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Assessment of Curcuminoid Bioactivity Containing in Different Varieties of Curcuma Longa

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Abstract

There are many brands of turmeric powder available for consumers to use in Pakistan, one of the most popular spices. Several forms of turmeric powder (homegrown, open-market, and two marketed packaged brands were examined for their anti-inflammatory, antioxidant and antibacterial properties. Despite being able to meet all the tested phytochemical properties, the marketed packed (MP I and MP II) and open-market Curcuma longa powder extracts lacked saponin terpenoid and tannins. Turmeric's primary chemical constituent, curcumin, has been shown to have a number of beneficial biological properties. Home grown turmeric contains large amount of curcumin (4.22±0.52%). Additionally, home-made turmeric powder shown notable anti-inflammatory activities (IC_{50}=117.42±0.41 g/mL). With a zone of inhibition of 17.07 ±0.27 mm and 17.03±0.30 mm, respectively, homegrown curcuma longa powder showed the most significant antibacterial activity against Staphylococcus aureus and Escherichia coli. Homegrown turmeric powder proved to be the best spice source of all the turmeric powders tested. This study provides information primarily on the therapeutic activities of turmeric, its derivatives, and potential medical uses for turmeric, as well as an assessment of their safety.

Keywords: curcuminoid bioactivity; curcuma longa; turmeric powder; antioxidant and antibacterial properties

Introduction

A perennial tropical plant in the *Zingiberaceae* family, turmeric (Curcuma longa) is whose rhizomes are used for several purposes [1]. It is among the most widely used spices in Pakistan and other Asian countries. In Pakistan and Southeast Asian nations, it has been used as a domestic remedy for various disorders due to its neuroprotective, anti-diabetic, anti-cancer and expectorant activities[2, 3]; to treat cough, sinusitis, anorexia and reduce the symptoms of various other diseases [4]. Curcuma longa linn extract is used as preservative and as a coloring agent in many nations of the world [4, 5]. Numerous curcuma longa species are known to have radical scavenging properties [6]. TNF-α (Tumor necrosis factor) production reduces following curcumin treatment, intercellular adhesion molecule 1, leukocyte adhesions, and gastric mucosal lesions are improved[7]. It is possible to cure degenerative eye disorders with curcuma longa as well as problems with the metabolism[8, 9]. Several significant phytochemicals, including saponins, tannins, terpenoids, flavonoids, phytosterols and alkaloids were found in extract of the Curcuma longa [10, 11]. A wide range of herbal products are available that contain turmeric extract. Turmeric, in the form of different crude extracts, has been shown to have remarkable antibacterial property against a range of different gram-negative and gram-positive strains, including Escherichia coli, Bacillus subtilis and Staphylococcus aureus [12]. Pakistan and other Asian countries use turmeric extract often as a seasoning, sources based on its biological activity have not yet been investigated. Because of this, the focus of this research was on a source-based comparative evaluation of the antioxidant, anti-inflammatory, and antibacterial activities of four different kinds of turmeric powder (MP I, MP II, open-market, and home-grown) that are accessible in Pakistan.

Materials and Methods

Chemicals and reagents used

Roche located in the Basel, Switzerland provided all of the reagents and chemicals utilized in this study. DMSO (Dimethyl sulfoxide) were from Merck,

Pakistan, nutrient broth media and nutrient agar media (Merck, Pakistan). BSA (bovine serum albumin) ethanol, ascorbic acid, hydrogen peroxide, sodium chloride, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), anhydrous sodium sulphate, salicylic acid (Fisher Scientific USA) was specifically used.

Collection and processing of samples

In Bahawalpur, Pakistan, samples of open-market turmeric powder (Curcuma longa) from two reliable brands (MP I and MP II) were gathered. Marketly packed I (MP I) and Marketly packed II were the study's two popular brands (MP II). The turmeric rhizomes were also obtained in the Okara region and a botanist from National Herbarium, Islamabad has recognized and confirmed them. The gathered rhizomes were cleaned in distilled water and allowed to dry at room temperature (about 27 °C) in the shade. Homegrown turmeric extract was prepared by pulverizing turmeric rhizomes into a powder form. Before performing solvent extraction, all of the obtained turmeric powder samples were kept at 6°C.

