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Research Article

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Potential Sources of Natural Antioxidants and Antimicrobials: Comparative Analysis of Turmeric (*Curcuma longa*) Extracts from Different Solvent Extraction Systems

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Abstract

Phytochemical content and activity of organic and aqueous extracts of turmeric (*Curcuma longa*) was studied to determine their potential for use as natural antioxidants and antimicrobials for therapeutic and food applications. Turmeric rhizomes were extracted with acetone, ethanol, methanol, water and analysed using standard procedures. Organic extracts exhibited significantly higher total phenolic and flavonoid content compared to aqueous extract; 1379.94, 515.60, 561.16, 307.45 mg /100g Gallic acid equivalence and 382.66, 411.88, 339.01, 158.11mg /100g quercetin equivalent in acetone, ethanol, methanol and water aqueous respectively ($p<0.05$). Tannins were highest in acetone and lowest in ethanol extracts (175.86 and 20.45 mg /100g Catechin equivalent respectively); while vitamin C levels varied significantly, being highest in water and lowest in acetone extract; 62.78 and 11.77 mg /100g ascorbic acid equivalent respectively ($p<0.05$). Free radical scavenging activity of the extracts was in decreasing order of acetone, ethanol, water, and methanol. Antioxidant activity of acetone extracts compared favourably with vitamin C (standard). Antimicrobial activity (Diameter of growth inhibition zones) varied significantly among the extracts. Antifungal activity of aqueous extracts (29.33 millimeters) was significantly higher than organic solvent (19-25.33 millimeters) extract ($p<0.05$). Ethanolic extract was the most effective against *Escherichia coli* (18.33 millimeters); while the efficacy against *Staphylococcus aureus* was not significantly different among the extracts ($p>0.05$) and *Candida albicans* was the most susceptible microorganism to all extracts. The study concluded that both organic and aqueous turmeric extracts exhibited varied antioxidant and antimicrobial activities owing to the level of major phytochemical groups in the extracts.

Keywords

Turmeric extracts, Phenolic content, Free radical scavenging, Antifungal activity

Introduction

The use of natural preservatives to replace synthetics is preferable and may be of great interest to the food industry. Consumers are increasingly demanding for low levels of synthetic preservatives in food; and yet there is also an increasing demand for wholesome and safe food with a long shelf life. For centuries, dietary herbs and spices have been traditionally used as food additives throughout the world, not only to improve the sensory characteristics of foods, but also to

extend their shelf life. Such additives are classified as GRAS (generally recognized as safe). They can extend the shelf life of foods by reducing or eliminating the survival of pathogenic bacteria and well as minimizing oxidation [1].

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae*. It is widely grown in the tropical and subtropical regions of the world [2]. In traditional food preparation, turmeric is used as a spice to impart a characteristic yellow color to the food along with its flavor and taste [3]. The yellow colour of turmeric is contributed by the polyphenolic compound curcumin. Turmeric is also reported to improve the keeping quality of food [4]. Spices such as turmeric are rich sources of polyphenolic compounds which have strong biological activities.

Curcumin and other curcuminoids constitute the major phytochemical compounds in turmeric [5]. These phytochemicals have made turmeric to gain a lot of attention due to its biological activities [6]. Curcumin and other curcuminiods in turmeric extracts are highly effective in neutralizing free radicals. For instance, turmeric oil has a free radical scavenging ability comparable to vitamin E and butylated hydroxytoluene (BHT) [7]. Additionally, turmeric extracts possess an excellent antimicrobial activity; extracts from different extraction solvents exhibited strong antibacterial effects against different pathogenic strains of microorganisms [8]. Efficacy of turmeric extracts from different solvents has also demonstrated potential for minimizing food, fungal related spoilage and fungal pathogens. The antioxidant and antifungal activity of turmeric extracts was associated with their bioactive components [9-11].

Antioxidant and antimicrobial components of turmeric may be extracted and concentrated as extracts, essential oils, or resins. Various solvents are employed for the extraction of bioactive compounds from food and medicinal plants. Suitability of solvents for extraction is based on the chemical nature and polarity of the bioactive components to be extracted [12]. Groups of compounds such as polyphenols, flavonoids, and anthocyanins are hydrosoluble. The polar and medium polar solvents, such as water, ethanol, methanol, propanol, acetone, and their aqueous combinations, are commonly used for their extraction [13].

