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Extraction of Curcumin

Anamika Bagchi
(Department of Food Technology, Techno India/ West Bengal University of Technology, India)

ABSTRACT : *Curcumin due to its various medicinal, biological, pharmacological activities is high on demand and has high market potential, high cost. Since curcumin has variety of uses, extracting it in a less expensive method other Super Critical Fluid Extraction is the main aim or objective of this work. Usage of food grade solvents is a main prerequisite of this work and optimization of the parameters in order to find an effective means of extraction sum ups the cause of this project work. Besides working on the extraction of curcumin, other properties such as curcumin's antioxidant, antimicrobial properties are to be envisaged upon. This project work mainly deals with the topic on 'extraction of curcumin' from its common source turmeric, using an effective low cost method of solvent extraction. Different solvents are used either in their pure form or being mixed in definite ratio's, while taking into consideration of other parameters such as particle size, time, temperature, solid: solvent ratio. The qualitative analysis of its antimicrobial property is also done along with the product development of Cake.*

Keywords - *Antiflatulent, Antifibrotic, Antimutagenic, Carcinogenesis, Ulcerogenic*

I. Introduction

The turmeric (Curcuma longa) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice. The characteristic yellow color of turmeric is due to the curcuminoids, first isolated by Vogel in 1842. Curcumin is an orange–yellow crystalline powder practically insoluble in water. The structure of curcumin (C 21 H 20 O 6) was first described in 1910 by Lampe and Milobedeska and shown to be diferuloylmethane. Turmeric is used as a dietary spice, coloring agent in foods and textiles, and a treatment for a wide variety of ailments. It is widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. Turmeric paste in slaked lime is a popular home remedy for the treatment of inflammation and wounds. For centuries, curcumin has been consumed as a dietary spice at doses up to 100 mg/d. Extensive investigation over the last five decades has indicated that curcumin reduces blood cholesterol. (Aggarwal *et al.*, 2006).Turmeric was described as C. longa by Linnaeus and its taxonomic position is as follows:

Class Liliopsida
Subclass Commelinids
Order Zingiberales
Family Zingiberaceae
Genus Curcuma
Species Curcuma longa

The wild turmeric is called C. aromatica and the domestic species is called C. longa. For the last few decades, extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. Its anticancer effect is mainly mediated through induction of apoptosis. It's anti-inflammatory, anticancer and antioxidant roles may be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis. Clinically, curcumin has already been used to reduce post-operative inflammation. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases. (Chattopadhyay *et al.*, 2004)

1.1 Chemical composition of turmeric

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%)5. Curcumin

(diferuloylmethane) (3–4%) is responsible for the yellow color, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%)6. Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated7 (Figure 1). Curcumin was first isolated in 1815 and its chemical structure was determined by Roughley and Whiting in 1973. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform. (Chattopadhyay *et al.*, 2004)

Curcumin (also known as curcumin I) occurs naturally in the rhizome of Curcuma longa, which is grown commercially and sold as turmeric, a yellow–orange dye. Turmeric contains curcumin along with other chemical constituents known as the "curcuminoids". The major curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III), and the recently identified cyclocurcumin. Commercial curcumin contains curcumin I (~77%), curcumin II (~17%), and curcumin III (~3%) as its major components. The curcuminoid complex is also referred to as Indian saffron, yellow ginger, yellow root, kacha haldi, ukon, or natural yellow. Spectrophotometrically, curcumin has a maximum absorption (λ max) in methanol at 430 nm, with a Beer's law range from 0.5 to 5 μ g/mL (Prasad, 1997). It absorbs maximally at 415 to 420 nm in acetone and a 1% solution of curcumin has 1650 absorbance units. Curcumin has a brilliant yellow hue at pH 2.5 to 7 and takes on a red hue at pH > 7. The spectral and photochemical properties of curcumin have been studied in different solvents by Chignell and coworkers. In toluene, the absorption spectrum of curcumin contains some structure, which disappears in more polar solvents such as ethanol and acetonitrile. The fluorescence of curcumin occurs as a broad band in acetonitrile (λ max = 524 nm), ethanol (λ max = 549 nm), or micellar solution (λ max = 557 nm), but has some structure in toluene (λ max = 460, 488 nm). In addition, curcumin was observed to produce singlet oxygen upon irradiation (λ max > 400 nm) in toluene or acetonitrile (phi = 0.11 for 50 μ M curcumin). Curcumin quenched singlet oxygen in acetonitrile (kq = 7 X 10 6 /M-s). Singlet oxygen production was about ten times lower in alcohols and was hardly detectable when curcumin was solubilized in an aqueous micellar solution of Triton X-100. However, in sodium dodecyl sulfate solution, no singlet oxygen phosphorescence could be observed for those micelles containing curcumin. Curcumin is also reported to be able to photogenerate superoxide in toluene and ethanol. (Aggarwal *et al.*,2006)

