A

Dissertation Report

On

ENZYMETIC DEGRADATION OF TURMERIC RHIZOME FOR THE EXTRACTION OF ESSENTIAL OIL

Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF SCIENCE
IN
CHEMISTRY

Submitted by

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(301602015)

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PATIALA-147004 (INDIA)

(June 2018)

Certificate

This is to certify that the dissertation entitled, **"ENZYMATIC DEGRADATION OF TURMERIC RHIZOME FOR THE EXTRACTION OF ESSENTIAL OIL"** being submitted by Mr. Dharminder in partial fulfillment for the requirements for the award of degree of Masters of Science in chemistry to the School of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala is a bonafide work carried by him under my supervision. The contents of this dissertation have not been submitted for the award of any other degree or diploma.

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Acknowledgment

My years of postgraduate study are finally nearing completion. This opportune moment to acknowledge all who have crossed my path during the journey and contributed directly or indirectly to reaching the light at the end of the tunnel.

Undoubtedly my supervisor Dr. Avinash Chandra (Department of Chemical Engineering) deserve to be at the top of the list. It was an excellent opportunity to work under the tutelage of two stalwarts of chemical engineering and chemistry department. These years have been helpful for my academic progress. I sincerely thank them for the kind guidance, helpful suggestion, encouragement. The fruitful discussion with them throughout the research work was great to work for successful completion of the present work.

It is a great pleasure to thank the authorities of Thapar Institute of Engineering and Technology, Patiala for providing the opportunity to carry out my research work successfully.

I owe sincere and earnest thankfulness to my family and my friend Anu who enthused, encouraged and fully supported me for every trial comes in my life. I acknowledge my sincere thanks to all my and friends for providing the companionship and making my stay pleasant and joyful at Thapar Institute of Engineering & Technology, Patiala.

Last but not the least, to god the father of all, I thank for the strength that keeps me standing and for the hope that keeps me believing that this affiliation would be possible and more interesting.

Dharminder

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Abbreviations

TPA = Aetradecanoylphorbol-13-acetate

NCD = A novel water soluble curcuminderivative

NF-KB = Nuclear transcription factor KB

AP-1= Activator protein 1

COX-2 = Cyclooxygenase-2

NOS = Nitric oxide synthase

LOX = Lipoxygenases,

MMP-9 = Matrix metallopeptidase 9,

uPA = Urokinase-type plasminogen activator

Egr-1 = Early growth response protein 1

CFTR = Cystic fibrosis transmembrane conductance regulator

ROS = Reactive oxygen species

RNS = Reactive nitrogen species

Wt = Weight

Hr = Hours

Abstract

Turmeric is one of the spices found in India. It has been used as flavoring, seasoning, and medicinal purpose from ancient time. Turmeric rhizomes were pretreated by five enzymes namely cellulase, pectinase, diastase, xylose, and lipase to obtain the turmeric oil. The operating temperature and pre-treatment time were optimized as incubation period four hours and five and half hour for the present work. It has been observed that all the enzymes are capable to degrade the turmeric rhizomes. The turmeric oil was obtained by hydrodistillation of the pre-treated rhizomes.The maximum and minimum oil yield were obtained in case of cellulose and lipase respectively. The maximum turmeric oil yield was obtained as 2.8 % and minimum yield of 0.7% and the minimum turmeric oil yield were obtained in case of lipase. The GC-MS is techniques used for the analysis of the turmeric oil. The major components of the turmeric oil are Monoterpenoid, Santalane, Bisabolane, Sesquiterpene, Bergamotane, Cedrane, Sesquisabinane, etc.

Chapter 1

1. Introduction

Turmeric is one of the spices of India from ancient times. “A spice is a seed, fruit, root, bark, or other plant substance primarily used for flavoring, coloring or preserving food”.

India cultivates about 75 out of 109 varieties of spices listed by International Organization for Standardization (ISO). Turmeric not only used for seasoning purpose but also imparts flavor, taste, aroma, and color to the food. It is well known for their medicinal properties and used in the cosmetic product, pharmaceuticals, and perfumery and religious rituals i.e. used in marriages and worship.

Turmeric is used in powdered form as well as the essential oil for the seasoning of the food product. Raw turmeric has certain disadvantages as they are unhygienic-often contaminated by filth (microorganism). Ground spices have poor flavor distribution and loss of flavor and degradation on storage happens. Dried herbs usually have an unacceptable hay-like aroma. They are also dusty and unpleasant to handle in bulk. Discoloration due to tannins also occurs. The production of oleoresin and extraction of essential oil from herbs is one of the best ways to store the spices for a long time without losing their aroma.

There are various techniques used for the extraction essential oil/oleoresin such as steam distillation in which autoclave generates the vapors with pressure control. These vapors are pass through the turmeric bed, steam was condensed by cooling water at room temperature and collected in separatory funnel together with the essential oil obtained **(Manzan et al., 2003).** Another technique of Solvent extraction by using soxhlet is used in which sample was placed in thimble-holder that filled with the condensed fresh solvent use d for extraction. As the liquid arriving at the overflow level a siphon aspirates the solute from the thimble-holder and put it back into the distillation flask, thus carrying the analyte into the bulk liquid repetition of operation occur until the extraction got completed. Assembly is operated in a batch system **(Saim et Al., 1997)** and mechanical expression. Since the use of turmeric has been used worldwide due to multicultural population residing in Europe and traveling to exotic places introduce the Indian food and flavors widespread and also due to the broad spectrum of medicinal value results in increasing its demand all over the world. We should consider some of the factors for future which can favor the production of the following isolates which are derived from the natural essential oils. However, Conversion and separation α-pinene and β-pinene from turpentine obtained from natural products to its desired basic derivatives like citronellal, geraniol, citral also require lots of energy and chemicals, which are obtained from petroleum feedstock. Its products can be derived synthetically but not economical as a large amount of energy is required which in turn obtained from petroleum fuel due to which cost of the product increases. These activities also add the hazardous pollutant to the environment.

One of the eco-friendly methods is the use of enzymes for the degradation of the raw material by treating it with different enzymes. These enzymes interact with the substrate (raw material) in various ways and lyse the cell wall and facilitate the extraction of different components of the cell.

Turmeric a native of South Asia is an ancient spice from classical time used for dye and condiment. Turmeric having botanical name Curcuma longa belongs to Zingiberaceae family is a rhizomatous perennial herb. It is indigenous to tropical southern Asia but now cultivated widely in the subtropical and tropical region of the world. Turmeric (deep orange-yellow powder) is extensively used as a spice for flavoring the foods and imparting color figure **1 (Aggarwal et al., 2007).**

Figure 1: Turmeric rhizome and powder.

India, Jamaica, Taiwan, Philippines, Indonesia, and China are the leading producer of turmeric. India is the largest producer cultivate about 90% turmeric of the total world's production about 70 variety of C. longa are cultivated in India.

Turmeric major constituents are phenols and terpenes including terpenes, sesquiterpenes, sterols, diarylheptanoids, diarylpentanoids, triterpenoids, alkaloids and diterpenes till now 235 compounds of turmeric are known **(Li et al., 2011).**

Table 1: List of probable Compounds found in essential oil. **(Li et al., 2011).**

No.	Compound name	Compound type
1	1,7-Bis(4-hydroxyphenyl)-1-heptene-3,5-dione	Diarylheptanoid
2	5-Hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
3	5-Hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
4	Tetrahydroxycurcumin	Diarylheptanoid
5	Bisdemethoxycurcumin (curcumin III)	Diarylheptanoid
6	1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione	Diarylheptanoid
7	1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-	Diarylheptanoid

	heptadiene-3, 5-dione	
8	Demethoxycurcumin (curcumin II)	Diarylheptanoid
9	Curcumin (curcumin I)	Diarylheptanoid
10	1,5-Bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one	Diarylheptanoid
11	1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1, 4-pentadiene-3-one	Diarylheptanoid
12	1,5-Bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one	Diarylheptanoid
13	1,7-Bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Diarylheptanoid
14	Cyclocurcumin	Diarylheptanoid
15	1,5-Epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene	Diarylheptanoid
16	1,5-Dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
17	1,5-Dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
18	1,5-Dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
19	1,5-Dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
20	1,5-Dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
21	3-Hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	Diarylheptanoid
22	5-Hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid
23	(*Z*)-Ferulic acid	Phenylpropene
24	(*E*)-Ferulic acid	Phenylpropene
25	(*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one	Phenylpropene
26	Calebin-A	Phenylpropene
27	4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate	Phenylpropene
28	4''-(4'''-hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate	Phenylpropene
29	Vanillin	Phenolic
30	Vanillic acid	Phenolic
31	*p*-Cymene	Monoterpenoid
32	*m-C*ymene	Monoterpenoid
33	*α*-Terpinene	Monoterpenoid

