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IN VITRO SCREENING OF PHYTO-METABOLITES WITH *ANTIOXIDANT CAPACITY AND SCAVENGING ACTIVITY OF* ZAPOTECA PORTORICENSIS *STEM BARK*

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Abstract

Without any scientific support, *Zapoteca portoricensis* is used to cure a variety of illnesses in Nigeria. This investigation of the *Z. portoricensis* stem's ethanol extract and its derivate fractions (n-hexane, ethyl acetate, and n-butanol) aimed to determine the total phenolic and flavonoid content as well as the antioxidant potential of the samples. Z. portoricensis stems were air dried for seven days before being ground into a coarse powder in a mill. *Z. portoricensis'* dried powdered stem was extracted with ethanol, and the resulting mixture was fractionated with n-hexane, ethyl acetate, and n-butanol—solvents with growing polarity. The existence of bioactive components such phenolic, flavonoids, and tannins in the ethanol extract and other fractions of *Z. portoricensis* was revealed by its qualitative phytochemical composition, with the exception of n-hexane fraction. For ethanol extract and different fractions, the total phenolic and flavonoid concentrations were calculated using the Folin-Ciocalteu method and the aluminum chloride colorimetric assay, respectively. The findings suggested that *Z. portoricensis* stem bark serves as a source of antioxidants. In general, the ethyl acetate fraction of *Z. portoricensis* stem had a high phenolic content (131.76.± 66 mg of gallic acid equivalent/g), as well as a high flavonoid content (433.33± 25.17 mg of quercetin equivalent/g). The n-hexane fraction had the most flavonoids (963.33± 37.87). According to this finding, phenolic and flavonoid content rose as extract and fraction concentrations rose. The highest concentration of phytochemicals was found in the n-butanol fraction (97.07 ± 0.09), which was followed by the n-hexane and ethyl acetate fractions. These results look at the potential of Z. portoricensis as a rich source of accepted antioxidants for the development of useful food supplements and health remedies.

Keywords: Phytochemistry, ethnomedicine, plant extract, antioxidant, and free radicals

1.0 Introduction

In Nigeria, stress and extensive labour always generate reactive oxygen species and free radicals. These free radicals are majorly responsible for rapid-aging, and disease causation. The use of traditional medicine and supplementation of herbs has help to balance, quenched, and trapped the free radicals. Molecules possessing an unpaired electron are known as free radicals. These compounds are extremely reactive because there is a free electron present (Okpashi *et al*., 2020). They are significant intermediates in biological processes that affect vascular tone, neurotransmission, and cytotoxicity. A potent technique for producing particular free radicals and determining their reactivity is known as radiolysis. Traditional medicine involves the use of herbs, and animal parts (Robert *et al.,* 2020; Obi-Abang *et al.,* 2019**).** Indeed, herbs are widely used in managing ailments, due to the current problem in the health issues, coupled with the adverse reactions associated with conventional drugs, the use of herbs as an alternative to eliminate stress caused by free radical was put in perspective. The used of herbs is not only applicable in adjuvant treatment, but also offers great nutritional benefits by reducing chronic degenerative diseases. This helps to support and advocate for the use of plants known to have medicinal effect in antioxidant and scavenging activity such as *zapoteca portoricensis* stem bark.

Ageing is progressively being recognized as a set of diseases in modern medicine (Agbo *et al*., 2010; Ukekpe *et al.,* 2019), and we can apply the anti-oxidant therapy, such as *Zapoteca Portoricensis*, to help slow down or protect against aging and age-related diseases. This study will validate the ability of *Zapoteca Portoricensis* to having useful phyto-metabolites with anti-aging properties. The outcome of this research will drive significant benefits in public health.