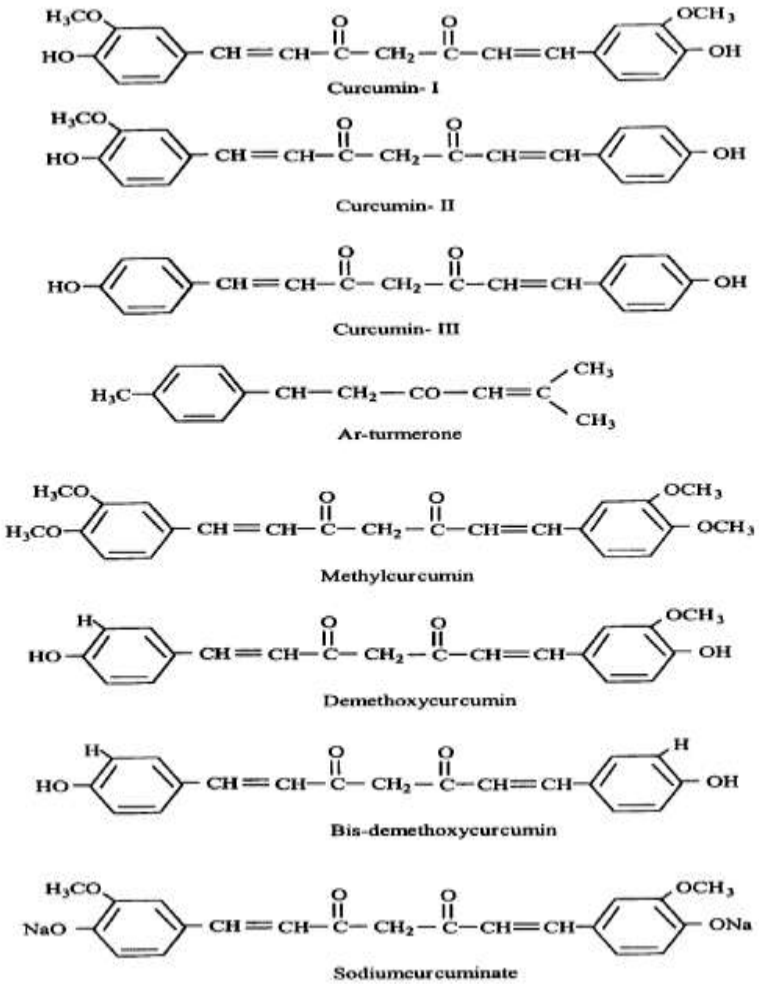

Fig 1: Structure of natural curcuminoids. (Chattopadhyay *et al.*, 2004)

1.2Biological activity of turmeric and its compounds

Turmeric powder, curcumin and its derivatives and many other extracts from the rhizomes were found to be bioactive. Turmeric powder has healing effect on both aseptic and septic wounds in rats and rabbits. It also shows adjuvant chemoprotection in experimental for stomach and oral cancer models of Swiss mice and Syrian golden hamsters. Curcumin also increases mucin secretion in rabbits. Curcumin, the ethanol extract of the rhizomes, sodium curcuminate, [feruloyl-(4-hydroxycinnamoyl)- methane] (FHM) and [bis-(4-hydroxycinnamoyl)- methane] (BHM) and their derivatives, have high anti-inflammatory activity against carrageen in induced rat paw oedema. Curcumin is also effective in formalin induced arthritis. Curcumin reduces intestinal gas formation and carbon tetrachloride and D-galactosamine induced glutamate oxaloacetate transaminase and glutamate pyruvate transaminase levels. It also increases bile secretion in anaesthetized dogs and rats, and elevates the activity of pancreatic lipase, amylase, trypsin and chymotrypsin. Curcumin protects isoproterenol-induced myocardial infarction in rats. Curcumin, FHM and BHM also have anticoagulant activity. Curcumin and an ether extract of C. longa have hypolipemic action in rats24 and lower cholesterol, fatty acids and triglycerides in alcohol induced toxicity. Curcumin is also reported to have antibacterial, antiamoebic and antiHIV activities. Curcumin also shows antioxidant activity. It also shows antitumour and anticarcinogenic activities. The volatile oil of C. longa shows anti-inflammatory, antibacterial and antifungal activities. The petroleum ether extract of C. longa is reported to have anti-inflammatory activity. Petroleum ether and aqueous extracts have 100% antifertility effects in rats. Fifty per cent ethanolic extract of C. longa shows hypolipemic action in rats. Ethanolic extract also possesses antitumour activity. Alcoholic extract and sodium curcuminate can also offer antibacterial activity. The crude ether and chloroform extracts of C. longa stem are also reported to have antifungal effects. A C. longa fraction containing ar-turmerone has potent antivenom activity. (Chattopadhyay *et al*., 2004)

1.3 Pharmacological action of turmeric and its extract

Turmeric powder has beneficial effect on the stomach. It increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants. However, controversy exists regarding antiulcer activity of curcumin. Both antiulcer and ulcerogenic effects of curcumin have been reported but detailed studies are still lacking. Curcumin has been shown to protect the stomach from ulcerogenic effects of phenylbutazone in guinea pigs at 50 mg/kg dose.

Curcumin has some good effects on the intestine also. Antispasmodic activity of sodium curcuminate was observed in isolated guinea pig ileum. Antiflatulent activity was also observed in both in vivo and in vitro experiments in rats. Curcumin also enhances intestinal lipase, sucrase and maltase activity.

Curcumin decreases the severity of pathological changes and thus protects from damage caused by myocardial infarction. Curcumin improves Ca^{2+}-transport and its slippage from the cardiac muscle sarcoplasmic reticulum, thereby raising the possibility of pharmacological interventions to correct the defective Ca^{2+} homeostasis in the cardiac muscle. Curcumin has significant hypocholesteremic effect in hypercholesteremic rats. Curcumin and manganese complex of curcumin offer protective action against vascular dementia by exerting antioxidant activity on nervous system.

Curcumin reduces low density lipoprotein and very low density lipoprotein significantly in plasma and total cholesterol level in liver along with an increase of a-tocopherol level in rat plasma, suggesting in vivo interaction between curcumin and a-tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol levels. Curcumin binds with egg and soy-phosphatidylcholine, which in turn binds divalent metal ions to offer antioxidant activity. The increase in fatty acid content after ethanol-induced liver damage is significantly decreased by curcumin treatment and arachidonic acid level is increased.

Curcumin is effective against carrageenin-induced oedema in rats and mice. The natural analogues of curcumin, viz. FHM and BHM, are also potent anti-inflammatory agents.

The volatile oil and also the petroleum ether, alcohol and water extracts of C. longa show anti-inflammatory effects. The antirheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin. That curcumin stimulates stress-induced expression of stress proteins and may act in a way similar to indomethacin and salicylate. Curcumin also enhances wound-healing in diabetic rats and mice, and in H^2O^2-induced damage in human keratinocytes and fibroblasts.

The antioxidant activity of curcumin was reported as early as 1975. It acts as a scavenger of oxygen free radicals. It can protect haemoglobin from oxidation. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H2O2 and nitrite radical generation by activated macrophages, which play an important role in inflammation. Curcumin also lowers the production of ROS in vivo. Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have antioxidant effect. Curcumin exerts powerful inhibitory effect against H2O2-induced damage in human keratinocytes and fibroblasts and in NG 108-15 cells.It also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates.The antioxidant mechanism of curcumin is attributed to its unique

conjugated structure, which includes two methoxylated phenols and an enol form of b-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant.

