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Extraction of Antioxidants and Phenolic Contents from Purslane (*Portulaca Oleracea L.*) using Ultrasound Assisted Extraction with Maceration

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Abstract: Purslane, or *Portulaca oleracea L.*, is an edible weed that is a rich source of natural antioxidants. Recently, dietary supplements and skincare products made from natural ingredients are highly in demand. Conventional extraction techniques have disadvantages, including lower extraction rate, high energy usage, and high solvent consumption. Thus, the effectiveness of the combination extraction method, ultrasound-assisted extraction with maceration was explored. The antioxidant activity and the total phenolic content of *Portulaca oleracea L.* extracts were analyzed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and Folin-Ciocalteu reagent method (FCR), respectively. The results showed that pulsed ultrasound assisted extraction at early part of the 24-hour maceration with 5 minutes, 100 W sonication was the most efficient compared to other techniques. Among the extraction methods of purslane extracts, 48-hour maceration without ultrasound displayed the highest total phenolic content (19.24 ± 1.01 mg GAE/g), whereas 72-hour maceration without ultrasound had the lowest with 14.30 ± 1.01 mg GAE/g. For purslane extracts, most of the samples give an inhibition percentage of 20 µg/mL, which ranges from 3.49 % to 58.70 %. Therefore, the combination of conventional and non-conventional extraction methods for purslane, which is ultrasound assisted extraction with maceration, has the potential to produce a higher extraction yield compared to maceration alone.

Keywords: *Portulaca oleracea L.*, purslane, maceration, ultrasound assisted extraction, antioxidants

1. Introduction

Purslane, *Portulaca oleracea L.*, is an edible weed that belongs to the *Portulacaceae* family. Purslane has been used in nutritious and medical field since thousands of years ago. Purslane which is also called khurfa in Arabic or hog weed has red stems and fleshy succulent leaves. The seeds grow in a tiny pod that rips open once it is matured. Purslane have been studied for their nutraceutical properties,

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as well as their role in promoting consumer health and preventing chronic degenerative diseases, primarily because they contain antioxidant compounds [1].

Purslane has a broad range of pharmacological effects, including neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory and anticancer. Due to these factors, it has traditionally been extensively used for medicinal purposes. In cosmetics, it is utilized to create new natural active ingredients for skincare. In fact, it contains antioxidants that can prevent ageing and wrinkles, as well as whitening properties that can remove stains caused by burns or excess melanin. Therefore, the study on the antioxidant and total phenolic content in the purslane is significant in order to explore the health benefits of this herb and move forward for natural antioxidants in the nutraceutical, pharmaceutical and cosmetic industries.

The conventional method in extracting bioactive compounds in purslane requires high consumption of organic solvents with longer extraction time at higher temperature [2]. There are limited research studies on the purslane extract. Previous studies more focus on the different solvents used for the extraction of antioxidants such as by using methanol, ethanol, acetone and ethyl acetate [3, 4], therefore this study was carried out to determine the effects of ultrasonic assisted maceration technique of extraction method and to evaluate the antioxidant properties in the purslane extracts.

2. Materials and Methods

In this part, the methods to extract *Portulaca oleracea L.* would be explained in detail. The purpose of this study is to evaluate extraction yield of purslane using ultrasound assisted extraction (UAE) with maceration and to determine the total phenolic contents and antioxidants in the extracts.

2.1 Materials

The research work was carried out in a laboratory on the Pagoh campus of Universiti Tun Hussein Onn Malaysia (UTHM). Fresh *Portulaca oleracea L.* was purchased from an online store. Ethanol, gallic acid, folin-ciocalteu reagent (FCR), ascorbic acid, sodium carbonate (7.5%), and 2,2-diphenyl-1picrylhydrazyl (DPPH) were prepared and provided by the Material Laboratory and Upstream Bioprocess Laboratory in UTHM. *Portulaca oleracea L.* was cleaned with distilled water and dried in an oven for 72 hours at 40°C.

