Characterization of Curcumin Extracted from *Curcuma longa* Cultivating in Pakistan

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Session 2021-2023

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A Thesis Submitted in partial fulfillment of the requirements for
the degree of
Master of Science in

Plant Biotechnology

Supervised by

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August 2023

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I certify that this research work titled "Characterization of curcumin extracted from *curcuma longa* cultivating in Pakistan" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged / referred.

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Dedication

I dedicate this thesis to my Beloved Father (Dr. Nusrat Pasha) and Grandmother (Razia Begum). May Allah Subhanahu-Wa-Ta'ala Grant them the Highest place in Jannah. (Ameen)

Their Love and Guidance continue to inspire me, and I am forever grateful for their presence in my life.

Acknowledgement

In the name of **"ALLAH"** the Most Merciful, the Most Gracious. All praise is due to **Allah**; we praise Him, seek His help, and seek His forgiveness. First and foremost, I extend my heartfelt Gratitude to **Allah**, who has bestowed upon me the gift of life and showered me with countless blessings.

I cannot forget the Ideal man of the world and most Respectable Personality for Whom Allah created the whole universe, **Prophet Muhammad (Peace Be Upon Him)**

اللّٰهمَّ صَلِّ عَلَىٰ سَيِّدِنَا مُحَمَّدٍ النَّبِيِّ الْأُمِّيِّ وَعَلَىٰ آلِهِ وَصَحْبِهِ وَسَلِّمْ

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Abstract

Curcumin is a polyphenolic bioactive compound found in the rhizomes of *Curcuma longa L.* which has garnered considerable interest due to its diverse medicinal properties. The bioactive compound is currently being used as a commercial ingredient. In Pakistan not many industries are using curcumin as an active ingredient in their products despite rich cultivation of *Curcuma longa* (turmeric) in Pakistan. This highlights the untapped potential of local turmeric. This study aims to characterize curcumin extracted from locally cultivated *Curcuma longa*. A distinctive aspect of this research lies in the adoption of the concept of "Whole Plant Medicine", where the entire turmeric extract was utilized for characterization, rather than isolating pure curcumin. The primary objectives of this study were to assess the efficiency of various solvents for curcumin extraction and to analyze the structural composition and chemical profile of different turmeric extracts. In this research, curcumin was extracted from Pakistani local turmeric using a Soxhlet apparatus with four different solvents (methanol, ethanol, acetone, and glycerol), and its chemical characterization was performed through UV spectrophotometry, FTIR spectroscopy, and GC-MS analysis. Among the solvents used, methanol exhibited optimal results, with a distinct absorption peak observed at 425 nm in the UV spectrum, indicative of curcumin's presence. Subsequently, FTIR analysis provided insights into the presence of characteristic functional groups associated with curcumin. GC-MS analysis of the methanol extract unveiled the presence of potential bioactive compounds with intriguing pharmacology demonstrating the complexity of the extract's chemical composition. Overall, the findings revealed that methanol is the most efficient solvent for curcumin extraction. The comprehensive chemical profile analysis of the extracts exhibited the diverse array of phytochemicals present in Pakistani turmeric, underscoring its remarkable significance in the field of natural medicine.

Key Words: Curcumin, Curcuma longa, Soxhlet extraction, UV-Vis Spectroscopy, FTIR, GCMS

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List of Abbreviations

ASAB	Atta-Ur-Rahman School of Applied Biosciences
UV	Ultraviolet
Vis	Visible
FT IR	Fourier Transform Infrared Radiation
GCMS	Gas Chromatography Mass Spectrometer
TLC	Thin Layer Chromatography
TEA	Techno-Economic Analysis
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
UAE	Ultrasound-Assisted Extraction
MAE	Microwave-Assisted Extraction
Rpm	Revolutions Per Minute

Chapter 1: Introduction

Organic plant products have been utilized by human beings as medicines since the beginning of history which are till date contributing to the health care sector. This is because plant-based medicines are more suitable for human use than other synthetic drugs manufactured using combinatorial chemistry. Most of these plant products are secondary metabolites released as a defense mechanism against certain plant infections or diseases. Experimental evaluation of natural plant products revealed that most of them are non-toxic or they have effective doses far beneath their lethal doses which depicts that the role of plant based products in human health care sector can't be undervalued (Goel, Kunnumakkara, & Aggarwal, 2008). *Curcuma longa L*., also known as turmeric is one of the many medicinal plants which has been used by our ancestors to treat injuries, ailments and skin infections although its antioxidant properties were discovered much later. This is the reason why it is often being called as spice of life.

1.1 Botanical Description

Curcuma is a genus name which further contains five species out of which *C. domestica* and *C. longa* entail rhizomes of turmeric(G. Singh, Singh, Maurya, & materials, 2002). *Curcuma longa* also commonly known as turmeric is a triploid specie (2n = 3x = 63) (Mirjanaik, Vishwanath, & Phytochemistry, 2020). It is a perennial herb usually utilized in food flavoring, health care, beauty care and natural dye. Turmeric is a rhizomatic crop with short stems and oblong shaped large leaves. Rhizomes grow underground and their shape is usually elliptical, oval, or pyriform (B. Jyotirmayee & G. J. A. S. Mahalik, 2022). The height of this crop is almost 60-90cm. There are short spikes in the tuft of leaves where flowers are born. Almost 71.1% fertility has been reported in turmeric (Mirjanaik et al., 2020). Turmeric is dried rhizome which has a distinct orange color in the inside and has extremely strong aroma. The rhizome is further powdered and refined into granular form to be used as a spice. Turmeric is a main ingredient here in Pakistani cuisine (Akram et al., 2010).

1.2 Different Names of *Curcuma longa L.*

Turmeric is mentioned with various names in different parts of world like Haldi, Indian saffron, Arab globe Curcuma, Haridra, Chinese yellow ginger Jianguang, Ayurvedic, Ukon or Japanese Koyo(B. Jyotirmayee & G. J. A. S. Mahalik, 2022).

1.3 Geographical Distribution

Curcuma longa is a tropical crop. It is distributed all over the world especially in the tropics of south east Asia and north Australia (Mirjanaik et al., 2020). In accordance with Kew

Gardens India is the biggest producer of turmeric, providing 94% of the world's interest (providing approximately 20,000 tons every year) (Saeed et al., 2017). Turmeric is also grown in other parts of world i.e. Bangladesh, China, Pakistan, Sri Lanka, Myanmar, Taiwan, West Indies, Jamaica, Nigeria, Peru and other Latin American and Caribbean nations (B. Jyotirmayee & G. J. A. S. Mahalik, 2022). It is cultivated on a commercial scale and enters the market usually in the form of dried rhizomes which are then given final touch according to their end use (Saeed et al., 2017)

1.4 Climate, Cultivation and Harvesting

Turmeric crops need humid and warm climate with temperature range between 20°C to 30°C. Temperature below 20°C cease the growth. Crop is usually grown at the altitude of 1200-1500 and requires annual rainfall of 70-225cm (Mirjanaik et al., 2020). The crop grows lushly in shade, but much bigger and better rhizomes produce in spacious ground under sunlight. Turmeric is cultivated in different kinds of soil like sandy loam, clay loam, stiff loam, red soils. Soil should be friable and rich. Crops are generally harvested between January and April. Early varieties become mature in just 7-8 months while medium varieties take 8-9 months. When the leaves start drying and turn yellow, it indicates the time of harvesting. while harvesting, leaves are sliced near the ground, land is furrowed and with handpicking rhizomes are gathered or sometimes clumps are lifted with the help of spade (Yadav, Tarun, & Phytochemistry, 2017).

Turmeric is cultivated on a commercial level and dried rhizomes enter the market to be further processed as per their utilization. Some of the rhizomes are retained which are used as seeds for replanting.

1.5 Turmeric Cultivation in Pakistan

Turmeric is considered as a significant agriculture in Pakistan. Districts with major turmeric production are Lahore, Kasur, Sialkot, and Okara in Punjab, Sanghar and Mirpurkhas in Sindh and Haripur and Banu in Khyber-Pakhtunkhwa. Punjab province is the main contributor of turmeric production. Pie chart 1.1 illustrates turmeric cultivation across the country (Saeed et al., 2017). District Kasur is leading in turmeric cultivation with annual production of 30569 metric tons from 3157 hectare and accounts for almost 80% of country’s output (Qazi et al., 2020). Changa Manga city of district Kasur is the main hub of turmeric marketing in Pakistan from where bulk of dried turmeric rhizomes or its powder form is purchased by exporters for

further export to USA, UK, UAE, Sri Lanka, North African countries and Japan (Saeed et al., 2017).

Pakistan is blessed with the climate which is ideal for turmeric cultivation. It is sown on Soils like sandy and clayey loam. The best cultivating period for turmeric is from April to May. 2500kg rhizome fingers per acre are used as seed. For better yield it takes almost 20-40 irrigations and 5 to 6 tons FYM (Farmyard manure) per acre. Yellowing of lower leaves indicates maturity which may differ in different varieties. Almost an average of 20 to 25 tons of turmeric rhizomes are obtained per acre (Saeed et al., 2017).

1.5.1 Turmeric Market of Pakistan

Turmeric marketing across the country is not managed properly. This is the reason why the costs of new (crude) turmeric varies habitually in each season. Turmeric is a minor crop therefore minimal empirical work has been carried out on studying opportunities and capabilities in value chain and financial matters of this crop. Also, there is not much comprehension about its cultivation, processing, and marketing. Recently domestic grinding units have been established which have additionally settled in the review region transforming turmeric cultivation to exceptionally beneficial business venture (Saeed et al., 2017).

Figure 1.1: Cultivation of *Curcuma longa L.* in Pakistan

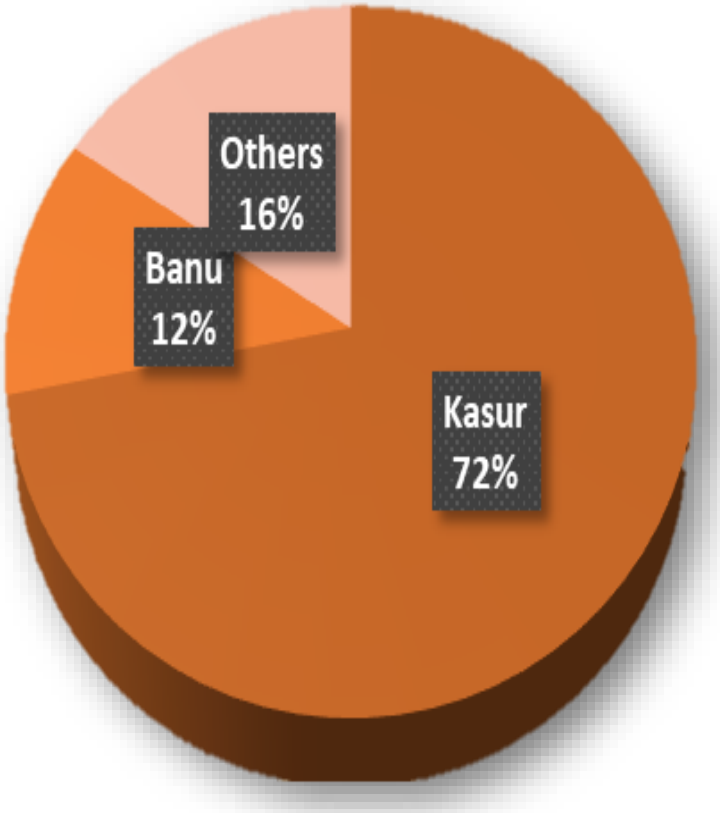

Figure 1.2: Major turmeric cultivating areas in Pakistan

1.6 Ethnobotany of Turmeric

Turmeric (*Curcuma longa L.*) is a spice which is widely cultivated since prehistoric times that traces all the way back to 4000 yrs. to Vedic culture in India where it was utilized as zest in culinary. Turmeric is called the "spice of life" and "golden spice". It is esteemed for its profound yellow tone (0.2-8% curcumin), aromatic flavor (1.5-5% volatile oils) and pungency (2.2-4.2% turmerol) (Mirjanaik et al., 2020). Also, turmeric is known to speed up the healing process in case of injury or ailment.

1.6.1 Common Household Remedy

Turmeric is typically used in a lot of household remedies to treat several ailments like cough, abdominal pain, ulcer, acidity, dysentery, skin infections, burns, bites, eye infections and dental disorders. "Haldi doodh" also known as "golden milk" is a very common remedy used by our ancestors to treat joint pains and inflammations. although the antioxidant properties of turmeric were discovered much later. After delivery, perineal laceration is treated with fresh turmeric to speed up the healing process. In rural areas of India fresh turmeric is applied as an antiseptic to clamped cord of infants Ladies are served with warm milk mixed with turmeric, ginger and honey to drink after labor (Gopinath, Karthikeyan, & leprology, 2018). Poultice, made with turmeric and slaked lime is scoured to the perineum, aids in the healing of lesions in birth canal. It also moderates the stimulating impacts of marijuana and other psychoactive medications (Fuloria et al., 2022). Turmeric with neem helps in treatment of chicken pox and small pox (Gopinath et al., 2018).

1.6.2 An Herbal Remedy

Turmeric (*Curcuma longa L*) has been widely used as a Traditional medicine for a lot of health conditions in Pakistan, India, Bangladesh, China and Middle Eastern nations over hundreds of years. It has been a significant component of Siddha, Chinese, Unani, Veterinary, folk medicine and Ayurveda (Gopinath et al., 2018). In Pakistan it is generally consumed as holistic medicine for healing wounds and treating acne (Fuloria et al., 2022). In traditional Ayurvedics, turmeric plant was considered as an astounding anti-inflammatory, antiseptic and analgesic. Simultaneously it was used in treating skin irritations and improving digestion. Likewise, in South Asia it has been utilized as a promptly accessible germicide for cuts, bruises, wounds and burns. Nonetheless, a few other helpful properties are accounted for in this folk medicine (Verma et al., 2018). In traditional Bhutanese medication, it's named Yung-ba, and is

utilized as an antidote, a tonic, an anti-inflammatory agent, an antiseptic and a preservative (Ayati et al., 2019).

1.7 Traditional Use of Turmeric

1.7.1 Condiment

Turmeric is popular in culinary recipes because of its bright color and aroma. Moreover, its preservative properties have expanded its appeal worldwide. It is an important part of curry powder (10-30% turmeric) used to season fish and meat. Almost 1.5g of turmeric is consumed each day by people of South east Asians (Gopinath et al., 2018). Vegan curry blends have less turmeric content because of the unpleasant taste. Oleoresins obtained after solvent extraction of powdered rhizomes are utilized in food industries.

1.7.2 Coloring Agent

Because of the deep yellow color of turmeric, it is used in the textile industry. It is utilized as a natural alternative to synthetic tartrazine because of similar colors. It is also used to color dairy items, cakes, soups, gravies, sauces, and cereals. Notwithstanding, it very well may be utilized exclusively for specific food items due to short storage capacity. This is because of its degradation and light sensitivity on heat exposures and synthetic oxidants (Gopinath et al., 2018).

1.7.3 Cosmetology

Turmeric might be the very first known cosmetic since women used to smear it on their skin. Still numerous ladies in Tamil Nadu apply turmeric all over their body each day prior to bath (Gopinath et al., 2018). It is known to lessen growth of facial hair, diminish skin break out and improve skin complexion. Moreover, it has also been used in skin health management items.

1.7.4 Cultural Significance

A piece of turmeric attached to a turmeric dyed string is used in numerous Indian weddings as matrimonial string. It is smeared to the whole body of both bride and groom on the earlier day of marriage. Garments colored or set apart with it are viewed as auspicious. It is applied on the neck, brow, and cheeks on propitious events. Ladies have been depicted as appealing according to Indian literature in the event if their bodies and faces are sparkling yellow. It is utilized in Hindu sanctuary practices and ceremonies, for example, "Pooja" and "Homa", and as a talisman to avert fiendish spirits (Gopinath et al., 2018).

1.8 Phyto-Constituents of Turmeric

Curcuma longa is a nutritionally rich plant. The rhizome of turmeric contains monosaccharides like arabinose, glucose, and fructose. Grains of starch are plentiful in these rhizomes. that is why it's widely utilized as a food zest (M. J. M. s. Jacob, 2016).

