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ORIGINAL ARTICLE

Comprehensive Evaluation of Phytochemical Composition, Antibacterial and Antioxidant Activities, and Genomic Variations among Different Turmeric Species and Commercially Available Turmeric Powders

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ABSTRACT:

This study aimed to extract curcuminoids from five turmeric varieties (*Curcuma longa*, *Curcuma aromatica*, *Curcuma caesia*, *Curcuma amada*, and *Curcuma angustifolia*) and investigate their phytochemical composition, antibacterial activity, and antioxidant potential. Curcuminoids were successfully extracted using acetone, butanol, and petroleum ether solvents, and their quantification was achieved using spectroscopic analysis. Phytochemical screening revealed the presence of various secondary metabolites. The extracted curcuminoids exhibited antimicrobial activity against selected bacterial strains and demonstrated antioxidant properties. Electrophoresis analysis allowed for genetic variation assessment among the turmeric varieties. This study provides valuable insights into the medicinal and culinary significance of turmeric and contributes to potential applications in the pharmaceutical and food industries.

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Introduction

Turmeric is a revered herb native to Asia and India which holds a great significance in traditional medicine such as Ayurveda. Turmeric is renowned for its anti-inflammatory, anti-infective, and digestive benefits. Its potent properties and minimal side effects exemplify the remarkable synergy between turmeric and the holistic principles of Ayurveda (Lantz et al., 2005).

The genus *Curcuma* comprises around 130 discovered species, with *Curcuma longa* (turmeric) being the most well-known and studied due to its therapeutic properties. Widely cultivated in India, *C. longa* is characterized by its large root, yellow tubers, and vibrant flowers. The *Curcuma* genus encompasses diverse species of economic and medicinal importance, with variations at both inter- and intraspecific levels. Turmeric is widely used as a flavoring agent and natural food colorant, and its curcumin content varies among different brands. Lesser-known *Curcuma* species like *C. amada Roxb*, *C. angustifolia* Roxb, *C. caesia* Roxb, and *C. aromatica* Salisb have significant roles in traditional medicine, offering potential treatments for various ailments. The rich diversity within the *Curcuma* genus highlights its valuable contributions beyond traditional medicine (Sharma et al., 2008; Sasikumar, 2005).

Plants synthesize secondary metabolites, encompassing alkaloids, terpenoids, phenols, flavonoids, tannins, and saponins, which exhibit diverse physiological effects on the human body. The phytochemical analysis of ethnomedicinal plants plays a pivotal role in uncovering therapeutic agents and novel reservoirs of bioactive compounds (Chin et al., 2006). Curcumin, the principal yellow bioactive constituent present in *Curcuma longa* and related species, demonstrates a broad spectrum of biological activities, including notable anticancer properties. Curcuminoids, comprising curcumin, bis -demethoxycurcumin, and demethoxycurcumin, contribute to the distinctive yellow-orange pigmentation observed in *curcuma* plants (Jayaprakasha et al., 2006). Notably, curcumin acts as a potent antioxidant by augmenting the levels of antioxidant enzymes and scavenging reactive oxygen species. The consumption of plant-derived antioxidants, such as curcumin, assumes significance in safeguarding the body against oxidative damage inflicted by reactive oxygen species (Ak & Gülçin, 2008; Jayaprakasha et al., 2006; Wright, 2002). These findings underscore the significance of curcumin in traditional medicine and its potential as a natural reservoir of valuable bioactive compounds.

Turmeric, a sterile, triploid species, is predominantly propagated vegetatively through its underground rhizomes due to its cross-pollinated nature (Corcolon et al., 2015). Genome size and chromosome numbers significantly influence various traits, the representation of such data is predominantly skewed towards tropical and subtropical regions, neglecting other geographic areas.

The current study aims to assess the genetic variation, secondary metabolite profiles, curcumin content, antibacterial activity, and antioxidant potential of five *Curcuma* species (*C. amada, C. angustifolia, C. caesia, C. longa, and C. aromatica*).