34	*γ*-Terpinene	Monoterpenoid
35	β-Phellandrene	Monoterpenoid
36	*p*-Mentha-1,4(8)-diene	Monoterpenoid
37	Terpinen-4-ol	Monoterpenoid
38	4-Terpinol	Monoterpenoid
39	Limonene	Monoterpenoid
40	Terpinolene	Monoterpenoid
41	Thymol	Monoterpenoid
42	Phellandrol	Monoterpenoid
43	Carvacrol	Monoterpenoid
44	(*E*)-Carveol	Monoterpenoid
45	*γ*-Terpineol	Monoterpenoid
46	Menthol	Monoterpenoid
47	1,3,8-Paramenthatriene	Monoterpenoid
48	p-Methylacetophenone	Monoterpenoid
49	Piperitone	Monoterpenoid
50	*o*-Cymene	Monoterpenoid
51	Carvone	Monoterpenoid
52	*p*-Menth-8-en-2-one	Monoterpenoid
53	*α*-Thujene	Monoterpenoid
54	*α*-Terpineol	Monoterpenoid
55	*p*-Cymen-8-ol	Monoterpenoid
56	*p*-Meth-8-en-2-one	Monoterpenoid
57	Piperitone epoxide	Monoterpenoid
58	Sylvestrene	Monoterpenoid
59	Menthofuran	Monoterpenoid
60	β,β-Dimethylstyrene	Monoterpenoid
61	Camphor	Monoterpenoid
62	Teresantalol	Monoterpenoid
63	Benzene, 1-methyl-4-(1-methylpropyl)	Monoterpenoid
64	2-Norpinanone	Monoterpenoid
65	Borneol	Monoterpenoid
66	Bornyl acetate	Monoterpenoid
67	(*E*)-Chrysanthenyl acetate	Monoterpenoid
68	(*Z*)-Cinerone	Monoterpenoid

69	(*Z*)-Sabinol	Monoterpenoid
70	2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid	Monoterpenoid
71	Camphene	Monoterpenoid
72	3-Carene	Monoterpenoid
73	2-Carene	Monoterpenoid
74	Ascaridole	Monoterpenoid
75	*α*-Pinene	Monoterpenoid
76	*β*-Pinene	Monoterpenoid
77	Cineole	Monoterpenoid
78	*cis*-Ocimene	Monoterpenoid
79	Citronellal	Monoterpenoid
80	Geranial	Monoterpenoid
81	Neral	Monoterpenoid
82	Myrcene	Monoterpenoid
83	*R*-Citronellene	Monoterpenoid
84	Citronellyl pentanoate	Monoterpenoid
84	Nerol	Monoterpenoid
85	Geraniol	Monoterpenoid
86	iso-Artemisia ketone	Monoterpenoid
87	*trans*-Ocimene	Monoterpenoid
88	Linalool	Monoterpenoid
89	Neryl acetate	Monoterpenoid
90	Geranic acid	Monoterpenoid
91	Geranyl acetate	Monoterpenoid
92	3-Bornanone	Monoterpenoid
93	4,8-Dimethyl-3,7-nonadien-2-ol	Monoterpenoid
94	3,4,5,6-Tetramethyl-2,5-octadiene	Monoterpenoid
95	3,7-Dimethyl-6-nonenal	Monoterpenoid
96	2,6-Dimethyl-2,6-octadiene-1,8-diol	Monoterpenoid
97	4,5-Dimethyl-2,6-octadiene	Monoterpenoid
98	Dehydrocurcumene	Bisabolane
99	Dihydro-ar-turmerone	Bisabolane
100	α-Bisabolol	Bisabolane
101	2,8-Epoxy-5-hydroxybisabola-3,10-diene-9-one	Bisabolane
102	*β*-Atlantone	Bisabolane

103	(6*R*)-[(1*R*)-1,5-Dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one	Bisabolane
104	2, 5-Dihydroxybisabola-3, 10-diene	Bisabolane
105	Curculonone A	Bisabolane
106	Curculonone B	Bisabolane
107	Curculonone D	Bisabolane
108	Curculonone C	Bisabolane
109	Curlone	Bisabolane
110	Curcuphenol	Bisabolane
111	(6*S*)-2-Methyl-6-[(1*R*,5*S*)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-One	Bisabolane
112	*(E)*-*γ*-Atlantone	Bisabolane
113	*(Z)*-*γ*-Atlantone	Bisabolane
114	*β*-Sesquiphellandrene	Bisabolane
115	*α*-Curcumene	Bisabolane
116	*β*-Curcumene	Bisabolane
117	*γ*-Curcumene	Bisabolane
118	*γ*-Cisabolene	Bisabolane
119	(6*S*,7*R*)-Bisabolene	Bisabolane
120	*β*-Bisabolene	Bisabolane
121	*(E)*-*α*-Atlantone	Bisabolane
122	*(Z)*-*α*-Atlantone	Bisabolane
123	Dehydrozingerone	Bisabolane
124	Zingerone	Bisabolane
125	Xanthorrhizol	Bisabolane
126	*α*-Zingiberene	Bisabolane
127	*α*-Oxobisabolene	Bisabolane
128	Turmeronol B	Bisabolane
129	Turmeronol A	Bisabolane
130	Bisacumol	Bisabolane
131	Bisabolone-9-one	Bisabolane
132	Bisacurone C	Bisabolane
133	Bisacurone B	Bisabolane
134	Bisacurone A	Bisabolane
135	Bisacurone	Bisabolane

136	4-Methoxy-5-hydroxy-bisabola-2,10-diene-9-one	Bisabolane
137	4-Hydroxybisabola-2,10-diene-9-one	Bisabolane
138	4, 5-Dihydroxybisabola-2,10-diene	Bisabolane
139	Bisabolone	Bisabolane
140	Bisabola-3,10-diene-2-one	Bisabolane
141	*ar*-Turmerol	Bisabolane
142	*ar*-Curcumene	Bisabolane
143	4-Methylene-5-hydroxybisabola-2,10-diene-9-one	Bisabolane
144	5-Hydroxyl-ar-turmerone	Bisabolane
145	2-Methyl-6-(4-formylphenyl)-2-hepten-4-one	Bisabolane
146	2-Methoxy-5-hydroxybisabola-3,10-diene-9-one	Bisabolane
147	2-Methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one	Bisabolane
148	2-Methyl-6-(4-hydroxyphenyl)-2-hepten-4-one	Bisabolane
149	β-Turmerone	Bisabolane
150	α-Turmerone	Bisabolane
151	*ar*-Turmerone	Bisabolane
152	(4*S*,5*S*)-Germacrone-4,5-epoxide	Germacrane
153	Dehydrocurdione	Germacrane
154	Germacrene D	Germacrane
155	Germacrone	Germacrane
156	Germacrone-13-al	Germacrane
157	β-Germacene	Germacrane
158	1,10-Dehydro-10-deoxy-9-oxozedoarondiol	Guaiane
159	Curcumenol	Guaiane
160	Epiprocurcumenol	Guaiane
161	Isoprocurcumenol	Guaiane
162	Zedoaronediol	Guaiane
163	Procurcumadiol	Guaiane
164	Procurcumenol	Guaiane
165	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)	Selinane
166	α-Selinene	Selinane
167	Juniper camphor	Selinane
168	Corymbolone	Selinane
169	α-Santalol	Santalane

170	*α*-Santalene	Santalane
171	*β*-Santalene	Santalane
172	(*E*)-Caryophyllene	Caryophyllane
173	Caryophyllene oxide	Caryophyllane
174	*β*-Elemene	Elemane
175	γ-Elemene	Elemane
176	Acoradiene	Acorane
177	Aristolene	Aristolene
178	(*Z*)-*α*-Bergamotene	Bergamotane
179	Curcumenone	Carabrane
180	Di-epi-cedrene	Cedrane
181	Himachalene	Himachalene
182	(*E*)-Sesquisabinene hydrate	Sesquisabinane
183	Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene)	Sesquiterpene
184	γ-Gurjunen epoxide	Sesquiterpene
185	1-Epi-cubenol	Sesquiterpene
186	Cubebene	Sesquiterpene
187	7-Epi-sesquithujene	Sesquiterpene
188	Caryophyllene	Sesquiterpene
189	6*α*-Hydroxycurcumanolide A	Sesquiterpene
190	Curcumanolide A	Sesquiterpene
191	Curcumanolide B	Sesquiterpene
192	Curcumin L	Sesquiterpene
193	*α*-Humulene	Sesquiterpene
194	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl	Sesquiterpene
195	Adoxal	Sesquiterpene
196	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	Sesquiterpene
197	(*E*,*E*)-*α*-Farnesene	Sesquiterpene
198	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)	Sesquiterpene
199	Hexadecane-1,2-diol	Sesquiterpene
200	Nerolidal	Sesquiterpene
201	(*Z*)-*β*-Farnesene	Sesquiterpene
202	Nerolidyl propionate	Sesquiterpene
203	Phytol	Diterpenoid
204	(*E*,*E*,*E*)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	Diterpenoid