There is limited scientific information on the phytochemical composition and activity of turmeric extracts. Due to the potential of turmeric extracts as new sources of natural food preservatives and an antimicrobial agent, this study was undertaken to investigate the effect of the aqueous and organic solvent extraction on the quantity of major groups of phytochemical compounds; antioxidant and antimicrobial activity of turmeric extracts.

Materials and Methods

Sample collection, processing and extraction

Fresh turmeric rhizomes (2.5 kg) were purchased from a local food market in Northern Uganda (2.2581° N, 32.8874° E); packed in air-tight bags and transported to the food biochemistry laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya. In the laboratory, samples were properly washed under tap water, drained to remove excess water, and grated for extraction.

Extraction of phytochemicals and sample preparation for antimicrobial analysis

Samples were extracted using acetone, ethanol, methanol, and water according to the procedure described [14]; with slight modifications. Briefly, 2 grams of fresh grated turmeric was put in 100 ml amber bottle and 30 ml of a particular solvent was added to each bottle. Aqueous extracts were also made by adding 30 ml of distilled water. Both organic and aqueous mixtures were shaken in a mechanical shaker (Labortechnik KS 250b, Germany) for 1 hour at 300 rpm and later kept in the dark for 72 hours to avoid the reaction of the flask content with light. The extracts were then filtered using Whatman filter paper No. 1 and analyzed for different phytochemical compounds.

Samples for antimicrobial assays were prepared by mixing grated turmeric paste with acetone, ethanol, methanol or water in a ratio of 1:4 (w/v); and shaken for 24 hours at 300 rpm in the dark. Subsequently, the solution was filtered using Whatman filter paper No 1. The filtrate of organic solvent extracts was collected, concentrated by rotary evaporation at 40 °C, while aqueous extracts were concentrated through evaporation in the water bath. The filtrate were collected and refrigerated at 4 ± 1°C in air-tight bottles for subsequent analysis.

Phytochemical analysis

Determination of total phenolic content

The total phenolic content (TPC) of the turmeric extracts was estimated using the Folin-Ciocalteu method, according to documented procedures [15]. Briefly, 10 mg of gallic acid was dissolved in 100 ml of 50% methanol and further diluted to 10, 20, 30, 40, 50, and 60 µg/ml. An aliquot (1 ml) of each dilution was transferred to a test tube and diluted with 10 ml of distilled water. Then, 2 ml of Folin Ciocalteu's reagent was added, vortexed, and allowed to incubate at room temperature for 5 min. Four ml of 0.7 M Na_2CO_3 was added in each test tube, adjusted with distilled water up to the mark of 25 ml, vortexed and left to stand for 30 min at room temperature.

Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan), against a blank (distilled water). A similar procedure was followed to prepare and analyze the sample extracts. A standard curve was then plotted using garlic acid; and total phenolic contents were determined from the linear equation of the standard curve. The total phenolic content was expressed as mg/100g gallic acid equivalent (GAE) of extract.

Determination of flavonoids

Aluminum chloride colorimetric method was used for determination of flavonoids [16, 17]. To a 10 ml volumetric flask, 4 ml of distilled water and 1 ml of turmeric extract were added; after 3 minutes, 0.3 ml of 5 % sodium nitrite solution was added. After 3 minutes, 0.3 ml of 10 % Aluminum chloride was added. After 5 minutes, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 ml with distilled

water. Absorbance was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan). The number of total flavonoids was calculated from the calibration curve of the standard prepared from quercetin and expressed as mg /100g quercetin equivalent of turmeric extract.

Determination of tannins

Tannin was assayed according to vanillin-hydrochloric acid method [18]; with modifications. Standards were prepared using catechin hydrate at 0, 10, 20, 40, 60, 80, and 100 µg/ml. Duplicate aliquots of 1 mL of each sample extract were put in test tubes, where one served as a sample blank; to the blanks were added 5 ml of 4 % hydrochloric acid (HCl) in methanol without the reagent (vanillin). On the other hand, samples and standards were reacted with 5 ml vanillin-HCl reagent (prepared by mixing equal volumes of 8% HCl in methanol and 1 % vanillin in methanol just before use) and allowed to stand for 20 min. Absorbance of the standards, samples, and blanks were read at 500 nm and tannin content calculated as percentage catechin equivalent (CE) using a standard calibration curve generated from the absorbance of catechin at various concentrations.

