"Design, Development & Evaluation of Curcumin-Gallic acid complex based OTF and Formulation development of Amla sachet with special reference to Anti-oxidant activity"

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CERTIFICATE

*This is to certify that the dissertation work entitled "**Design, Development & Evaluation of Curcumin-Gallic acid complex based OTF and Formulation development of Amla sachet with special reference to Anti-oxidant activity**" submitted by **MS. GADHIYA ANKITA H.** (13MPH501) in partial fulfillment for the award of Master of Pharmacy in "Phytopharmaceuticals and Natural products" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree to this or any other university or institution.*

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DECLARATION

I hereby declare that the dissertation entitled "Design, Development & Evaluation of Curcumin-Gallic acid complex based OTF and Formulation development of Amla sachet with special reference to Anti-oxidant activity", is based on the original work carried out by me under the guidance of Dr. Sanjeev Acharya, Associate professor, Department of Pharmacognosy, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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ABSTRACT

Curcuma longa (Zingiberaceae) is a plant well known as a spice and colouring agent with having uses like Anti-inflammatory activity, Antioxidant activity, wound healing etc having an active constituents such as Curcumin which is reported as very poorly soluble in water. Plants belonging to *Emblica officinalis* (Euphorbiaceae) mentioned in many traditional literaturers for it's as a wonder herb and a great source of Vitamin C. It is having Vitamin-C which is accepted by the human body and it is bonded with tannins that protect it from being destroyed by heat or light. Good source of tannins and polyphenols like Gallic acid, Ellagic acid etc are active constituents of Amla. Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, and a type of organic acid also known as 3, 4, 5-trihydroxybenzoic acid. It is found both in free and as part of hydrolysable tannins. It is having good water solubility. So, the major aim of this research was to "Design, Development & Evaluation of Curcumin-Gallic acid complex based OTF and Formulation development of Amla sachet with special reference to Anti-oxidant activity". So, these present study focuses on the Solubility and Antioxidant Property of both the drugs. The samples were collected and identified for the mechanism of Antioxidant activity. Initial estimation, evaluation and optimization were conducted on the powder samples of Curcumin, Gallic acid and Amla extract powder. There was developed method for forming complex of Curcumin and Gallic acid to enhanced Curcumin solubility. Then a ratio was optimized C:GA(9:1) of 4^{th} method. There were performed many Antioxidant assays on Curcumin, Gallic acid and C:GA(9:1) like DPPH, ABTS, TEAC etc. After observing the IC50 of all the drugs there was determined its Antioxidant Potential and the % Antioxidant Potential of C:GA(9:1) was more than Curcumin and Gallic acid. So, there was decided to make a formulation containing this C:GA(9:1) for increasing Bioavailability and Solubility of Curcumin in water. So, a Oral Thin Film was made by polymer PVA(4%), Plastzicer(PEG 20,000) 20% w/w of polymer with solvent ethanol and then it was evaluated by many parameters

There was to determine the Vitamin C content, Gallic acid and Antioxidant Potential of Fresh Amla Fruit and Amla extract. So, after estimation of all parameters there was observed that Amla extract is having more Vitamin C content, Gallic acid content and Antioxidant Potential the Fresh Amla fruits. So, there was to make a Antioxidant drink (Ready to drink) by using the Amla extract powder with some sweetening agent.

By observing the calculations and trying three ratios 1:1, 1:1.5 and 1.2. the optimized ratio was 1:2 and the Amla sachet was made of 10grams. The 10 grams of Amla sachet is having more Vitamin C, Gallic acid content and % Antioxidant Potential then 10 Fresh Amla Fruits.

AIM AND OBJECTIVES

AIM

AIM 1: Development and Evaluation of Oral Thin Film of Curcumin-Gallic acid complex to improve its solubility and Anti-oxidant Activity

AIM 2: Development and Evaluation of Amla Sachet as Antioxidant Drink

OBJECTIVES

- Identification of active ingredients using Physical Evaluation and Compatibility Studies.
- To develop analytical methods for estimation of Gallic acid and Curcumin using UV spectrophotometer.
- To find IC50 of Gallic acid and Curcumin using DPPH method.
- To study solubility profile using various approaches.
- To develop and optimize method of preparation for Curcumin Gallic acid complex based on its water solubility and to find IC50 of Gallic acid, Curcumin and its optimized ratio by different Antioxidant assays.
- Compatibility studies by using TLC, IR, MASS, NMR and DSC types of analytical methods.
- Mucoadhesive sustained release oral thin film of Gallic acid and Curcumin formulation.
- Optimization of formulation for an Amla sachet (Ready to Drin

1. INTRODUCTION

In the last few decades, the growth of the herbal medicine and its drugs are gaining popularity exponentially in the both developing and developed countries due to its natural origin. In past one decade, nearly 80% of the global population all over the world such as Europe, USA, Japan, China, Canada etc are still depended upon the herbal drugs for their health care. So, there is seen its increase demand for the pharmaceutical products of Ayurveda in all over the world. As this all herbal medicinal plants have played very vital role in the development of human culture. (Ajay K.M. et al 2009). In Germany, also they are using herbal medicines as phytoproducts and also the subject to the criteria for its efficacy, safety and quality of the drugs. There are 21,000 plants which are used as medicinal plants and they are by World Health Organization. India is the largest producer of the herbal medicinal plants and it is known as "Botanical Garden of the World".

Medicinal plants are used for curing various diseases, various treatments and many pharmaceutical herbal formulations. Medicinal plants are doing development of this traditional Indian system of medicines but its safety, efficacy, quality and to preserve the traditional heritage. So, in all over the world these herbal medicines are used as primary health care. They are also used in combination of two or more than two. Literally, the meaning of Ayurveda in ancient Indian time is "Science of life ". Still in this modern age also there is something in Ayurvedic medicines which proves the ancestors right that it is a "Science of life". Now a day's use of allopathic medicines cures the disease immediately but an Ayurvedic medicine cures slowly but perfectly from its roots.(M. Umadevi et al 2013).

1.1 Introduction to Anti-Oxidant Activity

The utilization of oxygen in humans has provided the benefit of metabolizing fats, protein and carbohydrates for energy. The highly reactive oxygen atom has a capability of becoming a part of potential damage of molecules commonly known as “Free radicals”.

They are capable of attacking the cells of the body, which caused them to lose their structure and location. Damage of the cell is caused by free radicals which appear to be a major contribution to aging and to degenerate diseases of aging such as cancer, cardiovascular disease, brain dysfunction cataracts and immune system decline. Free radicals are electrically charged molecules i.e. they are having unpaired electron, which causes them to seek out and capture electrons from other compounds to neutralize themselves.

Antioxidants are having capability to stabilize or deactivate the free radicals before they attack the cell. They are the compounds which are protecting cells from the damaged caused by unstable molecules which are known to be free radicals. They are interacting and stabilizing free radicals and sometimes it may also prevent some of the damage free radicals otherwise they might cause damage to the cells. The damage caused by free radicals also leads to cancer. Examples of anti-oxidants include beta-carotene, lycopene, Vitamin C, F and A (Mohsen A. Et al 2013.)

They delay oxidation and prolongs shelf life. They neutralize the effect of free radicals through different ways and prevent the body from many types of disease. There are many things which increases the body’s oxidant load, like vigorous exercise , chronic inflammation , infections , illness , cellular metabolism , exposure to drugs or toxins such as cigarette , smoke , pesticides , insecticides , pollution etc.

They are often acting as a reducing agent such as ascorbic acid, polyphenols, thiols etc. Because an anti-oxidant molecule is capable of slowing or preventing the oxidation of other molecules. Oxidation reactions are therefore those in which the central carbon of a functional group is transformed into a more highly oxidized form, and reduction reactions are those in which the central carbon is transformed into a more highly reduced form. Reactions done by oxidation are producing free radicals which damage the healthy cells by chain reaction (Cecila B. et al 2014). So, this anti-oxidant terminate the reactions by removing the intermediates of free radicals and that is why it is inhibiting other oxidation reactions by oxidizing themselves. So, they are known as reducing agent. Now a days they are also been widely used as ingredients in dietary supplements for maintaining health and prevention from the disease such as cancer and coronary heart disease. So, the uses of natural antioxidants are increasing in the food. They are abundantly found in fruits and vegetables. E.g.: Beta-carotene, Lutein, Lycopene. (Dr. Mark Pericival 1998).

1.2 Role of herbal drugs as anti-oxidants

"Herbal Medicine"- A growing field with a long lasting tradition. The knowledge, skills and practise based on experiences, theories, beliefs to different and various cultures used in the maintenance of health and in the prevention, diagnoses, improvement or treatment of physical and mental illness is said to be Traditional medicines. Vitamin-C is occurring naturally in citrus fruits like orange, lemon, amla etc.

They are highly water soluble in nature and having powerful anti-oxidant properties and has ability to regenerate other biological important anti-oxidant such as glutathione, Gallic acid and Vitamin-E. It also

functions as a reducing agent for oxidation involved in drug – metabolism by inactivating a wide variety of xenobiotic substances hormones. Therefore, it is used in supplementation of Vitamin-C could enhance many life – supporting biochemical systems (Swetha D. et al 2014).

CURCUMIN: Turmeric is a spice which is from the rhizomes which are belonging to the family of ginger (Zingiberaceae) component called Curcuma Longa. The principal curcuminoid found in turmeric is considered as its main constituent. It is a hydrophobic polyphenol.

The bright yellow colour of turmeric comes from fat-soluble polyphenols pigment known as curcumin. It was originally isolated 200 years ago.

It contains active ingredients like 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin.(Fig 1.1 Curcumin).

Fig. 1.1 Curcumin

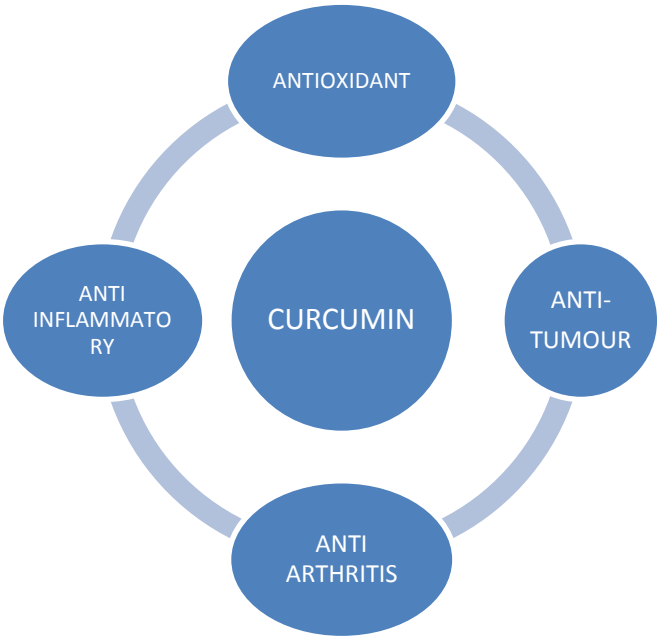

Fig. 1.2 USES OF CURCUMIN

There is a long history of medicinal use in India and Southeast Asia for different medical problems. It was reported as it is having many properties like Anti-Tumour, Anti-Oxidant, Anti-Arthritis and Anti-Inflammatory.

AMLA: It is the gift of nature to mankind and so it is known as wonder herb. It consists of fresh fruits of *Emblica officinalis* (Euphorbiaceae). It is rich in polyphenols, minerals and a great source of Vitamin-C. It has protein, fibres, carbohydrates, minerals and Gallic acid which is a potent polyphenols.(Fig 3.3 AMLA)

Fig 3.3 AMLA

Amla is providing strength and wellness to the body. It keeps us away from all the diseases by boosting our immune system. Because of its cooling nature it is used in treatments for burning sensation, inflammation, fever etc. It is having Vitamin-C which is easily accepted by the human body and it is bonded with tannins that protect it from being destroyed by heat or light (Ekta S. et al 2011). There is isolated a active constituents of Amla known as Gallic acid.

GALLIC ACID: It is extracted from citrus fruits like Orange, Amla, Grapes, Lemon that has shown to inhibit the formation of amyloid fibrils, one of the potential cause of Alzheimer's and Parkinson's disease. It is a trihydroxybenzoic acid, a type of phenolic acid, and a type of organic acid also known as 3, 4, 5-trihydroxybenzoic acid. It is found both in free and as part of hydrolysable tannins. It is a weak carbonic anhydrase inhibitor.

Autoxidation and oxidation of GA have been studied for many years due to its presence in various foods and its activity in living cells (mainly as antioxidant). The low hydrophobicity of gallic acid plays a vital role as it is poorly permeable across the intestinal epithelial cells and also minimize gastrointestinal tract absorption, leading to decreased oral bioavailability.(Singh et al 2010)

1.3 Problem Definition

As per the target, Curcumin is hydrophobic drug so, to increase its solubility in water by using some hydrophilic carriers like Piperine, Gallic acid etc. By making a complex of Curcumin with one carrier due to this there is increase in its solubility and also synergistically increase in its antioxidant potential.

2. REVIEW OF LITERATURE

The term "Medicinal plants "includes that it having various types of plants that are used as herbs and they also have its medicinal activities. These plants have been used in drug development and synthesis and these plays a critical role in the development of mankind. They are the plants which are having a promise with future because there are about half million plants in the world itself, and most of them are having medicinal use. It has been prove that the active constituents of the plants have properties to prevent and cure some disease (K.C. Velayudhan et al 2012).

2.1 Introduction and Role of plants *Curcuma longa*

(a) Curcuma longa

2.1.1 Biological Source: It is a spice derived from the rhizome of *Curcuma longa*

Fig. 2.1 *Curcuma longa*

2.1.2 Taxonomical Classification

Kingdom: Plantae – Plants
Family: Zingiberaceae
Class: Liliopsida
Genus: Curcuma
Species: longa

2.1.3 Vernacular name

Common name – Turmeric

English – Turmeric

Hindi – Haldi

Gujarati – Haldar

2.1.4 Introduction to genus *Curcuma*

The genus Curcuma (Zingiberaceae) is including more than 70 species having rhizomes as an herbal part of the plant. They are mostly grown in hilly areas of the world, some are cultivated in gardens and used as a food preservative, colouring agent, as a spice and medicinal plants. The volatile oil of this rhizomes and leaves of the plants have been obtained by solvent extraction or steam distillation while the essential oil is isolated from the rhizome of Curcuma zedoaria is showing good cytotoxicity, anti-oxidant and anti-microbial activities. Accordingly, the extracted essential oils from the dried rhizomes of curcuma longa, curcuma aromatic curcuma sichuanensis are having anti-oxidant properties of the essential oils (Nilanjana D. et al 2013).

2.1.5 Different species:

Curcuma longa, C.aromatica, C.sichuanesis, C.zedoaria, C.domestica, C.sattayasaii and C.alismatifolia (Hiroshi H. et al 2011)

2.1.6 Nutritional composition of Turmeric

Table 2.1 Nutritional composition of Turmeric

Serial No.	Constituents	Quantity per 100gm
1	Ascorbic acid(mg)	50.0
2	Ash(g)	6.8
3	Calcium(g)	0.2
4	Carbohydrate(g)	69.9
5	Fat(g)	8.9
6	Food energy(K Cal)	390.0
7	Iron(g)	47.5
8	Niacin(mg)	4.8

9	Potassium(mg)	200.0
10	Phosphorus(mg)	260.0
11	Protein(g)	8.5
12	Riboflavin(mg)	0.19
13	Sodium(mg)	30.0
14	Thiamine(mg)	0.09
15	Water(g)	6.0

2.1.7 Introduction to specie *Curcuma longa*

From the ancient immemorial time, the *Curcuma longa*, the yellow colour root, this has been cultivated in India, Southern China and other tropical and subtropical countries. This yellow colour roots are been obtained from East India, but it is not a wild plant. This is similar to ginger as its growth pattern is same as ginger. It can grow to a height of approximately 1 metre. They had grown in damp and warm areas. The underground parts of the plants are dug up in December and January. Root stocks are been separated from the side shoots of the plants. They are submerged in boiled water and then dried in presence of sun. By drying, the underground parts it forms its yellow colour as the heat of the sun causes the pigment from the glandular cells to the over the rootstocks and its rhizomes. Once these rhizomes are dried under the sunlight they form a powder which is called “Curcumin” or “Curcuma” and sold as “Turmeric”.

Morphology of rhizomes

The rhizomes are long or short and interconnected rhizomes are forming a series known as symposium which means ‘jointed feet’ in Greek. It is known as medicinal ginger because it is a golden spice of life and has been used in India which is having a very strong bonding with the socioculture life (Larsen *et al*., 1999).

Chemical constituents of curcuma longa

Essential oils(2-7%) consisting of 60% sesquiterpenes with methyl-p-tolyl carbinol, orange colorants such as curcumin (1.5-5.4%), monodesmethoxycurcumin(0.8%), didesmethoxycurcumin(0.5%) and others like turmeron, aturmeron, atlanton and zingiberene. The medicinal effect of curcumin is mainly due to the yellow-orange colour pigmented substance known as curcuminoids which provides the healing effect also. They are polyphenols with fat-soluble activity and a non-volatile substance in nature. There is 75-80% of curcumin divided in three parts like curcumin-A (diferuloylmethane), curcumin-B (demethoxycurcumin) and curcumin-3 (bisdemethoxycurcumin).