Zapoteca Portoricensis is a perennial-shrubby plant with slender branches, cream-coloured flowers and flat fruits. The genus *Zapoteca* belongst to *Fabaceae* family (Izo et al. 1995). It is a glabrous seasonal shrub with slender unarmed branches. The plant is ascribed to the West Africa, West Indies and the Atlantic coast of America. Different parts of *Zapoteca Portoricensis* are used in the treatment/management of several disorders in the Eastern Nigeria and other parts of the world. It is popularly called white stick pea in English, and "*Elugelu*" in Igbo language; its leaves are used to treat tonsillitis, spasm, prostate cancer and other gastrointestinal disorders. Its root possesses anti-inflammatory activity (Nwodo and Uzochukwu, 2008), anti-fungal and anti-bacterial activity (Esimone *et al*., 2009). The water and ethanol extract of *Z. portoricensis* stem bark, is used in the treatment of wounds due to its antioxidant potential (Agbafor *et al*., 2014). It increases proteinase activity and vitamins C concentration which indicates that the extracts possess wound healing potentials. Proteinases play vital role in the healing of acute and chronic wounds, via cellular invasiveness, apoptosis and remodeling (Puente *et al*., 2003). Proteinases enable cells participating in wound healing (examples; macrophages, mast cells, fibroblasts, etcetera) to digest the cellular barriers across their path to the site of injury (Harding *et al*., 2002). The exact chemical constituent(s) of the extracts responsible for this increase in proteinases activity and their

mode of action are currently under investigation. Vitamin C plays a key role in the synthesis of collagen (a structural protein required for the maintenance of connective tissues) by maintaining the necessary enzymes in their active forms. It is a cofactor for hydroxylase involved in the hydroxyl lation of proline residues of collagen. This hydroxylation is responsible for stability and tensile strength of collagen (Oakley, 1998; Kramer *et al*., 2006). Free radicals such as hydroxyl radical (OH^-) and superoxide radical are very important in the quenching of reactive oxygen species. The electron transport chain and several autoxidation events can both produce superoxide radicals, which are oxygen molecules in their one-electron reduction state. Going forward, this research will first deal with quantitative measurement of phytometabolites in *Z. portoricensis* stem bark extracted with different solvent systems. This will ultimately lead to *invitro* testing of the potential antioxidant capacities.

2.0 Materials and Methods

Collection of Plant Materials

stems bark The Bumaji forest in Cross River State, in Boki local government area is where *Z. portoricensis* stem bark was collected. Dr. S. Udo from the Department of Biological Science at Cross River University of Technology (CRUTECH), Calabar, identified and verified the stem bark sample.

2.1 Preparation and Extraction of Plant Material

The *Z. portoricensis* stem bark was dried for seven days in the air before being milled into coarse powders. For extraction, 500 grams (g) of plant matter were macerated with 99.7% aqueous ethanol. The filtrate was placed in a water bath and allowed to dry off. The dried extract was fractionated using a sequential solvent extraction technique with n-hexane, ethyl acetate, and n-butanol (Okpashi *et al*, 2014; Ushie *et al*., 2019).

2.2 Preparation of various concentrations of the extract

A 100 mg of the extract and fractions used to dissolve in 100 mL of methanol. This will give a stock solution (1000 mg/L or 1 mg/mL). Then various serial dilutions of 25, 50, 100, 200, 250 and 300 mg/L, respectively of each extract and fractions obtain from the solutions.

2.3 Qualitative Phytochemical Analysis

The Zapoteca portoricensis extract was subjected to a qualitative phytochemical examination using the techniques described by Trease and Evans in 1983 and by Harborne (1998).

2.3 Test for Phenol

A few drops of ferric chloride solution were added to 2 mL of the sample. The presence of phenol was detected as a blue green or red tint.

2.4 Test for Flavonoid

Five milliliters of diluted ammonia and a few drops of strong sulfuric acid were used to treat a produced extract. When standing, a yellow tint that was present vanishes.

2.5 Test for Tannins

0.5 g of the material was heated in 20 mL of distilled water, then filtered in a test tube. A few drops of ferric chloride solution containing 0.1 percent changed the color to brownish-green or blue-black.

2.6 Determination of Total Phenol Content

According to Saeed *et al*. (2012), the Folin-Ciocalteu technique was used to determine the total phenol concentration. In a volumetric flask, the reaction mixtures were made up to 1 mL of the produced extract and 9 mL of distilled water (25 mL). The mixture was combined with one milliliter of the Folin-Ciocalteu phenol reagent and mixed. 10 mL of a 7% sodium carbonate (Na2CO3) solution was added to the mixture after 5 minutes. A 25 mL volume was created. The test absorbance was measured against the reagent blank at 550 nm with a UV/Visible spectrophotometer after 90 minutes of incubation at room temperature with a set of standard solutions of gallic acid (25, 50, 100, 200, 250, and 300 ppm) that were made in the same way. Mg of GAE/g of extract will be used to express the total phenol content Okpashi *et al.* (2020).