Determination of ascorbic acid

The ascorbic acid content in the samples was determined by the HPLC method [19]. Briefly, extract from 2 g of sample was mixed with 0.8 % metaphosphoric acid, and the solution was centrifuged at 10000 rpm at 4 °C. The supernatant was filtered and diluted with 10 ml of 0.8 % metaphosphoric acid. This was passed through 0.45 µl filter and 20 µl injected into the HPLC machine, analysis was done using Shimadzu UV-VIS detector. The mobile phase was 0.8% metaphosphoric acid, at 1.2 ml/min flow rate and a wavelength of 266 nm. Various concentrations of ascorbic acid standards were also prepared and a calibration curve was plotted using their absorbance. Concentration of ascorbic acid in the sample was calculated and reported as mg/100g ascorbic acid equivalent (AE)

Determination of alkaloids

Alkaloid content was determined according to previous methods with modifications [15]. The extract from 2 g of sample was concentrated on a water bath (50 °C) for 4 hours to one quarter of the original volume. Absolute ammonium hydroxide (10 ml) was added drop wise to the concentrate until the precipitation was completed. The solution was allowed to settle, and the precipitate was collected, washed with dilute (ammonium hydroxide, 2 M, and filtered. The residue was then considered as alkaloid which was dried and weighed. The formula below was used to calculate the percentage of alkaloids in the sample.

$$\text{Alkaloid (\%)} = \left(\frac{W3\text{-}W2}{W1}\right) * 100$$

Where, W3= weight of sample residue before drying; W2=weight of residues after drying; W1= original sample weight.

Screening for terpenoids and saponin

Qualitative determination of terpenoids was done using the Salkowski test according to standard procedures [20]. Five ml of each turmeric extract was mixed in 2 ml of chloroform 100 % (v/v), and 3 ml of absolute sulphuric acid (H_2SO_4) was carefully added to form a layer. Formation of a reddish brown layer at the interface showed the presence of terpenoids. Saponins were qualitatively determined using the standard foam test [21]. Five ml of the extract was transferred into a test tube and diluted with 5 ml of distilled water; the mixture was shaken vigorously for 2 minutes. Persistent appearance of foam lasting for at least 15 minutes confirmed the presence of saponin.

Determination of antioxidant capacity by DPPH

The free radical scavenging activities of the turmeric extracts against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich, USA) were determined by UV spectrophotometer at 517 nm [22]. Different concentrations of the extracts; 0, 1, 2, 4, 6, 8, and 10 mg/ml in methanol (analytical grade) were prepared. Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was put in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbances were read at 517 nm after 5 minutes in UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The radical scavenging activity was calculated using the following formula:

$$\text{\% Inhibition of DPPH} = \left(\frac{AB - AA}{AB}\right) * 100$$

Where *AB*=absorption of the blank sample; *AA*= absorption of the tested extract solution.
The results were expressed as percentage inhibition of DPPH and mean inhibitory concentration at 50% (IC_{50}) determined from a plot of percentage inhibition of DPPH versus concentration.

Microbial culture and evaluation of the antimicrobial activity

Three food-borne microorganisms were used in this study: *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans* corresponding to Gram-positive bacteria, Gram-negative bacteria and yeasts respectively. Standard microbial cultures were obtained from the source culture collection at Food Microbiology Laboratory, JKUAT, and sub-cultured using recommended procedures to maintain the viability of cells. The concentrations of the actively growing broth cultures were adjusted using established procedures to obtain a working culture of 10^6cfu/ml.

Antimicrobial activity was investigated using the agar well diffusion method [23]. Agar media was prepared according to the manufactures instructions. Nutrient agar (NA) was used for bacteria and Sabouraud Dextrose Agar (SDA) for yeast. Twenty ml of the sterile agar media was transferred to the

respective sterile petri-dishes and allowed to solidify. Inoculums (100 µl) of each microbial suspension was dispensed and evenly spread on the surface of the media in the agar plates. Using a sterile cork borer, four 9 mm wells were cut into the inoculated media in each agar plate; and the bottom of the well was sealed with molten nutrient agar. Subsequently, 100 µl of each turmeric extract (25 mg/ml) was dispensed into two wells (treatment); negative control of the same amount of a particular solvent was dispensed in the remaining two wells.

Plates were then incubated at 37 °C for 24 hours for bacterial assays and 25 °C for 72 hours for yeast assay. After the incubation period, the observed zones of inhibition around the well were measured in mm using a Vernier caliper. The antimicrobial activity results were expressed in terms of the diameter of zone of growth inhibition in the agar plates, including the diameter of the well. Measurement of ≤10 mm zone was considered as inactive; 11-13 mm as partially active; while 14-19 mm as active and ≥ 20 mm as very active [24].