Generally, the nonenzymatic antioxidant process of the phenolic material is thought to be mediated through the following two stages:

S-OO° + AH → SOOH + A°,

A· + X· → Nonradical materials,

Where S is the substance oxidized, AH is the phenolic antioxidant, A· is the antioxidant radical and X· is another radical species or the same species as A·. A· and X· dimerize to form the non-radical product. Masuda et al.89 further studied the antioxidant mechanism of curcumin using linoleate as an oxidizable polyunsaturated lipid and proposed that the mechanism involves oxidative coupling reaction at the 3¢position of the curcumin with the lipid and a subsequent intramolecular Diels–Alder reaction.

Curcumin acts as a potent anticarcinogenic compound. Among various mechanisms, induction of apoptosis plays an important role in its anticarcinogenic effect. It induces apoptosis and inhibits cell-cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells.

Curcumin exerts both pro- and antimutagenic effects. At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in cyclophosphamide- induced chromosomal aberration in Wistar rats.In fact,the total effect of chromium and curcumin is additive in causing DNA breaks in human lymphocytes and gastric mucosal cells.

Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation in vitro as well as in vivo in rat thoracic aorta. Curcumin prevents galactose-induced cataract formation at very low doses. Both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat. Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus.

Both curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus, Staphylococcus, Lactobacillus,* etc.. The aqueous extract of turmeric rhizomes has antibacterial effects. Curcumin also prevents growth of Helicobacter pylori CagA+ strains in vitro. Ether and chloroform extracts and oil of C. longa have antifungal effects. Crude ethanol extract also possesses antifungal activity. Turmeric oil is also active against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme and Penicillium digitatum.* The ethanol extract of the rhizomes has anti-*Entamoeba histolytica* activity. Curcumin has anti-Leishmania activity in vitro. Several synthetic derivatives of curcumin have anti-L. amazonensis effect. Anti-*Plasmodium falciparum* and anti-L. major effects of curcumin have also been reported. Curcumin has been shown to have antiviral activity. It acts as an efficient inhibitor of Epstein-Barr virus (EBV) key activator Bam H fragment. Most importantly, curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1 integrase needed for viral replication. It also inhibits UV light induced HIV gene expression. Thus curcumin and its analogues may have the potential for novel drug development against HIV. (Chattopadhyay *et al.*, 2004)

II. Planning

The following shows the proposed work in a systematic manner:

1. Selection of dried turmeric and grinding them.
2. Separating the comminuted turmeric into their respective particle size of 250 µ, 44 mesh, 30 mesh and above 30 mesh size.
3. Selection of Food Grade solvents for solvent extraction.
4. Determination of moisture content of turmeric powder.
5. Selecting the parameters for solvent extraction and optimizing them subsequently which are:

- Particle size.
- Pure solvent.
- Mixture of solvents.
- Solid: Solvent ratio.
- Temperature.
- Time.

6. Selecting the effective parameter for highest extraction of curcumin from turmeric.
7. Determining the antimicrobial properties of curcumin.
8. Product Development.

III. Materials and Methods

3.1 Extraction of Curcumin

3.1.1 Materials

Medium: MRS Medium (Yeast Extract, Meat Extract, Glucose, Sodium Acetate, Dihydrogen Potassium Phosphate, Triammonium Citrate, Magnesium Sulphate, Manganese Sulphate, Agar, pH-5.7).

Solvent: Ethanol, Water, Pet ether.

Instrument: Mixer Grinder, Siever, Spectrophotometer, Dhona pan Balance, Water bath, Hot air oven, Shaker, Bidwell-Sterling.
Apparatus: Conical Flask, Funnel.
Sample: Comminuted Turmeric powder.

3.1.2 Methods.

3.1.2.1Grinding of dried turmeric and separation

500 g of dried turmeric is first grinded in a mixer grinder and then subjected to separation through a vibrating sieving machine. Four different particle sizes are separated as 250µ size, 44 mesh size, 30 mesh size and above 30 mesh size. The powders are accumulated and then sealed separately with labeling for further analysis.

3.1.2.2Solvent extraction of turmeric using ethanol and water

From each particle size 2 g of sample is taken and mixed with 30 ml of ethanol and 30 ml of water respectively, separately and then filtered. The concentration of each of the filtrate is kept same and then the absorbance is measured using spectrophotometer at 425nm. Curcumin content g/100 g is measured using this formula:

$$= \frac{0.0025 \times \text{Absorbance at 425 nm} \times \text{volume made up} \times \text{Dilution factor} \times 100}{0.42 \times \text{weight of sample} \times 1000}$$

Since 0.42 absorbance at 425 nm =0.0025 g of curcumin.

3.1.2.3 Solvent extraction of turmeric (250 µ size) using ethanol and water.

The above procedure is again experimented, with the exception of using timed interval of 2hour, 3hour and 4hour respectively for solvent extraction of turmeric using equivalent amounts of ethanol and water , that is 30 ml of each , separately filtering and keeping the concentration of the filtrate same. The filtrate is subsequently then taken for spectrophotometric analysis at 425nm and calculations are done accordingly with the above specified formula.

3.1.2.4 Moisture Analysis of dried turmeric powder

The moisture analysis is done for the sample of size of 250 micron. A Bidwell-Sterling moisture trap is used as apart of the apparatus for reflux distillation with a solvent less dense than water. Toluene is the desired solvent being used for moisture determination. When the toluene starts to boil , a hazy cloud is seen rising above the distillation flask. This is a vaporous emulsion of toluene in water. After a few cycle of refluxing two separate layers are seen to form and the amount of water extracted from the turmeric can be directly read from the calibrated trap.

3.1.2.5 Optimization of solvent extraction of turmeric at different time intervals

From the sample of 250 micron size, 2g of sample is taken for further solvent extraction of turmeric using separately for solvents ethanol and water respectively. The interval of time being optimized to 0.5 hour, 1 hour, 1.5 hour and 2 hour respectively. The amount of curcumin being extracted is then calculated using gravimetric method. After filtration 10 ml filtrate is taken from each set, using water as a solvent, and dried in hot air oven at 130°C for 1.5hours in a petriplate, where the weight of each empty petriplate was initially noted down. After taking them out of the oven they were kept in the dessicator to cool down and were measured until constant weight was obtained. Similarly for the set of ethanol extractives of curcumin, 10 ml of filtrate was taken and the solvent was evaporated under atmospheric pressure in water bath. The weight of petriplate with residual curcumin was noted down and the amount being extracted was calculated.