2.7 Determination of Total Flavonoids Content

The aluminum chloride colorimetric assay was used to determine the total flavonoid concentration. A 10 mL volumetric flask containing 4 mL of distilled water was added to an aliquot of the extract (1 mL) at various concentrations (25, 50, 100, 200, 250, and 300 mg/L). 0.3 mL of sodium nitrite (NaNO2) at 5% was added to the flask. 0.3 mL of 10% AlCl3.6H2O was added after five minutes. After waiting for five minutes, two milliliters of 1.0 M NaOH were added, and then 10 milliliters of distilled water were added. A UV spectrophotometer was used to measure absorbance at 510 nm while the fluid was stirred. The quantity of quercetin equivalents per gram (QE/g) of extract was used to express the overall flavonoid content.

2.8 Statistical Analysis

The outcomes of each experiment were performed in triplicate, and descriptive statistics were used to display the results as mean standard deviation. The mean variance of the samples was compared using a one-way analysis. At a 0.05 threshold, significance was considered to exist.

3.0 Results

3.1 Percentage Extract and Fraction Yield

Table 1 displays the total amounts of crude extract and fractions. When compared to the other fractions and solvents, the concentration of ethanol extract was clearly visible.

Table 1: The percentage (%) yield of the *Zapoteca portoricensis* extract and fractions

Yield	Ethanol extract	*n*-hexane	ethyl acetate	*n*-butanol
Total amount (yielded 500g of dry plant).	8.46%	0.90%	1.82%	3.41%
Percentage (relative to crude extract).	100 %	10.64%	21.51%	40.31%

3.2 Qualitative Composition of Extract and Fractions of *Z. portoricensis*

Table 2 displays the findings of the phytochemical examination of the crude

extract and fractions of the stem bark of Zapoteca portoricensis. Z. portoricensis' qualitative composition revealed the existence of bioactive substances as phenol, flavonoids, and tannins. The phenol, flavonoids, and tannin were detectable in the ethanol, n-hexane, ethyl acetate, and n-butanol fractions.

Table 2: Qualitative Assay of Extract and fractions of *Z. portoricensis*

Phytochemical constituents	Ethanol	*n*-hexane	Ethylacetate	n-butanol
Phenol	+	+	++	++
Flavonoid	++	+++	++	++
Tannins	+	-	++	++

− = not detected; + = present in low concentration; ++ = present in moderately high concentration; +++ = present in very high concentration.

Total Phenol Content of Z. Portoricensis Extract and Fractions

In general, the ethyl acetate fraction had a significant (p 0.05) high quantity of phenolic as compared to other fractions throughout the solvent extracts and fractions. The difference between 25–30 ppm in the ethanol extract and the n-hexane fraction was not statistically significant ($p > 0.05$). When ethanol extract is contrasted with the n-hexane, ethyl acetate, and n-butanol fractions, there is no discernible difference in 25, 100 ppm ($p > 0.05$) between the extract and the fractions Ethanol extract did not significantly ($p > 0.05$) rise or decrease in 25 ppm when compared to 50, 100, 200, and 250 ppm, respectively. Additionally, there was no difference in 25 ppm when compared to 50, 100, 200, and 300 ppm ($p > 0.05$). However, phenol levels in the ethyl acetate and n-butanol fractions were considerably (p 0.05) greater than those in the other concentrations, see Table 3.

Table 3: Total Phenolic Content (mg GAE/g dry weight of the extract plant extract)

Conc. (ppm)	Ethanol	*n*-Hexane	Ethyl acetate	*n*-Butanol
25	ND	ND	ND	ND
50	ND	ND	$8.73\pm1.58^{c(k)}$	$0.18\pm0.32^{b(l)}$
100	ND	ND	$36.61\pm3.19^{a(i)}$	$3.57\pm1.89^{c(j)}$
200	ND	ND	$90.24\pm4.29^{c(j)}$	$14.48\pm2.10^{a(k)}$
250	ND	ND	$114.19\pm8.94^{e(z)}$	$21.45\pm1.82^{d(z)}$
300	$4.48\pm2.29^{f(z)}$	$0.48\pm0.84^{f(j)}$	$131.76\pm4.66^{f(l)}$	$32.67\pm2.29^{f(r)}$

n=3. ND= Not Detected.

