ASSESSMENT OF PHYTOCHEMICAL AND THERAPEUTIC ACTIVITY OF DIFFERENT CULTIVARS OF *LAGENARIA SICERARIA*

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIRMENTS OF THE DEGREE OF MASTER OF PHARMACY

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KOLKATA

By

AMRENDRA KUMAR TIWARI
Reg. No. – 129118 of 2014- 2015

SCHOOL OF NATURAL PRODUCT STUDIES
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY
JADAVPUR UNIVERSITY
KOLKATA – 700032
INDIA

2015 – 2016
I

Certificate

This is to certify that Mr. Amrendra Kumar Tiwari, bearing the Regn. No. 129118 of 2014-15, has successfully completed the research work on the subject entitled “Assessment of phytochemical and therapeutic activity of different varieties of *Lagenaria Siceraria*” under the supervision of Dr. Pulok K. Mukherjee. He has incorporated findings of his research work in this thesis of the same title being submitted by him, in partial fulfillment of requirements for the degree of Master of Pharmacy of Jadavpur University. This thesis is absolutely based upon his own work and that neither this thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

Prof. Pulok K. Mukherjee Ph.D., FRSC
Director, School of Natural Product Studies
Department of Pharmaceutical Technology
Jadavpur University
Kolkata, India

Forwarded by,

Prof. Biswajit Mukherjee
Head
Department of Pharmaceutical Technology
Jadavpur University
Kolkata, India

Prof. Sivaji Bandyopadhyay
Dean
Faculty of Engineering & Technology
Jadavpur University
Kolkata, India

Declaration

I hereby declare that the thesis entitled “Assessment of phytochemical and therapeutic activity of different varieties of *Lagenaria Siceraria*” contains literature survey and original research work by the undersigned candidate.
All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.
I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are original to this work.

NAME	AMENDRA KUMAR TIWARI
EXAM ROLL NO	M4PHA1623
REGISTRATION NUMBER	129118 of 2014-15
THESIS TITLE	“Assessment of phytochemical and therapeutic activity of different varieties of *Lagenaria Siceraria”*

AMENDRA KUMAR TIWARI

This thesis is dedicated to my family and friends and respected teachers...

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Amrendra Kumar Tiwari

Preface

India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. Many food plants we eat are basically in many ways acts as medicine to keep our body homeostasis. The use of complementary and alternative medical therapies has become a common trend around the world and its utilization has been documented extensively over the last decade. Ayurveda, being one of the oldest traditional medicinal systems in India, is enriched with the use of various medicinal plants as the key components. *Lagenaria siceraria*, a food plant, almost 25 cultivars are present of different shapes and sizes throughout the world without a scientific differentiation having different compositions of metabolites, shows enormous potential as a medicine in our traditional, ethnobotanical and Ayurveda, but proper documentation and validation is very much necessary to not only assure the quality, authenticity and efficacy but also to come up with good safety profiling to evaluate the risk associated with beyond pharmacological and toxicological effects comparatively among these cultivars. This kind of medicinal plant must be studied and must be standardized for better understanding of biological activity parameters of different cultivars found. Product consistency and quality control issues such as misidentification of plants, processing method, product uniformity, batch-to-batch reproducibility, dose standardization, contamination, mislabeling and toxicity is the key concern of natural medicines.Pancreatic procaine lipase is a key enzyme responsible for lipid metabolism in the body. Lipid metabolism is elegantly balanced to maintain homeostasis. When the balance is lost, obesity or hyperlipidemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs. Therefore, the control of lipid metabolism by drugs could be used to prevent or treat these diseases. Pancreatic procaine lipase plays a key role for triglyceride absorption in the small intestine. This enzyme is secreted from the pancreas and hydrolyzes triglycerides into glycerol and fatty acids. Bearing this, the suppression of triacylglycerol absorption by inhibiting lipase is a major approach to avert obesity.

With these scopes, five cultivars of plant *Lagenaria siceraria* fruits were selected and each were studied through different chromatographic and spectrometric methods i.e. HPTLC, RP-HPLC and AAS etc with an aim of proper validation and documentation. Further, lipoprotein lipase inhibition potential was studied with the extracts (aqueous and methanolic) of these plant cultivars, for determination of their enzyme - extract interaction potentials and has been represented by various chapters in this thesis. A well proposed rat model *In –vivo* assay was done in order to get an *In vitro - In vivo* correlation of plant extract in order to validate its pharmacological activity.

Amrendra Kumar Tiwari

Abbreviations

AAS	:	Atomic absorption spectroscopy
CC	:	Calibration curve
Conc.	:	Concentration
ID	:	Identification
UV	:	Ultraviolet
mg	:	Milli gram
ml	:	Milli litre
No	:	Number
SOP	:	Standard operating procedure
DMSO	:	Dimethyl sulfoxide
HPLC	:	High-performance liquid chromatography
HPTLC	:	High performance thin layer chromatography
HTS	:	High-throughput screening
LDL	:	Low density lipid
STD	:	Standard
Vol.	:	Volume
%	:	Percentage
r2	:	Coefficient of determination
R_f	:	Retention factor
Rt	:	Retention time
RP-HPLC	:	Reverse Phase- High performance liquid chromatography
SD	:	Standard deviation
S.E.M	:	Standard error mean
TLC	:	Thin layer chromatography
TM	:	Traditional medicine
w/w	:	Weight by weight
mcg / µg	:	Microgram
µl	:	Micro liter
µM	:	Micro molar
LSFJE	:	*Lagenaria siceraria* fruit juice extract
LSME	:	*Lagenaria siceraria* methanolic extract
RSD	:	Relative standard deviation
RT	:	Retention time
LOD	:	Limit of detection
LOQ	:	Limit of quantitation

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Chapter – 1

Lagenaria siceraria and its cultivers

1.1. Introduction

1.2. Common names

1.3. Varieties present in nature

1.4. Botanical characterization and distribution

1.5. Traditional uses and ethnopharmacology

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1.7. Pharmacological activities

1.8. Toxicological assessment

1.9. Selected plant cultivers of *Lagenaria siceraria*

1.1. Introduction

Cucurbitaceae is among the most important plant families supplying humans with edible products and useful fibers. Cucurbitaceae is the major family name encompassing about 125 genera and 825 species. Cucurbitaceae is divided into two sub-families: Zanonioideae and Cucurbitoideae. The sub-family Cucurbitoideae contains the food producing plants, and will be the subject of our study. Varieties of both pumpkin, gourd and squash fall under the species (Deyo et al., 2008). The important genera belonging to the family are Trichosanthes, Lagenaria, Luffa, Benincasa, Momordica, Cucumis, Citrullus, Cucurbita, Bryonopsis and Corallocarpus. Some of the important plants that have been extensively studied are Momordica *charantia, Cucurbita pepo, Cucumis sativus, Cucumis melo, Citrullus colocynthis, Luffa echinata, Trichosanthes kirilowii, Lagenaria siceraria, Benincasa hispida etc* (Rajasree et al., 2016) Cucurbit plants were used actively as traditional herbal remedies for various diseases. They have demonstrated anti-inflammatory, antitumor, hepatoprotective, cardiovascular and immunoregulatory activities. In general, members of this family have always been considered as a subject of research due to the fact that they are rich source of proteins, with many biological activities like anti-fungal, anti-bacterial, antiviral, anti-diabetic, anti-tumor and anti-AIDS. It is also known to contain several bioactive compounds such as cucurbitacins, triterpenes, sterols and alkaloids (Rajashree et al., 2016)Seeds or fruit parts of some cucurbits are reported to possess purgatives, emetics and antihelmintics properties due to the secondary metabolite cucurbitacin content. A number of compounds of this group have been investigated for their cytotoxic, hepatoprotective, anti-inflammatory and cardiovascular effects. Cucurbitacins constitute a group of diverse triterpenoid substances which are well known for their bitterness and toxicity. They are highly oxygenated, tetracyclic triterpenes containing a cucurbitane skeleton characterized. The cucurbitacins are arbitrarily divided into twelve categories, incorporating cucurbitacins A to T (saboo et al., 2013).*Lagenaria siceraria* (molina) standley (family: cucurbitaceae) is an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans (Ghosh et al., 2009). *Lagenaria siceraria* is official in Ayurvedic pharmacopoeia of India. *L. Siceraria* fruits are traditionally used as a nutritive agent having cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, and cooling effects. It cures pain, ulcers, and fever and is used for pectoral cough, asthma and other bronchial disorders (Doere et al., 2010). *L. Siceraria* leaf contains hypolipidemic properties that can be used to decrease the cholesterol level and thus helpful in case of all elevated steroid level related diseases (Ghule et al., 2009). Plants of *Lagenaria siceraria* are very similar in above ground development, but they have high genetic diversity for fruit shape and other fruit characteristics, resulting in a variety of uses (Bisognin et al.,1996). A total of six species have been recognized as belonging to the genus Lagenaria or white flowered gourds. One is the domesticated monoecious species *L. Siceraria* while five of them are wild perennial, dioecious forms from Africa and Madagascar. The basic haploid chromosome number of the genus is 11 (2n = 22) (Singh. 1990). Bottle gourd was domesticated in Asia

and at the same time indigenous to Africa (Whitaker & Davis, 1962). Tropical Africa remains as the primary gene pool for this species (Singh. 1990). Bottle gourd was the most widely distributed plant in the world (Heiser et al., 1979) with a long history of use in both Old and New Worlds. In the Old World, bottle gourd cultivation was traced back over 5,000 years BP (Robinson et al., 1997). Archeological evidences showed that bottle gourd was cultivated in North America in 10,000-7,500 years BP and in South America in 6,000-5,000 years BP. There is no secure argument that can be used to resolve the unusual bi-hemispheric distribution of bottle gourd. Experimental evidence suggested that the early spread from Africa to the New World could occur through oceanic drift. At the present time, it is cultivated throughout the tropical and subtropical regions of the world for food and useful gourds (Whitaker et al., 1962).

The present study is based on the approach to evaluate the quality of the below mentioned cultivars of *Lagenaria siceraria* plant through different chromatographic and spectrometric method as well as to identify the heavy metal and mineral content of them. Later on this study focuses on the findings of lipoprotein lipase interaction potential of the above said medicinally active food plants with an intention to find out the probable anti hyperlipidemic activity of the plant *in -vitro*. Further in vivo studies was done to correlate the comparative effect of most active cultivars, which can be formulated as neutraceutical powder.

Table 1.1. Taxonomical Classification (Gorasiya et al., 2011)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Lagenaria
Species	Siceraria
Part used	Fruit, root, leaves and seed oil.

1.2. Common names

Lagenaria siceraria is a food plant so there are many common names given to this plant. Some of these are mentioned in Table 1.2.