Extraction of a sample

The technique used With a minor alterations for extraction of Curcuma longa powder [13]. About 750g of air-dried powdered open-market, home gardened and two Marketed curcuma (MP I and MP II) was taken, powdered curcumin was divided into up to 500 mL in four different conical flasks for the production of ethanol extracts. 350 mL of 100% ethanol was poured into each flask, which was then shaken for 74h at room temperature (32°C). Using filter paper (Whatman No.1) the solutions were thoroughly filtered. The resulting organic filtrates were then evaporated at 37 °C using a rotating vacuum evaporator (Ace Glass Incorporated, USA). The crude extract was placed into amber color jars, once it had completely evaporated, it was kept at 6°C until it was needed for additional examination.

Calculating the amount of curcumin in the extract

Curcumin ratio of turmeric extract in homegrown, two marketed packaged (MP I and MP II) and open-market were assessed in accordance with the methodology outlined by Pawar [14].

Phytochemical testing

The existence of several phytochemical elements was checked in the ethanolic extracts of homegrown, open market and two martetly packaged (MP I and MP II) turmeric powder[15].

Activation of antioxidants in vitro

An assay to measure antioxidant activity

The free radical scavenging activity was assessed using DPPH (2, 2-diphenyl-1-picryl-hydrazyl) anti-oxidant activity as outlined by the author in Ref. [16]. With the use of a vortex, extract solution and standard (L-ascorbic acid) were measured, then combined with 100% ethanol to obtain a homogeneous stock solution with the greatest concentration possible of 1 mg/mL. Following that, aliquots of L-ascorbic acid and curcuma longa powder, which provided as a control sample, were made at four concentrations (125, 250, 500, and 1000 g/mL). Measured DPPH was added to a 0.1 mM solution of 100% ethanol. After that, a micropipette was used to add 2 mL of 0.1 mM DPPH solution within each test tube that already contained 2 mL of the sample solution. The ultimate volume of the solution was 4 mL. After that, the test tube was left in the dark for half hour to allow the reaction to proceed. Furthermore, DPPH and an equal amount of ethanol were added to a clean test tube. Each test tube's absorption was determined at 418 nm using a UV spectrophotometer (Malvern analytical, UK). According to the log concentration of the sample extract, the IC_{50} value is the sample concentration that scavenges 50% of the DPPH free radicals.

Antioxidant action of hydrogen peroxide (H2O2)

Anti-oxidant activity of hydrogen peroxide of Curcuma longa extract was accessed with slight adjustments by the same method outlined by author in Ref.[17]. With basic pH of 7.4, a 0.1 M phosphate buffer solution was made using a 43 mM hydrogen peroxide solution. 4 distinct concentrations of the sample extract and L-ascorbic acid (125, 250, 500, and 1000 g/mL) were made. 3.6 mL of 0.1 M phosphate buffer was used to mix the sample solutions, and 0.8 mL of a 43 mM hydrogen peroxide (H_2O_2) solution was added. A UV spectrophotometer was used to test absorption at 250nm. Using a sodium phosphate buffer devoid of hydrogen peroxide (H_2O_2), a blank was produced. Equation-1 below was used to get the percentage of hydrogen peroxide (H_2O_2) free radical scavenging activity:

$$\text{Antioxidant effect\% (H2O2)} = \frac{1 - \text{AS (absorbance in the presence of a sample)}}{\text{AC (absorbance of the control)}} \times 100$$

Denaturation assay for BSA to determine anti-inflammatory activity

Protein denaturation inhibition was assessed by the technique employed in Ref.[18] . To achieve a concentration of 1 mg/mL for each experiment in this research, the obtained extracts were diluted in 100% ethanol. With 0.1 M phosphate buffer solution (basic pH of 7.6), the stock solution of test sample extracts and standard samples was diluted to various quantities (125–1000 g/mL) in various test tubes. 0.25 mL of 1% bovine albumin, 4.5 mL of phosphate-buffered saline (PBS, pH 6.6), and 0.028 mL of the extract made up the reaction mixture (4.8 mL). The reaction mixture was combined, heated to 69°C for few minutes, and then incubated for 17 minutes in a water bath (38 °C). A UV/VIS spectrometer (Malvern analytical, UK) was used to quantify turbidity at 599 nm after settling the reaction mixture. Using equation -2, the % of protein denaturation inhibition was determined:

$$\text{Inhibition of denatuaration (\%)} = \frac{AC - AS}{AC} \times 100$$

Whereas, AC is the absorbance of control and AS is the absorbance of sample in turmeric sample.