205	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene	Diterpenoid
206	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-,(E,E)	Diterpenoid
207	Hopenone I	Triterpenoid
208	Hop-17(21)-en-3β-ol	Triterpenoid
209	Hop-17(21)-en-3β-yl acetate	Triterpenoid
210	*β*-Sitosterol	Steroid
211	Stigmasterol	Steroid
212	Gitoxigenin	Steroid
213	20-Oxopregn-16-en-12-yl acetate	Steroid
214	Stearic acid	Fatty acid
215	Oleic acid	Fatty acid
216	Palmitic acid (n-hexadecanoic acid)	Fatty acid
217	8,11-Octadecadienoic acid, methyl ester	Fatty acid
218	Linoleic acid	Fatty acid
219	Curcuma-J	
220	2-(2'-methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline	
221	2,3,5-trimethylfuran	
222	(1,2,3-trimethyl-cyclopent-2-enyl)-methanol	
223	Dicumyl peroxide	
224	1-(3-cyclopentylpropyl)-2,4-dimethy-benzene,	
225	1,4-Dimethyl-2-(2-methylpropyl)-benzene	
226	2,2'-Oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran	
227	Cyclohexyl formate	
228	Methyleugenol	
229	3,3,5-Trimethyl-cyclohexanol acetate	
230	2,4-Dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one	
231	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde	
232	Bicyclo[3.3.1]nonan-9-one, 2,4-dimethyl-3-nitro- (exo)	
233	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	
234	Pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS)	
235	3,3,5-Trimethyl- peroxide	

Its Yellow color is due to the presence of three polyphenolic curcuminoids. These different types of curcuminoid are found in turmeric are curcumin having chemical formula $C_{21}H_{20}O_6$ (IUPAC) name 1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta1,6-diene-3,5-dione) 77%, demethoxycurcumin 17% and bis-demethoxycurcumin 6% figure 2. Curcumin is the major curcuminoid of Indian condiment turmeric. Antioxidant property is shown by all the curcuminoid **(Majeed et al., 1995).** Curcumin contains diferuloylmethane, p-hydroxycinnamoyl feruloylmethane and p- dihydroxydicinamoyl methane **(Srinivasan, 1952).**

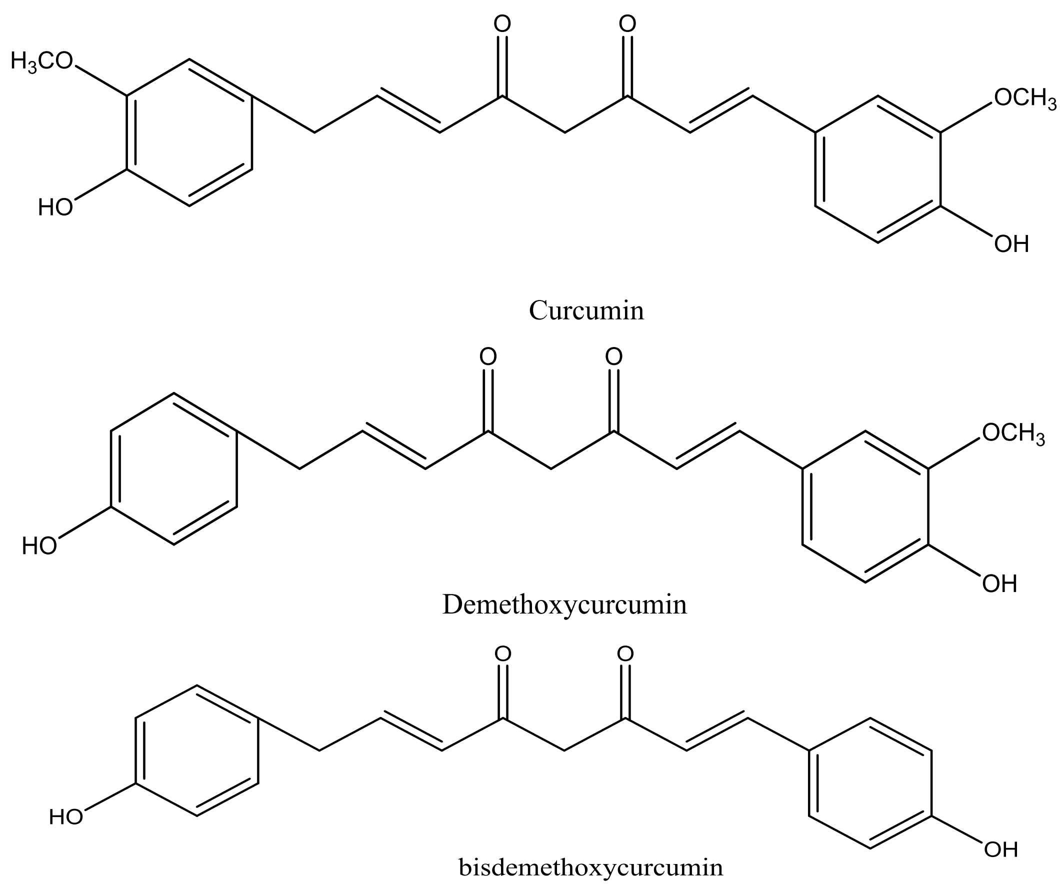


Figure 2: various structures of curcuminoids.

Curcumin exists in keto-enol form (tautomeric form). Analytically it has been shown that turmeric contains carbohydrates (69.4%), moisture (13.1%), protein (6.1%), fat (5.1%) and minerals (3.5%). Essential oil obtained from turmeric rhizome contains sesquiterpenes (53%), borneol (0.5%), cineol (1%), zingiberene (25%), α-phellandrene (1%), sabinene (0.6%) and curcumin (3-6%) given in **Table 1 (Prasad et al., 2014).** Different components of turmeric rhizome are used for various purpose as given blew in **Table 2.**

Curcumin is light sensitive soluble in organic solvent such as ethanol but insoluble in water and ether, highly stable to heat. It readily decomposes at pH higher than neutral but stable at acidic pH **(Deoghate and Ghate, 2015).** In Indian traditional Ayurveda medicine turmeric hold a place of honor. According to Unani, it is considered to be safest herbs of choice for all blood disorder as it stimulates, purifies and build blood. It is also used for the medicinal purpose as anthelmintic, laxative, carminative and cure for the liver ailment **(Srimal, 1997).** It used as a home remedy for the treatment swelling and sprains caused due to injury by mixing it with slaked lime. Turmeric could be the potent alternative to common antibiotics as the crude extract from it have both antimicrobial and antioxidant capacities **(Goel, 2009)**. Overall, the production of essential oil from plants is eco-friendly, cheaper and green.

Table 2: Various components of turmeric.

Product	Composition	Appearance	Uses
Turmeric Whole Rhizome	Carbohydrates(69.4%), moisture(13.1%), protein (6.1%), fat (5.1%), minerals (3.5%)	orange-brown, pale yellow or red-yellow	Medicinal uses (process to form other useful product)
Ground Turmeric	curcuminoids (curcumin 77%),demethoxycurcumin (17%),bis-demethoxycurcumin(6%) ,	yellow, reddish-yellow	Used as a spice for flavoring agent and also as a preservatives, dye stuff in textile industries, cosmetic, dietary supplement
Turmeric oil	sesquiturpens (53%), boneol (0.5%), cineol (1.0%), zingiberene (25%), α-phellandrene(1.0%), Sabinene (0.6%), curcumin (3-6%)	Greenish-yellow	Spice, medicine, diatary supplement, mosquito repellent, pesticide, bioherbcide
Turmeric oleoresin	Oil, resin and unisolable curcumin	Yellow-dark reddish brown oily fluids	Food coloring, medicine, and dietary supplement

Chapter 2

2. Literature survey

In Indian spices, turmeric rhizome has a very much rich history as it used as a cheaper food preservative and a coloring agent. It is also found biologically active against the broad spectrum of various issues and safe for use **(Majeed et al., 1995).** It has been used as a medicine in Ayurveda since a long time for liver problems, indigestion, arthritis and hypercholesterolemia **(Srimal, 1997).** It is considered that turmeric can improve digestion and nutrient metabolism related to atsiri oil and curcumin content **(Darwis et al., 1991).** Atsiri oil produced improves the digestive tracts function and production of digestive enzymes also stimulated which results in improvement of digestion and hence, improvement of nutrient metabolism takes place **(Al-Sultan and Gameel, 2004)**. Bile production is enhanced by curcuminoids which result in digestion of fat **(Sugiharto et al., 2011).**

It is used as a natural remedy in India and China for the cure of sprain and swelling caused by injury wound healing and abdominal problems since ancient time **(Surh, 1999).** Recent studies show that turmeric and its active principle have unique antimicrobial, antimutagenic, antitumorigenic, antioxidant, anti-inflammatory and anticarcinogenic properties **(Majeed et al., 1995)**.