2.2 Methods

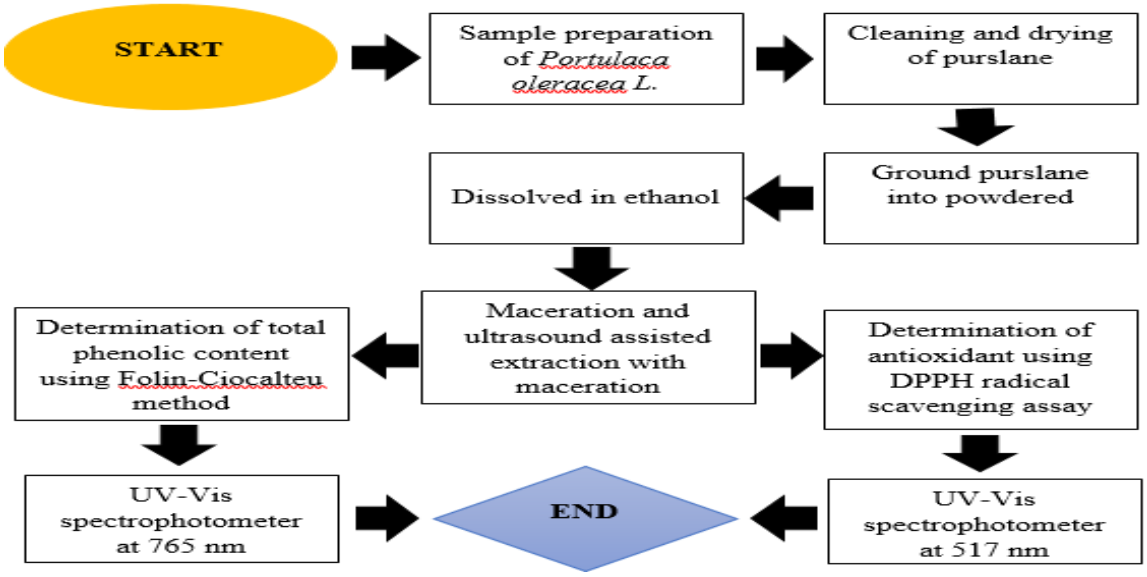

Figure 2.1: Flow charts of procedures for *Portulaca oleracea L.* extraction and analysis

Sample preparation of extracts

Portulaca oleracea L. was ground into powder, which was about 0.5 mm to 2 mm in size, using an analytical grinder for two minutes. The process was stopped at 15 seconds intervals to avoid the sample from heating. The powder was stored in an airtight container in a cold room at 4°C until further use [5].

Extraction by ultrasound assisted extraction with maceration

7 g of purslane powder is placed in 140 mL of 50% ethanol and extracted using direct sonication at 20 kHz and 50 W for one minute. The ultrasonic mode use is pulsed, with each pulse interval of 15 seconds. The solvent was then left at room temperature for 24 hours. The liquid is filtered using Whatman No. 1 filter paper to remove impurities after being left to macerate for a day. Then, the samples are extracted using a rotary evaporator with a vacuum system at 40°C for about 30 minutes. The extracts are kept in amber glass at 4°C until the antioxidant and total phenolic content can be determined. Ultrasound's effective power varies from 50 W, 70 W and 100 W for one, three and five minutes. The extraction yield of *Portulaca oleracea L.* was calculated using the following formula:

$$yield = \frac{X_1 \times 100}{X_0} \quad Eq.1$$

where X_1 is volume of sample after extraction (mL) while X_0 is volume of sample before extraction (mL).

Estimation of total phenolic contents by Folin-Ciocalteu's method

Total phenolic contents were estimated by modified Folin-Ciocalteu method using gallic acid as standard [5]. 0.25 mL of purslane extract was diluted with 2.25 mL of distilled water. Then, 0.25 mL of 0.2 N Folin-Ciocalteu reagent (2 N FCR was diluted with distilled water in the ratio of 1:10 v/v) was added. After 5 minutes, 2.5 mL of Na_2CO_3 was added and vortexed. The mixture was incubated in the dark at room temperature for 2 hours. The absorbance of the blue colour developed was read at 765 nm using a UV-Vis spectrophotometer. All reagents were added except the plant extract in 0.25 mL of ethanol, which was considered a blank. The calibration curve is made by using gallic acid solution with a concentration range of 100-500 µg/mL. The relationship between the concentration of gallic acid and its absorbance was plotted to produce a linear equation. For each analysis, the samples were prepared in triplicate. Total phenolic contents were calculated using the following formula:

$$\mathrm{T} = \frac{\mathrm{C} \times \mathrm{V}}{\mathrm{m}} \quad Eq.2$$

where T is total phenolic contents (mg/GAE/g), C is concentration of gallic acid obtained from calibration curve (mg/ml), V is volume of extract (ml), and m is mass of extract (g).