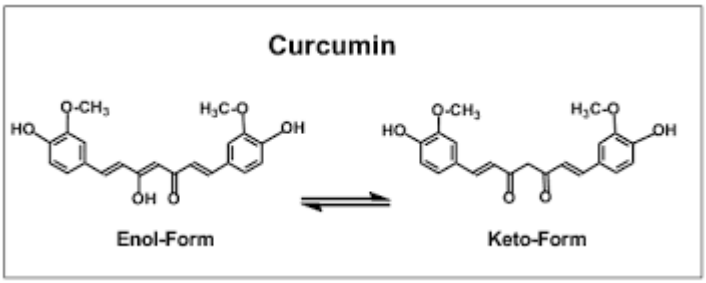

Fig. 2.2 Curcumin

Uses

Most useful as a spice and colouring substance , Antioxidant, pro-inflammatory eicosanoids, immunomudulation, inflammatory intestinal disorder, infections, depression, psoriasis, stomach ulcers, wound healing, rheumatoid arthritis, atherosclerosis, asthma, cataracts, Alzheimer's disease etc (M. Akram et al 2010).

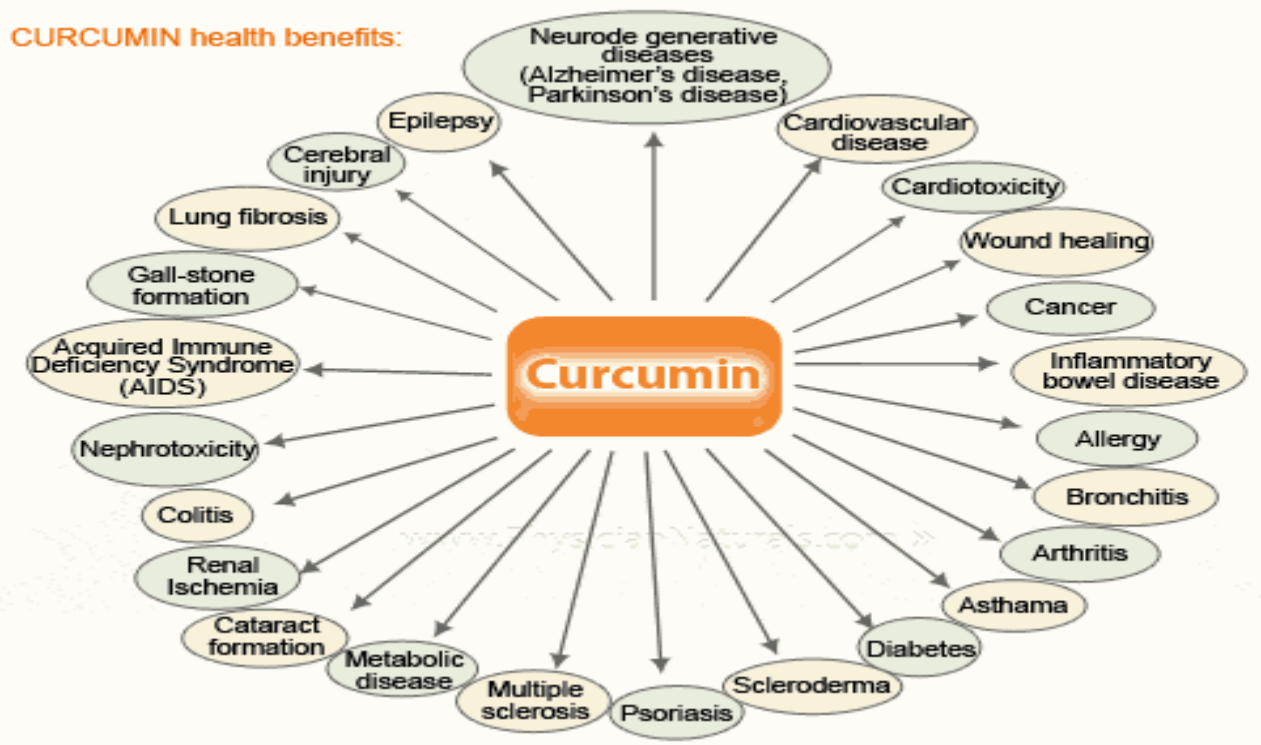

Fig. 2.3 Curcumin Health Benefits

2.2 Literature review of *Curcuma longa*

2.2.1 Pharmacognostic review

Policegoudra R.S et al.,(2011) reported that mango ginger which is having a typical exotic flavour, coolant and promote digestion. Also, giving wide variety of biological activities like anti-oxidant, anti-bacterial, anti-cancer, anti-depressant etc. This species may be concluded that *Curcuma amada* is having number of active constituents in which one is responsible for enhancing the action of other ingredient. So, this species is used in Ayurveda and also in other traditional medicines.

Sharad et al., (2006) they reported pharmacognostic evaluation of the drug *Curcuma haritha* Linn. By concluding that the macroscopical and microscopical parameters of this drug are very useful for

identification of the species this will be helpful in pharmaceutical industries for the authentication of commercial samples.

Preetha Anand et al.,(2008) they reported on the status of approaches in which generated a "Super Curcumin". As it is a yellow colour Indian spice which is useful for various disease but its utility is limited sometimes due to its colour, lack of water solubility and relatively very low bioavailability. So, they used "man-made" Curcumin analogues to overcome these problems.

M.S. Brewer (2011) have shown the overview on Natural Anti-oxidants which are playing a vital role as sources, compounds, mechanism of action and potential applications in human life.

Jaggi Lal (2012) concluded that it is a extraordinary molecular structure which is imparting colour and flavour to the food while it shows strong anti-oxidative, as well as anti-inflammatory properties. So, it was known as Kitchen Queen.

More Minokshi S. et al.,(2012) they concluded that a herbosome which is formed by liposome act as a bridge between novel drug delivery system and conventional system. It is used foe enhancement of bioavailability of herbal extract for medical applications because a molecule of phospholipids are acting as a carrier which is made up of water soluble head and two fat soluble tails, due to this nature they are having dual solubility and thus acting as an effective emulsifier.

Nilanjana et al.,(2013) examined that the extracts of *curcuma longa* have neuropharmacological activity as evident by its reduction in locomotor activity and coordination of muscles. By its all preformulation studies they gave the result that the aqueous extract has greater CNS depression activity in comparison with the ethanolic extract.

Tasneef Ahmad. et al.,(2013) they conclude that the phytochemical examination of a plant involves the selection, collection, identification, extraction and authentication of the plant material and the biosynthetic pathways are examined by particular compounds, quantitative evaluation and pharmacological activities.

2.2.2 Phytochemical reviews

Guide shoba. et al.,(1997) reported that the influence of piperine is on the pharmacokinetics of curcumin in animals and human volunteers. As, piperine increases the serum concentration which enhances the absorption and bioavailability of curcumin in rats and humans by giving no adverse effects.

Deepthi V., Jatiraju .et al.,(2013) concluded about natural bio enhancers which are a type of chemical entities which promote the bioavailability of the drugs by mixing with them and do not give synergistic effect.

Vedamurthy Joshi et al., (2010) reported a comparative study on solution stability and dissolution behaviour of solid dispersions of curcumin by using carriers like PEG-4000, PEG-6000 and PVP K-30. In presence of Ascorbic acid, Tartaric acid and Citric acid there is increase in curcumin aqueous stability relatively by 3 folds in pH 7.4

Sushma Drabu et al., (2011) showed the herbal plants as bio enhancers in Ayurveda. These natural bio enhancers reduce the dose and reduce the drug-resistance problems. Natural bio enhancers like piperine, ascorbic acid, Gallic acid, sinigrin etc. This when used in combinations with drug classes like anti-viral, antibiotics, anti-fungal etc they are quite effective while it also improves the oral absorption of minerals, vitamins, amino-acids etc. So, mainly they affect absorption, drug metabolism and drug – target action.

Aude Munin et al., (2011) reviewed on encapsulation of Natural polyphenolic compounds which possess scavenging properties towards oxygen species and proteins. So, polyphenols are encapsulated for food, cosmetics and pharmaceutical industries.

Ghanshyam B. Dudhatra et al.,(2012) gave some review on mechanism of action of bio enhancers and its role as a bioavailability and bio efficacy enhancer.

Umesh K. Patel et al., (2011) explained the role of piperine as a bioavailability enhancer by its increase level of serum and lengthen the serum half lives of some nutritional substances, such as beta-carotene and co-enzyme Q10. So, when piperine is given along with various drugs and nutrients it shows its best bio enhancing ability.

Cretu R.et al., (2011) they improved solubilisation of curcumin with a micro emulsification formulation. So, there was concluded that there was seen enhancement of solubility.

Sav Ajay et al., (2012) wrote the review article on solubility and dissolution rate enhancement of curcumin using Kollidon V A64 by solid dispersion technique.

Modasiya M. et al., (2012) studies on solubility of curcumin they concluded that using solid dispersion technique to increase its water solubility.

Kritika Kesarwani et al., (2013) gave a review on the bioavailability enhancers of herbal origin with the mechanism of action and improvement in drug bioavailability, exhibited particularly by natural compounds.

Ehab A. Abourashed (2013) gave a brief introduction on the bioavailability related to natural products having anti-oxidant activity. So, this research includes more than 40 compounds belonging to the above mentioned classes of natural anti-oxidants.

Kumavat Suresh. et al., (2013) showed enhancement of solubility and dissolution rate of curcumin by solid dispersion technique by using PEG(4000,6000) in the ratio of 1:3,1:5 and 1:10 respectively which attempt to increase the solubility and dissolution by fusion method.

2.3 Solubility profile of curcumin in water

Curcuminoids plays a great role in the treatment of various traditional and allopathic system of medicine. Solid dispersion techniques has been used to enhance the absorption of poorly soluble drugs and its dissolution by dispersing the drug in a highly water soluble carrier in a solid state.

It is having very low solubility in water while highly soluble in acetone, dichloromethane and diethyl sulfoxide. In various studies it has been proved that safety and efficacy of curcumin at very high doses, however the relative bioavailability of curcumin is of major concern and still it is unclear when metabolizes into active or inactive metabolites because it is having extremely very low water solubility. So, some novel drug delivery systems are used to overcome these problems.

Due to this problem curcumin is not been approved as therapeutic agent. So, there are done many attempts to increase its bioavailability via solubility, stability and permeability.

Curcumin is having ability to develop as a therapeutic agent through its improvement in formulation properties or delivery systems, enabling its enhanced absorption and cellular uptake (Sav A. et al 2012).

2.4 Introduction and role of *Emblica officinalis*

(b) Emblica officinalis

2.4.1 Biological source: It consists of fresh fruits of *Emblica officinalis.*

2.4.2 Taxonomical classification

Kingdom: Plantae

Division: Angiospermae

Family: Euphorbiaceae

Genus: Emblica

Species: Officinalis

Fig. 2.4 *Emblica officinalis*

2.4.3 Vernacular names

English: Indian gooseberry

Sanskrit: Aamalaki

Hindi: Amla

Marathi: Amla

Gujarati: Ambla

2.4.4 Introduction to genus *Emblica*

In India, the herbal plants are used in Ayurvedic medicines as they are having most common ingredients for making ayurvedic preparations like Lehya, Churna etc. The methanolic extract of Amla is having anti-microbial activity than chloroform and diethyl ether extracts. In rural areas also this drug is used for anti-inflammatory and anti-pyretic treatments. It is used to formulate new and more anti-microbial drugs of natural origin by observing this study. The content

of ascorbic acid is more citrus lemon than in *Phyllanthus acidus* and *Phyllanthus emblica*. Amount of cholesterol is more in *Phyllanthus emblica* and level of total carbohydrates, starch and protein is more in *Phyllanthus emblica*.(Anil K. Et al 2012).

2.4.5 Nutritional composition of Amla

Table 4.2 Nutritional composition of Amla

Serial No.	Constituents	Quantity in %
1	Moisture	81.1
2	Protein	0.5
3	Fat	0.1
4	Mineral Matter	0.7
5	Carbohydrates	14.2
6	Fibre	3.4
7	Calcium	0.05
8	Phosphorus	0.02
9	Iron	1.2mg /100g
10	Nicotinic Acid	0.2mg /100g
11	Vitamin-C	600mg /100g

4.4.6 Introduction to specie *Emblica officinalis*

There are many studies done on Amla to show its anti-microbial and anti-oxidant activity, therefore it also can be further explored for the isolation of its bioactive compounds. The phytochemical test of the extract of *Emblica officinalis* has reported the presence of flavonoids, terpenoids, tannins and phenolic compounds. Leaves of this plant have been used for anti-inflammatory and anti-pyretic treatments. The milky juice of the leaves is also good for the treatment of sores while its infusion is used for eyes sores.

The fresh fruit is also used as laxative so occasionally it is preserved in sugar. Decoction of leaves and seeds are used in treatment of diabetes. Juice of the bark with honey and turmeric is very much useful in treatment of gonorrhoea. The juice or extract is used to stop painful respiration and hiccoughs. As used as anti-oxidant also used to either lighten or dipigment the skin by playing its role as skin lightens (D.A. Dhale 2012).

Morphology of Fruits

They are round or oblate in shape but almost sessile. They are having diameter 2.5cm with 3 celled drupes, indented at the base and smooth through 6 to 8 pole lines, extending from the base to the apex by appearing into segments and lobes. When they becomes mature there colour is whitish or dull greenish yellow or occasionally brick red. The skin is translucent, thin and sticked with a juicy pulp, but ripe fruits are astringent, extremely acid and some are distinctly bitter.

Chemical constituents

An Indian scientist has designated “Phyllemblic” the active ingredient which is significantly having pharmacological action. The fruit of Amla is rich in quercetin, phyllemblic compounds, Gallic acid, tannins, flavanoid, pectin and Vitamin-C with some polyphenolic compounds. The fruits, leaves and bark are rich in tannins. The seeds are having brownish yellow colour oil which is fixed oil (16%). Many fatty acids like linolenic (8.8%), linoleic (44%), oleic (28.4%), stearic (2.15%), palmitic (3.07%) and myristic (1%). Phytochemicals like ellagic acid, Gallic acid, corilagin (ellagitannins), furosin and geraniin (Dehydroellagitannin). Flavanoids like quercetin while alkaloids like phyllantine, phyllantidine. Highest concentration of Vitamin-C is (478.56mg / 100ml). Hydrolysable tannins include Emblicanin A and B, pedunculagin, punigluconin, chebulinic acid (Ellagitannin), chebulagic acid (Benzopyran tannin) and Ellagotannin. Organic acids such as citric acid (Khan et al 2009).

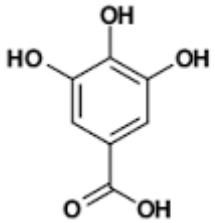

Fig. 2.5 Structure of Gallic acid

Uses

This is a plant with plenty of uses like energy refiller, anaemia therapy, fights with acidity, prevents from tannins, urinary trouble frustration agent, relieves leucorrhoea, effects on urinary stone, improves body weight, used as febrifuge, as aperients(stimulate the appetite), anti-fungal, anti-bacterial, anti-viral, anti-oxidant, aphrodisiac, used for boils and spots, chelating agent, in constipation, dental problems, diabetes, diarrhoea, diuretic, fever, gonorrhoea(inflammatory discharge from the urethra or vagina), hair growth, headache, indigestion, inflammation, mouth ulcers, nausea, nose bleed, perfumery, pruritis (marked by itching), respiratory problems, scurvy, skin sores, skin whitening, sore eyes, tonic, rheumatism, heart disease, treats hypertension, remedy for menstrual cycle, liver tonic, vaginal complaints, water purification, in ageing and also as a vermifuge (Ekta S. et al 2011).

2.5 Literature review of *Emblica officinalis*

2.5.1 Pharmacognostic reviews

Anthony C. Dweck gave some new ideas for the ayurvedic wonder known as Amla because of its miscellaneous uses. So, this reviewed that it justifies the use of this plant in a wide range of personal care applications.

K.H. Khan (2009) observed the role of *Emblica officinalis* in Medicine. He observed many vernacular names and chemical constituents of Amla. It is beneficial for various diseases with many uses in variety of formulations.

Shaista Akhund et al., (2010) studied on the fungal species composition on retail Amla fruits obtained from local market of three localities of Hala, Tando Allah Yar and Hyderabad.

Ekta Singh.et al., (2011) they studied on phytochemistry, traditional uses and chemo preventive activity of Amla. It was observed that Vitamin-C content of Amla increases in the sun dried amla and this concluded that Indian gooseberry is a traditionally and clinically proven fruit for its usefulness and efficacy.

Ragh H.S. and Ravindra P. studied the anti-microbial and phytochemical studies of *Phyllanthus emblica* by comparing extract of Amla in methanol, diethyl ether and chloroform and observed that methanolic extract show more anti-microbial activity than the diethyl ether and chloroform extracts.