Data analysis

All measurements were done in triplicate and values reported as mean ± standard deviation (SD). ANOVA tests were performed using GenStat software to determine significant differences among extracts. Statistical comparisons were separated using Bonferroni posthoc test with Least Significant Difference (LSD) considered at $P \leq 0.05$.

Results and Discussion

Phenolic content

The phenolic content of the organic solvent (acetone, ethanol, and methanol) extracts was significantly higher than the corresponding aqueous extracts ($P<0.05$) (Table 1). Values obtained in this study are in the range of those reported previously for ethanol, methanol, and water extracts of turmeric [9]. On the contrary, higher values were reported for turmeric extract; 22790, 17210, 9010, 380 mg in GAE/100g in acetone, ethanol, methanol and water respectively [25]; although the trend is similar, being high in acetone extracts, and low in water extracts.

Variation in total phenolic content is attributed to the extraction method, nature, and concentration of the solvent as well as the source/nature of the raw material for extraction. In a previous study, 70 % acetone was considered as the most efficient solvent for extracting TPC in a wide range of raw vegetables [26]. Solubility of phenolic compounds is generally higher in mixtures of aqueous organic solvents compared to the absolute aqueous or organic solvent system [27]. The high efficiency of acetone in extracting total phenolic content could be due to its ability to prevent the protein-polyphenol binding which forms an insoluble complex in the food matrix [28]. It is suggested that acetone is able to inhibit the formation of protein-polyphenol complexes during extraction, or perhaps breakdown the interaction between the functional groups of polyphenols (-OH) and the carbonyl group of proteins [29].

Flavonoid content

In this study, the total flavonoid content was significantly high in ethanol and acetone extracts (411.88 and 382.66 mg QE/100 g, respectively) compared to 58.11 mg QE/100 g in water extracts ($p<0.05$) (Table 1). Flavonoid is reported to form the largest proportion of dietary polyphenols (up to 60 %) [30]. As a result of its abundant presence in plant materials and good biological functions, flavonoids continue to be investigated as a potential source of drug or food supplements [27]. Previous reports have presented turmeric as a good source of flavonoids possessing an antioxidant/ free radical scavenging ability [2].

Lower quantities of flavonoid were reported for turmeric extracts from Malaysia compared to the results of this study [31]. The high flavonoid content in organic solvent is corresponding to the phenolic content in the respective extracts. This trend was also observed between the organic and aqueous solvents of different turmeric varieties in Bangladesh [32]. The low flavonoid content in water extracts may be due to the fact that curcuminiods are insoluble in water [33]; although curcuminiods are not classified as flavonoids, they are known to act the same way as flavonoids compounds when reacted with Aluminium chloride, which is the procedure involved in the determination of total flavonoid content [25].

Taninn content

Tannin was significantly highest in acetone extract and lowest in the ethanol extracts (175.86 and 20.45 mg CE/100g respectively). Methanol and water extracts had a non- significant difference in tannin content ($p> 0.05$) (Table 1). This finding is in agreement with previous reports where water extracts of most turmeric varieties contained higher levels of tannins compared to the ethanolic extracts [32]. Tannins are important water-soluble plant secondary metabolites which

Table 1: Total phenolic, flavonoid, and tannin content of different Turmeric (*Curcuma longa*) solvent extracts.

Solvent	TPC (mg QAE/100g)	TFC (mg GE/100g)	TC (mg CE/100g)
Acetone	1379.94 ± 62.93^{c}	382.66 ± 20.83^{c}	175.86 ± 3.24 ^{c}
Ethanol	515.60 ± 37.78^{b}	411.88 ± 29.2^{c}	20.45 ± 3.02^{a}
Methanol	561.16 ± 8.511^{b}	339.01 ± 18.62^{b}	96.19 ± 0.19^{b}
Water	307.45 ± 43.91^{a}	58.11 ± 6.44^{a}	71.56 ± 10.31^{b}
p	***< 0.001***	***< 0.001***	***< 0.001***

Results are expressed as the mean ± Standard deviation of triplicate determination. Different superscript letters along the column show significant difference ($P < 0.05$). TPC=Total phenolic content, TFC=Total Flavonoid content, TC=tannin content.

have been reported to have stringent, antioxidant, anti-inflammatory, and antimicrobial property [34]. Their strong anti-oxidative action is reflected in the free radical scavenging activity, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation [27].