Until now, almost 719 Phyto-constituents have been recognized and isolated from a total of 32 *Curcuma* species. these Phyto-constituents include Alkaloids, diphenyl alkaloids, flavonoids, steroids, terpenoids, phenylpropene derivatives and others (Sun et al., 2017). Single rhizome of *C. longa* contains more than 235 phytoconstituents, most of which are terpenoids and polyphenols (Table 1) (Fuloria et al., 2022). This herbal plant likewise contains essential oils in it which have colossal worth. As per the most recent Gas chromatography analysis, total content of essential oil in rhizome was roughly 3.97%, with , α-turmerone, ar-turmerone and curlone as major components (table 2) (Guimarães, Vinhas, Gomes, Souza, & Krepsky, 2020)

Table 1.1: Phytochemical constituents of turmeric

Sr no.	**Phyto-chemical Constituents of Turmeric Rhizome**	**Total amount**
1	Sesquiterpenes	109
2	Monoterpenes	68
3	Diarylheptanoids	22
4	Phenolics	8
5	Diterpenes	5
6	Sterols	4
7	Terpenoids	3
8	Alkaloids	2
9	Others	14

Table 1.2: Major essential oil constituents of turmeric

Sr no.	Major Essential oil Constituents of turmeric rhizome	Total Percentage
1	α-turmerone	10%
2	Ar turmerone	40%
3	Curlone	23%

1.8.1 Curcuminoids

Major phytochemical constituents of turmeric are curcuminoids i.e. a concoction of curcumins such as curlone, curcuphenol, turmerone and cyclocurcumin (M. J. M. s. Jacob, 2016). Curcuminoids get their name from 'Curcuma' the genera. They are the most prominent class of polyphenol compounds. Curcuminoids are responsible for bright yellow orange color of turmeric (Nair, Nair, Agronomy, Turmeric, & Ginger, 2019). Almost all the medicinal activities of turmeric are attributed to these curcuminoids. Total curcuminoid content of turmeric varies between 2%-9% relying on topographical circumstances. There are three major compounds of curcuminoids i.e. Curcumin, Bisdemethoxycurcumin (BDMC) and demethoxycurcumin (DMC) and they are considered as natural analog of turmeric (Nebrisi, 2021).

Table 1.3: Major bioactive constituents of turmeric

Sr. no.	Compound	Molecular Formula	Molecular Weight
1.	Demethoxycurcumin	$C_{20}H_{18}O_5$	338.4g/mol
2.	Curcumin	$C_{21}H_{20}O_6$	368.38g/mol

3.	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	308.3g/mol

1.9 Curcumin

The most bioactive compound of turmeric, curcumin is alluded to as "Holy Grail" of all the organic molecules because no other compound is know which entails such a broad array of health benefits (J. J. S. i. n. p. c. Jacob, 2016). Curcumin makes up almost 2%-5% of turmeric (B. Jyotirmayee & G. J. I. J. o. F. P. Mahalik, 2022). This compound has gained the attention of many researchers working all over the world.

1.9.1 Chemistry of Curcumin

Curcumin is a symmetric molecule also known as Di-feruloyl methane. The IUPAC name of Curcumin is [1, 7-bis (4-hydroxy-3-methoxyphenyl)- 1, 6-heptadiene-3, 5-dione]. The structure of curcumin comprises of two ortho-methoxy phenols, linked through a heptadiene chain containing α, β-unsaturated β-diketone moiety. Radical scavenging activity of Phenolic compounds is due to position and number of hydroxyl groups. Numerous experimental and hypothetical examinations revealed the pivotal role of hydroxyl groups of phenol in the antioxidant property of curcumin (Purushothaman et al., 2022). Curcumin also exhibits Structurally Curcumin is almost like other curcuminoids, i.e. bisdemethoxycurcumin and desmethoxycurcumin, that vary just with total number of methoxy functional group on aromatic rings (Den Hartogh, Gabriel, & Tsiani, 2020). Curcumin also shows keto-enol tautomerism, with keto structures dominating in neutral or acidic conditions and enol structures dominating in alkaline conditions (Nebrisi, 2021).

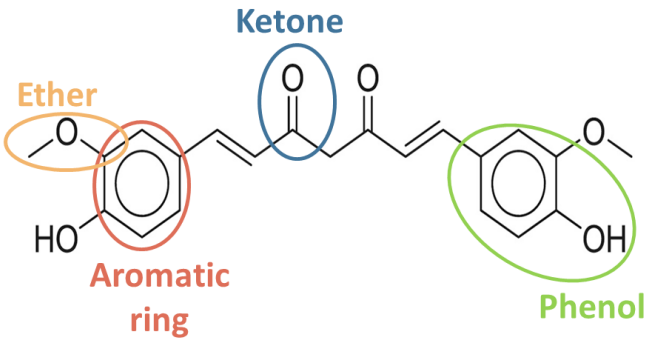

Figure 1.3: Chemical structure of curcumin

1.9.2 Solubility of Curcumin

Curcumin is a non-polar compound which doesn't dissolve in water at acidic or neutral pH and soluble in organic, alkali solvents which makes it a hydrophobic compound. It is a lipid soluble pigment (Nebrisi, 2021). Curcumin is soluble in different nonpolar and polar solvents. Some of them are mentioned below.

- **Polar solvents:** DMSO, Ethanol, Methanol, Acetone, Propanol, Acetonitrile
- **Non-Polar Solvents:** Ethyl acetate, Chloroform
- It is sparingly soluble in **hydrocarbons** like hexane and cyclohexane.

1.9.3 Pharmacological Activity of Curcumin

Studies reveal that turmeric has shown a wide array of pharmacological activities whether it's in powder form or an extract. the methoxy group of phenyl ring and 1,3 diketones of curcumin have critical impact in its various pharmacological properties (Jyotirmayee & Mahalik, 2022). Curcumin is a powerful natural compound which is non-toxic and harmless unlike other phytochemicals. The Joint FAO/WHO Expert Committee on Food Additives has set the acceptable daily intake of curcumin to 0–3 mg/kg/day.

Table 1.4: Pharmacological activities of curcumin

Sr. no.	Pharmacological Activity	Mechanism	Reference
1	**Antioxidant**	Hydroxyl groups of phenol are responsible for radical scavenging property of curcumin	(Purushothaman et al., 2022)
2	**Anti inflammatory**	Curcumin inhibits the formation of inflammatory mediators by regulating inflammatory signaling pathways	(Peng et al., 2021)

3	**Anti-cancer**	Curcumin focuses on numerous pathways engaged with the development, initiation, and growth of tumors. Factors that have significant role in developing multiple kinds of malignancies like Transcription factors, growth factors, genes involving in apoptosis, cytokines and protein kinases are targeted by curcumin.	(Zoi et al., 2021)
4	**Anti Diabetic**	Curcumin can inhibit oxidative stress, inflammations and hyperglycemia caused because of Diabetes Mellitus. Likewise, it also represses systemic complications such as endothelial dysfunction, neuropathy, dyslipidemia, nephropathy, and hypertension	(Marton et al., 2021)
5	**Cardiovascular protection**	curcumin is exhibited to have antihypertensive impacts while bringing down pulse, likewise it increments myocardial trophic blood stream. Curcumin helps in reducing blood viscosity and formation of thrombosis by inhibiting formation of thromboxane A2 (TXA2) and controlling calcium signals to forestall platelet activation and accumulation.	(Zhou et al., 2021)
6	**Antiaging**	Curcumin has ability to Inhibit telomerase activity	(Benameur et al., 2021)
7	**Neuroprotective**	Curcumin regulates multiple signaling pathways and their functions which are responsible for reducing disease progression..	(Nebrisi, 2021)

8	**Antiviral**	Curcumin and its derivatives have ability to hinder the replication of a multiple groups of viruses via different mechanisms	(Jennings & Parks, 2020)
9	**Anti-Obesity**	Curcumin reduces inflammations caused by obesity and other diseases. It helps in restoring the balance among anti- and pro- inflammatory factory by interacting with different factors i.e., cytokines, chemokines, transcription and growth factors, cellular receptors, and enzymes.	(Varì, Scazzocchio, Silenzi, Giovannini, & Masella, 2021)

1.9.4 Extraction Methods of Curcumin

The first step for recovering curcumin from plants is extraction. There are various extraction methods, but all are based on the same objectives i.e.

- Recovery of targeted compounds
- Expand the selectivity of the extraction methods
- Further improvement in extraction efficiency
- Providing a steady and reproducible methodology

These methods can also cause alteration in chemical structure and properties of curcumin. Generally, extraction methods are divided into two categories i.e. conventional/traditional methods and modern/novel methods (Jiang, Ghosh, Charcosset, & technology, 2021). In Conventional methods solvents are used to extract targeted substance, based on solubility principle. All of them are very affordable but their limitations are that they are time consuming and can cause damage to heat sensitive compounds (R. Zhang, Li, Zhu, He, & Technology, 2019). Modern methods on the other hand not only combat these limitations but are more efficient and considered as ecofriendly green technologies (Jiang et al., 2021). Conventional methods are sometimes coupled with modern methods to enhance their productivity i.e., microwave assisted Soxhlet extraction, ultrasound assisted Soxhlet extraction and ultrasonic maceration extraction etc.

Table 1.5: Extraction Methods for Curcumin

Sr no.	Extraction Methods	Working Principle
	Conventional Methods	
1	**Maceration**	The solute is placed in a container with solvent for 3-7 days until it is solubilized completely. The mixture is then strained
2	**Soxhlet extraction**	It involves solvent reflux and siphon principle which helps in continuous extraction of targeted substance by pure solvent.
3	**Hydro-distillation**	It is used mainly for oil extraction. Aromatic plant is placed in sufficient quantity of boiling water and live steam is injected which extracts essential oils from oil glands of plant tissue
	Novel Methods	
4	**Ultrasound assisted extraction**	It involves use of ultrasound energy which passes in the form of wave through solvent containing solute particles
5	**Microwave assisted extraction**	It utilizes microwave energy to heat up the solvent containing solutes which helps in dissolving targeted substance in solvent.
6	**Super critical fluid extraction**	It is the process of separating substances by using supercritical fluids as extracting solvents.

7	**Enzyme assisted extraction**	It involves the use of enzymes to facilitate the recovery of oils, proteins, or any other targeted substance from plant sample
8	**Pressurized liquid extraction**	It is a green technology that utilize solvents in liquid form at a temperature more than their atmospheric boiling point for extraction purpose.
9	**Ionic liquid-based extraction**	It is the concept of using green solvents i.e., ionic liquids for extraction instead of using volatile organic compounds as extractants or diluents.

1.9.5 Commercial Value of Curcumin

Curcumin is a useful substance that is derived from the rhizomes of the curcuma longa plant and has the potential to treat a wide range of illnesses. In three of the most well-known industrial areas, namely food, medicines, and cosmetics, this bioactive molecule has become a potential ingredient. Today, curcumin is sold commercially in a variety of products, including Energy drinks, Pills, Ointments, Tablets, Cleansers, and Cosmetics.

1.9.5.1 Food Industry

To improve food products' nutritional value, curcumin is added. Tea, drinks, curries, sauces, chips, and spread all contain it as an active ingredient. Additionally, it is used as a preservative and dye. A coffee with curcumin was developed by the international coffee company "Starbucks" which quickly gained popularity among consumers.

1.9.5.2 Pharmaceutical Industry

Curcumin is regarded as a fortunate "new medication" today. It is utilized as a supplement in many nations, especially in India, Pakistan, Malaysia, Thailand, Korea, Japan, China, and Japan (Fuloria et al., 2022). Due to its limited bioavailability, curcumin is usually encapsulated with Bioprene or Black pepper. Several companies, including Nature Made, BioSchwartz, and Nature Wise, sell these capsules. Only Nutrifactor sells curcumin pills in

Pakistan. Curcumin and ginger immunity booster shots during the Covid Pandemic were particularly successful in warding off the illness.

1.9.5.3 Cosmetic Industry

In cosmetic industry curcumin shines as a beauty enhancer. Considering the antioxidant property and anti-inflammatory properties of curcumin, many Cleansers, Skin gels, Face washes, Waxing creams and other derma products have curcumin as an active ingredient.

Although curcumin entails a plethora of medicinal properties, the items produced using it are yet lacking.

1.9.6 Local Market of Curcumin

The local market of curcumin in Pakistan presents an interesting landscape with certain challenges and opportunities. Despite being one of the largest producers of turmeric, Pakistan predominantly relies on imports for curcumin, limiting the country's economic benefits from its abundant turmeric cultivation. Additionally, there is a lack of widespread use of pure curcumin in various industries, even if they are incorporating turmeric in their products.

One of the primary reasons for the import dependence is the absence of extensive curcumin extraction and processing facilities within the country. As a result, Pakistan exports raw turmeric while importing the more refined curcumin from other countries, missing out on value addition and potential export opportunities. Furthermore, local industries using turmeric or turmeric extracts in their products often face uncertainty about the exact curcumin content and its contribution to the desired attributes. This uncertainty arises due to the lack of standardized extraction processes and quality assessment procedures.

Without reliable data on the curcumin concentration, it becomes challenging to harness its full potential in various applications, ranging from pharmaceuticals to functional food products. To tap into the vast potential of curcumin in Pakistan, there is a need to invest in local curcumin extraction facilities, leveraging the country's abundant turmeric resources. Establishing standardized extraction methods and quality control protocols can ensure consistent and high-quality curcumin production. This would not only reduce import dependence but also open new avenues for exports, boosting the local economy.

Justification of Work

Due to a variety of its medicinal properties, Curcumin soon will be one of the most sought after Phyto-components from a plant. Understanding the potential of curcumin present in Pakistani turmeric holds immense promise and value across various domains. Since Pakistan is blessed with the climate that is ideal for turmeric cultivation, delving into the curcumin content of this indigenous variety could unlock a plethora of benefits for both scientific and societal advancements. Understanding the quality and composition of curcumin extracted from this indigenous source is crucial for promoting its use in various applications, including herbal medicine and food additives.

Objectives

- Evaluate the efficiency of different solvents for curcumin extraction.
- Investigate the chemical profile and structural composition of the extracts.
- Explore the pharmacological and industrial potential of local turmeric.

Chapter 2: Literature Review

2.1 Extraction Techniques

Curcumin can be extracted using different methods that involve the use of various extraction techniques. These techniques may include processes like solvent extraction, steam distillation, or supercritical fluid extraction. Each method aims to isolate curcumin from turmeric efficiently and effectively, catering to different research or industrial needs.

2.1.1 Soxhlet Extraction

(Wakte et al., 2011) extracted curcuminoids to study the activity of its anti-inflammatory effect using Soxhlet extraction. In this study dried 40g of oleo resinous powder was taken. Methanol was selected as the extracting solvent. The extraction time was kept to 6 hrs. After the extraction was completed, the solvent was evaporated from the mother liquor using rotary vacuum. Upon cooling, yellow crystals of curcuminoid were obtained. The method yielded a good quality of curcuminoid i.e., 75% of curcuminoids known to be present in a turmeric rhizome of the respective quantity which was later tested for anti-inflammatory activity. The identity of curcuminoids was confirmed using thin layer chromatography.

(Popuri & Pagala, 2013) performed Soxhlet extraction using the turmeric roots. The study undertook numerous solvents and changed different parameters to find out which set of parameters works best for curcumin extraction. Turmeric roots used were ground using a mortar and then dried at room temperature in air as opposed to using a grinder for the former. The solvents used in this study were acetone, methanol, ethanol, ethyl acetate, and isopropanol. The solid to solvent ratio was maintained at 1:8. The extraction duration was kept 1-4 hours for each solvent. Extraction was followed by distillation at the respective solvent's boiling point then drying and washing to obtain the curcumin crystals. The study concluded Acetone to be the best solvent for curcumin extraction.

Another similar study was performed by (Nabati, Mahkam, & Heidari, 2014) where Soxhlet assembly was chosen as the extraction method for curcumin isolation. However, what distinct this series of extraction apart from other of the similar kind is the first step i.e., addition of 25g of powder to n-Hexane using a magnetic stirring rod. The mixture was agitated for a period of 3 days afterwards of which the solution was decanted off to isolate the suspension at the bottom of the beaker. This suspension was then used in place of granulated oleo resin in the Soxhlet apparatus. The solvent used here was methanol. The Soxhlet extraction was continued for 3 days. The extraction was followed by evaporation of the solvent in the rotary evaporator.

This was followed by a series of acidifying steps and extractions which eventually led to the formation of yellow crystals of curcuminoids that were isolated using thin layer chromatography. The process yielded 208mg form 25g of the turmeric powder.

Along with *Curcuma longa*, (Dutta, 2015) chose Soxhlet assembly for the extraction of curcumin from additional *Curcuma* species. The extraction solvent utilized was ethanol, and the rhizomes were pulverized. The ratio of solid to solvent was 1:1. The Soxhlet extraction was place over the course of two days that were 48 hours apart. To confirm the presence of curcumin, the ethanolic extract was dried before being subjected to phytochemical analysis and spectrophotometry. 100g of the ground turmeric yielded 125mg of curcumin.