D.A. Dhale and U.P. Mogle (2011) studied on the phytochemical screening and anti-bacterial activity. Amla is also used by rural population for disorders like Scurvy, Cancer and Heart disease.

M. Suriyavathana and P. Subha (2011) analysis on biochemical study of *Phyllanthus acidus, Phyllanthus emblica* and citrus lemon which shows that level of polysaccharides and total proteins are higher in *Phyllanthus emblica*, Ascorbic acid content was found to be higher in citrus lemon while amount of cholesterol is more in *Phyllanthus acidus*.

K.P. Sampath Kumar.et al., (2012) concluded that *Emblica officinalis* is having many medicinal importance in traditional herbal drugs and useful for bulk of diseases.

Anil Kumar.et al., (2012) gave some very important perspectives for *Emblica officinalis* as it is having applications in hepatoprotective, anti-oxidant, anti-inflammatory, dental problems, respiratory problems etc.

D.A. Dhale (2011) gave some pharmacognostic evaluation of *Emblica officinalis* by different parts of the plants like leaves, stem and fruits. Therefore, these studies helped the scientist, Ayurvedic pharmaceuticals companies and others for identification and authentication of this drug.

Pankaj R. Mali (2012) studied on anti-diabetic activity of *Phyllanthus emblica* and *Curcuma longa* on alloxan induced mice. So, this investigation indicates that the extract of the fruit of Amla and rhizome extract of *Curcuma longa* have hypoglycaemic effect on alloxan induced diabetic mice. Therefore, this plant could be selected further for the work to isolate active principle having anti-diabetic activity with less side-effect.

Mohanapriya M. and Lalitha R. (2012) reported Amla- "The Wonder of Ayurvedic Medicine". It is an interesting trend in the use of food as medicine and so, it is known as super fruits with benefits that go for beyond the level of nourishment alone.

Sunil Pareek and R.A. Kaushik (2012) show the effect of drying methods on quantity of Indian gooseberry powder during storage. So, they concluded that it can be dried with fluidized bed drying at a temperature of 65^0 C with air velocity of 90m/min.

Gupta Priya.et al., (2012) done a review on anti-microbial and anti-oxidant activity of *Emblica officinalis* seed extract. By its methanolic extract it shows that the seed is having high anti-bacterial and anti-oxidant property and so is explored further for the isolation of bioactive.

Parminder Nain.et al., (2012) studied on preparation, characterization and anti-oxidant activity of *Emblica officinalis* leaves extract. So, this was observed because of doing preliminary phytochemical tests of Amla and it was considered as a potential source of natural anti-microbial and anti-oxidant activity.

Nikhil K. Sachan.et al., (2013) done an investigation into phytochemical profile and nutraceutical value of Amla. They explored physico-chemical, phytochemical and nutraceutical profile of the drug and its ethanomedicinal fruit through basic scientific research to establish the rational scientific foundation of its utility as a rejuvenating food and tonic.

2.5.4 Phytochemical reviews

Charis M. Galanakis investigated on the activity co-efficient of 15 natural phenols from that one is Gallic acid which is having appropriate solvent s water.

Daneshfar A.et al., (2008) concluded the solubility of Gallic acid in methanol, ethanol, ethyl acetate and water by relatively comparing the each solvent respectively.

Hamid K.et al., (2011) shows anti-oxidant cytotoxic activities and structure of Gallic acid based on Indole derivatives.

Kuamwat Radhey Shyam.et al., (2012) studied on preparation, characterization and anti-oxidant activities of Gallic acid – phospholipids complex. So, they concluded that the complex was on effective scavenger of DPPH radicals and showed the strong anti-oxidant activity.

Noubigh A.et al., (2012) studied on solubility of Gallic acid in liquid mixture and it shows that Gallic acid is extremely soluble in water.

2.6 Solubility profile of *Emblica officinalis*

Many of the bioactive constituents of herbal drugs are having good water solubility. However, water soluble polyphenols are poorly absorbed. Therefore water-soluble phytoconstituents molecules can be converted into lipid-compatible molecular complexes known as Phytosomes. Gallic acid and its derivatives are a group of naturally occurring polyphenols anti-oxidants. So, after developing Gallic acid-phospholipids complex it was a strong anti-oxidant molecule.

Solvents are classified for their ability to dissolve phenols. So, water is perfect solvent for dissolving Gallic acid. There was done a relative comparison of solubility of Gallic acid in methanol, ethanol, water and ethyl acetate. By the solubility of Gallic acid it shows its anti-oxidant and cytotoxic activities as the structure of Gallic acid is based on Indole derivatives.

2.7 Literature review of Anti-oxidants

An antioxidant is a molecule which prevents the oxidation of other chemicals. They prevent the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism. Free radicals are formed when oxygen is metabolized in the body and are chemical species that posse an unpaired electron in the outer (valance) shell of the molecule.

Therefore, the free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearest stable molecules, taking its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, finally resulting in the description of a living cell. Methods available for the measurement of antioxidant capacity are reviewed, presenting the general chemistry underlying the assays, the types of molecules detected, and the most important advantages and shortcomings of each method. This overview provides a basis and rationale for developing standardized antioxidant capacity methods for the food, nutraceutical, and dietary supplement industries.

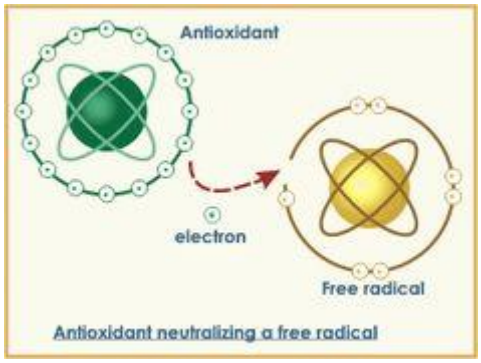

Fig. 2.6 Mechanism of Action of Antioxidants

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. There are various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to

trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (A. Hamid et al 2010).

2.7.1 Mechanism of Action of Antioxidants

LMWAs (low molecular weight antioxidants) are small molecules that frequently infiltrate cells, accumulate (at high concentrations) in specific compartments associated with oxidative damage, and then are regenerated by the the cell. In human tissues, cellular LMWAs are obtained from various sources. Glutathione (GSH), nicotinamide adenine dinucleotide (reduced form), and carnosine are synthesized by the cells; uric acid (UA) and bilirubin are waste products of cellular metabolism; ascorbic acid (AA) , tocopherols and polyphenols are antioxidants obtained from the diet.

Among these LMWAs, a considerable attention was focused on ascorbic acid (AA), known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks; it is also important for therapeutic purposes and biological metabolism. Ascorbic acid is an antioxidant with therapeutic properties, which plays an important role in activating the immune response, in wound healing, in osteogenesis, in detoxifying the organism, in iron absorption, in collagen biosynthesis, in preventing the clotting of blood vessels, and in many other metabolic processes. Vitamin C can be easily oxidized, its degradation being accelerated by heat, light and the presence of heavy metal cations. Thus, due to its content variation, vitamin C represents an important quality indicator of foodstuffs and contributes to the antioxidant properties of food (Vivek G. et al 2006).

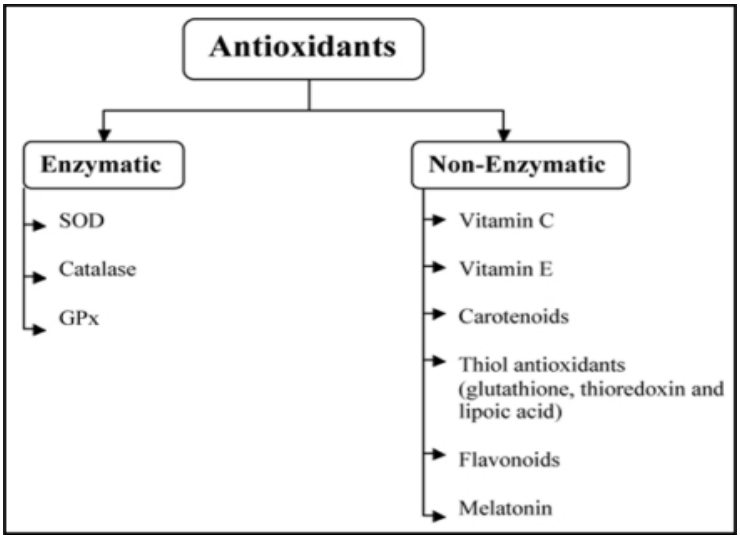

Fig. 2.7 Classification of Anti-oxidants

2.7.2 List of Drugs having Anti-oxidant activity (Vivek K. G. et al., 2006)

Sr. No.	Biological source	Common name/ Part Used	Chemical constituents	Therapeutic uses
1	Curcuma longa Linn. (Zingeberaceae)	Turmeric (Rhizome)	Curcumin, β-Pinene, Camphene, eugenol, β-sitosterol	Antioxidant, Antimalaria, Anti-inflammatory
2	Custcuta reflexa Roxb. (Convolvulaceae)	Akashabel (Stem)	Flavanoids, dulcitol, coumarins, lactone	Expectorant, Carminative, Purgetive, Diuretic
3	Daucus carota Linn. (Apiaceae)	Carrot (Root)	Carotenes, arotenoids, glycosides	Bronchitis, urinary problems, lepory
4	Emblica officinalis Gaertn. (Euphorbiaceae)	Amla (Fruit)	Ascorbic acid, Gallic acid, Ellagic acid	Antioxidant, vomiting, constipation
5	Foeniculum vulgare	Sanuf, Fennel (Fruit oil)	Fenchone, volatile oil,	Stimulant, aromatic,

	Mill. (Apiaceae)		anethole	vermicide, diuretic
6	Glycyrrhiza glabra Linn. (Fabaceae)	Liquorice (Root)	Glycyrrhizin, liquiritin, iso-liquiritin	Diuretic, asthma, peptic ulcer
7	Magnifier indicia Linn, (Anacardiaceae)	Mango (Fruit root)	Mangiferin, cynogentic glycosides, gallic acid	Leucorrhea, dysentery, bronchitis
8	Mamoridaca charantia Linn. (Curcubitaceae)	Bitter Melon (Root, leaf)	Stearic acid, tritepene, glycosides	Laxative, antipyretic, asthma, cholera
9	Ocimum sanctum Linn. (Lamiaceae)	Tulsi (leaf)	Volatile oil, terpenoids, eugenol	Expectorant, ringworm, stomachic
10	Psoralea corylifolia Linn. (Fabaceae)	Babchi (Seed)	Essential oil, fixed oil, resin	Purgative, stimulant, scabies
11	Santalum album Linn. Santalaceae	Sandal wood (Heart wood)	Santalol, α-santalol, β-santalol	Antipyretic, aphrodisiac
12	Solanum nigrum Linn. Solanaceae	Common Nightshade (Leaf)	Polyphenlic compounds, Flavonoids	Diuretic, laxative, malaria
13	Swertia chirayita Roxb. (Gentianaceae)	Chirayita (Whole plant)	Xanthones, chiratin, arginine	Febrifuge, anthelmentic, laxative
14	Withania somnifera Dunal (Solanaceae)	Ashwagandha (Root)	Steroidal lactone, withaniolides	Analgesic, hepatoprotective

2.9 Literature of review of Anti-oxidant assays

Ronald L. Prior.et al., (2005) studied the standardized methods for the determination of Anti-oxidant capacity and phenolics in foods and Dietary supplements.

Dejlan Huang.et al., (2005) reported the chemistry behind the anti-oxidant assays and so on the basis of this analysis, it is suggested that the total phenols assay by FCR been used to quantify and anti-oxidant's reducing capacity and the ORAC assay to quantify peroxyl radical scavenging capacity.

Muhammed Nadam Asghar.et al., (2008) defined a modified 2,2' Azinobis (3- Ethylbenzo Thiazoline)-6'-Sulphonic Acid Radical cation Decolorization Assay for Anti-oxidant Activity of Human Plasma and Extracts of Traditional Medicinal Plants.

Gunars Tirzitis and Grzegorz Bartosz (2010) they have studied the determination of anti-radical and anti-oxidant activity with its new ideas and basic principles.

A.V. Badarinath.et al., (2010) a review on In-vitro anti-oxidant Methods – Comparisions, Correlations and Considerations by carrying out accurate methods for exact results which also act as a good reference for the further researches.

G.Marinova and V.Batchvarav (2011) they gave the evaluation of the methods for determination of the free radical scavenging activity by DPPH.

Aurelia Magdalena Pisoschi and Gheorghe Petre Negulescu (2011) studied about the all different methods of anti-oxidant assays.

Eugenia Jose Garcia.et al., (2012) observed that Anti-oxidant activity by DPPH Assay of Potential Solutions to be applied on Bleached Teeth.

Ammor A. Temimi and Ruplal Choudhary (2013) determine the anti-oxidant activity in different kinds of plants In vivo and In vitro by Diverse Technical Methods.

2.10 Literature reviews on Oral Thin Film

Recent developments have been presented in the technology were various dosage alternatives from oral route for geriatric, paediatrics, bedridden, nauseous or non-compliant patients. Buccal drug delivery system is a very important route of administration. Many bioadhesive mucosal dosage forms have been developed such as adhesive tablets, gels, patches, ointments and more recently the use of polymeric films for buccal delivery, also known as mouth dissolving films.

Mouth dissolving films, a new drug delivery system for the oral delivery of the drug, was developed based on the technology of the transdermal patch. Pharmaceuticals companies and consumers alike have embraced OTFs as a practical and accepted alternative to tradictional OTC medicine forms such as tablets, capsules and liquids.

- Special features of OTF:
  Thin elegant film
  Available in various size and shape
  Excellent mucoadhesion
  Fast disintegration
  Rapid release

- Advantages:

  Convenient dosing

  No water needed

  No risk of chocking

  Taste masking

  Enhanced stability

  Improved patient compliance

- Characteristics of an ideal orally soluble film drug delivery system

  Do not required water to swallow and should dissolve and disintegrate in the mouth within few seconds.

  They possess pleasant mouth feel

  Resistant to environment conditions like temperature and humidity

  Processing and packing can be done at low prices

- Choice of drug candidate

  No bitter taste drug should be there

  Good stability in water and saliva

  Dose should be low as possible

  Short half-life and frequent dosing

  Required control or sustained release

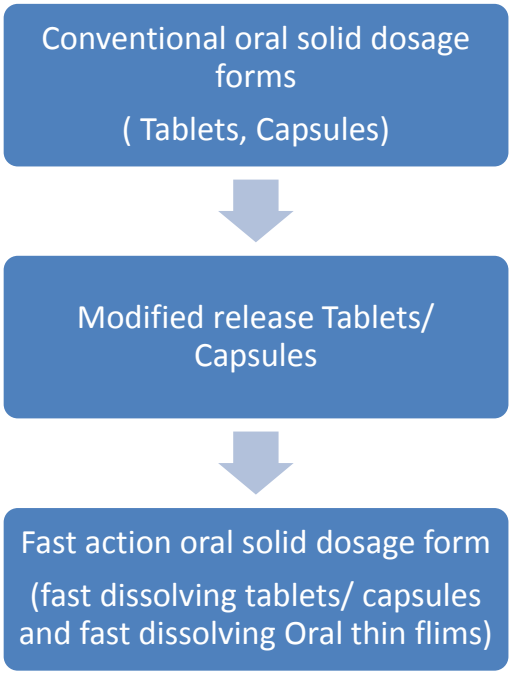

Fig.2.8 Flow chart for the development of oral thin film

- Classification of oral thin films
- There are three types of OTFs

1) Flash release
2) Mucoadhesive melt-away wafer
3) Mucoadhesive sustained-release wafer

Sub type	Flash release wafer	Mucoadhesive melt-away wafer	Mucoadhesive sustained release wafer
Area (cm^2)	2-8	2-7	2-4
Thickness (μm^2)	20-7	50-500	50-250
Structure	Film: Single layer	Single or multilayer system	Multi layer sustem
Excipients	Soluble, highly hydrophilic polymers	Soluble, hydrophilic Polymers	Low/Non-soluble Polymers
Drug phase	Solid solution	Solid solution or suspended drug particles	Suspension and/or solid Solution
Application	Tongue(upper palate)	Gingival or buccal Region	Gingival, (other region in a oral cavity)
Dissolution	Maximum 60 seconds	Disintegration in a few minutes, forming gel	Maximum 8-10 hours
Site of action	Systemic or local	Systemic or local	Systemic or local

Table 2.4 Types of Oral Thin Films

➢ Formulation consideration

Formulation of oral film involves the intricate application of aesthetic and performance characteristics such as taste masking, fast dissolving, physical appearance, mouth feel etc. The excipients used in formulation of Oral film are given below as per the categories. Film should be Generally Regarded as Safe (i.e. GRAS listed) and should be approved in the use of oral thin film (N. G. Raghavendra Rao et al 2013).

