

Media Optimization in immobilized culture to enhance the content of Curcumin in *Curcuma longa* (Zingiberaceae) L and Protein profile of treated samples in static culture

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Scientific Classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Monocots
(unranked):	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	*Curcuma*
Species:	***C. longa***
Binomial name	
Curcuma longa	



Turmeric : The Miracle Stem

- Reduce side effect of chemotherapy
- Anti-arthrites
- Skin Tonic
- Speed up wound healing
- Natural Analgesic
- Natural Anti-Inflammatory
- Lower Cholesterol
- Prevent many cancers
- Improves asthma
- Improves digestion
- Aids in Fat Metabolism

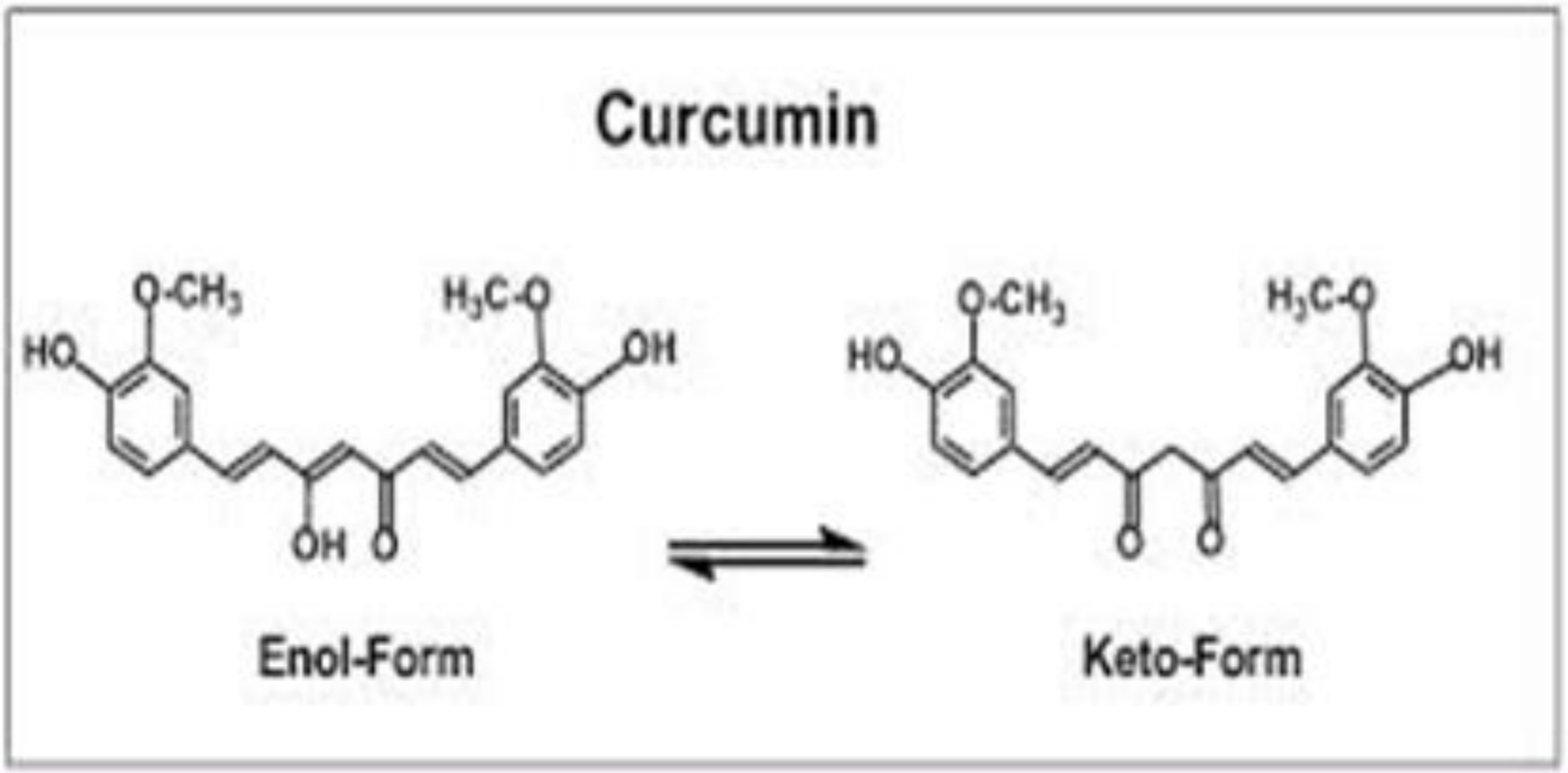
Phyto-constituents of Turmeric

- The main components of turmeric are Curcuminoids and essential oil.

- Natural curcumin, from *Curcuma longa*, contains …
  1. Curcumin I (diferuloylmethane 4-8%)
  2. Curcumin II (6%)
  3. Curcumin III (0.3%)

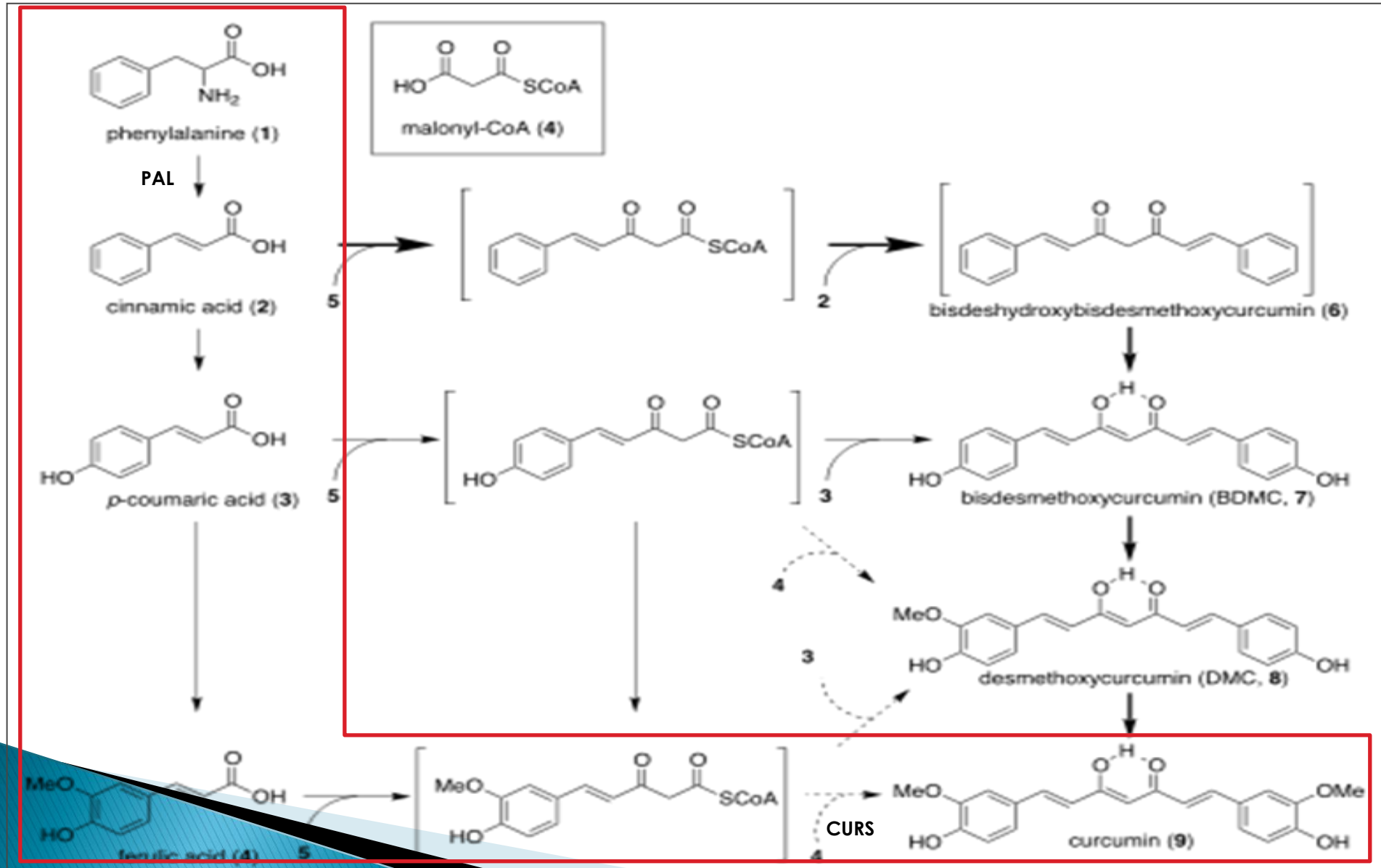
Other components

- Moisture (>9%),
- Extraneous matter (<0.5% by weight),
- Volatile oils (<3.5%).
  d-a-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes .
- Aroma of turmeric due to turmerone, arturmerone, and zingiberene.

