studied and several methods have been developed to extract bioactive polysaccharides from their natural sources. Hot water extraction is the convectional and cheapest method while ultrasound-assisted extraction is currently gaining grounds due to its high extract yield. Only little information is available about *Vernonia amygdalina* polysaccharide and its extraction method. In this study, we aimed to used hot water extraction and ultrasound assisted extraction to extract polysaccharides from *Vernonia amygdalina*. The extract yield of both methods was determined and the physical and chemical properties of the extracted polysacharied were analysed. The results showed that polysaccharide yield with ultrasound extraction was higher (4.19%) than the yield from hot water extraction (3.38%). The extracted polysaccharides were water soluble and tested positive to Fehling reagent. The protein contents of the polysaccharides determined from Coomassie Blue reaction were 1.23% and 1.32% respectively. The extracts were negative to $FeCl_3$ reagents, Carbazole reaction and α-napthol reagents indicating that both polysaccharides were without polyphenols, did not contain uronic acid and are either trioses or tetroses. The results showed that the two extraction methods were suitable for extracting useful polysaccharides from *Vernonia amygdalina.*

Keywords: *Vernonia amygdalina*, Polysaccharides, Ultrasound, Bitter leaf, Extraction, Hot water.

INTRODUCTION

Thanks to the potential applications of natural polysaccharides in the drug and food industry[1,2], research on the extraction of plant's polysaccharides and the associated study of their biological function has become a hot spot and in the last decade, has gained significant interest in the field of biochemistry. As a result, many methods have been developed and applied to extract important polysaccharides from plants[3,4], mushrooms[5,6] and even from microorganisms including endophytes of medicinal plants[1,7,8]. Some of these methods includes treatment with enzymes[9], alkali extraction[10], ultrasound-assisted extraction[11,12], microwave assisted extraction[13,14], pulse electric energy extraction[15], pressurized hot water extraction[16] and hot water extraction and alcohol precipitation. While some of these methods such as the acid-base extraction method, are notable for being suitable to extract specific types of polysaccharides such as acidic polysaccharides, others like the microwave assisted extraction, and ultrasound assisted extraction are gaining grounds due to their high yield, purity level and short extraction time[17,18]. However, hot water extraction and alcohol precipitation method remains the classic, most convenient and the cheapest method of extraction and is commonly used in laboratories and suitable for industrial production[19]. Till date, there is no one particular extraction method that is considered best for extracting polysaccharides hence the method chosen often depends on the availability of materials and the types of polysaccharides sorted. Sometimes the extraction process are also modified to suit the researcher's need. For example, [20] in their study, extracted pectin polysaccharides from the seeded fruit of oil pumpkin using a series of processes that included classical extraction and short ultrasound-assisted extraction methods. It is important to choose an extraction method that minimize the distortion of the polysaccharide structure or its existing form.[21] This is because extraction methods affect the structural orientation of polysaccharides which in turn influences the physicochemical and biological activities of the polysaccharides.

Plant polysaccharides as natural carbon sources have important uses in the pharmaceutical industries, food industries and in other related industries. Recent reports have shown that polysaccharides extracted from medicinal plants possesses various therapeutic activities including antioxidant, anti-aging, anticancer, antimicrobial, anti-inflammatory, immunological activities, etc.[6,11,13] Plant polysaccharides also find uses as thickening or gelling agents in food either in their single forms or as hydrocolloids[22]. They are also sometimes modified into hydrogels and/or scaffolds and used as carriers for drug delivery or soft tissues engineering applications[23].

Vernonia amygdalina also known as bitter leaf plant is a famous Africa perennial plant that is commonly used both as food and in Africa traditional medicines[24]. *Vernonia amygdalina* is from the family Asteraceae and is locally called bitter leaf in West Africa due to its bitter taste. Although originally predominant to the African tropics especially Nigeria and Cameroon, it has become