A typical composition contains the following:

- Drug – 0.5-30%
- Water soluble polymers – 45%
- Plasticizers – 0-20%
- Surfactants – q.s.
- Sweetening agent – 3-6%
- Saliva stimulating agent – 2-6%
- Fillers, colours, flavours – q.s.

Fig. 2.9 Oral Thin Film

- **Manufacturing of OTF by Solvent Casting Method**

  In this method of manufacturing there is taken water soluble polymers first then dissolved it in water then keep that solution in room temperature for overnight. In another beaker take drug along with plasticizers, surfactants, saliva stimulating agent, colour, flavour etc. Then mix both of the solution on magnetic stirrer for 1 hour. Then keep the solution on sonicator for degassing it. After the solution becomes bubble free pour it or caste it into a petri dish. Then dry it in oven at 50° C for 24 hours.

Figure 1

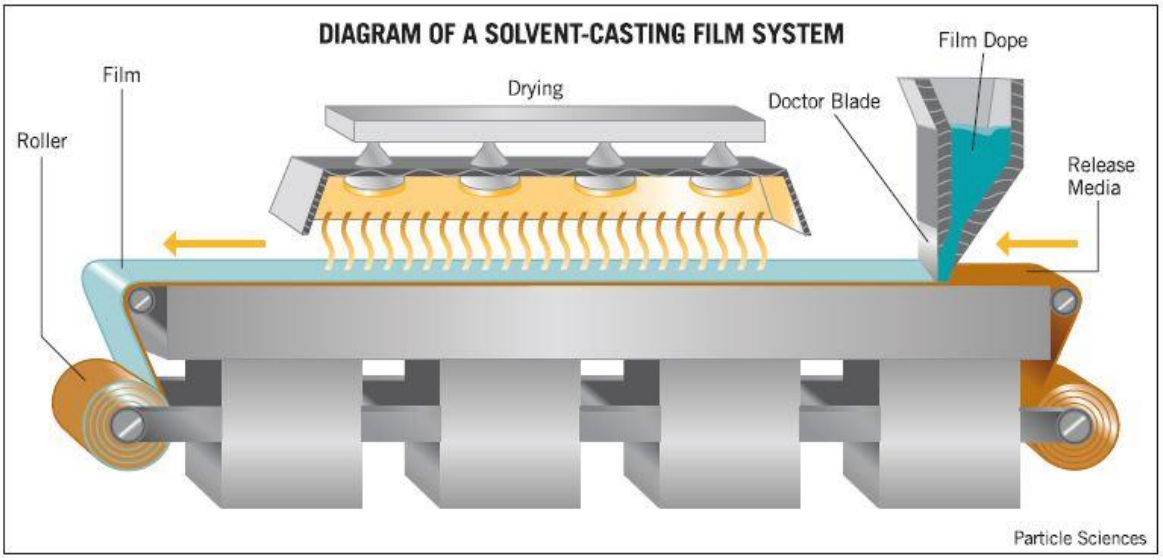

Fig. 2.10 Solvent Casting Method

2.10.1 Pharmacognostic reviews:

Arun Arya.et al., (2010) they have studied that "fast dissolving Oral films"- An Innovative drug delivary system and Dosage form. Also they proved OTF a accepted technology for the systematic delivery of API for over-the-counter (OTC) medications and are in the early to method development stages for prescription drugs.

Rathi Varun.et al., (2011) they gave a brief review on the oral film technology and observed its applications like taste masking, immediate release and sustained release formulation.

B.P. Panda.et al., (2012) tudied about the development of innovative orally fast disintegrating film Dosage forms and it has a large future scenario on a global market as a pharmaceutical dosage forms.

N.G. Raghuvendra Rao.et al., (2013) has overview on the fast dissolving oral films which are solid dosage forms which disintegrate or dissolve within 1 min when placed in the mouth without drinking water or chewing.

Deepthi A.et al., (2014) have showed the formulation and evaluation of fast dissolving oral films of Zolmitriptan which releases 98.5% of drug while has a tensile strength of 1.80 MPa.

3. Formulation of Oral Thin Film

3.1 Collection and authentification of sample drugs

The samples extract of Curcumin has been supplied with the help K. Patel Phyto Extractions Pvt. Ltd. while Gallic acid was available from the PG Pharmacognosy laboratory, Institute of Pharmacy, Nirma University.

3.2 Physical properties of drug powder samples

3.2.1 Physical evaluation

A. Loss on Drying (%) (Anonymous, 1, 2014, 96-277)

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any king that can be driven off under specified conditions. The test is carried out on a well mixed sample of the substance. If the substance is in the form of large crystals, reduce the size by rapid crushing to a powder.

Where the drying temperature is indicated by a single value other than a range, drying is carried out at the prescribed temperature ± 2^o.

Unless otherwise specified in the individual monograph, use Method A. Method A:

Weigh a glass-stopper, shallow weighing bottle that has been dried under the same conditions to be employed in the determination. Transfer to the bottle the quantity of the sample specified in the individual monograph, cover it and accurately weigh the bottle contents. Distribute the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10mm.

Dry the substance by placing the loaded bottle in the drying chamber as directed in the monograph, remove the stopper and leave it also in the chamber. Dry the sample to constant weight or for the specified time and at

the temperature indicated in the monograph. Dry by one of the following procedures. After drying is completed, open the drying chamber, close the bottle promptly and allow it to cool to room temperature (where applicable) in a desiccators before weighing. Weigh the bottle and the contents.

B. pH (Anonymous, 1, 2014, 96-277)

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In the Pharmacopeia, standards and limits of pH have been provided for those pharmacopoeia substances in which pH as a measure of the hydrogen-ion activity is important from the standpoint of stability or physiological suitability. The determination is carried out at a temperature of 25^{o} + or – 2^{o}, unless otherwise specified in the individual graph.

Apparatus

The pH value of a solution is determined potentiometer by means of a glass electrode, a reference electrode and a pH meter either of the digital or analogue type.

Operate the pH meter according to the manufacturer's instructions. Calibrate the apparatus using *buffer solution D* as the primary standard, adjusting the meter to read the appropriate pH value given in the table, corresponding to the temperature of the solution. To set the scale, use a second reference buffer solution, either *buffer solution* A, *buffer solution E or buffer solution G* and carry out a check with a third buffer solution of intermediate solution must not differ by more than 0.05 from the corresponding value indicated in the table.

C. Flow properties (Anonymous, 1, 2014, 96-277).

1. Bulk density

It is defined as mass of powder divided by the bulk volume. It depends on particle size distribution, particle shape, and tendency of particle to adhere to each other. A quantity of weighed granules was introduced in a 25ml

measuring cylinder and the initial volume was noted. The Loose bulk density (LBD) was calculated using:

LBD = Mass of the powder / untapped volume

2. Tapped density

After measuring the bulk density the same measuring cylinder was set into tap density apparatus. It was set at 100 taps drop per minute. The tap density is calculated by,

TD = Mass of the powder / tapped volume

3. Carr's Index

It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder.

%Carr's Index = ((TD – BD)) / TD * 100

4. Hausner's Ratio

It is also known as the Packing factor and is the ratio of tap density upon bulk density.

Hausner's Ratio: Tapped density / Bulk density

5. Angle of Repose

The frictional force in loose microparticles can be measured by angle of repose. It is defined as angle possible between the surface of a pile of the powder and the horizontal plane.

$\mathbf{Tan\theta = h/r \text{ or } \theta = \tan^{-1} h/r}$

Where θ = angle of repose

h= height of pile

r= radius of the base of pile

A funnel was filled to the brim and the test sample was allowed to flow smoothly through the orifice under gravity. From the cone formed on the graph sheet the height and the radius of the pile was determined for the calculation of the angle of repose by the above mentioned formula.

Table 3.1 Scale of Flowability

FLOW PROPERTY	AR(Θ°)	CI(%)	HR
Excellent	25-30	≤ 10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair (aid not added)	36-40	16-20	1.19-1.25
Passable (may hanged up)	41-45	21-25	1.26-1.34
Poor (must agitate, vibrate)	46-55	26-31	1.35-1.45
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

D. Extractive value (Anonymous, 1, 2014, 96-277)

Alcohol soluble extractive value

Macerate 5 g of the dried drug, coarsely powdered, with 100ml of ethanol or methanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing or at least 18 hours. Thereafter, filter rapidly taking care against loss of ethanol or methanol, evaporate 25ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105° and weigh. Calculate the percentage of alcohol soluble extractive with reference to the air-dried drug.

Water soluble extractive value

Add 5g to 50ml of water at 80° in a stopper flask. Shake well and allow standing for 10 minutes, cool, add 2g of kieselghur and filter. Transfer 5ml to porcelain dish and evaporate the solvent on water-bath, finally dry in a steam oven for 2 hours and weigh the residue. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

3.3 Estimation of Gallic acid and Curcumin and its Antioxidant DPPH assay.

3.3.1 Estimation of Gallic acid

Procedure

200 of supernatant A was transferred to a test tube (4 tube / sample). Sample was dried under vacuum 600 µl of 0.2N sulphuric acid was added to the 4 tubes. So, 900 µl of rhodanine solution (0.667%) was added to 3 test tubes while to 4^{th} tube methanol was added. 4^{th} tube was set as blank. Then after 9 min. There was added 600 µl of 0.5N potassium hydroxide solution was added to all 4 test tubes. Later on after 6 min. 12.9 ml distilled water was added. After keeping the solution for 25 min. There was taken absorbance of red-purple colour solution which was measured at λ_{max} = 520 nm against blank. (Pawar et al 2013)

3.3.2 Estimation of Curcumin

Procedure

There was dissolved 10mg of Curcumin powder extract in 10ml of distilled water So, the concentration was 1mg/ml. Then, the absorbance of these solutions was taken at λ_{max} = 420 nm in UV Spectrophotometer.

3.3.3 Anti-oxidant DPPH assay for Curcumin and Gallic acid

Procedure

To make 0.4% DPPH solution with methanol. Control reading of DPPH is obtained by adding 3ml of methanol in a test tube with 10µl of 0.4% DPPH solution till the absorbance should be more than 1. The concentration of sample is 1mg/ml. The absorbance of these samples is taken adding the solution as 10µl of sample, freeze µl of DPPH solution and 3ml of methanol. Then absorbance of this solution is taken in sequence at λ_{max} = 517 nm wavelength after incubating this mixture for 30 min. at 30° C. There is obtained purple colour but after adding the sample there will be colour change of the solution to light yellow this is

due to the effect of anti-oxidant property of the particular sample. Because when DPPH reacts with anti-oxidant compound, which can donate hydrogen so it is reduced.(Marinova et al 2011). There is a formula for Percentage inhibition (I %):

$I\% = A_{CONTROL} - A_{TEST} / A_{CONTROL} * 100$

Where,

$A_{CONTROL}$ = absorbance of control reading

A_{TEST} = absorbance of sample reading

3.4 Solubility profile of Curcumin and Gallic acid

Curcumin is having very poor solubility in water compare to acetone low solubility of Cur cumin is identified by estimation of Curcumin respectively. So to improve its solubility in water various hydrophilic carriers are used. One of them is Gallic acid. Many techniques are used to enhance the solubility and dissolution rate such as solid dispersion technique etc. So, there are developed some methods for increasing the solubility of Curcumin in water.

3.5 Development and optimization of method of preparation of Curcumin-Gallic acid complex.

3.5.1 Methods:

1) There is physically mixed powder sample of Curcumin and Gallic acid in the ratio of 9:1, 8:2 and 7:3.
2) Curcumin is dissolved in acetone and Gallic acid sample powders is mixed directly without dissolving it in the ratio of 9:1, 8:2 and 7:3 and then evaporate it to dryness.
3) Gallic acid is dissolved in methanol and Curcumin sample powder is mixed directly without dissolving it in the ratio of 9:1, 8:2 and 7:3 and then evaporates it to dryness.
4) Gallic acid is dissolved in water and some amount of methanol while Curcumin is dissolved in acetone. Then, Gallic acid solution is added to Curcumin solution and then evaporates it to dryness.

5) Gallic acid and Curcumin are mixed and dissolved in methanol and add drop by drop distilled water and then evaporate it to dryness.

3.5.2 Solubility profile by developing method in UV Spectrophotometer

There is dissolved each and every ratio of each method as 1mg/ml and then taken absorbance in UV at λ_{max} = 420nm to observe its solubility with Gallic acid. There is taken absorbance at the interval of 3 hours.

3.6 Other Antioxidants assays

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals. Endogenous antioxidants are enzymes, like superoxide dismutase, glutathione peroxidase or non enzymatic compounds, such as uric acid, bilirubin, albumin, and metallothioneins. When endogenous factors cannot ensure a rigurous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound.

Amongst the most important exogenous antioxidants, Vitamin E, vitamin C, β-carotene, Vitamin E, Flavonoids, minerals etc are derived from natural sources but it can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc.

There is great number of methods for determination of antioxidant capacity of foods and beverages based on different principles: peroxyl radical scavenging (Oxygen Radical Absorbance capacity, ORAC); Total Radical-

trapping Antioxidant Power (TRAP); metal reducing power (Ferric Reducing Antioxidant Power, FRAP); Cupric Reducing Antioxidant Power (CUPRAC); hydroxyl radical scavenging (deoxiribose assay); organic radical scavenging (2,2-Azino-bis(3-ethylbenz-thiazoline- 6-sulfonic acid, ABTS); 2,2-Diphenyl-1- picrylhydrazyl, DPPH); quantification of the products formed during the lipid peroxidation (Thiobarbituric Acid Reactive Substances, TRAPS); Low-density Lipoproteins (LDLs) oxidation, etc.

3.6.1 DPPH Scavenging antioxidant assay

Procedure

To make 0.4% DPPH solution with methanol. Control reading of DPPH is obtained by adding 3ml of methanol in a test tube with 10µl of 0.4% DPPH solution till the absorbance should be more than 1. The concentration of sample is 1mg/ml. The absorbance of these samples is taken adding the solution as 10µl of sample, freeze 90µl of DPPH solution and 3ml of methanol. Then absorbance of this solution is taken in sequence at λ_{max} = 517 nm wavelength after incubating this mixture for 30 min at 30° C. There is obtained purple colour but after adding the sample there will be colour change of the solution to light yellow this is due to the effect of anti-oxidant property of the particular sample. Because when DPPH reacts with anti-oxidant compound, which can donate hydrogen so it is reduced. There is a formula for Percentage inhibition (I %)

$I\% = A_{CONTROL} - A_{TEST} / A_{CONTROL} * 100$

Where,

$A_{CONTROL}$ = absorbance of control reading

A_{TEST} = absorbance of sample reading

3.6.2 ABTS Antioxidant activity

Procedure

ABTS was dissolved in double distilled water to a 7 mM concentration. $ABTS^{\circ+}$ was formed by reaction with ABTS stock solution which was (20mM sodium acetate buffer pH-6.5 and 2.45mM potassium per sulphate) then this mixture is allowed to stand in dark room for 12-16 hrs. To study the antioxidant activity there was taken control reading of ABTS solution is obtained by adding 3ml of double distilled water in a test tube with 10µl of ABTS this the absorbance should be 0.7 ± 0.2. The concentration of sample is 1mg/ml. The absorbance of these samples is taken adding the solution as 10µl of sample and 3ml of double distilled water. Then absorbance of this solution is taken in sequence at λ_{max} = 734 nm wavelength. (Mohammad N. et al(2008). The percentage inhibition of absorbance at 734 nm (*I*734) was calculated as follows.

$I_{734} = (1- A_f / A_o) * 100$

Where, A_f = absorbance of control

A_0 = absorbance of sample

3.6.3 TROLOX EQUIVALENT ANTIOXIDANT CAPACITY ASSAY(TEAC)

Procedure

ABTS was dissolved in double distilled water to a 7 mM concentration. $ABTS^{\circ+}$ was formed by reaction with ABTS stock solution which was (20mM sodium acetate buffer pH-6.5 and 2.45 mM potassium per sulphate) then this mixture is allowed to stand in dark room for 12-16 hrs. To study the antioxidant activity there was taken control reading of ABTS solution is obtained by adding 3ml of double distilled water in a test tube with 10µl of ABTS this the absorbance should be 0.7 ± 0.2. The concentration of sample is 1mg/ml. The absorbance of these samples is taken adding the solution as 10µl of sample and 3ml of double distilled water. But the reading should be taken at 1min., 4 min. and 6 min. after mixing it at 30ºC and absorbance of this solutions are taken at λ_{max} = 734 nm. The difference of the absorbance reading is plotted versus the antioxidant

concentrations to give a straight line. The concentration of antioxidants giving the same percentage change of absorbance of the ABTS as that of 1 mM Trolox was regarded as TEAC. (Dejian H. et al (2005)

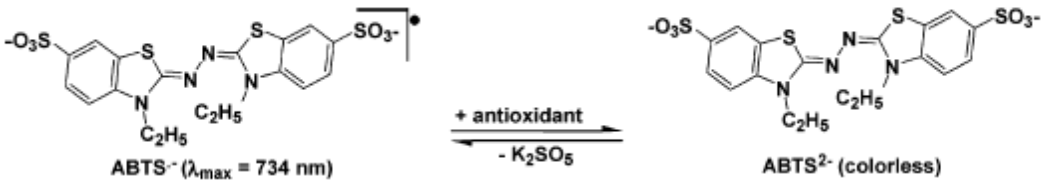

Fig. 5.1 Mechanism of ABTS

3.6.4 β-CAROTENE ANTIOXIDANT ASSAY

Procedure

The antioxidant activity of extract was evaluated by the b-carotene-linoleate model system. A solution of b-carotene was prepared by dissolving 2 mg of b-carotene in 10 ml of chloroform. Two millilitres of this solution were pipette into a 100 ml round bottom flask. After chloroform was removed under vacuum, 40 mg of purified linoleic acid, 400 mg of Tween 40 emulsifier, and 100 ml of aerated distilled water were added to the flask with vigorous shaking to form an emulsion. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing different concentrations of the extract (250 and 500mg/3ml). As soon as the emulsion was added to each tube, the zero time absorbance was measured at λ_{max} = 470 nm, using a spectrophotometer. The tubes were placed, at 50 ^{0}C, in a constant temperature controlled water bath and measurement of absorbance was recorded after 2 h; a blank, devoid of b-carotene, was prepared for background subtraction. Measurement of absorbance was continued until the colour of β-carotene disappeared in the control reaction (t = 120 min). (Onder Y et al(2011).Antioxidant activity (A_A) was determined as percent inhibition relative to control sample.