Multiple extraction methods were utilized by (Shirsath et al., 2017) to determine which yielded the best results. 10g of the powder was extracted with 250mL of the solvent for the Soxhlet assembly. The solvent of choice was ethanol. After a predetermined range of intervals, samples were taken out and subjected to HPLC analysis to track the extraction period that yielded the best results. The extraction took place for 8 hours. 12.75mg/g of curcumin was produced using this procedure.

The Soxhlet assembly was employed by (Pawar, Gavasane, & Choudhary, 2018) for the extraction of curcumin. Since curcuminoids are liposoluble, they can be found in turmeric essential oils. As the oils solubilize the phytochemical, it becomes more challenging to extract pure curcumin from the oleo resin. Therefore, (Pawar et al., 2018) tested out a number of solvent mixes that might be employed for curcumin extraction in Soxhlet assembly in order to derive the pure curcumin out of the oil fraction. Isopropanol with water, isopropanol with hexane, ethanol with water, ethanol with hexane, methanol with water, and methanol with hexane were the solvents tested in this study. The combination of isopropanol and hexane produced the best results. FT IR analysis and TLC were used to confirm the curcumin crystals that were produced.

2.1.2 Ultrasound Assisted Extraction

Using ultrasound aided extraction, (Pawar et al., 2018) improved the curcumin extraction procedure. The ethanol-water mixture with the solvent volume adjusted to 20mL produced the highest yield when several solvent mixes were tried. The extraction procedure significantly shortened the extraction time to just 70 minutes. Soxhlet and maceration procedures were also used for comparison. 200mL of acetone and 2g of turmeric powder were combined in a Soxhlet. After the procedure had been running for 4 hours, the solvent was

expelled, and then methanol was added. A magnetic stirrer was utilized for maceration. 50mL of acetone and 2g of powder were combined, and the mixture was continuously swirled for 24 hours. The solvent was evaporated after extraction, and methanol was then added. HPLC analysis was performed on each of the three extracted curcumin samples. In terms of curcumin yield, ultrasound assisted extraction outperformed the two conventional methods.

Ultrasonic aided extraction was carried out by (Shirsath et al., 2017), 250mL of the solvent and 6g of the powder were consumed. There were several different solvents used, including acetone, ethanol, methanol, and ethyl acetate. Also changed were the particle sizes, which were 0.09, 0.10, 0.21, and 0.85mm. Each of these solvents was used for extraction. The solid and solvent mixture was taken and placed in a flask before being submerged in water. For a variety of situations, the water bath's temperature was adjusted from 25 to 55°C. The study's ultrasonic horn had a 250W power output and a 22kHz frequency. One hour was spent on the extraction process. To establish the ideal irradiation time, samples were taken out at regular intervals and their curcumin percentages were checked. The ideal extraction parameters for UAE were ethanol as the solvent, 0.09 mm particle size, and 1 hour of extraction time. As the temperature rose, the yield of the extraction increased.

Curcuma longa rhizomes were subjected to ultrasonic extraction of curcuminoids by (Binello et al., 2020). Ethanol was chosen as the extraction solvent. The amount of powdered oleo resin used was 10g, and the temperature was held constant at 40°C. The solid to solvent ratio used was 1:5. The employed ultrasonic horn had a frequency of 20.5 kHz and could produce 350–500W of power. The obtained curcumin was analyzed using HPLC. In the same study, the UAE approach and a conventional technique were also contrasted. Maceration was the method used for comparison. The solvent and the oleo resinous particles were blended using a magnetic stirrer. For maceration, the same set of conditions were applied. With the earlier technique for extracting curcumin, the HPLC analysis produced superior results while taking less time.

2.1.3 Maceration

Curcumin extraction was carried out using the maceration approach by (Wulandari, Martono, & Rohman, 2018). Ethanol was used as the solvent for extraction. The *Curcuma longa* root was employed in its ground form. The ratio of solid to solvent was 1:6. The extraction was carried out for a continuous 12 hours at a fixed temperature of 80°C. A 30 rpm agitation speed was maintained.

(Shirsath et al., 2017) employed batch or maceration extraction. 6g of powdered oleo resin and 150 ml of the solvent were used in this study. The solvent for this extraction was decided upon as being ethanol. A glass reactor was filled with the solution. The agitator was activated so that it could mix continually. Agitation was programmed to occur at a speed of 420 rpm. The extraction process took place for 8 hours at a constant 30°C. To avoid the oleo resinous particles from settling at the bottom of the glass reactor, it was ensured that the agitator rotated quickly enough. The samples from the mother liquor were submitted to HPLC at regular intervals to establish the ideal time for extraction.

2.1.4 Microwave Assisted Extraction

(Dandekar, Gaikar, & Technology, 2002) used Microwave Assisted Extraction (MAE), another cutting-edge technique, to isolate curcuminoids. Acetone was chosen as the extracting solvent for this study. A modified microwave system was employed. It incorporated sparging gaseous nitrogen to keep the atmosphere as neutral as possible. Additionally, the device included an outlet to allow the gas to exit the microwave after it has completed its task. This setup required 6 days and around 10 hours each day to complete the extraction procedure. Acetone and oleo resinous powder were utilized in quantities of 30g and 200mL, respectively. At the beginning of each day of the extraction, fresh acetone was added. The percentage yield of curcuminoids was 5.8%, according to the study using High-Performance Thin Layer Chromatography (HPTLC).

To determine whether microwave aided extraction approach is superior to traditional extraction techniques, (Mandal, Maity, Dewanjee, & Mandal, 2008) employed it. Acetone was selected as the extraction agent for this study. In contrast to earlier methods, the methanol-dipped solid plant material was used as a process modification. 40mL of acetone and 2g of turmeric powder were used together. The best process parameters were determined using the Taguchi design technique. A dual heating mechanism used in the microwave aided extraction method ensures a higher yield from the feed volume of the solid or powdered material. A Soxhlet extraction and maceration process was used as a benchmark. 2g of turmeric powder and 100ml of acetone were added to the solvent flask for Soxhlet assembly. The extraction took place for three days and eight hours each day. Every day, a new extracting solvent of the same volume was substituted. Methanolic extract was made from the mother liquor that was obtained. Using a magnetic stirrer was used in the maceration process. 40ml of acetone and 2g of the powder were combined, and the mixture was left to blend for an entire day. After the solvent had

evaporated, methanolic extract was created. The three extracts' HPLC analyses revealed that microwave aided extraction was the most successful method for removing curcumin from the powdered oleoresin.

(Wakte et al., 2011) chose to extract curcumin from *Curcuma longa* using microwave assistance. The study's 140 W microwave was employed. To accomplish the objective, two experiments were conducted. 20g of powdered turmeric was taken for the first experiment and spread out flat on a glass dish. After that, the powder was exposed to irradiation for 1 to 7 minutes (a variable amount of time for each batch of the experiment). The sample powder was added to the solvent (ethanol or acetone) after it had dried, maintaining a solid to solvent ratio of 1:5. The extraction chamber, which included nine cylindrical jars, received the mixture next. A 400 rpm agitation speed was kept throughout the extraction process. The procedure was performed at two different powers, 60 W and 90 W. 90 W of electricity was utilized for the extraction process when ethanol was the solvent, whereas only 60 W was used when acetone was the solvent. The solid to solvent ratio utilized in the following set of experiments was 1:2. The temperature was maintained at 20°C throughout the entire extraction process. In this experiment, the solvents employed were ethanol and water. The power supply for the ethanol experiment was increased to 270 W whereas it was kept at 50 W for the water-based experiment. The two sets of extracts from the experiments were then examined.

For the comparison, ultrasonic aided extraction was also used (Wakte et al., 2011). A 150 W ultrasonic horn was employed for it. The retrieved parameters for the UAE were retained the same as those for the MAE. The oleo resinous powder was dipped into the solvent following an initial irradiation of around 5 minutes at 21**°C**. Here, ethanol and acetone were also used as solvents. The second set of tests were carried out using the UAE approach, but this time the extracting solvents were water and ethanol. Using HPLC, the extracts from MAE and UAE were examined. The findings indicated that both sets of trials had successful yields and that organic solvents outperformed inorganic ones.

2.1.5 Super Critical CO2 Extraction

The extraction of curcumin and turmeric oils was done using the supercritical CO_2 extraction method (Wakte et al., 2011). A straightforward supercritical CO_2 extraction system was used for the experiment. Supercritical CO_2 serves as a solvent in the procedure. The cosolvent chosen was ethanol. The procedure involved pumping CO_2 through the extraction chamber that held the requisite particle size of solid plant material. Ethanol was combined with

supercritical CO_2. Prior to extraction, the former had to be mixed in the vessel that contained the ethanol. A Bourdon-type gauge was employed to maintain and track the chamber's pressure. The oleo resinous particles were condensed, and the extracted fraction's solvent was then removed using a rotary evaporator. After that, the extract was measured and examined with HPLC. Using this technique, a maximum yield of 22.5% was attained.

Curcumin was extracted from *C. longa* by ultrasonic supercritical CO_2 assisted extraction (Chhouk et al., 2018). It led to a substantial improvement in curcumin output. The experiment was conducted at a temperature between 40°C and 60°C. 10%, 15%, and 20% of cosolvent were used, respectively. For determining the ideal time for curcumin extraction, the full extraction procedure was carried out over periods of 30, 60, and 90 minutes. After the procedure was finished, a rotary evaporator was used to dry the extract's solvent. For further precision, duplicates or triplicates were run for each parameter value that fluctuated. To examine the quality of curcumin, Fourier Transform Infrared (FTIR) analyses were carried out. A maximum of 7.17% yield was reached at the best circumstances of 90 minutes, 10% cosolvent, and the CO_2 flow rate of 3mL/min after studying the effects of each set of parameters..

In the extraction column, (Gopalan, Goto, Kodama, Hirose, & Chemistry, 2000) used 9g of turmeric powder. The extraction chamber was kept at the desired temperature and pressure. After passage, compressed CO_2 produced the extract. Curcumin and turmeric oils were both present in the yield. You can change the variables and cosolvents to produce turmeric oils.

2.2 Previous Work on Turmeric Cultivating in Pakistan

(Charan et al., 2022) attempted to show the adequacy and potential of indigenous turmeric powder from Kasur, Pakistan by comparing it with international grade turmeric powder. For this different analysis were performed like, phytochemical testing, anti-microbial activities and presence of curcuminoid content i.e. curcumin which was extracted through four different solvents which are methanol, ethanol, acetone and chloroform. Results were verified using TLC and FTIR spectroscopy. This study legitimizes that local turmeric has better potential and quality as it gives a greatest curcuminoid yield of around 25% in Ethanol and showed extreme zone of restraint against Staphylococcus aureus E.coli around 16mm and 14mm against E.coli. While Antifungal action was likewise noticed high in contrast with international grade turmeric extract. Further, electro-spun nanofibers effectively created from curcuminoid of Kasur, Pakistan with PVA, generated smooth and uniform nanofibers with 227. 49 nm diameter. Study revealed that turmeric of Kasur has comparatively more potential than

international grade turmeric and can be utilized/swapped in nanotechnology labs for different applications.

(Mushtaq et al., 2019) investigated bioactive profile and antioxidant activities of ginger and turmeric. Both ginger and turmeric were obtained from University of Agriculture, Faisalabad, Punjab-Pakistan, Mineral profile, and chemical composition of both spices were compared. With the help of solvent extraction, bioactive compounds from both spices were isolated which were quantified through the high-pressure liquid chromatography. Antioxidant profile was analyzed including DPPH assay, FRAP assay and total phenolic content. Results uncovered that antioxidant activity including DPPH (80.16 ± 0.23%) and free radical scavenging activity (47.67 ± 0.19 mg/100 g) was much higher in turmeric ginger powder extract. Also, flavonoids (4.27 ± 0.05 mg CE/100 g) and all out phenolics content (103.39 ± 0.58 mg of GAE/g) were a lot higher in turmeric ginger powder when contrasted with ginger powder and turmeric powder, separately which concludes high anti-oxidant potential of turmeric ginger powder.

(Irshad, Muazzam, Shahid, & Dalrymple, 2018) performed in vitro assessment of turmeric extracts in ethanol, methanol and water through phytochemical analysis, disc diffusion and agar well technique. Silica gel, HPLC and TLC were used to purify curcumin from turmeric and its DNA protection activity and antioxidant activity were evaluated. It was observed that sensitivity of alcohol extracts against bacterial species varied, yet Bacillus subtilis and Staphylococcus aureus subsp. Aureus both showed articulated hindrance in agar well and disc diffusion methods respectively. Ethanol extract had more inhibitory impact on bacterial growth with a mean of 9.4±1.00 mm contrasted with 8.8±0.58 mm in methanol extract. Phytochemical examination affirmed the presence of flavonoids, carbohydrates, steroids, tannins, phenols and saponins. HPLC purification o curcumin gave the fundamental peak at retention time of 61-65 minutes with 55% of acetonitrile. Lower curcumin concentrations had protective impacts on human DNA as compared to increased concentrations which had damaging impacts. Percentage scavenging activity was observed to be highest (91.84%) at 45 µg with per unit increase in concentration provoked 6 units increase in hindrance rate with a linear regression, R2= 0.914. This large number of attributes support its importance as herbal medicine.

2.3 Analytical Characterization of Curcumin

2.3.1 UV-Vis Spectroscopy

The identification and quantification of curcumin in various samples are crucial for pharmaceutical, nutraceutical, and biomedical applications. Among various analytical techniques, UV analysis has emerged as a widely used and reliable method to detect and quantify curcumin due to its simplicity, sensitivity, and cost-effectiveness. UV analysis is based on the principle of absorption spectroscopy, wherein molecules absorb light at specific wavelengths corresponding to their electronic transitions. Curcumin exhibits characteristic absorption peaks in the UV spectrum due to its conjugated double bonds and phenolic groups, making it suitable for UV analysis. The main absorption peak of curcumin in the UV spectrum is typically observed around 425 nm, which corresponds to the π-π* transition of its conjugated double bonds. This specific absorption peak is a key characteristic used to identify curcumin in various extracts and formulations.

In the study (Subhan, Alam, Rahaman, Rahman, & Awal, 2014), two solvents, methanol and DMSO, were used to capture the electronic spectra of curcumin. Curcumin showed a wide range of UV-visible absorption in methanol, from 300 to 500 nm, with the strongest absorption band seen at 424 nm. Furthermore, lesser absorption bands at 262 nm were seen, and shoulder peaks were visible between 360 nm and 460 nm. A lesser absorption band was discovered at 268 nm while a maximum absorption band was seen at 435 nm in DMSO. The prominent maximum absorption in both instances can be attributed to the extended conjugation system of curcumin's electronic dipole allowing -* type excitation.

According to (Zaghary, Hanna, Zanoun, Abdallah, & Sakr, 2019) in the field of curcumin analysis, researchers have been investigating the estimation of curcumin content in various solvents. They use the absorption of light by curcumin at specific wavelengths, usually between 420 and 430 nm, to determine its presence in the solvents. However, the absorption band of curcumin in non-polar solvents, such as chloroform, acetic acid, toluene, and carbon tetrachloride, exhibits an irregular shape. The type and nature of the solvent plays a significant role in how curcumin absorbs light. When comparing non-polar solvents like n-hexane to polar solvents like methanol, only a minor shift towards longer wavelengths (red-shift) of approximately 0 to 20 nm occurs in the absorption band. This shift indicates a slight change in the way curcumin interacts with light in different solvent environments.

In the majority of the sample matrices, methanol was discovered to be an effective solvent for spectrophotometric measurements of curcumin (Kotra, Satyabanta, Goswami, & technology, 2019). Furthermore, the pH level of the solvent also affects the absorption spectrum of curcumin. This means that the acidity or basicity of the solvent influences how curcumin absorbs light, potentially leading to variations in the measured absorption intensity at specific wavelengths (Zaghary et al., 2019). Following points depicts maximum absorbance value of curcumin in different studies.

- Curcumin exhibits its maximum absorption in ethanol at approximately 426 nm (PubChem CID 969516).
- (Kadam, Yadav, Bhingare, Patil, & Phytochemistry, 2018) examined curcumin solution using a UV spectrophotometer. The analysis covered a wavelength range from 200 to 800 nm, with methanol used as a blank reference. The researchers observed that the maximum absorption of curcumin in methanol occurred at a wavelength of 424 nm.
- (Hazra et al., 2015) detected Curcumin at an absorption maximum of 421nm using methanol as the solvent.
- (A. Singh & Avupati, 2017) observed the wavelength of maximum absorption (λmax) of curcumin by scanning curcumin solution in the UV-visible range of 400-600 nm. Ethyl acetate was used as the blank solvent. The maximum absorbance value of curcumin was observed at 418 nm.