Estimation of antioxidants by DPPH method

The antioxidant activity of plant samples was estimated using the modified DPPH method [6]. 0.1 mL of the sample was taken and made up to 5 mL by adding ethanol. Then, 1 mL of DPPH solution (4 mg of DPPH dissolved in 100 ml of ethanol) was added, mixed well, and incubated in the dark at room temperature for 30 minutes. The absorbance was read at 517 nm using a UV-Vis spectrophotometer. Ethanol was taken as a blank, while ascorbic acid was taken as a standard. Measurements were tested for three times. The percentage of DPPH inhibition was calculated using Equation 3.

$$\mathrm{I}\% = \frac{\mathrm{Ac} - \mathrm{A}_0}{\mathrm{A_C}} \times 100 \quad Eq.3$$

where A_C is the absorbance of the control, A_O is the absorbance of the sample.

3. Results and Discussion

The result from this study shows that all objectives, from the extraction of *Portulaca oleracea L.* to the analysis of antioxidants, were successfully achieved.

3.1 Extraction yield of *Portulaca oleracea L.*by different extraction methods

Figure 3.1 shows that ultrasound assisted extraction with maceration for 24 hours, 5 minutes, 100 W (ua5) had the highest extraction yield (22%) compared to the least at 5.71% by maceration for 24 hours (ma). Among maceration methods, 72 hours have the highest yield, which is 19.09%, followed by 48 hours and 24 hours, at 7.88% and 5.71%, respectively. A previous study by Handayani et al. [7] showed that maceration using ethanol for 3 days gave 8.30%, while Chen et al. [4] stated that maceration with ethanol for 3 days gave 33.20%. Therefore, the results of 19.09% for maceration of 24, 48 and 72 hours is within the range reported by other researcher.

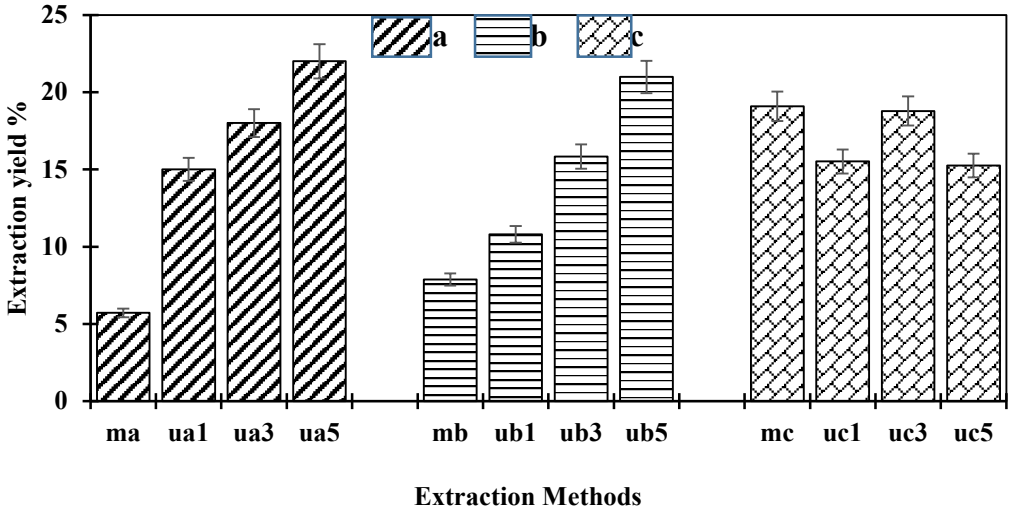

Figure 3.1: Extraction yield for different extraction methods, where m= maceration; u= UAE with maceration; a= 24 hours; b= 48 hours; c= 72 hours; 1= 1minute, 50 W treatment; 3= 3 minutes, 70 W treatment; 5= 5 minutes, 100 W treatment)