Table 1.2. Common names (Tyagi et al., 2012)

Language	Names used
Sanskrit	Alabu, Tumbi Ishavaaku, Katutumbi, Tiktaalaabu, Alaabu
Hindi	Lauki, Ghia
Bengali	Laus, Lokitumbi
English	Bottle Gourd
Gujrati	Dudi, Tumbadi
Kannad	Isugumbala, Tumbi
Malyalam	Chorakka, Churan, Choraikka, Piccura, Tumburini, Cura, Tumburu
Marathi	Phopia
Punjabi	Tumbi, Dani
Tamil	Shorakkai, Surai, Suraikkai
Telugu	Sorakaya, Anapakaya
Urdu	Ghiya, Lauki

1.3. Varieties present in nature

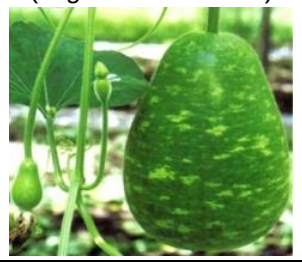
Lagenaria siceraria, a food plant, almost 25 cultivars are present of different shapes and sizes throughout the world without a scientific differentiation having different compositions of metabolites, shows enormous potential as a medicine in our traditional, ethnobotanical and Ayurveda, but proper documentation and validation is very much necessary to not only assure the quality, authenticity. Till now throughout the literature almost 25 cultivars were sorted out and these are mentioned in Table 1.3

Table 1.3. Varieties present in nature

Plant name	Botanical description	Reference
Big Apple Gourd (*Lagenaria siceraria*) 	Category: Vegetables, Vines and Climbers. Heighted almost 20-30 ft. (6-9 m). Needs full sun exposure. The bloom color is white/near white and in midsummer blooms repeatedly. Foliage is Veined and needs average water but regularly. This plant has been said to grow in the following regions: Hereford, Arizona; Brooksville, Florida; Orlando, Florida, Commerce, Georgia, Lawrenceville, Georgia.	Essien et al., 2013.
Blister Gourd (*Lagenaria siceraria*) 	New and unusual. 110 days. Apple shaped fruit covered in warts, weight 4-7 lbs. and measuring 9 x 12 inches when mature, excellent fall decoration. Larger and more warted	Essien et al., 2013.
Indian Serpent Gourd (*Lagenaria siceraria*) 	Actually an unusual cucumber with long, white speckled fruits that resembles snakes. Long used in India and the orient as a vegetable, quite delicious when young. Roots and seeds are also used to expel worms and treat diarrhea and syphilis	Kim, 2012.

Bottle Gourd (*Lagenaria siceraria*) 	The legendary bulb shaped gourd used so widely for decorations. Dries to a buff brown.	Chimonyo et al., 2013.
Giant Bottle Gourd (*Lagenaria siceraria*) 	This is the largest bottle gourd ever grown. Standard bottle gourds may reach 16 in. tall in good conditions, this one reaches 24 inches tall regularly.After the mature fruit has dried and the seeds are removed, this bottle gourd can be used as a container or for decorative purposes. The plant requires a long, warm growing season. For ornamental use only.	Kim, 2012.
Chinese Bottle Gourd (*Lagenaria siceraria*) 	These pear shaped gourds grow about 5 inches tall by 4 inches across. It has a flat bottom, making it an excellent craft gourd. The Chinese Bottle Gourd is used by many cultures around the world for many different purposes (food, drink, container, garment, healing).	Kim, 2012.

India Long Hybrid (*Lagenaria siceraria*) 	Fruits are cylindrical can be harvested at small size as well as full size stages for cooking and eating. White soft flesh is very tender and delicious, suitable for stir-fry and soup cooking. Plants are very vigorous and should be well watered in warm summer. Plants can be grown along supports or on the ground.	Chimonyo et al., 2013.
Big Green Sausage Gourd (*Lagenaria siceraria*) 	A new gourd that lives up to its name, the fruits do indeed look like big green sausages. Plant produces good yields of large 20 inches long light green gourds. Best if grown on trellis or fence.	Anonymous, 1940.
Harvest Bowl Gourd (*Lagenaria siceraria*) 	Also known as tobacco box gourd and basket gourd, this plant produces good yields of round bowl shaped gourds that have unlimited crafting possibilities.	Chimonyo et al., 2013.;

Penguin Gourd (*Lagenaria siceraria*) 	A favorite with craftsmen, 12 inches long by 5 inches wide.	Shivraj et al., 2005; Morimoto et al., 2005.; www.seedman.com
Long Handle Dipper Gourd (*Lagenaria siceraria*) 	Beautiful gourd with long handle. Handles can be trained into different shapes as they are growing, if so desired. Dipper Gourds can grow on trellis (straight gourds) or on the ground (various shapes). Green in color and great for crafts	Morimoto et al., 2005.; www.centeroftheweb b.ecrater.com
Green Bell Hybrid (*Lagenaria siceraria*) 	These are found as annuals, vegetables, vines and climbers having height of 6- 12 in (15-30 cm). Green Bell is a new high quality hybrid variety developed by Kaoshung Horticulature Research Center in Taiwan. Fruits with green skin and white stripes, in bell shape and 1.5 Lb. in weight. Plants grow very well in subtropical climates. Plants are vigorous and tolerant to disease attacks. Very productive and easy to grow.	Essien et al., 2013.; www.davesgarden.co m

Speckled Swan (*Lagenaria siceraria*) 	It is a highly attractive ornamental gourd, the long neck of which curves elegantly downward resembling a Swan's head. Dark green flesh is speckled white. Inedible.	Essien et al., 2013,; www.victoriananursery.co.uk
Cave Man's Club (*Lagenaria siceraria*) 	These rare and unique club shaped gourds are edible when young (about 8 inches long) and great for crafts when more mature 15-18 inches long with wrinkled knobby bulb end about 6 inches in diameter. Has average water needs.	Essien et al., 2013.; www.centeroftheweb b.ecrater.com
Dinosaur Gourd (*Lagenaria siceraria*) 	It belongs to a Category vines and climbers. Possess height of almost 6-12 in. (15-30 cm) 12-18 in. (30-45 cm). Traditionally used to decorate sweat lodges. Solid-green fruits have curved necks and wing-like projections, perfect for gourd craft swans. Total length of 18-24 in. with an 8 in. bowl and unique serpentine projections.	Essien et al., 2013.; www.davesgarden.co m

Powder Horn (*Lagenaria siceraria*) 	Belongs to a category of annuals, vegetables, vines and climbers. Possess Height of 12-18 in. (30-45 cm). Needs average water but regularly. Propagated directly from seeds which are directly sown after last frost. Unblemished fruit must be significantly overripe before harvesting. This plant has been said to grow in the following regions: Saucier, Mississippi and Hulbert, Oklahoma.	Kim, 2012.; www.davesgarden.com
Long Siphon (*Lagenaria siceraria*) 	Extra-long 36 inch hollow handle with 10 inch diameter ball end.	Shivraj et al., 2005.; www.seedman.com
Basket Gourd (*Lagenaria siceraria*) 	A slightly smaller size gourd than the bushel, with a more rounded basket shape. Green weight is about 20 lbs, this is the perfect size gourd for novelty baskets and decorations.	Chimonyo et al., 2013.; www.seedman.com

Large Kentucky Bushel Gourd (*Lagenaria siceraria*) 	A large variety that can grow 24-30" in diameter. We have seen larger, this is about the norm in good soil, to get largest gourds, remove all but one or two gourds per plant. Has a good hard shell for crafting. Not quite as large as the 100 pound bushel gourd, but this one makes a very consistent harvest.	Chimonyo et al., 2013.; www.seedman.com
Sugar Bowl (*Lagenaria siceraria*) 	Produces round gourds with flattened tops and bottoms averaging about 8 to 10 inches wide by 4 to 5 inches tall, that are ideal for making bowls or gift baskets, each gourd when divided will make two bowls. Well suited for crafting.	Shivraj et al., 2005.; www.seedman.com
Bushel Gourd (*Lagenaria siceraria*) 	Annual vine. These have been selected to produce large, globular fruits which may be crafted into containers for holding all types of goods. Originally from Africa, these were the main market container for carrying beans or other goods (prior to the invention of plastic).Still these are employed quite extensively in Africa--they are useful, comely, and biodegradable.	Shivraj et al., 2005.; www.horizonherbs.com

Martin House Gourd (*Lagenaria siceraria*) 	Annuals, vines and climbers, having height of almost 6-12 in (15-30 cm) of fruit and 10-12 ft. (3-3.6 m) of plant. The foliage are herbaceous, blue-green, velvet/fuzzy-textured.	Shivraj et al., 2005.; www.davesgarden.com
Bule (*Lagenaria siceraria*) 	Apple-shaped French gourd is 6-8 inches tall and 5-6 inches across, with a very hard, light green shell covered in small warty bumps. Excellent for crafts or ornamental displays, the gourds turn brown (pictured) as they dry. Long vigorous vines.	Essien et al., 2013.; www.loghouseplants.com
Opo Long Bottle Gourd (*Lagenaria siceraria*) 	This cylindrical bottle gourd has flesh that is sweet, tender, and delicious when harvested young. The fruits have a smooth, light green skin, and mature to about 12 to 15" long. Very productive plants.	Kim, 2012; Shivraj et al., 2005.; www.seedman.com

Asia Short Hybrid Bottle Gourd *(Lagenaria siceraria)* 	This hybrid variety is a vigorous plant with prolific bearing habit, producing fruits continuously for a long time. Relatively short cylindrical fruits with 25-30 cm. In length and 300-350 grams in weight are very tender and delicious. Light green skin with white flesh fruits with slow seed maturity. Plants grow vigorously in warm climates, starting to set fruits in 45 days after the transplanting. Easy to grow.	Shivraj et al., 2005.; www.davesgarden.com

1.4. Botanical characterization and distribution

1.4.1. Habitat

Lagenaria siceraria has both wild and cultivated forms. The latter is native to Africa and Asia. *Lagenaria siceraria* is cultivated throughout the year. All types of soil are suitable for cultivation but the best yield is obtained in heavily manured soil. The herb grows best in warm and humid climate. If *Lagenaria siceraria* is cultivated during dry weather then plenty of watering is required (Aslam et al., 2013). Seeds may be sown in nursery beds and seedlings transplanted when they have put forth 2-3 leaves. They may be also sown directly, 4-5 seeds together, in manure beds or pits 5-6ft. apart; the strongest among the seedlings is retained, while others are removed and transplanted. Seedling transplantation is where an early crop is desired, generally two crop raised in India; the summer crop is sown from the middle of October to the middle of March and the later crop, from the beginning of March to the Middle of July. Round fruit types are usually sown for the early crop and bottle–shaped types for the second crop. Vines are allowed to trail on the ground or trained over walls. Trees, or other support; trailing over give high yield of fruit (Kubde et al., 2010).

