Cost-effective Extraction of Curcumin from locally grown *Curcuma longa* for Pharmaceutical, Food processing, and Cosmetics industry

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In

Plant Biotechnology

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Dedicated to my beloved parents and sister for their immense love and support throughout my educational career.

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Amnah Asghar

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List of Abbreviations

ASAB	Atta-ur-Rahman School of Applied Biosciences
FT IR	Fourier Transform Infrared Radiation
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

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Abstract

Curcumin is an important polyphenolic bioactive compound with a plethora of medicinal properties including its anti-inflammatory, anti-viral, anti-bacterial, hepatoprotective, and anti-cancerous action. As the raw material for its extraction i.e., *Curcuma longa* (turmeric) is widely cultivated in Pakistan, its cost-effective extraction method with optimal conditions were narrowed down. The extraction method was limited to Soxhlet assembly and hot plate method. The optimal conditions while taking the cost effectivity aspect into account included extraction through Soxhlet assembly for 3-4 hours with acetone and ethanol performing best as solvents. The optimal number of hexanes washing steps were 3-4 times. Technoeconomic analysis for its bulk production at lab scale was done to provide a bird's eye view of how much revenue could be generated if its production starts locally. To elucidate further, sensitivity analysis with extraction percentage yield has the key variable was done. With Asian countries being the active player in supplementing the turmeric crop needs of the western market, it will not be long before the local industries start to think about the curcumin extraction on a commercial scale.

1. Introduction

1.1 *Curcuma longa*

Curcuma is a genus with five species that belongs to the family *Zingiberaceae* (Araujo et al, 2001). The popular family is home to two of the most widely consumed spices of the world especially in Asia i.e., ginger and turmeric (Hazra et al., 2000). While ginger belongs to the genus Zingiber, turmeric belongs to Curcuma but they both have rhizomes that appear very similar to each other (Nair, 2019). When cut, turmeric rhizomes are distinctively orange in color with a strong smell. Out of the five species of this genus, *C. longa* and *C. domestica* entail turmeric rhizomes (Singh et at., 2002). Both the species are often used synonymously by a layman as the difference between the two lies at the genetic level. When we talk about turmeric as a spice, it is *Curcuma longa* that is primarily being used.

Curcuma longa is largely grown in south and southeast tropical Asia i.e., Pakistan, India, Bangladesh, as well as China (Omosa et al., 2017). The herbaceous plant requires 1500-2250mm of annual rainfall with a growth temperature range of 20-30^{0}C. It thrives well in clayey loam soil rich in hummus content (Lee et al., 2020). Climate and soils of Pakistan favors its growth therefore the dried rhizomes of turmeric are available all year round across the entire country (Akram et al., 2010).

Turmeric is basically dried rhizome of *Curcuma longa*. The rhizomes are granulated and further refined into powder form to be used as a common spice widely used in curries and gravies in Pakistani and Indian cuisine (Akram et al., 2010). Turmeric powder is also known to speed up the healing process in a plethora of injuries and ailments. ‘Haldi doodh’ also referred to as ‘Golden milk’ is a decades old remedy used to treat joint pain, inflammations, and skin injuries. Its antioxidant properties, although discovered much later, validated this traditional remedy used by our ancestors (Tilak et al., 2004; Prasad et al., 2011). Starbucks, an international coffee company, introduced Golden turmeric latte just recently that gained the interest of its consumers and scientists alike. Immunity booster shots containing turmeric, and ginger got very popular amidst the recent pandemic. Its health benefits are not unknown to many as using turmeric is a part of South Asian culture especially in Pakistan and India (Tilak et al., 2004).

1.2 Herbal substance

Turmeric is an herbaceous plant. Its complete rhizome is of medicinal significance as defined by European pharmacopeia (Amel, 2015). Its rhizomes are dried and cured to be used

as spice and for extracting medicinal bioactive compounds the herbal plant entails (Maheshwari et al., 2006). The spice comes in powder form packaged by various companies. Only a fraction of population uses fresh turmeric rhizomes majority of whom are those who either do not have access to commercial scale product or require it for medicinal purposes as the processed form takes the medicinal properties or essence out of it.

1.3 Common name(s)

Turmeric as a plant as numerous products by the names of which it is often referred as (Menniti-Ippolito et al., 2020). In India, many people call it Indian saffron. It also goes by the name of 'Kumkum,' sindoor/red alkalized turmeric powder applied to the parting of a bride's hair by the groom to officiate the wedding (Velayudhan et al., 2012). In Southeast Asian, it is most called as 'haldi.'

1.4 Constituents

Curcuma longa comprises of a prominent class of polyphenol compounds known as curcuminoids. Curcuminoids derive their name from the genera 'Curcuma.' It is these curcuminoids that give turmeric its distinct yellowish orange color (Nair, 2019b; Prasad et al., 2011). This yellowish-orange color of turmeric makes it ideal for use in textile and dyeing industries. Curcuminoids present in turmeric include curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Lal, 2012 ; Niranjan et al., 2008; Pfieffer et al., 2003; Soleimani et al., 2018). Out of these, curcumin is the most bioactive (Soleimani et al., 2018). It makes up around 0.3-5.4% of a fresh turmeric rhizome (Akram et al., 2010). Multiple studies and preclinical trials suggest the anti-cancer properties of curcumin (Agarwal et al., 2003; Perrone et al., 2015) The polyphenol has gained the interest of numerous researchers around the world working of cancerous cell lines especially pancreatic cancer. Curcumin is also the subject of interest for numerous scientists working on antiaging agents around the world (Shailaja et al., 2017; Zia et al., 2021) Curcumin is also being used as a food additive because of its numerous medicinal properties (Additives & Food, 2010; Zaki Ahmad et al., 2014).

The herbal plant also contains essential oils in it. Turmeric oil has immense value. The exact percentage of Turmeric essential oil (TEO) is predicated upon the method used for the oil extraction. Hydrodistillation is its preferred oil extraction method as it generally gives the highest yield if done properly (Zaibunnisa et al., 2009). The volatile oils include atlatone,

tumerone, and zingiberone (Jayaprakasha et al., 2001). The herbal plant has the highest percentage of carbohydrates out of its components. The literature suggests carbohydrates >60% of its total mass. Its moisture content depends on the climate and varies with the locality and variety of the rhizomes. Moreover, raw turmeric also contains a small percentage of proteins, resins, and fatty oils in it (Ikpeama et al., 2014; Li et al., 2011).

1.5 Medicinal properties

Turmeric is an integral part of Chinese medicines and Indian/Pakistani Ayurvedic medicines due to a plethora of its medicinal properties (Chaturvedi, 2009; Shang et al., 2019). The herbal plant being used as a traditional medicine to heal numerous ailments have achieved the status of a medicinal plant (Chaturvedi, 2009).

1.5.1 Antioxidant activity:

One of the most prominent of its medicinal properties, turmeric is an antioxidant in nature. The presence of hydroxyl and methyl groups on the phenyl ring in curcumin's structure give explanation of the antioxidant activity (Tilak et al., 2004). Some studies insinuate towards the carbon-centered radicals being responsible for it. DPPH assays confirm its antioxidant activity (Maizura et al., 2011). This activity has yielded its use in various antiaging formulas and drugs for diseases that cause oxidative damage. Curcumin stops the free radicals inside our body from interacting with the proteins that can cause serious damage under certain conditions (Zahra et al., 2016).

1.5.2 Anti-cancer effects:

Several re searchers have studied and are still working with curcumin on cancerous cell lines. The previous studies have shown curcumin to be effective in carcinogenesis of pancreas, liver, stomach, and skin (Akram et al., 2010). The breast carcinoma is also known to be prevented by curcumin in a prominent study (Ramachandran et al., 2002). Curcumin imparts its anti-cancerous activity at various stages of carcinogenesis including tumor initiation and promotion. The bioactive compound regulates normal functioning of the cells by initiating apoptosis of tumorous tissues (Giordano & Tommonaro, 2019; Nagabhushan & Bhide, 1992).

1.5.3 Anti-inflammatory activity:

Curcumin's anti-inflammatory activity is linked with its ability to act on NF-Kb (Nuclear Factor kappa-light-chain-enhancer of activated B cells). NF-kB is an inducible transcription

factor that is responsible for the expression of genes that result in inflammation (Akram et al., 2010). Curcumin suppresses its activity thereby inhibiting inflammation (Kohli et al., 2005).

1.5.4 Hepatoprotective activity:

In chronic hepatitis, curcumin plays a vital role in stopping it from developing into liver cirrhosis, a more fatal condition. A study was conducted on the rat liver and curcumin's effect on the damaged liver condition were observed using the magnetic resonance-based electronic conductivity imaging method (Kyung et al., 2018). Damaged liver tissues showed an improvement when treated with curcumin which confirmed its hepatoprotective activity. Numerous other studies also reiterate towards its impact on cirrhotic liver tissues (Marinda, 2014; Tung et al., 2017).

1.5.5 Lipid digestion

Lipid digestion is one of curcumin's most intriguing medicinal properties. Curcumin attaches with the bile acids and results in the formation of an emulsion that aids in the digests of lipids or fats by absorption mechanism (Akram et al., 2010; Zhang et al., 2016).

1.5.6 Other medicinal effects

Its other noteworthy therapeutic effects include its antiviral, antibacterial, and antifungal activity among many more (Akram et al., 2010; Zorofchian Moghadamtousi et al., 2014). Curcumin has been the subject of being used in textile materials for its antimicrobial activity (Sun et al., 2021). It is also being used as an active component of antimicrobial topical gels along with other antimicrobial agents (Patel et al., 2009). To prove its antiviral activity, different bioconjugates of curcumin were checked against prominent viruses including herpes simplex virus (HSV), respiratory syncytial virus (RSV), and flock house virus (FHV)(Singh et al., 2010). The MTT test results showed significant levels of antiviral activity (Nabila et al., 2020). Another study conducted on curcumin proved its antifungal activity upon its addition to plant tissue culture. Its methanolic extract has effectively inhibited the proliferation of *Candida albicans* (Dovigo et al., 2011).