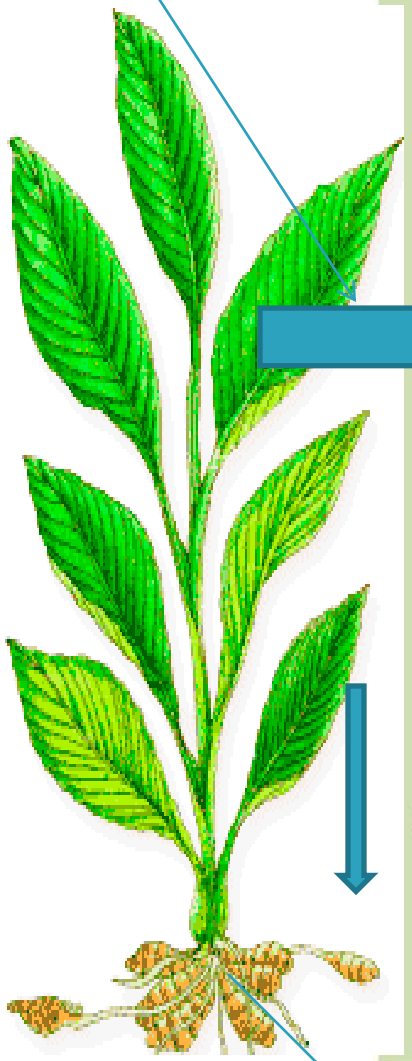


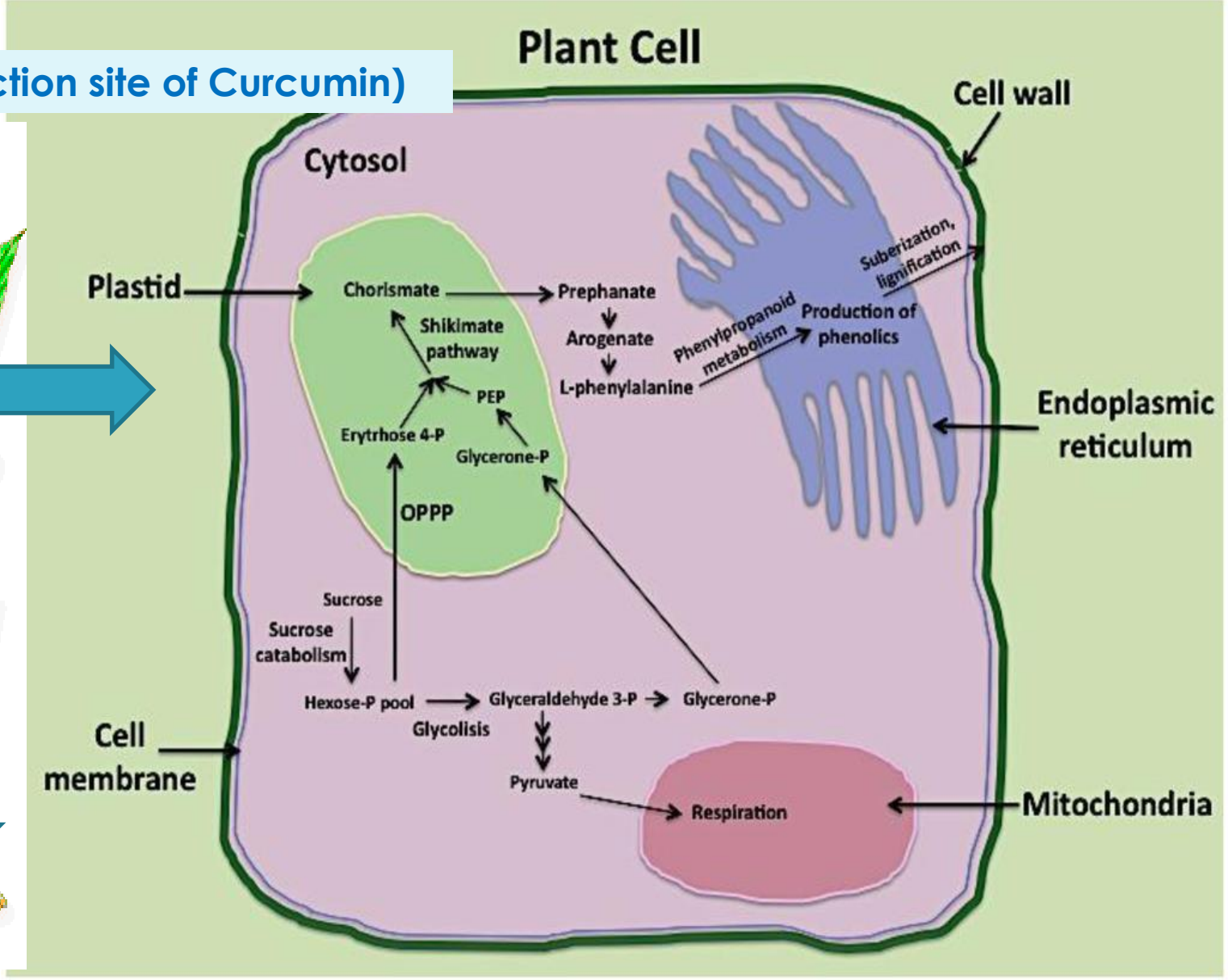
Tautomeric forms of Curcumin

Phenyl propanoid biosynthetic pathway of Curcumin



Leaves (production site of Curcumin)





Rhizome(Accumulation site of Curcumin)

Curcumin Sources



Curcuma aromatica
(Wild turmeric, Vanarisrita, Jangali Haldi, Aranyaharidra)



Curcuma longa
(Haldi, Turmeric, Ukon, Woolgum, Kunyit, Oendre, Rame, Temu kuning, Temu kunyit, Goeratji, Kakoenji, Koenjet, Kondin, Tius, Kunir, Gianghuang)

3-8 %



Curcuma Phaeocaulis
(Ezhu, Zedoary rhizome, Gajutsu)

3 %



Curcuma zedoaria
(White turmeric, Zedoary root)

0.1%



Etlingera elatior
(Torch ginger, eka, dgushi, pua vao)

1-2 %



Curcuma mangga
(Mango ginger)



Curcuma xanthorrhiza
(Temu Lawak, Ubat Jamu, Ubat maaju)