Based on the results, maceration and UAE with maceration increase linearly with time and power. However, UAE with maceration for 72 hours, 5 minutes, 100 W method shows decreases in extraction yield. According to Kumar et al. [8], when power was boosted to an extremely high level, more bubbles were created. At increasing bubble volume concentrations, the cavitation impact is reduced. A greater deformation, inter-bubble collision, and non-spherical collapse are caused by an increase in the number of bubbles, which lessens the impact of the bubble implosion. Additionally, the layer of cavitation bubbles that has formed around the probe tip limits yield by preventing energy from entering the extraction liquid. This is in coherent with the findings that shows reduce number of extract was produced when high power sonication was used.

Based on the findings, it is suggested that the combination method of UAE and maceration has showed significant increases in the yield. Ultrasound speeds up the mass transfer from the cell to the solvent by mechanically breaking the cell wall through cavitation shear stress. This causes the tissue to fracture, develop pores, and mix, which increases diffusivity and improves extraction yield. In addition, the increased solid-solvent contact area caused by the mechanical vibration of the ultrasonic probe promotes solvent penetration, which increases the yield [8].

3.2 Total Phenolic Contents (TPC)

Figure 3.2 demonstrated that as the concentration of gallic acid increases, the measured absorbance increases. These results are consistent with previous studies by Dugawale et al. [9].

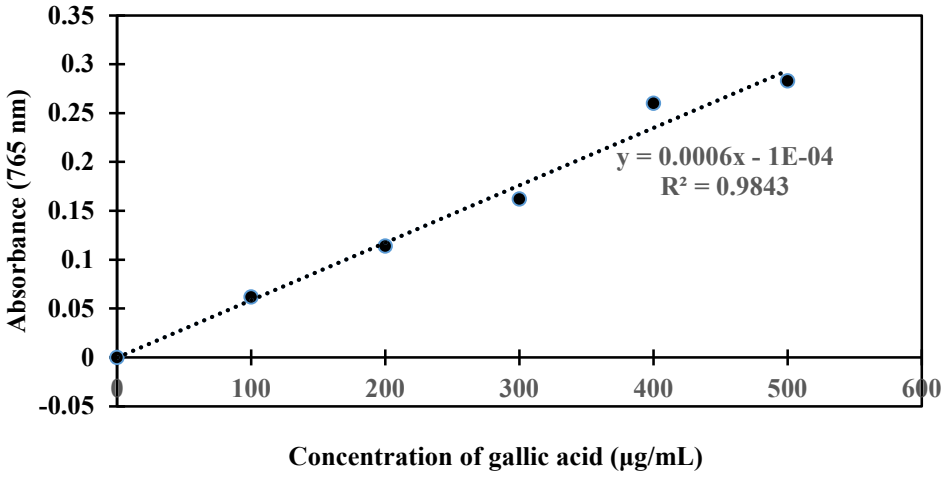

Figure 3.2: Calibration curve of standard gallic acid against absorbance

The greatest TPC value was observed from maceration for 48 hours (19.24 ± 1.01 mg GAE/g), and the smallest was for maceration of 72 hours (14.30 ± 1.01 mg GAE/g). The phenolic content of purslane varies depending on extraction methods and solvent used. The results are supported by Dugawale et al. [9], who found that the total phenolic content of purslane obtained by using microwave assisted extraction varieties ranged from 6.19 mg/g to 15.67 mg/g. Chen et al. [4], who found that the total phenolic content varied between samples in the range from 19.67 to 219.27 mg/g.

Table 3.1: Total phenolic content of different extracts expressed as mg GAE/g

Sample	Absorbance	Total phenolic content (mg GAE/g)
Ma	0.962	19.24 ± 1.01
Mb	0.965	19.30 ± 0.04
Mc	0.715	14.30 ± 1.01
Ua1	0.844	16.87 ± 0.40
Ua3	0.830	16.60 ± 1.13
Ua5	0.799	15.97 ± 0.09
Ub1	0.914	18.27 ± 0.21
Ub3	0.870	17.40 ± 0.21
Ub5	0.839	16.77 ± 0.47
Uc1	0.749	14.97 ± 1.98
Uc3	0.875	17.50 ± 0.12
Uc5	0.825	16.50 ± 1.46