1.6 Cultural significance

Turmeric holds immense value in traditions of Southeast Asia (Velayudhan et al., 2012). In Pakistan and India, the herbal plant is more than just a spice. It is an important part of South

Ethnobotany of Curcuma Longa

Sr. No.	Disease cure	Country	Traditional recipe/ Plant part used	References
1.	Urticaria	India	Roots; a bit is taken orally for some days.	(Saikia, Ryakala et al. 2006)
2.	Ringworm	India	The roots are crushed and applied directly on the infected skin after scratching it	(Saikia, Ryakala et al. 2006)
3.	Scabies	India	The extracted juice is mixed with milk and ghee and the mixture is orally taken	(Saikia, Ryakala et al. 2006)
4.	Dry skin	India	The crushed form is applied directly on the skin. The extracted juice is orally taken too.	(Saikia, Ryakala et al. 2006)
5.	Measles	India	The juice and the dried powder are applied on the infection	(Saikia, Ryakala et al. 2006)
6.	Wrinkled skin	India	The crushed form is applied directly on the skin	(Saikia, Ryakala et al. 2006)
7.	Jaundice	Nepal	Rhizome powder given orally with luke warm water	(Singh, Kumar et al. 2012)
8.	Impure blood	Nepal	Rhizome decoction given as a stimulant, tonic, and blood purifier.	(Singh, Kumar et al. 2012), (Uddin, Hassan et al. 2006)
9.	Wounds and injuries	Nepal	Rhizome paste externally applied on wounds and injuries.	(Singh, Kumar et al. 2012)
10.	Parasitic worms	Nepal	Fresh rhizome juice	(Singh, Kumar et al. 2012)
11.	Diabetes	Karnataka, India	A pinch of dried rhizome powder is mixed with half a cup of cow's milk and taken daily	(Bhandary, Chandrashekar et al. 1995), (Jayakumar, Ajithabai et al. 2010)
12.	Diphtheria	Karnataka, India	An infusion of the rhizome is sweetened with sugar and taken orally 3-4 times a day, for diphtheria	(Bhandary, Chandrashekar et al. 1995)

Table 1. 1: Biologically active compounds from *Curcuma Longa*

Sr. No.	Chemical class	Compound	Molecular formula	Molecular weight	References
1.	Curcuminoid	Demethoxycurcumin	$C_{20}H_{18}O_5$	338.4g/mol	(Jayaprakasha Jagan Mohan Rao et al. 2002)
2.	Curcuminoid	Curcumin	$C_{21}H_{20}O_6$	368.38g/mol	(Jayaprakasha, Jagan Mohan Rao et al. 2002)
3.	Curcuminoid	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	308.3g/mol	(Jayaprakasha, Jagan Mohan Rao et al. 2002)

Table 1. 2: Pharmacological activities from bioactive compounds from *Curcuma Longa*

Sr. No.	Compounds	Pharmacological activities	References
1.	Demethoxycurcumin	Antitumor	(Ramkumar, Rajasankar et al. 2019), (Huang, Ma et al. 1995) (Mehanny, Hathout et al. 2016)
2.	Curcumin Demethoxycurcumin Bisdemethoxycurcumin	Antioxidant	(Jayaprakasha, Rao et al. 2006) (Ahmad, Umar et al. 2013)
3.	Curcumin Demethoxycurcumin Bisdemethoxycurcumin	Anti-inflammatory	(Sandur, Pandey et al. 2007) (Guo, Cai et al. 2008) (Kim, Jeon et al. 2010)
4.	Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin	Hepatoprotective	(Cheon, Park et al. 2007)
5.	Bisdemethoxycurcumin	Acaricidal	(Luo, Ding et al. 2013)

Asian wedding festivities. The wedding festivities begin with a ‘rasam’ or tradition of applying ‘haldi’ or turmeric to the bride and groom to give them a vibrant glow before the big day.

In a Hindu wedding, applying a dash of ‘sindoor’ on the parting of bride’s hair is an important nuptial without which the wedding cannot be officiated. This ‘sindoor’ or red powder is made with turmeric powder and either lime juice or any other forms of lime. The yellowish-orange turmeric powder is alkalized to turn it into a bright red color.

Cuisine forms an important part of every culture. When it comes to Pakistani and Indian cuisine gravies and curries are what set the two very similar cuisines apart from others. Meat and vegetable dishes are mostly made in the curry or gravy form to be consumed with ‘chapati’ or bread. With each gravy/curry dish different in taste, the one thing that brings them together is the distinct yellow-orange color of the broth. This yellow-orange color is due to the addition of turmeric powder. Turmeric powder is believed to add color as well as flavor to the Southeast Asian gravy/curry dishes. Certain rice dishes like ‘biryani’ also have turmeric powder as an ingredient in their traditional recipe.

1.7 Curcumin as an emerging commercial product

With numerous health benefits and potential to treat a range of ailments, curcumin as emerges as a valuable polyphenol compound extracted from turmeric rhizomes. The bioactive compound is currently being used as an ingredient in many commercial products. Immunity shots containing curcumin along with ginger and black pepper gained a lot of popularity during the coronavirus pandemic.

Curcumin in capsulated form is a medicine available in all major countries across the globe. Since the polyphenol compound has a low bioavailability, it is majorly sold with the addition of Bioperine or simply black pepper. The capsules are sold by various brands some of which include Nature Made©, BioSchwartz©, and Nature wise©. Some of the local brands selling curcumin in Pakistan only include Nutrifactor©. Not many pharmaceutical companies are preparing curcumin in Pakistan. Most of it is imported although Pakistan is rich in the treasured herbal plant.

Curcumin finds its applications in three prominent industrial sectors i.e., pharmaceutical, food and cosmetics. The three industries in Pakistan are using curcumin one way or another. For instance, in pharmaceuticals we have curcumin capsules being made, although not many companies are extracting it locally. In foods, turmeric powder is largely being used. As for the cosmetics industry, since curcumin is an antioxidant many topical gels, face washes, scrubs, waxing creams and other applicatory products have curcumin as their active ingredient. Given the number of medicinal properties the polyphenol compound entails, the products made from it are still inadequate.

1.8 Extraction of curcumin

There are various methods that can be used to extract curcumin. Curcumin is a polyphenolic compound that does not dissolve in water. It is soluble in lipids. Extraction methods include Soxhlet assembly, microwave-assisted extraction, maceration, hot magnetic plate method, and ultrasonic extraction. Out of these methods, Soxhlet assembly is most actively employed in the lab scale preparation of curcumin.

1.8.1 Soxhlet extraction

Soxhlet assembly uses different solvents for curcumin extraction. The solvents used include Ethanol, Propyl alcohol, Methanol, Acetone, and Hexane. The granulated or powdered turmeric rhizome is put in the thimble and extracted with the solvent for about 4-8 hours as the duration depends on the effectivity of the solvent to extract curcumin. The extracted fraction is later washed with Hexane to obtain pure curcumin.

1.8.2 Hot plate method

Hot plate method uses magnetic stirrer to stir the turmeric powder/granules into the solvent over high temperature. The temperature is usually set as the boiling point of the solvent. The solution is mixed at high temperature for about 1-2 hours. After filtration, the solution is washed with hexane to get curcumin.

1.8.3 Microwave-assisted extraction

The same solution with a suitable solvent like Methanol or Acetone is prepared for microwave-assisted extraction. Granulated or powdered turmeric is used as the solute. Microwave extractor with suitable features including appropriate power supply ranging

between 600-1200W is taken. The solution kept on vacuum in the extractor from 30 minutes to 2 hours.

1.8.4 Maceration

Century old technique for extracting bioactive compounds from plants, maceration is the simplest method used to extract curcumin. The solution containing powdered turmeric and Methanol is taken in a dark bottle and put in a dark cabinet for 24 hours. The solution can then be treated with column chromatography to get the curcumin fraction.

1.9 Market of curcumin in local industries

Curcumin's potential is recognized across various industries. There are not many industries using pure curcumin as an ingredient in their products. Most of them use turmeric as extraction of curcumin from turmeric is an arduous task. Moreover, many of these industries do not realize that it is curcumin's presence in turmeric that yields to its significant properties like, antioxidant activity, anti-inflammatory, wound healing, etc. Using pure curcumin can enhance the effect of these properties.

Pakistan is blessed with a climate that is ideal for turmeric cultivation. The plant is grown across numerous regions of the country. Most of it is being used to produce turmeric powder. Fresh turmeric rhizomes are available in vegetable markets while the dried and cured ones at 'pansari' shops. There is hardly a small percentage of population that buys them. People prefer refined products over the raw turmeric. Upon doctor's recommendation, people consume turmeric that too in the capsulated form. Like mentioned earlier, the capsulated form of curcumin is only being produced by a couple of local brands. Researchers studying the efficacy of curcumin on cancerous cell lines get it imported to avoid having to extract it themselves. Part of the reason why researchers get it imported is because it is not locally produced with as much purity as is required for the experiments as well as the lack of cost effective and time saving protocol for its extraction on lab scale.

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. Currently, Pakistan imports it and produces only a meager amount of it to be used in only a few pharmaceutical products. A cost-efficient method for its extraction can encourage pharmaceutical companies and food industries to produce it on a commercial scale and import it to global markets where it is currently in high demand.

Objectives

1. Cost-effective extraction of curcumin from turmeric rhizomes
2. Technoeconomic analysis for the bulk production of locally produced curcumin to be largely used for medicinal and research purposes

2. Literature Review

Turmeric is one of the most popular spices in the south Asian food culture. The spice comes from the rhizomes of Curcuma longa commonly known as Turmeric (Akram et al., 2010). Turmeric is majorly grown in India (Omosa et al., 2017) and Pakistan as the climate of the two South Asian countries is ideal for its growth. The plant has a lot of sacred value in Hinduism and forms a significant part of Indian culture.

Turmeric was recognized as a medicinal plant decades ago, but it was not until the presence of curcuminoids that the plant became the focal point of research for scientists working on medicinal plants. Curcuminoids form a vital part of turmeric rhizomes and it is the presence of this class of polyphenols that gives turmeric the healing properties for which it has largely been used by our ancestors.

Curcumin extraction from *Curcuma longa* rhizomes has captivated the researchers and pharmacists due to the medicinal properties the phytochemical entails. It is antibacterial, antiviral, antioxidant, anti-angiogenic, anti-inflammatory, antifungal, anti-coagulant, and has inhibitory effects on numerous types of cancers. The rhizome is also used to extract turmeric oil and converted into powder form to be used as a condiment widely used in South Asian cuisine. The plant is also used as a common household remedy for healing numerous injuries in the same region.

Turmeric is widely grown in Asia as the climatic conditions favor its growth. The crop requires well-drained sandy or loamy clayey soil and farm yard manure for its growth. Turmeric prefers 20-30C temperature and 1500-2250mm annual rainfall. Due to its feasible cultivation and wide usage as a condiment in South and Southeast Asia, the plant is grown in almost every part of the region especially in Pakistan and India.

In the context of pharmacological research, the rhizomes are used for obtaining curcuminoids, out of which curcumin is the most bioactive. The liposoluble compound is extracted to be used as a basis in medicinal capsules for healing purposes. The phytochemical has low bioavailability and is paired with piperidine for increasing its bioavailability.

Curcumin, the major curcuminoid present in turmeric can be extracted from the rhizomes using various techniques including soxhlet assembly, maceration, microwave-assisted extraction, hot plate method, and Supercritical CO_2 extraction among others. Out of all the

extraction processes, soxhlet assembly has been used by the researchers the most. Each of these techniques have their own benefits.

2.1 Soxhlet extraction

(Patil et al., 2011) extracted curcuminoids to study the activity of its anti-inflammatory effect using soxhlet extraction. In this study dried 40g of the 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. (Popuri & Pagala, 2013) kept the extraction duration to 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 et al., 2014) where soxhlet assembly was chosen as the extraction method for curcumin isolation. However, what distincts 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.

(Dutta, 2015) also opted for soxhlet assembly for curcumin extraction from other species of *Curcuma* along with *Curcuma longa*. The ground rhizomes were used with ethanol as the extraction solvent. the solid to solvent ratio used was 1:4. The soxhlet extraction was carried out for 2 consecutive days (48hrs). The ethanolic extract was dried and then subjected to phytochemical analysis and spectrophotometry to confirm the presence of curcumin. 125mg of curcumin was obtained from 100g of the ground turmeric.