Analyzing antibacterial activity

Preparation of pure culture of bacterial strains

Bacillus cereus ATCC14579, Escherichia coli O157: H7, Staphylococcus aureus NCTC8325, and Enterococcus faecium NCTC 7171 were isolated bacterial strains that were acquired from the Department of Microbiology at the Islamia University of Bahawalpur, Punjab, Pakistan. On nutrient broth and nutrient agar medium, different bacterial strains were grown. A 250 mL conical flask containing 50 mL of nutrient broth medium was inoculated with 100 µl of frozen stock culture for the antibacterial test and subsequent production of the stock culture. This flask was then incubated at 38 °C. The bacteria were cultured in it by subjecting it to continuous shaking at 100rpm until the mid-log phase of absorbance at 550 nm was attained. For the measurement of bacterial broth culture, a UV spectrophotometer was used.

Disc-diffusion method

The disc diffusion technique, was used to evaluate the antibacterial activity as published by author Ref. [19]. In this test, filter paper discs (Whatman No. 1) 8 mm were placed in a micro vial and autoclaved for 17 minutes at 120 degrees Celsius under 15 lb/inch2 pressure. The discs were then thoroughly dried in an oven at 60 degrees Celsius. Each disc of filter paper (Whatman No. 1) was immersed in 10 µL of 200µg/disc of curcuma longa extract before being air-dried in the laminar flow cabinet and applied for the antibacterial experiment. In nutrient broth medium, bacterial strains were grown for 24 hours. On nutrient agar media, 100 µL of each concentration of bacteria was then added. For 24 hours, all of the plates were incubated at 35-38°C. The zone of inhibition was measured in millimetres to evaluate the antibacterial activity. In separate research, the antibacterial efficacy of the antibiotic's erythromycin (15 µg/disc) and ampicillin (25 µg/disc) was also assessed in order to monitor the susceptibility of the investigated microorganisms. Each assessment was carried out three times (n = 3).

Table 1: The results of phytochemical analysis of samples of Curcuma longa extract from the four distinct sources (open market, home-made, MP I and MP II).

Analyzed Samples	Curcumin% (mg/ 100 mg)
Open Market	3.44 ± 0.24
Home grown	4.34 ± 0.39***
MP I	3.49 ± 0.78
MP II	3.35± 0.41

Values are mean ± SD, very highly significant (***). MP I indicate marketly packed I, MP II indicate marketly packed II.

Results

The phytochemical examination of the alcholic extract from the several curcuma longa powder source materials used in this study (MPI, MP II, open-market, and home-grown) uncovered the presence of a numberof the phytochemicals mentioned in table 1. As opposed to the MP II and open-market, the homegrown and MP I curcuma longa extract included

all of the evaluated medicinally significant phytoconstituents. Table 1 and Table 2 showed that the home-grown curcuma longa extract contained a significant level of curcumin ($p < 0.001$). The free radical-scavenging capacity of the ethanolic extracts was assessed using the DPPH test.

Table 2: The determination of the amount of curcumin in turmeric (Curcuma longa) powder samples from the four various sources (MP I, homegrown, MP II, and open market).

Tests	Phytochemicals	Analyzed samples			
		Open Market	MP I	MPII	Homegrown
Xanthoproteic Test	Protein	+ve	+ve	+ve	**+ve**
Lead Test	Tannin	-ve	+ve	+ve	-
Molish's Test	Carbohydrate	+ve	+ve	+ve	**+ve**
Killer-Kilani test	Glycoside	-ve	+ve	+ve	**+ve**
Wagner Test	Alkaloids	+ve	+ve	+ve	**+ve**
Foam test	Saponin	-ve	+ve	-ve	**+ve**
Ferric Chloride Test	Phenol	+ve	+ve	+ve	**+ve**
Alkaline Reagent test	Flavonoid	+ve	+ve	+ve	**+ve**

Positive Sign (+ve) shows the presence and negative sign (-ve) shows the absence. MP I shows marketly packed I, MP II shows marketly packed II.