3.1.2.6 Optimization of solvent extraction method of turmeric.

Keeping the particle size constant, 2 g of sample was taken and solvent extraction was done separately using ethanol and water as solvent. The solvents were kept at different time intervals, in order to ably extract the maximized portion of curcumin from turmeric, keeping the set of ethanol at 1.5 hour and set of water at 1hour. The respective volumes were taken as 30 ml, 40 ml and 50 ml respectively for each case. The amount of extractives was again calculated using the same above method of gravimetric measurement.

3.1.2.7 Solvent extraction using mixture of solvent of ethanol and water.

Keeping the particle size, amount and time constant at 1.5 hour different ratio of solvents were utilized to find the effective extraction parameter. Two different sets were made with water and ethanol separately of 30 ml each respectively and three sets of mixed solvents were used with 80%, 70% and 50% ethanol in ratio with

water, keeping the total volume at 30 ml of each set constant respectively. The amount of curcumin extracted was again calculated using the gravimetric method mentioned earlier for the two different set of water and ethanol while another approach was taken into account for the mixed solvents. The filtrate from the mixed solvents were taken 10 ml each, same as that for the pure solvents, and were left to evaporate the ethanol in water bath and subsequently transferring the petriplates into hot air oven at 130°C for 1.5 hours and then repeating the previously mentioned method again.

3.1.2.8 Solvent extraction of turmeric using pet ether.

Keeping time constant at 1 hour, particle size at 250 micron and sample weight at 2 g, solvent extraction of turmeric is carried out using a different solvent of pet ether with successive volume of 50 ml, 60ml, 70ml and 80 ml respectively. Filtration is carried out and the filtrates are collected in a petriplate each of 10 ml quantity and the solvent is evaporated at atmospheric pressure in water bath. After drying the residual weight is noted down and subtracted from the initial empty weight of the petriplate, and the amount of curcumin being extracted is calculated.

3.1.2.9 Qualitative analysis of antimicrobial activity of curcumin against E.coli

The MRS medium was prepared and autoclaved. Then *E.coli* suspension was prepared with one or two ml sterilized distilled water. From the heavy suspension 0.1 ml of solution was taken and spreaded over the petriplate, then 18-20 ml of agar solution was poured into the petriplate. 20-25 minutes standing time was given to the agar to solidify. Then using a cylindrical sterilized object a small groove was made where 0.1 ml of curcumin solution was added in order to examine its antimicrobial activity against *E.coli.* The petriplate was then kept in incubator for 24 hours for 42ºC, after which the zone of inhibition against *E.coli* was seen.

3.1.3 Result and Discussion.

3.1.3.1Solvent extraction of turmeric using ethanol and water

Table 1: showing the values of curcumin extracted

Particle size	Curcumin Content g/100g	
	Solvent ---Ethanol	Solvent ----Water
250 µ	0.01238	0.01327
44 mesh	0.01337	0.01274
30 mesh	0.01239	0.01087
Above 30 mesh	0.01139	0.01099

3.1.3.2 Solvent extraction of turmeric (250 µ size) using ethanol and water.

Table 2: Showing the different percentages of curcumin extracted at different time intervals.

Time (Hour)	Curcumin Content g/100g	
	Solvent ---Ethanol	Solvent ----Water
2	0.01682	0.01706
3	0.01679	0.01693
4	0.01652	0.01693

3.1.3.3 Moisture Analysis of dried turmeric powder

100 gm of turmeric contains 5 % moisture.

3.1.3.4 Optimization of solvent extraction of turmeric at different time intervals

Table 3: Showing percentage of curcumin extracted using water and ethanol as solvent for optimized time intervals

Time(Hour)	Percentage of curcumin extracted (%)	
	Solvent ---Water	Solvent----Ethanol
0.5	20.68	2.24
1	23.11	0.04
1.5	12.46	32.28
2	19.07	14.47

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3.1.3.5 Optimization of solvent extraction method of turmeric.

Table 4: showing the percentage of curcumin extracted using different volume of solvent.

Volume of Solvent (ml)	Percentage of curcumin extracted (%)	
	Solvent – Ethanol (at 1.5 hour)	Solvent-Water (at 1 hour)
30	49.095	20.015
40	0.120	2.49
50	0.185	7.25

3.1.3.6 Solvent extraction using mixture of solvent of ethanol and water

Table 5: Showing the percentage of curcumin extracted using mixture of solvents in ratio.

Volume of Water(ml)	Volume of Ethanol(ml)	Total(ml)	Percentage of curcumin extracted (%)
30	---	30	9.890
----	30	30	28.656

6	24	30	1.380
9	21	30	0.265
15	15	30	29.625

3.1.3.7 Solvent extraction of turmeric using pet ether

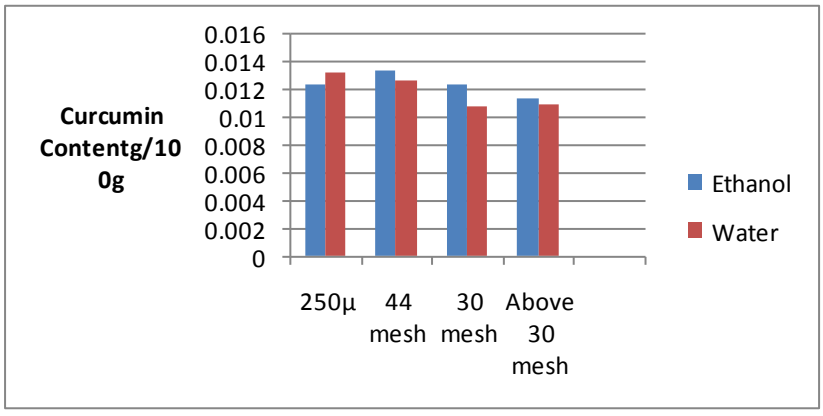
Table 6: Showing the percentage of curcumin extracted using pet ether as a solvent

Volume of Pet ether used(ml)	Percentage of Curcumin Extracted (%)
50	0.1
60	0.63
70	1.56
80	1.79

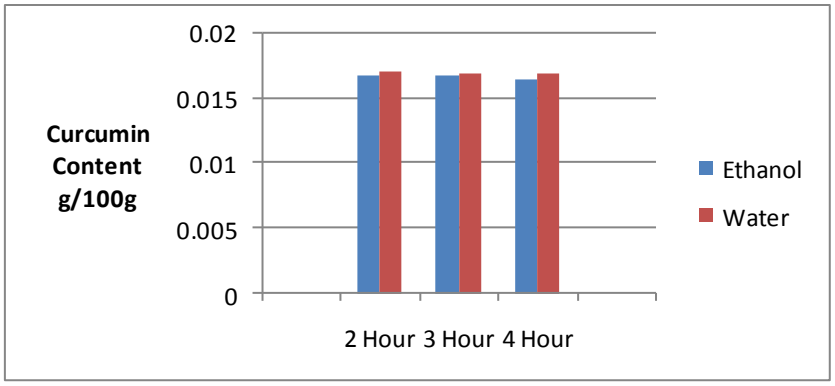
3.1.3.8 Qualitative analysis of antimicrobial activity of curcumin against E.coli

The following image shows the qualitative analysis of antimicrobial activity of curcumin against *E.coli* showing that it is effective against gram negative micro organisms.