2.3.2 FTIR Analysis

Fourier-transform infrared (FTIR) spectroscopy is a powerful analytical technique used to identify functional groups and molecular vibrations in organic compounds, including curcumin. Several research studies have employed FTIR analysis to characterize the molecular structure of curcumin and its interactions in different solvents and formulations. In FTIR analysis of curcumin, characteristic peaks have been observed at specific wavenumbers, providing valuable information about its functional groups.

Overall, FTIR analysis of curcumin provides valuable information about its molecular structure and functional groups in different solvents. The presence and intensity of specific peaks in the spectra vary depending on the solvent used, indicating the different interactions and conformations of curcumin in different environments. Researchers can utilize FTIR analysis to assess the purity of curcumin in various formulations, monitor its stability, and understand its

interactions with other compounds. The technique's ability to provide detailed molecular information makes it a valuable tool for the pharmaceutical and biomedical industries, aiding in the development and optimization of curcumin-based therapies and formulations.

Table 2.1: FTIR wavenumber values for functional groups of curcumin from literature

Sr no.	**Functional Groups**	**(Pawar et al., 2018)**	**(Charan et al., 2022)**	**(Gunathilake, Ching, Uyama, Hai, & Chuah, 2022)**
1	O-H (Hydroxyl Stretching)	3512 cm^{-1}	3303 cm^{-1}	3464 cm^{-1}
2	C-H (Aliphatic Hydrocarbon Stretching)	2922 cm^{-1}	2929 cm^{-1}	2939 cm^{-1}
3	C=C (Aromatic Stretching)	1602 cm^{-1}	1622 cm^{-1}	1628 cm^{-1}
4	C=O (Carbonyl Stretching)	1510 cm^{-1}	1510 cm^{-1}	1513 cm^{-1}
5	C-O (Enol Stretching)	1280 cm^{-1}	1270 cm^{-1}	1280 cm^{-1}

2.3.3 GCMS Analysis

GC-MS analysis involves the separation and identification of its individual components based on their mass-to-charge ratio and fragmentation patterns. It is commonly used to analyze the volatile and semi-volatile compounds present in curcumin extracts and essential oils. Turmeric, the rhizomatous herb of *Curcuma longa*, has been widely recognized for its medicinal and culinary uses, owing to its rich bioactive compounds, especially curcuminoids. Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as a powerful analytical tool for identifying and quantifying the diverse chemical constituents present in turmeric extracts. One of the main advantages of GC-MS analysis is its ability to provide detailed information about the chemical constituents of turmeric. This allows researchers to identify and quantify various bioactive molecules present in turmeric extracts and study their potential pharmacological activities. GC-MS analysis has also been employed to assess the purity and authenticity of

curcumin samples, especially in commercial products. By comparing the GC-MS data of curcumin samples with reference spectra or databases, researchers can confirm the presence of curcumin and detect any adulterants or contaminants.

(Arivoli et al., 2019) findings from GC-MS analysis provide valuable insights into the chemical composition of turmeric extracts, highlighting the presence of curcumin and other bioactive compounds that contribute to its wide range of health-promoting effects.

In (Chen et al., 2019) study Gas Chromatography-Mass Spectrometry (GC-MS) was performed to examine the volatile oil of turmeric, and a total ion flow graph was produced (Fig. 4). Researcher found 45 different chemicals in the volatile oil by comparing the mass spectra of the observed substances with NIST14.L and published research. The area normalization approach was used to determine the relative content of each component. Total 45 distinct chemical compounds were found in this study. AR-Turmerone (36.43%), Curlone (13.33%), and Tumerone (8.52%) made up the majority of the mixture.

Moreover, GC-MS analysis has been used to study the effect of different extraction methods and solvents on the chemical composition of curcumin extracts. This information is crucial for optimizing the extraction process to obtain curcumin-rich extracts with desired bioactive components. Despite the potential of GC-MS analysis, it is essential to consider its limitations, such as the thermal instability of curcumin and its potential to undergo thermal degradation during the GC-MS process. Careful selection of derivatization methods and GC-MS conditions is necessary to minimize degradation and obtain accurate results.

Chapter 3: Materials and Methods

3.1 Chemicals and Reagents

Extraction solvents were obtained from ASAB's chemical vendor. Four different solvents were used for extraction i.e., Acetone, Ethanol, Methanol and Glycerol.

3.2 Plant Sample

Fresh rhizomes of turmeric were purchased from the local market of Multan.

3.3 Soxhlet Assembly

A Soxhlet apparatus has primarily three parts:

- percolator (heater and reflux) in which solvent circulates.
- a thimble (made up of thick filter paper) which holds the sample (solute) to be extracted.
- a siphon instrument, which intermittently empties the thimble.

Solvent is boiled to reflux. Vapors of boiling solvent goes up in distillation arm, and floods back into the chamber containing thimble with sample material. The condenser guarantees that vapors of solvent cool and trickle down into the chamber with thimble. Gradually the chamber containing sample material loads up with warm solvent. A portion of the targeted substance solubilizes in warm solvent. At the point when the Soxhlet chamber is practically full, the chamber is exhausted by the siphon. The solvent is returned to the distillation flask. Thimble makes sure that rapidly moving solvent does not carry sample material to still pot. This cycle continues and repeats for hours or days.

In each cycle, some portions of compounds(non-volatile) solubilize in solvent. After numerous cycles the targeted substance is moved inside distillation flask. The benefit of this apparatus is that rather than multiple portions of solvent being gone through the sample material, only one clump of solvent is reused.

Figure 3.1: Soxhlet apparatus

3.4 Rhizome Pretreatment

Fresh rhizomes were washed thoroughly with distilled water. While washing it was made sure to remove all the dirt by gently rubbing each rhizome with fingers. After washing, rhizomes were cut into dainty cuts of roughly 5mm thickness.

3.4.1 Rhizome Drying

The sliced turmeric rhizomes were spread on paper sheets for drying. They were shade dried first for almost two days in an open atmosphere on a bright day. On the very next day, they were dried in a food dehydrator i.e., Arshia FD-130 for around two hours.

3.4.2 Turmeric Powder Formation

Dried pieces of rhizomes had an exceptionally crispy texture and could be effortlessly ground with the help of a food processor. WestPoint food processor was used for this purpose. The ground powder was further grinded through pestle and mortar to make it even fine powder.

3.4.3 Mesh Size of Powder

Sieving or screening is a technique for sorting a particle size of powder by running it through a particular sized sieve. Ground turmeric powder was run through the sieve of 0.4mm or 40 mesh. The rest of the powder that could not pass through the sieve was grinded again using pestle and mortar and afterward run again through the same sieve.

Figure 3.2: Thinly sliced turmeric rhizome

Figure 3.3: Dried slices of turmeric rhizome

Figure 3.4: Turmeric rhizome powder

Figure 3.5: Powder Refining

3.5 Method

3.5.1 Solvent Selection

For choosing the right solvent, writing was counseled, and a couple of choices were looked for. These include.

- Acetone,
- 95% Methanol
- 95% Ethanol
- Glycerol/Water (50%/50%)

Extraction was proceeded with each of these solvents. Extraction procedure and conditions were same for all the solvents as described below.

3.5.2 Solute to Solvent Ratio

Solute to solvent ratio is very important for any extraction. In this study the solute to solvent ratio was kept at 1:10 for each solvent. For this 20g of ground turmeric powder and 200ml of extraction solvent was taken.

3.5.3 Soxhlet Extraction

A 250 mL round bottom flask was poured with 200mL of extraction solvent. 20g of turmeric powder was added in thimble which was placed inside chamber. The temperature of the heating mantle was set at 55ºC. When the temperature of condenser reaches 4ºC, the heating mantle was turned on to begin extraction. Extraction continued for almost 4.5 to 6 hrs.

3.5.4 Solvent Evaporation

After extraction, mother liquor was cooled down and poured in petri dishes for solvent evaporation. Just a little amount was layered in petri dishes so it can dry out rapidly. Since glycerol does not evaporate at room temperature, the extract remained as it is. Other alcoholic solvents are volatile, so they evaporate at room temperature. Petri dishes with mother liquor were half covered with aluminum foil and placed in dark for 24 hours. Prior to scraping, petri dishes with turmeric extract were placed in food dehydrator i.e., Arshia FD-130 for almost 2 hours to eliminate all the remaining moisture content.

3.5.5 Scraping

After solvent evaporation, curcumin dries out and adheres to the petri plates. It is then scraped off utilizing a steel spatula. It gets very tricky when scraping extract powder from petri plates. It ought to be ensured that there is no moisture content left otherwise extract will come out tacky. A very light stroke was applied with steel spatula while scraping. Scraped powder was saved in a dark cabinet for later use.

Figure 3.6: Soxhlet extraction of curcumin (first hour of extraction)

Figure 3.7: Soxhlet extraction of curcumin (last hour of extraction)

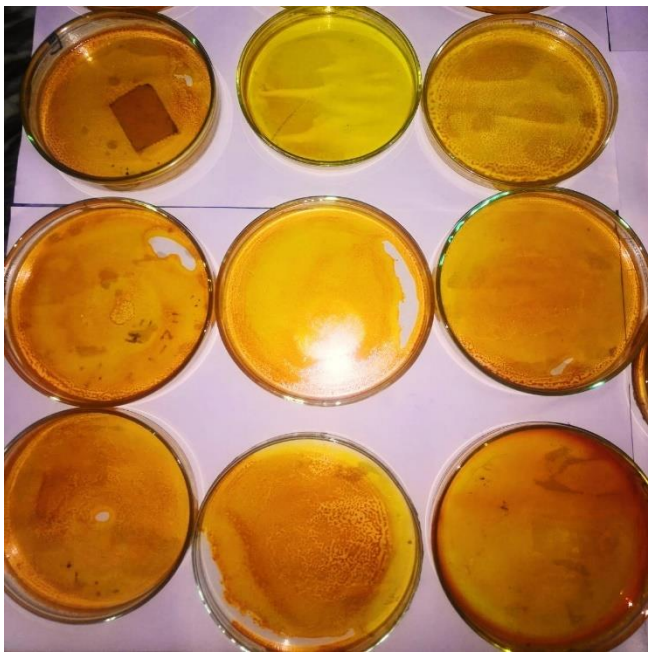

Figure 3.8: Extract layered in petri plates for air drying

Figure 3.9: Dried turmeric extract

3.6 pH Measurement

Measuring pH (potential of hydrogen) is a procedure to detect acidity or alkalinity of solution. pH of all four different curcumin extract solution was measured. For this, the pH electrode was immersed into sample solution, ensuring it is completely submerged without contacting walls or base of container. The electrode was stirred gently until the pH reading stabilize display.

3.7 Density Measurement

The density of any liquid sample is determined by dividing mass by volume. First of all, the weight of the empty falcon tube was calculated through weighing balance i.e., 6.67g. Then 10ml of liquid sample was poured in the tube, again its weight was calculated, and weight of empty falcon tube was deducted from the resultant value to figure out final mass of liquid sample.

$$density = \frac{mass}{\text{volume}}$$

3.8 Characterization Techniques

3.8.1 UV/Vis Spectrophotometer

UV Spectroscopy helps in identification of sample constituents based on their absorption wavelength. This technique is the simplest and most common method for determination of curcumin in multiple sample matrices. UV spectrophotometry of all four turmeric extracts from different solvents were performed. UV analysis of the turmeric extracts was performed using the "SPECORD Plus UV spectrophotometer". The analysis was conducted within a wavelength range of 200 to 600 nm. The prepared turmeric extracts were placed in suitable cuvettes and inserted into the spectrophotometer for measurement. The absorbance spectra were recorded, and the data were collected for further analysis. The instrument settings, including wavelength range and other parameters, were kept consistent throughout the analysis to ensure accurate and reliable results. Graphs were generated through SpectraGryph software for data visualization and analysis.

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Figure 3.10: SPECORD Plus UV

3.8.2 FTIR (Fourier Transform Infrared Spectrophotometry)

Fourier Transform infrared (FTIR) analysis is one of the most profoundly used techniques of infrared spectroscopy to verify the presence of functional group in extract or compound. FTIR analysis of all 4 curcumin extracts were performed. All the extracted curcumin samples were taken in powder form except for glycerol extract which was used in liquid form. FTIR analysis of turmeric extract powder was performed using the "Carry 630 FTIR spectrophotometer" by Agilent Technologies within the wavenumber range of 600-4000 cm^{-1}. To prepare the sample, the turmeric extract powder was mixed with infrared grade potassium bromide (KBr) and pressed into a pellet. The resulting pellet was then used for FTIR analysis to obtain the infrared absorption spectrum of the turmeric extract. Graphs depicting the characteristic peaks and absorption bands were plotted using SpectraGryph software for data visualization and interpretation.

Figure 3.11: Carry 630 FTIR Spectrophotometer

3.8.3 GCMS (Gas Chromatography Mass Spectrometer)

GCMS or Gas Chromatography/Mass Spectrometry analysis is a technique that consolidates the elements of gas chromatography and mass spectrometry to recognize various substances inside a sample matrix. GC/MS investigation is by and large viewed as one of the most accurate analytical techniques available. This technique was performed to investigate the volatile compounds of extracted sample along with their structural analysis. GCMS analysis of only methanol extract was performed.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract was performed following the running specifications described in the study by Arivoli et al. (2019). The analysis was performed using a Shimadzu GCMS QP2020 equipped with a Shimadzu SH-Rxi-5Sil MS column (L=30m, ID=0.25, DF=0.25). The column was temperature-programmed, and the temperature range was optimized to achieve separation of the target compounds. The injector and detector temperatures were set accordingly. The methanolic extract powder was dissolved in GCMS grade methanol solvent and injected into the GCMS system. The running time of the analysis was 90 minutes to ensure comprehensive compound detection.

Figure 3.12: Shimadzu GCMS QP2020

Chapter 4: Results

4.1 Color of Turmeric Extract

It was observed that the color of all turmeric extracts of different solvents turned out to be different. The following figure shows variation in color of extracted sample.

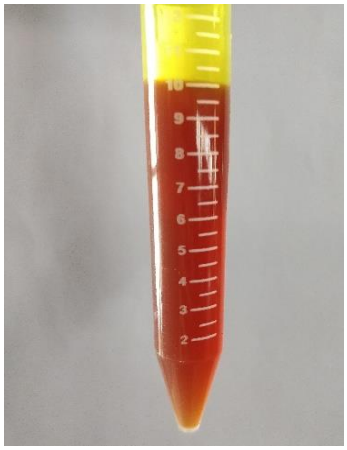


Figure 4.1: Ethanol extract

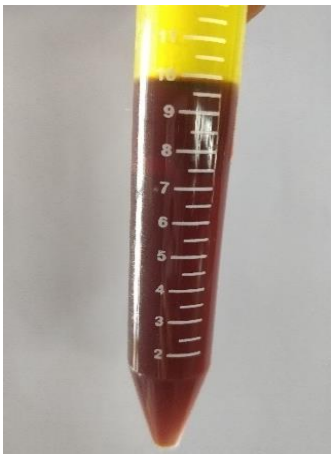


Figure 4.2: Methanol extract

Figure 4.3: Acetone extract

Figure 4.4: Glycerol extract

4.2 pH Measurement

Results show that pH value of turmeric extracts varies depending upon the type of extraction solvent. The pH value of turmeric extract from different solvents indicates that all of them are slightly acidic table. Glycerol/Water extract is the most acidic compared to others.

Table 4.1: The pH of turmeric extracts in different solvents

Sr no.	Turmeric Extracts	pH Value
1	Ethanol extract	6.53
2	Methanol extract	6.81
3	Acetone extract	6.29
4	Glycerol/Water extract	5.86

4.3 Density Measurement

Out of all four turmeric extracts glycerol/water extract turned most dense i.e., 1.27g/ml. While acetone extract is less dense of them all i.e., 0.787g/ml. Density of ethanol and methanol extract is practically the same.