Ascorbic acid content

Ascorbic acid content varied significantly among the different extracts ($P<0.05$). Water extracts had the highest content followed by methanol, ethanol, and acetone, respectively (Table 2). This trend of results is expected as ascorbic acid is a water soluble vitamin. According to previous reports, ascorbic acid content was in the range of 0.08-5.5 mg AE/g for turmeric and other dry spices from India [35]. This finding is of great interest as vitamin C is a strong antioxidant that directly interacts with a broad spectrum of harmful reactive oxygen species to terminate the reaction initiated by free radicals via electron transfer; it is also involved in the regeneration of other antioxidants, such as tocopherol, to their functional state [32, 35].

Alkaloids

In this study, the alkaloid content was in the range of 14.97 to 8.15 % for water and ethanol extracts, respectively (Table 2). This is in the range of that reported for other spices; pepper (13.44%), ginger (11.21%) and 2.54 % in garlic [36]. The basic nature of alkaloids facilitates the formation of salts with minerals or organic acids; these alkaloid salts are soluble in water and dilute alcohols.

Saponins and terpenoids

Saponin was present in aqueous, acetone and methanol extracts, but not detected in ethanolic extracts (Table 2). Terpenoids on the other hand, were present in both the aqueous and organic solvent extracts of turmeric. In other studies, saponins were not detected in ethanolic and aqueous extracts; whereas terpenoids was detected in aqueous extract and not in ethanolic extracts of turmeric [37]. Plant Saponins and terpenoids are secondary metabolites that are mainly involved in defense mechanisms and are related to the repair of damaged tissues. This qualifies terpenoids as natural antibiotics [36]. Large quantities of terpenoids in turmeric extracts could be contributing to their antimicrobial activity. Previous research has shown that terpenoids have anticancer, antibacterial, antifungal, antimalarial, and anti-inflammatory properties [24].

Antioxidant activity

The antioxidant activity when determined according to 2,2-diphenyl-1- picryl hydrazyl (DPPH) free radical scavenging activity, designates the ability of the extracts to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution; due to the formation of non-radical form of DPPH-H in the reaction solution. The purple color of DPPH will reduce to yellow-colored α, α-diphenyl-β-picrylhydrazine; scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate the scavenging activity of antioxidants [29].

Turmeric extracts exhibited varied antioxidant activity, reported as percentage inhibition against different concentrations. The DPPH radical scavenging activity of the turmeric extracts increased in a concentration-dependent manner from 1-10 mg/ml (Figure 1). Free radical scavenging activity is denoted by inhibitory concentration at 50 (IC_{50}); defined as the concentration of the extract required to decrease DPPH free radical absorbance by 50%.

Acetone extract had a significantly highest free radical scavenging ability (IC_{50}= 0.10 mg/ml), which compared favorably with Vitamin C; followed by ethanol (1.72 mg/ml), water (6.98 mg/ml) and methanol (7.24 mg/ml) extracts respectively (Figure 2). This suggests that acetone and ethanol are more efficient in extracting compounds with strong free radical scavenging activity compared to methanol. This trend was also reported for most medicinal plant species. When acetone extracts were compared to the methanol extracts; for instance, IC_{50} of *Thymelaea hirsuta* in acetone (0.17mg/ml) was almost 11 times lower than in the methanol (1.90 mg/ml) extracts [11]. The free radical scavenging abilities of the ethanolic and aqueous extract (IC_{50}: 1.72; 6.98 mg/ml) in this study were lower than those reported for ethanolic and aqueous extracts (IC_{50}: 1.08 to 3.03; 5.31 to 16.55 μg/ml) of popular turmeric varieties in Bangladesh [32]. However, variations in the antioxidant activity of ethanolic and aqueous extracts followed a similar trend, thus indicating the influence of extraction solvent on antioxidant properties of extracts.

The relationship between Inhibitory concentration (IC_{50}) and TPC is shown in figure 2. Higher phenolic content in acetone extracts resulted in a significantly lower IC_{50}, hence higher free radical scavenging activity. Ethanol extracts had a lower phenolic content, yet with a low IC_{50} (high free radical scavenging ability). This is attributed to the effectiveness of

Table 2: Vitamin C, alkaloid, saponin, and terpenoids content of different Turmeric (*Curcuma longa*) solvent extracts.

Solvent	Vitamin C (mg AE/100g)	Alkaloid (%)	Saponin	Terpenoids
Acetone	11.77 ± 0.06^{a}	11.68 ± 1.34^{b}	+	+++
Ethanol	19.97 ± 0.15^{b}	8.15 ± 0.13^{a}	ND	+++
Methanol	26.82 ± 1.41^{c}	9.83 ± 0.99^{ab}	+	++
Water	62.78 ± 0.03^{d}	14.97 ± 0.69^{c}	+	+++
p	*< 0.001*	*< 0.001*		

Results are expressed as the mean ± Standard deviation of triplicate determination. Different superscript letters along the column show significant difference ($P < 0.05$). Key +: present in minute quantity, ++: present in medium quantity, +++: present in large quantity, ND: Not detected.