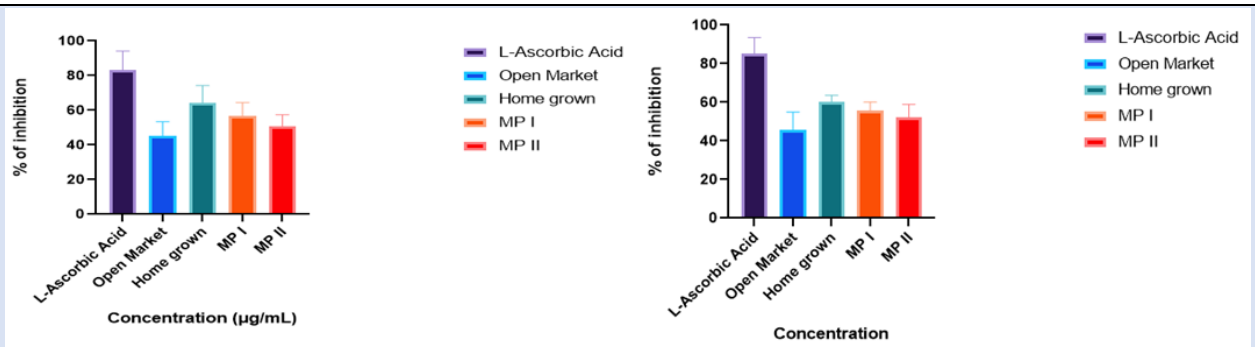

Figure 1: Comparison of the antioxidant properties of turmeric samples from the four distinct sources (MP I, home-grown, MP II, and open-market).
(a) Concentration-dependent DPPH (2, 2-diphenyl-1-picryl-hydrazyl) scavenging activities of Curcuma longa powder.
(b) Concentration-dependent hydrogen peroxide (H_2O_2) scavenging activities of Curcuma longa powder. Values are presented as Mean ± SD (n = 3) for n values. L-ascorbic acid is utilised as a standard.

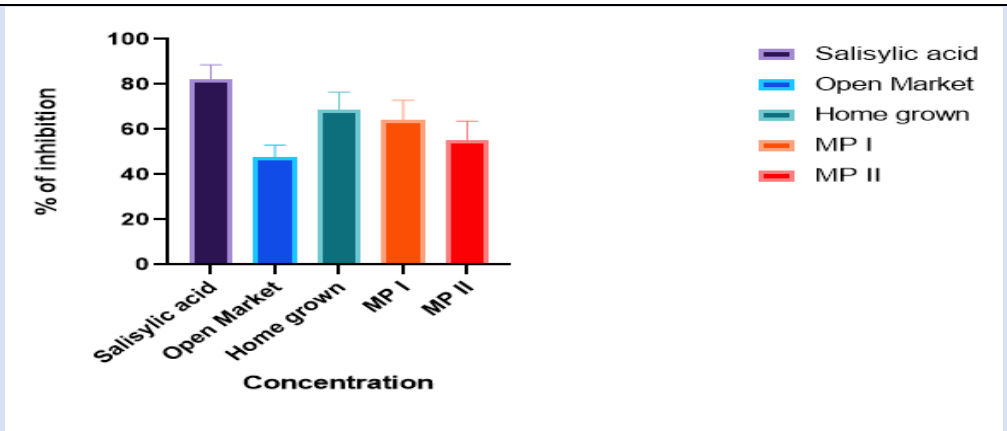

Figure 2: Comparison of the concentration-dependent anti-inflammatory properties measured of the ethnolic extracts from the 4 distinct sources of C. longa samples (MP I, home-grown, MP II, and open-market). The degree of significance was indicated as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ for several C. longa extracts that differ from the standard (Salicylic acid). Values are presented as Mean ± SD (n = 3).

Comparing the open market, MP I, and MP II sources to the C. longa powder grown at home, antioxidant activity was shown to be higher. The home-grown extract had the most substantial DPPH free radical

scavenging properties, despite the fact that all examined samples of the extract showed antioxidant properties. Home-grown and MP I, in that order, were more dominant than the other two samples (MP II and open-market), according to scavenging activity of hydrogen peroxide (H_2O_2).

Table 3: IC_{50} values of the ethanolic extracts of four different sources Curcuma longa powder samples in the 2 distinct antioxidant determinations.