Free radicals are produced in the body by various biological processes and are capable of destroying many biological molecules and cells. The free radical such as reactive oxygen and nitrogen species (ROS and RNS) can be blocked by antioxidant present in turmeric which removes the free radicals and detoxifies the organisms. The antioxidant action occurs as it either prevents the formation of free radical or neutralizing the already formed radical **(Majeed et al., 1995; Miquel et al., 2002).**

Curcumin present in it found to be active against different bacterial strain like salmonella paratyphi, trichophyton, gypsum, Staphylococcus aureus and Mycobacterium tuberculosis **(Schraufstatter and Bernt, 1949).**

There are four different types of metabolites of curcumin are found namely dihydro-curcumin (DHC), trihydro-curcumin (THC), hexahydro-curcumin (HHC) and octahydro-cucumin (OHC) are the various metabolites of curcumin. THC is the main metabolite of curcumin and has been found to be more stable **(Pen et al., 1999).** THC a partially reduced product of curcumin has anti-inflammatory, anti-oxidative and anticancer activities **(Khopde et al., 2000).**THC induces autophagic (Cell's own death due to microenvironment stress) cell death by increasing autophage marker acidic vascular organelle formation (AVO) in human HL-60promyelocytic leukemia cell **(Wu et al., 2011).** The antioxidant and anti-inflammatory property has also been shown by OHC **(Pen et al., 1999).**

Bioavailability in our body of curcumin is very low due to high metabolism speed, inadequate absorption and high elimination rate from the body. Because of its limited bioavailability, it has the less therapeutic effect **(Sanlier, 2017).** Despite fact that its bioavailability is very low when measured in the human clinical trial **(Sharma et al., 2004).** To improve the bioavailability of curcumin the various strategies have been used such as the change in the route of administration, by changing the medium of administration, by blocking the metabolic pathway etc. One of the major steps taken to improve the bioavailability is by making derivatives of curcumin like nanocurcumin, encapsulated curcumin, and liposomal-encapsulated curcumin. Nanoglobule based nanoemulsion show much higher release of curcumin as compared to the curcumin suspension **(Kumar et al., 2012).** The nanoparticle of curcumin has greater concentration in plasma and higher residence time as compared to regular curcumin when tested in mice **(Cheng et al., 2013)**. Liposomes spherical sac of phospholipid molecule enclosing water droplet is considered to be as effective drug carrier. Liposome curcumin derivatives are formulated to improve the bioavailability of it inside living organism **(Takahashi et al., 2009).** In other study, cells are inhibitidTumor promotion and initiation is inhibited by 12-0-tetradecanoylphorbol-13-acetate and benxo[a] pyrene respectively **(Majeed et al., 1999).**

It is effective against different types of cancer such as leukemia, melanoma, sarcoma, and lymphoma as well as genitourinary, ovarian, breast, head and neck, lung, gastrointestinal and neurological cancer **(Anand et al., 2008).** It prevents tumor at various stages like invasion, transformation, promotion, initiation, angiogenesis, and metastasis. The curcumin inhibits (in vitro) the wide variety of proliferation and suppress the carcinogenesis **(Aggarwal et al., 2003).** Nano micelles (cur micelles) of curcumin showed anti-proliferative properties against colon cancer **(Zheng et al., 2013).** TPA-induced tumor promotion can be inhibited by a combination of 100nmol curcumin with 5nmol TPA by taking 18 weeks 2 times in a week it is due to strong inhibitory action on RAN and DNA synthesis **(Miquel et al., 2002).**

Diacetyl curcumin (a lipophilic derivative of curcumin) and digiutaryl curcumin (hydrophilic derivative of curcumin) derivatives of curcumin show anti-inflammatory and analgesic effect. Diacetyl curcumin is found to be more effective as compare to the curcumin **(Jacob et al., 2013).**

Antioxidant properties of curcumin are well known even though several derivatives of it are synthesized to improve its antioxidant property **(Dolai et al., 2011).**

The derivative (NCD) showed the hypoglycemic effect by lowering the plasma glucose by 27.5% and increased plasma insulin 66.67% **(Abdel Aziz et al., 2012).** It also shows antipsoriatic effect this property of curcumin is based on that modifying phosphoryl-kinase (a multimeric enzyme having i.e α, β, γ, and δ) four subunits activity integrates multiple calcium/calmodulin-dependent signaling pathway **(Heng et al., 2000).** Antioxidant property of curcumin showed effectiveness against atherogenesis i.e. it is anti-atherogenic **(Miquel et al., 2002).**

It also found effective against myocardial infraction by inhibiting the aggregation of platelets and rapid increase in the number of vascular smooth muscle cells(VSMCs) and peripheral blood mononuclear cells (PBMCs) atherosclerosis i.e. show cardioprotective properties **(Aggarwal and Shishodia, 2004).** Several skin diseases also treated by it such as psoriasis, skin carcinogenesis, scleroderma, and dermatitis. Psoriasis is a chronic skin disease in which keratinocyte hyperproliferation and abnormal differentiation occur **(Sun et al., 2013).** It has antidiabetic properties if taken orally prevent the loss of body weight reduces glycosylated haemoglobin, hemoglobin and level of glucose in the body **(Arun and Nalini, 2002)** improve insulin sensitivity by THC present in curcumin **(Murgan and Pari, 2007).**

It inhibits the inflammatory molecule such as NF-KB, AP-1, COX2, NOS, LOX, MMP-9, uPA, NF, chemokines, and Egr-1 hence show antirheumatic and antiarthritic effect **(Aggarwal et al., 2003)**. Curcumin is found to be safe and improve the disease condition when effectiveness is measured with the patient of rheumatoid arthritis **(Chandran and Goel, 2012).** Destruction of oligodendrocytes (provide support and insulation to axons in the central nervous system of some vertebrates) and myelin sheath in the central nervous system is a characteristic of multiple sclerosis. Curcumin's anti-inflammatory and neuroprotective effect is found to be effective against multiple sclerosis **(Tegenge et al., 2013).** It blocks the interleukin (IL)-12 signaling in T cells and inhibits the allergic encephalomyelitis suggesting that it is effective against multiple sclerosis **(Aggarwal et al., 2003).**

It has potential to inhibit the neurodegenerative disease since it acts against Aβ fibril formation **(Kim et al., 2001).** In Alzheimer disease, it suppresses the inflammation, cognitive deficit, oxidative damage and amyloid accumulation **(Yang et al., 2005).** According to these studies, curcumin improves the disease condition interfering with plaque formation. Inflammatory bowel disease is an inflammatory condition of the colon and small intestine curcumin is effective in preventing and treating this disease **(Ukil et al., 2003).** It may be the possibility that it inhibits the free radical, increase antioxidant and influence multiple signaling pathways and hence prevent the inflammatory bowel disease **(Baliga et al., 2012).**

Cystic fibrosis a genetic disorder of lungs also occur in liver, intestine, and pancreas caused by the mutation in trans membrane conductor gene in cystic fibrosis (CFTR). Curcumin inhibits the sarcoplasmic/endoplasmic reticulum calcium (SERCA) pump and hence prevent the CFTR degradation and improve the manifestation of cystic fibrosis **(Bilmen et al., 2001).** In atherosclerosis, it acts as a chemopreventive agent **(Soni and Kuttan, 1992).** Pieces of evidence show that curcumin is safe and effective and it is bioavailable in serum and plasma when taken orally **(Chang et al., 2013; Yang et al., 2007).**

Essential oils are the secondary metabolites of plants and are volatile natural compounds stored in secretory cells, cavities, canals, epidermis cells or glandular trichomes having strong odors obtained by steam or hydro-distillation known for their antiseptic, fragrance and medicinal property. It is also added to food as a preservative. Fresh rhizomes contain about 0.16% to 1.94% essential oil.

It obtained from it is a mixture of more than one hundred components in which few components make the major part of it. These components are essentially terpenes and terpenoids (monoterpenes(C_{10}) or sesquiterpenes(C_{15})). Usually, Ar-termeron, α-termeron and β-termeron are the major components present in oil **(Li. et al., 2011)**. Two isoprene units combine to form terpene. Terpenoid is oxygen-containing terpene. The essential oil is obtained is used for fragrance, flavor and perfumery purpose. Essential oil guards the plant against fungus, bacteria, viruses, and insects and also from herbivores by reducing their appetite for this type of plants. It removes the free radical and inactivates the mutagens hence inhibit the penetration of mutagens across the cell and act as antimutagenic **(Bakkali et al., 2008).**

Antimicrobial and antiseptic properties of the essential oil are based on cytotoxic capacity due to prooxidant activity **(Hammer et al., 1999).** Environmental issues and long-term health effect of pesticides which are sprays on the food is the biggest concern nowadays. The United States made food quality protection act in 1996 and also some countries made some laws which restrict the use of many conventional pesticides and insecticide on which growers are dependent form decades. This will make a huge opportunity in the market to find out the alternatives to reduce the risk of these harmful chemicals. Plant essential oils can use as a natural pesticide in place of chemical pesticides. Essential oil from plants can be obtained from any part or as a whole. The essential oil is one of the natural substitutes for chemical pesticides **(Isman 2000).**

Investigation of essential oil's antifungal property is done by **(Kurita et al., 1981)** in which they screened 40 components against 7 food spoiling fungi and by (**Singh et al., 1980)** in which they screened essential oil against 22 species of fungi including plant pathogen and human. The insecticidal and antifungal action is due to the monoterpenic phenols **(Sangwan et al., 1990).**

Some essential oil has also been found to inhibit the germination of plant suggesting the bioherbicidal activity **(Dudai et al., 1999).** A large number of Plastic material used for the food packaging harmful for the environment, therefore, an alternative source for packaging is required which is biodegradable. Essential oils can be used as packaging by coating on food products they are of biodegradable and protect because of their antibiotic antimicrobial properties **(Loren et al., 2016).**

Turmeric volatile oil contains aromatic turmerone(20-30%) a major compound **(Govindranjan, 1980)**, is a mosquito repellent **(Twastin et al., 2001**) and may be an effective drug for dermatophytosis **(Apisariyakul et al., 1995)** and for the treatment of respiratory disease **(Li et al., 1998).** Termeron isolated from turmeric has also been reported for anti-venom activity **(Ferreira et al., 1992).**

Chapter 3

3. Method and materials

3.1. Collection of turmeric rhizomes and enzymes

Fresh turmeric rhizomes were collected from the markets of Patiala, Chandigarh, and Haryana in the month of March, in the spring season. Rhizomes were unhygienic soil particles were present on them. It was kept in the well-ventilated area. It has been assured from the farmers that the turmeric was cultivated in these regions. The enzymes cellulase, pectinase, lipase, diastase, and xylose were purchased from the market. These enzymes were used for pre-treatment of turmeric rhizomes. During pre-treatment process, these enzyme were used to degrade the cellulose, hemicelluloses, pectin layer and to convert the xylem and phloem into glucose.