(Shirsath et al., 2017) used multiple extraction techniques to check which one gives the better yield. For soxhlet assembly, 10g of the powder was extracted with 250mL of the solvent. Ethanol was chosen as the solvent. To monitor what extraction duration gives best yield, samples were taken out after a set range of intervals and analyzed using HPLC. The extraction was carried out for 8 hrs. Curcumin yield obtained using this protocol was 12.75mg/g.

(Pawar et al., 2018) used the soxhlet assembly for curcumin extraction. Curcuminoids being liposoluble are present with the turmeric essential oils. It becomes difficult to obtain pure curcumin from the oleo resin as the oils solubilize the phytochemical. So, for deriving the pure curcumin out of the oil fraction (Pawar et al., 2018) tested out a series of solvent mixtures that could be used for curcumin extraction in soxhlet assembly. The solvents tried in this study included isopropanol with water, isopropanol with hexane, ethanol with water, ethanol with hexane, methanol with water, and methanol with hexane. Isopropanol with hexane gave the most fruitful results. The curcumin crystals obtained were confirmed using FT IR analysis and TLC.

2.2 Ultrasound assisted extraction

(Mandal et al., 2009) optimized the extraction protocol for curcumin using the Ultrasound assisted extraction. Different solvent mixtures were tested out of which ethanol-water mixture with the solvent volume set to 20mL gave the most yield. The extraction method drastically reduced the extraction duration to 70 minutes. For comparison, soxhlet and maceration techniques were also performed. 2g of the turmeric powder was taken with 200mL of acetone in soxhlet assembly. The process was carried out for 4hrs afterwards of which the solvent was

evaporated followed by the addition of methanol. For maceration, magnetic stirrer was used. 2g of powder was added to 50mL of acetone and the solution was constantly stirred for 24 hrs. Extraction was followed by the evaporation of the solvent and then addition of methanol. All of the three curcumin extracts obtained were subjected to HPLC analysis. Ultrasound assisted extracted trumped the two conventional techniques in terms of curcumin yield.

(Shirsath et al., 2017) performed ultrasonic assisted extraction. 6g of the powder was taken with 250mL of the solvent. Multiple solvents including acetone, ethanol, methanol, and ethyl acetate were used. Particle size was also varied i.e., 0.09, 0.10, 0.21, and 0.85mm. The extraction was performed with each of these solvents. The mixture of solvent and the solid was taken in a flask and then put into the water bath. The temperature of the water bath was varied between 25 to 55ºC for different set of conditions. The ultrasonic horn used in this study operated at 250W power and 22kHz frequency. The extraction was performed for 1 hr. The samples were taken out at regular intervals to check for curcumin percentage in order to determine the optimal irradiation time period. Optimal conditions for extraction using UAE were 0.09mm particle size, ethanol as the solvent, and 1hr as the extraction time period. The extraction yield increased with the increase in temperature.

(Binello et al., 2020) performed ultrasonic extraction of curcuminoids from *Curcuma longa* rhizomes. The extracting solvent selected was Ethanol. The solid to solvent ratio taken was 1:5. 10g of the powdered oleo resin was taken and the temperature was maintained at 40ºC. The ultrasonic horn used had the frequency of 20.5 kHz with a power range of 350-500W. The curcumin obtained underwent HPLC for analysis. The UAE technique was compared with a conventional technique as well in the same study. The comparative technique chosen was maceration. Magnetic stirrer was used to mix the solvent with the oleo resinous particles. The same set of conditions were used in maceration. The HPLC analysis gave better results with the former method of curcumin extraction while reducing the overall extraction time.

2.3 Maceration

(Paulucci et al., 2018) performed curcumin extraction using maceration technique. the extraction was done using Ethanol as the solvent. The ground form of *Curcuma longa* was used. The solid to solvent ratio used was 1:6. The extraction was performed for 12hrs straight at the fixed temperature of 80ºC. the agitation speed was kept at 30rpm.

(Shirsath et al., 2017) used maceration or batch extraction to check if the process gives a better yield of curcumin compared to soxhlet assembly and ultrasonic assisted extraction (UAE). For this study, 6g of the powdered oleo resin was taken with 150 ml of the solvent. ethanol was selected as the solvent for this extraction. The solution was poured in a glass reactor. In order for it to mix continuously, agitator was turned on. The speed for agitation was set to be 420rpm. The extraction was performed for 8 hrs and the temperature was fixed at 30ºC. It was made sure that the agitator spun fast enough to prevent the oleo resinous particles from settling at the bottom of the glass reactor. To confirm the optimal time for extraction, the samples from the mother liquor were subjected to HPLC after regular intervals.

2.4 Microwave assisted extraction

Another advanced method, Microwave Assisted Extraction (MAE) for isolation curcuminoids was used by (Dandekar & Gaikar, 2002) In this study acetone was selected as the extracting solvent. Modified system for the microwave was used. It included sparging nitrogen in gaseous form to maintain the atmosphere as inert as possible. The system was also equipped with an outlet to let the gas leave the microwave after it has served its purpose. The extraction process using this set up was carried out for about 10hr per day for a total of 6 days. The amount of oleo resinous powder and acetone used was 30g and 200mL respectively. Throughout the extraction process, fresh acetone was added at the start of every day. The High-Performance Thin Layer Chromatography (HPTLC) analysis revealed the percentage yield of curcuminoids to be 5.8%.

(Mandal et al., 2008) used microwave assisted extraction technique to find out if it is better than the conventional extraction techniques. The extracting solvent chosen for this study was acetone. Unlike other techniques, the solid plant material dipped in the solvent i.e., methanol was taken as a modifier for the process. 2g of turmeric powder was used with 40mL of acetone. Taguchi design approach was used for finding out the optimal parameters for the process. microwave assisted extraction technique uses a dual heating mechanism that promises a better yield out of the feed volume of the solid/powdered material. For comparison, soxhlet extraction and maceration technique were carried out. In soxhlet assembly, 2g of the turmeric powder was taken with 100ml of acetone in the solvent flask. The extraction was performed for a period of 8hrs for 3 days. The extracting solvent was changed with the fresh solvent of the

same volume every day. From the mother liquor obtained, methanolic extract was prepared. In maceration technique, magnetic stirrer was used. 2g of the powder was added to 40ml of acetone and the solution was allowed to mix for a complete day. The solvent was later evaporated and methanolic extract was prepared. HPLC analysis for the three extracts showed microwave assisted extraction to be the most effective in extracting curcumin from the powdered oleoresin.

(Wakte et al., 2011) Opted for microwave assisted extraction of curcumin from *Curcuma longa*. The microwave used for this study was 140 W power. Two experiments were performed to achieve the goal. For the first experiment, 20g of the powdered turmeric was taken and laid flat on a glass dish. The powder was then subjected to irradiation for 1-7minutes (a different time for each batch of experiment). Once dried, the sample powder was added to the solvent (either ethanol or acetone) keeping a 1:5 solid to solvent ratio. The mixture was then added to the extracting chamber that comprised of nine cylindrical vessels. For the entire extraction process, an agitation speed of 400 rpm was maintained. The process was carried out at two varied powers i.e., 60 W and 90 W. For extraction process where ethanol was used as a solvent, 90 W power was used whereas 60 W was kept in the case of Acetone. In the next set of experiment, the solid to solvent ratio used was 1:2. The extraction was carried out for a complete day and the temperature was kept at 20ºC. The solvents used in this experiment were water and ethanol. For water-based experiment the power supply was kept at 50 W while for ethanol it was raised to 270 W. The extracts obtained from the two sets of experiments were then analyzed.

For the comparative (Wakte et al., 2011) did ultrasonic assisted extraction as well. For it an ultrasonic horn with a 150 W power was used. The parameters for UAE based extracted were kept the same as the MAE ones. After initial irradiation of about 5 minutes at 21ºC, the oleo resinous powder was dipped into the solvent. The solvents used here were also ethanol and acetone. Under UAE method, the second set of experiments were performed but this time using water and ethanol as extracting solvents. The extracts from MAE and UAE were analyzed using HPLC. The results showed effective yields for both set of experiments and organic solvents trumped the inorganic ones.

2.5 Super critical CO_2 extraction

(Mendez et al., 2000) used supercritical CO_2 extraction technique for the extraction of curcumin and turmeric oils. For the experiment, a basic supercritical CO_2 extraction setup was used. In the process supercritical CO_2 acts as a solvent. Ethanol was taken as the cosolvent. The process entailed flowing CO_2 through the extraction chamber that contained the solid plant material of required particle size. Supercritical CO_2 was mixed with Ethanol. The former passed the vessel containing ethanol first for mixing before extraction could take place. The pressure of the chamber was maintained, and Bourdon-type gauge was used to monitor it. The oleo resinous particles were condensed, and then rotary evaporator was used to remove the solvent from the extracted fraction. The extract was then quantified and analyzed using HPLC. A maximum yield of 22.5% was obtained using this method.

(Chhouk et al., 2017) used ultrasonic supercritical CO_2 assisted extraction of curcumin from C. *longa*. It resulted in drastically improving the yield of curcumin. The temperature for the experiment was kept in the range of 40-60ºC. The amount of cosolvent was varied from 10%, 15%, and 20%. The entire extraction process was performed at 30-, 60-, and 90-minutes duration to find out the optimal time period for curcumin extraction. After the process was complete, the solvent from the extract was dried with the help of rotary evaporator. For each of the varied value for parameters, triplicated or duplicates were performed for better accuracy. Fourier Transform Infrared (FT IR) analysis were performed to check the quality of curcumin. Effect of each set of conditions were studied and a maximum of 7.17% yield was obtained at the optimal conditions of 90 minutes, 10% cosolvent, and the CO_2 flow rate of 3mL/min.

(Gopalan et al., 2000) took 9g of turmeric powder in the extraction column. The desired temperature and pressure were maintained in the extraction chamber. Compressed CO_2 upon passing, yielded the extract. The yield obtained contained turmeric oils along with curcumin. The parameters and cosolvents can be adjusted to yield turmeric oils.

3.Material and Method

3.1 Chemicals and reagent

Analytical grade reagents including Acetone, Ethanol, Propanol, Hexane, and Methanol were obtained from the ASAB's chemical vendor to be used as solvents for extraction.

3.2 Plant sample

Turmeric rhizomes were obtained from a local market in Rawalpindi. The rhizomes were fresh. They were washed and dried before further processing.

3.3 Soxhlet assembly

For extraction, two apparatus set ups were used. The first and preferred one was soxhlet assembly. The apparatus comprises of a heater that holds the solvent flask, a column connected to condenser at one end and the other end going into the round bottom flask above the heater. The column has a siphon mechanism for holding the thimble in place. Thimble is filled with the sample subjected to extraction. The heater has a temperature controller.

3.4 Magnetic stir plate

Magnetic stir plate is used to mix solutions. The plate has two controls i.e., temperature control and a revolutions control. It comes with a small magnet that stirs the solution. The magnet moves when the magnetic field is turned on. This apparatus is used to prepare plant extracts.

3.5 Rotary evaporator

Rotary evaporator works by separating the moieties present in the solution. It is used to evaporate the solvent that can be recovered from the collection flask. Its complete assembly comprises of a motor that is responsible for rotating the flask containing the subject solution, a water bath for maintaining a constant temperature, a vacuum system for reducing pressure, and a condenser for cooling the solvent vapors.