Analyzed Samples	H2O2 scavenging efficiency (IC_{50}) µg/mL	DPPH scavenging efficiency (IC_{50}) µg/mL
L-Ascorbic acid	89.18 ± 1.00	90.38 ± 0.42
Home grown	122.87 ± 2.00 ***	150.99 ± 2.99
MP I	148.79 ± 3.11	169.34 ± 7.56
MP II	180.64 ± 5.55	201.86 ± 7.44
Open Market	280.48 ± 2.56	250.99 ± 9.64

significant (***). Values are mean ± SD, MP I indicate marketly packed I, MP II indicate marketly packed II.

To investigate the anti-inflammatory properties of the extract from the four distinct sources of C.longa powder, the BSA denaturation experiment was carried out. Home-grown and MP I sample, in that order, were more significant than the other two samples (MP II and open market), according to anti-inflammatory properties.

Table 4: IC_{50} values of the ethanolic extracts of four different sources C. longa powder samples in the 2 distinct anti-inflammatory determination.

Analyzed Samples	Denaturation assay for BSA to determine anti-inflammatory activity (IC_{50}) µg/mL
Salicylic Acid	96.28 ± 0.29
Home grown	120.67 ± 0.38***
MP I	122.47 ± 0.99***
MP II	154.88 ± 4.90***
Open Market	253.68 ± 7.92***

Highly significant (***), MP I indicate marketly packed I, MP II indicate marketly packed II, Values are mean ± SD.

The 4 comcentrations presented the significant anti-bacterial properties against g+ve and g-ve bacteria. The maximum zone of inhibition 17.19 ± 0.44 mm against S.aureus was found in the homegrown sample extract. The zone of inhibition was determined to be 12.98 ± 0.45 mm and 11.82 ± 0.38 mm against B. cereus and E.faecium respectively. With a zone of inhibition ranging from 6.44 ± 0.24 mm to 15.83 ± 0.67 mm, the ethanolic extracts of the four distinct samples at a dosage of 500 mg/mL showed strong high antibacterial action against E. coli. The homegrown sample has the largest zone of inhibition (19.22 ± 0.18 mm) out of the four samples. As a good control, the standard antibiotic erythromycin (15 g/disc) displayed a zone of inhibition ranging from 19.22 ±0.45 mm to 28.49 ± 0.351 mm, respectively.

Discussion

Turmeric powder serves as one of the most frequently and commonly bought spices in Pakistan. According to investigations, turmeric powder has health advantages, but using counterfeit turmeric powder can have disastrous results against chronic diseases such as cancer, diabetes, ulcer, etc. Turmeric powder is sold in the market from a variety of sources. The current research compared the sources-based bioactivities of turmeric powder's ethanolic extract by comparing its anti-inflammatory, free radical scavenging and antibacterial effects. The majority of the phytochemical components are found in all samples, according to the investigation of phytoconstituents screening of the ethanolic extract of the various sources of turmeric powder (MP I, MP II, homegrown, and open-market). Although, the MP II and open market samples did not contain any detectable amounts of saponin, terpenoid, tannin, or glycoside. MP I and homegrown samples have high content and are enriched with alkaloids, terpenoid, saponin and tannins. Plant extracts' phytochemicals, which are their active elements, can have a variety of medicinal effects. Flavonoid and phenolic