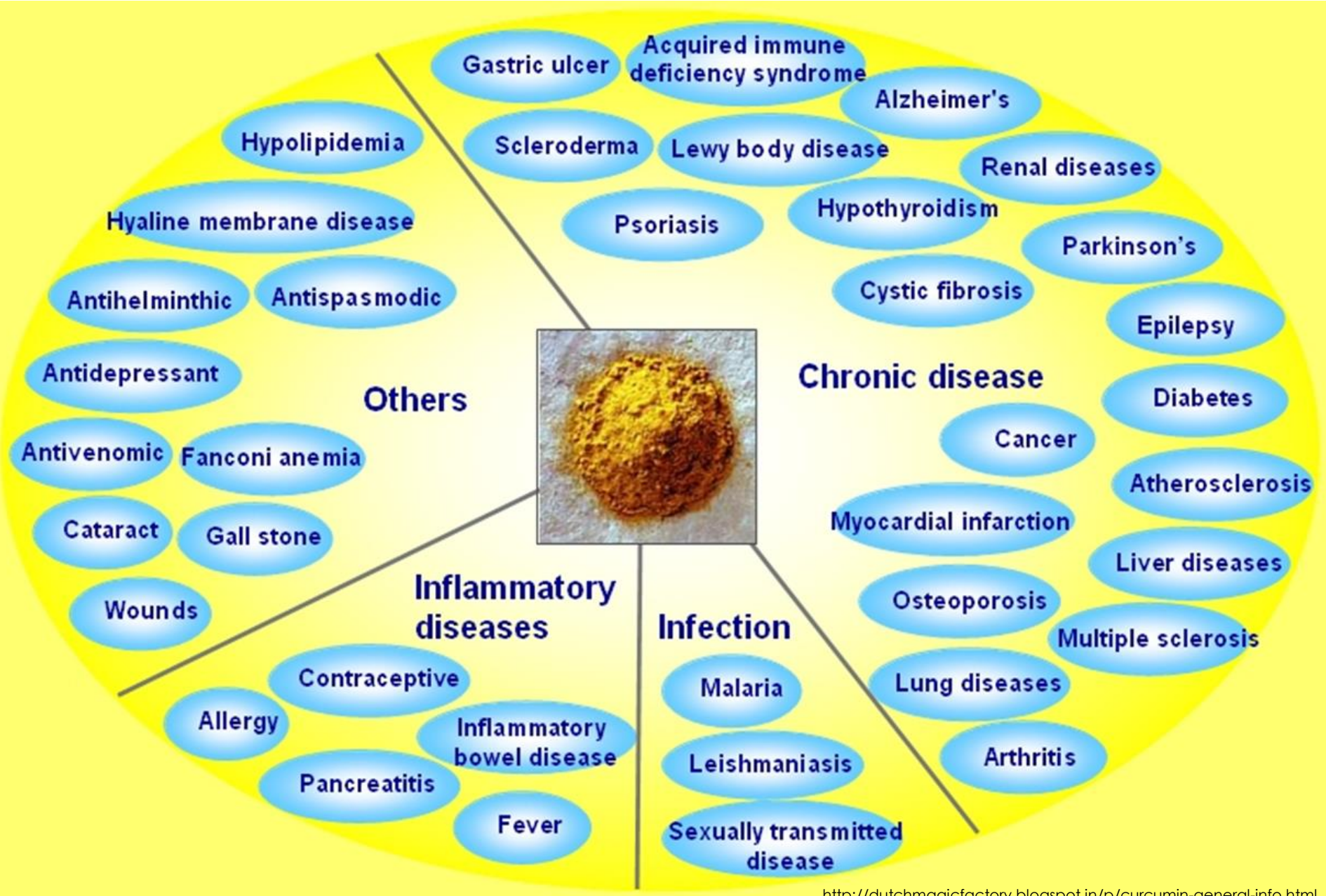


Costus speciosus
(Cane Reed, Crepe ginger, Wild ginger, Keokand)



Zingiber cassumunar
(Cassumunar ginger)

Medicinal use of Curcumin



http://dutchmagicfactory.blogspot.in/p/curcumin-general-info.html

Advantage of Plant Tissue Culture for sec metabolites production

- Provide efficient sytem for study biosynthesis, productivity, biotransformation, regulation of metabolites synthesis.
- It is independent of seasonal and geographical variation.
- Continuous, reliable source of plant pharmaceuticals and could be used for the large-scale culture of plant cells for sec.metab production.(due to Totipotent nature)*
- In the present study Dual strategy (Zenk 1975)*was used to enhance Curcumin in *Curcuma longa* tissue culture (Static and Immobilized).
- Two different range of Tyrosin and cinnamic acid concentrations were selected for curcumin enhancement in *Curcuma longa.*

Objective

- **To develop a novel economical potential method for enhancement the Curcumin content *in vitro* tissue culture (static and immobilize culture)of *Curcuma longa* (Zingiberaceae).**

- **To evaluate the protein profile of treated samples *in vitro***

Material and Methods

Plant material of *Curcuma longa* rhizome was collected from local market Mumbai.(Latitude: 18°96′50″N Longitude: 72°82′53″E) in the month of March .The identification was done by Botanist ,Haffkine Institute and the voucher specimen of *C.longa* preserved for reference(ZNS/HITRT/2014/02)

Static Culture

1. • Identified rhizome was washed with teepol and surface sterilized with 0.1%HgCl2 for three min., washed with 5 times with sterile water ,cut pieces of 1cm long and inoculated in the sterilized media aseptically.

2. • The inoculation was done into autoclaved Zenk Production media supplemented with different concentration of Tyrosin and cinnamic acid separately.

3. • The cultures were incubated in 16 hr of photoperiod and 8 hr of dark period with 70% humidity in 1000 lux light. The cultures were harvested separately at the time duration of 1 week.

Extraction and quantification for Curcumin

1.
   - The cold extraction in ethanol ,dried ,weighed and subjected for HPTLC with Standard Curcumin (Sigma Alderich)
   - (Rf value 0.52).

2.
   - Inert gas as spray gas and Chloroform :Methanol(9:1) was used as mobile phase

Immobilized Culture of *Curcuma longa*

Continue…

1. • Calcium Alaginate beads were formed.

2. • Calcium alginate entrapped cells (300 beads) were kept in Zenk suspension media supplemented with 40mg/100ml, 60mg/100ml of Tyrosine and 5mg/100ml, 7.5mg/100ml of cinnamic acid were kept in 90 rpm and 25± 20^{o}c for one week duration.

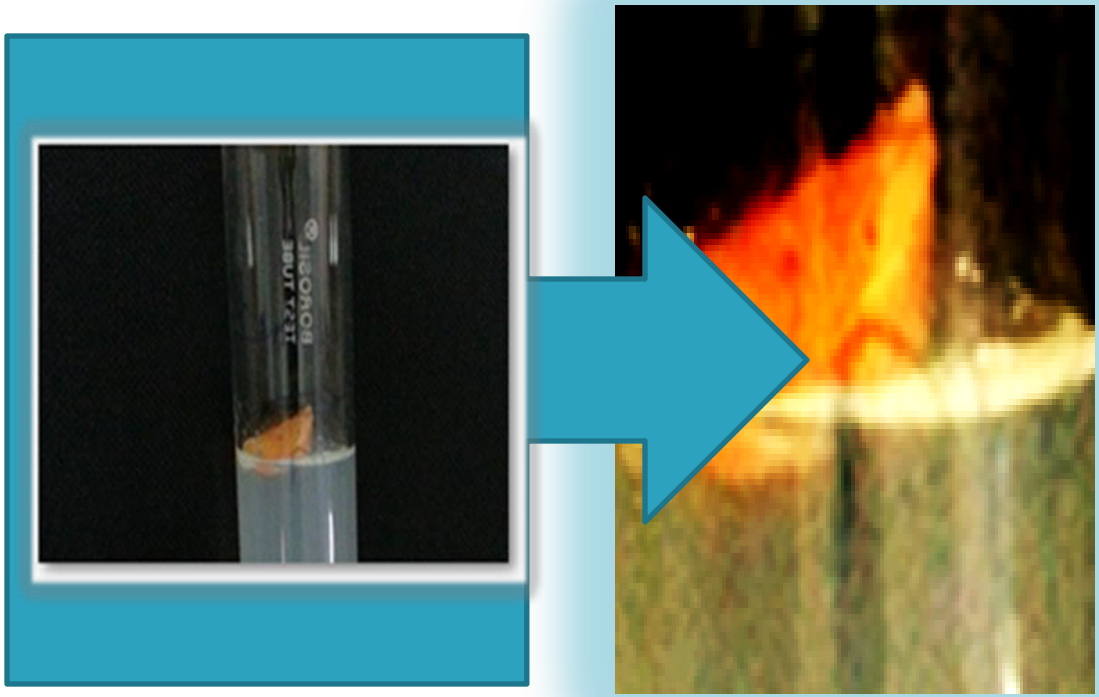
3. • One week old samples were harvested, extracted in ethanol and centrifuged .The supernatant of centrifuged extracts were dried, weighed and subjected to HPTLC for qualitative as also quantitative estimation of Curcumin content.