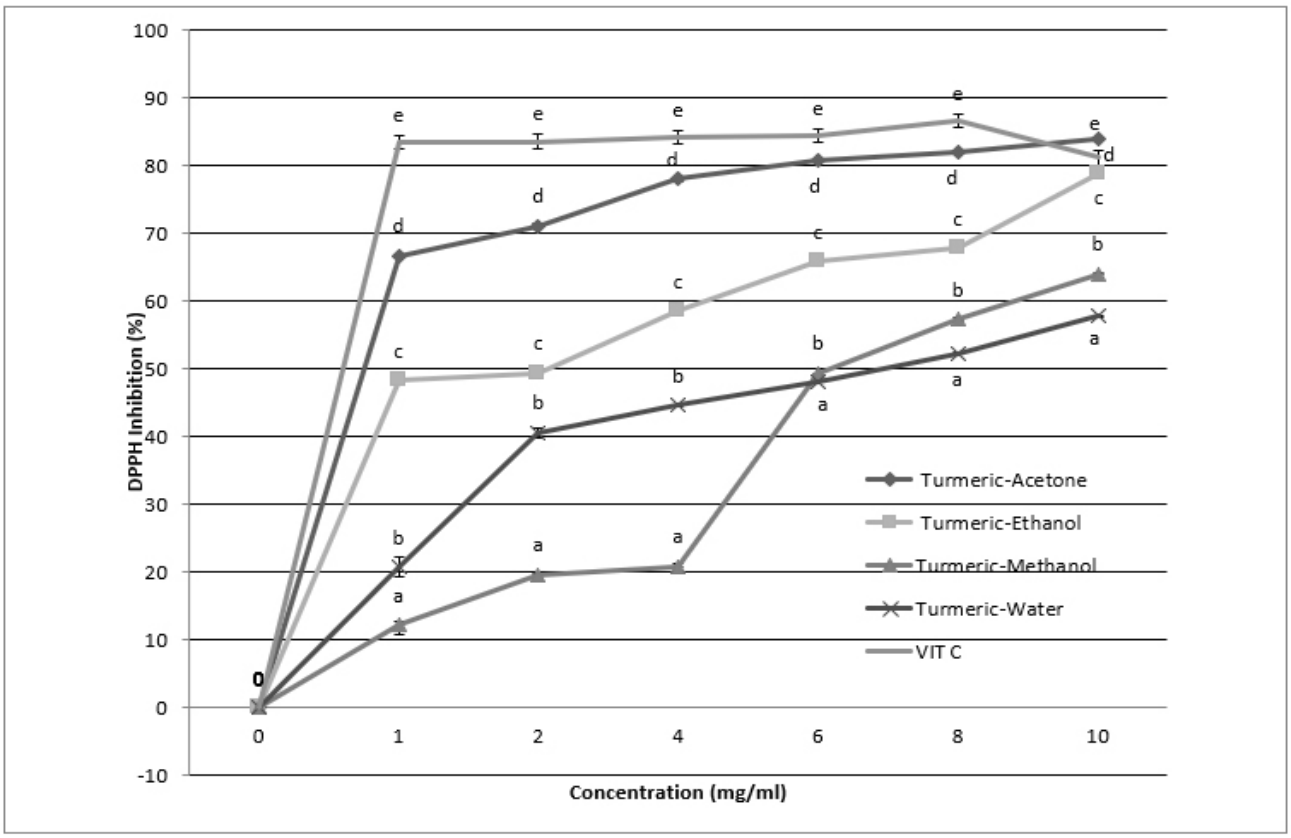

Figure 1: DPPH Inhibition of aqueous and organic solvent turmeric (*curcuma longa*) extracts at different concentrations. Results are expressed as the mean ± Standard deviation. Different letters (a, b, c, d, and e) show significant differences at the same concentration ($P < 0.05$).