1.4.2. Botanical description

Part	Description	Reference
Plant	Large pubescent, climbing or tailing herb with stout 5- angled stems and bifid tendrils, found throughout India, either wild or cultivated.	Kubde et al., 2010
Leaves	Leaves are long, petioled, 3-5 lobed, 10-40 cm wide, slightly hairy on both sides.	Gorasiya et al., 2011
Fruits	Large, up to 1.8m. Long, fruit bottle shaped with a hard shell-like epicarp when ripe, numerous seeds, long, white, smooth, 1.6- 2.0 cm long, horizontally compressed with marginal groove.	Kubde et al., 2010
Flowers	White, solitary, auxiliary, unisexual. Male flowers possess botanical description of calyx and campanulate, narrow tube, 5 linear lobes, 5 free and white petals, 3stamens. Female flowers possess botanical description of calyx and carola as in male flowers. Ovary densely villous, style thick, 3 stigmas blobbed.	Kubde et al., 2010

1.4.3. Microscopy

Transverse section of *Lagenaria siceraria* leaf showed following features:

Part	Description	Reference
Upper epidermis	Consists of elongated parenchymatous cells, covered by cuticle. It shows few stomata, which are of anisocytic type.	Tyagi et al., 2012
Palisade cells	Present at upper and lower epidermis. It shows hexagonal to polygonal, large, thin walled colorless cells, and may be water storing.	Saha et al., 2010
Mesophyll	Mesophyll is made up of 3-4 layered chloroplast containing, compactly arranged, oval to circular cells. It is interrupted by vascular bundles of various sizes.	Saha et al., 2010
Vascular bundles	Surrounded by 2-3 layered sclerenchyma. They are conjoint, collateral and closed. Xylem is placed towards upper epidermis and phloem towards lower epidermis.	Saha et al., 2010
Lower epidermis	Contains elongated wavy walled parenchymatous cells covered by cuticle. Number of Covering and collapsed trichomes are present, while very few glandular trichomes are also present.	Saha et al., 2010

1.5. Traditional uses and ethnopharmacology

1.5.1. Uses described in traditional and folklore medicine

The fruits, leaves, stem, seeds and oil of *Lagenaria siceraria* are traditionally used in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic, antibilious, and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge (Kumar et al., 2012). In tribal communities it is extensively used as mentioned in table 1.4.

Table 1.4. Uses described in traditional and folklore medicine

Name of the tribe	Location of tribe	Name ascribed by the tribal community	Uses by tribal community	References
Koyas, Gutti koyas and Lambadas	Northern Telan-gana zone	sorakaya, anapakaya, anamgapkaya, burrakaya, and tumri	Use the dry hard shells of bottle gourd fruits for various purposes. Domestic utensils like bottles, bowls, milk pots, spoons, and containers of several types are made out of the dried shells.	Kumar, et al., 2012
The Ethnic groups	Khamma m district	-	Using the dry shells for carrying country liquor (mahua drink, toddy), honey, and water. It is also used for making stringed and wind musical instruments and pipes.	Upaganlawa r, et al., 2009
Koya communit y	Andra pradesh region	sorakaya, anapakaya, anamgapkaya, burrakaya, and tumri	Uses the fruits of the wild types for medicinal purposes (purgatives).	Upaganlawa r, et al., 2009
Gutti Koya tribals	Andra pradesh region	sorakaya, anapakaya, anamgapkaya, burrakaya, and tumri	Use the bottle gourd as a cure for headache by mixing the seed oil with castor oil. The pulp of the fruit is considered cool and diuretic. Leaves of *Lagenaria siceraria* are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in Jaundice. Crushed leaves are used for baldness and applied on the head for the headache. Leaves are also used as alternative purgative. Flowers are also mentioned as antidote in certain kind of poisons. Stem bark is diuretic. Roots are emetic and used in dropsy.	Kumar, et al., 2012

1.5.2. Ethanobotanical uses (Upaganlawar et al., 2009; Rehman et al., 2003)

Ethnobotanical uses of the fruit are mentioned below in table 1.5.

Table 1.5. . Ethanobotanical uses of *Lagenaria siceraria*

Sr. No	System	Uses
1.	Gastrointestinal	Adenopathy, Diuretic, Dropsy, Laxative, Litholytic, Lithontriptic, Purgative
2.	Cardio vascular system	Dropsy, Diuretic, Hydropsy
3.	Central nervous system	Ache (Head), Emetic, Ache (Tooth), Bilious, Convulsion, insanity
4.	Genito-urinary system	Dropsy, Diuretic, Litholytic, Lithontriptic
5.	Infections	Alexiteric, Alopecia, Sore throat, Boil, Burn, Cancer, Fever, Depurative, refrigerant, Rheumatism, Tetanus, Tumor, Wound
6.	Respiratory system	Asthma, Cough
7.	Ear, Nose, Throat	Gum problem, Hoarseness of voice
8.	Immunology	Cancer, Scrofula, Tetanus, Tumor
9.	Skin	Alopecia, Leucoderma, Anasarca, Boil, Burn, Depurative, Pimple, Wound
10.	Metabolism	Refrigerant
11.	Musculo-skeleton	Pectoral Rheumatism
12.	Poison	Alexiteric, Antidote

1.6. Chemical constituents

Lagenaria siceraria is a rich source of different classes of natural products with varying structural patterns. In the past few decades, a lot of primary and secondary metabolites have been isolated from *Lagenaria siceraria*, including alkaloids, flavone c- glycosides, triterpenoids, flavonoids, polysterols, phenolic compounds, tanins and steroids like campesterol and fucosterol.

1.6.1. Flavonoids

The flavonoid complex occuring in *Lagenaria siceraria* were reported to be mainly flavone C-glycosides. The C-glycoside flavonoids includes saponarin, vitexin (22), isovitexin (18), lutonarin (21), saponarin (20), saponarin 4- O- glucoside (17), saponarin caffeic ester (19), were isolated and identified. These flavonoids were obtained from methanolic extract of flowering herbs. (Baranowska et al., 1994).Other than these Isoquercitrin (13), kaempferol (14) are also reported (Gangwal et al., 2010).

1.6.2. Triterpenoids

Triterpenoids are the main active constituents of *Lagenaria siceraria.* The fruits of *Lagenaria siceraria* contains cucurbitacins B (27), D, H and G, leaves contains cucurbitacin B and seeds contains cucurbitacin B, D and E (28). These are present as aglycones. (Tyagi et al., 2012).Other than these nine triterpines have been isolated and identified from *Lagenaria siceraria*. Four new D:C-friedooleanane-type triterpenes, 3b -O-(E)- feruloyl-D:C friedooleana-7,9 (11)-dien-29-ol (1), 3b -O-(E)- coumaroyl-D:C friedooleana -7,9(11)-dien-29-ol (2), 3b-O-(E)-coumaroyl-D:Cfriedooleana-7,9(11)-dien-29-oic acid (3), and methyl 2b ,3b -dihydroxy-D:C-friedo olean-8-en-29-oate (6), isolated from methanolic extract from bark of *Lagenaria siceraria,* together with five known triterpenes with the same skeleton, 3-epikarounidiol (4), 3-oxo-D:C-friedoolena-7,9(11)-dien-29-oic acid (5),4) bryonolol (7), bryononic acid (8), and 20-epibryonolic acid (9) (Chen et al., 2008).

1.6.3. Sterols

The presence of mixture of sterols were reported from methanolic extract of *Lagenaria siceraria.* The major sterols identified were β - sitosterol (15), campesterol (23), fucosterol (24), stigmasterol (25), racemosol (26) (Shirwaikar, et al., 1996). Other than these spinasterol, racemosol, avenasterol, codisterol, elesterol, isofucasterol, stigmasta-7,22-dien-3β,4β-diol have been reported (Kalsait et al., 2011).

1.6.4. Polyphenols

In *Lagenaria siceraria* ellagitanins were reported to be present (Deshpande et al., 2007).

1.6.5. Volatile essential oils

Till now eight essential oils were reported including octanal, nonanal, decanal, hydroquinone, 2-pentadecyn-1-ol, 9, 12-octadecadienal, palmitic acid and stearic acid (Chatterjee et al., 2009).

1.6.6. Vitamins

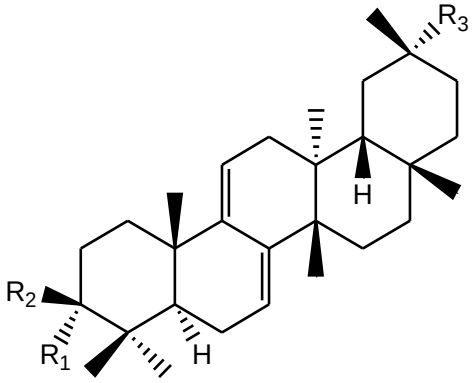
So far β-carotene, retinol, thiamine, riboflavin, niacin, choline, ascorbic acid were reported to be present in *Lagenaria siceraria* (Kumar et al., 2013).

1.6.7. Polysaccharides

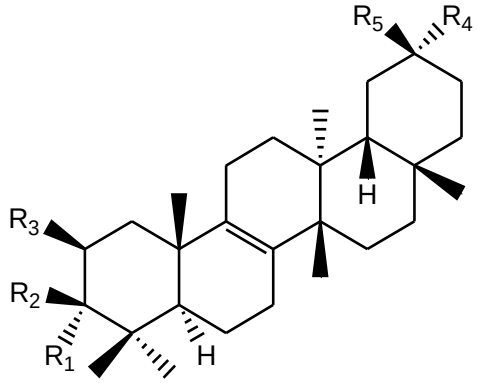
Lagenaria siceraria contains water soluble polysaccharides. These Polysaccharides includes methyl alpha-d-galacturonate, 3-O-acetyl methyl-alpha-d galacturonate, and beta-d-galactose (Ghosh et al., 2007).

1.6.8. Other compounds

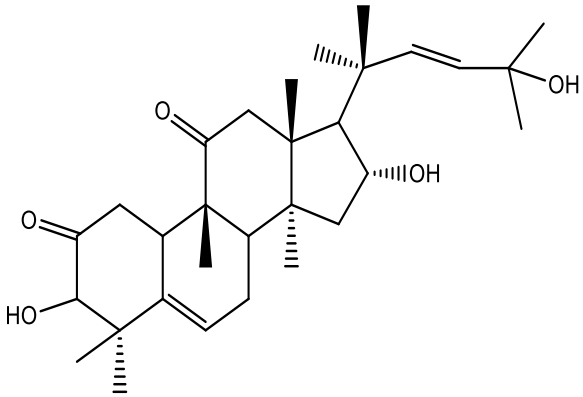
A range of other compounds were also reported to be present in *Lagenaria siceraria,* such as free fatty acid, lagenin, saponins, protein, carbohydrates, moisture, inorganic elements (like phosphorus, calcium, iron, sodium, potassium, iodine) and amino acids (like phenylalanine, leucines, valine, tyrosine, alanine, glutamic acid, serine, aspartic acid, cystine, arginine, proline, threonine). Monosaccharide such as glucose, fructose and traces of sucrose were reported (Gorasiya et al., 2011; Kubde et al., 2010; Aslam et al., 2013; Kumar et al., 2012; Rahman et al., 2003).