Materials and Methods:

Materials:

Fresh and dried turmeric rhizomes were collected from five distinct species, namely *Curcuma longa, Curcuma aromatica, Curcuma*

amada, Curcuma caesia, and *Curcuma angustifolia*, in the Idukki district of Kerala, India. Nutrient agar medium from HiMedia was used for antibacterial activity verification, along with laboratory-grade solvents including acetone, petroleum ether, ethanol, and butanol etc.

Methodology:

Collection and Preparation of Turmeric Rhizomes:

Fresh rhizomes of *Curcuma longa, Curcuma aromatica, Curcuma casiea, Curcuma angustifolia,* and *Curcuma amada* were collected from Peermade, Idukki district. The rhizomes were cleaned, washed with deionized water, peeled off, sliced and dried in the shade for a week. Dried rhizomes were then cut into small pieces and powdered for further analysis.

Extraction and Concentration of Turmeric Extracts:

A total of 50g of the coarse powder of dried rhizomes from each species (*C. longa, C. aromatica, C. amada, C. caesia,* and *C. angustifolia*) was mixed with 100 ml of solvents including acetone, butanol, and petroleum ether. The mixture was allowed to soak for three days with occasional shaking. The extracts were then filtered and the excess solvent was evaporated to concentrate the extracts. The concentrated extracts were stored in closed conical flasks under refrigeration until further use.

Antimicrobial Activity and Quantitative Analysis of Curcuminoids:

The prepared extracts were used for the assessment of antimicrobial activity and quantitative analysis of curcuminoids. The

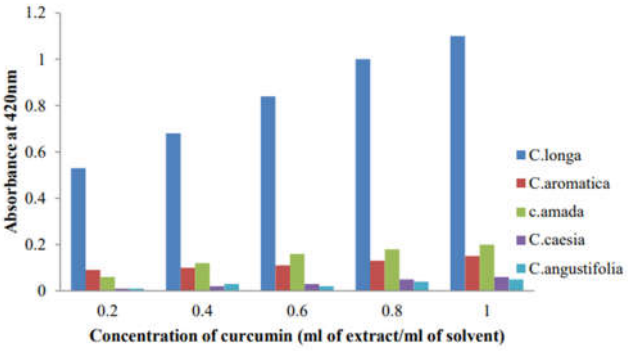

Fig.1. Solubility of curcumin in acetone

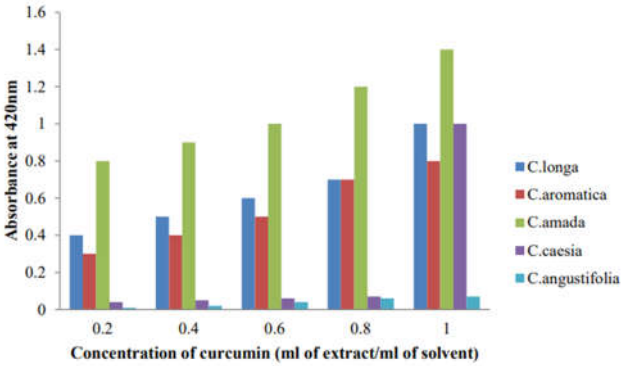

Fig.2. Solubility of curcumin in butanol

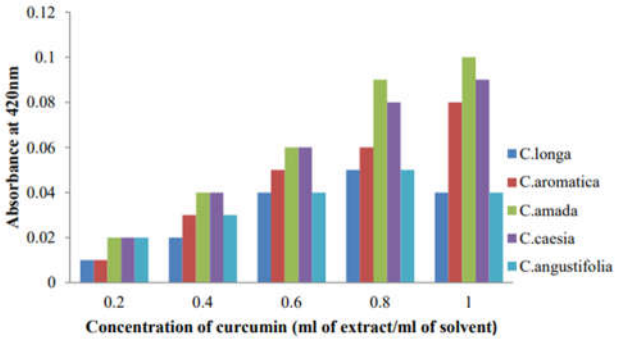

Fig.3. Solubility of curcumin in petroleum ether

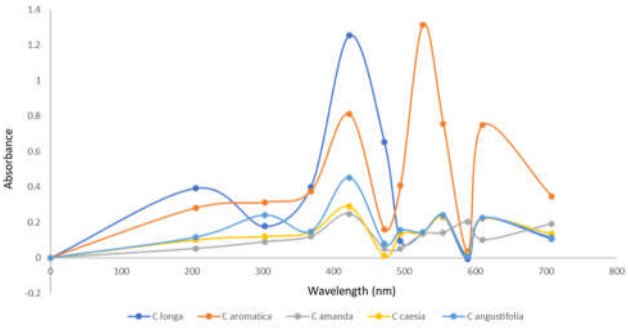

Fig.4. UV Visible spectra of curcumins

absorbances of the extracts were measured at 420nm using a spectrophotometer, against the blank