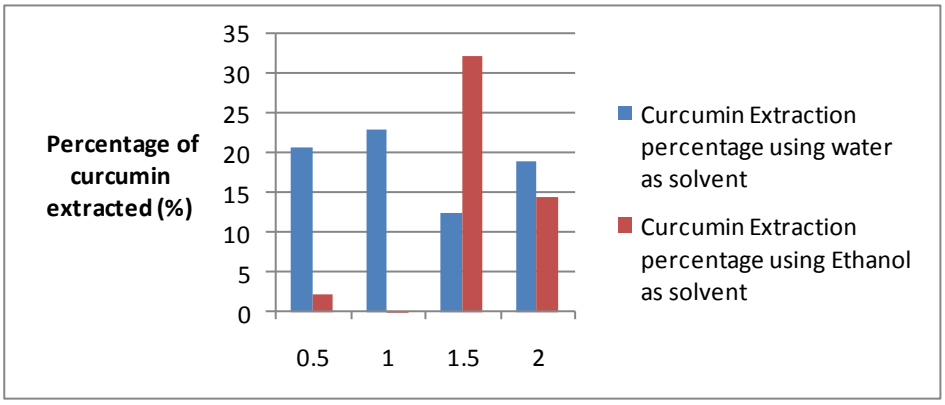
3.1.3.1Solvent extraction of turmeric using ethanol and water



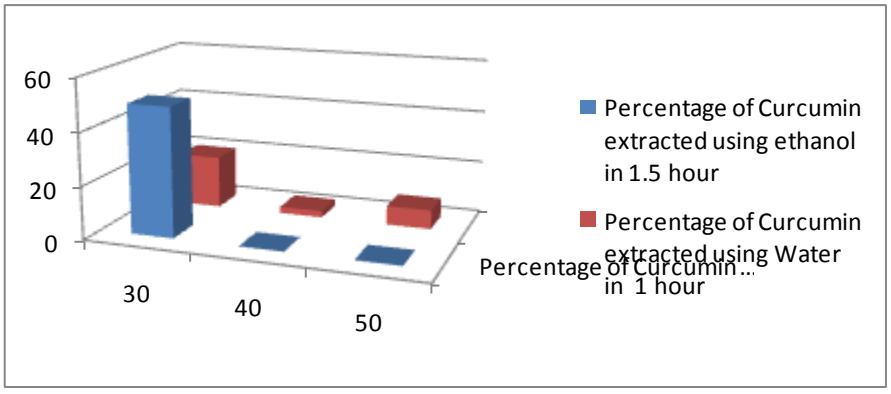
3.1.3.2 Solvent extraction of turmeric (250 µ size) using ethanol and water.



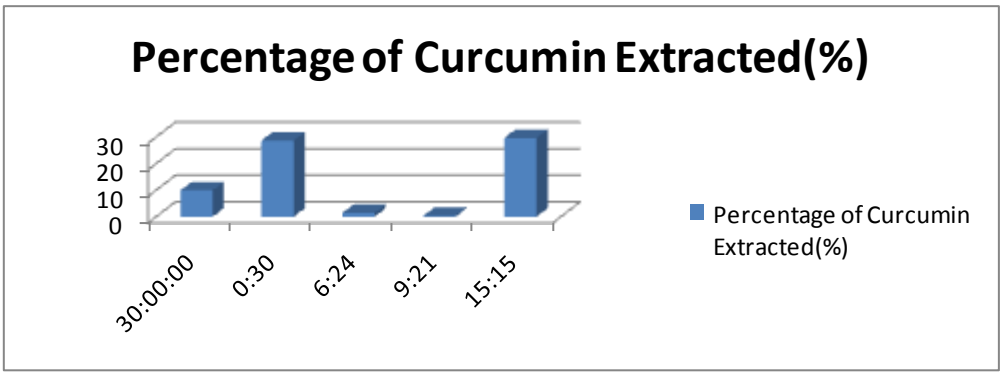
3.1.3.4 Optimization of solvent extraction of turmeric at different time intervals



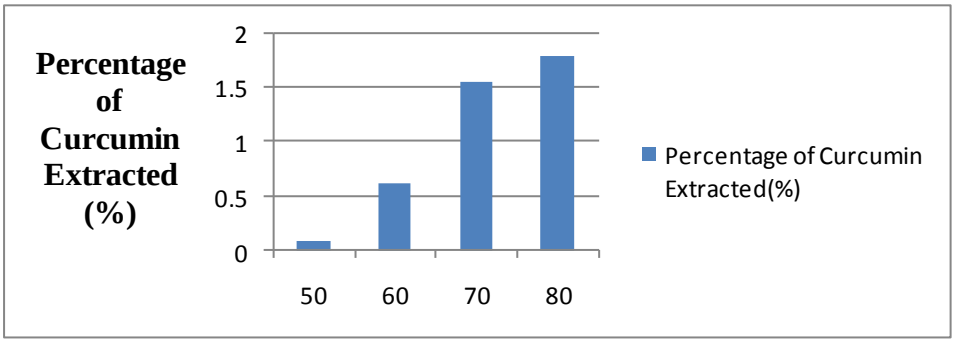
3.1.3.5 Optimization of solvent extraction method of turmeric.