distributed almost all round the world[25]. In Africa traditional medicines, the roots, stems, and leaves extracts are used against a wide range of tropical diseases including gastrointestinal problems, venereal diseases and malaria[24]. *V.amygdalina* have been reported to contains important phytochemicals such flavonoids, xanthones, edotides, phenolic acids, coumarins, fatty acids, saponins and sesquiterpen[26]. Recent studies have indicated that extracts of *V. amygdalina* have varying degree of bioactivities including antioxidant, anti-malaria, anti-obesity and also the ability to interfere with the biochemical processes of cancerous cells[25]. It was reported that aqueous extracts of *V. amygdalina* have activity of ERKs in vitro and show inhibition against human breast cancers[25]. Important bioactive polysaccharides have been reported from a wide range of medicinal plants, however, despite the wide use of *V. amygdalina* as food and medicine in Africa and many part of Asia, most of the available scientific work on *V. amygdalina* till date only focus on the bioactivities of its aqueous extracts and to the best of our knowledge, there is no report on extraction of polysaccharides from *V.amygdalina*. Hence, in this study, we aimed to investigate the extraction of polysaccharides from *V. amydalina* using hot water extraction and ultrasound-assisted method, so as to provide a guide for further research on extraction of *V. amygdalina* polysaccharides and the study of their molecular structure and biological functions.

MATERIALS AND METHODS

Plant materials

Fresh stems and leaves of V. *amydalina* were collected from Aguda, Surulere, Lagos State, Nigeria and air dried. Then the sample was crushed into power using a pulverizer (Tianjin Taisite Instrument Co. Ltd. Tianjin, China) and sifted through a 20 mesh sieve. The fine powder was then placed in a clean flask and labeled for polysaccharide extraction.

Polysaccharide extraction

Hot water treatment: 2 g each of sample powder was mixed with 20 ml of distilled water in a 250 ml triangular flask. The sample was extracted in a hot water bath (HH-4, Beijing Kowei Yongxing Instrument Co. Ltd., Beijing, China) under the following preset extraction conditions: extraction time 80 min, extraction temperature 91 ^{o}C and solid/liquid ratio 1:10 respectively. The mixture was centrifuged at 3000 r/min for 15 min and the supernatant collected. The supernatant was labeled as hot water polysaccharide (HTP) and then stored for further analysis. The experiment was performed in triplicate and mean value gotten.

Ultrasonic-assisted treatment: For the ultrasonic extraction, the extraction conditions were preset at extraction time 52 min, extraction temperature 70 ^{o}C, solid/liquid ratio 1:10 and ultrasonic power fixed at 210 watt respectively. Then, each sample powder (2 g) was mixed with 20 ml of distilled water in a 250 ml triangular flask. The sample was extracted with an ultrasonic processor (KQ-250DB, Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China). The extract was centrifuged at 3000 r/min for 15min and the supernatant was collected, named Ultrasound Polysaccharide (USP) and stored for further analysis. The test was performed in triplicate and mean value gotten.

Determination of polysaccharides content

0.01 g of standard glucose was dissolved with 100 ml distilled water. Then the standard solution was diluted to different concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL. 0.5 ml phenol was added and mixed up. Then 2.5 ml sulfuric acid was added and mixed. Solution was allowed to stand for 20 min. The absorbance of the solution was measured at 485 nm. 1 ml of distilled water mixed with 0.5 ml of phenol and 2.5 ml of sulfuric acid was used as blank. The total polysaccharide was determined by phenol-sulfuric method. (27). The content of *V. amygdalina* was calculated according to the linear regression equation ($Y = 0.5776x - 0.0131$, $R^2 = 0.9973$) based on the standard curve. The horizontal coordinated denoted the concentration of glucose (mg/mL) while the vertical coordinate denoted the absorbance (OD_{485}). The polysaccharide yield (%) was calculated using the following equation:

$$\text{Polysaccharide yield (\%)} = \frac{C \times N \times V}{W \times 1000}$$

Where C is concentration of polysaccharide calculated from the calibrated regression equation (mg/mL), N is the dilution factor, V is the total volume of the extraction solution (mL), W is the weight (g) of the *V. amygdalina* powder.

Physical and chemical properties analysis of HTP and USP

The Solubility test and colour observation of HTP and USP were respectively carried out according to the method described by[28]. The presence of phenol was determined by the ferric chloride test as described by[29]. Fehling reagent was used to determine reducing sugar according to Schneider[30]. Protein was measured by Coomassie Blue reaction according to the process outlined by[31]. Uronic acid was evaluated by Carbazole Reaction following[32] procedures. Molisch's Test was done to confirm the presence of carbohydrate. 2ml of the crude polysaccharide was placed in a test tube and 2 drops of α-naphthol solution was added. Using a dropper, conc. H_2SO_4 was carefully dropped along the sides of the tubes and the junction of the two liquids was observed for a colour change. The presence of violet colour at the junction of the two liquid indicates a positive reaction.