	R_1	R_2	R_3
1	H	O-(E)-feruloyl	CH_2OH
2	H	O-(E)-coumaroyl	CH_2OH
3	H	O-(E)-coumaroyl	COOH
4	H	OH	CH_2OH
5	= O		COOH



	R_1	R_2	R_3	R_4	R_5
6	H	OH	OH	COOCH3	CH_3
7	H	OH	H	CH_2OH	CH_3
8	= O		H	COOH	CH_3
9	H	OH	H	CH_3	COOH
10	H	OH	H	$COOCH_3$	CH_3



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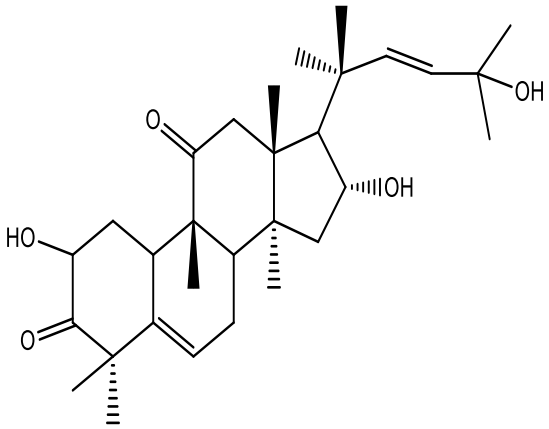

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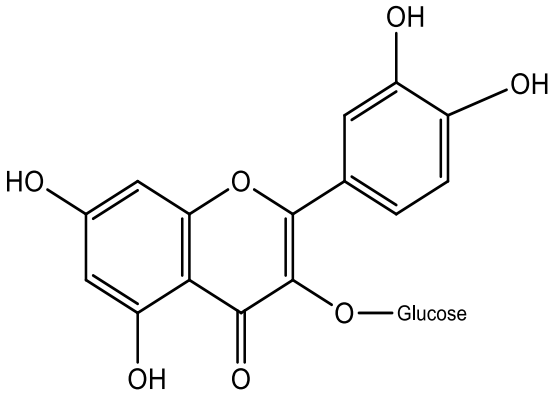

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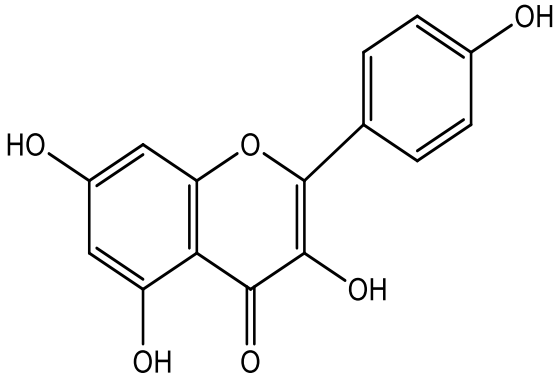

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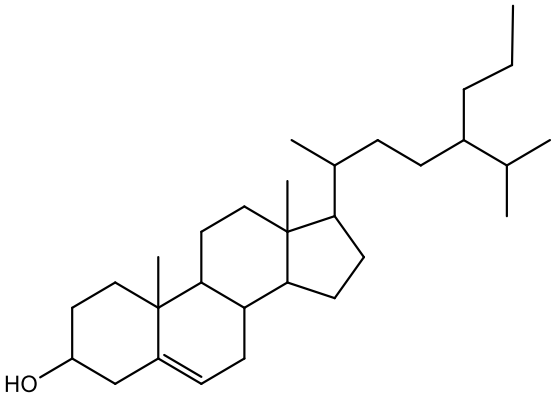

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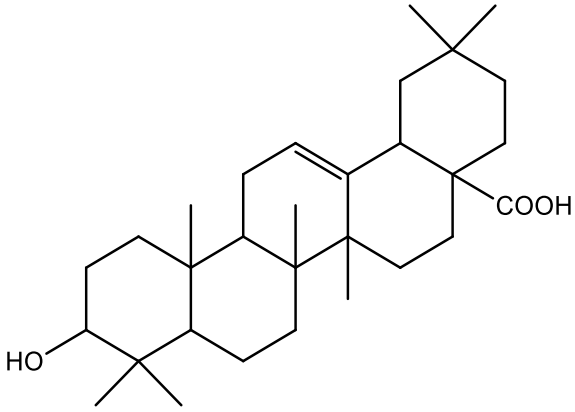

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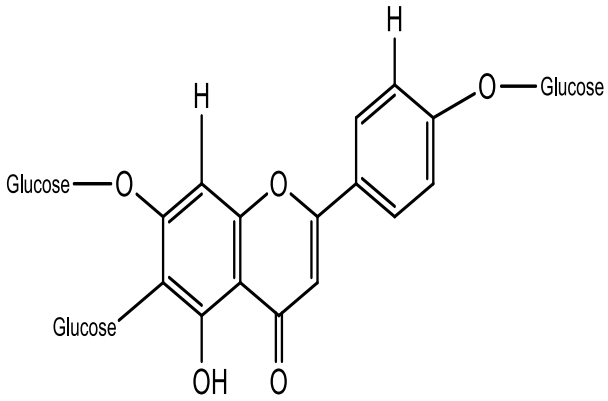

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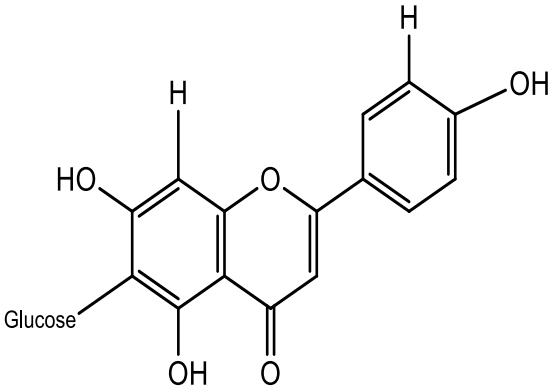

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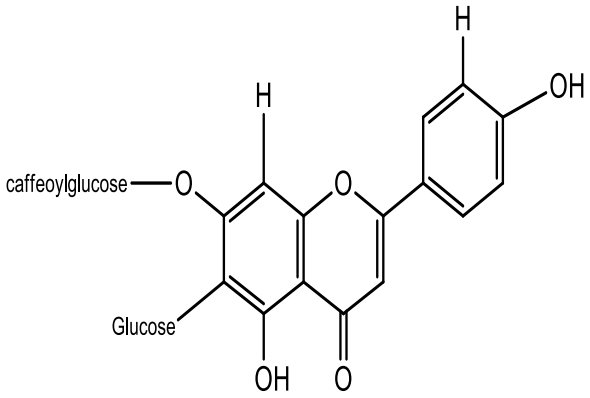

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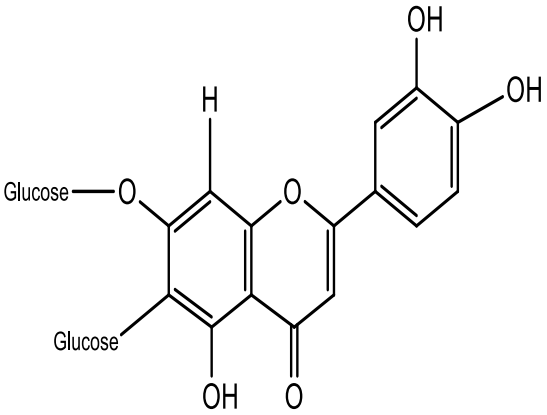

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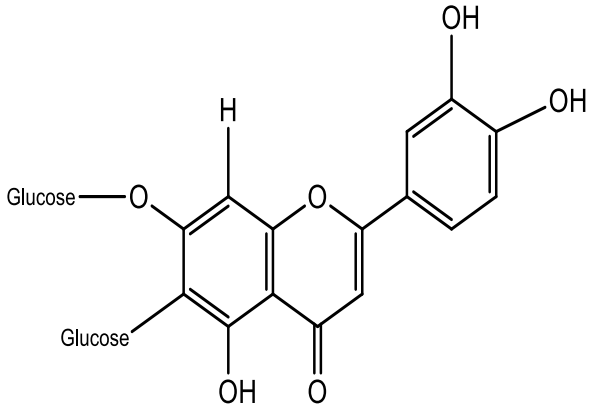

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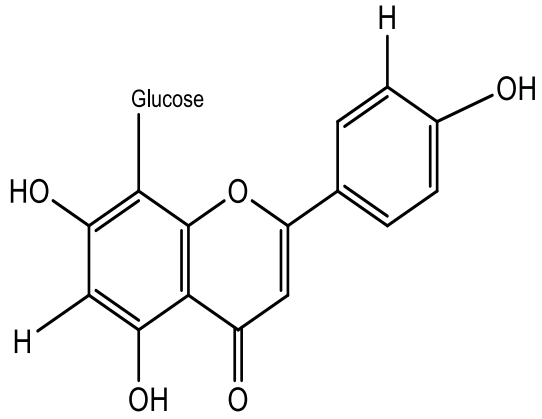

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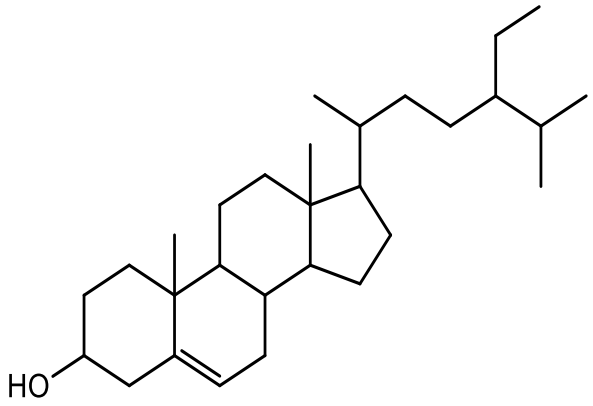

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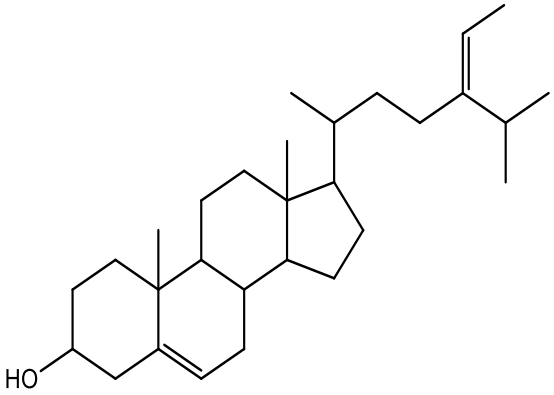

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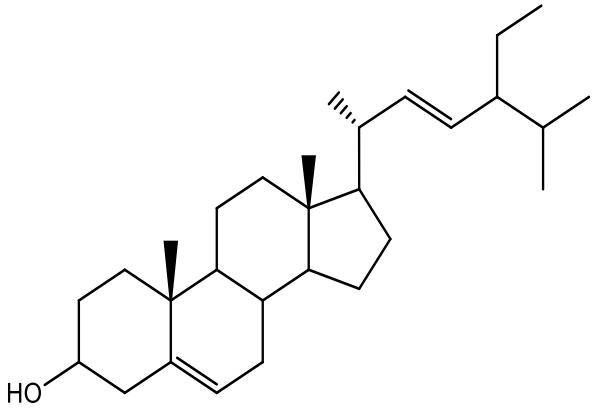

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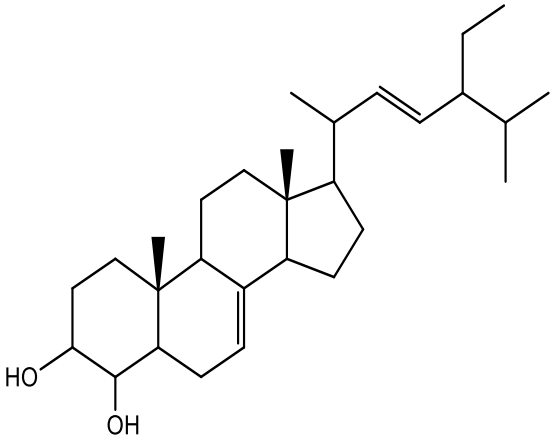