Protein profiling

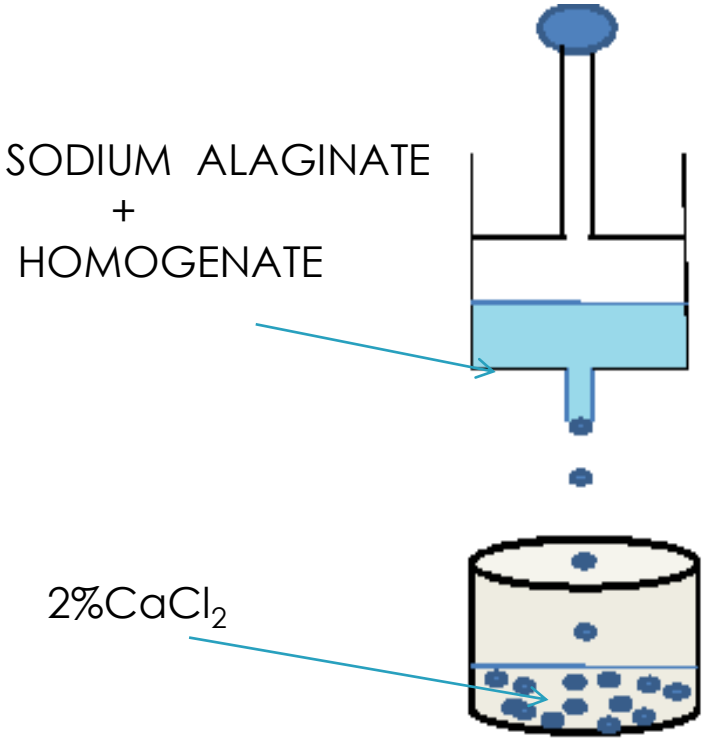
1. .Protein of treated samples of one week age were extracted by using kit method purchased from Pierce.

2. Protein profiling of extracted protein was done by using 1D SDS PAGE

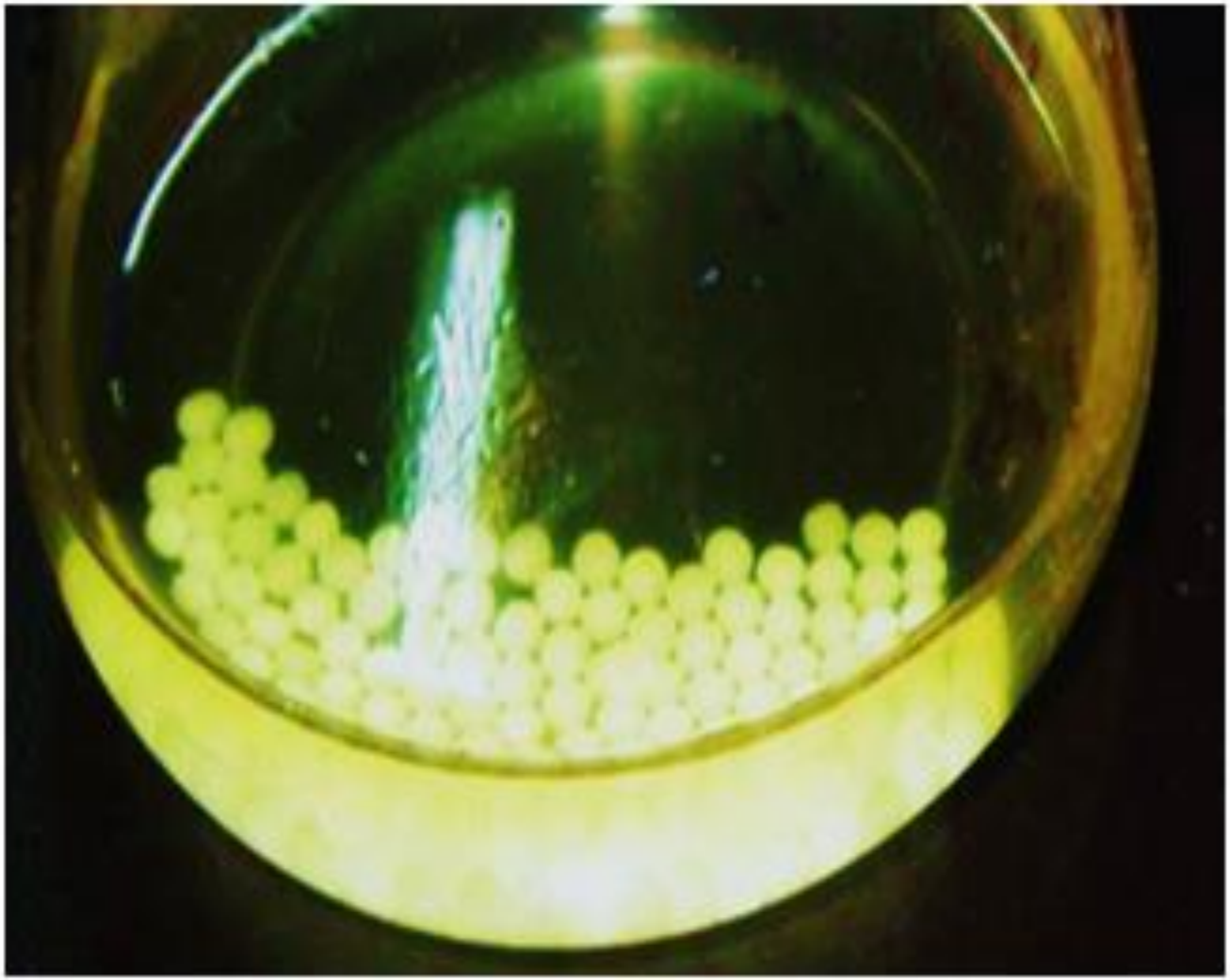
Static culture of *Curcuma longa*



Preparation of beads



Immobilized culture of *C.longa*



Results

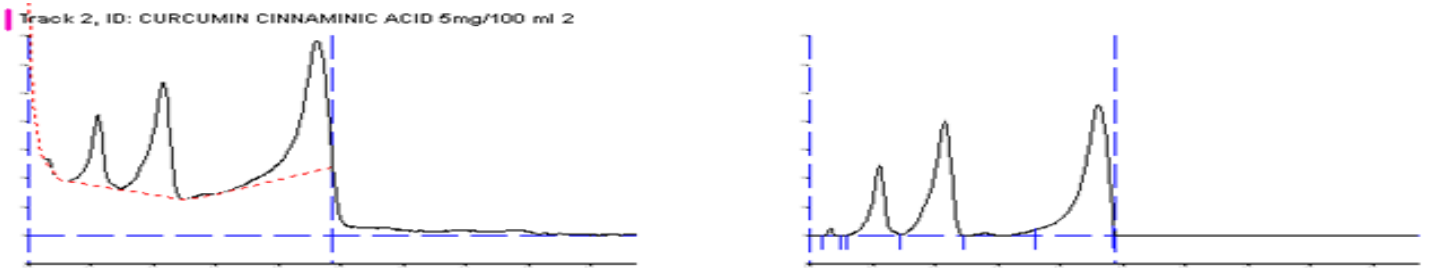
1.Seven fold enhancements in Curcumin content was observed in one-week old static culture, which was maintained on Zenk production media incorporated with 5 mg/100ml of Cinnamic acid (Control 1.57% and induced 8.717%).