A_A = **$R_{CONTROL} - R_{SAMPLE} / R_{CONTROL}$ * 100**

$R_{control}$ and R_{sample} represent the bleaching rates of b-carotene without and with the addition of antioxidant, respectively. Degradation rates(R_D) were calculated according to first-order kinetics

3.6.5 SUPEROXIDE RADICAL SCAVENGING ANTIOXIDANT ACTIVITY

Procedure

The assay was based on the capacity of the aqueous extract to inhibit formazan ion formation by scavenging the super oxide radicals generated in a riboflavin-light-NBT system. The method was used for the determination of super oxide ion. The reaction mixture contained 50 mM phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, and NBT(Nitro Blue Tetrazolium), 0.1mg/3 ml, added in that sequence. Reaction was started by illuminating (fluorescent lamp) the reaction mixture with various concentrations of extracts for 150 seconds. Immediately after illumination, the absorbance was measured at λ_{max} = 590 nm. The entire reaction assembly without drug served as control. The entire reaction assembly was enclosed wooden box. Identical tubes, with reaction mixture, were kept in the dark and served as blanks. Ascorbic acid was used as a standard antioxidant.(Palash Mandal et al(2009). The percentage inhibition of super oxide anion generation was calculated using the following formula:% Inhibition $= \frac{(A_0 - A_1)}{A_0} * 100$;

Where, A_0 was the absorbance of the control.

A_1 was the absorbance of the drug extract/standard

3.7 COMPATIBILITY STUDIES

3.7.1 TLC

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g)

quantities can be analyzed by TLC - and it takes little time for an analysis (about 5-10 minutes). TLC consists of three steps - spotting, development, and visualization. Photographs of each step are shown on the course website. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipette to transfer a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic sheet. The spotting solvent quickly evaporates and leaves behind a small spot of the material.

TLC of C:GA (9:1)

Sample solution: Curcumin, Gallic acid and Curcumin: Gallic acid complex (9:1) is taken1mg and dissolved in 2ml of Methanol.

Solvent system**:**

Toluene: Ethyl acetate: Formic acid (2 : 8 : 0.1)

Procedure: Apply a spot of these samples one by one on a TLC plate in the sequence (C, GA, and C: GA)

Visualization: In UV Cabinet at 254nm (Short UV) and 365nm (Long UV) there is observed the separation of pigments

5.7.2 IR Spectroscopy

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibres, gases and surfaces can all be examined with a judicious choice of sampling technique. As a consequence of the improved instrumentation, a variety of new sensitive techniques have now been developed in order to examine formerly intractable samples. Infrared refers to that part of the electromagnetic spectrum between the visible and microwave regions. Electromagnetic spectrum refers to the seemingly diverse collection of radiant energy, from cosmic rays to X-rays to visible light to microwaves, each of which

can be considered as a wave or particle travelling at the speed of light. These waves differ from each other in the length and frequency.

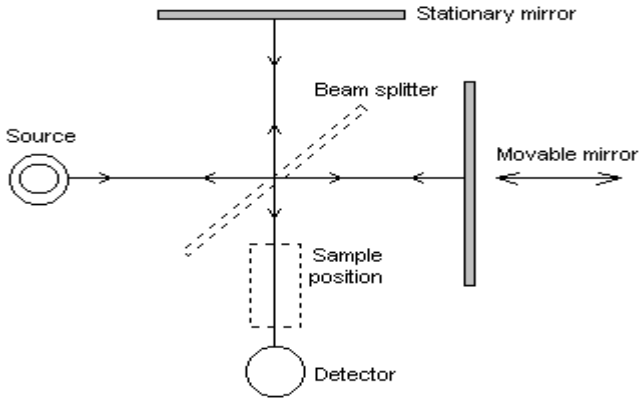

Fig. 5.2 IR Spectroscopy

5.7.3 MASS SPECTROSCOPY

A mass spectrometer especially a multi-sector instrument is one of the most complex electronic and mechanical devices one encounters as a chemist. Therefore this means high costs at purchase and maintenance besides a specialized training for the operator. Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them. Therefore, mass spectroscopy allows quantization of atoms or molecules and provides structural information by the identification of distinctive fragmentation patterns. The general operation of a mass spectrometer is: 1. Create gas-phase ions 2. Separate the ions in space or time based on their mass-to-charge ratio 3. Measure the quantity of ions of each mass-to-charge ratio.

3.8 FORMULATION APPOROCH

DESIGN AND DEVELOPMENT OF ORAL THIN FILM

3.8.1 Equipments and Materials

Table 3.2 List of Equipments

Equipments	Manufacturer
Magnetic stirrer with hot plate	Labotech
Sonicator Bath	EIE Instruments Pvt. Ltd.
Digital pH Meter	ANALAB Scientific Instruments Pvt. Ltd.
Weighing Balance	NOVA Instrument Pvt. Ltd.
UV/Visible Spectrophotometer	UV 2450 Shimadzu scientific instrument, Japan
Hot Air Oven	EIE Instruments Pvt. Ltd.
UV Cabinet	KOMPAKT

Table 3.3 List of Materials

Materials		Manufacturers
Polymer	Polyvinyl alcohol	CDH Pvt. Ltd.
Plasticizer	Polyethylene Glycol (PEG 20,000)	CDH Pvt. Ltd.
Starch	Potato starch	CDH Pvt. Ltd.
Saliva stimulating agent	Citric acid	S.D. Fine Chem. Ltd
Solvent	Ethanol	Ureca Consumers Co-op. Stores Ltd.
	Acetone	CDH Pvt. Ltd.

3.8.2 Formulation of Oral Thin Film By Solvent Casting Method

Procedure:

Dissolve water soluble polymer in specified amount of water after mixing by magnetic stirrer for 1 hour. Then keep that solution for overnight at room temperature. Drug was dissolved in specified amount of acetone and plasticizers, surfactants; saliva stimulating agent was dissolved in ethanol. Ethanolic solution was added in aqueous solution and mixes this solution by magnetic stirrer for 20 minutes. Then pour that solution in Petri dish after degassing it in sonicator and keep it for 24 hours in oven for drying at 50ºC. Then next day the film was peeled off from Petri dish.

3.8.3 EVALUATION PARAMETERS

1. **Physical Appearance**

   The films are observed for their physical appearance such as colour, surface and transparency.

2. **Weight variation of the film**

   There was cut 2.25 cm^2 films from various sides of films and then the weight of each film was taken and then weight variation was calculated in grams.

3. **Thickness of the film**

   The thickness of the film was measured by Digital Vernier Callipers with the count of 0.01 m at different spots of the film. The thickness was measured from three different spots of the film and average was taken and SD was calculated respectively.

4. Tensile strength

Tensile strength (TS) Tensile strength is the maximum stress applied to a point at which the film specimen breaks and can be calculated by dividing the maximum load by the original cross-sectional area of the specimen and it was expressed in force per unit area.

Tensile Strength = Force at break (N) / Cross sectional area (mm2)

5. Percent elongation

On application of the stress, a strip sample stretches and this is known as strain. Deformation of film divided by original dimension of the sample is referred as Strain. When concentration of plasticizers increases elongation of strips also increases. (Dhire et al 2011)

% Elongation = increase in length of strip / Initial length of strip * 100

Fig. 3.3 Tensile strength

6. Content Uniformity

The particular size of film pieces are kept in a 100ml stimulated wound fluid. The it was mixed under magnetic stirrer for at least 2 hrs for getting a homogenous solution and filtered. Then sample solutions were prepared by diluting to different concentrations.

Content Uniformity = Actual amount of drug in film / Theoretical amount of drug present in film * 100

7. pH

These is measured by keeping the particular size of film in contact to distilled water , after 1 hour, the pH of the solution s measured. (Khurana et al 2000)

8. Folding endurance

The folding endurance is expressed as the number of folds (number of times of film is folded at the same plain) required breaking the specimen or developing visible cracks. It gives an indication of brittleness of the film. A small strip was subjected to this test by folding the film at the same plane repeatedly several times until a visible crack was observed.

9. Mouth dissolving time

The mouth dissolving time was determined by placing the film manually into a Petri dish containing 25 ml of 7.4 pH phosphate buffer. Time required by the film to dissolve was noted.

10. Moisture uptake

This test was performed by taking pre weighted film and keep that film in dessicator at particular temperature and humidity. After three days, the film was taken out and the again weighed to determined its % Moisture uptake.

(Saxena et al 2006)

% Moisture uptake = Final weight – Initial weight / Initial weight *100

11. Moisture content

The pre weighted film was kept in dessicator for 24 hours and then again weighed to determine its % Moisture content. (Saxena et al 2006).

% Moisture content = Final weight – Initial weight / Initial weight * 100

3.8.4 Optimization of Oral Thin Film

There was used water soluble polymers like 5% HPMC(Hydroxyl propyl methyl cellulose (15 cps) with 20% w/w PEG 20,000 and PVC(Poly Vinyl Alcohol) 4 % with PEG 20,000 was prepared. Prepared Films were evaluated for physical characterization. After characterization the films having 5% HPMC was having very poor transparency while film having 4% PVA was having good transparency. Both of the films were prepared by using solvent as ethanol. From the physical evaluation the polymer and plasticizer were selected with solvent and gave the good transparency and stability of the film. Selected proportion of the polymer was used to prepare final batch with drug loaded film using the different concentration of complex drug.

3.9 RESULT AND DISCUSSION

Table 3.4 % LOD

Drug sample	LOD%
Curcumin	2.16%
Gallic acid	2.45%

Table 3.5 pH

Drug sample	Solvent	
	Water	Methanol
Curcumin	6.5 ± 0.01	6.27 ± 0.02
Gallic acid	2.02 ± 0.03	7.1 ± 0.03

Table 3.6 Flow properties

Drugs	Bulk density (g/ml)	Tapped Density (g/ml)	Carr's index (%)	Hausner's ratio	Angle of reprose (Aº)
Curcumin	0.5	0.65	23.12	1.30	77.63
Gallic acid	0.4	0.61	35.39	1.54	76.07

Table 3.7 Extractive value

Drugs	Water soluble Extractive value	Alcohol soluble extractive value
Curcumin	N.A.	46.1%
Gallic acid	41.6%	89%

By studying the physical proprety of powder sample of Curcumin and Gallic acid there was observed that Curcumin is having less % LOD then Gallic acid, Curcumin is having neutral pH in both solvent water and methanol while Gallic acid is acidic in water and neutral in methanol. Both powder sample flow property is good and passable and water soluble extractive value of Curcumin is not available while Gallic acid is 41%and alcohol soluble extractive value is 46.1% and 89 % repectively.

Table 3.8 Linearity of Calibration

Concentration (ng/ml)	µg/ml	Average	Standard Deviation
1000	1	0.342333	0.011 ± 0.03
1250	1.25	0.463667	0.040 ± 0.04
1500	1.5	0.542667	0.027 ±0.05
1750	1.75	0.615667	0.040 ±0.05
2000	2	0.709333	0.056 ± 0.06
2250	2.25	0.807333	0.045 ±0.07
2500	2.5	0.897	0.054 ±0.08
2750	2.75	0.996667	0.005 ±0.09
3000	3	1.102	0.049 ±0.03

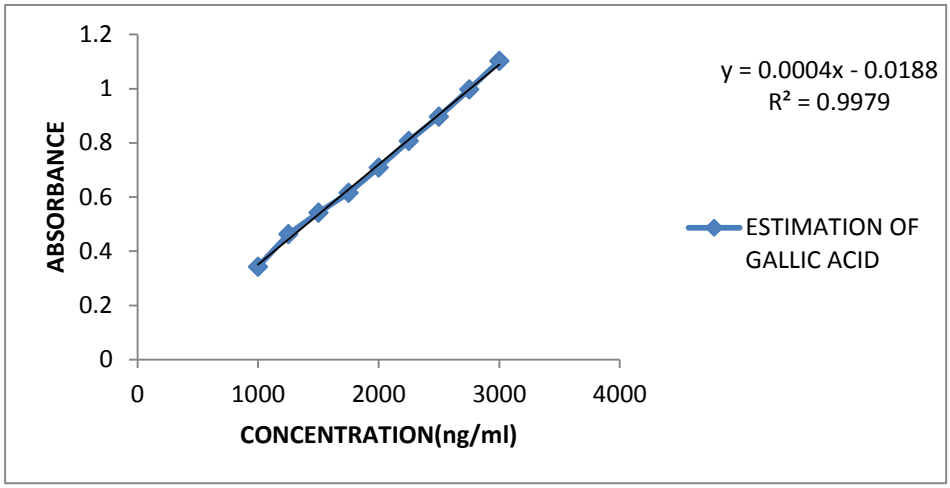

Fig. 3.4 Calibration Curve of Gallic acid

Table 3.9 Estimation of Curcumin

Solution (µg / ml)	**Absorbance**
	WATER
1	0.125
2	0.205
3	0.256
4	0.311
5	0.355
6	0.421
7	0.524
8	0.614
9	0.664
10	0.702

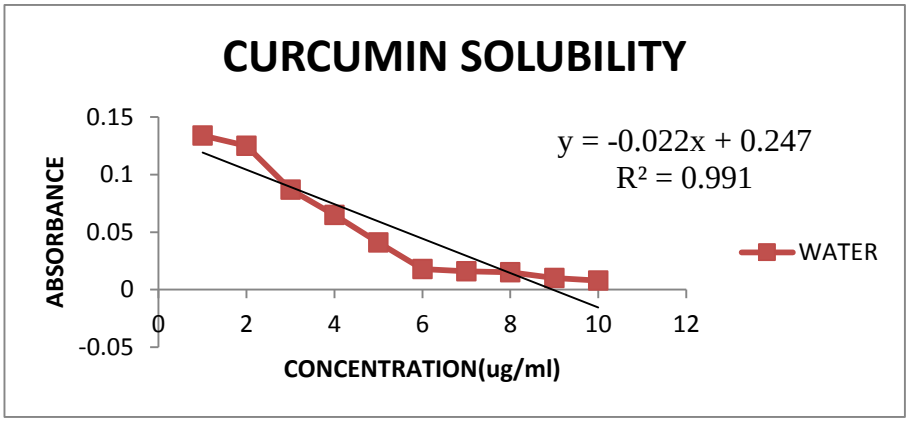

Fig. 3.5 Solubility profile of Curcumin in water

By estimation of Gallic acid there was seen the linearity in graph and a standard curve was obtained while by estimation of curcumin there was observed that curcumin is poorly soluble in water and highly soluble in acetone.

Table 3.10 DPPH IC50

DRUGS	IC 50 µg/ml
CURCUMIN	15.31
GALLIC ACID	11.94

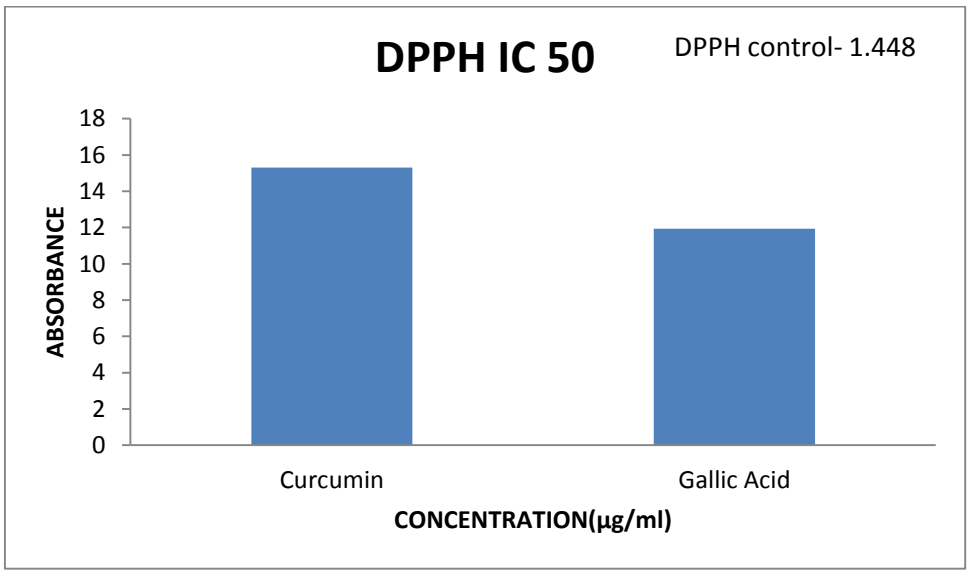

Fig 5.6 DPPH Antioxidant assay of Curcumin and Gallic acid

By performing the DPPH Antioxidant assay of Curcumin and Gallic acid it was observed that Gallic acid is more potent Antioxidant than Curcumin.