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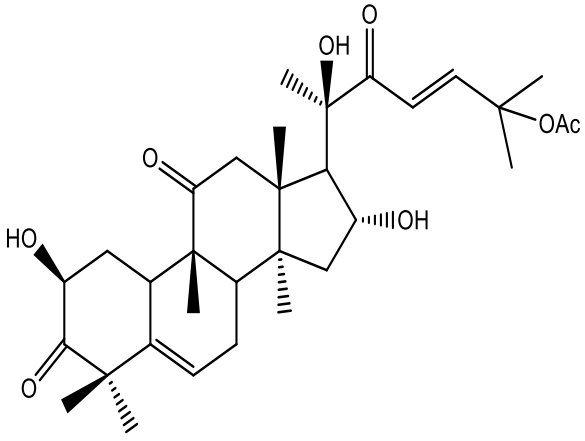

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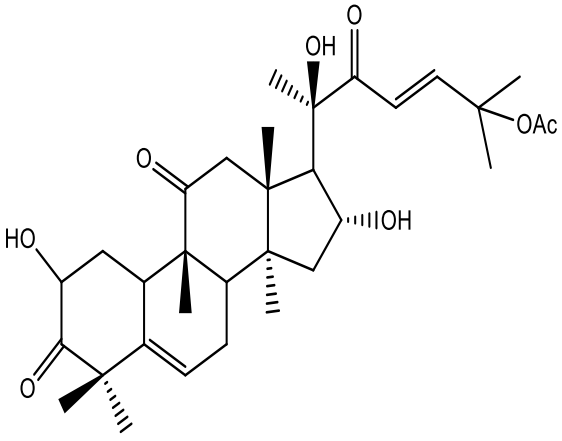

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1.7. Pharmacological activities

Lagenaria siceraria exhibits a variety of pharmacological activity till yet reported such as:

1.7.1. Anti- hyperglycemic activity

The antihyperglycemic activity of methanol extract of *Lagenaria siceraria* aerial parts (MELS) was reported for its purported use in diabetes. Hyperglycemia was induced by streptozotocin in rats. Treatment was done by methanolic extract of *Lagenaria siceraria*. They observed significant reduction in fasting blood glucose levels during treatment. The study explored the potent antihyperglycemic activity of extract (Saha et al., 2011). In another experimental study a notable reduction in blood glucose levels was observed in diabetic subjects on juice therapy (Katarea et al., 2013).

1.7.2. Anti- hyperlipidemic effect

Methanolic extract of *Lagenaria siceraria* fruit (LSFE) was administered to the high fat-diet-induced hyperlipidemic rats to evaluate its hypolipidemic activity. They obtained that there was a significant reduction in lipid levels in the LSFE treated rats as compared to the rats fed with high-fat diet (Ghule et al., 2009). Sterol crystals obtained from methanolic extract of *Lagenaria siceraria* fruit or phytosterols were reported to possess antihyperlipidemic activity to treat the hyperlipidemic conditions (Kalsait et al., 2011).

1.7.3. Anti- oxidant activity

The study the antioxidant effect of fresh and dried fruits of *Lagenaria siceraria* was evaluated by comparing the 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging and reducing capacity of ethyl acetate and n-butanol extracts of fresh and dried fruits. Results indicated that ethyl acetate (EA) extract of the fresh fruits exhibited higher DPPH radical scavenging activity than other samples. In the reducing capacity assay, fresh fruits extract exhibited higher reducing power than all test samples. (Erasto et al., 2009). A new phenolic glycoside (E)-4-hydroxymethyl-phenyl-6-O-caffeoyl-b-D-glucopyranoside was isolated and identified together with chemicals showing antioxidant activity (Mohan et al., 2012).

1.7.4. Cardio protective activity

The cardioprotective activity of *Lagenaria siceraria* fruit powder in isoprenalin induced cardiotoxicity in rats was experimentally proved. They obtained that Isoprenalin showed cardiotoxicity, manifested by increase in heart rate and serum marker enzyme. *Lagenaria siceraria* alone produced bradycardia. In LS pretreated rats isoprenalin neither increased heart rate nor produced the hypotensive effect (Mali et al., 2010).in another study the cardioprotective activity of *Lagenaria siceraria* fruit powder was reported against Doxorubicin induced cardiotoxicity. (Fard et al., 2008).

1.7.5. Immunomodulatory effects

The immunomodulatory effect of n-butanol soluble and ethyl acetate soluble fractions of successive methanolic extract of *Lagenaria siceraria* fruits in rats was reported. It inhibited delayed type hypersensitivity reaction in rats. The immunomodulatory effects were indicated by decrease in foot pad thickness of rats. The results suggested that test fractions possess promising immunomodulatory activity (Rana et al., 2008). A dose-dependent increase in both primary and secondary antibody titer was observed. Fractions also significantly increased both white blood cell and lymphocyte count (Gangwal et al., 2008).

1.7.6. Analgesic activity

The analgesic activity of methanolic and aqueous extract of fruit of *Lagenaria siceraria* was reported in an experimental study using the tail immersion method in rats. The results support the traditional use of this plant in some painful and inflammatory conditions (Saha et al., 2010). *L. siceraria* seeds are used in migraine type headache and pain (Rahman., 2003).

1.7.7. Diuretic activity

The evaluation of *Lagenaria siceraria* for its diuretic activity in albino rats have been reported. The rats treated with vacuum dried *Lagenaria siceraria* juice extracted (LSJE) and *Lagenaria siceraria* methanol extract (LSME), showed higher urine volume when compared to the respective control. The excretion of sodium, potassium and chloride has also been significantly increased. The elevated diuretic potential of LSFE and LSME was statistically compared to that of the standard diuretic agent furosemide .The results obtained in that study indicated that LSJE and LSME act as effective hypernatremic , hyperchloremic and hyperkalemic diuretics (Ghule et al., 2007).

1.7.8. Hyperthyroidism and lipid peroxidation

The hyperthyroidism and lipid peroxidation activity have been reported by the study of *Lagenaria siceraria* peel extract, in mice. In an *in-vitro* study the quenching potential of the peel extract on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH)- dependent free radicals was examined. In another experiment, an *in-vivo* study was performed considering three different concentrations of the test peel extract to select its most effective and safe dose for the regulation of hepatic lipid peroxidation, thyroid function and glucose metabolism. After treatment, a decrease in the concentrations of serum thyroid hormones, glucose as well as in hepatic lipid peroxidation with a parallel increase in antioxidants such as superoxide dismutase, catalase and glutathione indicated the efficacy of the test peel in the amelioration of hyperthyroidism, hyperglycemia and hepatic lipid peroxidation (Dixit et al., 2008).

1.7.9. Cytotoxic activity

It was reported D: C-Friedooleanane-type triterpenoids from *lagenaria siceraria* showed cytotoxic activity. The methanolic extract was evaporated in vacuum to give a black residue, which was suspended in H_2O and then partitioned sequentially using EtOAc and n- BuOH. The EtOAc fraction was chromatographed over silica gel, using mixtures of nhexane and EtOAc of increasing polarity as eluents. The cytotoxicity of compounds 1-9 was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazoliumbromide] colorimetric method based procedure. Compounds 3b -O-(E)-coumaroyl-D: C-friedooleana-7, 9 (11)-dien-29-oic acids and 20- epibryonolic acid showed significant cytotoxic activity against the SK-Hep 1 cell line (Chen et al., 2008).

1. 7. 10. Hepato protective activity

Study was carried out to evaluate the hepatoprotective activity of the aerial parts of *L. siceraria* methanol extract (MELS). Hepatoprotective activity of the extract was investigated against carbon tetrachloride induced hepatotoxicity in rats. The potent hepatoprotective activity in rat was reported from its significant effect on the level of serum biomarker enzymes and total protein and bilurubin and improvement of the endogenous antioxidant status, which was finally substantiated by histological studies of the liver tissue. (Saha et al., 2011). In another experiment hepatoprotective activity was reported by examining the influence of the ethanolic extract of *Lagenaria siceraria* (EELS) on hepatotoxicity induced by administration of CCl_4 (Shirwaikar et al., 1996).

1.7.11. Anti- inflammatory activity

The anti-inflammatory effects were investigated employing the acute inflammatory models, i.e. ethyl phenylpropionate-induced ear edema, carrageenin- and arachidonic acid-induced hind paw edema, and also the albumin-induced paw edema in rats. *Lagenaria siceraria* fruit juice extract (LSFJE) elicited significant inhibitory effect on the ear edema formation after EPP-injection. In other acute inflammatory models, the extract significantly inhibited carrageenan- and arachidonic acid-induced hind paw edema. LSFJE also caused inhibition of albumin-induced paw edema over a period of 90 min. There was a significant, dose-dependent inhibition of the formalin induced pain response in mice. (Kubde et al., 2010).

1.7.12. Anti- diabetic activity

The anti-diabetic potential evaluation of *Lagenaria siceraria* pulp extract (LSPE) and *Lagenaria siceraria* seed extract (LSSE) against the pancreatic damage from alloxan-induced diabetes in rats related to diabetes mellitus was shown. *Lagenaria siceraria* induced, significant reduction in blood glucose and increase in serum insulin, there data indicate that the level of glucose in the animals that were subjected with alloxan was three times comparing with normal, the level

of blood glucose in diabetic group when subjected with *Lagenaria siceraria* extract decreased to almost twice of normal. These findings suggest that LSPE and LSSE treatment exerts therapeutic protective effect in diabetes by preserving pancreatic cell integrity and significant activity (Bhattacharya et al.,2012).

1.7.13. Central nervous system activity

Among cucurbits, the bottle gourd, *Lagenaria siceraria* (Mol.) Standl. is the only plant which contains highest choline level along with required metabolites/metabolic precursors for brain function (Rehman., 2003). The central nervous system activity of *Lagenaria siceraria* was reported. In the study the methanolic extract significantly reduces spontaneous motor activity at higher doses than petroleum ether extract. The fall off time (motor coordination) was also decreased. A potentiation in the pentobarbitone induced sleep due to the sedative effect of the methanolic extract was observed. The result shows that petroleum ether extract and methanolic extract shows CNS depressant activity is due to the presence of different chemical compounds present in that extracts (Jayashree et al., 2010).

1.7.14. Anti- microbial activity

The antibacterial and antifungal potency of *Lagenaria siceraria* (Mol.) Standley have been reported. Five extracts Petroleum Ether, Chloroform, Methanol, Absolute alcohol and Water were prepared by using soxhlet apparatus and the extracts showed moderate to potent antimicrobial activity against the bacterial strains*: Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* and antifungal strains: such as *Aspergillus flavus, Aspergillus oryzae* and *Trichoderma harzianum*. (Nagaraja et al., 2011). In another study Petroleum ether, Chloroform, ethanol and water extracts of *Lagenaria siceraria* were prepared separately and evaluated for antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebisella* species by cup-plate method (lawn and pour) and Disc diffusion method (Sharma et al., 2013).