3.6 Separating funnel

Separating funnel is useful in separating the two liquid fractions with different densities. When the mother liquor is washed with hexane, curcumin settles down at the bottom of the

funnel. Two different colored fractions are clearly seen and tap at the bottom can be used to separate the fractions.

3.7 Rhizome pretreatment

Fresh turmeric rhizomes were taken from the local sabzi mandi/market and washed with water while gently rubbing with fingers to remove all the dirt from it. The rhizomes were thoroughly checked and only the soft ones were used. After washing, the rhizomes were cut into thin slices of approximately 5mm thickness.

Figure 3. 1: Thinly sliced fresh turmeric rhizomes

3.8 Rhizome drying

The thin slices were then spread on newspaper sheets and were first shade dried for a complete day period in an open environment on a sunny day. The rhizomes were then dried using Arshia FD-130 food dehydrator the very next day for about 2 hours.

3.9 Powder formation

The dried slices of rhizomes had a very crispy texture and can now be easily ground using a food grinder. The sample was ground using a WestPoint food processor. Once ground the powder was put in a glass jar.

3.10 Method

3.10.1 Solvent selection

For selecting the right solvent, literature was consulted and a few of the options were sought. These include Propyl alcohol, Ethanol, Methanol, Hexane, and Acetone. Extraction was proceeded with each of these solvents and maximum yield was given by Acetone. Therefore, only extraction using Acetone as a solvent is described.

3.10.2 Soxhlet extraction

In the round bottom flask, 200mL of Acetone was taken. The temperature of the heater was set at 55ºC. 20g of ground turmeric was added in thimble. Once the condenser was cold enough, the heater was turned on to begin the extraction. The extraction continued for 4.5 hours.

3.10.3 Hexane washing

After the extraction is complete, the mother liquor was put aside to cool down a bit. Afterwards, 500mL fractionating column was taken. A small quantity, almost 5-10mL of it was added to the column. Hexane was then poured over it. It was poured until a slight color change was observed. The column was capped and shaken gently. Two fractions could be seen. A darker and denser fraction at the bottom and a lighter one on top. The darker fraction was collected. The collected fraction was washed again with hexane. The fraction got lighter and denser. The same washing step was repeated until a fine orange powdery fraction was seen. It took a different number of washing steps for a different extraction. The orange fraction was taken out in petri dishes.

3.10.4 Drying and scraping

Only a small amount of curcumin was taken in a petri dish so that it dries out quickly. The petri dishes were covered and put in dark for 24hrs. The curcumin dries out and sticks to the petri plates. It is then scraped out using a steel spatula.

3.11 Method

3.11.1 Solvent selection

The same solvent, Acetone, was used for the magnetic plate extraction.

3.11.2 Solution preparation

20g of ground turmeric was taken in 200mL beaker. To it, 100mL of Acetone was added.

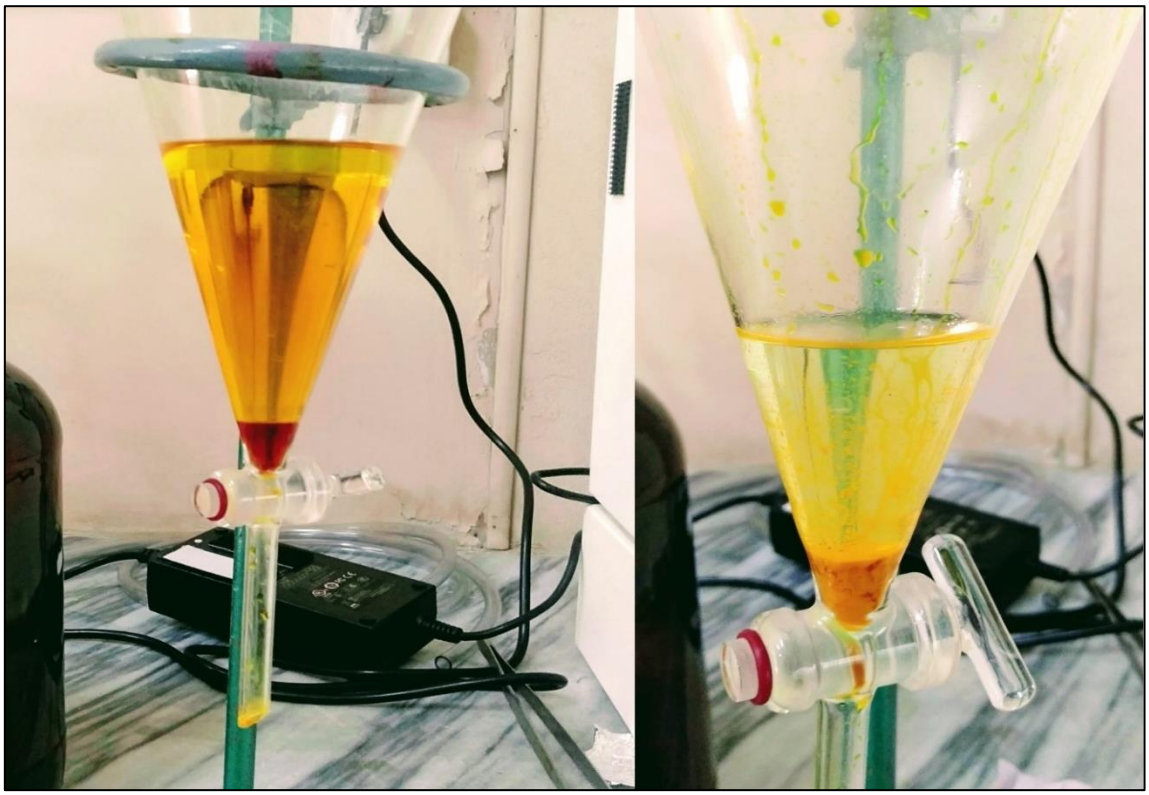


Figure 3. 2: Left; curcumin before hexane wash, right; curcumin after 4 hexane washes

3.11.3 Extraction

Hot magnetic plate was turned on. It was used as a hot plate as the magnetic field was kept off. The temperature was set at 55ºC. The solution was mixed with a glass stirrer by hand for about 1.5 hours. Magnet was not used as the volatile oils extracted with curcumin stick to the magnet's Teflon coating. After extraction is complete, the solution was decanted off and then sieved.

3.12 Rotary vacuum drying

To concentrate the solution, it was dried under vacuum on rotary at 55-60ºC for 20 minutes. The solution turned darker.

3.13 Hexane washing

After the extraction is complete, the mother liquor was put aside to cool down a bit. Afterwards, 500mL fractionating column was taken. A small quantity, almost 5-10mL of it was added to the column. Hexane was then poured over it. It was poured until a slight color change

was observed. The column was capped and shaken gently. Two fractions could be seen. A darker and denser fraction at the bottom and a lighter one on top. The darker fraction was collected. The collected fraction was washed again with hexane. The fraction got lighter and denser. The same washing step was repeated until a fine orange powdery fraction was seen. It took a different number of washing steps for a different extraction. The orange fraction was taken out in petri dishes.

3.14 Drying and scraping

When small amount of liquid curcumin fraction taken in petri dishes it dries out quickly. The petri dishes were covered and put in dark for 24hrs. The curcumin dries out and sticks to the petri plates. It is then scraped out using a steel spatula and saved for later use in a dark cabinet.

Table 3. 1: Experiment trials for optimal conditions

Trial No.	Parameter changed in the protocol	Observation
1	Rhizome powder extracted with Ethyl acetate, Ethanol, Acetone, Methanol, Ethyl-propanol	Acetone and Ethanol turned out to be good solvents
2	Rhizome powder extracted for 4hrs, 6hrs, 10hrs, 8hrs, 12hrs	4-6hrs is optimal extraction time
3	Rhizome powder extracted using Soxhlet assembly and hot magnetic plate method	Soxhlet assembly gives better yield
4	Solvent evaporated using rotary vacuum, air dried	Rotary vacuum is the preferable for drying
5	Obtained fraction washed with hexane 0, 2, 3, 4, and 5 times	3-4 times works
6	Solute to solvent ratio set to 1:10, 1:20, and 2:5	1:10 is ideal
7	Mother liquor set aside overnight, for 2 nights, 3 nights and not at all	Did not have much effect

3.15 Confirmatory tests/analysis

3.15.1 Fourier Transform Infrared (FTIR) analysis

Fourier Transform infrared (FTIR) analysis is one of the most highly used methods of infrared spectroscopy when it comes to identification of a chemical constituent in an extract or compound.

In order to confirm that it is curcumin that is extracted, FTIR analysis of the two samples were performed. Curcuminoid from Neurology lab of Atta-ur-Rahman School of Applied Biosciences (ASAB) was obtained and used as a standard to run with the two experimental samples. The sample was taken in a powdered form. The sample was mixed with infrared grade potassium bromide was pressed to form a pellet. This pellet was then used further for FTIR analysis.

3.15.2 Antioxidant (DPPH) test

2,2 diphenyl-1-picrylhydrazyl is a free radical with a Nitrogen center that has a Hydrogen accepting capability. This Hydrogen accepting capability makes it ideal for antioxidants as they can get reduced by the former. It can act as an indicator for identifying compounds with antioxidant activity as the compound itself can get reduced by accommodating a Hydrogen ion leaving the other reacting substance oxidized. The compound is violet in color and changes to light yellow upon reduction. The lighter the yellow color, the more is the antioxidant activity. The compound is widely used to check the antioxidant activity of medicinal plants.

For confirming the antioxidant activity of the extracted curcumin DPPH test was performed. DPPH (Alizadeh et al., 2020) was prepared in Ethanol. 3 ml of DPPH was added in 0.5 ml extract or standard. The 2 differently extracted lab samples were taken along with the standard. For each of the three samples, four dilutions were prepared in ethanol. The samples were named A, B, and C. The A series further had A1, A2, A3, and A4. Each had 5 ml of Ethanol. A1 had 1mg of the sample, A2 had 2mg of the sample, and so on. Likewise, other 2 sample dilutions were also prepared. C was the standard while A and B were experimental samples. For the scavenging activity, Ascorbic acid was used as a standard. Its dilutions were made under the D series i.e., D1, D2, D3, and D4.

3.16 Total antioxidant test

For the total antioxidant test Phosphomolybdate method of (Prieto et al., 1999) was used. The working principle of this method is the reduction of Mo(VI) to Mo (V). Mo(V) is a green color complex that is formed upon reduction of Mo(VI) by the sample. For this method, Ascorbic acid was used as the standard. The Phosphomolybdate shows absorbance at 695nm. Therefore, the absorbance was checked at 695nm. The sample dilutions from the free radical

scavenging activity were used. The dilution tubes were incubated for 90 minutes at 95℃ before measuring the absorbance. These dilutions consisted of 1-4mg of the sample particles in 5ml of Ethanol. The higher the absorbance, the higher is the antioxidant activity.