Table 3.11 Solubility profile of Curcumin and Gallic acid

Drugs	Solubility (30-37C°)	Time (min.)
Curcumin	10mg - 50ml	9.5 min
Gallic acid	200mg – 50 ml	4.15min.

Curcumin is hydrophobic drug and Gallic acid is hydrophilic drug can be observed by this solubility profile.

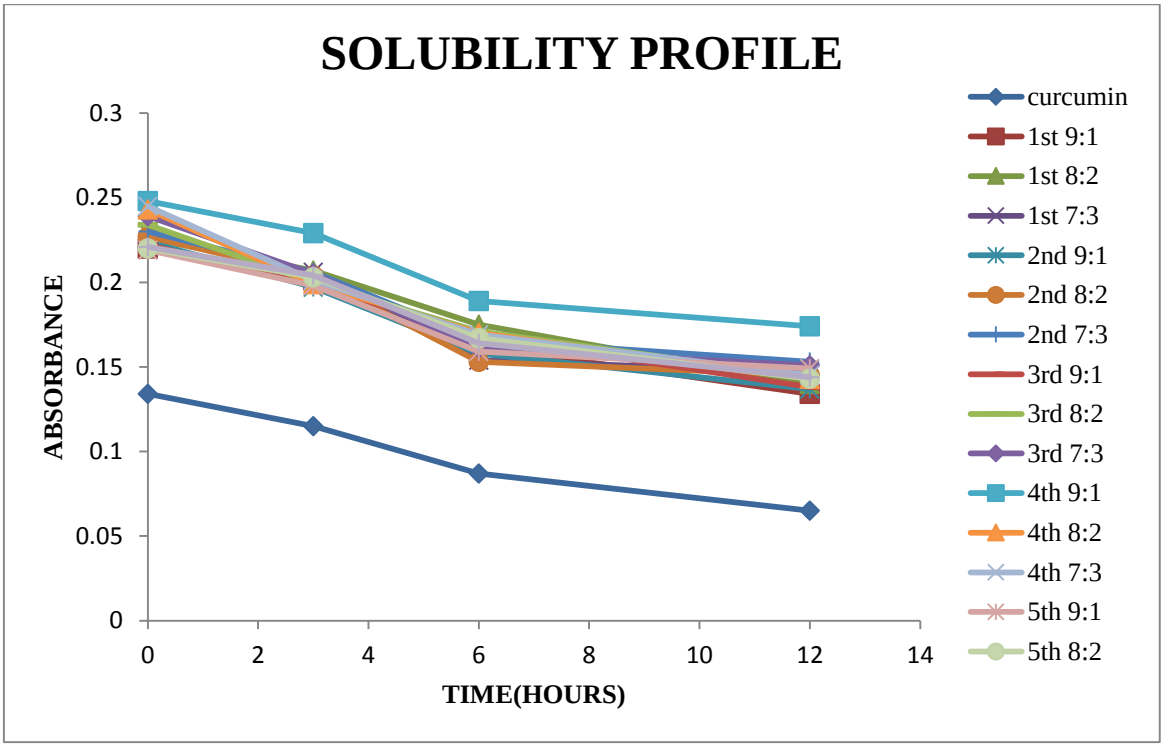

Fig 3.7 Solubility profile of all ratios

By this solubility profile of all ratios of all developed methods for incerasng solubility of curcumin, it was observed that the highest solubility is seen in C:GA(9:1) of 4th method and is more soluble then Curcumin. By adding 9 part of Curcumin and 1 part of Gallic

acid. So, there is **106 times increase** in solubility of Curcumin in water by using Gallic acid.

Table 3.12 DPPH IC50 with C:GA (9:1)

DRUGS	IC 50(µg/ml)
CURCUMIN	15.34
GALLIC ACID	11.94
C:GA(9:1)	8.706

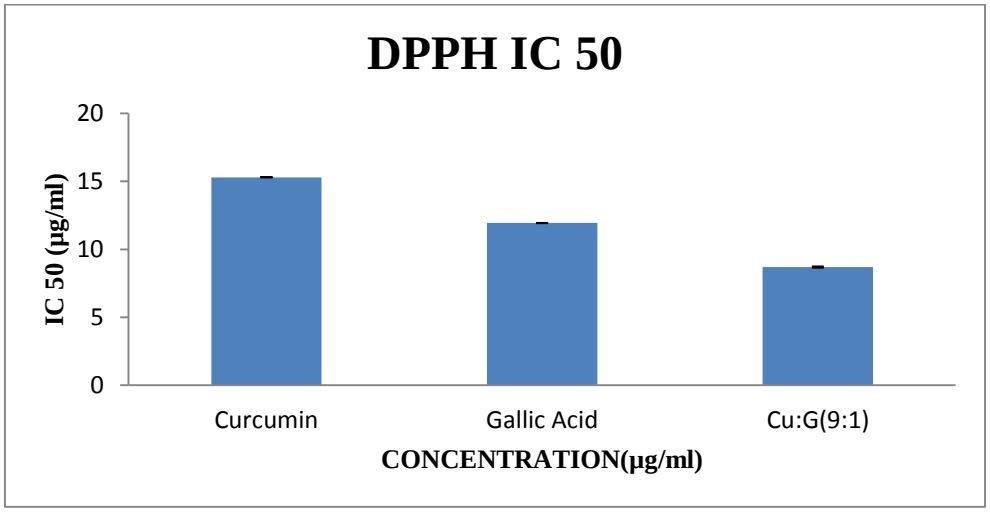

Fig 5.8 DPPH IC50 with C:GA (9:1)

Table 5.13 ABTS IC50 with C:GA (9:1)

DRUGS	IC 50(µg/ml)
CURCUMIN	5.097
GALLIC ACID	0.98179
C:GA(9:1)	2.782

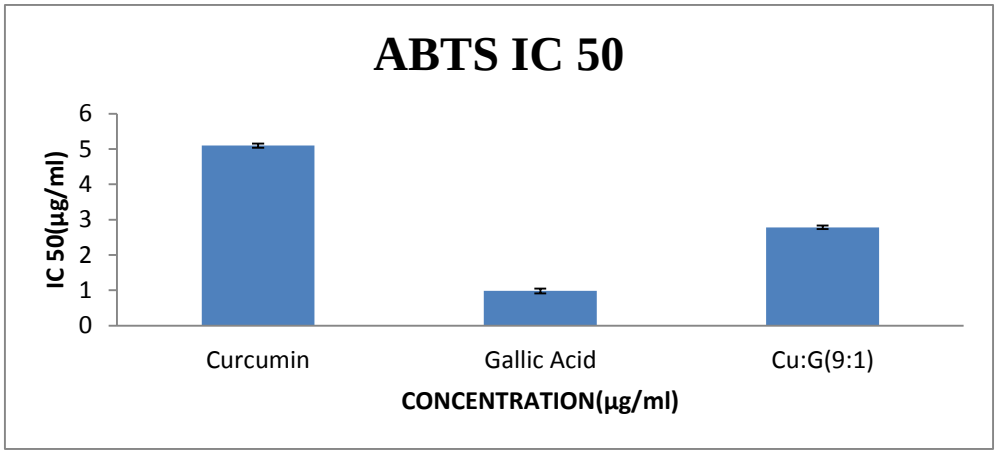

Fig 3.9 ABTS IC50 with C:GA(9:1)

Table 3.14 TEAC IC50 with C:GA (9:1)

DRUGS	TEAC
CURCUMIN	7.247098
GALLIC ACID	0.861933
C:GA(9:1)	3.379737

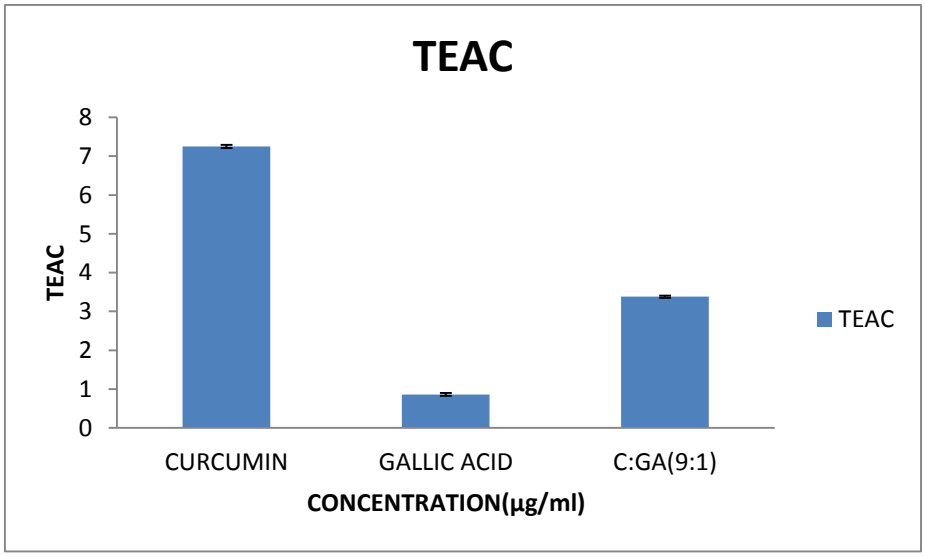

Fig 3.10 TEAC IC50 with C:GA (9:1)

Table 3.15 β-CAROTENE IC50 with C:GA(9:1)

DRUGS	IC 50(µg/ml)
CURCUMIN	76.54883
GALLIC ACID	58.15291
C:GA(9:1)	71.01433

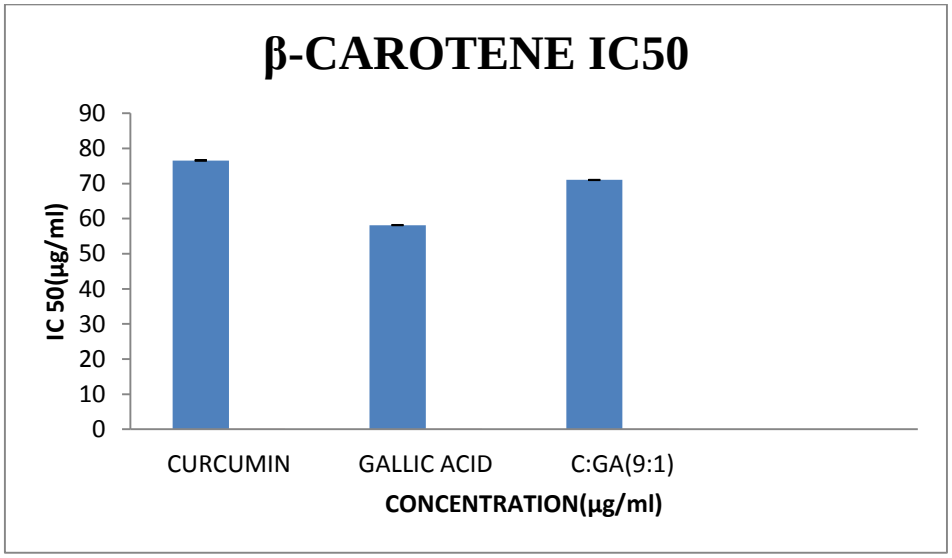

Fig 3.11 β-CAROTENE IC50 with C:GA(9:1)

Table 3.19 SUPEROXIDE IC50 with C:GA(9:1)

DRUGS	IC 50(µg/ml)
ASCORBIC ACID	66.83278
CURCUMIN	543.6937
GALLIC ACID	693.6782
C:GA(9:1)	168.4813

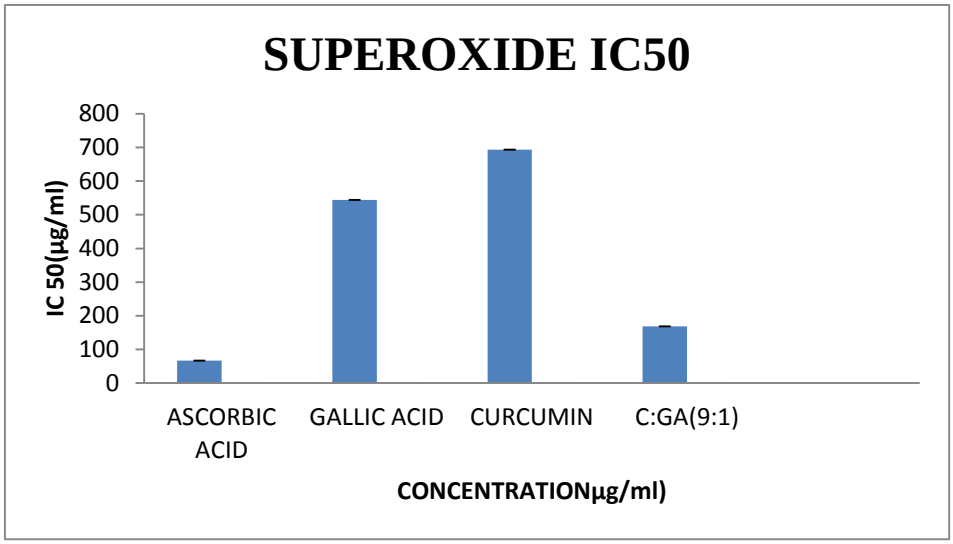

Fig 3.12 SUPEROXIDE IC50 with C:GA(9:1)

After observing the IC50 value of all the five Antioxidant assays there was observed that % Antioxidant potential of C:GA(9:1) is increasing synergistically then Curcumin. This is due to the enhancement in solubility of Curcumin in water.

TLC

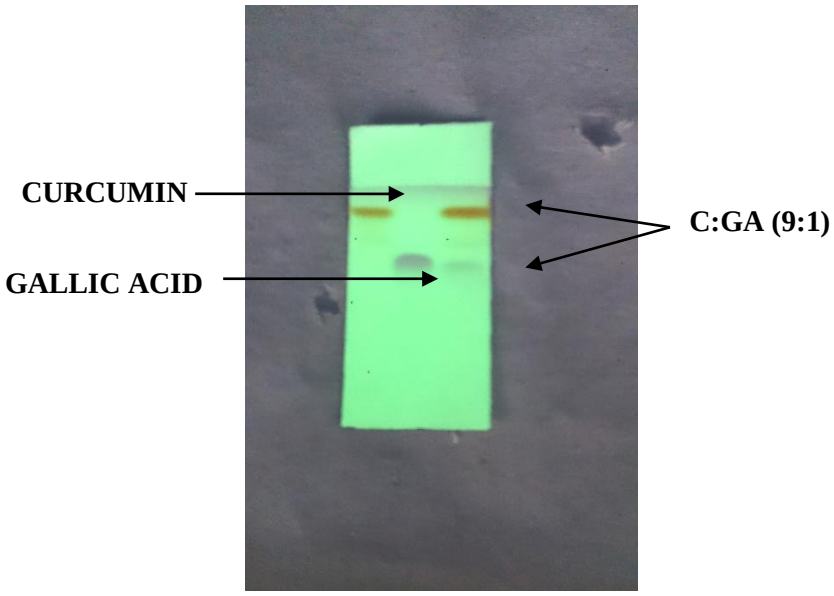

Fig 3.13 TLC of C:GA(9:1)

By spotting the samples on the TLC plate it was observed that on running off the solvent system (Toluene: Ethyl acetate: Formic acid) in the ratio of 2:8:0.1on to the TLC plate there is separation of Curcumin and Gallic acid from the complex C:GA(9:1).