RESULT AND DISCUSSION

Plants cell walls are composed of macromolecules like polysaccharides, proteins, lignin etc. However, polysaccharides makes up a larger part of the content of the cell wall[33]. The extractability of a given solvent is majorly dependent on the solubility of the targeted compound in the solvent, the kinetics of mass transfer of

the product and the strength of interaction between the solute and the matrix. But in ultrasound-assisted extraction, it is the propagation and interaction of sound waves with the cell wall that brings about the physical and chemical alteration of the plant cell wall, thus, facilitating release of targeted compounds from the plants matrices[34].

Fig. 1 shows the glucose standard curve with linear regression equation, Y = 0.5776x – 0.0131, and correlation coefficient R^2 = 0.9973 which signifies that the linear relationship between the absorbance and concentration of glucose was good. This is in accordance with Lambert-Beer's law.

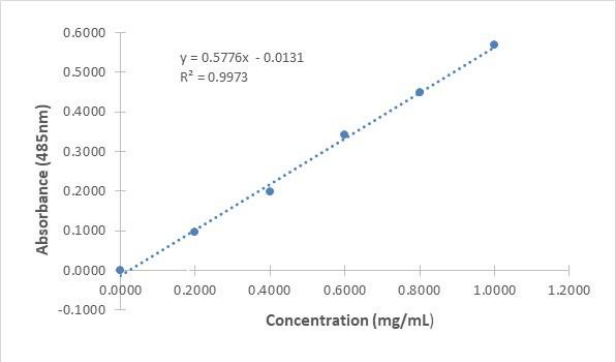

Figure 1: plot of the glucose standard curve

In this study, the yield of polysaccharides from *V.amygdalina* using hot water extraction and ultrasound assisted extraction was determined. Polysaccharide yield of ultrasonic extraction (4.19%) was higher than the polysaccharide yield using hot water extraction (3.38%). This obtained result is in agreement with previous scientific reports that stated that ultrasonic assisted extraction and microwave extraction have the advantage of higher polysaccharide yield over conventional methods like hot water extraction[21,22,35]. The higher extract yield in ultrasonic assisted extraction could be attributed to the cavitation of the cell due to the effect of high intensity ultrasound on the cell wall. This promote the dissolution and release of the active ingredients within the cell[35,36]. Another reason could be due to the fact that ultrasound waves have the ability to facilitate the swelling and hydration of the cell, thus causing the enlargement of the cell wall pores. This enhances the diffusion process bringing about the transfer of mass[37]. Besides the higher polysaccharide yield, the ultrasound assisted extraction also had a shorter extraction time (52 min) and a lower temperature (70 ℃) compare to 80 min extraction time and 91 ℃ extraction temperature of hot water extraction which also agree with previous reports[21,22].

The polysaccharide (HTP and USP) were green and water soluble, dissolving in cold and hot water. Their positive reaction to Fehling reagent indicates that they contain reducing sugar (a monosaccharide). Negative reaction to α-naphthol signifies that the polysaccharides are either trioses or tetroses. The result of Carbazole reaction was negative, which showed that uronic acid was absent in both HTP and USP. The Coomassie blue reaction results for HTP and USP and their negative test to ferric chloride show that the samples contained some protein without polyphenols and the content was 1.23% and 1.32% respectively. The standard curve for Coomassie Blue reaction and its linear regression of Y = 0.4362x + 0.0220, with R^2 = 0.9917 indicated that Beer lambert Law was obeyed.

CONCLUSION

Today, plant polysaccahrides play vita roles as lead product for drug discovery in many pharmaceutical industries and are also very important in the food processing industries and tissue engineering industries. This research studied the extraction of polysaccharide from *V.amygdalina*, an important Africa medicinal plant using hot water and ultrasound assisted extraction methods. The experimental results suggests that useful polysaccharides can be extracted from *V.amygdalina* and that hot water extraction and ultrasound assisted extraction are suitable methods for extracting these polysaccharides from *V. amygdalina*. This experiment lays a foundation for further research of polysaccharide extraction from *V.amygdalina*. Further studies on the optimization of the extraction process and the physicochemical and biological properties of the polysaccharides are currently underway.

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