2.In immobilized culture studies, Curcumin content was higher in one-week old (2.03%/ 300 beads) immobilized suspension culture supplemented with Zenk basal media and 5 mg/100 ml of cinnamic acid .

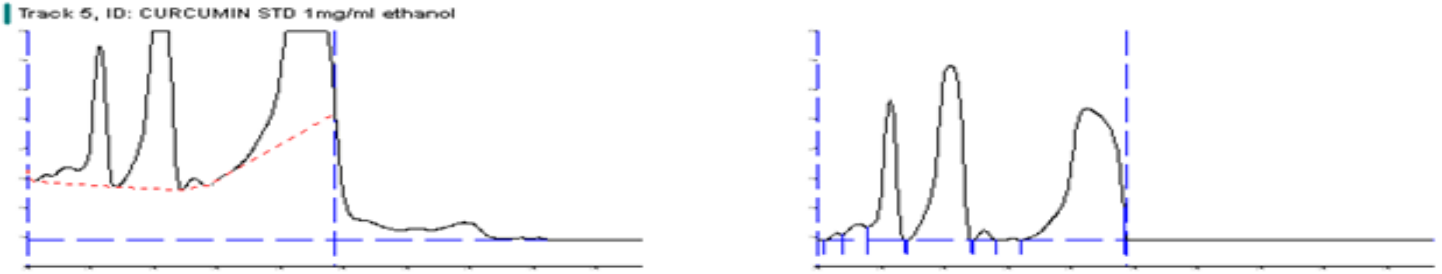
3.In Protein profile studies, all the treated samples were analysed for their proteomic profile using 1D SDS PAGE and it was observed that protein of 23,420 D was most prominent in all samples .

4.Maximum intensity is expressed in tissue grown in Tyrosine 40 mg/100ml (Relative intensity of possible Glycine rich protein: 65.53% w.r.t control)

HPTLC Chromatogram of immobilize culture at 525 nm with Standard of Curcumin

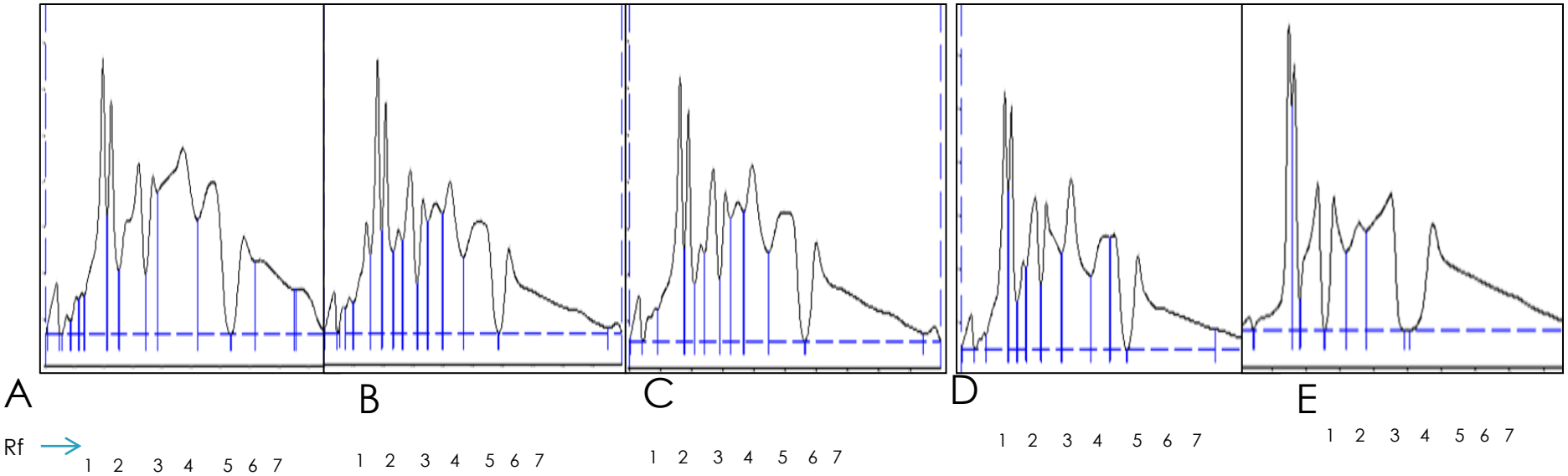


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.0	0.06	11.8	2.08	0.07	0.0	75.0	0.44	unknown *
2	0.08	1.2	0.14	123.9	21.93	0.17	2.1	2428.3	14.19	unknown *
3	0.17	2.1	0.24	200.0	35.42	0.27	0.2	5737.3	33.53	unknown *
4	0.39	11.0	0.49	229.1	40.57	0.51	15.6	8868.4	51.83	unknown *



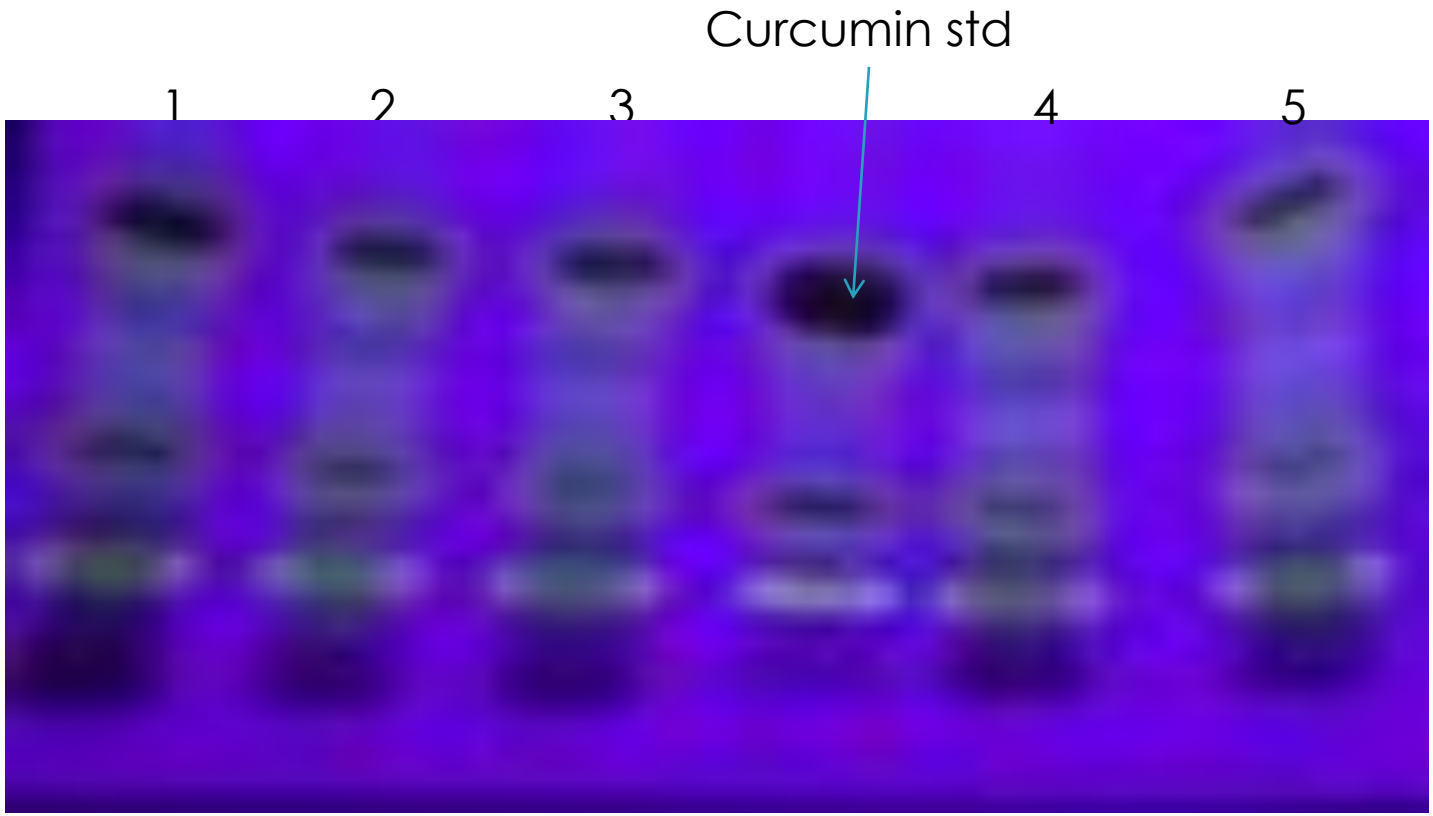
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.2	0.05	24.5	1.51	0.06	20.0	347.6	0.54	unknown *
2	0.06	20.1	0.09	55.7	3.43	0.10	45.8	1464.2	2.28	unknown *
3	0.10	46.3	0.14	471.5	29.01	0.16	0.4	9419.5	14.76	unknown *
4	0.17	0.1	0.23	593.2	36.50	0.27	0.0	21868.0	34.25	unknown *
5	0.27	0.5	0.29	33.7	2.07	0.31	0.5	534.1	0.84	unknown *
6	0.35	2.7	0.45	446.4	27.47	0.51	109.1	30215.8	47.33	unknown *