4.3.1 Ethanol Extract

$$density = \frac{mass}{\text{volume}} = \frac{8.21g}{10\text{ml}} = 0.821g/ml$$

4.3.2 Methanol Extract

$$density = \frac{mass}{\text{volume}} = \frac{8.58g}{10\text{ml}} = 0.858g/ml$$

4.3.3 Acetone Extract

$$density = \frac{mass}{\text{volume}} = \frac{7.87g}{10\text{ml}} = 0.787g/ml$$

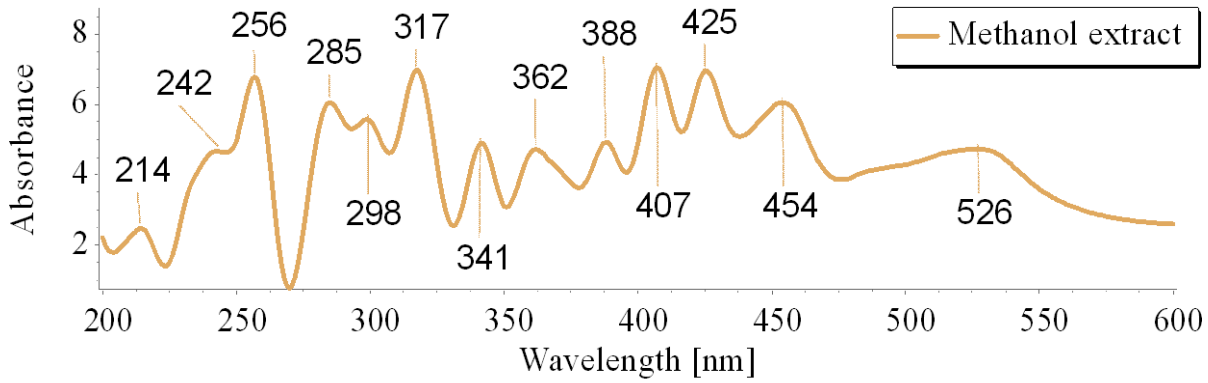
4.3.4 Glycerol/Water Extract

$$density = \frac{mass}{\text{volume}} = \frac{12.7g}{10\text{ml}} = 1.27g/ml$$

4.4 UV Spectra of Turmeric Extracts

UV-Vis spectroscopy was performed on turmeric extracts obtained using four different solvents: ethanol, methanol, glycerol, and acetone. The UV-Vis spectra revealed distinct variations in the absorption profiles among the solvents. Notably, the turmeric extract in methanol exhibited the highest and most distinct peak at approximately 425nm, with an absorption value of 7. In contrast, the other three solvents (ethanol, glycerol, and acetone) did not exhibit a prominent peak.

4.4.1 UV-Vis Spectra of Turmeric Extract in Methanol

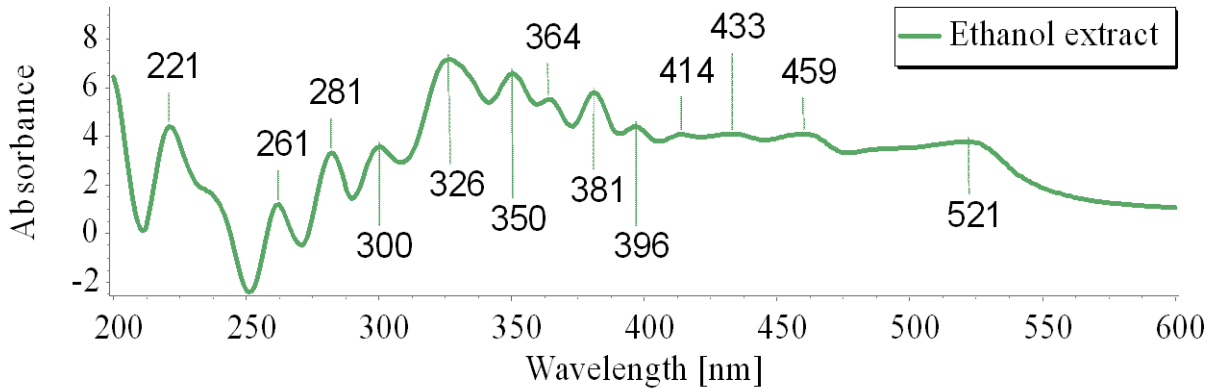


Graph 4.1: UV-Vis Spectra of turmeric extract in methanol

Table 4.2: Maximum UV-Vis absorbance wavelengths of turmeric extract in methanol

Sr no.	Wavelength (nm)	Absorbance
1	242.00	4.6634
2	256.00	6.7837
3	285.00	6.0544
4	317.00	6.9705
5	341.00	4.8819
6	362.00	4.7304
7	388.00	4.9272
8	407.00	7.0478
9	425.00	6.9587
10	454.00	6.0572
11	527.00	4.7329

4.4.2 UV-Vis Spectra of Turmeric Extract in Ethanol

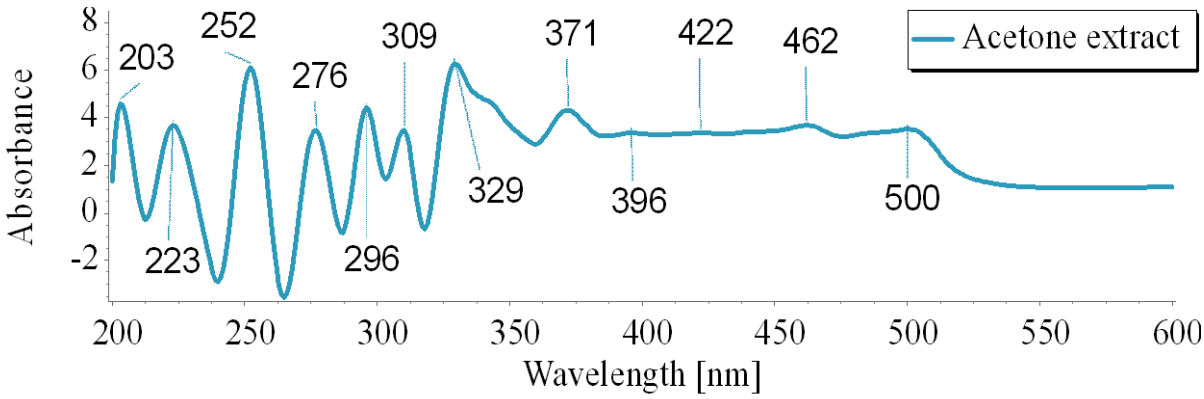


Graph 4.2: UV-Vis Spectra of turmeric extract in ethanol

Table 4.3: Maximum UV-Vis absorbance wavelengths of turmeric extract in ethanol

Sr no.	Wavelength (nm)	Absorbance
1	221.00	4.3959
2	261.00	1.2225
3	281.00	3.3217
4	300.0	3.5690
5	326.00	7.1441
6	350.00	6.5576
7	381.00	5.7983
8	396.00	4.3830
9	433.00	4.0943
10	459.00	4.0844
11	521.00	3.7727

4.4.3 UV-Vis Spectra of Turmeric Extract in Acetone

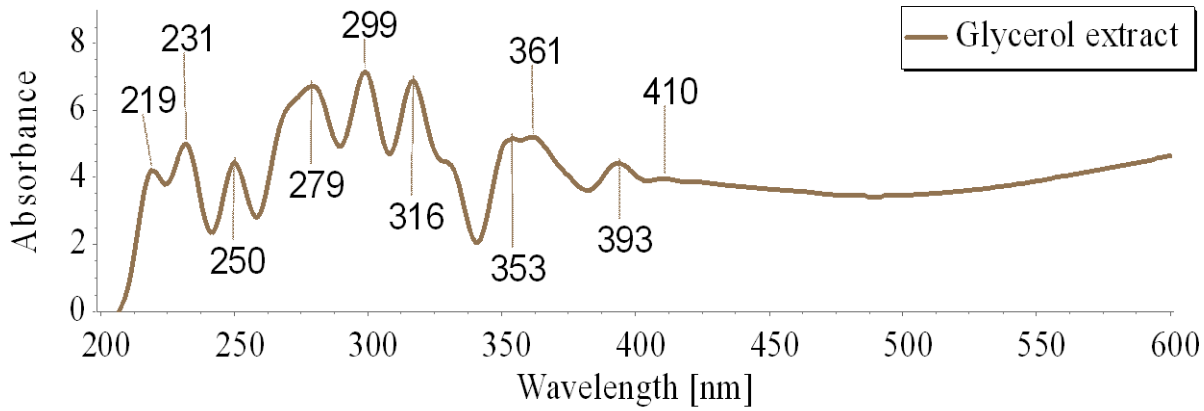


Graph 4.3: UV-Vis Spectra of turmeric extract in acetone

Table 4.4: Maximum UV-Vis absorbance wavelengths of turmeric extract in acetone

Sr no.	Wavelength (nm)	Absorbance
1	223.00	3.6920
2	252.00	6.1073
3	277.00	3.4835
4	296.00	4.4244
5	310.00	3.4748
6	329.00	6.2789
7	372.00	4.3187
8	396.00	3.3559
9	422.00	3.3502
10	462.00	3.6822
11	500.0	3.5157

4.4.4 UV-Vis Spectra of Turmeric Extract in Glycerol



Graph 1.4: UV-Vis Spectra of turmeric extract in glycerol

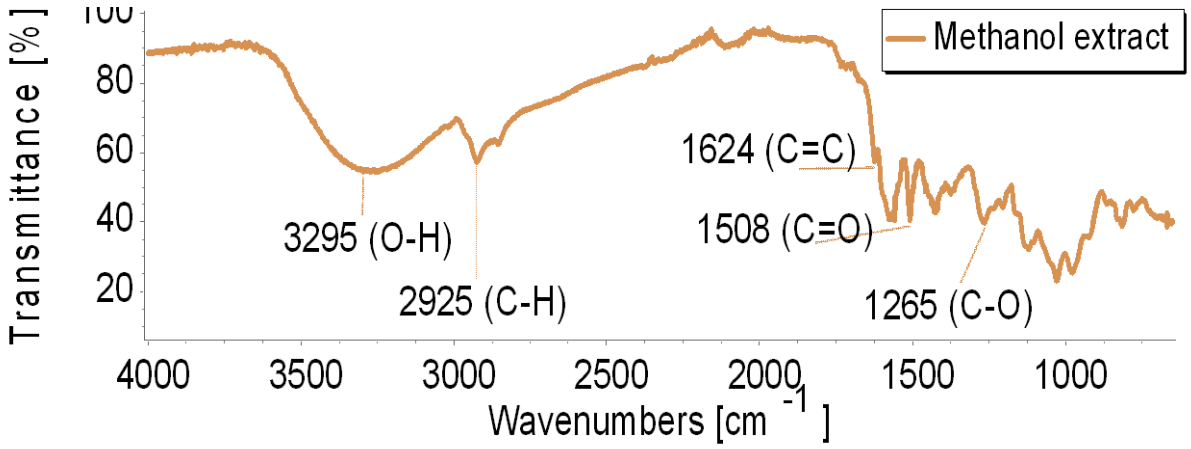
Table 4.5: Maximum UV-Vis absorbance wavelengths of turmeric extract in glycerol

Sr no.	Wavelength (nm)	Absorbance
1	219.00	4.1979
2	232.00	5.0093
3	250.00	4.4437
4	279.00	6.7390
5	299.00	7.1615
6	317.00	6.8747
7	354.00	5.1448
8	362.00	5.1935
9	394.00	4.4256
10	411.00	3.9657
11	219.00	4.1979

4.5 FTIR Analysis

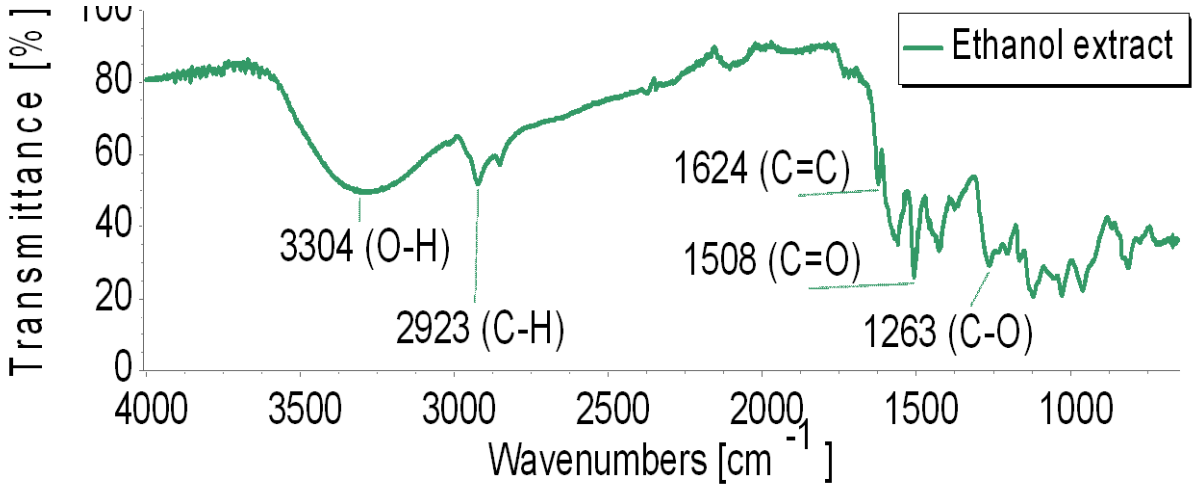
FTIR (Fourier Transform Infrared Spectroscopy) analysis was performed on four different turmeric extracts obtained using different solvents: ethanol, methanol, glycerol, and acetone. The FTIR spectra were obtained to characterize the functional groups present in each extract and to compare the molecular structure of curcumin in different solvent environments. The FTIR analysis of four different turmeric extracts (ethanol, methanol, acetone, and glycerol) revealed distinctive variations in peak intensities, reflecting the presence of specific functional groups in each solvent.

4.5.1 FTIR Spectra of Methanol Extract



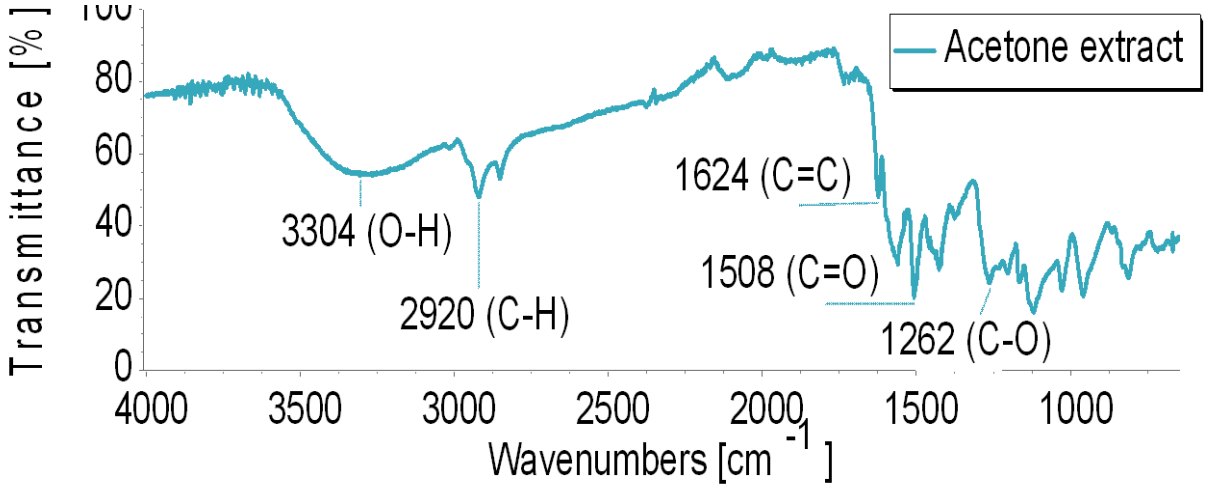
Graph 4.5: FTIR spectra of curcumin extracted through methanol

4.5.2 FTIR Spectra of Ethanol Extract



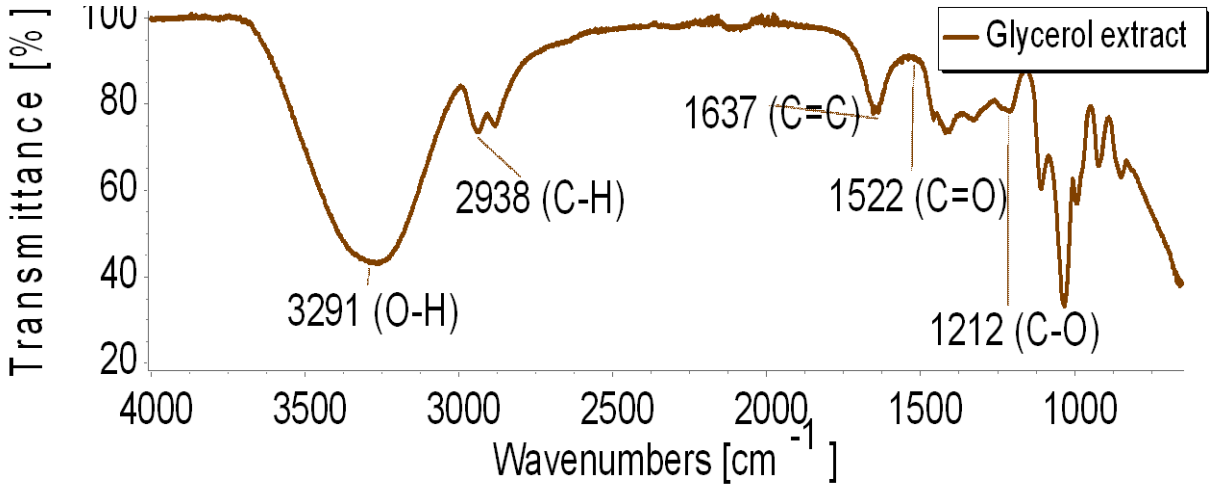
Graph 4.6: FTIR spectra of curcumin extracted through ethanol

4.5.3 FTIR Spectra of Acetone Extract



Graph 4.7: FTIR spectra of curcumin extracted through acetone

4.5.4 FTIR Spectra of Glycerol Extract



Graph 4.8: FTIR spectra of curcumin extracted through glycerol

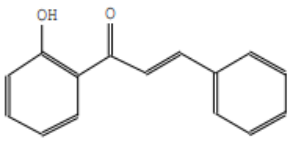
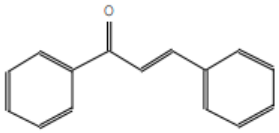
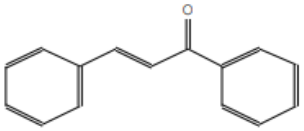
4.6 GCMS Analysis

The GCMS analysis of the methanolic extract of turmeric revealed a comprehensive profile of chemical constituents present in the sample. A total of 734 compounds were detected and identified through this analysis.