3.1 Moisture content of turmeric rhizome

The moisture contents of the turmeric rhizome are calculated by keeping the fixed weight of turmeric rhizome for 4 hours at 105^0C. The loss in of weight gives the % moisture contents.

$$\% \text{ moisture contents} = \frac{Initial\ weight - Final\ weight \times 100}{Initial\ weight}$$

3.2 Preperation of feed

Fresh turmeric was weighed on the weighing balance and then washed thoroughly to remove the dirt with tap water. As the soil particle present on the turmeric rhizomes may cause the deactivation the enzymes. Turmeric was chopped finely into small pieces as small as possible on the chopper to increase the surface area of the substrate. A petri dish taken and washed with distilled water and dried, turmeric was put into it. Petri dish was kept in the water bath by making sufficient amount of water level in the water bath for pretreatment by covering it with the aluminum foil.

3.3 Pre-treatment of turmeric rhizomes

We started our work with demo experiment in which 10gm of chopped turmeric was exposed for pre-treatment with enzymes (all five) at temperature ranging from 27 to 55 ^{o}C **(Yoon et al., 1974; Ceci et al., 1998; Murici et al., 2012; Alam et al 2016).** The enzyme concentration was also varied from 10-50mg to obtain the optimum enzyme concentration. At 40^{o}C all the enzymes show activity with 50mg concentration. It has been observed that, all enzymes worked well at low pH (acidic condition) except lipase. The literature shows that the enzyme Lipase works well at pH higher than the neutral pH (>7)**(Yoon et al., 1974; Ceci et al., 1998; Murici et al., 2012; Alam et al, 2016).**

In further experiments, we have optimized the incubation time for pre-treatment.

3.4 Yield estimation of turmeric oil

The oil obtained from the turmeric rhizome is measured in percentage by using the following expression.

$$\% \text{ oil yield} = \frac{\text{essential oil obtained} \times 100}{\text{Fresh feed weight x } (1 - \% \text{ moisture content} / 100)}$$

4 GC-MS details

The analysis of the essential oil of turmeric was carried out by the technique by GC-MS (Gas chromatography-Mass Spectrometer) spectroscopy using Perkin Elmer having model number Clarus 500. The length of the fused silica capillary column is 25 meter having thickness 20 µm. The flow was maintained 1l/min. The helium gas was used as a mobile phase and inlet temperature was maintained at 280^0C.The temperature in the oven is maintained at 40^0C for 2 minutes, 200^0C for 5 minutes, 300^0C for a minutes.

Chapter 4

5 Result and discussion

5.1 Incubation time Optimization

Incubation time is the time to which substrate is exposed to the enzyme activity. The incubation is optimized by conducting various experiments for the time range of 1 to 5 hours at 1 hour time interval **(Table 3)**. Table 3 shows that the oil was obtained for all samples. The enzyme concentration was taken as 50 mg/l. It is also observe that the maximum yield oil was obtained at 3 and 5 hour. For sample no. 4 (4 hour incubation time) the oil yield reduces up to 15.4%. This reduction in the yield with increase in incubation time shows revers pattern. This may be due to the absorption of oil by upper layer extracted from the subsequent bottom layer of the raw turmeric. The more than 5 hour time is not economical hence we have chosen 3 hour as optimum incubation time.

Table 3: Effect of incubation period on the Yield of turmeric oil.

Sample no.	Incubation Time(hour)	Oil yield (ml)
1	1	0.8
2	2	0.9
3	3	1.3
4	4	1.1
5	5	1.3

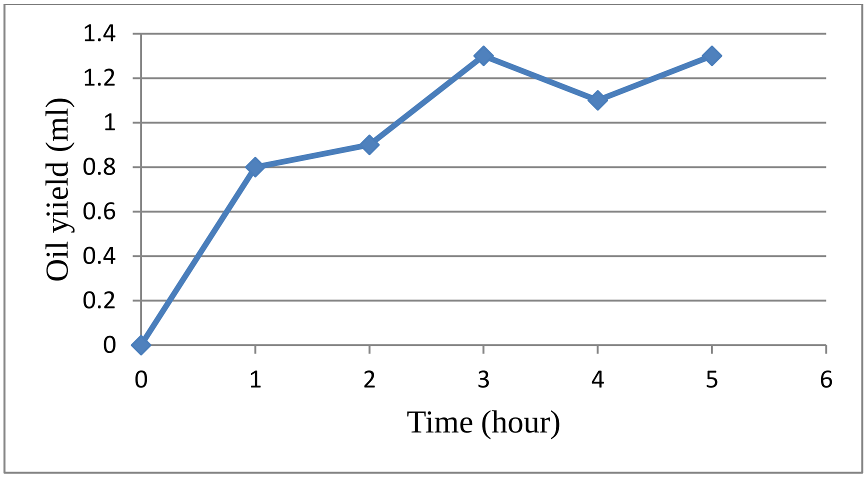


Figure3: Percentage yield of turmeric oil obtained at various incubation periods.

5.2 Distillation Time Optimization

After the pretreatment (incubation), the pretreated turmeric rhizomes was taken out from water bath, water was drained off and put it into round bottom flask. The pretreated rhizomes were feed into distillation assembly to extract essential oil.

Table 4: Effect of distillation time on the yield of turmeric oil.

S. No.	Time (min)	Oil yield (ml)
1	30	0.3
2	60	0.5
3	90	0.9
4	120	1.3
5	150	1.5
6	180	1.8
7	210	2.0
8	240	2.4
9	270	2.7
10	300	2.7

11	330	2.7

. The distillation time was also optimized and shown **Table 4 (figure 4).** The observation of table 4 shows that the optimum time for distillation is 270 min (4 ½ hour).

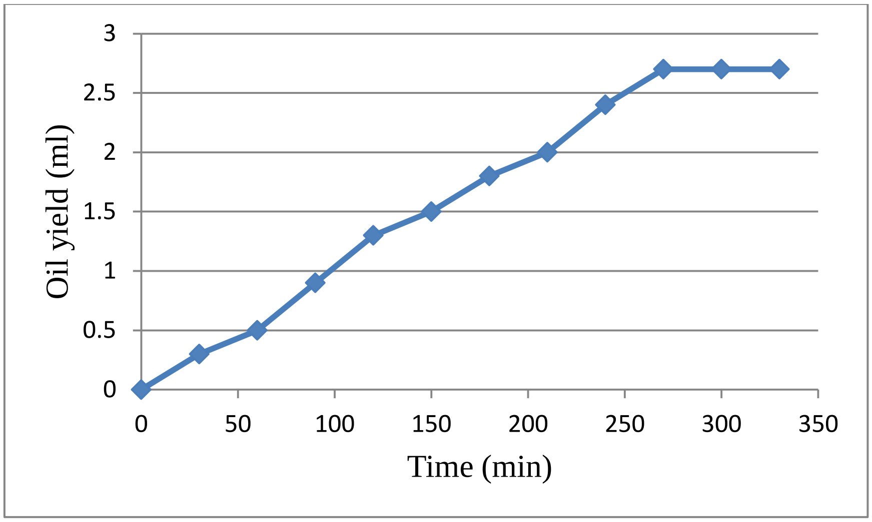


Figure 4: Percentage yield of turmeric oil obtained at various distillation times.

After the incubation time and distillation time optimization, further experiments were performed on above optimum condition for all five enzymes. The influence of feed weight was also observed by taking samples of 200 gm and 400 gm.

The essential oil present in the turmeric is trapped in biological cells. The percentage of oil yield is directly proportional to the rupture of cells. The use of enzymes facilitates the cell disruption and the cell disruptions provide the increase yield of essential oil. The use of enzymes to disrupt the cell may be the eco-friendly way to extract essential oil with increase yield than conventional methods.

The cell disruption was carried as pretreatment with the help of five different enzymes namely cellulase, lipase, diastase, xylose, and pectinase. These enzymes are able to burst the cells of turmeric rhizome to release the oil contents of the cell.