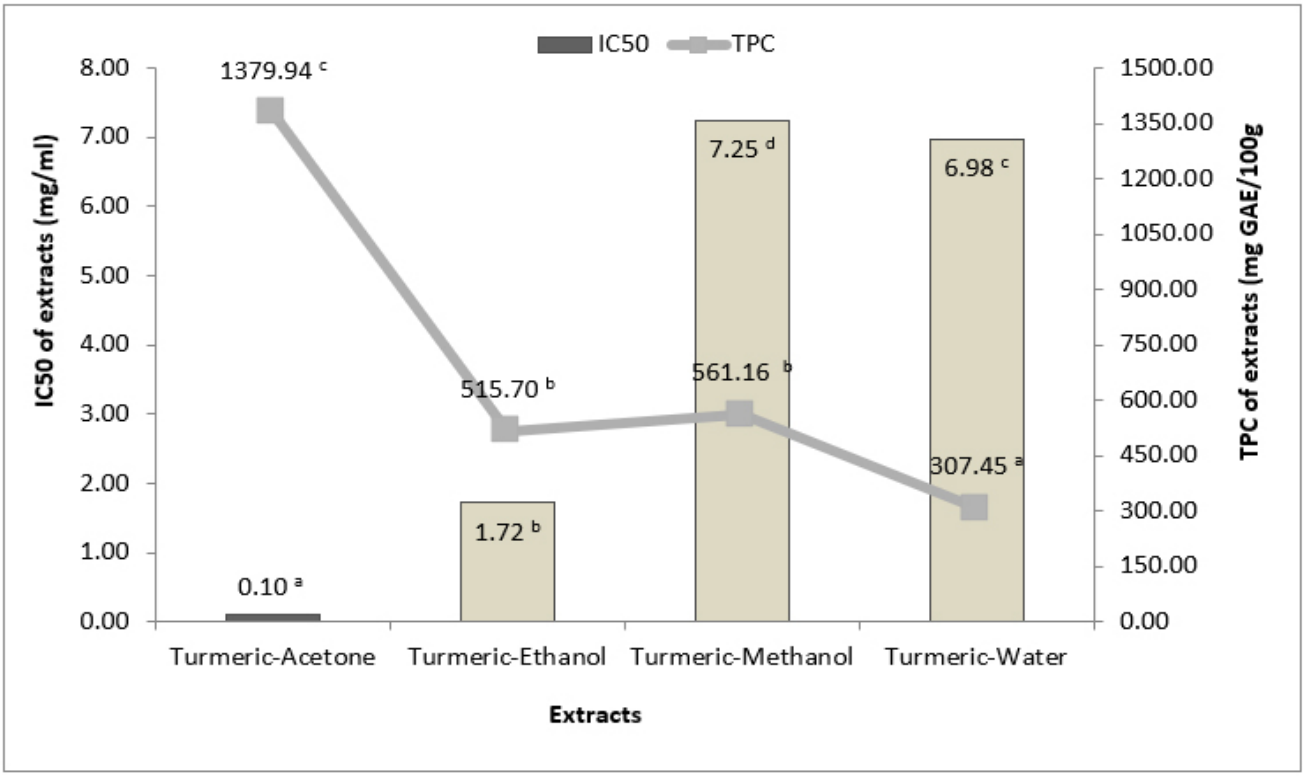

Figure 2: Relationship between total phenolic content and IC $_{50}$ of different turmeric (*curcuma longa*) extracts. Results are expressed as mean ± Standard deviation. Different letters (a, b, c, d) show significant differences in IC_{50} and phenolic content ($P < 0.05$).

ethanol in extracting compounds that possess strong antioxidant activities. A similar trend was observed for ethanolic extracts of turmeric in the DPPH assay with IC_{50} of 200 μg/ml [3]. This result suggests the suitability of turmeric for replacing/supplementing the use of synthetic antioxidants in food systems, with additional health benefits [38]. The antioxidant activity of extracts with high phenolic content is attributed to their ability to donate hydrogen atoms or electrons and to capture free radicals; phenolic compounds in spices and herbs are reported to have significantly contributed to their antioxidant properties [4].

Effect of solvent extraction on the antimicrobial activity of turmeric extracts

Turmeric extracts exhibited varied antimicrobial activity against the tested microorganisms. Antibacterial activity of raw juices against *S. aureus* (17.00 mm) was significantly higher than solvent extracts (14.33 to 14.67 mm) at 25 mg/ml. Ethanol extracts had the widest inhibition zone against *E. coli,* while water extract had the lowest inhibition zone against *E. coli* and the highest against *C. albicans* (Table 3).

These results were in the range reported in which all organic and aqueous extracts of turmeric effectively inhibited the growth of the clinical *Staphylococcus* strains and standard *S. aureus* with inhibition zones ranging from 10-20 mm and 10-19 mm at 50 mg/ml for organic and aqueous extracts [8]. Differences in the diameter of the zones of inhibition are attributed to the higher concentration of extracts in the previous study. It can be envisaged that the use of turmeric extracts in food application can aid in preventing the growth of food-borne spoilage organisms.