HPTLC chromatogram of bio enhancement of Curcumin in *Curcuma longa* static culture A-1Wk old treated with Cinnaamic acid 5 MG/100ML,B-1Wk old treated with Cinnamic acid 7.5 MG/100MC-1Wk old treated with Tyrosin 40 MG/100ML, Std,D-1Wk old treated with Tyrosin 60 MG/100ML,E STD. Curcumin shows at 525nm



HPTLC Fingerprinting of ethanolic extracts of induced samples of *Curcuma longa* static culturewith standard compound of Curcumin at 525 nm (1-5)

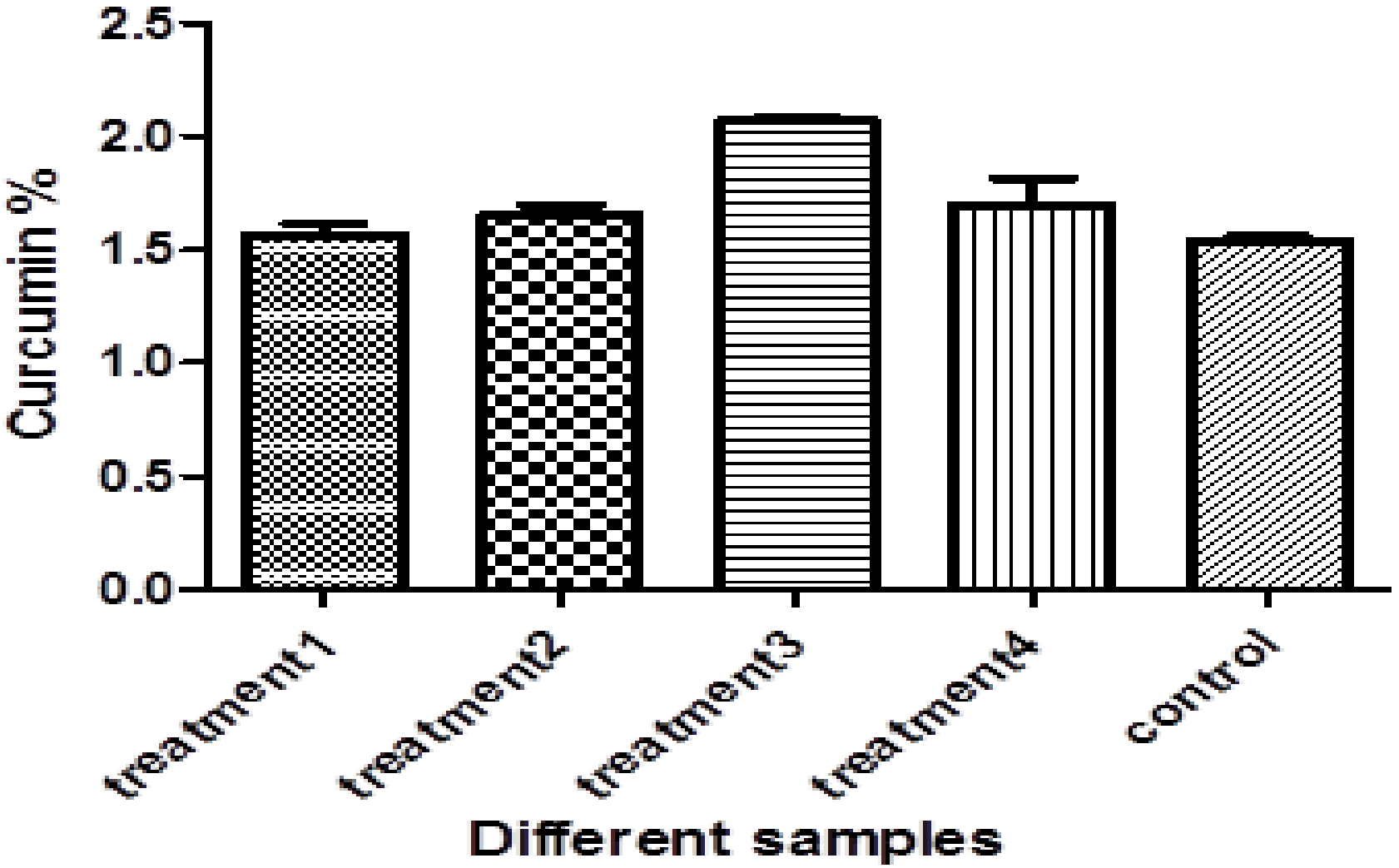
1-5mg/100ml cinnamicacid; 2-7.5mg/100ml; 3-40mg/100ml; std; 4-40mg/100ml; 5-6omg/100ml