1.8. Toxicological assessment

The ingestion of bottle gourd can cause rapid onset diarrhea, vomiting, gastrointestinal bleeding, and hypotension due to release of a substance named cucurbitacin. If the bottle gourd juice becomes bitter it is considered toxic (Ho et al., 2014) A report of 15 patients, who developed toxicity due to drinking bitter bottle gourd juice, Patients presented with abdominal pain, vomiting, hematemesis, diarrhea and hypotension within 15 min to 6-h after ingestion of bottle gourd juice. Endoscopy showed esophagitis, gastric erosions, ulcers and duodenitis. All patients recovered in 1–4 days. Endoscopically the lesions healed in 2 weeks. Bitter bottle gourd can cause gastrointestinal toxicity with hematemesis and hypotension. Supportive management is the treatment and all patients recover within 1 week (Puri et al., 2010).

Table 1.9. Selected plant cultivars of *Lagenaria siceraria*

S no.	Plant Names	Botanical description	Reference
1	*Lagenaria siceraria* (Green Bell Hybrid) 	These are found as annuals, vegetables, vines and climbers having height of 6-12 in (15-30 cm). Green Bell is a new high quality hybrid variety developed by Kaoshung Horticulature Research Center in Taiwan. Fruits with green skin and white stripes, in bell shape and 1.5 Lb. in weight. Plants grow very well in subtropical climates. Plants are vigorous and tolerant to disease attacks. Very productive and easy to grow.	Essien et al., 2013.; www.davesgarden.com
2	*Lagenaria siceraria* (Kettle Gourd) 	Annuals, vines and climbers, having height of almost 6-12 in (15-30 cm) of fruit and 10-12 ft. (3-3.6 m) of plant. The foliage are herbaceous, blue-green, velvet/fuzzy-textured.	Shivraj et al., 2005.; www.davesgarden.com

3	*Lagenaria siceraria* (Round Gourd) 	Annual vine. These have been selected to produce large, globular fruits which may be crafted into containers for holding all types of goods. Originally from Africa, these were the main market container for carrying beans or other goods (prior to the invention of plastic).Still these are employed quite extensively in Africa--they are useful, comely, and biodegradable.	Shivraj et al., 2005.; www.horizonherbs.com
4	*Lagenaria siceraria* (Long Sousage Gourd) 	A new gourd that lives up to its name, the fruits do indeed look like big green sausages. Plant produces good yields of large 20 inches long light green gourds. Best if grown on trellis or fence.	Anonymous, 1940.

5	*Lagenaria siceraria* (Indian Hybrid) 	Fruits are cylindrical can be harvested at small size as well as full size stages for cooking and eating. White soft flesh is very tender and delicious, suitable for stir-fry and soup cooking. Plants are very vigorous and should be well watered in warm summer. Plants can be grown along supports or on the ground.	Chimonyo et al., 2013.

Chapter – 2

Hyperlipidemia and its evaluation

Hyperlipidaemia and its evaluation

Hyperlipidaemia is a condition of abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood. It is ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases, stroke and atherosclerosis, thus the case of hyperlipidaemia is the primary cause of death (Reddy et al., 2014). American heart association defined hyperlipidaemia as a high level of fatty molecules in the blood. These fatty substances, called lipids include cholesterol and triglycerides. There are different types of hyperlipidaemia depending on which lipid levels are high in the blood. Hyperlipidaemia may be classified as either familial (also called primary) caused by specific genetic abnormalities, and acquired (also called secondary) when resulting from Decreased lipoprotein lipase, Altered ApoC2, LDL receptor deficiency, Increased VLDL production and Decreased elimination etc. that leads to alterations in plasma lipid and lipoprotein metabolism. Also, hyperlipidaemia may be idiopathic, that is without known cause (Harikumar et al., 2013).

LDL cholesterol normally circulates in the body for 2.5 days, and subsequently binds to the LDL receptor on the liver cells, undergoes endocytosis, and is digested. LDL is removed, and synthesis of cholesterol by the liver is suppressed in the HMG-CoA reductase pathway In familial hyperlipidaemia, the function of LDL receptors are reduced or absent, and LDL circulates for more time in blood, resulting in significantly increased level of LDL cholesterol in the blood. In mutations of ApoB, reduced binding of LDL particles to the receptor causes the increased level of LDL cholesterol. Although disorders occurs to a certain degree in all people, familial hyperlipidaemia patients may develop disorders more frequently due to the excess level of LDL. The occurrence of diseases approximately depends of the number of LDL receptors still expressed and the functionality of these receptors. In many heterozygous forms of FH, the receptor function is only mildly impaired, and LDL levels will remain relatively low. In the more serious homozygous forms, the receptor is not expressed at all. In addition to the classic risk factors such as smoking, high blood pressure, and diabetes, genetic studies have shown that a common abnormality in the prothrombin gene (G20210A) increases the risk of cardiovascular events in patients with familial hyperlipidaemia (Garciaotin et al., 1999). Several studies showed that the high level of lipoprotein was major risk factor for ischemic heart disease (Bhatnagar et al., 2008). Persons with hypercholesterolemia are at high risk of dying because of heart disease or stroke. Many studies have been done to draw a relationship between elevated cholesterol levels and increased risk for heart attack and death. In one research investigation of relatively young males who had no known heart disease, cholesterol levels were measured and volunteers were followed for 6 years. During this time, all heart attacks and deaths that occurred among those volunteers were recorded. As serum cholesterol levels increased, so did the risk of experiencing a heart attack. The risk of a fatal heart attack was approximately five times higher in persons having cholesterol levels of 300 g/dl or more compared to those with cholesterol levels below 200 mg/dl. The Framingham Heart Study is an ongoing research effort. Cholesterol levels, smoking habits, heart attack rates, and deaths in the population have been recorded for over 40 years. After 30 years, more than 85% of persons with cholesterol levels of 180 mg/dl or less were still alive; almost a third of those with cholesterol levels greater than 260 mg/dl had died (Ray et al., 2010) High

lipid levels can speed up a process called atherosclerosis, or hardening of the arteries which is the primary cause of heart disease and stroke (Harikumar et al., 2013). The fat-protein complexes in the blood are called lipoproteins. The best-known lipoproteins are LDL (low-density lipoprotein) and HDL (high-density lipoprotein). Excess LDL cholesterol contributes to the blockage of arteries, which eventually leads to heart attack. Higher the level of LDL cholesterol, the greater the risk of heart disease. In contrast the lower the level of HDL cholesterol, the greater the risk of coronary heart disease. Low HDL cholesterol levels are typically accompanied by an increase in blood triglyceride levels.

Studies have shown that high triglyceride levels are associated with an increased risk of coronary heart disease. People with coronary disease develop thickened or hardened arteries in the heart muscle. This can cause chest pain, a heart attack, or both. Because of these risks, treatment is often recommended for people with hyperlipidaemia. The World Health Organization (WHO) reports that high cholesterol contributes to 56% of cases of coronary heart disease (CHD) worldwide and causes about 4.4 million deaths each year. During the past years, several studies and clinical trials have revealed the adverse effects of high blood lipid levels on the progression of atherosclerosis and consequently the development of cardiovascular disease (Chanmee et al., 2013). The most recent cholesterol management guidelines (the third report of the adult treatment panel APT III), which are issued by the national cholesterol education program (NCEP) in May 2001, redefine the levels at which blood cholesterol should be treated. These new evidence-based recommendations are departure from the NCEP's previous guidelines (ATP II) in several ways (Harikumar et al., 2013).

The medicines currently used to treat cardiac disorders have many side effects. The major side effects of anti-hyperlipidaemia agents include muscle toxicity, rhabdomyolysis, psychiatric adverse reactions which include depression, memory loss, confusion and aggressive reactions. Hence it is the needed to investigate herbal drugs for treatment of hyperlipidaemia which are devoid or can show less of the above side effects (Ghori et al., 2015). Dietary factors play a key role in the development of various human diseases, including cardiovascular diseases. Common belief that, herbal formulations are safer than modern drugs increased the use of herbal formulations. The prophylactic and therapeutic effect of many plant extracts such as *Azadirachta indica*, green tea, garlic, *Ginkgo biloba*, Tender coconut water, *Withania somnifera* etc (Saoji et al., 2009). Medicinal plants, fruits used in day-to-day preparation of food in Indian kitchens have been identified as hypolipidaemic in Ayurveda (Mohale et al., 2008). Hyperlipidaemia is a condition which characterized by abnormal elevation of lipid such as (triglyceride and cholesterol) and lipoproteins such as (LDL, VLDL) levels in the blood. Lipid metabolism is elegantly balanced to maintain homeostasis. When the balance is lost, obesity or hyperlipidaemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs. Therefore, the control of lipid metabolism by drugs could be used to prevent or treat these diseases (Birari et al., 2007). LS fruit is consumed as vegetable in all over world. The vegetable is stomach filling and thus proves to be a major component of weight reducing diet. The high mineral content present, fulfils the need of body. In addition

high dietary fibres increases mass and relieve constipation (Mali et al., 2010). Some studies have reported that *Lagenaria siceraria* fruit is effective on decreasing the levels of total lipids. Early work showed that semi purified dietary fibres isolated from *Lagenaria siceraria* fruits affects faecal excretion of steroids (Nadeem et al., 2012). LS fruits are frequently used to treat a large variety of ailments and symptoms of hyperlipidaemia and atherosclerotic impasse. LS fruits are traditionally used for its cardioprotective, cardiotonic and diuretic properties. It also cures pain, ulcers, jaundice and fever, and used to treat pectoral cough, asthma and other bronchial disorders (Ghule et al., 2009). It is a commonly used vegetable in India is described as cardiotonic (Deshpande et al., 2008). It is well-known that meal that is rich in vegetables and fruits reduces chances of cardiovascular diseases. LS fruits are used to treat a large variety of ailments and symptoms of hyperlipidaemia and atherosclerotic impasse and as a cardioprotective drug (Sivarajan and Balachandran, 1981),

In the present thesis lipid lowering activity of plant cultivars is investigated by experimentally induction of hyperlipidaemia. Therefore, the most potent plant cultivar was given to rats by oral gavage along with high fat diet to investigate the antihyperlipidaemia effects by: (a) estimating serum lipid profile; (b) estimating biochemical parameters and (c) determining the weight gain by experimental animals.

Chapter – 3

Scope, objective and plan of work

3.1. Scope and rationale of the present work

3.2. Objective of the study

3.3. Framework of the study

3.1. Scope and rationale of the present work

Many developing countries are taking herbal medicines as herbal formulation and dietary supplements due to safe, cost sufficient. Assurance of the quality, safety and efficacy of herbals and herbal products has now become a key issue in developed and in developing countries. The increased usage of herbals made it essential to develop the clinical and scientific data (Mukherjee, 2004; Mukherjee et al., 2007). If a product is too complex, it must be standardized for better understanding of biological activity parameters. *Lagenaria siceraria* is a food plant showing a huge number of therapeutical applications. A lot of study had been done by many researchers on this particular plant. There are almost 25 cultivars of *Lagenaria siceraria* are reported throughout world but none of these are official. The chemical constituents and their amounts in herbs can be different, due to growing conditions, such as climate and soil, the drying process, the harvest season, etc. Thus the Identification is needed to avoid fraud and adulteration (Goodarzi et al., 2013). Marker compound analysis and estimation of secondary metabolites, not only helps in establishing the correct botanical identity but also in regulating the chemical sanctity of the medicinal products. Indian systems of medicine are considering new methodology for development of existing traditional drugs. These efforts will benefit the studies of plants and natural products to accelerate the evidence-based pharmaceutical research and to revitalize traditional medicine (Mukherjee et al., 2012).