IR Spectroscopy

1. CURCUMIN

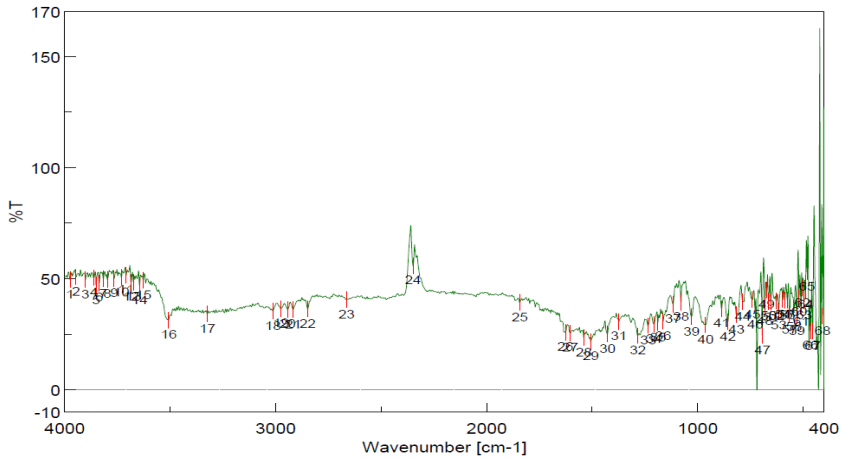

Fig 3.14 IR Spectra of Curcumin

Table 3.14 Interpretation of Curcumin

Peak Frequencies	Functional Group
1250–1020 (m)	C–N stretch(aliphatic amines)
1370–1350 (m)	C–H rock (alkanes)
1500–1400 (m)	C–C stretch (in–ring)(aromatics)
1760–1665 (s)	C=O stretch carbonyls (general)
2830–2695 (m)	H–C=O: C–H stretch (aldehydes)
3500–3200 (s ,b)	O–H stretch, H–bonded (alcohols,phenols)

2. GALLIC ACID

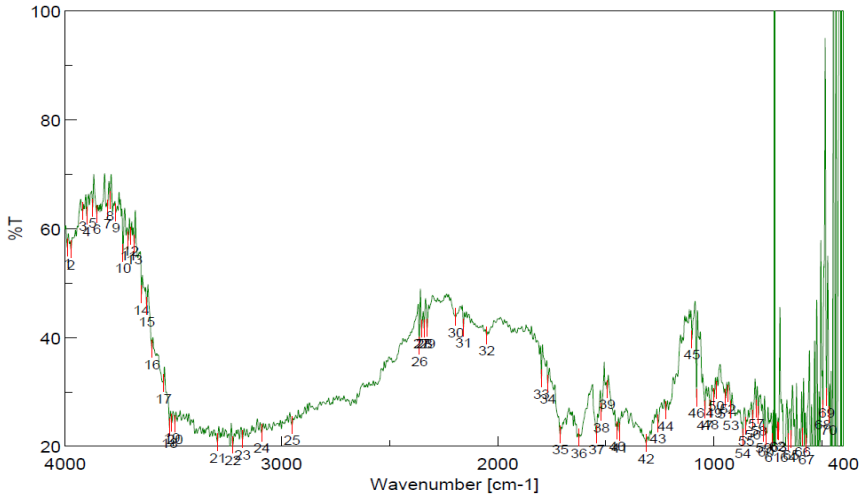

Fig 3.15 IR Spectra of Gallic acid

Table 3.15 Interpretation of Gallic acid

Peak Frequencies	Functional Group
3300–2500 (m)	O–H stretch carboxylic acids
2260–2100 (w)	–C= C– stretch alkynes
1710–1665 (s)	C=O stretch α–unsaturated aldehydes, ketones
1600–1585 (m)	C–C stretch (in–ring) aromatics

3. CURCUMIN :GALLIC ACID (9:1)

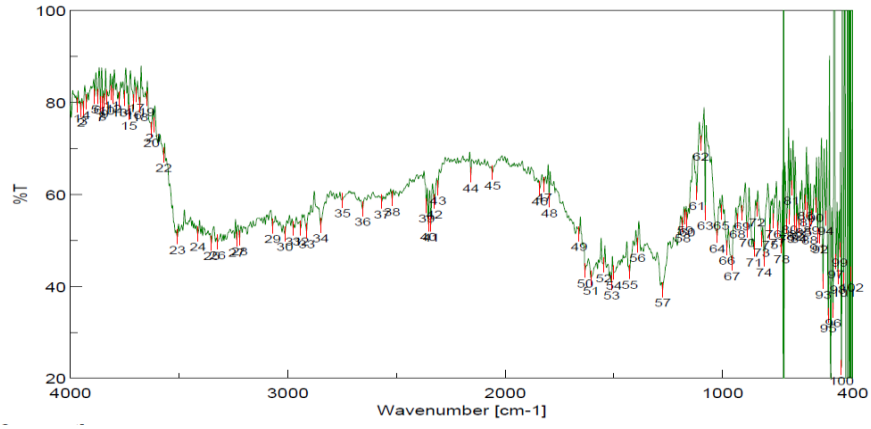

Fig 3.16 IR Spectra of C:GA (9:1)

Table 3.19 Interpretation of C:GA (9:1)

Peak Frequencies	Functional Group
3500–3200 (s,b)	O–H stretch, H–bonded alcohols, phenols
3000–2850 (m)	C–H stretch alkanes
2260–2100 (w)	–C=C– stretch alkynes
1250–1020 (m)	C–N stretch aliphatic amines

MASS spectroscopy

1. CURCUMIN

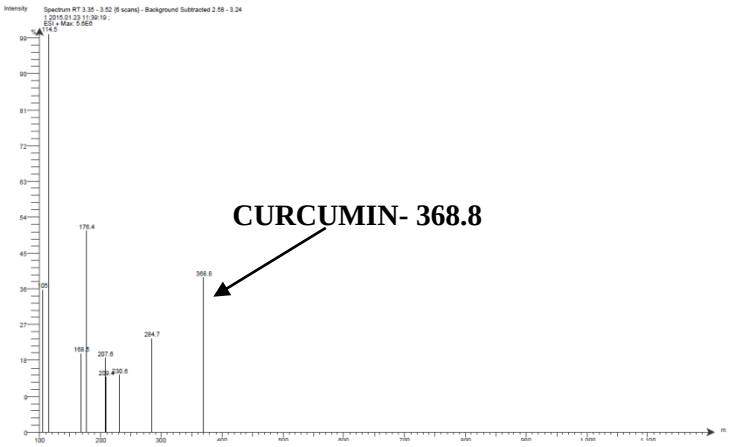

Fig 3.17 Mass Spectra of Curcumin

2. GALLIC ACID

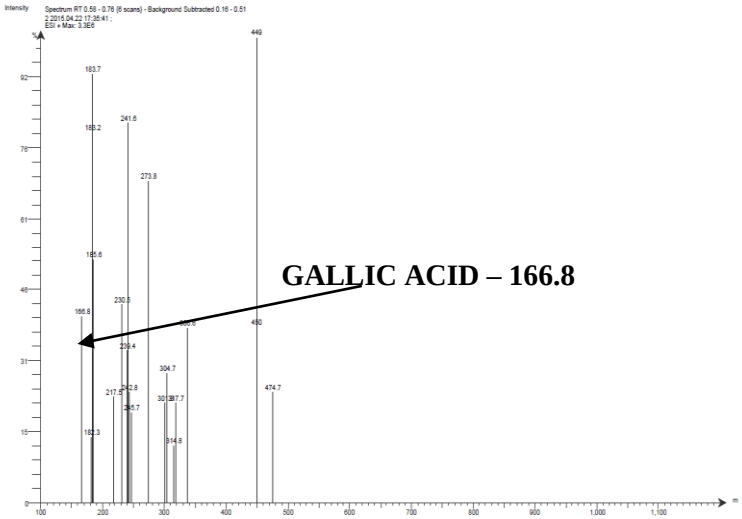

Fig 3.18 Mass Spectra of Gallic acid

3. CURCUMIN:GALLIC ACID (9:1)

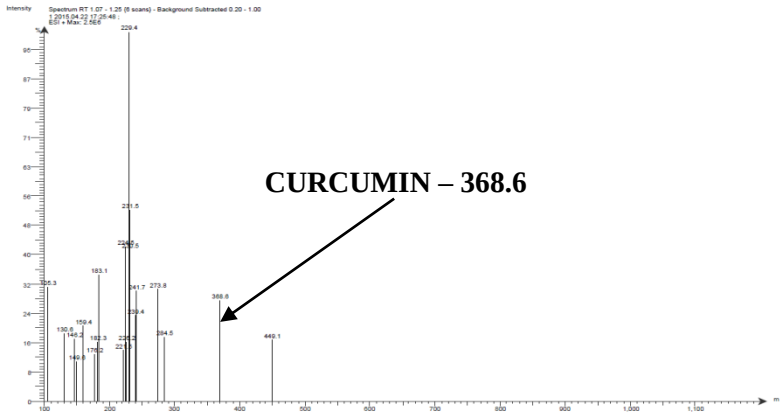

Fig. 3.19 MASS Spectra of C:GA (9:1)

By observing the MASS Spectra of Curcumin, Gallic acid and C:GA(9:1) there is concluded that thre is formation of complex of Curcumin and Gallic acid. So, there is not seen peak of Gallic acid in Mass spectra of C:GA(9:1).

Table 3.20 %Antioxidant Potential

DRUGS	DPPH	ABTS	TEAC	B-CAROTENE	SUPEROXIDE
CURCUMIN	15.34	5.097	7.247	76.54	543.69
GALLIC ACID	11.94	0.981	0.861	58.15	693.67
C:GA(9:1)	8.706	2.782	3.379	60.00	168.48
Average %A.O.P. increase in compared to Curcumin	175 %	183%	214%	127%	322%
Average %A.O.P. increase in compared to Gallic acid	137%	35%	25%	96%	411%

There was observed that % Antioxidant potential of C:GA(9:1) is synergistically increases in compared to Curcumin by 175%, 183%, 214%, 127% and 322% in DPPH, ABTS, TEAC, β-CAROTENE and SUPEROXIDE respectively and highest Antioxidant potential increases in Superoxide assay and % Antioxidant potential of C:GA(9:1) is synergistically increases in compared to Gallic acid by 137%, 35%, 25%, 96% and 411% in DPPH, ABTS, TEAC, β-CAROTENE and SUPEROXIDE respectively and highest Antioxidant potential increases in Superoxide assay.

Optimization of Oral Thin Film with PVA Polymer

Table 3.21 Optimization of OTF

WATER SOLUBLPOLYMER	**DISTILLED WATER**
PVA	4%
PLASTICIZERS	
PEG 20,000	20% w/w of Polymer

Evaluation of Placebo films using PVA as polymer with plasticizer PEG 20,000

Table 3.22 Evaluation of Placebo Films

Film	**Tensile Strength ($N/cm^{2)}$**	**%Elongation**	**Folding Endurance**	**Thickness**	**Moisture uptake**	**Moisture content**
4% PVA with PEG	0.734	10	56	0.03	2.78%	15%

The prepared placebo film by using 4% PVA and 20% w/w PEG 20,000 was having good physical appearance and observed that 20% plasticizers increases tensile strength and % elongation also increases. This increase in percentage elongation was due to the bond formed between polymer and plasticizers. The film formed using 4% PVA and 20% w/w PEG 20,000 in water showed good plasticity, good elasticity and best tensile strength. So, the preparation of drug loaded film was done by using PVA was finalized with polymer to drug ratio loaded film.

Formulation of Drug Loaded Oral Thin Film

FILM of C:GA(9:1) with PEG

Polymer used: Polyvinyl alcohol (4%)

Plasticizer used: Polyethylene glycol(PEG 20,000)(20% w/w of polymer)

Solvent used: Ethanol

Active ingredients: Curcumin - Gallic acid complex(9:1)

Table 3.23 Formulation of Drug loaded OTF

Polymer	4%	2gm
Plasticizers	20% w/w of polymer	800mg
Starch	5%	2.5gm
Saliva stimulating agent	1%	0.5gm
Drug	2%	1gm
Solvent	Ethanol	30ml

EVALUATION

Table 3.24 Evaluation of Drug loaded OTF

Parameters	**Results**
WEIGHT VARIATION OF THE FILM	0.083 g
THICKNESS OF THE FILM	0.17mm (170 µg)
TENSILE STRENGTH	5.735 N/cm^2
%ELONGATION	20.9%

pH	6.8
FOLDING ENDURANCE	85 folds at one place
MOUTH DISSOLVING CAPACITY	1800 seconds
% MOISTURE UPTAKE	0.0888%
% MOISTURE CONTENT	0.0277%

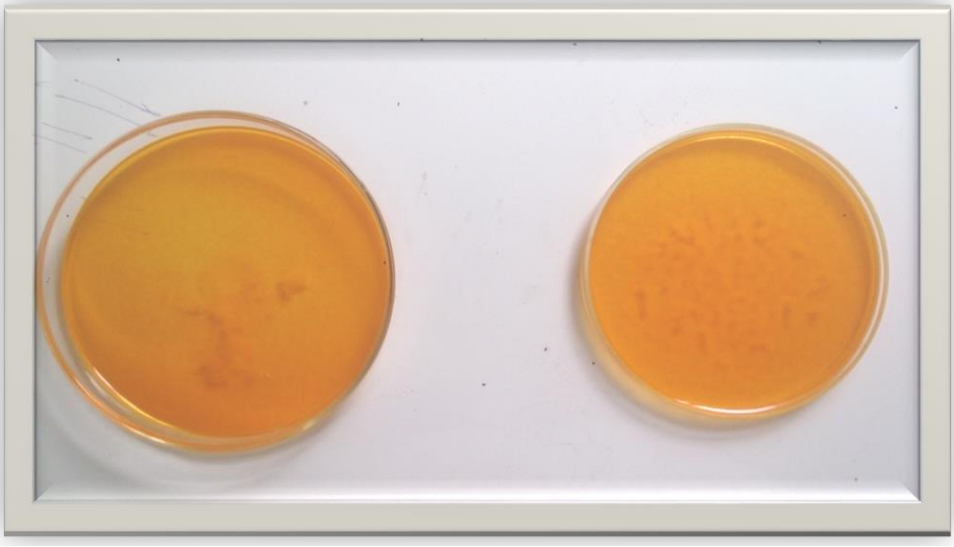

Fig. 3.20 Formulated Oral Thin Film of C:GA(9:1)

Fig. 3.21 Small Pieces of OTF C:GA (9:1)

The optimized batch of film with polymer to drug (4:1%) were evaluated for the characterization of C:GA(9:1), it was found that 4% of PVA as a polymer using ethanol as a solvent when used, with the 20% w/w of PEG 20,000 was used to formulated with

desired flexibility and optimum tensile strength in the prepared film. The prepared films were evaluated weight variation, folding endurance, thickness, tensile strength, pH, % elongation, and moisture uptake and moisture content observed good result.

4. Development and Evaluation of Amla Sachet as Antioxidant Drink

Sachet is a small cloth scented bag filled with herbs or aromatic ingredients A. sachet is also a small porous bag or packet containing a material intended to interact with its atmosphere; for example desiccants are usually packed in sachets which are then placed in larger packages. A sachet is a small packet or a bag that it has some items that are used as food or cosmetics items.There was no amla based ready to drink sachets available in market. The drink is focused for validated Antioxidant activity, Vitamin C content and Gallic acid content. The focus is also to design one sachet equivalent to 10 fresh amla fruits.

To find equivalent quantity of vitamin C, gallic acid and antioxidant potential 25 fresh amla fruits were taken from various places and of different sizes and quality were procured from vegetable market. Each Amla, juice was prepared in same method. Gallic acid content, DPPH Antioxidant Activity and Vitamin-C content was determined for each fresh amla and calculated. Average values of all three parameters were established and equivalent to 10 fresh amla fruits quantity of extract was taken for preparation of sachet. The said approach is novel in preparation of powdered form sachet.

4.1 Collection and authentification of sample drugs

The samples extract of Amla powder has been supplied with the help K. Patel Phyto Extractions Pvt. Ltd.

4.2 Physical properties of drug powder samples

As mentioned in Chapter 5

4.3 Solubility profile of Amla extract powder

As mentioned in Chapter 5

4.4 Anti-oxidant DPPH assay for Amla extract powder

As mentioned in Chapter 5

4.5 Preparation of each Amla juice

1. AMLA FRUIT

Take one Amla fruit. Cut into pieces and make paste in a mixer. Dilute the juice up to 25 ml. filter the juice. Collect filtrate. Take 2 ml of filtrate and dilute it up to 20 ml. From that 20ml, 0.4 ml was taken for estimation.

2. AMLA EXTRACT

Take 25-100mg of Vigour powder in 25 ml of water. Take 2ml and dilute it up to 20ml. From that 20ml, 0.4ml was taken for estimation.

4.6 Estimation of Ascorbic acid (Vitamin C)

Procedure

20mg of standard was dissolved in 100ml of distilled water on a volumetric flask to get a concentration of 200µg/ml. Various dilutions of different concentrations were prepared in test tubes using the standard stock solution i.e.2, 4, 6, 8, 10, 12µg/ml. In this all test tubes then add 0.8ml of concentrated Sulphuric acid and 1.6ml of Ammonium molybdate. Then keep these solutions aside for 1 hour, then after volume was make up to 10ml with distilled water. Then absorbance of this blue color solution was taken at λ_{max} = 650nm

4.7 FORMULATION APPROCH

Design and Development of Amla Sachet

4.7.1 Materials and Manufacturers

Table 4.1 List of Materials

Materials	Manufacturer
Glucose	CDH Pvt. Ltd
Mannitol	S.D.Fine Chem.Ltd.
Fructose	Nice Chemicals Pvt. Ltd.
Icying sugar	Gujarat General Food Chem. Pvt. Ltd.