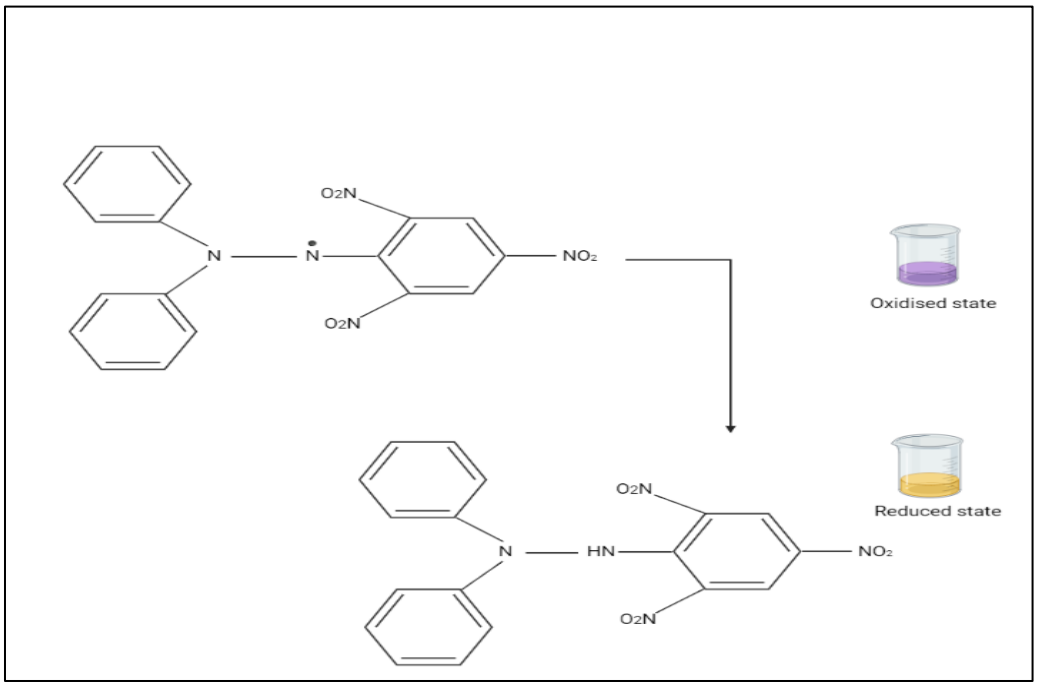


Figure 3. 3: DPPH changes color from purple to yellow when reduced by an antioxidant

$$\text{Percentage activity} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

3.17 Techno-economic Analysis

Techno-economic analysis (TEA) indicates the feasibility of a certain process, technology, or the overall manufacturing of a product. When estimating the cost-effectiveness of a process, technoeconomic analysis becomes relevant as it lays down the effect of all the parameters in contributing to the overall cost.

In order to perform technoeconomic analysis for the proposed extraction method i.e., Soxhlet extraction, (Kunta, 2018)'s work was taken as the reference study. A detailed study into the available literature revealed that the first step to a successful TEA is an adequate understanding about the process of equipment/machinery to be used. Since the extraction had already been carried out on lab-scale, finding bulk scale production equivalents for the apparatus was quite straightforward. An equipment/machinery list was made against the

individual processes in the extraction method. The machinery costs were estimated from the renowned global vendor [Alibaba.com].

The next step was setting a schedule for the entire process and evaluate if the process altogether yielded significant product. For this evaluation, the volume of each part of the apparatus was noted and analyzed for per batch production i.e., the time it took for the entire feed volume, the volume of the raw materials and solvents to be processed before more raw material could be added for another batch.

Table 3. 2: Volume of the equipment selected for bulk scale extraction

Equipment	Volume
Soxhlet apparatus	25L
Industrial rotary	50L
Separating funnel (3)	15L (5L each)
Dehydrator	100L

A brief look into the market of curcumin, global as well as local, can give useful insights about the potential of the country's local industry to produce it given how much turmeric is grown by the agriculture sector every year. The market analysis were performed by finding the various applications of curcumin throughout different industries including food, cosmetics, pharmaceutical, and others. The figure below highlights the regions using curcumin by its various application.

3.18 Selected Equipment

The entire process requires three industrial scale equipment for the bulk-scale production of curcumin. For a better understanding of their efficiency, it is important to discuss the features of each briefly.

3.19 Machinery depreciation & expense

To further strengthen the cost effectiveness analysis, machinery depreciation expense was also calculated. Every machinery comes with a useful lifespan in which it can work properly. After the useful lifespan of the machine is over, it holds little significant value, monetarily speaking. This value is called its salvage value. The machine cold be sold by its salvage value.

At times, certain equipment is exhausted so much in their useful lifespan that their salvage value becomes zero.

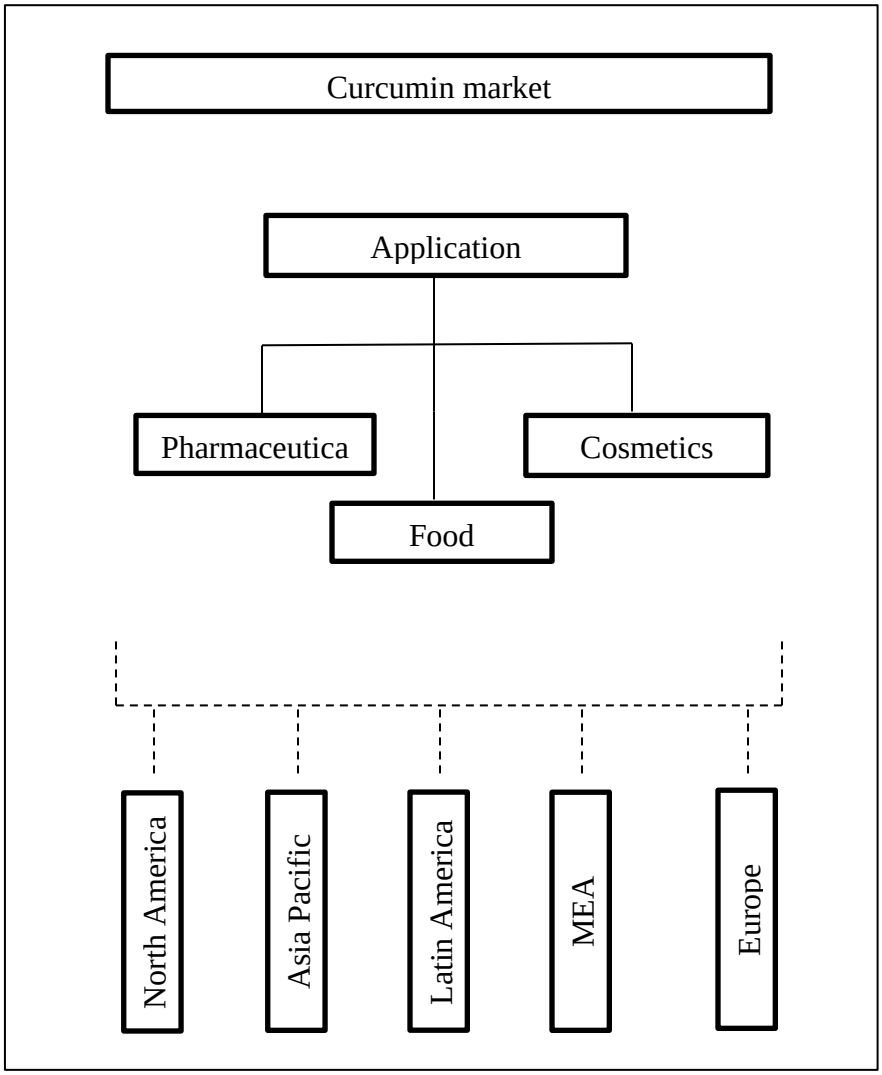


Figure 3. 4: Curcumin market across the globe, MEA; Middle East Asia (Source: Global Market Insights)

Finding out the depreciation expense of the machinery or equipment to be used in the extraction process will enable us to further our Techno-economic analysis. The depreciation expense can tell us about the total cycles that can be extracted out of the machinery. The number of cycles will corelate to the number of batches that could be prepared from it. Table 3.5 lays down the depreciation expense details of every machinery to be used in the extraction process. Straight-line depreciation analysis was used to calculate the depreciation.

3.20 Cost estimation

For the cost estimation, the market value of the product was already available. The cost of all the raw materials and chemicals along with the required machinery were taken from [Alibaba.com]. The costs were divided into fixed and variable cost. Two types of variable costs were identified i.e., for Ethanol as the extracting solvent and Acetone as the extracting solvent. The fixed cost comprises of the overall price of the machinery and apparatus to be used. Whereas the variable cost entails the cost of feed i.e., raw material and chemicals that are to be added before every batch preparation.

Using these costs along with the cost of the available market product as a benchmark, revenue and gross margin were calculated.

For these calculations following formulas were used.

Revenue = Price x Quantity

Gross margin = Revenue – Total variable cost

Table 3. 3: Breakdown of the variable and fixed cost of the entire extraction process

Unit operation	Material/Equipment	Amount/Volume	Variable cost (E) PKR	Variable cost (A) PKR	Fixed cost PKR
Loading	Turmeric rhizomes	1 KG	125	125	--
Extraction	Ethanol/Acetone	25 L	225	18,000	--
	Soxhlet assembly		--	--	194,460
Evaporation	*Rotary vacuum*		--	--	4,25,000
Washing	Hexane	50 L	2,186	2,186	--
	Separating funnel		--	--	38,350
	PolyP funnel		--	--	7,000
Drying	*Dehydrator*		--	--	75,000
Labor	*17,500 x 5/20		4,375	4,375	--
Total	**Product per cycle**	225 mg	2,536	20,311	739,810
Revenue	Price*Quantity		23,700 x 225/10	23,700 x 225/10	533,250
Gross margin	Revenue-Total Variable		533,250-20,311	533,250-20,311	512,939

These calculations helped us study how changing price of the valuable product will impact the revenue and gross margin. Using the Excel's basic functions, bar graphs for the effect of price on the revenue as well as gross margin were plotted.

Table 3. 4: Total depreciation and the salvage value of each piece of machinery to be used in the extraction process

Equipment	Original cost	Salvage value	Useful lifespan	Depreciation/year	Total Dep.
Rotary	4,25,000	1,27,500	7	42,500	297,500
Year 1	4,25,000	1,27,500	6	-42,500	382,500
Year 2	4,25,000	1,27,500	5	-42,500	340,000
Year 3	4,25,000	1,27,500	4	-42,500	297,500
Year 4	4,25,000	1,27,500	3	-42,500	255,000
Year 5	4,25,000	1,27,500	2	-42,500	212,500
Year 6	4,25,000	1,27,500	1	-42,500	170,500
Year 7	4,25,000	1,27,500	0	0	127,500
Soxhlet	194,460	0	10	19446	194460
Year 1	194,460	0	9	-19446	175,014
Year 2	194,460	0	8	-19446	155,568
Year 3	194,460	0	7	-19446	136,122
Year 4	194,460	0	6	-19446	116,676
Year 5	194,460	0	5	-19446	97,230
Year 6	194,460	0	4	-19446	77,784
Year 7	194,460	0	3	-19446	58,338
Year 8	194,460	0	2	-19446	38,892
Year 9	194,460	0	1	-19446	19,446
Year 10	194,460	0	0	-19446	0
Dehydrator	75,000	45000	4	7,500	30,000
Year 1	75,000	45000	3	-7,500	67,500
Year 2	75,000	45000	2	-7,500	60,000
Year 3	75,000	45000	1	-7,500	52,500
Year 4	75,000	45000	0	-7,500	45,000

3.21 Process time

For a streamlined process flow, the time to produce one batch of curcumin that comprises of 225mg of the product was noted. The time was approximated using the lab scale optimized protocol while taking (Kunta, 2018)'s work into account. The calculations were based on the production of the first batch of the product as once the process starts more raw material could be fed to as the production progresses.