Results were reported as mean standard deviation. The difference between mean values with different letters as superscripts across rows and columns and those with the same letters as superscripts across rows and columns was declared significant (p 0.05), whilst the difference between those two was considered non-significant (p > 0.05).

Total Flavonoid Content of *Z. Portoricensis* Extract and Fractions

Overall, n-butanol had a considerably (p 0.05) lower level of total flavonoid concentration than ethanol extract, n-hexane, and ethyl acetate fractions across all of the solvents employed. The total flavonoid content of 25 ppm revealed that there was no difference between the ethanol extract and the ethyl acetate and n-butanol fractions or between the ethanol extract and the n-hexane fractions when compared to the ethyl acetate and n-butanol fractions (p > 0.05). In contrast to n-hexane and ethyl acetate fractions, there was neither a significant (p > 0.05) increase nor a decrease in the total flavonoid content in the ethanol extract and n-butanol fraction for values of 50 and 100 ppm. There was not a significant difference in the overall flavonoid content, which was 200–300ppm. The ethanol extract did not differ significantly (p > 0.05) between 25 ppm and 50 ppm, or between 50 ppm and 100 ppm, at the various concentrations of the extract and solvent fractions. N-hexane, on the other hand, revealed that there was no discernible change between 25 ppm and 50 ppm (p > 0.05). Additionally, the ethyl acetate percentage did not differ significantly (p > 0.05) between 25 ppm and 50 ppm, however a larger amount of TFC (total flavonoid content) was found in the various values between 100 and 300 ppm. No significant (p > 0.05) difference was found between 25, 50, 100, 200, and 250 ppm in the n-butanol fraction. For illustration, see Table 4.

Table 4: Total Flavonoids Content (mg QE/g plant extract)

Conc. (ppm)	Ethanol	*n*-Hexane	Ethyl acetate	*n*-Butanol
25	$103.33\pm15.28^{a(i)}$	$73.33\pm15.28^{by(j)}$	$96.67\pm15.28^{ay(k)}$	$76.67\pm15.28^{ay(l)}$
50	$120.00\pm26.46^{b(ir)}$	$156.67\pm20.82^{ax(j)}$	$126.67\pm5.77^{bx(k)}$	$103.33\pm5.78^{b(lx)}$
100	$170.00\pm20.00^{c(kr)}$	$320.00\pm26.46^{bm(k)}$	$220.00\pm20.00^{cm(l)}$	$130.00\pm10.00^{c(ixy)}$
200	$256.67\pm28.87^{d(l)}$	$646.67\pm15.28^{b(i)}$	$280.00\pm20.00^{c(j)}$	$153.33\pm11.55^{a(kys)}$
250	$316.67\pm32.15^{e(jx)}$	$773.33\pm20.82^{b(r)}$	$340.00\pm10.00^{e(i)}$	$166.67\pm15.28^{d(rs)}$
300	$366.67\pm20.82^{f(zx)}$	$963.33\pm37.87^{b(w)}$	$433.33\pm25.17^{f(z)}$	$240.00\pm36.06^{d(v)}$

n=3. Results were given as a mean standard deviation. Mean values across rows and columns that were superscripted with different letters were deemed significant (p 0.05), but mean values

across rows and columns that were superscripted with the same letters were deemed non-significant ($p > 0.05$).

Flavonoids-phenol ratio of *Z. portoricensis* extract and fractions

According to the aforementioned findings, when compared to the ethyl acetate fraction, the flavonoids-phenolic ratio was highest for the n-hexane fraction, n-butanol fraction, and ethanol extract. However, the flavonoid-phenol ratio of the ethyl acetate fraction was subpar.

Table 5: Flavonoids-phenolic ratio for extract and various solvent fractions

Concentration (ppm)	Ethanol	*n*-Hexane	Ethyl acetate	*n*-Butanol
25	ND	ND	ND	ND
50	ND	ND	14.48±3.65	574.06±18.06
100	ND	ND	6.01±6.27	36.41±5.29
200	ND	ND	3.10±4.66	10.59±5.5
250	ND	ND	2.98±1.12	7.77±8.40
300	81.85±9.09	2006.94±45.08	3.29±5.40	7.35±15.75

n=3. ND= Detected.