solvents. The curcumin content in the extracts was determined and expressed as a percentage.

Spectrophotometric Estimation of Curcumin Content in Turmeric Extracts:

Curcumin was quantitatively extracted from the turmeric samples by refluxing the material in acetone. The curcumin content was estimated spectro-photometrically using a spectrophotometer within the wavelength range of 200 to 700nm. The absorbance of curcumin exhibits a strong broad peak around 420 nm. Therefore, the curcumin content was estimated spectro-photometrically at approximately 420 nm for all the turmeric extracts.

Phytochemical Screening:

Phytochemical screening of the turmeric samples involved conducting various tests to determine the presence of specific phytoconstituents. Commonly employed precipitation and coloration reactions were employed for this purpose, following standard procedures described in reference books. The aqueous extracts were prepared by soaking the powdered samples in distilled water, followed by filtration. Tests were performed to detect the presence of carbohydrates (Molish's test), proteins (Biuret test), phlobatannins (boiling with 1% aqueous HCl), phenols (reaction with ferric chloride), flavonoids (alkaline reagent test), coumarin (reaction with NaOH), emodins (reaction with NH_4OH and benzene), syringyl groups (treatment with $KMnO_4$, HCl, and NH_4OH), terpenoids (reaction with chloroform and con. H_2SO_4), diterpenes (reaction with copper acetate), cardiac glycosides (Keller-Killani test), tannins (reaction with ferric chloride), carotenoids (treatment with con. HCl and phenol), alkaloids (reaction with Wagner's reagent), and saponins (boiling with water and observation of frothing). The appearance of characteristic colors, precipitates, or frothing indicated the presence of specific phyto-constituents.

Antioxidant assay:

For the antioxidant assay, the reducing power and superoxide dismutase (SOD) activity of the turmeric extracts were determined. The reducing power assay involved mixing the extracts (100-500µg/ml) with phosphate buffer and potassium ferricyanide, followed by incubation and termination of the reaction. The absorbance of the resulting mixture was measured at 700nm, and increased absorbance indicated higher reductive capacity. Ascorbic acid was used as a reference material. For the SOD assay, the extract was mixed with ethanol and chloroform, and the supernatant was collected after centrifugation. The reaction mixture containing pyrogallol was prepared, and the rate of auto-oxidation was measured at 470nm using a spectrophotometer at regular intervals.

Results and discussions.

Curcumin Content.

The spectrophotometric analysis revealed that the turmeric samples (*Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia,* and *Curcuma angustifolia*) exhibited varying curcumin content. Measurements of absorbance at 420nm, corresponding to the λmax of curcuminoids, were obtained for each sample in different solvents (acetone, butanol, and petroleum ether) as shown in Fig.1,2 and 3. Furthermore, notable differences in curcumin solubility were observed across the solvents tested. These findings highlight the impact of genetic and environmental factors on curcumin levels and solubility in

Table 1: Results of Phytochemical Screening in Different Curcuma Species

S.No	SPECIES	CARBOHYDRATE	PROTEIN	ALKALOIDS*	TERPENOIDS	FLAVONOIDS*	PHENOL*	PHLOBATANNINS	TANNINS	DITERPENES	CAROTENOIDS	GLYCOSIDES	SAPONINS	EMODINS	SYRINGYL GROUPS	COUMARINS
1	*Curcuma longa*	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+
2	*Curcuma aromatica*	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
3	*Curcuma amada*	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-
4	*Curcuma caesia*	+	+	+	+	+	-	+	-	-	+	-	+	-	+	-
5	*Curcuma angustifolia*	+	+	+	-	+	-	+	-	-	+	-	+	-	+	-

different *Curcuma* species. Further investigations are essential to identify and characterize the specific compounds responsible for these variations and explore their potential bioactive properties within each species. The spectrophotometric analysis of three different brands of commercially available turmeric powder was conducted to compare their curcumin content. Pure curcumin exhibited an absorbance of 1.12 at 420nm, while brand A showed an absorbance of 1.76, brand B showed 1.32, and brand C showed 1.66. These results indicate the presence of coloring agents in the samples, as the spectrophotometer measures the colored substances in the sample (Fig. 4).