Against each step, time for its complete processing was noted. The schedule of the process time can provide valuable insight about the overall production capacity of the chosen extraction. The same schedule will form the basis for the calculation of bulk production per week/month or annually.

Table 3. 5: Breakdown of the process time for the unit operations of the extraction

Equipment	Unit operation	Start time	Process time
Soxhlet	Loading oleo resin	0	0.25
	Loading solvent	0.25	0.25
	Extraction	0.25	4
Rotary	Drying	4	0.5
	Cooling	4.5	0.5
Glass tray	Drying	5	0.5
Separating funnel	Washing	5.5	1.5
Glass tray	Drying	7	12
	Scraping	12	1
		hr.	hr.
Total time for one batch	Production	20.5	0.83
25 liter--225 mg		hr.	Days

3.22 Geographical Data

Turmeric crop is widely grown across different areas of Pakistan. The total turmeric production as per the latest study is 67,807 ton [Agristatistics]. Out of the said production, about 54,673 ton is contributed by Kasur alone. Kasur district has emerged out to be the main area of the crop's production.

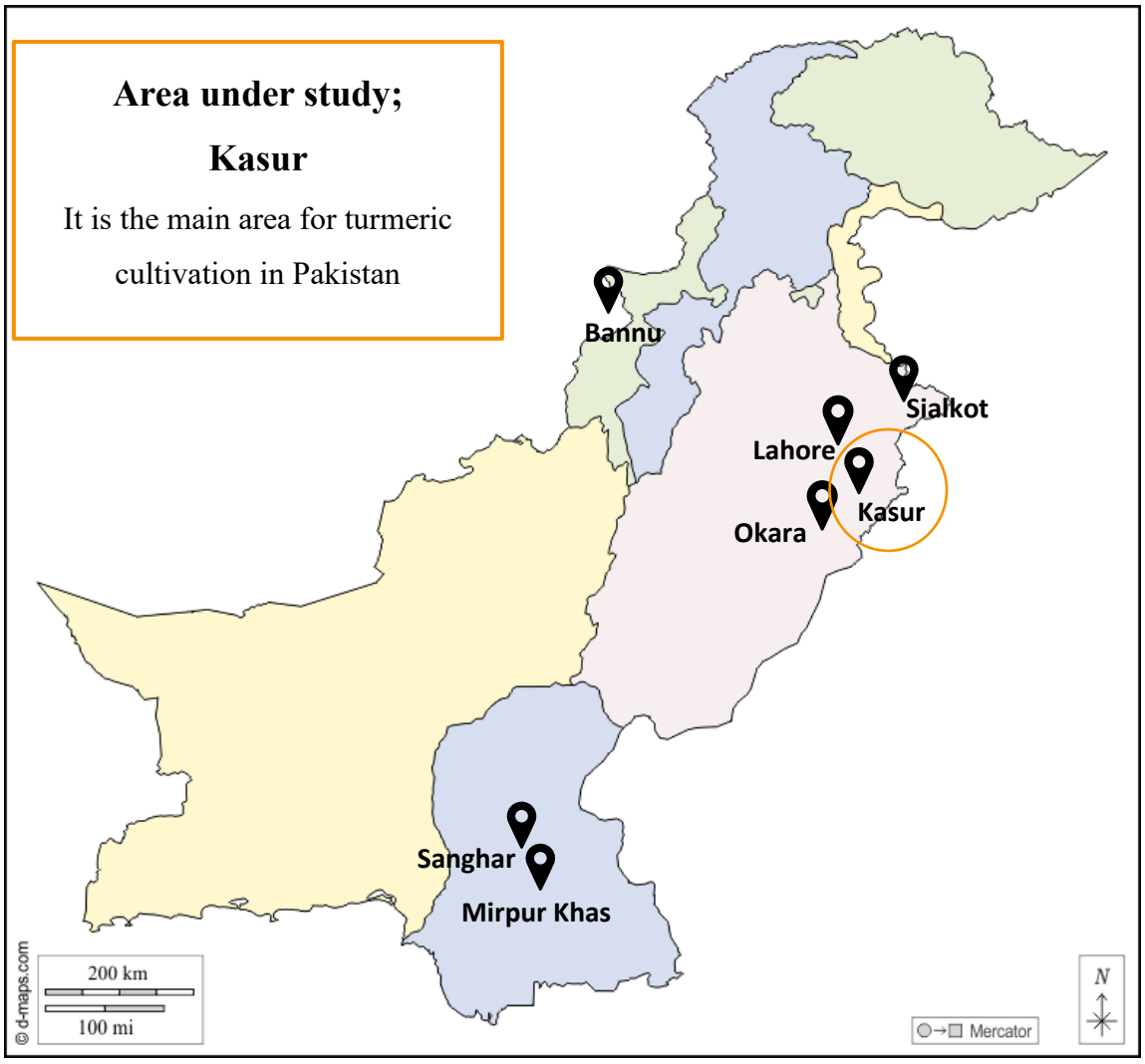


Figure 3. 5: Geographical map highlighting the turmeric cultivation districts of Pakistan

Upon making calls to the local vendors, it was confirmed that the majority of the market needs of turmeric are being met through Kasur district crop production. Therefore, Kasur was identified as the major area for turmeric crop production.

3.23 Trade value data

In order to proceed further with finding out the benefit of extracting curcumin from turmeric locally, the trade data for the crop was consulted. It was found out that a small percentage of the total crop produced is exported. After the local crop needs are met, the remaining 108 ton is exported which stands at a trade value of 313,000 USD (53,210,000 PKR). This data is from the most recent data survey [Agristatistics, 2018]. The previous patterns suggest an increase in the annual growth of turmeric.

Production (2018)	67,807 ton
Export (2018)	108 ton
Consumption	67,699 ton
Trade Value (1000USD)	313.55

4. Results

4.1 Antioxidant (DPPH) activity test:

Radical scavenging activity for each of the dilutions was checked against the absorbance of DPPH. Using the formula, absorbances were calculated. The pattern observed revealed that as the concentration of the sample particles increased in the given dilution, the scavenging activity also increased. Ascorbic acid, the standard used for this activity, validated this trend.
Maximum scavenging activity was observed in the most concentrated dilution from the A series i.e. A4 which denoted the lab extracted curcumin. Minimum scavenging activity was also observed in the same series in the least concentrated dilution i.e. A1.

Table 4. 1: Antioxidant percentage activity of the samples by DPPH method

Sample	Absorbance value (AU)	Percentage activity (%)
DPPH	0.9	0
A1	0.42	53.3
A2	0.38	57.7
A3	0.25	72.2
A4	0.12	86.6
B1	0.47	47.7
B2	0.46	48.8
B3	0.3	66.6
B4	0.2	77.7
C1	0.22	75.6
C2	0.18	80
C3	0.17	81
C4	0.17	81
D1	0.35	61
D2	0.18	80
D3	0.1	88.8
D4	0.09	90

4.2 Total antioxidant activity:

The total antioxidant activity of curcumin by Phosphomolybdate showed staggering diversity. For the lab extracted sample A, the total antioxidant activity increased with the increase in the concentration of the particles. This trend was confirmed against Ascorbic acid

that showed increasing total antioxidant activity with the gradual increase in its concentration. However, the sample B (lab extracted), showed the maximum total antioxidant activity at 2mg and 4mg with minimal values at 1mg and 3mg. This divergence from the trend can be explained

Table 4. 2: The absorbance value of each of the samples by phosphomolybdate method

Sample	Absorbance value (AU)
A1	0.6
A2	0.48
A3	0.55
A4	1.01
B1	0.4
B2	1.49
B3	0.9
B4	1.499
C1	0.9
C2	0.8
C3	0.85
C4	0.99
D1	3.4
D2	2.4
D3	1.5
D4	1.01

4.3 FTIR analysis:

FTIR analysis revealed the following spectrum. The spectrum showed a noticeable stretching at 3300cm^{-1} which corelates to the OH stretching in the curcumin structure. The peak observed at 2900cm^{-1} corelates to the C-H bond present in the curcumin structure. For the peak at 1513cm^{-1}, C=C bond can be identified. The aromatic rings within the curcumin structure were confirmed by the peak at 1600cm^{-1}. The peak at 1116cm^{-1} corelated with Ether.

For drawing a comparison between the absorbance, a lab standard for curcuminoids was used. The orange curve in fig is for the curcumin standard. A higher absorbance was observed in the extracted samples. Maximum absorbance was noted at 0.89 by the completely extracted fraction of curcumin. All the structural peaks in curcumin standard corelate with the completely as well as partially extracted curcumin samples. However, the three subjects differed in their absorbance. The lab standard curcumin showed absorbance at 0.721.

From the obtained curves, it was inferred that the extracted fractions were of curcumin. The polyphenolic compound’s structure verified its identity. The obtained FT IR spectrum for the

two extracted samples also matched with the FT IR sample reported in the literature which further validated the identity of the extracted fractions.