The mean standard deviation was used to express the results. Mean values across rows and columns with distinct letter superscripts were deemed significant (p 0.05), but mean values across rows and columns with the same letter superscripts were deemed non-significant ($p > 0.05$).

Discussion

A greater knowledge of plants' priceless therapeutic properties and their subsequent exploitation in the management of diseases have so far resulted from the rising quest for plants with high medicinal values. Utilizing plant resources and goods is recommended over synthetic ones since they are allegedly safer to consume, more accessible, and more affordable. These traits are related to the phytochemical components of plants (Ikpeme *et al.,* 2013, 2014, 2015; Ekaluo *et al*., 2015). According to Padmanabhan and Jangle (2012), antioxidant molecules, which are plentiful in medicinal plants, are responsible for these plants' therapeutic capabilities. These anti-oxidants have a reputation for scavenging free radicals and treating diseases and conditions including oxidative stress that are linked to free radicals.

Plant phytochemicals are extracted using a number of processes, including milling, grinding, homogenization, and extraction. The primary stage for recovering and separating phytochemicals from plant

sources is extraction. The chemical makeup of phytochemicals, the extraction technique, sample particle size, the solvent employed, and the presence of interfering compounds all have an impact on extraction efficiency (Stalikas, 2007). The extraction yield is affected by the solvent's polarity, pH, temperature, extraction time, and sample makeup. Solvent and sample composition are considered to be the most crucial variables at the same extraction time and temperature. Differences in extraction yield and antioxidant activity may be explained by variations in the polarity (and subsequently the extractability) of antioxidants. In addition, solvent polarity is important. Additionally, a significant factor in enhancing phenolic solubility is solvent polarity (Naczk and Shahidi, 2006). As a result, defining a standardized process for the extraction of plant phenols is challenging. Unless very high pressure is employed, the least polar solvents are typically thought to be useful for extracting lipophilic phenols, and polar solvents are used for extracting hydrophilic phenols (Alothman *et al*., 2009). Their physiological stage can have an impact on the composition and number of polyphenols as well as their biological activity. Table 2 lists the quantities of dry extract and fractions extracted from *Z. portoricensis* plant stem. These findings demonstrated the ability of polar solvents to generate enormous fractions with various metabolites. The ethanol extract and solvent fractions of Z *portoricensis* stem bark fractions and extracts were used in the current study. In the current study, the total phenolic, and total flavonoid of ethanol extract and solvent fractions of *Z. portoricensis* stem bark were assessed. The extraction yields for different solvents ranged from 0.90 g for n-hexane to 8.46 g for ethanol. In decreasing order, ethanol, n-butanol, ethyl acetate, and then n-hexane were used to extract different substances. This demonstrates that extraction yield rises as extraction solvent polarity increases. Our finding is consistent with Milan's (2010) findings. The *Z. portoricensis* stem's qualitative phytochemical analysis revealed a very high concentration of active substances in the ethanol extract and various solvent fractions, including n-hexane, ethyl acetate, and n-butanol. The findings showed that tannins, flavonoids, and phenol were present in the ethanol extract, ethyl acetate, and n-butanol fractions. This finding is in line with findings made by (Ukwe *et al.,* 2010). The presence of these phytochemicals in the aqueous extract was validated in the Agbafor *et al.* (2014) publication on the phytochemical screening of *Z. portoricensis* leaves. A growing body of research has been done on polyphenols due to some fascinating recent discoveries on their biological functions. These phytochemical concentrations suggest that a properly extracted *Z. portoricensis* stem employing ethyl acetate as the extraction solvent may provide consumers with therapeutic and chemoprotective effects. The many therapeutic uses of *Z. portoricensis* stem are facilitated by these compounds. According to Abubakar *et al*. (2019), the therapeutic properties of medicinal plants are due to their secondary metabolites (phytochemicals) and other chemical components.