Hence, the pretreatment of turmeric rhizomes with the help of enzymes followed by hydro-distillation were performed. The obtained oil was collected and obtained yields are tabulated in **Table 5**. The observation of file shows that maximum yield was obtained by cellulase and least in the case of lipase. Since, lipase works on basic environment pH (>7) whereas turmeric shows acidic behavior, hence the reaction (conversion) goes down due to reduce activity of lipase enzyme in acidic media **(Yoon et al., 1974; Ceci et al., 1998; Murici et al., 2012; Alam et al, 2016).**

The influence of various enzymes on the same quantity of turmeric rhizome is tabulated in **Table 5**. There are various compounds in the oil contents of turmeric rhizomes are present such as monoterpenoid, sesquiterpenoid, bisabolane, caryophellane, sesquisabinane, bergamotane, Cedrane, Salinane, Santalane which were identified by using GC-MS spectroscopy shown in **Table 6**. It has been observe that with increase in distillation time yield of the essential oil was increased as shown in **Table 4.** It was shown by **Srivastava et al., 2001** that essential oil content of turmeric rhizomes is very low about 2.2% (v/wt). The pretreatment of the turmeric rhizome with the enzyme with cellulase increase the yield of the oil contents 2.8% (v/wt). This is due to the fact that the major component of the turmeric rhizome is carbohydrates (69.4%) which is made up of cellulosic material that was degraded by enzyme. The moisture contents of the turmeric rhizome are comes out be 76% when stored at 105^0C for 4 hr.

Table 5: Variation of yield with different enzymes with different amount of turmeric.

Enzymes	Amount (gm)	volume (ml)	% yield (ml/gm)
Pectinase	200	0.70	1.4
	400	0.80	0.8
Diastase	200	0.60	1.25
	400	0.70	0.7
Lipase	200	0.50	1.0
	400	0.75	0.7
Cellulase	200	0.90	1.8
	400	1.15	1.1
Xylose	200	0.50	1.0
	400	0.70	0.7

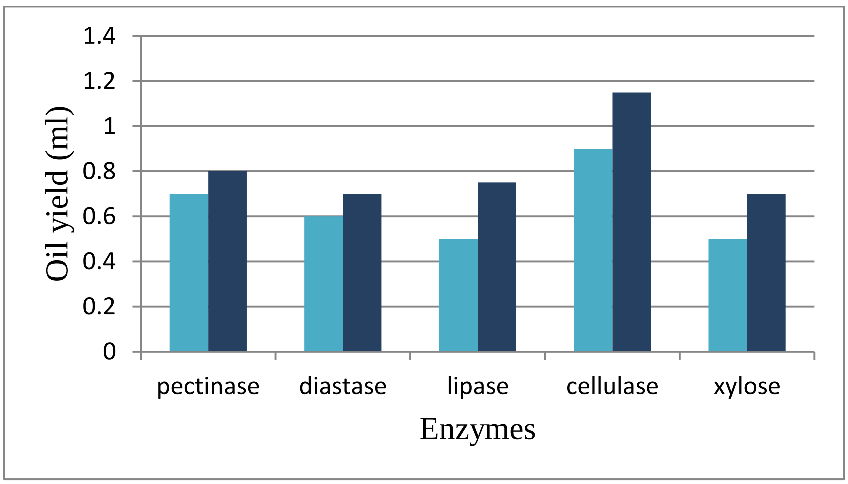


Figure 5: Percentage yield of turmeric oil obtained with different amount of rhizomes.

Figure 6: Turmeric oil extracted with turmeric rhizome.

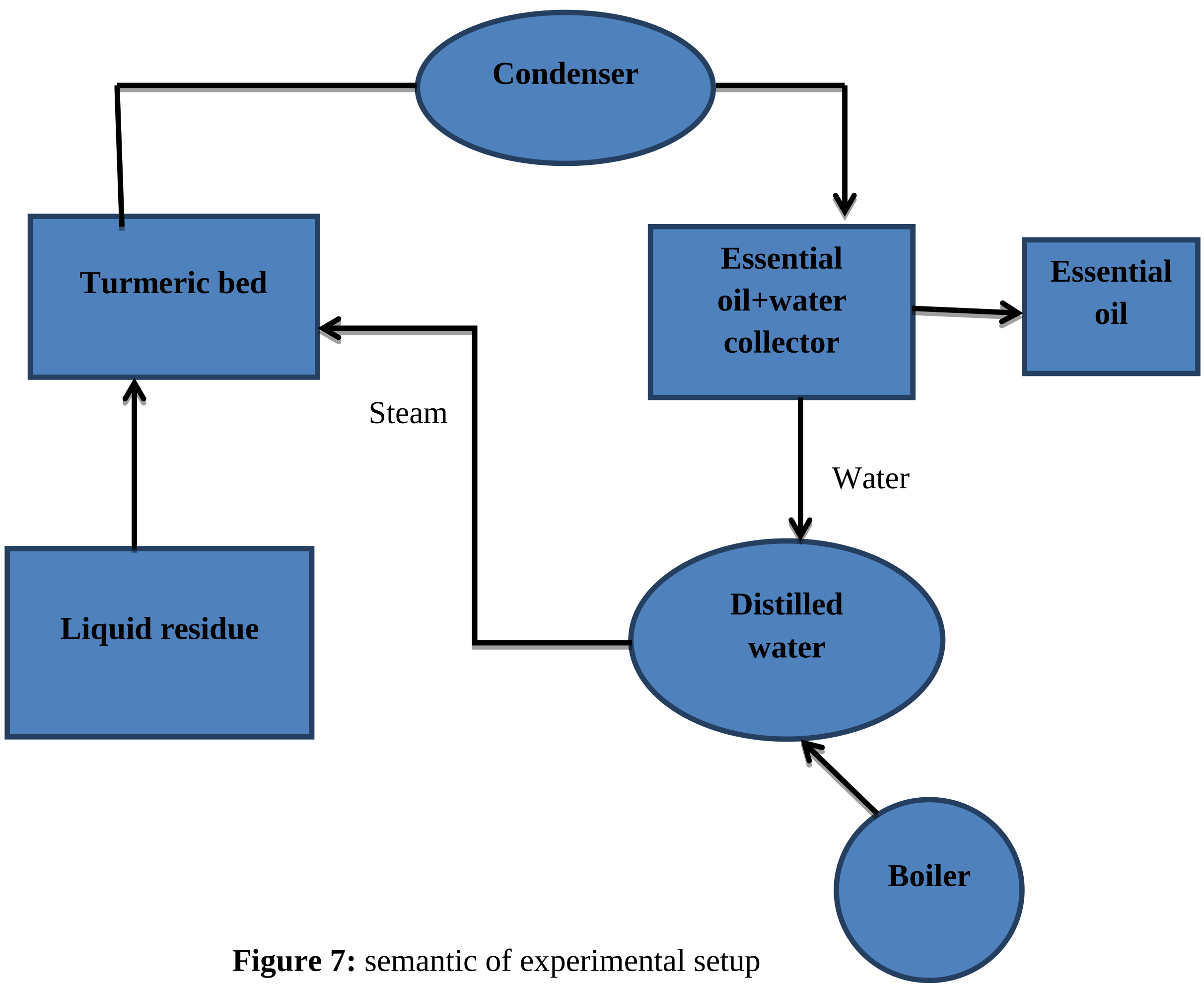
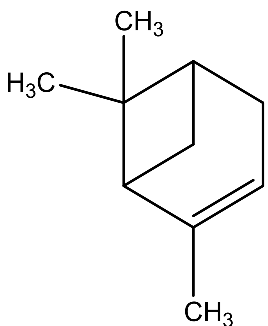


Figure 7: semantic of experimental setup

5.3 GC-MS Analysis

The GC-MS (Gas chromatography-Mass spectrometer spectroscopy) technique is used for the identification of components of turmeric rhizome's essential oil. The GC-MS based on the principle that the compound elute from the capillary column is ionizes into different molecular fragments. A compound having more abundance is considered as the base component with 100% abundance. The GC-MS has been performed at sophisticated analytical lab Thapar Institute of Engineering & Technology, Patiala was identified by matching the obtained result with the GC-MS library. The list of compounds obtained with each enzyme is given in **Table 6 .** The observation of **Table 5** shows that the compounds are present in all case such as β-atlantone, ar-termerone, β-sesquiphellenderene, cineole, α-terpinolene, α-terpinene, α-humelene, γ-terpinene, α-thujene, α-phellenderene, geraniol, β-myrcene, γ-carene, α-pinene. However, the other compounds are specifically found in particular type of enzymes such as nerol, linalool, careen, caryophellene, zingebrene, α-bergamotene, α-turmerone in case of cellulase; β-pinene ,xanthorrhizole, p-cymen-8-ol, caryophellene, zingebrene, β-bisabolane, α-bergamotene, α-termerone, α-atlantone, ascaridole, geranyl acetate these found in case of diastase; β-pinene, carene, p-cymen-8-ol, α-turmerone, α-atlantone, ascaridole, α-cubenene in case of pectinase; trans-sesquisabinene hydrate, p-cymen-8-ol, caryophellene, di-epi-α-cadrene, zingebrene, β-bisabolane, α-bergamotene, α-atlantone, α-santalol, ascaridole, α-cubenene in case of xylose; trans-sesquisabinene hydrate, careen, p-cymen-8-ol, caryophellene, zingebrene, β-bisabolane, α-bergamotene, α-atlantone, α-santalol, ascaridole in case of lipase. The structure of various components are given in Figure 6 and the structure number are corresponding to the serial number of the compound listed in table 6. The all the available turmeric oil compound are broadly categorized in six categorized namely monoterpenoid, bisabolene,sesquisabinane, sesquiturpene cadrene bergamotene, and santalane (**Table 6**). These categorized based upon isoprene unit.