3.1.3.6 Solvent extraction using mixture of solvent of ethanol and water



3.1.3.7 Solvent extraction of turmeric using pet ether



Effect of 4 independent variables — temperature (50–90°C), particle size (0.42–0.85 mm), mixing time (10–50 min) and solvent (ethanol) to meal ratio (10–50) on curcumin yield from turmeric (Curcuma longa L) was studied using central composite rotatable design. The experimental value of curcumin yield ranged between 4.49 and 12.89%. The maximum curcumin yield was obtained when temperature, particle size, mixing time and solvent to meal ratio were 60°C, 0.42 mm, 30 min and 50, respectively. (Sogi *et al.*, 2009)
In our experiment all the values of curcumin found were of crude content, so further purification is necessary in order to determine the exact value of curcumin determined.

3.2 Product Development of Cake using Curcumin.
3.2.1 Materials and Equipments.

Materials used:-Flour, Sugar, Fat, Eggs, Baking Powder, and Vanilla Essence.
Apparatus Used: - Mixing Bowls, Baking Oven, Gas Oven.
Sample Used: -Curcumin in soluble complex Water: Ethanol ratio.

3.2.2 Method.
Trial 1: Production of Cake using 120 gm sugar and 5 ml Curcumin Sample.

All the ingredients required to be made for production of cake are measured accordingly with respect to 150 gm flour. Then two eggs are whipped to a frothy texture, while 120 gm of fat including equal amount of Amul Butter and Vanaspati is melted in a bowl on gas oven. Measured amount of 120 gm of sugar is comminuted in a mixer grinder and then added to the melted fat in the bowl and is constantly mixed until a white creamy and smooth texture is obtained. After obtaining the creamy texture the whipped eggs mixture is added to it with continuous stirring. Initially the baking powder is mixed with the flour and sequentially the flour is added slowly to it with continuous stirring. When all the ingredients are mixed few drops of vanilla essence is added along with 5 ml of curcumin solution. The batter was poured into a rectangular baking pan with butter paper on all its sides. The baking oven was preheated to 186 ºC and the cake was inserted to bake for 45 minutes until the tops are golden and a toothpick poked into the center of the layer comes out clean. The cake was then kept outside to cool and was then analyzed.
Observation: Less Sweet, light yellow crumb color, Taste of Curcumin was not recognizable; Texture was crumbly with a crack on the surface.
Recommendation: Curcumin amount should be increased, with increase in the amount of sugar.

Trial 2: Production of Cake using 125 gm sugar and 10 ml Curcumin Sample.

All the ingredients required to be made for production of cake are measured accordingly with respect to 150 gm flour. Then two eggs are whipped to a frothy texture, while 125 gm of fat including equal amount of Amul Butter and Vanaspati is melted in a bowl on gas oven. Measured amount of 120 gm of sugar is comminuted in a mixer grinder and then added to the melted fat in the bowl and is constantly mixed until a white creamy and smooth texture is obtained. After obtaining the creamy texture the whipped eggs mixture is added to it with continuous stirring. Initially the baking powder is mixed with the flour and sequentially the flour is added slowly to it with continuous stirring. When all the ingredients are mixed few drops of vanilla essence is added along with 10ml of curcumin solution. The batter was poured into a rectangular baking pan with butter paper on all its sides. The baking oven was preheated to 186 ºC and the cake was inserted to bake for 45 minutes until the tops are golden and a toothpick poked into the center of the layer comes out clean. The cake was then kept outside to cool and was then analyzed.
Observation: Moderately sweet in taste, with no recognizable taste of curcumin. Crumb color was moderately yellow, crumbly texture with crack on the surface.

Recommendation: Decreasing the amount of sugar in order to produce a diabetic cake but with slowly increasing the curcumin content in order to understand the maximum level of color which can be obtained.
Trial 3: Production of Cake using 120 gm sugar and 15 ml Curcumin Sample.

All the ingredients required to be made for production of cake are measured accordingly with respect to 150 gm flour. Then two eggs are whipped to a frothy texture, while 120 gm of fat including equal amount of Amul Butter and Vanaspati is melted in a bowl on gas oven. Measured amount of 120 gm of sugar is comminuted in a mixer grinder and then added to the melted fat in the bowl and is constantly mixed until a white creamy and smooth texture is obtained. After obtaining the creamy texture the whipped eggs mixture is added to it with continuous stirring. Initially the baking powder is mixed with the flour and sequentially the flour is added slowly to it with continuous stirring. When all the ingredients are mixed few drops of vanilla essence is added along with 15ml of curcumin solution. The batter was poured into a rectangular baking pan with butter paper on all its sides. The baking oven was preheated to 186 ºC and the cake was inserted to bake for 45 minutes until the tops are golden and a toothpick poked into the center of the layer comes out clean. The cake was then kept outside to cool and was then analyzed.
Observation: Crumbly texture with high yellow crumb color, Soft and fluffy in texture with a crack on the surface, moderately sweet in taste.
Recommendation: Decreasing the amount of curcumin while keeping the sugar contents same.

Trial 4: Production of Cake using 120 gm sugar and 12 ml Curcumin Sample.

All the ingredients required to be made for production of cake are measured accordingly with respect to 150 gm flour. Then two eggs are whipped to a frothy texture, while 120 gm of fat including equal amount of Amul Butter and Vanaspati is melted in a bowl on gas oven. Measured amount of 120 gm of sugar is comminuted in a mixer grinder and then added to the melted fat in the bowl and is constantly mixed until a white creamy and smooth texture is obtained. After obtaining the creamy texture the whipped eggs mixture is added to it with continuous stirring. Initially the baking powder is mixed with the flour and sequentially the flour is added slowly to it with continuous stirring. When all the ingredients are mixed few drops of vanilla essence is added along with 12ml of curcumin solution. The batter was poured into a rectangular baking pan with butter paper on all its sides. The baking oven was preheated to 186 ºC and the cake was inserted to bake for 45 minutes until the tops are golden and a toothpick poked into the center of the layer comes out clean. The cake was then kept outside to cool and was then analyzed.
Observation: The texture was crumbly with slightly less yellow crumb color, moderately sweet in taste with a crack on the surface.
Recommendation: Decreasing both the sugar and curcumin content so as to produce a diabetic cake with the properties of curcumin.

Trial 5: Production of Cake using 115 gm sugar and 12 ml Curcumin Sample.