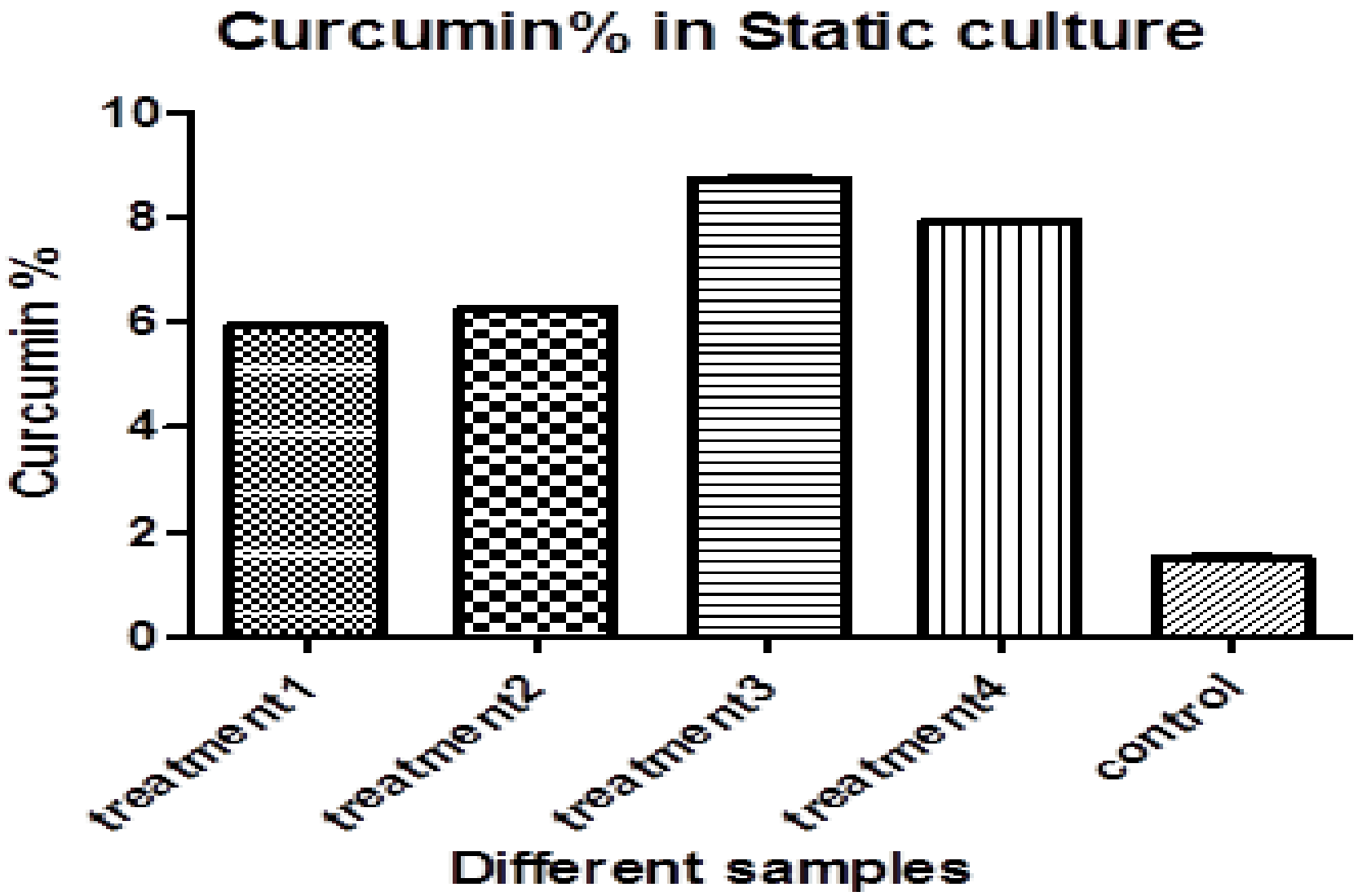


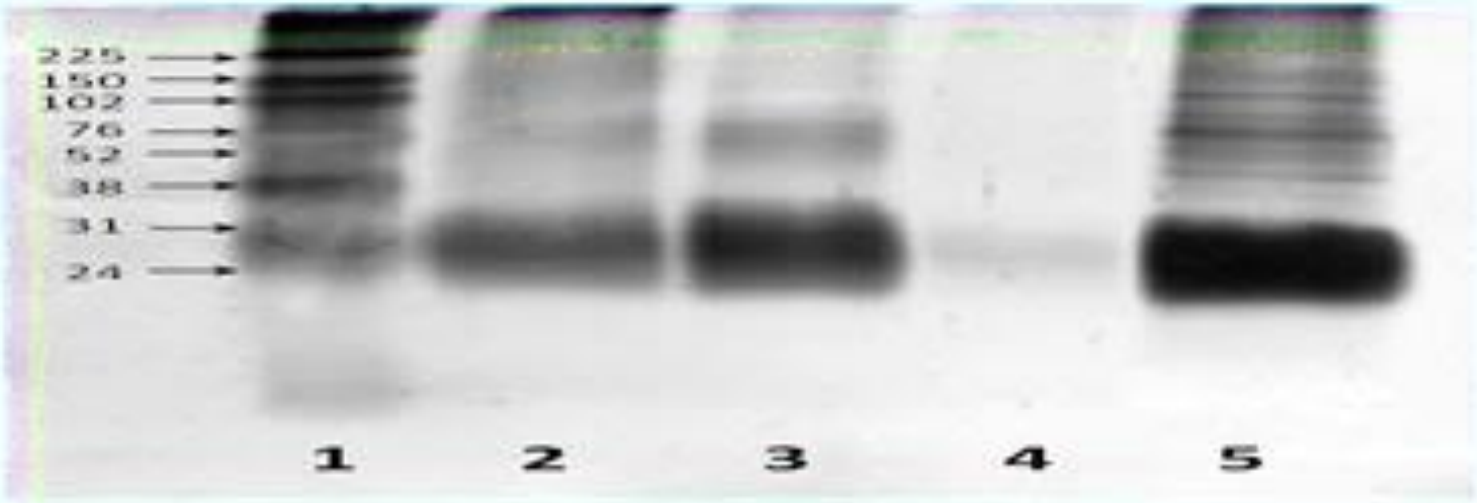
S.no.	Media content	Curcumin content% of Curcuma longa/300 beads in immobilize culture	Curcumin content% of Curcuma longa in static culture FW
		One week	One week
1.(treatment1)	Tyrosine 40mg/100ml	1.4±0.081	5.9±0.030
2.(treatment2)	Tyrosine 60mg/100ml	1.66±0.0780	6.22±0.036
3.(treatment3)	Cinnamic acid5mg/100ml	2.03±0.03	8.71±0.035
4(treatment4)	Cinnamic acid 7.5 mg/100ml	1.7±0.2	7.91±0.030
5.	Control	1.5±0.035	

.Effect of different compounds on Curcumin production in immobilized culture and static culture of *Curcuma longa*± Mean S.D.(Annova test) of three replicates statistically significant $P < 0.05$FW-Fresh weight

Curcumin% in Immobilized culture




Curcumin% in Static culture
Curcumin %
10
8
6
4
2
0
treatment1
treatment2
treatment3
treatment4
control
Different samples



Figour showing the protein profile of induce samples 2,3,4(Tyrosine60mg/100ml,Tyrosine 40mg/100ml,Cinnamic acid 7.5mg/100ml)with ladder 1 and 5 control. Figure showing the expression of 24 KD band in all the instances which is most prominent in Curcuma longa tissue fed with Tyrosine 40 mg/100ml of Zenk media in static culture. However the tissue fed with 5mg/100ml of Cinnamic acid in Zenk media did not express any band.

Sr. No.	Media used	Relative Band Intensity
1.	Tyrosine 60 mg/100ml	41.79%
2.	Tyrosine 40 mg/100ml	65.53%
3.	Cinnamic acid 7.5 mg/100ml	1.64%
4.	Control	100.00%

Showing the band intensity (%) of possibly Glycine Rich Protein in induced samples of *Curcuma longa* tissue culture.

Sno	Description	Expenditure in normal practice (USD) 6 months	Expenditure in present model (USD) one wk
1.	Light	700	30
2.	Chemicals	300	15
3.	Man Power	200	9
4.	Miscellaneous	140	55
5.	Total	1340	105
Saving(USD)	1235/-(92%)		