Where m= maceration; u= UAE with maceration; a= 24 hours; b= 48 hours; c= 72 hours; 1= 1minute, 50 W; 3= 3 minutes, 70 W; and 5= 5 minutes, 100 W. The data was expressed as Mean ± Standard Deviation (SD); $p < 0.05$; $n = 3$

Based on Table 3.1, the TPC value was 16.87 ± 0.40 mg GAE/g for ua1 and 15.97 ± 0.09 for ua5. For ub1, the TPC value was 18.27 ± 0.21 mg GAE/g, whereas it was 16.77 ± 0.47 mg GAE/g for ub5. For the UAE with maceration, treatment for 1 minute, 50 W shows the highest TPC compared to the other treatments at 3 minutes, 70 W and 5 minutes, 100 W. Only UAE with maceration for 72 hours showed the highest concentration in 3 minutes, 70 W (17.50 ± 0.12 mg GAE/g) treatment, compared to 1 minute, 50 W (14.97 ± 1.98 mg GAE/g) treatment.

The TPC values in this study were higher in maceration method compared to UAE with maceration. The comparison of the data obtained with previous findings is difficult as *Portulaca oleracea L.* studies reported various types and conditions of extraction and the findings were computed differently. Nevertheless, these findings are consistent with a previous study by Gallo et al. [10], in which TPC value of maceration (115.5 GAE mg/8 g dry weight) was higher than maceration with an ultrasound bath (44.6 GAE mg/8 g dry weight). Maceration resulted in higher TPC levels (117.42 ±

0.14 mg GAE/100 g dry weight) according to Nemzer et al. [11]. TPC may be reduced as a result of longer sonication times, which cause degradation of some target compounds, resulting in lower concentrations.

3.3 DPPH Method

The antioxidant activity of *Portulaca oleracea L.* was determined by a DPPH radical scavenging assay. Antioxidants are plant elements that can be seen quenching the stable purple DPPH radical to the yellow DPPH radical. In an aqueous or ethanol solution, DPPH is comparatively stable. In order to eliminate free radicals, antioxidants may react with the DPPH radical and transfer an electron or hydrogen atom. The DPPH could be effectively scavenged by ascorbic acid and all purslane extracts.

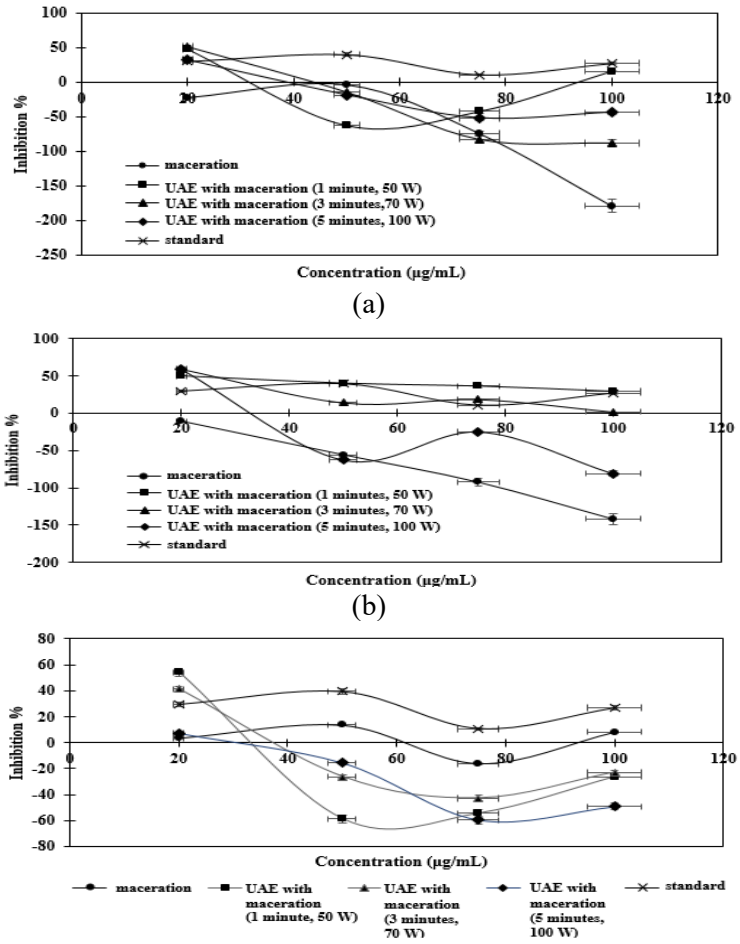

Figure 3.3: Percentage inhibition of DPPH radical by purslane extracts and ascorbic acid for: (a) 24-hour; (b) 48-hour; (c) 72-hour of extraction. The data were presented as mean values ± standard deviation; $p > 0.05$; $n = 3$.