constituents of plants are said to have strong anti-oxidant, astringent and antimicrobial properties[18, 20]. Tannin is a secondary metabolite with antibacterial, anti-inflammatory, and antioxidant effects[21]. As the MP I and homegrown C. longa samples in this study are higher in phenol, alkaloids, glycosides, flavonoid, terpenoids and tannin than the MP II and open-market ones, the MP I and homegrown turmeric extracts displayed stronger anti-diabetic [22, 23], anti-diarrhoeal [24], anti-oxidant, astringent, anti-inflammatory [23], anti-cancer [25] and antibacterial activity[20, 21]. Colour, quality, medicinal value, and consequently cost are all influenced by the amount of curcumin it contains. Therefore, the curcumin content in turmeric is crucial for both pharmacological and commercial purposes. The turmeric powder grown at home contained the most curcumin (Table 2). This research would suggest that the real source has a big influence on the amount of curcumin in C. longa powder. The curcumin amount in rhizome may be influenced by climatic circumstances[14]. In order to establish the anti-oxidant properties based by a single electron transferring process, DPPH and Hydrogen peroxide evaluations of the ethanolic extracts of the four multiple sources of samples were carried out concurrently in this investigation. The homegrown sample was found to have considerable anti-oxidant potential ($p < 0.001$) even though the ethanolic extracts of the 4 turmeric samples demonstrated antioxidant activity when compared to the IC_{50} values of the four distinct samples. This might occur as a result of the presence of all the phytoconstituents necessary for antioxidant action (Table 1 and Table 2). Accordingly, the findings imply that the four turmeric ethanolic extract include substances that can donate H to a free radical in order to eliminate an odd electron, that gives the reactivity to radicals. Alcoholic extracts of Curcuma longa linn were found to have greatest level of anti-oxidant action[26, 27]. The conclusions of this report's analysis of antioxidant activity agree with their results. Protein denaturation is the major cause of inflammation. Inflammatory illnesses can be treated with substances that can stop protein denaturation [28]. The potential ethanolic extract of the distinct samples of C. longa to suppress protein denaturation was researched as a portion of the research into the process of anti-inflammatory action. When particularly in comparison with other samples, the homegrown sample showed superior at different concentrations BSA denaturation inhibition. The IC_{50} for the homegrown turmeric extract was 121.89 ± 0.67 µg/mL, while the corresponding values for the MP I, MP II, and open-market turmeric extractss were 144.72 ± 2.88, 164.09 ± 6.19, and 244.09 ± 8.92 µg/mL. This shows, Curcuma longa extract prepared at home had the strongest anti-inflammatory effects. Different f four preparations strongly prevented BSA denaturation, according to anti-inflammatory data, although the homegrown version dominated. Parallel to aspirin, a common anti-inflammatory medication, inhibited BSA denaturation, that inhibition was identical [28]. It can be because the homegrown extract has a lot more biologically active compounds than other extract because it contains a unique component. In this research, E. coli-0157 H7 and S. aureus NCTC 8325, B. cereus ATCC 14579, and E. faecium NCTC 7171 were employed to test the antibacterial effects of four separate turmeric samples (MP I, homegrown, MP II, and open-market). Although the homegrown Clonga samples repeatedly displayed the remarkable antibacterial property, all samples of the ethanolic extracts demonstrated strong anti-bacterial action against bacteria (gram negative and gram positive) [29]. The inclusion of significant phytochemical compounds and the maximum level of flavonoids and curcumin contributed to the potent antibacterial action in homegrown turmeric extract. According to observations, turmeric exhibits potent antibacterial properties against a number of bacterial strains [28,30]. Due to the presence of flvanoids and curcumin; a phenolic molecule, Curcuma longa linn has reportedly been shown to be beneficial against the actions of gramme positive and gramme negative bacterias [31,32]. By employing the disc diffusion technique it was observed that the ethanolic extracts of turmeric had strong antibacterial properties against many pathogenic bacteria [33]. When pathogenic bacteria were tested with both ethanolic and aqueous extracts turmeric, it has reported similar outcomes [34]. Bacillus, Sarcinia, Corynebacterium, Gaffkya, Streptococcus strains among the majority of bacteria found in cholecystitis—were all suppressed by an ethanolic extract of turmeric [35].

Conclusions

While comparing with the four different samples, phytochemical analyses revealed that homegrown and MP I sample have highest levels

of phytoconstituents than open market and MP II. All different samples that were used in this study, MP I, homegrown, MP II, and open-market presented high level of significant anti-inflammation, anti-bacterial, anti-cancer and anti-oxidant property, however the homegrown extract performed the best. Homegrown sample showed considerable antibacterial activity against Enterococcus faecium NCTC 7171, E. coli-0157 H7, B. cereus ATCC 14579, and S. aureus NCTC 8325 at 500 mg/mL doses, with the zone of inhibition spanning 17.19 ± 0.44 mm to 19.22 ±0.45 mm. It will be considerably easier for individuals to choose beneficial Curcuma longa according to their health needs if they are aware of its bioactive compounds depend on its source. Lastly, this study recommended turmeric powder producers to capitalise on the bioactive compounds that has been assessed in their good products so that consumers are aware of them, particularly in marketing and preserving initiatives.

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