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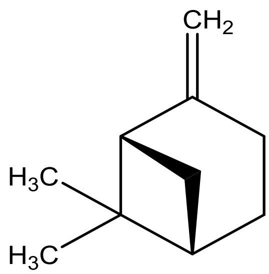

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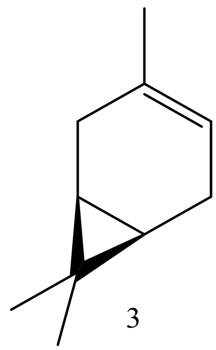

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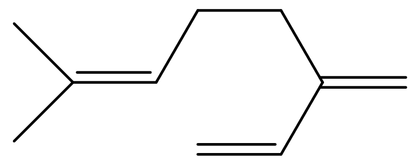

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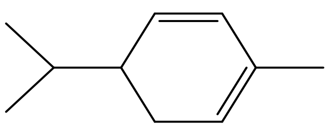

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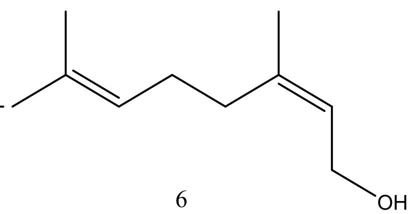

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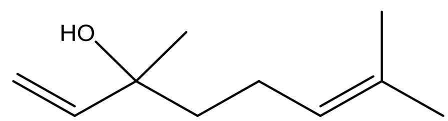

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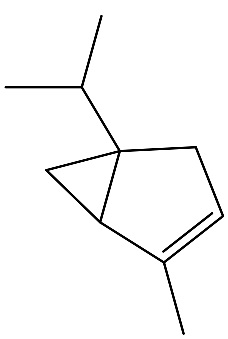

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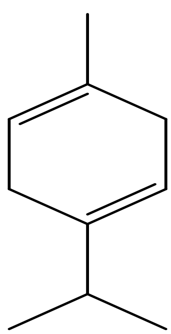

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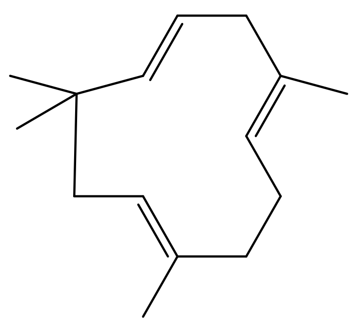

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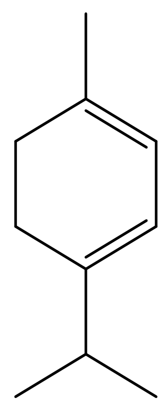

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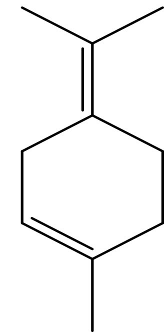
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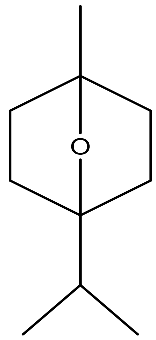
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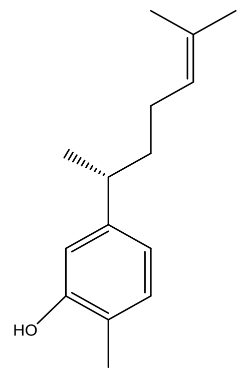
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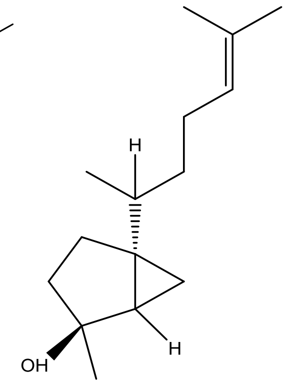
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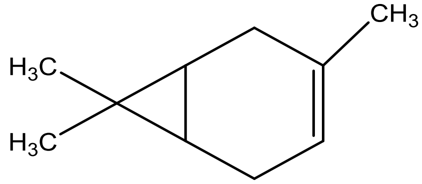
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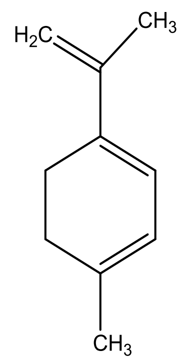
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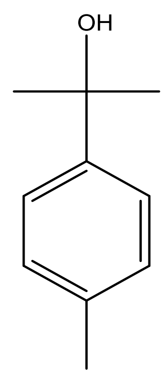
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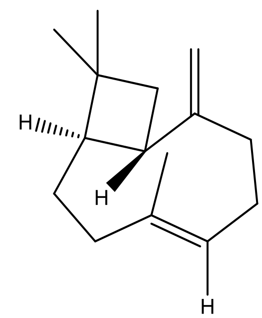
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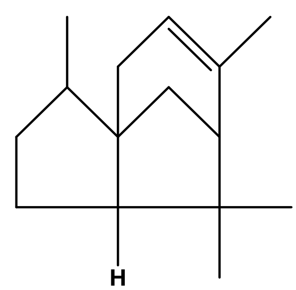
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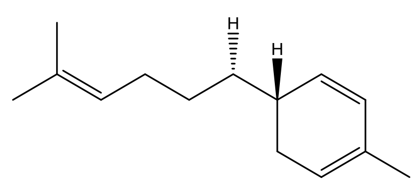
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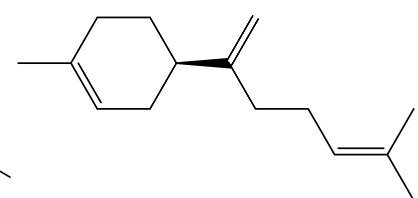
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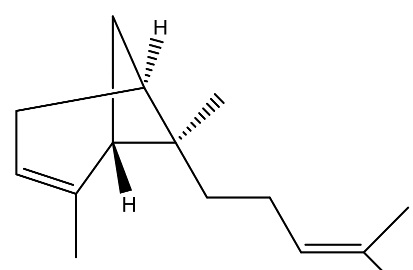
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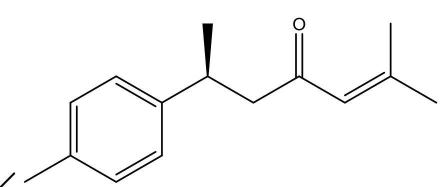
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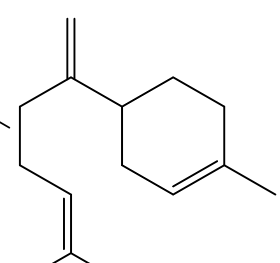
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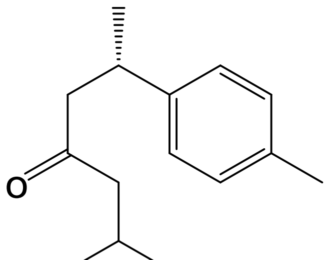

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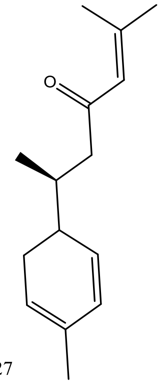

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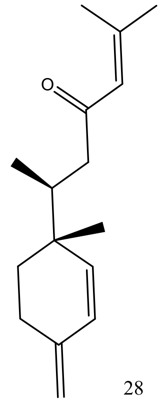

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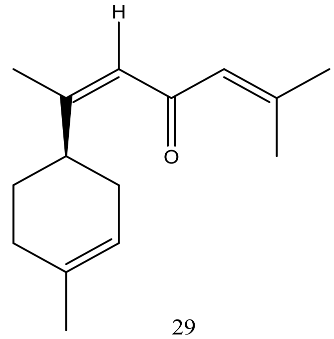

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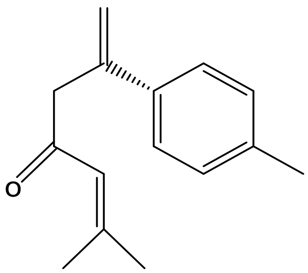

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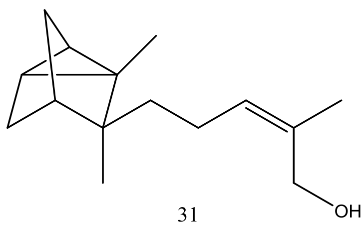

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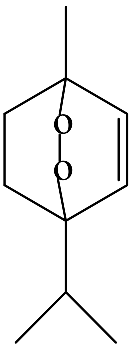

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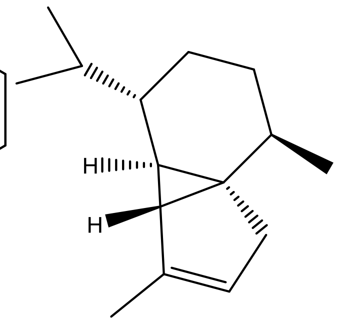

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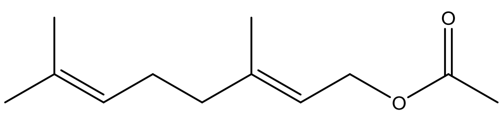

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Figure 8: structure of different components of turmeric oil.