4.6.1 Curcumin related compounds

The GC-MS analysis of the methanolic extract revealed the presence of several chalcones, which are structurally related to curcumin. While the exact structure of curcumin was not identified in the results, the presence of these chalcones indicates the potential presence of curcumin or curcumin-like compounds in the extract. The identified chalcones add to the complexity and diversity of the chemical profile of the extract, offering insights into the possible presence of bioactive components with similar structural features to curcumin.

Table 4.6: Chalcones Identified in Turmeric Extract GC-MS Analysis (Methanol)

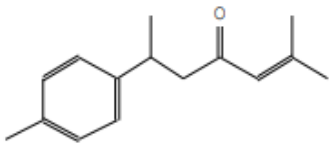
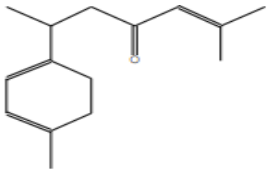
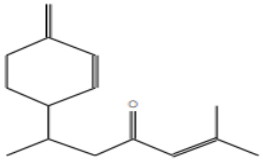
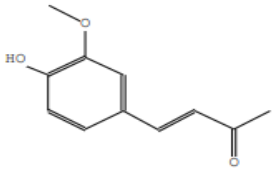
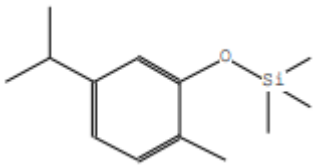
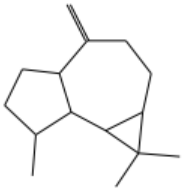
Sr no.	Chalcones	Chemical structure	Molecular formula	Molecular weight g/mol
1	2-Propen-1-one, 1-(2-hydroxyphenyl)-3-phenyl		$C_{12}H_{20}O_2$	224
2	(E)-1,3-diphenylprop-2-en-1-one		$C_{12}H_{20}O$	208
3	2-Propen-1-one, 1,3-diphenyl-, (E)-		$C_{12}H_{20}O$	208

4.6.2 Major Bioactive Compounds

Among the 734 compounds identified, a significant number were found to possess bioactive properties with potential medicinal significance. These bioactive compounds exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial properties, among others. The detection of these bioactive compounds underscores the potential health benefits of the methanolic extract of curcumin and its possible applications in various therapeutic areas. Some of them are listed in table 13.

Table 4.7: Major bioactive compounds Identified in Turmeric Extract

GC-MS Analysis (Methanol)

Sr no.	Compound Name	Chemical Structure	Molecular Formula	Molecular weight g/mol
1	aR-Turmerone		$C_{15}H_{20}O$	216
2	Tumerone		$C_{15}H_{20}O$	216
3	Curlone		$C_{15}H_{22}O$	218
4	Atlantone		$C_{11}H_{12}O_3$	192
5	Carvacrol		$C_{15}H_{22}O$	218
6	Alloaromadendrene		$C_{15}H_{24}$	204

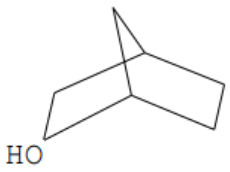
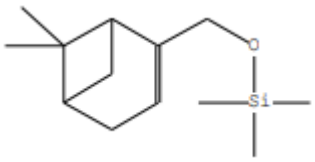
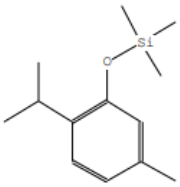
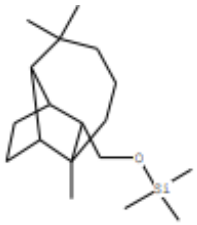
7	Exo-Norbornyl alcohol		$C_7H_{12}O$	112
8	Myrtenol		$C_{13}H_{24}OSi$	224
9	Epiglobulol		$C_{15}H_{26}O$	222
10	Thymol		$C_{13}H_{22}OSi$	222
11	Isolongifolol		$C_{18}H_{34}OSi$	294

Table 4.8: Pharmacology of major bioactive compounds identified in GCMS analysis of turmeric extract (methanol)

Sr no.	Compound Name	Pharmacology	Reference
1	aR-Turmerone	Anti-oxidant,Anti-inflammotary, Anti-cancer, Neuroprotective, Wound healing	(Orellana-Paucar & Machado-Orellana, 2022)
2	Tumerone	Anti-microbial, Anti-oxidant,Anti-inflammotary, Neuroprotective	(Orellana-Paucar & Machado-Orellana, 2022)
3	Atlantone	Skin health, Anti-oxidanti, Anti-inflammatory	(Nadeem, Khalid, Jilani, Rahman, & Sciences, 2019)
4	Chalcone	Anti-diabetic, Cardiovascular, anti-inflammatory, antioxidant	(Nadeem et al., 2019)
5	Alloaromadendrene	Analgesic, gastrointestinal effect, respiratory benefits, anti-microbial	(S Metwally et al., 2020)
6	Minoxidil	Stimulate hair follicles, Increase blood flow	(Ohko et al., 2020)
7	Camphor	Anti-septic, anti-pruritic, anti-infective	(Sharma, Tiwari, Das, & Parmananda, 2021)

8	Norborneol	Anti-cancer activity	(Calvo-Martín et al., 2022; Jha et al., 2023)
9	Carvacrol	Antioxidant, antimicrobial, and anti-inflammatory	(de Carvalho et al., 2020; Silva et al., 2018)
10	Thymol	Antimicrobial, antioxidant, and anti-inflammatory, gastroprotective, respiratory health benefits, anti-cancer	(Sahoo, Paidesetty, & Padhy, 2021)
11	Isolongifolol	Antimicrobial and anti-inflammatory properties,	(Wahab et al., 2023)
12	Patchouli alcohol	Antimicrobial, anti-inflammatory, and antioxidant, anti-cancer	(Xie et al., 2020; W. ZHANG, ZHANG, GUO, CHEN, & YIN, 2020)
12	Myrtenol	Antimicrobial and antioxidant	(de Britto et al., 2018; Sousa et al., 2022)
14	Epiglobulol	Antioxidant, anti-inflammatory	(Jayaprakash, Johns, Haneef, Radhamany, & Industries, 2019; Majumder, Ghosh, & Bhattacharya, 2020)

4.6.3 Other Compounds in GCMS Analysis of Turmeric Extract (methanol)

Due to the extensive nature of the analysis, only select compounds from each phytochemical class are presented here. The identified compounds were categorized into various classes, such as phenols, terpenes, terpenoids, sterols, aliphatic hydrocarbons, flavonoids, coumarins, alkaloids, and carboxylic acids.

4.6.3.1 Phenols

Phenolic compounds are characterized by the presence of a phenol group, which consists of a benzene ring (aromatic ring) attached to a hydroxyl (-OH) group. The phenol group is responsible for the distinctive properties of these compounds. They are the most important bioactive compounds found in a wide range of plant-based foods and herbal medicines. They have antioxidant, anti-inflammatory, and anti-cancer properties. They protect cells from damage, reduce inflammation, and may help prevent chronic diseases.

Table 4.9: Phenols in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular Formula	Molecular weight g/mol
1	cis-3-Hexenyl salicylate	$C_{13}H_{16}O_3$	220
2	Hexanoic acid, 6-salicylidenamino	$C_{13}H_{17}NO_3$	235
3	2-Propen-1-one, 1-(2-hydroxyphenyl)-3-phenyl	$C_{15}H_{12}O_2$	224
4	2-Hydroxychalcone	$C_{15}H_{12}O_2$	224
5	cis-3-Hexenyl salicylate	$C_{13}H_{16}O_3$	220
6	2-Hydroxy-5-(2-hydroxybenzylideneamino) benzoic acid	$C_{14}H_{11}NO_4$	257

7	Phenol, 4-(2-amino-5-nitrophenyliminomethyl)-2-methoxy	$C_{14}H_{13}N_3O_4$	287
8	Cinnamyl angelate,	$C_{14}H_{16}O_2$	216

4.6.3.2 Terpenes

Terpenes are a diverse group of organic compounds found in plants, and they exhibit a wide range of pharmacological activities. Their structures are based on the repetition of isoprene units (C5H8), which can be arranged in different ways to form various terpene classes, such as monoterpenes, diterpenes, triterpenes, tetraterpenes. They exhibit various pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. Some terpenes have immunomodulatory properties and support eye health. Terpenes play a crucial role in traditional medicine and have potential as sources for new drug development.

Table 4.10 Terpenes in turmeric extract (methanol) GCMS analysis

Sr no.	**Compound Name**	**Molecular Formula**	**Molecular Weight g/mol**
1	cis-Verbenol	$C_{10}H_{16}O$	152
2	(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	$C_{10}H_{16}O$	152
3	2-Hepten-4-one, 6-hydroxy-2-methyl-6-(4-methyl-3-cyclohexen-1-yl)-	$C_{15}H_{24}O_2$	236
4	p-Mentha-1,5-dien-8-ol	$C_{10}H_{16}O$	152
5	Carvacrol	$C_{13}H_{22}OSi$	222
6	Thymol	$C_{13}H_{22}OSi$	222
7	Verbenyl angelate, cis (E)	$C_{15}H_{22}O_2$	234

4.6.3.3 Sesquiterpenes

Sesquiterpenes are a class of terpenes, which are natural compounds derived from the isoprene unit and composed of three isoprene units (15 carbons). They have been studied for their anti-inflammatory, antimicrobial, antioxidant, and anticancer activities, among others, highlighting their potential therapeutic applications.

Table 4.11: Sesquiterpenes in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular Formula	Molecular Weight g/mol
1	Tumerone	$C_{15}H_{20}O$	216
2	aR-Turmerone	$C_{15}H_{20}O$	216
3	Ar-tumerone	$C_{15}H_{20}O$	216
4	(Z)-.gamma.-Atlantone	$C_{15}H_{22}O$	218
5	Patchouli alcohol	$C_{15}H_{26}O$	222
6	(-)-Isolongifolol, TMS derivative	$C_{18}H_{34}OSi$	29444
7	Epiglobulol	$C_{15}H_{26}O$	222

4.6.3.4 Terpenoids

Terpenoids are a subclass of terpenes, consisting of modified terpene compounds that may undergo oxidation or other chemical modifications. Pharmacologically, terpenoids exhibit a wide range of activities, including antioxidant, antimicrobial, anti-inflammatory, antiviral, and anticancer effects. Their potential therapeutic applications have led to extensive research and exploration of terpenoids for drug development and alternative medicine.

Table 4.12: Terpenoids in turmeric extract (methanol) GCMS analysis

Sr no.	**Compound Name**	**Molecular Formula**	**Molecular Weight g/mol**
1	(-)-Myrtenol, TMS derivative	$C_{13}H_{24}OSi$	224
2	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans	C10H16O	152
3	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{31}H_{52}O$	440
4	9,19-Cyclolanostan-3-ol, 24-methylene-, acetate, (3.beta.)-	$C_{33}H_{54}O_2$	482
5	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$	426
6	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-	$C_{31}H_{52}O$	440
7	Isosteviol	$C_{20}H_{30}O_3$	318
8	(7S,8R,S)-7-Hydroxymethyl-8-ethoxy-cis-bicyclo[4.3.0]-3-nonene	$C_{12}H_{20}O_2$	196
9	5-Isopropenyl-1,2-dimethylcyclohex-2-enol	$C_{11}H_{18}O$	166
10	3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	$C_{10}H_{16}O$	152

4.6.3.5 Coumarins

Coumarins are a class of aromatic organic compounds with a benzene ring fused to an alpha-pyrone ring. it exhibits various pharmacological activities. It is known for its anticoagulant, anti-inflammatory, antioxidant, antimicrobial, antitumor, vasodilatory, and hepatoprotective properties.

Table 4.13: Coumarins in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular Formula	Molecular Weight g/mol
1	Benzofuran-2,3-dione, 4,7-dimethyl-2,3-dihydro	$C_{10}H_8O_3$	176
2	1,3-Isobenzofurandione, 4,5-methylenedioxy	C9H4O5	192
3	2-Methyl-2-phenyl-5-(1,4-dihydropyridin-4-ylidene)-1,3-dioxan-4,6-dione	$C_{16}H_{13}NO_4$	283
4	4H-1-benzopyran, 4,4'-oxybis[2-phenyl	$C_{30}H_{22}O_3$	430

4.6.3.6 Sterols

Plant sterols, also known as phytosterols, have a steroidal structure with four interconnected rings of carbon atoms. They have various potential health benefits, including anti-inflammatory and anticancer properties. Some plant steroids have been studied for their therapeutic potential in certain medical conditions.

Table 4.14: Sterols in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular Formula	Molecular Weight g/mol
1	Androsta-3,5-dien-7-one	$C_{19}H_{26}O$	270
2	Pregna-4,16-diene-3,20-dione, 16-methyl	$C_{22}H_{30}O_2$	326
3	Cyclopropa[16,17]pregn-4-ene-3,20-dione, 3',16-dihydro-, (16.beta.)-	$C_{22}H_{30}O_2$	326
4	9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-, (3.beta.,5Z,7E)-	C24H36O2	356

4.6.3.7 Alkaloids

Alkaloids are a class of naturally occurring compounds that contain nitrogen and have a pronounced basic nature. They have diverse structures but commonly include nitrogen atoms in rings, aromatic or heterocyclic rings, and various functional groups. Alkaloids have a wide range of biological activities, including analgesic (pain-relieving), stimulant, sedative, anti-inflammatory, and antimalarial properties.

Table 4.15: Alkaloids in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular formula	Molecular Weight g/mol
1	Tetrahydropyrazine	$C_5H_{10}N_2$	98
2	Morphinan-3,14-diol, 4,5-epoxy-, (5.alpha.)-	$C_{16}H_{19}NO_3$	273
3	Morphinan-3,14-diol, 4,5-epoxy-, (5.alpha.)-	$C_{16}H_{19}NO_3$	273

4.6.3.8 Aliphatic Hydrocarbons

In plants, aliphatic hydrocarbons are a diverse group of compounds that play essential roles in various biological processes. They are used for essential oil production, traditional medicine, emollients in cosmetics, flavor enhancement in food, and formulation of insecticides.

Table 4.16: Aliphatic Hydrocarbons in turmeric extract (methanol) GCMS analysis

Sr no.	Compound Name	Molecular formula	Molecular Weight g/mol
1	3-Methyladipic acid	$C_7H_{12}O_4$	160
2	3-Hexenoic acid, (E)-	$C_6H_{10}O_2$	114
3	5-Nonynoic acid	$C_9H_{14}O_2$	154

4	Ethyl 5,8,11,14-eicosatetraenoate	$C_{22}H_{36}O_2$	332
5	Undec-10-ynoic acid, octyl ester	$C_{19}H_{34}O_2$	294
6	Adipic acid, 1-phenylpropyl propyl ester	$C_{18}H_{26}O_4$	306
7	3-Octyn-1-ol	$C_8H_{14}O$	126
8	Phenacetic acid, 2-carboxy-3-methyl	$C_{10}H_{10}O_4$	194
9	n-Heptyl hexanoate	$C_{13}H_{26}O_2$	214
10	Hexanoic acid, 1-methylhexyl ester	$C_{13}H_{26}O_2$	214
11	Exo-Norbornyl alcohol	$C_7H_{12}O$	112
12	Exo-Norbornyl propionate	$C_{10}H_{16}O_2$	168
13	Exo-Norborneol, methyl ether	$C_8H_{14}O$	126

Chapter 5: Discussion

5.1 Extraction Method

The extraction of curcumin from *Curcuma longa* using the Soxhlet extraction method with four different solvents (ethanol, methanol, acetone, and glycerol) yielded valuable insights into the efficiency and selectivity of the extraction process. Curcumin, being a non-polar compound, requires careful consideration of the solvent selection.