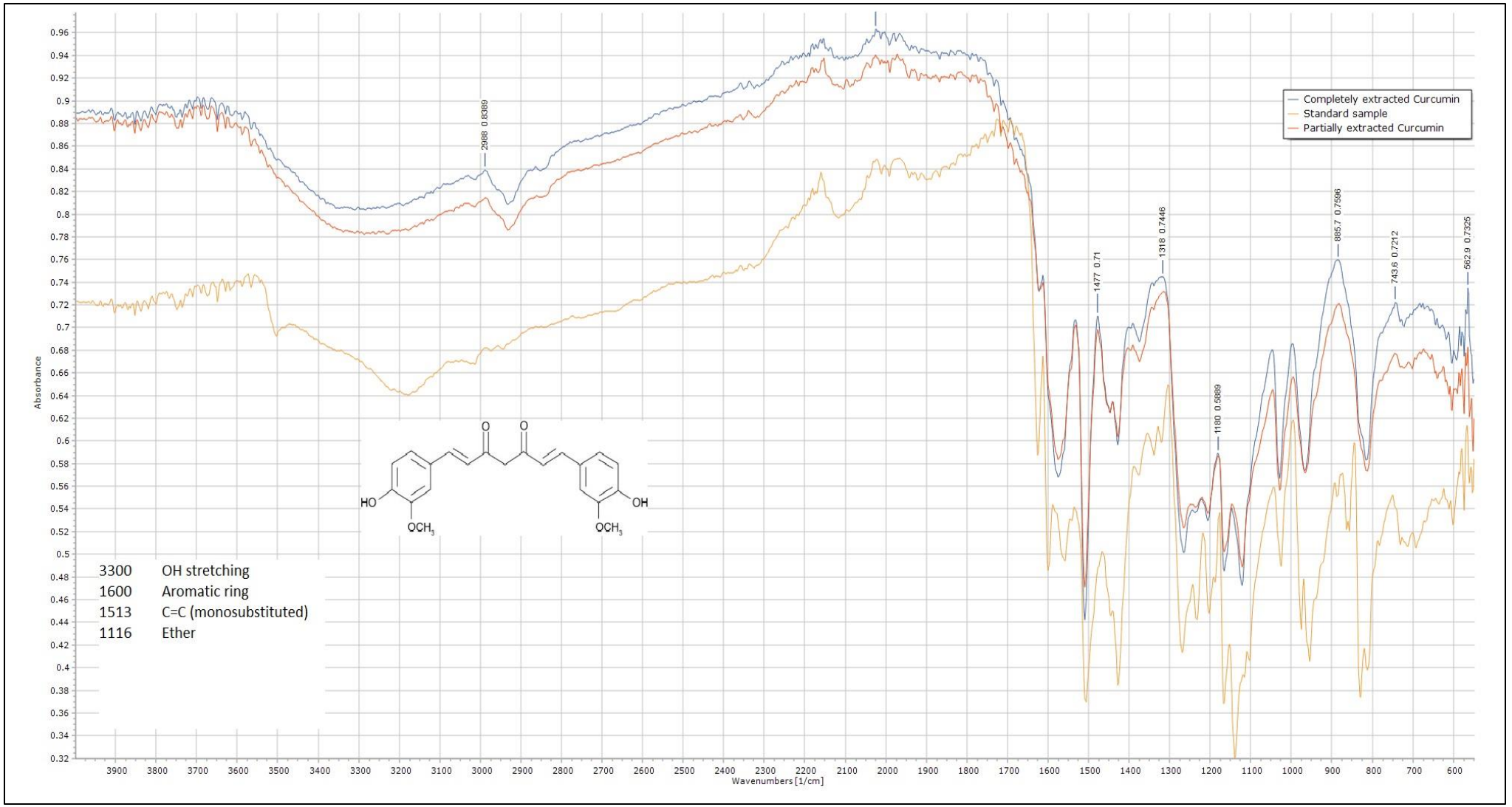


Figure 4. 1: FTIR graph of the curcumin standard with two experimental samples

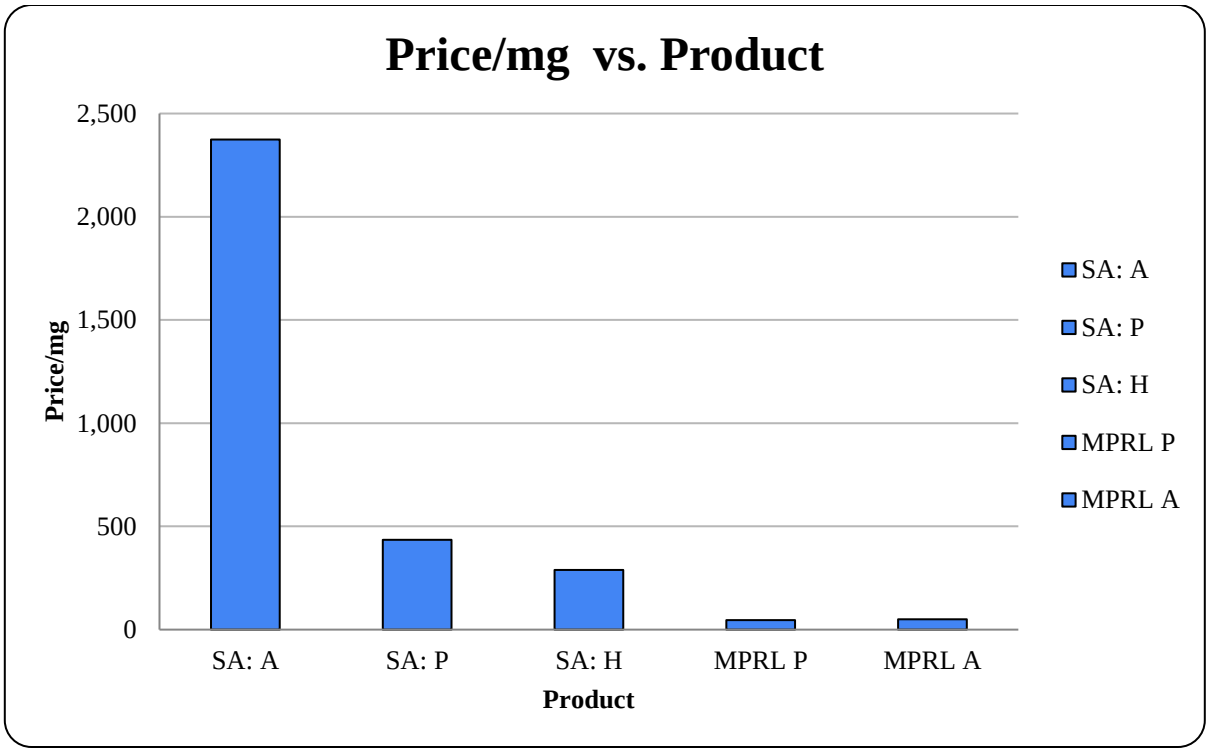
4.4 Techno-economic analysis

After establishing the optimized protocol and completing process modelling of the equipment to be used in the extraction process, techno-economic analysis was proceeded. The cost of bulk production of lab extracted curcumin with its local and global market parallel was calculated. The calculations were primarily based on the lab parallel of extraction using the same chemicals and methodology. The cost for producing 225 mg of curcumin with ethanol as the extraction solvent came out to be PKR 2,536 (USD 15.85-16). This extraction used food grade hexane which is cheaper than the analytical grade hexane. For the pharmaceutical industry grade curcumin extracted with acetone, the cost for the same amount of curcumin i.e., 225 mg was PKR 20,311 (USD 127). These are the costs of the production of curcumin.

To elucidate the cost-effectiveness of curcumin extracted through the optimized lab protocol, techno-economic analysis as briefed by (Kunta, 2018) was performed. For it, multiple market products by the name of curcumin were considered to draw a parallel with. Upon extensive outreach calls to the vendors, it was confirmed that the local market imports curcumin to fulfil the lab-based application needs of curcuminoids. Sigma Aldrich came out to be the major source of the local vendors.

For approximating the revenue, cost of the labor and the price mark-up were also taken into account. It was inferred that the cost of producing curcumin in bulk, if borne by the local industry, has the potential to reduce the dependency on the international companies for its import. The proposed techno-economic analysis could serve as a means to further estimate the cost of its commercial production by a local industry.

As for the local market products for the food industry, only one local manufacturer worth mentioning was found. The market products go by the formulation of ‘Turmeric curcumin.’ The product labels were vague in giving the information about the exact formulation.



Graph 4. 1: Price analysis of available curcumin with the lab extracted two types

Table 4. 3: Price of various types of curcumin

Company/brand	Price/mg (PKR)
Sigma Aldrich Analytical grade (SA: A)	2,374
Sigma Aldrich Pharmaceutical grade (SA:P)	435
Sigma Aldrich HPLC grade (SA:H)	289
Lab extracted pharmaceutical grade (MPRL P)	46
Lab extracted Analytical grade (MPRL A)	50

The cost of all major equipment to be used for its production are analyzed in Table 4.4. Numerous sources including [Alibaba.com] were used for cost estimation. Figure 4.3 shows the overall share of the equipment, materials, and human resources in the net cost of the entire process.

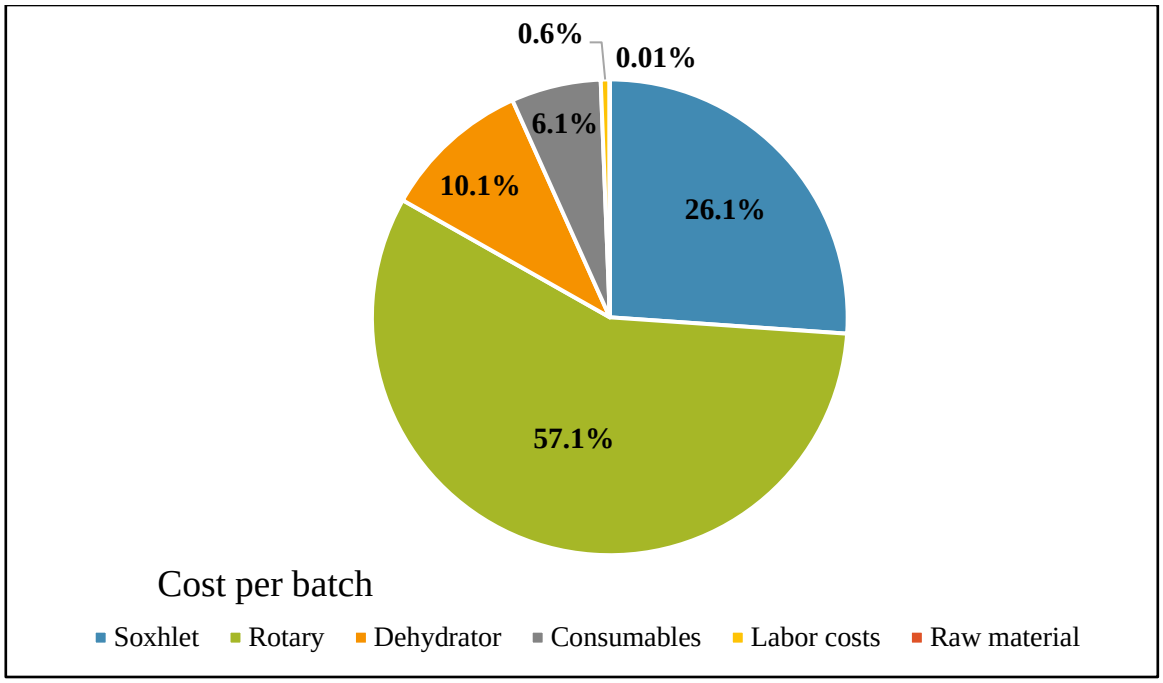


Figure 4. 2: Share of each equipment/material in total cost of single curcumin batch

Table 4. 4: Cost of the equipment for extraction

Equipment/Material	Cost (PKR)
Soxhlet apparatus	194,460
Industrial rotary vacuum evaporator	4,25,000
Dehydrator	75,000
Consumables	45,350
Turmeric rhizomes	125
Labor	4,375

Using Excel's formulas, effect of price change on revenue and gross margin was plotted with a bar graph (Figure 4.4). The graphs revealed a direct relationship of revenue and gross margin with the changing price. An increase in the price increased revenue as well as gross margin and vice versa. It gave useful insights into how much price change could be afforded. The set price i.e., PKR 23,743 was used as a benchmark. Any change below it was considered as a decline in the revenue.

The same analysis was applied for the Gross margin and similar pattern was observed insinuating that both behaved similarly when encountered with a price change.