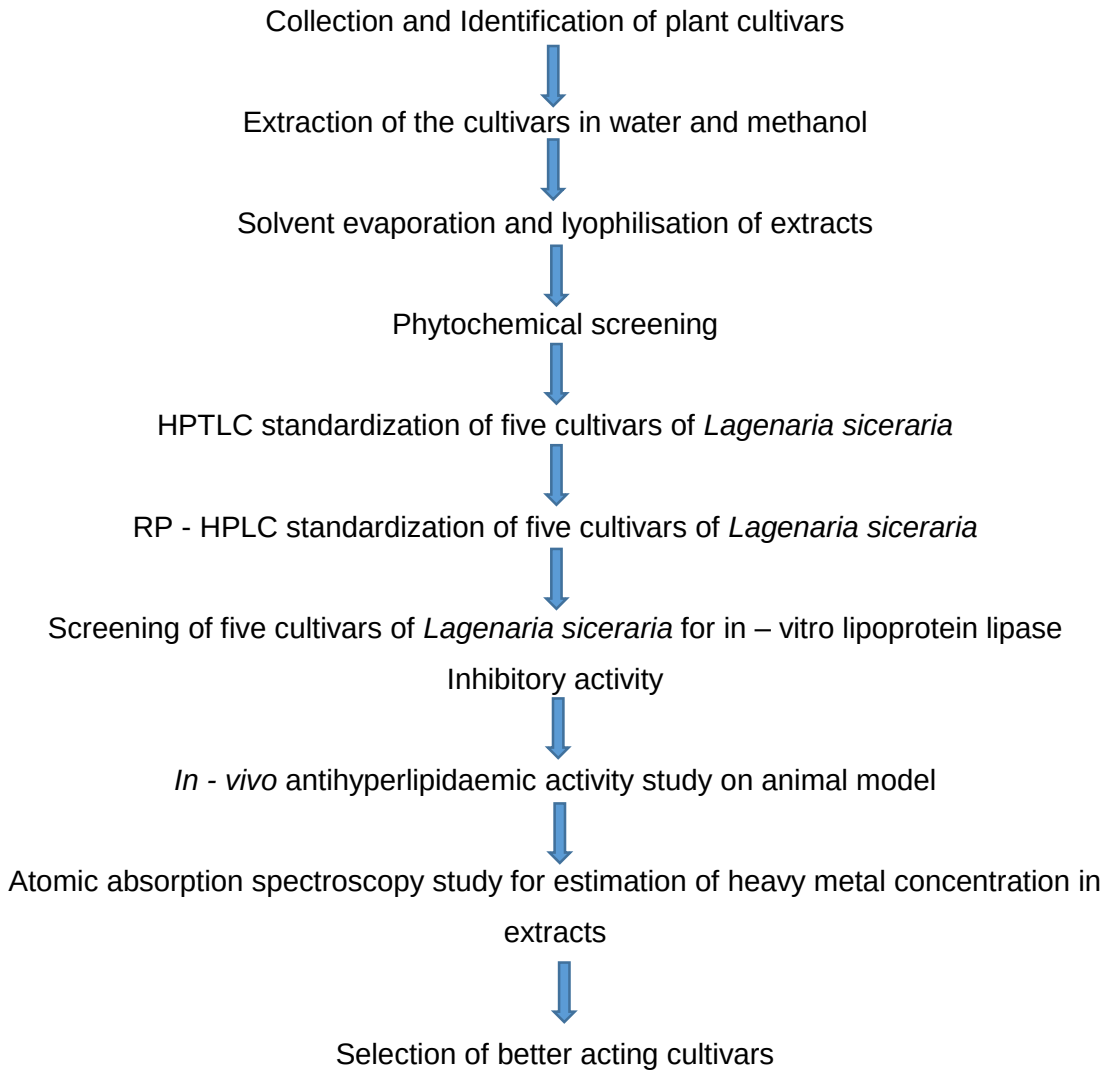
The study mainly deals with the collection, extraction and standardization along with the investigation of probable anti hyperlipidaemia activity by means of lipoprotein lipase enzyme inhibition assay of the five cultivars of plant *Lagenaria siceraria*. A *In- vivo* anti hyperlipidaemic assay in rat model will be done so as to get the *in -vivo – in -vitro* comparative correlation of plant cultivars of *Lagenaria siceraria* extracts.

So the rationale of the following work has a great significance in promotion and dissemination knowledge about our food plant *Lagenaria siceraria* and its cultivars in front of scientific logic.

3.2. Objective of the study

- Phytochemical evaluation of five cultivars of *Lagenaria siceraria.*
- Standardization of five cultivars of *Lagenaria siceraria* with suitable markers through HPLC and HPTLC, in both qualitative and quantitative manner.
- Investigation of the probable anti hyperlipidaemic activity by means of studying the lipoprotein lipase enzyme inhibition assay of five cultivars of *Lagenaria siceraria*
- Heavy metal analysis of the five cultivars of plant *Lagenaria siceraria* to ensure the safety and efficacy comparatively.
- *In- vivo* antihyperlipidaemia assay of plant cultivars of *Lagenaria siceraria* aqueous extract in rat model.

3.3. Framework of the study



Chapter – 4

Collection, extraction and qualitative evaluation of plants

4.1. Collection

4.2. Extraction procedure

4.3. Results of extraction

4.4. Qualitative evaluation of plant materials

4.5. Results of qualitative evaluation of the selected plants

4.6. Discussion and conclusion

4.1. Collection

The five cultivars of *Lagenaria siceraria* fruits were purchased from local vendor present in Jadavpur supermarket near Jadavpur University Kolkata. The voucher specimens (specimen no. SNPS/JU/2015/1091 - 1095) was deposited at School of Natural Product Studies, Jadavpur University, and Kolkata, India for future references. The plant material was authenticated by Dr. S. Rajan, Msc, DPIM, DCA, PhD, Field Botanist and the voucher specimen number was issued. The collected cultivars are:

4.1.1 Plant cultivars collected

1. *Round Gourd* - A slightly smaller size gourd than the bushel, with a more rounded shape. Green weight is about 20 lbs, this is the perfect size gourd for novelty baskets and decorations (Chimonyo et al., 2013).

2. *Long sausage gourd* - Fruits are cylindrical can be harvested at small size as well as full size stages for cooking and eating. White soft flesh is very tender and delicious, suitable for stir-fry and soup cooking. Plants are very vigorous and should be well watered in warm summer. Plants can be grown along supports or on the ground (Chimonyo et al., 2013).

3. *Green bell hybrid* - These are found as annuals, vegetables, vines and climbers having height of 6- 12 in (15-30 cm). Green Bell is a new high quality hybrid variety developed by Kaoshung Horticulature Research Center in Taiwan. Fruits with green skin and white stripes, in bell shape and 1.5 lb. in weight. Plants grow very well in subtropical climates. Plants are vigorous and tolerant to disease attacks. Very productive and easy to grow (Essien et al., 2013).

4. *Kettle gourd* - Annuals, vines and climbers, having height of almost 6-12 in (15-30 cm) of fruit and 10-12 ft. (3-3.6 m) of plant. The foliage is herbaceous, blue-green, velvet/fuzzy-textured (Shivraj et al., 2005)

5. *Indian hybrid* - This hybrid variety is a vigorous plant with prolific bearing habit, producing fruits continuously for a long time. Relatively short cylindrical fruits with 25-30 cm. In length and 300-350 grams in weight are very tender and delicious. Light green skin with white flesh fruits with slow seed maturity. Plants grow vigorously in warm climates, starting to set fruits in 45 days after the transplanting. Easy to grow (Shivraj et al., 2005).

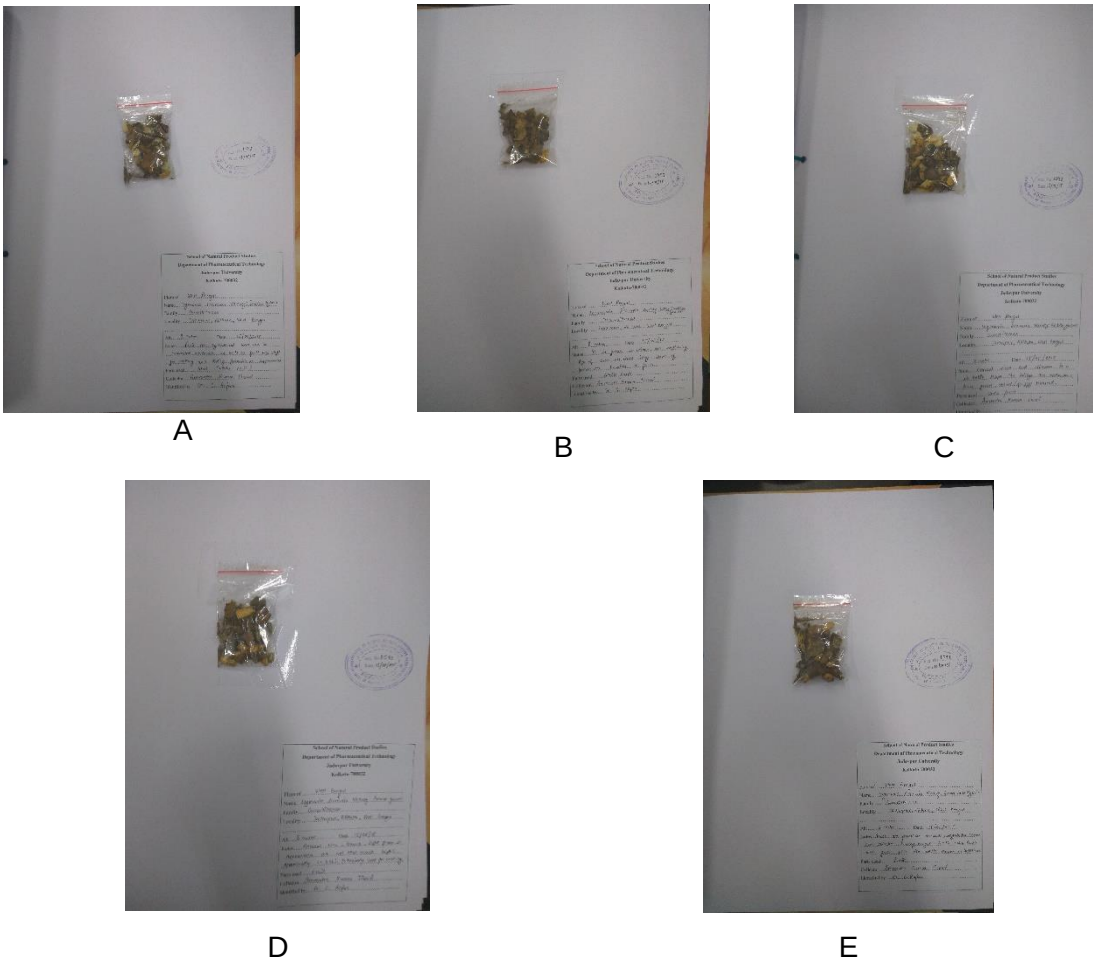


Figure 4.1.Voucher Specimen of five cultivers of Lagenaria siceraria. (A – Indian Hybrid B- Long Sausage Gourd; C- Kettle Gourd; D- Round Gourd; E- Green Bell Hybrid.)