5.1.1 Solvent Selection

Curcumin is a lipophilic compound and exhibits limited solubility in polar solvents. Therefore, the use of non-polar or moderately polar solvents is more suitable for efficient extraction. Ethanol and methanol are polar solvents, and they still extract some curcumin due to their ability to solubilize small amounts of non-polar compounds. Acetone, being moderately polar, strikes a better balance between solvating curcumin and other non-polar compounds in turmeric. Glycerol, although non-polar, is viscous and non-volatile, which complicates the evaporation process and necessitates alternative drying methods.

5.1.2 Solvent Evaporation

The solvent evaporation step is crucial in obtaining a dry curcumin extract. Ethanol and methanol, being volatile, readily evaporated at room temperature, allowing for the recovery of a concentrated curcumin extract. Acetone also demonstrated good volatility, aiding in the extraction process. However, the non-volatility of glycerol posed challenges, as it remained as a viscous residue. For glycerol extracts, alternative drying techniques like freeze-drying or vacuum drying are recommended to remove glycerol completely and obtain a dry curcumin extract. When considering industrial applications or large-scale production, factors like solvent cost, safety, and environmental impact should be considered. Ethanol and methanol are commonly used solvents on a large scale due to their affordability and ease of handling. Acetone is also a viable option for scale-up. However, the non-volatile nature of glycerol makes it impractical for large-scale extraction.

5.1.3 Concept of “Whole Plant Medicine”:

In this study, it is important to note that curcumin was not purified from the extract intentionally. The decision to retain the other phytochemicals present in the extract was deliberate, as these compounds may contribute to the overall therapeutic potential of the extract. Curcumin is just one of the numerous bioactive compounds found in turmeric, and its biological effects can be influenced by the synergistic action of other phytochemicals. The presence of

other bioactive compounds, such as other chalcones, terpenoids, flavonoids, and alkaloids, in the crude extract could enhance the extract's pharmacological activities. Studies have shown that these compounds can exhibit anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Furthermore, the combination of different compounds may result in a broader spectrum of activity, leading to potential benefits in a variety of health conditions. Indeed, the concept of "whole plant medicine" has gained attention in recent years, emphasizing that the therapeutic potential of a plant extract can be greater than that of isolated compounds. The synergistic effects of multiple compounds, known as the "entourage effect," have been proposed to enhance the overall efficacy of the extract. However, it is essential to recognize that this approach may also introduce complexity in understanding the specific contributions of individual compounds to the observed effects. Purified curcumin is often used in research to isolate its effects and establish clear cause-and-effect relationships. Nonetheless, the use of the crude extract, with its diverse phytochemical profile, reflects the more holistic way in which herbal medicine has been traditionally employed.

In light of these considerations, further investigation into the biological activities of the crude extract, containing curcumin and other phytochemicals, would be valuable. Animal studies and in vitro assays can shed light on the potential synergistic effects and therapeutic applications of the whole extract. Moreover, exploring potential interactions between different compounds could provide insights into how the extract's components work together to achieve therapeutic outcomes.

5.2 Color Variation of Turmeric Extracts

The color variation observed in the turmeric extracts obtained from different solvents reflects the varying solubilities of curcuminoids and other phytochemicals. The intense orange-red color in the methanol extract indicates a higher concentration of curcuminoids, while the lighter yellow-orange color in the acetone extract suggests a lower concentration. The brownish-yellow color in the glycerol extract is a result of the lower solubility of curcuminoids in this non-polar solvent. It is important to note that the color variation is not solely dependent on the concentration of curcuminoids in the extract but also influenced by the presence of other pigments and phytochemicals. Turmeric contains other compounds such as carotenoids and polyphenols, which can also contribute to the overall color of the extract. Furthermore, the interaction between different compounds in the crude extract may lead to color modifications.

5.3 Difference in pH and Density measurements of Turmeric Extracts

The discussion on different pH and density values of turmeric extracts provides valuable insights into the chemical composition and physical characteristics of the extracted compounds using various solvents.

5.3.1 pH Measurements

The pH values of the extracts, ranging from 5.86 to 6.81, indicate that the extracts are slightly acidic to neutral in nature. These pH variations can be attributed to the presence of different phytochemicals in the extracts. Turmeric contains a variety of compounds, including curcuminoids, which are known to have acidic properties.

5.3.2 Density Measurements

Regarding density, the values ranging from 0.787 to 1.27 g/cm³ indicate differences in the concentration of dissolved solids and compounds in the extracts. The higher density of glycerol extract (1.27 g/cm³) compared to other solvents might be attributed to the presence of non-volatile and viscous components like glycerol itself, which contributes to the higher mass per unit volume.

5.4 UV-Vis Analysis of Turmeric Extracts

The UV analysis of curcumin extracts was performed to verify the presence of curcumin and identify its absorption wavelength in different solvents. The obtained UV spectra showed multiple peaks in all four extracts, indicating the presence of other phytochemicals alongside curcumin. However, the methanol extract stood out with distinct and well-defined peaks in the absorption spectra of curcumin, ranging from 350 to 500 nm, suggesting a higher concentration of curcumin in this solvent compared to the others. In contrast, the UV spectra of curcumin in the remaining solvents (ethanol, acetone, and glycerol) displayed absorbance, but there were no distinct peaks. The relatively lower intensity of these spectra suggests a lower concentration of curcumin in these solvents. The absence of well-defined peaks in these solvents could be attributed to the presence of interfering compounds that might overlap with the absorption bands of curcumin, leading to a more complex and less pronounced spectrum. The high concentration of curcumin in the methanol extract is evident from the distinct peak at 425 nm, which aligns with the primary absorption wavelength of curcumin. This peak not only confirmed the presence of curcumin in the methanol extract but also suggests a higher concentration of this bioactive compound compared to the other solvents.

Considering the UV results, methanol appears to be the most suitable solvent for investigating curcumin due to its ability to yield a well-defined peak with high intensity, making it easier to identify and quantify curcumin. The other solvents, while still showing some absorbance related to curcumin, lack the distinctness of the peak seen in the methanol extract, which can hinder accurate identification and quantification of curcumin content.

5.5 FTIR Analysis of Turmeric Extracts

The FTIR analysis was conducted to identify and assess the important functional groups of curcumin in different extracts. The hydroxyl group, characteristic of alcohols and phenols, was observed in all four solvents. The higher peak intensity in glycerol suggests that this solvent might be more effective in extracting compounds with hydroxyl groups, potentially enhancing the therapeutic properties of the turmeric extract. The C-H stretching peaks, typical of aliphatic compounds, were present in all four solvents, indicating the presence of aliphatic hydrocarbons in the extracts. This suggests that the choice of solvent does not significantly affect the extraction of aliphatic compounds. The aromatic group peak was noticeable in methanol, ethanol, and acetone, indicating the presence of aromatic compounds in these extracts. However, the less distinct peaks in these solvents suggest that other functional groups may dominate the spectra. The absence of the carbonyl group peak in glycerol is intriguing, as the carbonyl group is a significant functional group in curcumin, suggesting that glycerol may not be the most effective solvent for curcumin extraction. The results reveal that solvents Ethanol, methanol, and acetone demonstrate more noticeable interactions with functional groups of curcumin compared to glycerol which exhibits the most favorable interaction with the hydroxyl group but has lower interaction with rest of the functional groups.

Table 1 presents the FTIR wavenumber ranges for different functional groups of curcumin, along with the corresponding literature values and the experimental values obtained from the extracts in methanol, ethanol, acetone, and glycerol.

Table 5.1: FTIR Wavenumber Ranges and Comparative Values for Curcumin Functional Groups in turmeric extracts

Functional groups	**FTIR range**	**Wavenumber (cm^{-1})**				
		Literature	**Experimental curcumin extracts**			
		(Charan et al., 2022)	**MetoH extract**	**EtoH extract**	**Acetone extract**	**Glycerol extract**
O-H	3200-3600cm^{-1}	3303cm^{-1}	3301cm^{-1}	3283cm^{-1}	3305cm^{-1}	3318cm^{-1}
C-H	2800-3000cm^{-1}	2929cm^{-1}	2925cm^{-1}	2923cm^{-1}	2920cm^{-1}	2938cm^{-1}
C=C	1600-1680cm^{-1}	1622cm^{-1}	1624cm^{-1}	1624cm^{-1}	1600cm^{-1}	1637cm^{-1}
C-O	1000-1300cm^{-1}	1270cm^{-1}	1265cm^{-1}	1263cm^{-1}	1262cm^{-1}	1212cm^{-1}
C=O	1500-1530cm^{-1}	1510cm^{-1}	1508cm^{-1}	1508cm^{-1}	1508cm^{-1}	1522cm^{-1}

5.6 GCMS Analysis

During the GCMS analysis, various phenolic compounds and chalcones were detected, indicating the rich phenolic content of the turmeric extract. However, it is essential to note that curcumin, the primary phenolic compound of interest, was not directly identified in the chromatograms. This may be due to various reasons, including its low concentration in the methanolic extract or interference from other co-eluting compounds. Although the chromatographic analysis did not directly detect curcumin, its presence can still be inferred from the detection of related phenolic compounds and chalcones. Curcumin is a known precursor for chalcones, and its conversion to these compounds may have occurred during the extraction and sample preparation process. The absence of the specific curcumin structure might also be due to its potential degradation during the GCMS analysis or the presence of derivatives with slightly different retention times.

Despite the challenges in directly identifying curcumin, the GCMS analysis successfully revealed the complexity and diversity of the phenolic compounds present in the turmeric extract. Many of these compounds have been reported to possess significant pharmacological activities, including antioxidants, anti-inflammatory, and anticancer properties. It also uncovered the presence of other bioactive compounds with intriguing biological activities. Thus, the methanolic extract of turmeric shows great potential for various medicinal and therapeutic applications. Future studies employing additional analytical techniques can further validate the presence and concentration of curcumin in the turmeric extract, contributing to a comprehensive understanding of its medicinal potential.

Chapter 6: Conclusion

The golden spice, Curcuma longa, commonly known as turmeric, has been revered for its vibrant color, distinct flavor, and historical significance in traditional medicine. In recent years, the spotlight has shifted towards one of its most prominent bioactive compounds – curcumin. This polyphenolic compound has garnered attention due to its potential health-promoting properties, ranging from anti-inflammatory and antioxidant effects to potential anticancer activities. As a staple in Pakistani culinary traditions and a natural resource abundantly cultivated in the region, turmeric holds the promise of not only flavoring dishes but also contributing to holistic well-being. This comprehensive research seeks to delve into the characterization of curcumin extracted from locally cultivated Curcuma longa in Pakistan, shedding light on its chemical intricacies, medicinal potential, and commercial implications. Through a meticulous examination of various extraction solvents and analytical techniques, this study offers a deeper understanding of the diverse facets of curcumin and its applications, further uncovering the untapped potential of this golden gem.

- **Comprehensive Insights:** This research provides valuable insights into the potential and applications of curcumin extracted from locally cultivated Curcuma longa in Pakistan.

- **Solvent Efficiency:** UV-Vis spectroscopy revealed methanol as the most efficient solvent, with a distinct peak at 425nm, optimizing curcumin extraction protocols for industrial applications.

- **Authenticity Confirmation:** FTIR analysis confirmed the presence of characteristic functional groups of curcumin in all extracts, validating their authenticity and potential medicinal properties.

- **GCMS Discovery:** GCMS analysis of the methanol extract unveiled an astounding 734 compounds, including curcumin fractions and essential oil constituents of turmeric, highlighting the extract's complexity.

- **Bioactive Potential:** The presence of numerous bioactive compounds with potential medicinal properties opens avenues for further research in drug discovery and development.

- **Therapeutic Potential:** The diverse chemical profile of Pakistani turmeric underscores its remarkable therapeutic potential, offering promising opportunities for harnessing its bioactivity in various therapeutic applications.

Future Prospects

The journey embarked upon in this study serves as a stepping stone to untapped potential and uncharted territories. Here are some compelling future prospects

- **Exploring Synergistic Effects:** Further investigations can delve into the potential synergistic effects of curcumin in conjunction with other compounds within the turmeric extract. This approach could reveal unique combinations that amplify the therapeutic benefits beyond what individual compounds could offer.
- **Clinical Validation:** Translating the promising bioactive compounds identified in this research into clinical trials holds immense potential. Rigorous clinical studies could validate the therapeutic effects observed in vitro, potentially leading to novel treatments and interventions.
- **Biotechnological Advancements:** With the growing importance of biotechnology, the optimization of extraction techniques and cultivation methods for high-yield curcumin-rich turmeric varieties could become a cornerstone of research. This could enhance the availability and affordability of curcumin-rich extracts.
- **Tailored Formulations:** The diverse compounds identified in this study could inspire the development of tailored formulations for specific health conditions. Formulating combinations that harness the unique attributes of different phytochemical classes could create highly targeted and effective interventions.
- **Novel Delivery Systems:** Exploring innovative delivery systems, such as nanoparticles or encapsulation, could enhance the bioavailability and efficacy of curcumin and other bioactive compounds. This could address challenges related to absorption and utilization in the body.
- **Functional Food Development:** Integrating curcumin-rich extracts into functional foods or beverages could offer a convenient and enjoyable way to incorporate its health benefits into daily life, catering to consumer preferences for natural and holistic wellness solutions.

- **Eco-friendly Extraction:** As sustainability gains momentum, developing environmentally friendly extraction methods using greener solvents or innovative technologies could align with the global shift towards eco-conscious practices.

References

Akram, M., Shahab-Uddin, A. A., Usmanghani, K., Hannan, A., Mohiuddin, E., & Asif, M. J. R. J. B. P. B. (2010). Curcuma longa and curcumin: a review article. *55*(2), 65-70.

Arivoli, S., Tennyson, S., Divya, S., Rani, S., Marin, G. J. J. o. P., & Phytochemistry. (2019). GC-MS analysis of bioactive compounds of Curcuma longa Linnaeus (Zingiberaceae) rhizome extract. *8*(6), 49-52.

Ayati, Z., Ramezani, M., Amiri, M. S., Moghadam, A. T., Rahimi, H., Abdollahzade, A., . . . Emami, S. A. J. C. p. d. (2019). Ethnobotany, phytochemistry and traditional uses of Curcuma spp. and pharmacological profile of two important species (C. longa and C. zedoaria): a review. *25*(8), 871-935.

Benameur, T., Soleti, R., Panaro, M. A., La Torre, M. E., Monda, V., Messina, G., & Porro, C. J. M. (2021). Curcumin as prospective anti-aging natural compound: focus on brain. *26*(16), 4794.

Binello, A., Grillo, G., Barge, A., Allegrini, P., Ciceri, D., & Cravotto, G. J. F. (2020). A cross-flow ultrasound-assisted extraction of curcuminoids from Curcuma longa L.: Process design to avoid degradation. *9*(6), 743.

Calvo-Martín, G., Plano, D., Martínez-Sáez, N., Aydillo, C., Moreno, E., Espuelas, S., & Sanmartín, C. J. P. (2022). Norbornene and Related Structures as Scaffolds in the Search for New Cancer Treatments. *15*(12), 1465.

Charan, T. R., Bhutto, M. A., Bhutto, M. A., Tunio, A. A., Murtaza, G., Aftab, U., . . . Khaskheli, S. A. J. J. o. I. T. (2022). Comparative analysis by total yield, antimicrobial and phytochemical evaluation of curcuminoid of district Kasur: With its potential use and characterization in electrospinning nanofibers. *52*, 15280837221111457.

Chen, Z., Quan, L., Zhou, H., Zhao, Y., Chen, P., Hu, L., . . . Cao, D. J. J. o. e. (2019). Screening of active fractions from Curcuma Longa Radix isolated by HPLC and GC-MS for promotion of blood circulation and relief of pain. *234*, 68-75.

Chhouk, K., Wahyudiono, Kanda, H., Kawasaki, S.-I., Goto, M. J. F. o. C. S., & Engineering. (2018). Micronization of curcumin with biodegradable polymer by supercritical anti-solvent using micro swirl mixer. *12*, 184-193.