Table 4. 5: Effect of Price on the revenue

Price	Revenue
PKR 32,743.00	736717.5
PKR 29,743.00	669217.5
PKR 26,743.00	601717.5
PKR 23,743.00	534217.5
PKR 20,743.00	466717.5
PKR 17,743.00	399217.5

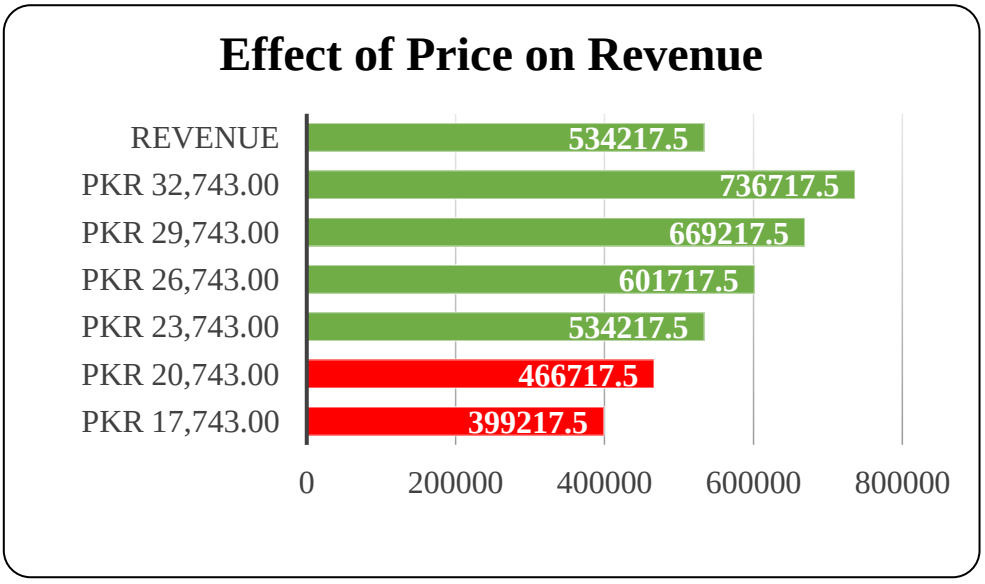


Figure 4. 3: Effect of price on the revenue

Table 4. 6: Effect of price on the gross margin

Price	Gross margin
PKR 32,743.00	716406.5
PKR 29,743.00	648906.5
PKR 26,743.00	581406.5
PKR 23,743.00	513906.5
PKR 20,743.00	446406.5
PKR 17,743.00	378906.5

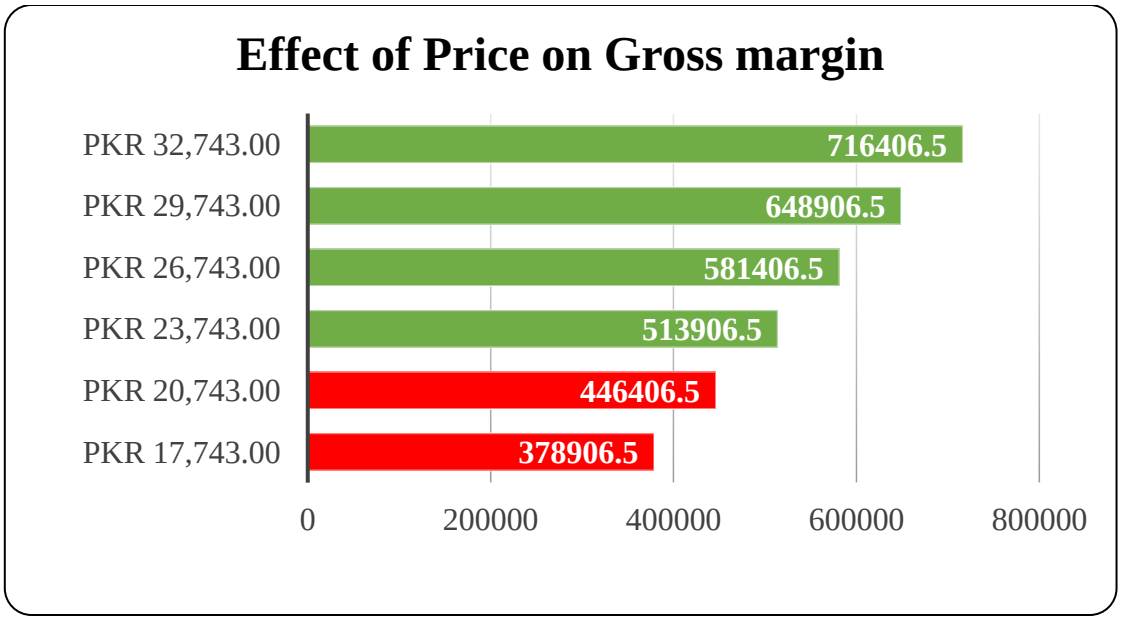


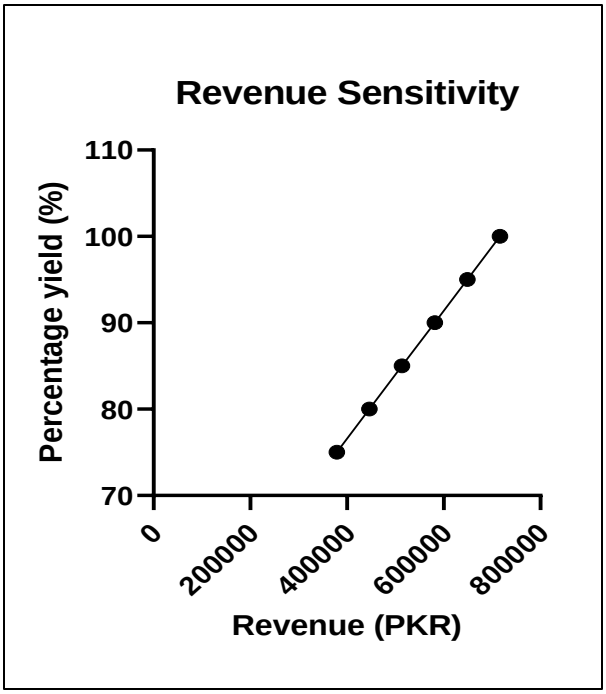
Figure 4. 4: Effect of price on the gross margin

4.5 Sensitivity analysis

Sensitivity analysis gives us useful insight about the key variable affecting the cost of the bulk production of curcumin. Since the major parameter that determines the cost of the bulk production is the percentage yield of the extracted fraction, it was identified as the key parameter. The sensitivity analysis also revealed how much could it impact the revenue.

Figure 4. 5: Effect of percentage change in the curcumin yield on the revenue

Extraction rate change	Revenue PKR
+15	716406.5
+10	648906.5
+5	581406.5
85	513,907
-5	446406.5
-10	378906.5



Graph 4. 2: Sensitivity analysis of extraction percentage yield on the revenue

The revenue sensitivity analysis shows a sharp decline in revenue when the percentage yield starts to go below the threshold value of 85%. Any value above 85% is considered ideal case as obtaining more than that is not practically possible (Kunta, 2018). If the bulk production of curcumin is to be started then any percentage yield below 85% could mean a significant loss in the revenue.

4.6 Trade value intervention

The [Agristatistics, 2018] trade data suggests that the export of just the raw form of the crop brings in 313 (1000USD) of revenue. This data was further used to make valid intervention about the production of curcumin from the crop that is widely grown in Pakistan. For the intervention, it is proposed that the amount of turmeric crop that is otherwise exported could be used for the extraction of curcumin from it.

If curcumin is extracted from the same amount of crop that is otherwise exported, the trade value (excluding market profit) could increase up to 5 folds. Once the costs of setting up a local extraction unit are borne, a highly profitable revenue cycle could be initiated.

Table 4. 7: Trade value of Curcumin after the proposed intervention

Production (2018)	**67,807 ton**
Allotted for curcumin	108 ton
Curcumin Production	24 Kg
Trade Value (1000USD)	1588.24

5. Discussions

Extraction of curcumin from Curcuma longa grown in Pakistan has not been previously reported. However, a comparative study by (Sahne et al., 2016) and reviews expounding on its medicinal properties inlcuding (Akbar et al., 2018) among many could be found. Therefore, the only available literature to compare with it includes curcumin extracted in other parts of the world.

The optimal conditions mentioned by (Pawar et al., 2018) used Ethyl acetate as the extraction solvent. While this solvent was checked, it did not come out to be a good solvent for our study. The extraction method used was the same. However, the two solvents that gave the best yield were Acetone and Ethanol. Both the solvents have been reported as good solvents previously (Altunay et al., 2020) under different other conditions.

5.1 Identity confirmation

Comparing the FT IR analysis graph of the extracted fraction with the previously extracted fractions by (Pawar et al., 2018), the fraction showed an absorbance at 0.9 AU with a standard curve. With the established optimised protocol by this study, the fraction showed an absorbance at 0.89 AU with characteristic peaks of O-H, C=C, and aromatic ring at $3300cm^{-1}$, $1513cm^{-1}$, and $1600cm^{-1}$ respectively. These peaks were in line with the studied literature (Pawar et al., 2018).

5.2 Qualitative and Quantitative analysis

The antioxidant and total antioxidant assays gave significant results. The percentage antioxidant activity recorded by the literature ranged from 26-58% by (Ak & Gülçin, 2008) and 57-79% by (Borra et al., 2013). The maximum experimental value for the DPPH assay was observed to be 86% with ascorbic acid as a standard showing a maximum of 90% antioxidant activity. This indicates that the curcumin fraction can have its antioxidant activity intact under optimal conditions. The narrowed down optimal conditions proved to be ideal for extracting good quality curcumin.

5.3 Technoeconomic analysis

Technoeconomic analysis of curcumin has only been reported in the literature once (Kunta, 2018). Given the quality of extracted curcumin and wide scale availability of the raw material, the technoeconomic analysis gave a bird’s eye view of the bulk production of curcumin.

The approximation of revenue and gross margin can be used for further cash flow calculation that could then be used (after further analysis) for replicating the same extraction model on a commercial level. As per the market and business analysis, it is only a matter of a few years that the growing need of curcumin would be largely felt across the US as well as Europe. In such scenario, countries like India and Pakistan can emerge as vital players in the local production and export of curcumin to the first world nations. The current trade value for the export of turmeric crop could be increased immensely if it the crop is commercialized. The surplus production of the crop in Pakistan is adequate to meet the needs of the local populace as well as enter the global market.

6. Conclusion

Curcumin entails numerous medicinal benefits that find their application across multiple industries. This study revealed that *Curcuma longa* crop grown here in Pakistan has adequate amount of curcumin present in it. The polyphenolic compound has its biological activities intact when extracted under the optimal conditions **(**Soxhlet extraction for 4-6hrs with ethanol/acetone as extracting solvent, fraction washing with hexane 3-4 times and drying under rotary vacuum**)** as proven by the qualitative and quantitative assays.

With an increased customer consciousness related to natural or organic products and growing debate on sustainability, the market for plant-based products is continuously expanding. With Asian countries being the active player in supplementing the turmeric crop demands of the western market, it will be even more beneficial if the production of a polyphenolic compound as vital as curcumin starts locally. This study would be vital in outlining the optimal conditions for its extraction.

However, to further improve the efficiency of its extraction on a commercial scale, techniques as advanced as supercritical CO_2 extraction and microwave or ultrasound assisted methods could be tried out. The methods are energy intensive but give the highest yield of curcumin extract without requiring to be continuously fed with the extraction raw materials.

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