Table 4 displays the total phenolic content of *Z. portoricensis* stem using four different solvent solutions. The total phenolic content

was calculated using the Folin-Ciocalteu technique. Our research shows that the phenolic concentration is influenced by the extract and fraction concentrations. In other words, when extract and fraction concentrations rise, so does the concentration of phenol. Values for the total phenolic content were derived using the gallic acid standard's calibration curve. *Z. portoricensis* extract and fractional phenolic content values dropped in the following order: ethyl acetate > n-butanol > ethanol extract > n-hexane. In general, the ethyl acetate fraction had a significant (p 0.05) high quantity of phenolicswhen compared to other solvent extract and fractions. The amounts of phenols in ethanol extract and n-hexane fraction are much smaller. The phenolic contents of *Z. portoricensis* stem bark is comparable to those of other medicinal plants collected from Meghalaya state, India, such as Morus indica (24.94 ±0.58 mg GAE/g), Paria*roxburghil* (49.39 ±0.25 mg GAE/g), and *Prunus nepalensis* (10.49± 0.14 mg GAE). The n-butanol fraction was also found to contain a significant number of phenolic compounds. The total phenolic content of *Z. portoricensis* plant extracts vary according to the type of extract, which is determined by the polarity of the extraction solvent. Because phenol is soluble in polar solvents, extracts made with polar solvents for extraction have significant concentrations of these chemicals (Pougoue *et al*., 2020). According to the available information, ethyl acetate has proven to be the most effective solvent solution for extracting total phenol from the stem bark of the plant under study.

Colorimetric analysis was used to determine the total flavonoids. It is necessary to extract flavonoids from plant materials using preferably polar solvents because numerous combinations of various groups, such as hydroxyl, sugars, oxygen, and methyl attached to the basic flavonoids moiety, result in various classes of flavonoids, such as flavanols, flavanones, flavones, flavonols, and anthocyanins. Our research revealed that *Z. portoricensis* extract has a high concentration of flavonoids, which could indicate that the extract has potent antioxidant effects. The total flavonoid content of this plant was ascertained using the aluminum chloride colorimetric technique. Flavonoid content was described in terms of quercetin equivalent. The *Z. portoricensis* n-hexane fraction has the highest level of flavonoid concentration. The extract and *Z. portoricensis* fractions' total flavonoid content values declined in the following order: n-hexane > ethyl acetate > ethanol extract > n-butanol. The various solvents utilized during the extraction procedure are to blame for this difference. Our finding is consistent with what Khan *et al.* (2012) reported. This finding makes it clear that phenolic play a significant role in the composition of this plant. Plant molecules known as flavonoids are significant phytochemicals because they have been shown to have powerful antioxidant effects (Yusuf *et al.,* 2021). The ethanol extract of *Z. portoricensis* had the lowest absorbance (94.55 0.17 mg EAA/g). This indicates that for each fraction to display antioxidant characteristics, there must be some level of polyphenolic concentration. This characteristic also suggests that this phenomenon could be explained by differences in the locations of

OH groups in the flavonoids (Spiridon *et al*., 2011). Numerous flavonoids and related polyphenols have been demonstrated in recent investigations to significantly contribute to the phosphomolybdate scavenging action of medicinal plants (Khan *et al.*, 2012). The findings of the current study concur with those of Shukla *et al*. (2008) who screened the ethanol leaf extract of Stevia Rebaudiana Bert for *in vitro* antioxidant activity and total phenolic content. Overall, this investigation show that phenolic compounds are primarily responsible for the presence of antioxidants in the extracts and fractions, albeit the involvement of additional phyto-metabolites including triterpenes, steroids, and terpenoids cannot be completely ruled out. Another possibility is that the bio-actives work together synergistically, increasing the plant components' overall antioxidant activity. The structure-activity relationship of phenolics and flavoniods are very important, and it is possible that some phenolics and flavonoids have high antioxidant activity while others do not, or that their actions take place through different mechanisms, which can be used to explain a random correlation of total phenolics with some assays.

Conclusion

This study's findings revealed significant diversity in phytometabolite chemicals and extraction solvents, which made it necessary to optimize extraction techniques in order to extract the most nutritional value possible from plant extracts. Ethyl acetate and n-hexane fractions, which contain the most phenolics and a sizable number of flavonoids, had greater levels of phenolics and flavonoids overall. Based on the findings of the current study, it is possible to investigate this medicinal plant as a potential source for the extraction of high-valued bioactive and natural antioxidants, which could provide a starting point for the discovery of new antioxidants and bioactive for the production of functional foods, nutraceuticals, and pharmaceuticals.

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