Dandekar, D. V., Gaikar, V. J. S. S., & Technology. (2002). Microwave assisted extraction of curcuminoids from Curcuma longa. *37*(11), 2669-2690.

de Britto, R. M., da Silva-Neto, J. A., Mesquita, T. R. R., de Vasconcelos, C. M. L., de Almeida, G. K. M., de Jesus, I. C. G., . . . Toxicology, C. (2018). Myrtenol protects against myocardial ischemia-reperfusion injury through antioxidant and anti-apoptotic dependent mechanisms. *111*, 557-566.

de Carvalho, F. O., Silva, É. R., Gomes, I. A., Santana, H. S. R., do Nascimento Santos, D., de Oliveira Souza, G. P., . . . de Souza Araújo, A. A. J. P. R. (2020). Anti-inflammatory and antioxidant activity of carvacrol in the respiratory system: A systematic review and meta-analysis. *34*(9), 2214-2229.

Den Hartogh, D. J., Gabriel, A., & Tsiani, E. J. N. (2020). Antidiabetic properties of curcumin I: Evidence from in vitro studies. *12*(1), 118.

Dutta, B. J. J. M. P. S. (2015). Study of secondary metabolite constituents and curcumin contents of six different species of genus Curcuma. *3*(5), 116-119.

Fuloria, S., Mehta, J., Chandel, A., Sekar, M., Rani, N. N. I. M., Begum, M. Y., . . . Nordin, R. J. F. i. P. (2022). A comprehensive review on the therapeutic potential of Curcuma longa Linn. in relation to its major active constituent curcumin. *13*, 820806.

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. J. B. p. (2008). Curcumin as "Curecumin": from kitchen to clinic. *75*(4), 787-809.

Gopalan, B., Goto, M., Kodama, A., Hirose, T. J. J. o. A., & Chemistry, F. (2000). Supercritical carbon dioxide extraction of turmeric (Curcuma longa). *48*(6), 2189-2192.

Gopinath, H., Karthikeyan, K. J. I. j. o. d., venereology, & leprology. (2018). Turmeric: A condiment, cosmetic and cure. *84*, 16.

Guimarães, A. F., Vinhas, A. C. A., Gomes, A. F., Souza, L. H., & Krepsky, P. B. J. Q. N. (2020). Essential oil of Curcuma longa L. rhizomes chemical composition, yield variation and stability. *43*, 909-913.

Gunathilake, T. M. S. U., Ching, Y. C., Uyama, H., Hai, N. D., & Chuah, C. H. J. C. (2022). Enhanced curcumin loaded nanocellulose: A possible inhalable nanotherapeutic to treat COVID-19. *29*(3), 1821-1840.

Hazra, K., Kumar, R., Sarkar, B. K., Chowdary, Y. A., Devgan, M., & Ramaiah, M. J. I. j. o. p. (2015). UV-visible spectrophotometric estimation of curcumin in nanoformulation. *2*(3), 127-130.

Irshad, S., Muazzam, A., Shahid, Z., & Dalrymple, M. B. J. P. J. P. S. (2018). Curcuma longa (Turmeric): An auspicious spice for antibacterial, phytochemical and antioxidant activities. *31*(6), 2689-2696.

Jacob, J. J. S. i. n. p. c. (2016). Comparative studies in relation to the structure and biochemical properties of the active compounds in the volatile and nonvolatile fractions of turmeric (C. longa) and ginger (Z. officinale). *48*, 101-135.

Jacob, M. J. M. s. (2016). Everything's coming up turmeric. *129*(31), 7-7.

Jayaprakash, A., Johns, A. E., Haneef, F. K., Radhamany, P. J. M. P.-I. J. o. P., & Industries, R. (2019). GC-MS analysis and in silico molecular docking studies of anti-inflammatory compounds from Thottea barberi (gamble) ding Hou root. *11*(3), 286-291.

Jennings, M. R., & Parks, R. J. J. V. (2020). Curcumin as an antiviral agent. *12*(11), 1242.

Jha, V., Kadam, P., Jain, T., Bhargava, A., Marick, A., Saiya, B., . . . Jadhav, N. J. J. o. U. A.-Q. U. f. A. S. (2023). Investigation of physico-chemical properties and evaluation of the biological potential of essential oil extracted from Artemisia pallens. 1-14.

Jiang, T., Ghosh, R., Charcosset, C. J. T. i. f. s., & technology. (2021). Extraction, purification and applications of curcumin from plant materials-A comprehensive review. *112*, 419-430.

Jyotirmayee, B., & Mahalik, G. J. A. S. (2022). Traditional Uses and Variation in Curcumin Contentin Varieties of Curcuma–the Saffron of India. *9*(1), 06-12.

Jyotirmayee, B., & Mahalik, G. J. I. J. o. F. P. (2022). A review on selected pharmacological activities of Curcuma longa L. *25*(1), 1377-1398.

Kadam, P. V., Yadav, K. N., Bhingare, C. L., Patil, M. J. J. J. o. P., & Phytochemistry. (2018). Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. *7*(5), 1913-1918.

Kotra, V. S. R., Satyabanta, L., Goswami, T. K. J. J. o. f. s., & technology. (2019). A critical review of analytical methods for determination of curcuminoids in turmeric. *56*, 5153-5166.

Majumder, S., Ghosh, A., & Bhattacharya, M. J. B. o. t. N. R. C. (2020). Natural anti-inflammatory terpenoids in Camellia japonica leaf and probable biosynthesis pathways of the metabolome. *44*(1), 1-14.

Mandal, V., Maity, S., Dewanjee, S., & Mandal, S. J. P. M. (2008). Effect of matrix pretreatment on microwave assisted extraction of curcumin as an alternative to conventional solid liquid extraction. *74*(09), SL46.

Marton, L. T., Pescinini-e-Salzedas, L. M., Camargo, M. E. C., Barbalho, S. M., Haber, J. F. d. S., Sinatora, R. V., . . . Cincotto dos Santos Bueno, P. J. F. i. e. (2021). The effects of curcumin on diabetes mellitus: a systematic review. *12*, 669448.

Mirjanaik, R. H., Vishwanath, Y. J. J. o. P., & Phytochemistry. (2020). Advances in production technology of turmeric. *9*(4), 1198-1203.

Mushtaq, Z., Tahir Nadeem, M., Arshad, M. U., Saeed, F., Ahmed, M. H., Bader Ul Ain, H., . . . Hussain, S. J. I. J. o. F. P. (2019). Exploring the biochemical and antioxidant potential of ginger (Adric) and turmeric (Haldi). *22*(1), 1642-1651.

Nabati, M., Mahkam, M., & Heidari, H. J. I. c. c. (2014). Isolation and characterization of curcumin from powdered rhizomes of turmeric plant marketed in Maragheh city of Iran with soxhlet technique. *2*(4, pp. 236-325, Serial No. 5), 236-243.

Nadeem, F., Khalid, T., Jilani, M. I., Rahman, S. J. I. J. o. C., & Sciences, B. (2019). A review on ethnobotanical, phytochemical and pharmacological potentials of Cedrus deodara. *15*, 28-34.

Nair, K. P., Nair, K. P. J. T., Agronomy, G.-W. s. I. M. S. T., Turmeric, E. o., & Ginger. (2019). Ginger physiology. 367-373.

Nebrisi, E. E. J. I. j. o. m. s. (2021). Neuroprotective activities of curcumin in Parkinson's disease: A review of the literature. *22*(20), 11248.

Ohko, K., Nakajima, K., Nakajima, H., Hiraki, Y., Kubota, K., Fukao, T., . . . Sano, S. J. T. J. o. D. (2020). Skin and hair abnormalities of Cantu syndrome: a congenital hypertrichosis due to a genetic alteration mimicking the pharmacological effect of minoxidil. *47*(3), 306-310.

Orellana-Paucar, A. M., & Machado-Orellana, M. G. J. M. (2022). Pharmacological profile, bioactivities, and safety of turmeric oil. *27*(16), 5055.

Pawar, H. A., Gavasane, A. J., & Choudhary, P. D. J. N. P. C. R. (2018). A novel and simple approach for extraction and isolation of curcuminoids from turmeric rhizomes. *6*(1).

Peng, Y., Ao, M., Dong, B., Jiang, Y., Yu, L., Chen, Z., . . . therapy. (2021). Anti-inflammatory effects of curcumin in the inflammatory diseases: Status, limitations and countermeasures. 4503-4525.

Popuri, A. K., & Pagala, B. J. I. J. I. R. S. (2013). Extraction of curcumin from turmeric roots. *2*, 289-299.

Purushothaman, A., Rose, K. T., Jacob, J. M., Varatharaj, R., Shashikala, K., & Janardanan, D. J. F. C. (2022). Curcumin analogues with improved antioxidant properties: A theoretical exploration. *373*, 131499.

Qazi, M., Iqbal, M., Ashraf, M., Khan, N., Ahmad, R., Umar, F., . . . Iqbal, Z. J. P. J. o. S. (2020). NPK REQUIREMENTS OF TURMERIC FOR CULTIVATION IN PUNJAB. *72*(4).

S Metwally, A., A El-Naggar, H., A El-Damhougy, K., AE Bashar, M., Ashour, M., AH Abo-Taleb, H. J. E. J. o. A. B., & Fisheries. (2020). GC-MS analysis of bioactive components in six different crude extracts from the Soft Coral (Sinularia maxim) collected from Ras Mohamed, Aqaba Gulf, Red Sea, Egypt. *24*(6), 425-434.

Saeed, R., Bashir, A., Khan, S., Bakhsh, K., Qasim, M. J. P. J. o. A., Agricultural Engineering, & Sciences, V. (2017). An economic assessment of turmeric production in Punjab-Pakistan. *33*(1), 85-99.

Sahoo, C. R., Paidesetty, S. K., & Padhy, R. N. J. D. D. R. (2021). The recent development of thymol derivative as a promising pharmacological scaffold. *82*(8), 1079-1095.

Sharma, J., Tiwari, I., Das, D., & Parmananda, P. J. P. R. E. (2021). Chimeralike states in a minimal network of active camphor ribbons. *103*(1), 012214.

Shirsath, S., Sable, S., Gaikwad, S., Sonawane, S., Saini, D., & Gogate, P. J. U. S. (2017). Intensification of extraction of curcumin from Curcuma amada using ultrasound assisted approach: Effect of different operating parameters. *38*, 437-445.

Silva, E. R., de Carvalho, F. O., Teixeira, L. G., Santos, N. G. L., Felipe, F. A., Santana, H. S. R., . . . Nunes, P. S. J. C. p. d. (2018). Pharmacological effects of carvacrol in in vitro studies: a review. *24*(29), 3454-3465.

Singh, A., & Avupati, V. R. J. J. o. Y. P. (2017). Development and validation of UV-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulations. *9*(4), 491.

Singh, G., Singh, O. P., Maurya, S. J. P. i. C. G., & materials, C. o. (2002). Chemical and biocidal investigations on essential oils of some Indian Curcuma species. *45*(1-2), 75-81.

Sousa, D. B. d., Silva, G. S. d., Guedes, J. A., Serrano, L. A., Martins, M. V., Rodrigues, T. H., . . . Zocolo, G. J. J. J. o. t. B. C. S. (2022). Volatile Metabolomics from Cashew Leaves: Assessment of Resistance Biomarkers Associated with Black Mold (Pilgeriella anacardii Arx & Müller). *33*, 1423-1440.

Subhan, M., Alam, K., Rahaman, M., Rahman, M., & Awal, R. J. J. S. R. (2014). Synthesis and characterization of metal complexes containing curcumin (C21H20O6) and study of their anti-microbial activities and DNA binding properties. *6*(1), 97-109.

Sun, W., Wang, S., Zhao, W., Wu, C., Guo, S., Gao, H., . . . nutrition. (2017). Chemical constituents and biological research on plants in the genus Curcuma. *57*(7), 1451-1523.

Varì, R., Scazzocchio, B., Silenzi, A., Giovannini, C., & Masella, R. J. N. (2021). Obesity-associated inflammation: does curcumin exert a beneficial role? *, 13*(3), 1021.

Verma, R. K., Kumari, P., Maurya, R. K., Kumar, V., Verma, R., & Singh, R. K. J. I. J. C. S. (2018). Medicinal properties of turmeric (Curcuma longa L.): A review. *6*(4), 1354-1357.

Wahab, R. A., Ahmed, Q. U., Khatib, A., Ibrahim, Z., Nipun, T. S., Nawawi, H., . . . Alkahtani, S. J. J. o. K. S. U.-S. (2023). Potential anticancer agents identification of Hystrix brachyura bezoar through gas chromatography-mass spectrometry-based metabolomics and protein-ligand interaction with molecular docking analyses. *35*(6), 102727.

Wakte, P. S., Sachin, B., Patil, A., Mohato, D., Band, T., Shinde, D. J. S., & technology, p. (2011). Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. *79*(1), 50-55.

Wulandari, R., Martono, S., & Rohman, A. J. J. o. A. P. S. (2018). Liquid chromatography and fourier transform infrared spectroscopy for quantitative analysis of individual and total curcuminoid in Curcuma longa extract. *8*(9), 107-113.

Xie, L., Guo, Y.-l., Chen, Y.-r., Zhang, L.-y., Wang, Z.-c., Zhang, T., & Wang, B. J. I. i. (2020). A potential drug combination of omeprazole and patchouli alcohol significantly normalizes oxidative stress and inflammatory responses against gastric ulcer in ethanol-induced rat model. *85*, 106660.

Yadav, R. P., Tarun, G. J. J. o. P., & Phytochemistry. (2017). Versatility of turmeric: A review the golden spice of life. *6*(1), 41-46.

Zaghary, W., Hanna, E., Zanoun, M., Abdallah, N., & Sakr, T. J. J. o. A. P. R. (2019). Curcumin: analysis and stability. *3*(2), 47-58.

Zhang, R., Li, S., Zhu, Z., He, J. J. T. i. F. S., & Technology. (2019). Recent advances in valorization of Chaenomeles fruit: A review of botanical profile, phytochemistry, advanced extraction technologies and bioactivities. *91*, 467-482.

ZHANG, W., ZHANG, J.-j., GUO, Q.-f., CHEN, L., & YIN, Z.-h. J. C. J. o. E. T. M. F. (2020). Advances in pharmacological studies of patchouli alcohol. 213-221.

Zhou, F., Hu, X., Feng, W., Li, M., Yu, B., Fu, C., & Ou, C. J. E. J. o. P. (2021). LncRNA H19 abrogates the protective effects of curcumin on rat carotid balloon injury via activating Wnt/β-catenin signaling pathway. *910*, 174485.

Zoi, V., Galani, V., Lianos, G. D., Voulgaris, S., Kyritsis, A. P., & Alexiou, G. A. J. B. (2021). The role of curcumin in cancer treatment. *9*(9), 1086.

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Abstract

Curcumin is a polyphenolic bioactive compound found in the rhizomes of *Curcuma longa* L. which has garnered considerable interest due to its diverse medicinal properties. The bioactive compound is currently being used as a commercial ingredient. In Pakistan not many industries are using curcumin as an active ingredient in their products despite rich cultivation of *Curcuma longa* (turmeric) in Pakistan. This highlights the untapped potential of local turmeric. This study aims to characterize curcumin extracted from locally cultivated *Curcuma longa*. A distinctive aspect of this research lies in the adoption of the concept of "Whole Plant Medicine", where the entire turmeric extract was utilized for characterization, rather than isolating pure curcumin. The primary objectives of this study were to assess the efficiency of various solvents for curcumin extraction and to analyze the structural composition and chemical profile of different turmeric extracts. In this research, curcumin was extracted from Pakistani local turmeric using a Soxhlet apparatus with four different solvents (methanol, ethanol, acetone, and glycerol), and its chemical characterization was performed through UV spectrophotometry, FTIR spectroscopy, and GC-MS analysis. Among the solvents used, methanol exhibited optimal results, with a distinct absorption peak observed at 425 nm in the UV spectrum, indicative of curcumin's presence. Subsequently, FTIR analysis provided insights into the presence of characteristic functional groups associated with curcumin. GC-MS analysis of the methanol extract unveiled the presence of potential bioactive compounds with intriguing pharmacology, demonstrating the complexity of the extract's chemical composition. Overall, the findings revealed that methanol is the most efficient solvent for curcumin extraction. The comprehensive chemical profile analysis of the extracts exhibited the diverse array of phytochemicals present in Pakistani turmeric, underscoring its remarkable significance in the field of natural medicine.

Key Words: Curcumin, *Curcuma longa*, Soxhlet extraction, UV/Vis Spectroscopy, FTIR, GC/MS

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