Ethanol extract exhibited a significantly high antibacterial activity (18.33 mm) compared to acetone (15.00 mm) and methanol (16.00 mm) against *E. coli;* whereas water extract was partially active. This is in line with previous reports where organic solvent extracts of turmeric were more active; methanol (26 to 28 mm) and ethanol (22 to 24 mm) against *E. coli* compared to aqueous extracts (17 to 18 mm) [24]. Efficacy of organic solvents extracts is attributed to the ability of the solvent to dissolve organic compounds in turmeric; hence liberating the antimicrobial components for activity. There are reports that phenols, alkaloids, and flavonoids are responsible for the activity. In a previous study, alkaloids inhibited the growth of *S. aureus* and *E. coli* [24]. Other reports on the activity of turmeric extracts against *E. coli* have suggested that the activity is due to the presence of curcumin and other curcuminiods, which are phenolic compound [10].

Effectiveness of turmeric extracts against *E. coli* and *S. aureus* is remarkable for the control of food borne pathogens in the food industry; which is reported to cause several diseases with significant effect on the human health [39]. Additionally, this provides an alternative for handling the current challenge of antimicrobial resistance reported in the food chain [40]. Moreover, most Gram positive bacteria are known to be more resistant to synthetic antibiotics than Gram negative bacteria.

In this study, turmeric extracts exhibited very high activity against *C. albicans.* Water extract had the highest inhibition zone (29.33 mm), followed by ethanol and methanol extracts (25.33 mm). Activity of curcumin, a major component in turmeric, against clinical and standard strains of Candida was previously reported [41]. The antifungal activity of aqueous extracts is probably due to the anionic components such as thiocyanate, nitrate, chlorides, and sulphates among other water-soluble components which are naturally occurring in the plant material. Among the mechanisms suggested for the antifungal effect of spices are cytoplasm granulation, cytoplasmic membrane rapture and inactivation and/or inhibition of intracellular and extracellular enzymes [24].

Conclusion

According to the study, the extraction solvent had an impact on the phytochemical content, antioxidant capacity, and antimicrobial activity of the turmeric extracts. While ethanol performed better for the extraction of flavonoids, acetone was the best solvent for extracting total phenolic compounds, flavonoids, and tannins. Acetone was least effective for extracting vitamin C, while water was most effective for extracting vitamin C and alkaloids; high amounts of terpernoids were extracted using both aqueous and organic solvents. Acetone and ethanol extracts were more potent in scavenging free radicals than methanol and water extracts. Different polarity of the extraction solvent and the solubility of the plant materials in the solvents were thought to cause variations in extraction ability and activity.

At a concentration of 25 mg/ml, the turmeric extracts had varying effects on the different microorganisms; being more potent as antifungal than antibacterial agents. Water extracts

Table 3: Antimicrobial activity of aqueous and organic solvent turmeric *(Curcuma longa)* extracts.

Solvent	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Raw	17.00 ± 1.00^{b}	16.00 ± 1.00^{b}	17.67 ± 0.58^{a}
Acetone	14.67 ± 1.15^{a}	15.00 ± 1.00^{ab}	19.00 ± 1.00^{a}
Ethanol	14.67 ± 0.58^{a}	18.33 ± 2.08^{c}	25.33 ± 3.51^{b}
Methanol	14.33 ± 0.58^{a}	16.00 ± 1.00^{b}	25.33 ± 0.58^{b}
water	15.67 ± 1.15^{ab}	13.00 ± 1.00^{a}	29.33 ± 1.15^{c}
P value	***0.014***	***0.006***	***<.001***

Results are expressed as the mean ± Standard deviation of triplicate determination, including the diameter of the well (9.00 mm). Different superscript letters along the column show significant difference among extracts ($P < 0.05$). Diameter of Inhibition zone ≤ 10.00 mm is considered as no activity.

had the highest activity against *C. albicans* and ethanolic extracts was the most effective against *E. coli*. Activity against *S. aureus* was comparable for all extracts.

The study concluded that, turmeric extracts in both aqueous and organic solvents are possible sources of natural antioxidants and antimicrobial agents. To give more information needed to support the use of turmeric as a natural preservative in the food industry, additional research is required to examine the in vivo efficacy against free radicals and food-borne bacteria.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Author Contribution

All Authors contributed equally to the work.

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