All the ingredients required to be made for production of cake are measured accordingly with respect to 150 gm flour. Then two eggs are whipped to a frothy texture, while 115 gm of fat including equal amount of Amul Butter and Vanaspati is melted in a bowl on gas oven. Measured amount of 120 gm of sugar is comminuted in a mixer grinder and then added to the melted fat in the bowl and is constantly mixed until a white creamy and smooth texture is obtained. After obtaining the creamy texture the whipped eggs mixture is added to it with continuous stirring. Initially the baking powder is mixed with the flour and sequentially the flour is added slowly to it with continuous stirring. When all the ingredients are mixed few drops of vanilla essence is added along with 12ml of curcumin solution. The batter was poured into a rectangular baking pan with butter paper on all its sides. The baking oven was preheated to 186 ºC and the cake was inserted to bake for 45 minutes until the tops are golden and a toothpick poked into the center of the layer comes out clean. The cake was then kept outside to cool and was then analyzed.
Observation: Compact crumb texture with good browning in all sides, Crack on the upper surface of the cake with optimum sweetness and color of the cake.
Recommendation: Further analysis is to be done.

Trial 1
Production of Cake using 120 gm sugar Curcumin Sample.

Trial 2
Production of Cake using 125 gm sugar and 10 ml and 5 ml Curcumin Sample

Trial 3
Production of Cake using 120 gm sugar Curcumin Sample.

Trial 4
Production of Cake using 120 gm sugar and 12 ml and 15 ml Curcumin Sample.

Trial 5 Production of Cake using 115 gm sugar and 12 ml Curcumin Sample.

IV. Plant Profile

- Name of the product and its uses:

The name of the product is curcumin powder and it has variety of uses in medicinal, biological, pharmacological and food industry.

- Name and address of the entrepreneur with his or her background:

Anamika Bagchi, 69/10 M.C Garden Road East Sinthee DumDum Kolkata-30, Bachelor of Technology in Food Technology under West Bengal University of Technology.

- Location of the project with address:

Falta Phase I, Industrial Sector-II, South 24 Parganas, 50 kms away from Kolkata towards South-West direction, near Falta Special Economic Zone.

- Name of the Bankers with address:

Bank of India
111, C R Avenue, Raja Bhavan
Kolkata-73
State Bank of India
150, Chittaranjan Avenue,
Kolkata -73

- Type of Business:

Sole Proprietorship.

- Market potential:

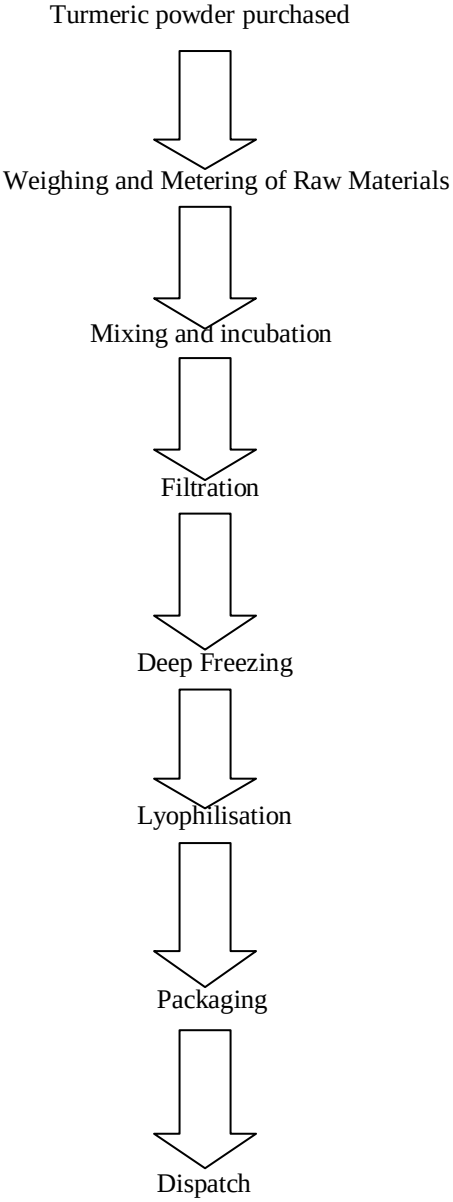
For centuries, Curcumin has been used as a food additive, medicinal agent, and in addition a cosmetic and fabric dye, without harboring known side effects. The record of safety has been one of the deciding factors that allowed the FAO/WHO expert committee on food additives to approve it as a natural food coloring substance. Curcumin is also listed by the U.S. FDA as an herb Generally Recognized As Safe (GRAS) for its intended use as a seasoning and flavoring agent. Curcumin's safety is further indicated since this natural substance is consumed in the food supply of populations in many developing regions in the world, and in the United States. In Asia this substance in crude mixtures, is consumed extensively in food. In its natural form in crude mixtures, it is also used as a treatment against inflammation, skin wounds and tumors. Curcumin has been

reported to reduce the size of tumors and pain of human oral cancer. Credible preliminary studies in animal models have demonstrated it is very effective in preventing tumors of the colon, breast, small intestine, mouth and skin. All of these biological effects of the natural molecule are also caused by the primary pharmacological actions of Curcumin as both an antioxidant and a broad anti-inflammatory substance with many anti-inflammatory properties. As an antioxidant, Curcumin scavenges active oxygen species such as hydroxyl radicals, superoxide anions, and singlet oxygen molecules that cause damage to cells; it also interferes with lipid per oxidation, xanthine oxidase activity and nitrate/nitrogen oxide production.

- Annual Production Target:

150 kg of curcumin per year.

- Process of Manufacture:



- Expense for land and building:
  The expense for land and building is around 50 Lakhs.
- Expense for Machine and Equipment with office furniture:
  The expense for machine and equipment with office furniture is 1, 08,460,00 rupees
- Expense for staff and labour per month:
  The expense is 1,26,000 rupees permonth.
- Expense for raw materials per month:
  The expense is 5,80,736 rupees per month.
- Expenses for Utilities per month:
  The expense is 30,000 rupees per month.

- Other Expenses:
  The other expenses with rents, postages, telephones, stationary, consumable expenses amount to rupees 10,000 per month.
- Future and Plant Expansion:
  Some of the area is left for future expansion if required.
- List of machine and equipment supplier.

SS GAS LAB ASIA PVT LTD,Vega Conveyors & Automation Ltd.,Blenzor (India)

- List of Raw material supplier

Some of the name of Raw material suppliers of Turmeric powder is:-
Satikuwar Exports PVT LTD, Airan Tradecom Pvt Ltd.,.Accord Trade.Some of the name of Raw material suppliers of solvents used are:-International Biological laboratories, Harshit Exports.