Figure 3.3 displays the mean percentage of DPPH free radical scavenging activity at different extract concentrations. In this study, a best-fit line for the ascorbic acid standard curve could not be achieved. The percentage of inhibition depends on the concentration of the extracts. Ascorbic acid is used as the standard, and the different extracts show variable antioxidant properties. In general, the graph of percentage inhibition against concentration should be increasing linearly [3]. However, in this study, only non-linear regression can be achieved. When compared to purslane extracts at the same concentrations, ascorbic acid had the highest inhibition activity against DPPH at most concentrations. For purslane extracts, most of the samples give an inhibition percentage of 20 µg/mL, which ranges from 3.49 % to 58.70 %. In this study, the ub3 extraction method gave the highest inhibition percentage

(58.70%), followed by ub5 (58.26%), ua3 (50.66%), uc1 (41.13%), ub1 (49.57%), ua1 (47.16%), uc3 (41.13%), ua5 (32.31%) and mc gave the lowest inhibition percentage (3.49%).

In a trial to get decreasing linearly absorbance, seven different concentrations of ascorbic acid were prepared (10, 20, 30, 40, 50, 75 and 100 µg/mL). However, at 30 µg/ml and 40 µg/mL the absorbance increase, then decrease at 50 µg/ml and continue increase at 75 µg/mL and decrease at 100 µg/mL. Based on previous study, percentage inhibition was calculated from absorbance which the graph should be increasing linearly. Then, IC_{50} values were calculated using the regression equation (y= mx + c) from calibration curve. IC_{50} is the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH radicals. The lower the IC_{50} value, the more potent is the substance is at scavenging DPPH, and this implies a higher antioxidant activity.

According to Aryal et al. [12], the DPPH radical scavenging activity for *Portulaca oleracea L.* was 41.48% at a concentration of 50 µg/mL, whereas maceration with an ultrasound bath shows an inhibition percentage of 65.4%, while maceration at 70°C was 52.7% [10]. In theory, in sonication processes, the extraction of compounds results from the breakup of cavitation bubbles. This is consistent with the findings that show a lower inhibition percentage for the combined extraction method compared to maceration.

Bahmani et al. [13] reported that higher ultrasonic power had a negative impact on antioxidant activity. Another study claimed that the lower percentage of DPPH radical scavenging activity was due to excessive exposure or thermal degradation by cavitation. On the other hand, a study by Martinez-Morales et al. [14] recommended that it is important to use standardized units for accurate interpretation of the IC_{50} value resulting from natural antioxidants' inhibition of the DPPH radical. Furthermore, De Menezes et al. [15] conducted a critical examination of the DPPH method and presented a mathematical model to improve DPPH determination. The study has claimed that much research has reported misconceptions about the DPPH method, making it impossible to compare the results.

4. Conclusion

This study found that ultrasound assisted extraction with maceration gives a significant, higher extraction yield (22%) compared to maceration without ultrasound (19.09%). However, for total phenolic content, maceration gives greater total phenolic contents (19.30 $\pm$ 0.04 mg GAE/g) than ultrasound assisted extraction with maceration (18.27 $\pm$ 0.21 mg GAE/g). Total phenolic content increases linearly with absorbance, whereas percentage inhibition decreases linearly with absorbance. In this study, the DPPH method should be reevaluated. Alternatives to the current extraction method should be investigated in the future to evaluate yield and extraction efficiency using different extraction methods. The correlation between extraction method and antioxidant properties could help lay the groundwork for future research focusing on extraction method in order to increase the use of purslane extract in a variety of fields, including the cosmetic, pharmaceutical, and nutraceutical industries.

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