Table 6: Composition of turmeric oil with different enzymes**.**

No.	Compound	Type	Without enzymes	(Cellulase)	(Lipase)	(xylose)	(pectinase)	(diastase)
1.	α-Pinene	Monoterpenoid	✗	✓	✓	✓	✓	✓
2.	β –Pinene	Monoterpenoid	✗	✗	✗	✗	✓	✓
3.	δ-carene	Monoterpenoid	✓	✓	✓	✓	✓	✓
4.	β-myrcene	Monoterpenoid	✗	✓	✓	✓	✓	✓
5.	Geraniol	Monoterpenoid	✗	✓	✓	✓	✓	✓
7.	α –Phellandrene	Monoterpenoid	✓	✓	✓	✓	✓	✓
8.	Nerol	Monoterpenoid	✗	✓	✗	✗	✗	✗
9.	Linalool	Monoterpenoid	✗	✓	✗	✗	✗	✗
10.	α-Thujene	Monoterpenoid	✓	✓	✓	✓	✓	✓
11.	γ-Terpinene	Monoterpenoid	✓	✓	✓	✓	✓	✓
12.	α –Humulene	Sesquiterpene	✗	✓	✓	✓	✓	✓
13.	α- Terpinene	Monoterpenoid	✗	✓	✓	✓	✓	✓
14.	α-Terpinolene	Monoterpenoid	✓	✓	✓	✓	✓	✓
15.	Cineole	Monoterpenoid	✓	✓	✓	✓	✓	✓
16.	Xanthorrhizol	Bisabolane	✗	✗	✗	✗	✗	✓
17.	tans-sesquisabinene hydrat	Sesquisabinane	✗	✗	✓	✓	✗	✗
18.	Carene	Monoterpenoid	✗	✓	✓	✗	✓	✗
19.	P-Cymen-8-ol	Monoterpenoid	✓	✗	✓	✓	✓	✓
20.	Caryophyllene	Sesquiterpene	✓	✓	✓	✓	✗	✓
21.	di-epi- α –Cedrene	Cedrane	✗	✗	✗	✓	✗	✗

22.	Zingebrene	Bisabolane	✓	✓	✓	✓	×	✓
23.	β-sesquiphellenderen	Bisabolane	✓	✓	✓	✓	✓	✓
24.	β –bisabolene	Bisabolane	×	×	✓	✓	×	✓
25.	α-bergamotene	Bergamotane	✓	✓	✓	✓	×	✓
26.	Ar-turmerone	Bisabolane	✓	✓	✓	✓	✓	✓
27.	α-turmerone	Bisabolane	✓	×	✓	×	×	✓
28.	β –Turmerone	Bisabolane	×	×	✓	✓	×	×
29.	α –Atlantone	Bisabolane	×	×	✓	✓	✓	✓
30.	β-Atlantone	Bisabolane	×	✓	✓	✓	✓	✓
31.	α –Santalol	Santalane	×	×	✓	✓	×	×
32.	Ascaridole	Monoterpenoid	×	×	✓	✓	✓	✓
33.	α –Cubebene	Sesquiterpene	×	×	×	✓	✓	×
34.	Geranyl acetate	Monoterpenoid	×	×	×	×	×	✓
35	β-cedrene	Cadrane	✓	×	×	×	×	×
36	Himachalene	Himachalene	✓	×	×	×	×	×

The turmeric oil obtained by enzymatic pretreatment process is found rich as compare to the oil obtained by without pretreatment. It may possible that the rich turmeric oil obtained by enzymatic process will have more medicinal properties than other traditional essential oil extraction methods. The maximum number of compounds were obtained in case of cellulase pretreatment whereas lipase is the next one. The table 6 also shows that the minimum number of compounds were found in case of pectinase which is the main finding of this work. Hence, the cellulase is very good for the pretreatment of turmeric rhizome over other enzymes considered for present work. The chromatogram of turmeric pretreated with cellulase is Given in **Appendix A**.

Chapter 5

Conclusion

The present work was aimed to find out the possibility of enzymatic degradation as pretreatment to obtain enhanced turmeric oil yield over the conventional process such as hydro distillation, steam distillation, cold pressing etc. The turmeric oil was successfully obtained from the fresh turmeric rhizomes by pretreatment with enzymes and followed by hydro distillation. The obtained turmeric oil by enzymatic pretreatment process is found rich in terms of medicinal components than the other traditional methods. The maximum yield was obtained with cellulase enzyme which is 25-27% higher than the yield obtained by traditional hydro distillation process reported in the literature. The quality of turmeric oil (essential oil) are usually estimated with the chemical composition of obtained essential oil. In case of enzymetic pretreatment with cellulase, the obtained oil is rich in terms of composition. Although some of the compound are not present in case enzymatic processing, which are present in case of hydro-distillation.

The major identified compounds are monoterpenoid, sesquiterpene, santalane, bisabolane, bergamotane, cedrane, sesquisabinane. The medicinal activity of the obtained oil is needed to be investigating in future. Overall the present process is suitable for obtaining turmeric oil using enzymes and the cellulase is identified as more effective enzyme for the pretreatment of turmeric rhizomes. Furthermore, the present process can be concluded as the less energy-intensive, efficient, economical and ecofriendly.

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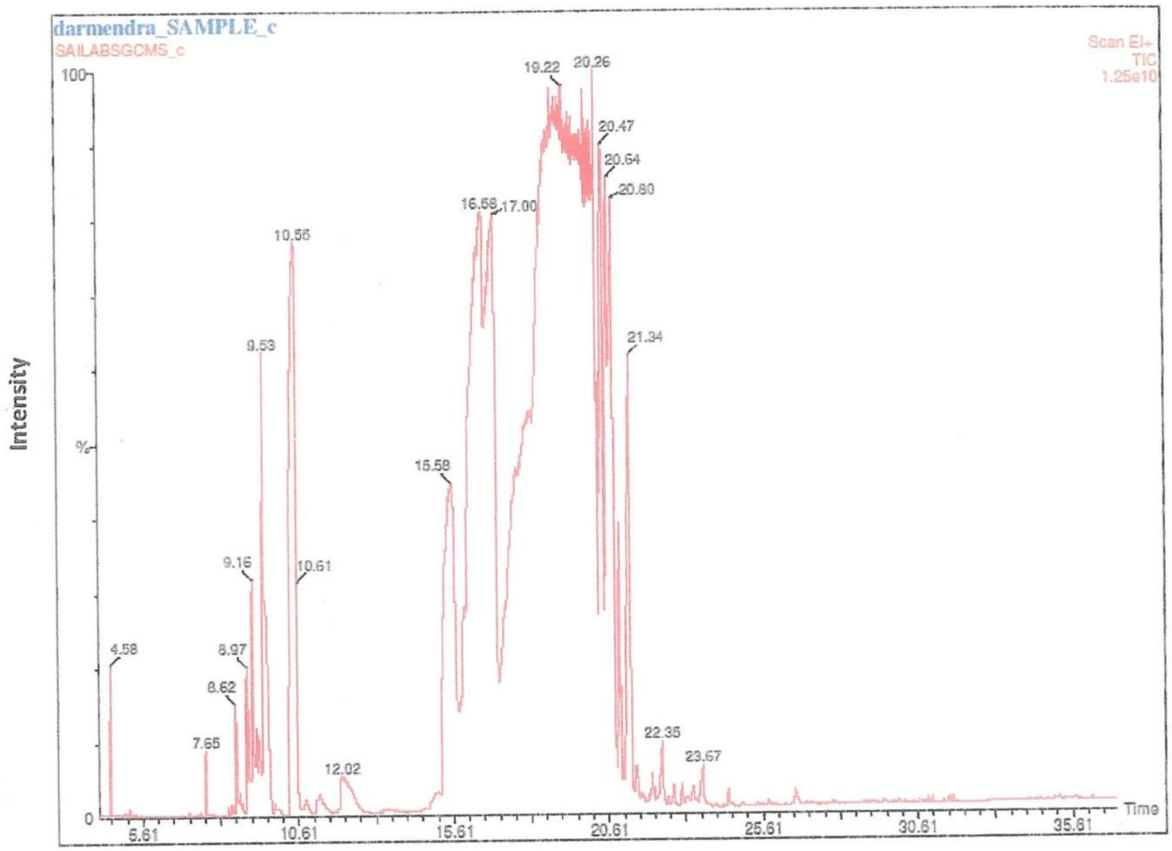
Appendix A



Retention time (min)

Figure 6: chromatogram of sample pretreated with cellulose.