Phytochemical screening

The phytochemical screening reveals that the methanolic and aqueous extracts of the rhizomes from *Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia,* and *Curcuma angustifolia* contain various pharmacologically active compounds.

The results of the phytochemical screening revealed the presence or absence of various compounds in the different species of Curcuma. All species showed the presence of carbohydrates, proteins, alkaloids, terpenoids, flavonoids, phenols, and diterpenes. However, *Curcuma amada* and *Curcuma angustifolia* lacked alkaloids and diterpenes, respectively. Phlobatannins were present in *Curcuma longa, Curcuma aromatica*, and *Curcuma caesia* but absent in *Curcuma amada* and *Curcuma angustifolia*. Tannins were found in *Curcuma longa* and *Curcuma aromatica* only. Carotenoids were present in *Curcuma longa* and *Curcuma caesia*, while glycosides were detected in *Curcuma longa* and *Curcuma amada.* Saponins were found in *Curcuma longa*, *Curcuma aromatica*, and *Curcuma amada*, but absent in *Curcuma caesia* and *Curcuma angustifolia*. Emodins were present in *Curcuma longa*, *Curcuma aromatica*, and *Curcuma amada*. Syringyl group compounds were detected in *Curcuma longa* and *Curcuma caesia*, whereas

Table 2: Antibacterial Activity of Turmeric Varieties against Selected Bacterial Strains

		Bacterial Species			
Turmeric Variety	**Solvent Used**	***Klebsiella***	***Bacillus***	***Proteus***	***E. coli***
Curcuma longa	Acetone	+	+	+	-
	Butanol	-	+	-	-
	Petroleum ether	+	+	+	+
Curcuma aromatica	Acetone	+	-	+	-
	Butanol	+	-	-	-
	Petroleum ether	+	+	+	+
Curcuma amada	Acetone	-	-	+	-
	Butanol	+	-	+	-
	Petroleum ether	+	+	+	+
Curcuma caesia	Acetone	-	-	+	-
	Butanol	+	-	-	+
	Petroleum ether	+	+	+	+
Curcuma angustifolia	Acetone	-	-	-	-
	Butanol	+	-	-	+
	Petroleum ether	+	+	+	+

Note: "+" indicates presence of zone of inhibition against the respective bacterial strain, while "-" indicates absence of zone of inhibition.

Table 3: Results of Superoxide Dismutase (SOD) Activity at 470nm

Samples	**Acetone**	**Butanol**	**Petroleum ether**
Blank	0	0	0
C. longa	2.81	0.43	0.74
C. aromatic	2.36	0.54	0.331
C. amada	1.56	0.46	0.32
C. caesia	1.65	0.36	0.24
C. angustifolia	0.98	0.25	0.13

Table 4: Results of Reducing Power Assay at 700nm

Turmeric Species	**OD at 700nm**
Curcuma longa	0.74
Curcuma aromatica	1.42
Curcuma amada	0.97
Curcuma caesia	1
Curcuma angustifolia	1.03

coumarins were absent in all species.

The results indicate that the different species of *Curcuma* possess varying profiles of phytochemical compounds. These compounds are known to contribute to the biological activities and potential health benefits associated with *Curcuma* species. The variations observed in the presence or absence of specific compounds among the species suggest differences in their potential therapeutic properties and pharmacological activities. Further investigation and analysis of these phytochemical constituents can provide valuable insights into the medicinal and nutritional value of each *Curcuma* species.