V. Price List

Raw Materials and Manpower required
Raw Materials: 1) Turmeric powder.
2) Petroleum Ether Solvent.
Raw Materials Cost:

Serial No.	Name of Raw materials	Amount Required in one month	Price/month(Rupees)	Annual price(in rupees)
1	Turmeric powder	560 kg	56,000	6,72,000
2	Petroleum ether Solvent	2235 L	5,31,036	63,72,432
			Total=5,870,36	Total=70,444,32

Manpower:
Employee Salary:

Serial no.	Employee Designation	No. of employees	Salary per person(in rupees)	Total Salary per month (in rupees)
1	Manager	1	15,000	15,000
2	Skilled Labour	4	7,000	28,000
3	Unskilled Labour	8	5,000	40,000
4	Store Keeper	6	3,000	18,000
5	Reception	1	4,000	4000
6	Peon	2	3500	7000
7	Laboratory Technician	2	7,000	14,000
				1,26,000

Equipment Cost:
A Plant Instruments:

Serial No.	Name of Equipment	Number of Equipment	Cost per Unit(Rupees)	Total Cost =No. of Equipments x Cost per unit
1	Weighing & Metering Machine	1	20,000	20,000
2	Mixing Tank	1	200,000	200,000
3	Holding Tank	1	50,000	50,000
4	Press Filter	1	450,000	450,000
5	Evaporator	1	8,00,000	8,00,000
6	Deep freezer	1	20,00,000	20,00,000
7	Lyophilizer	1	20,00,000	20,00,000
8	Packaging Unit	1	50,00,000	50,00,000
	Total	8	10,500,000	10,500,000

B. Laboratory Equipments:

Serial No.	Name of Equipment	Number of Equipment	Cost per Unit(Rupees)	Total Cost =No. of Equipments x Cost per unit
1	Balance	1	1,50,000	1,50,000
2	Spectrophotometer	1	1,50,000	1,50,000
3	Water Bath	1	10,000	10,000
4	Hot air Oven	1	6,000	6,000
	Total	4	3,16,000	3,16,000

VI. Cost Analysis

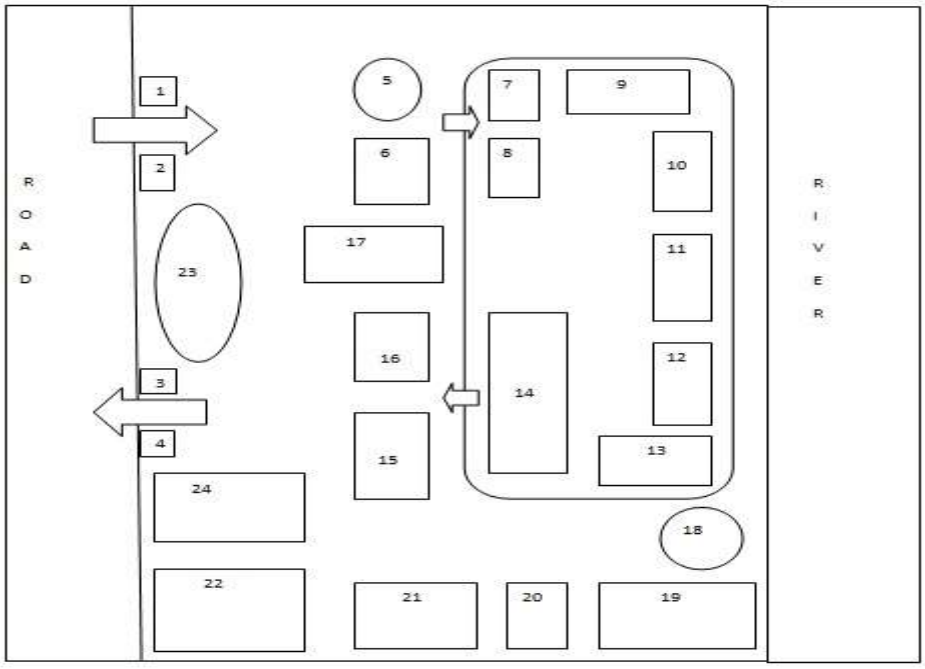
Cost calculation is the way to determine the investment to be done for making and selling a product on providing a service. Cost analysis benefits us in many ways:-

(a) Costing helps to set prices.
(b) Costing helps to set control and reduce prices.
(c) Costing helps plan for the future.
(d) Costing helps making a better decision.
(e) Costing helps to get credit from financial institution and marked credit.

Thus cost calculation is done in following ways:-

1) Fixed Capital (A) = 83, 876, 00 Rupees
2) Recurring Capital (B) = 18, 506, 36 Rupees.
3) Total Capital = A+ 3B= 1.3939508 Crore Rupees.
4) Cost of production / annum (D) = 12 x B+ Depreciation Cost
= 2.3295232 crore Rupees.
5) Turn Over/Year (E) = Total Sales X Rate= 2.4 crore Rupees.
6) Profit/year (F) = E- D= 7, 047, 68 Rupees.
7) Profit percentage (G) = (F/D) X 100= 3.025 %
8) Return on Investment / year (H) = (F/A) X 100= 8.40
9) Fixed Expense (I) = 713036 Rupees.
10) Break even percentage (J) = [I/ (I +H)] X 100 = 99.
11) No. of payback period in year = J/G= 33.05

VII. Plant layout.



1. Guard Room.
2. Guard Room.
3. Guard Room.

4. Guard Room.
5. Solvent Tank storage.
6. Raw Material Storage.
7. Weighing Section.
8. Metering Section.
9. Mixing Tank.
10. Filtration Unit.
11. Evaporator.
12. Deep freezing unit.
13. Lyophilizing unit.
14. Packaging Unit.
15. Storage Section.
16. Dispatch Section.
17. Reception.
18. Boiler.
19. Water Treatment Plant.
20. Toilet.
21. Effluent Treatment plant.
22. Refreshment Area.
23. Garden.
24. Parking area.

VIII. Future Scope

Curcumin has a variety of uses, so after recognizing the effective parameter for its extraction, its purification is to be highly emphasized upon. Freeze drying of the product and then consequently determining its antioxidative and antimicrobial properties will be the main work. There by, optimization of the properties will be of relatively high importance. Since its usage in product development has been found out, various other product developments may be done.

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