Comparison of expenditure between normal model and present static culture model

Discussion

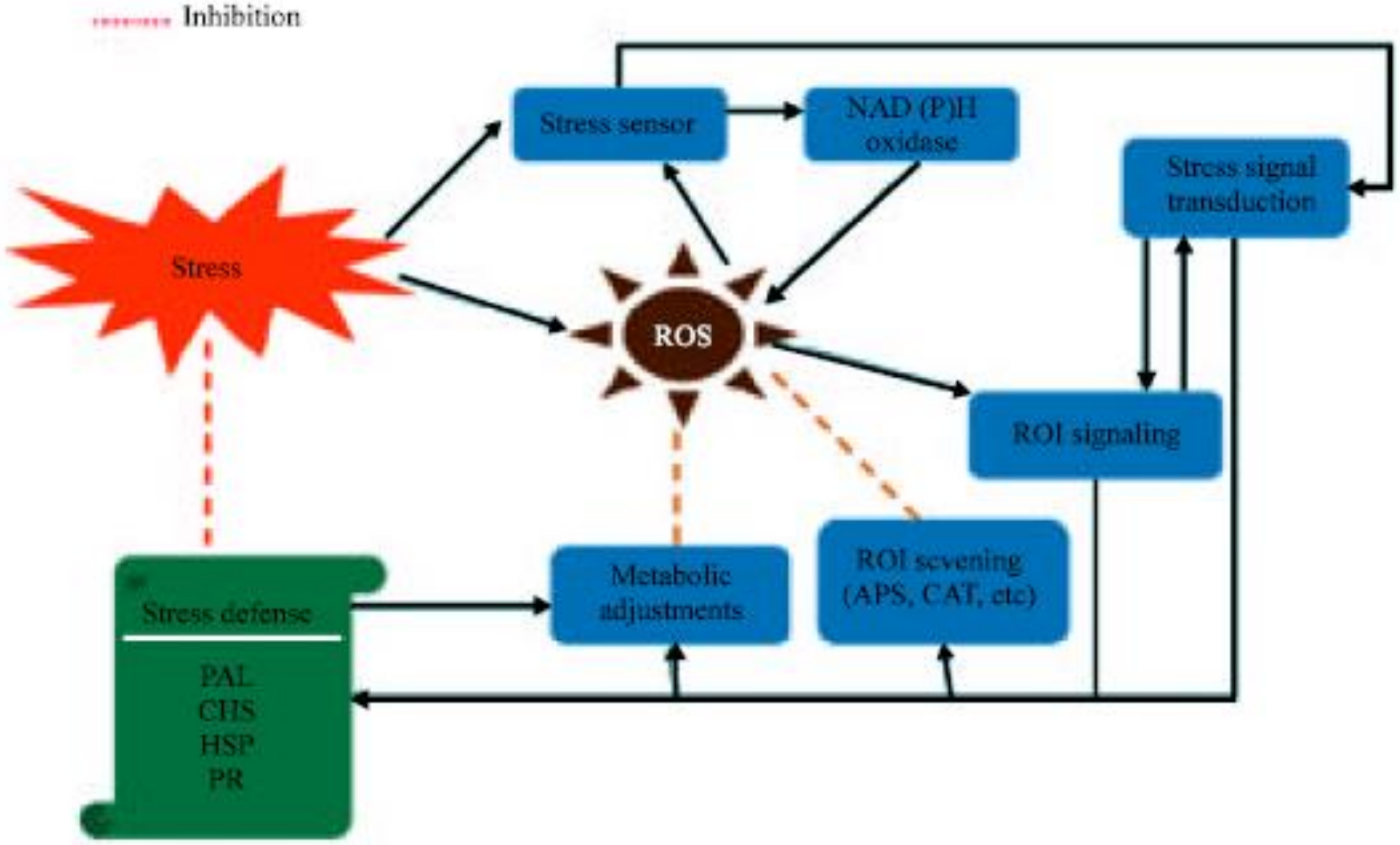
- Cinnamic acid and Tyrosine compounds are intermediate compounds in biosynthetic pathway of Curcumin. Secondary metabolites in cell culture have been enhanced by many scientists by following this strategy .

- The expression of protein (based on intensity of the bands) was reduced in treated samples as compared to control; this may be due to the formation of Reactive Oxygen species (ROS) in culture condition and which modified the protein.(pKa value of cinnamic acid :4.44;Tyrosin : 10)

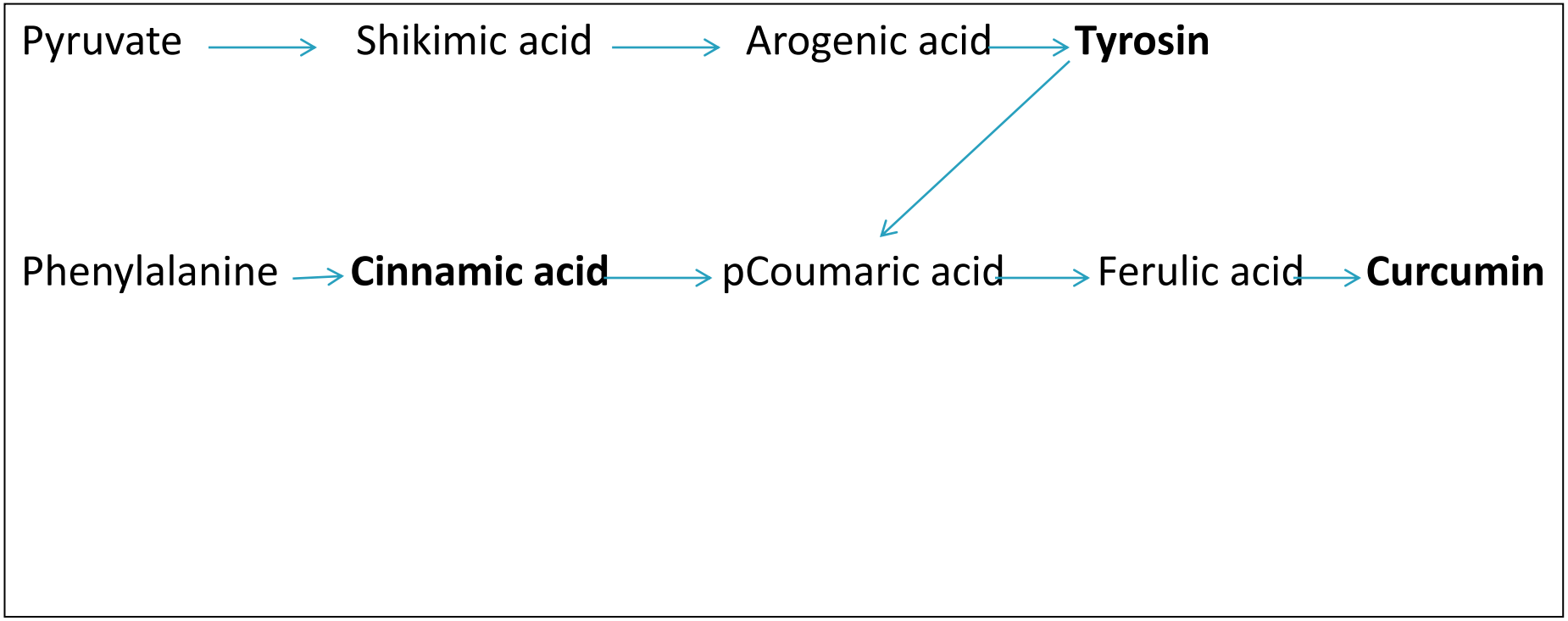
- Increased level of sucrose than MS media (5%) has been added to *Curcuma longa* culture media, that is known inducer of oxidative stress (abiotic stress) and eventually the Curcumin production is increased.

Accumulation of compounds depends upon…

- Amplitude of ΔpH
- Permeability co-efficient at the tonoplast
- pK_a value
- Binding site in vacuole(Storage site)
- Specific carrier
- Development of Organ, Tissue and Cell
- Size of vacuole



Mechanisms of ROS production and scavenging during biotic or abiotic stresses. phenylalanine ammonia-lyaze (PAL), chalcone synthase (CHS); nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H oxidase) are some of the important stress related genes



Proposed Biosynthetic pathway of Curcumin

Significance out come

1.Static culture is better source than immobilized culture for Curcumin production *in vitro* tissue culture of *Curcuma longa*.

2.Zenk media incorporated with 5mg/100ml of cinnamic acid with 5% sucrose is the best media for Curcumin production(Abiotic stress).

3.Created ROS *in vitro* condition can reduce the protein profile in treated samples. Glycine rich protein may have role in Curcumin production in tissue culture.

4.Study made easy to understand Curcumin Biosynthetic pathway.

5.The present study deals with the enhancement of Curcumin using directly plant tissue rather than callus which saved time and expenditure.

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You Must Be The Change You Want To See In The World"

- Mahatma Gandhi



The Mahatma accompanied by Sardar Patel and Sir Sahib Singh Sokhey at Haffkine Institute in 1935

Alleviating Human Suffering Through Science and Thereby Serving the Welfare of the People



THANK YOU FOR YOUR ATTENTION