The results of the analysis are summarized in Table 1, indicating the presence or absence of specific constituents in the extracts. The legend for

the table of phytochemical screening indicates that "+" represents the presence of phytochemical compounds, "-" represents their absence, and "*" signifies samples that were extracted using ethanol.

Antimicrobial activity

The antimicrobial activity of different turmeric varieties (*Curcuma longa, Curcuma aromatica*, *Curcuma amada, Curcuma caesia,* and *Curcuma angustifolia*) was evaluated against selected bacterial strains (*Klebsiella, Bacillus, Proteus*, and *E. coli*). The results showed variations in the antibacterial activity among the turmeric extracts, depending on the solvent used and the bacterial strain tested. The zone of inhibition indicated the effectiveness of turmeric against specific bacteria. A summary of the antimicrobial results can be found in Table 2.

Antioxidant properties

The antioxidant activity of five different species of *Curcuma* (*Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia,* and *Curcuma angustifolia*) was evaluated through Super Oxide Dismutase (SOD) activity and reducing power assay. The results showed that a complex of three curcuminoids exhibited notable antioxidant activity, with *Curcuma longa* displaying the highest SOD activity. The reducing power assay revealed that *Curcuma aromatica* had the highest reducing power among the tested species. These findings suggest that the *Curcuma* species possess antioxidant potential, which can be attributed to their varying levels of bioactive compounds. Further exploration of these compounds and their potential therapeutic applications is warranted.

The table presents the absorbance values at 470nm for different samples in various solvents. The blank and solvents (acetone, butanol, petroleum ether) showed negligible absorbance, indicating their minimal contribution to the measured absorbance. The *Curcuma* species, namely *Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia*, and *Curcuma angustifolia*, exhibited varying levels of absorbance in the respective solvents. These absorbance values reflect the Super Oxide Dismutase (SOD) activity, with higher values indicating increased antioxidant potential.

Table 4 presents the absorbance values at 700nm for the acetone extracts of different species of turmeric. The higher the absorbance value, the greater the reducing power, indicating stronger antioxidant activity. The results indicate that *Curcuma aromatica* exhibited the highest reducing power with an absorbance of 1.42, followed by *Curcuma angustifolia* (1.03), *Curcuma caesia* (1.00), *Curcuma amada* (0.97), and *Curcuma longa* (0.74). These findings suggest variations in the antioxidant potential among the different species of turmeric.

Summary

In this study, various analyses were conducted to evaluate the differences and properties of different turmeric samples. Phytochemical screening revealed the presence or absence of various phytochemical compounds, with *Curcuma longa* and *Curcuma aromatica* showing positive results for all tested compounds, while other species exhibited variations in their phytochemical profiles. The assessment of antioxidant activity revealed differences in superoxide dismutase activity and reducing power among the turmeric species, with *Curcuma longa* showing the highest superoxide dismutase activity and *Curcuma aromatica* exhibiting the highest reducing power.

Conclusion

This comprehensive analysis highlighted the variations in phytochemical composition, curcumin content, antibacterial activity and antioxidant activity among different turmeric species and commercially available turmeric powder samples. The presence of diverse phytochemical compounds and variation in curcumin content suggest the potential health benefits associated with different turmeric species. The antibacterial activity demonstrated the potential of turmeric powder samples in combating certain bacterial strains. The variation in antioxidant activity suggests differences in the free radical scavenging abilities of different turmeric species.

Future Prospects:

Further investigations could include more extensive phytochemical profiling to identify additional compounds responsible for the observed activities and explore their synergistic effects. In-depth characterization of curcuminoids and other bioactive compounds could provide a better understanding of their individual contributions to the overall properties of turmeric. Additionally, exploring the mechanisms underlying antibacterial and antioxidant activities would provide insights into their potential applications in medicine and functional foods. Further genomic studies could uncover genetic markers associated with specific traits and facilitate breeding programs for turmeric improvement. Moreover, clinical studies and bioavailability assessments would contribute to understanding the health benefits and optimal utilization of turmeric. These future prospects would advance the knowledge and utilization of turmeric in various fields, including pharmaceuticals, nutraceuticals, and functional foods.

Declaration of Conflict of Interest:

The authors declare no conflict of interest in conducting this research and publishing the findings.

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