4.8 Manufacturing Process

Procedure

Take specified gram of Amla powder and triturate it properly in glass mortar-pestle. Then weigh sweetening agent accordingly to its ratio. Weigh specified amount of Glucose, mannitol, fructose and of icying sugar and powder it properly Then mix Amla powder with sugars and add isopropyl alcohol for formation of granules. Take sieve no. 44 and form granules. Keep the granules in hot air oven at 50ºC temperature for drying.

4.9 Evaluation parameters of Amla Sachet

4.9.1 Flow properties

1. Bulk density

It is defined as mass of powder divided by the bulk volume. It depends on particle size distribution, particle shape, and tendency of particle to adhere to each other. A quantity of weighed granules was introduced in a 25ml measuring cylinder and the initial volume was noted. The Loose bulk density (LBD) was calculated using:

LBD = Mass of the powder / untapped volume

2. Tapped density:

After measuring the bulk density the same measuring cylinder was set into tap density apparatus. It was set at 100 taps drop per minute. The tap density is calculated by,

TD = Mass of the powder / tapped volume

3. Carr's Index:

It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder.

%Carr's Index = ((TD – BD)) / TD * 100

4. Hausner's Ratio:

It is also known as the Packing factor and is the ratio of tap density upon bulk density.

Hausner's Ratio: Tapped density / Bulk density

5. Angle of Repose:

The frictional force in loose micro particles can be measured by angle of repose. It is defined as angle possible between the surface of a pile of the powder and the horizontal plane.

$$\tan\theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where θ = angle of repose

h= height of pile

r= radius of the base of pi

A funnel was filled to the brim and the test sample was allowed to flow smoothly through the orifice under gravity. From the cone formed on the graph sheet the height and the radius of the pile was determined for the calculation of the angle of repose by the above mentioned formula.(Anonymous, 1, 2014, 96-277).

Table 4.2 Scale of Flowability

FLOW PROPERTY	AR(Θ°)	CI(%)	HR
Excellent	25-30	≤ 10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair (aid not added)	36-40	16-20	1.19-1.25
Passable (may hanged up)	41-45	21-25	1.26-1.34
Poor (must agitate, vibrate)	46-55	26-31	1.35-1.45
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

4.10 Formulation of Amla Sachet

After observing this there was decided to make a Antioxidant drink from this Amla extract with some sweetening agent. First there was decided to optimized the ratio of Amla extract with sugars So, there was taken 3 ratios i.e. 1:1, 1:1.5 and 1:2(Amla : Sugars) respectively

4.11 RESULT AND DISCUSSION

4.10.1 PHYSICAL PROPERTIES OF AMLA EXTRACT POWDER

Table 4.3 Physical properties of Amla extract

PHYSICAL PROPERTIES		AMLA EXTRACT
%LOD		2.05%
pH	Water	2.07 ± 0.01
	Methanol	6.9 ± 0.02
Bulk Density		0.5 ± 0.01
Tapped Density		0.64 ± 0.01
Carr's Index		21.87
Hausner's Ratio		1.28
Angle of Repose		75.27
Water soluble Extractive value		39.4% ± 0.01
Alcohol soluble Extractive value		86% ±0.3

4.10.2 SOLUBILITY OF AMLA EXTRACT

200mg of Amla extract powder was dissolved in 50ml water in4.45 minutes at room temperature (30-37 C°). By observing this it was concluded that the Amla extract powder is having good flow properties and good solubility in water.

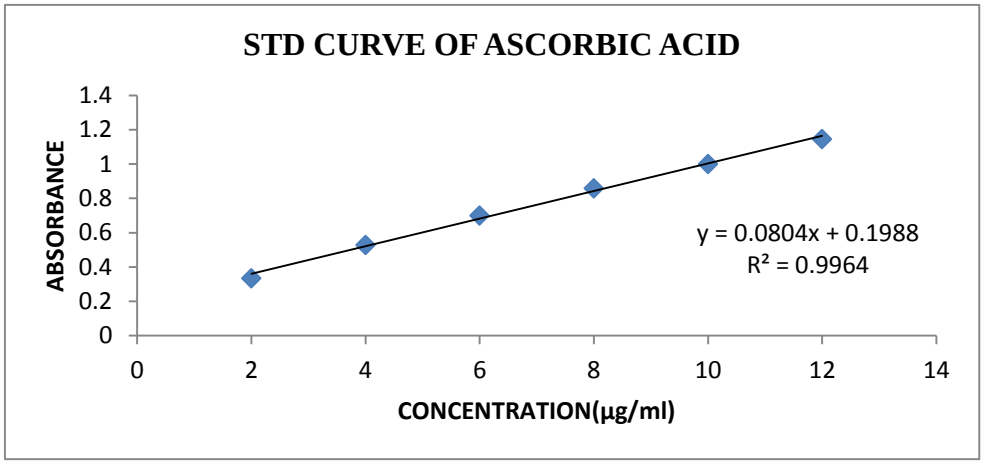

Fig. 4.4 Calibration curve of Ascorbic acid

4.10.3 ANTIOXIDANT ASSAYS OF AMLA EXTRACT

Table 4.4 Antioxidant assays of Amla extract

AMLA	DPPH	ABTS	B-CAROTENE	SUPEROXIDE
	11.073	5.472	71.014	620.03

4.10.4 IR SPECTROSCOPY

AMLA EXTRACT

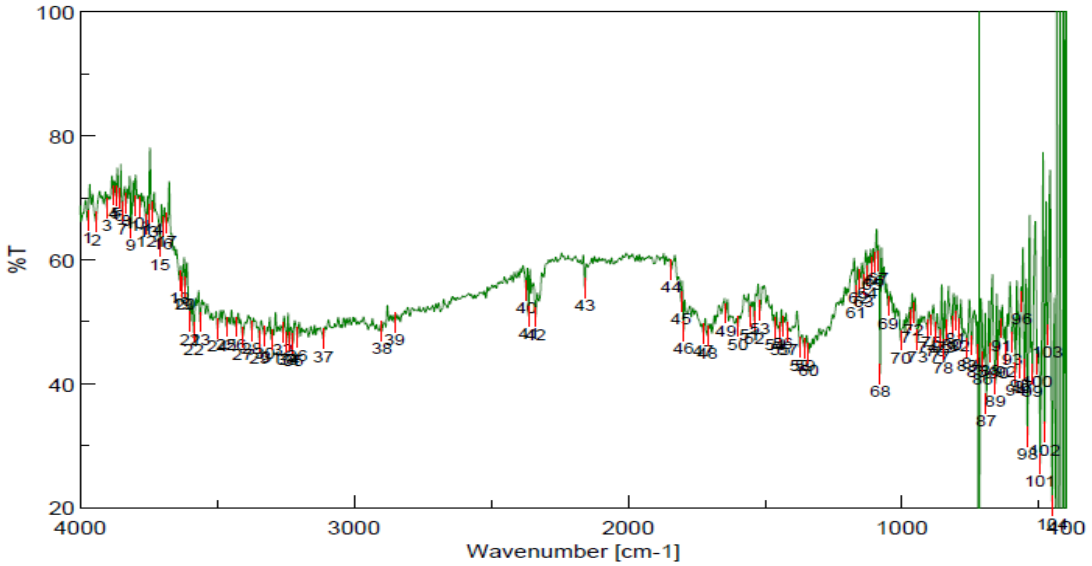

Fig. 4.5 IR Spectra of Amla

Table 4.5 Interpretation of Amla extract powder

Peak Frequrncies	Functional Group
3640–3610 (s, sh)	O–H stretch, free hydroxyl alcohols, phenols
3100–3000 (s)	C–H stretch aromatics
2260–2100 (w)	–C=C– stretch alkynes
1250–1020 (m)	C–N stretch aliphatic amines

4.10.5 CONTENTS OF VITAMIN C, GALLIC ACID AND ANTIOXIDANT POTENTIAL IN 10 AMLA FRUIT

Table 4.6 Content of Vitamin C, Gallic acid and % Antioxidant potential

	VITAMIN C CONTENT	GALLIC ACID CONTENT	ANTIOXIDANT POTENTIAL
AMLA FRUIT	3.568 ± 0.03 mg / amla fruit	1.334 ± 0.01 mg / amla fruit	IC 50 = 0.814 ± 0.2 mg
	0.1057 ± 0.006 mg / g of amla fruit	0.0395 ± 0.004 mg / g of amla fruit	
AMLA EXTRACT	21.80 ± 0.02 mg / g of amla extract	3.227 ± 0.05 mg / g of amla extract	IC 50 = 0.011 ± 0.05 mg
EXTRACT : FRUIT	206 times	81 times	70 times
EXTRACT ≈ TO 10 AMLA FRUIT	**1.637 g / 10 amla fruit**	**4.13 g / 10 amla fruit**	**N.A.**

By observing and calculating the 1.637 g of Vitamin C and 4.13 g of Gallic acid in 10 Amla fruit the summation is 5.737 g divided by 2 is 2.8835 g. **So, approximately 3 g of Amla extract contains 1.637 g of Vitamin C and 4.13 g of Gallic acid per 10 Amla fruit. So there was decided to make 10 grams Amla sachet with Amla extract and sweeteners.**

4.10.6 OPTIMIZATION OF AMLA SACHET

Table 4.7 Optimized ratio of Amla sachet

Drug (Amla extract powder)	3.33 g (33.3 %)
Sweetening agent	6.66 g (66. 6 %)
Glucose	0.66 g (6.66 %)
Mannitol	0.66 g (6.66 %)
Fructose	2.66 g (26.6 %)
Icying sugar	2.66 g (26.6 %)

4.10.7 EVALUATION PARAMETERS

Table 4.8 Evaluation of Amla sachet

EVALUATION PARAMETRS	**RESULTS**
DRUG SOLUBILITY	10mg- 25ml- 45 seconds at room temp.
BUL DENSITY	0.34 g/ml
TAPPED DENSITY	0.421 g/ml
CARR'S INDEX	19.23 %
HAUSNER'S RATIO	1.2352
ANGLE OF REPOSE	89.89º

Fig. 4.6 Formulated Amla sachet

4.10.8 IR SPECTROSCOPY

AMLA SACHET

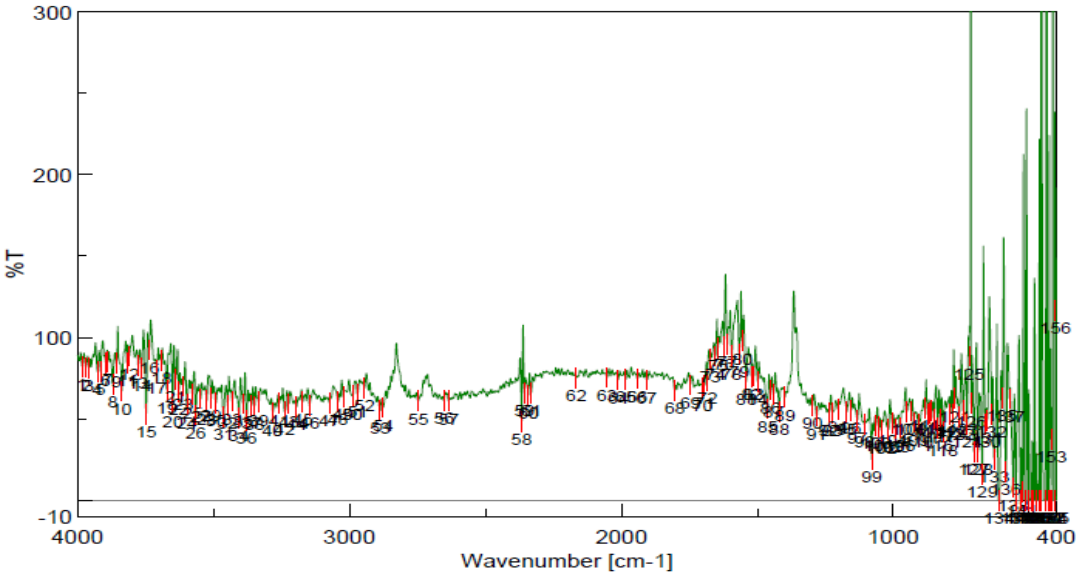

Fig. 4.7 IR Spectra of Amla Sachet

Table 4.9 Interpretation of Amla Sachet

Peak Frequencies	Functional Group
3640–3610 (s, sh)	O–H stretch, free hydroxyl alcohols, phenols
2830–2695 (m)	H–C=O: C–H stretch aldehydes
2260–2100 (w)	–C=C– stretch alkynes
1250–1020 (m)	C–N stretch aliphatic amines

By using the Amla extract in a ready to drink sachet there is optimized that the 10gram of Amla Sachet is having more Vitamin C , Gallic acid content and % Antioxidant Potential than 10 fresh Amla fruits.

STABILITY STUDIES

Stability was measured every month for 6 months(Oct 2014- Mar 2015) by observing its antioxidant DPPH by UV spectrophotometer method of Curcumin, Gallic acid, Amla and 3 months (Jan 2015- Mar 2015) of C:GA(9:1) Its antioxidant activity does not change as long period of time. So, its stability is good enough.

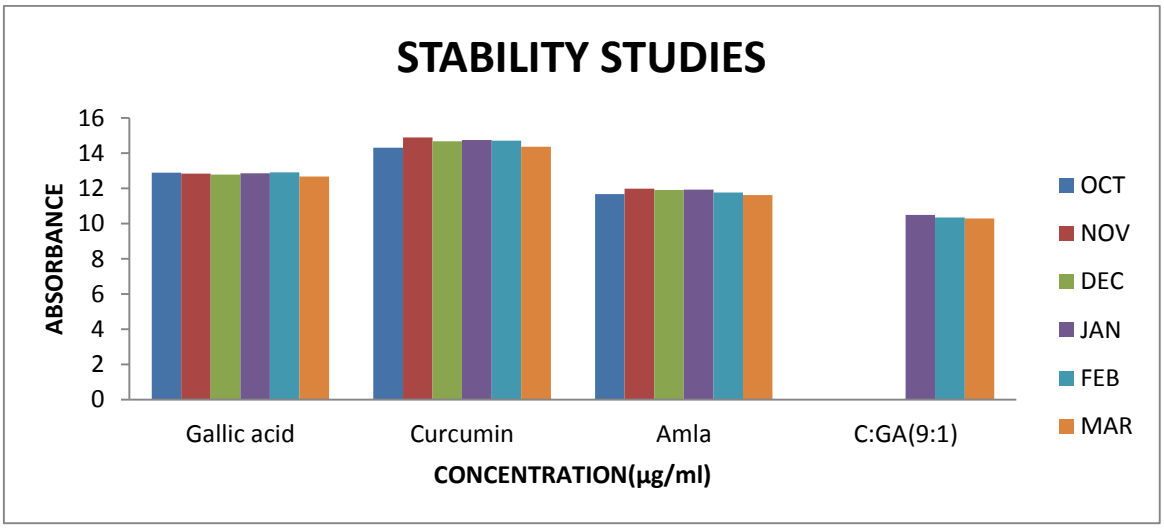

Fig. 6.8 Stability studies

7. Conclusion

C:GA(9:1)

Antioxidants are having capability to stabilize or deactivate the free radicals before they attack the cell. They are the compounds which are protecting cells from the damaged caused by unstable molecules which are known to be free radicals. They are interacting and stabilizing free radicals and sometimes it may also prevent some of the damage free radicals otherwise they might cause damage to the cells. The damage caused by free radicals also leads to cancer. Examples of anti-oxidants include beta-carotene, lycopene, Vitamin C, F and A. The bright yellow colour of turmeric comes from fat-soluble polyphenols pigment known as curcumin. Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, and a type of organic acid also known as 3, 4, 5-trihydroxybenzoic acid. It is found both in free and as part of hydrolysable tannins.tje solubility is the problem for Curcumin in water, so to cure this problem Gallic acid was used with Curcumin and formed a ratio C:GA (9:1) due to which there is 106 times increase in solubility of Curcumin in water.

The antioxidant potential of its complex is also synergistically increases with special reference to Anti-oxidant assays. Therefore, % Antioxidant Potential in C: GA (9:1) is 200 % more than of Curcumin and 150 % more than of Gallic acid. After Observing this there was formulated an Oral Thin Film by using C: GA (9:1) Complex which would be known as Antioxidant Film to enhanced its Solubility and Antioxidant Potential.

AMLA SACHET

- Amla is the gift of nature to mankind and so it is known as wonder herb. It is rich in polyphenols, minerals and a great source of Vitamin-C. It has protein, fibres, carbohydrates, minerals and Gallic acid which is a potent polyphenols. Amla extract was used to make the Amla Sachet(1:2) there is observed that 10 gram of Amla sachet is equal to more than 10 fresh Amla fruits. By observing its Vitamin C content, Gallic acid content and Antioxidant Potential there is observed 206 times, 81 times and 70 times increase in its folds respectively

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