4.2. Extraction procedure

4.2.1. Aqueous extraction

Initially 200 grams of *Lagenaria siceraria* fruits was taken then *Lagenaria siceraria* fruits were grinded by mixer grinder into juice. The aqueous extract was filtered and subjected to lyophillization to get a semisolid mass. These lyophilized were weighed and the percentage yield was calculated. Then the extracts were stored in glass vials in air tight condition at room temperature with proper labels.

4.2.2. Methanolic extraction

4.2.2.1. Soxhlation:

The fresh fruits were collected and were chopped into small pieces. These chopped pieces of fruits were packed to form thimble. The round bottom flask was filled with methanol and apparatus was set up. The solvent was heated at 63˚C (boiling point of

methanol). The vapours of solvent get condensed and then pass though the packed thimble. As the solvent level rises it reached to the mark in siphon tube (solvent + extract) went back to the solvent pool present in round bottom flask. This solvent evaporation was repeated for 8 hours resulting in concentrated hydro alcoholic solution. The end point of soxhlet extraction was determined by spot test. The extract solution was taken and subjected to rotary evaporator for concentration.

4.2.2.2. Concentration:

The concentration was done by using rotary evaporator. Instrument used was Eyela CCA -1111. The instrument was switched on and the refrigerator was set at temperature 4˚C. Then the refrigerator was allowed to reach to the set temperature. The collecting flask and rotating flask (it contains sample) was attached to the instrument for proper attachment to the instrument clamp was put. The rotating flask containing sample solution was put in the Eyela N – 1100 (heating instrument containing water heated at specific temperature as for methanol 45 – 55˚C). When the temperature of refrigerator was reached to 4˚C, pump and rotator was started. The optimum speed of rotation was maintained at 30 -40 revolutions per minutes. The pressure was maintained at 700 mm hg /20 in hg.

After 30 – 40 minutes concentrated extract was obtained in rotatory flask and the solvent (methanol) was recovered in recovery flask. The extract obtained further heated to increase its consistency to semi solid form this semisolid form is scrapped and stored as methanolic extract.

4.3. Results of extraction

The extracts were obtained, weighed and the percentage yield for five cultivars of Lagenaria *sieraria* for both methanolic and aqueous extracts are mentioned in Table 4.1. The percentage yield (%) was calculated by formula:

Percentage yield (%) = weight of extract / weight of plant sample taken * 100

Table 4.1: The percentage yield of the extraction of cultivars of *Lagenaria siceraria*. (Aq. = Aqueous; Meth. = Methanolic)

S no.	Cultivar's name	Initial weight of the plant material (g)		Final weight of the extract (g)		Percentage yield (%)(w/v)	
		Aq.	Meth.	Aq.	Meth.	Aq.	Meth.
1	Round gourd	200	200	7.66	5.5688	3.83	2.7844
2	Kettle gourd	200	200	11.762	2.3044	5.881	1.1522
3	Long sausage gourd	200	200	14.498	3.5086	7.249	1.7543
4	Green bell hybrid	200	200	8.16	4.744	4.08	2.372
5	Indian hybrid	200	200	8.402	3.886	4.201	1.943

4.4. Qualitative Evaluation of Plant Materials

4.4.1. Preparation of reagents

4.4.1.1. Mayer's reagent

1.36 gm. of mercuric iodide in 60 ml of water mixed with a solution which contains 5gm of potassium iodide in 20 ml of water.

4.4.1.2. Libermann-Burchard reagent

5 gm. of acetic anhydride was carefully mixed under cooling with 5 ml concentrated sulphuric acid; this mixture was added continuously to 50 ml of absolute ethanol with cooling.

4.4.1.3. Dragendorff's reagent

1.7 gm. basic bismuth nitrate and 20 gm tartaric acid are dissolved in 80 ml of water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml of water.

4.4.1.4. Fehling's solution A

34.64 gm. copper sulphate was dissolved in a mixture of 0.5 ml of sulphuric acid and sufficient water to produce 500 ml.

4.4.1.5. Fehling's solution B

176 gm. of sodium potassium tartarate and 77gm of NaOH are dissolved in sufficient water to produce 500ml. Equal volumes of solution A & B are mixed at the time of use.

4.4.1.6. Benedict's reagent

1.73 gm. of cupric sulphate, 1.73 gm of sodium citrate and 10 gm anhydrous sodium carbonate are dissolved in water and the volume was made up to 100 ml with water.

4.4.1.7. Molish's reagent

2.5 gm. of pure α-naphthol was dissolved in 25 ml of ethanol.

4.4.2. Methodology

4.4.2.1. Detection of alkaloid

4.4.2.1.1. Mayer's test

1.2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff colored precipitate gives positive test for alkaloid.

4.4.2.1.2. Dragendroff's test

0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orange brown colored precipitate suggested the presence of alkaloid.

4.4.2.1.3. Wagner's test

2 ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicated the positive response for alkaloid.

4.4.2.2. Detection of glycosides

4.4.2.2.1. Legal test

Extract was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. Pink red color was produced.

4.4.2.2.2. Baljet test

To a drug extract, sodium picrate solution was added. Yellow to orange color was produced.

4.4.2.2.3. Borntrager's test

Few ml of dil. sulphuric acid added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink red color was produced in organic layer.

4.4.2.2.4. Keller Killiani test

Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulphuric acid. At the junction of liquid reddish brown color was produced which gradually becomes blue. Detection of phenolic compounds and tannins

4.4.2.2.5. Ferric chloride test

5 ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

4.4.2.3. Detection of saponins

4.4.2.3.1. Foam test

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. Development of stable foam suggested the presence of saponins.

4.4.2.3.2. Potassium dichromate test

1 ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

4.4.2.4. Detection of phytosterols

4.4.2.4.1. Libermann-Burchard Test

10 mg of extract was dissolved in 1ml of chloroform. 1 ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid, a reddish violet color developed, indicating the presence of steroids.

4.4.2.4.2. Salkowski Test

1 ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish-blue color exhibited by chloroform layer and green fluorescence by the acid layer suggested the presence of steroids.

4.4.2.5. Detection of triterpenoids

4.4.2.5.1. Nollar's test

In the test tube 2 ml of 0.01% anhydrous stannous chloride in thionyl chloride solution and test solution was added. Purple colour formed changed to deep red colour after few minutes indicates the presence of triterpenoids. Detection of flavonoids

4.4.2.5.2. Shinoda test

To the extract magnesium turnings and then conc. hydrochloric acid was added. Red color was produced.

4.4.2.6. Detection of protein and amino acids

4.4.2.6.1. Ninhydrin test

Extract solution was treated with ninhydrin (Triketohydrindene hydrate) at the pH range of 4-8. Development of purple color indicated the positive response for amino acids.

4.4.2.6.2. Biuret test

1 ml of 40% NaOH mixed with 2 drops of 1% copper sulphate was added to the extract, a violet color indicated the presence of proteins. Detection of deoxy sugars

4.4.2.6.3. Keller Kiliani test

To 1gm of the sample, 10 ml of 70% ethanol were added and boiled for 2-3 min. it was filtered and to the 5 ml of the filtrate, 5 ml of distilled water and 0.5 ml strong lead acetate solution were added. It was filtered and 5 ml of chloroform were added to the filtrate. Excess chloroform was pipetted off and gentle evaporation of chloroform was done on a porcelain dish. It was cooled and to the residue, 3 ml of glacial acetic acid and 2 drops of

5% ferric chloride were added. The solution was transferred to the surface of 2 ml concentrated sulphuric acid. Reddish brown color (which changed to bluish green to dark on standing) at the junction confirmed the presence of deoxy sugars in the sample.

4.4.2.7. Detection of reducing sugars

4.4.2.7.1. Fehling's test

5 ml of the extract solution, mixed with 5 ml of Fehling's solution was boiled for 5 minutes. Formation of brick red colored precipitate demonstrated the positive test for reducing sugars.

4.4.2.7.2. Benedict's test

To 5 ml of the extract solution, 5 ml of Benedict's solution was added in a test tube and boiled for few min. Development of brick red precipitate confirmed the presence of reducing sugars.

4.5. Results of Qualitative Evaluation of the selected plants

Table 4.2. Results of Qualitative Evaluation of the selected plant cultivers of *Lagenaria siceraria.* By different above mentioned tests; (+ : present; - : absent) (aq. : aqueous; meth : methanolic)

Phytoconstituents	**Round Gourd**		**Kettle Gourd**		**Long Sausage Gourd**		**Green Bell Hybrid**		**Indian Hybrid**	
	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.
Carbohydrate	-	+	-	+	-	+	-	+	-	+
Phytosterol	-	+	-	-	-	+	-	+	-	+
Alkaloids	+	-	+	-	+	-	+	-	+	-
Terpenoids	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	+	-	+	-	+	-	+	-
Glycosides	+	+	+	+	+	+	+	+	+	+
Tannins & Phenolics	+	-	+	-	+	-	+	-	+	-
Saponins	+	+	+	+	+	+	+	+	+	+
Proteins	+	-	+	-	+	-	+	-	+	-
Volatile Oils	-	-	-	-	-	-	-	-	-	-

4.6. Discussion and conclusion

The collection, different extraction and phytochemical screening of the selected plant cultivars was done. The lyophilisation and methanolic extraction method gave adequate amount of extracts e.g. Round Gourd (3.83, 2.7844), Long Sausage Gourd (7.249, 1.7543), Indian Hybrid (4.201, 1.943), Green Bell Hybrid (4.08, 2.372), Kettle Gourd (5.881, 1.1522). Among lyophilised extract the Indian Hybrid extract showed maximum percentage yield and in methanolic extracts Round Gourd showed maximum yield followed by Green Bell Hybrid Moreover the phytochemical study showed the presence

of different phytoconstituent like alkaloids, glycosides, phytosterols, flavoniods, tannins and saponins in the selected plant cultivars. Which gave the information about different phytoconstituents present in respective plant cultivar

Chapter- 5

Standardization and quality evaluation of plant extract; HPTLC, HPLC and AAS study.

5.1. HPTLC standardization the plant extracts (aqueous and methanolic)

5.2. RP-HPLC standardization plant extracts (aqueous and methanolic)

5.3. AAS analysis for detection of heavy metals and minerals in plant extracts.

5.1. HPTLC standardization of five cultivars of *Lagenaria siceraria*

Thin layer chromatography (TLC) is a readily available technique used for fast screening of samples to identify herbal products and to differentiate between related herbal species. One of the major advantages of TLC resides in its flexibility to optimize operational parameters, such as the sample application, plate development, documentation and derivatization. Recent developments and modifications of the technique significantly increased its reproducibility, resolution and sensitivity which combined TLC with improved digital scanning and documentation software, resulted in a more adequate extraction of the information as well as the comprehensive identification and assessment of herbal products improved to a great extent. High performance thin layer chromatography (HPTLC) increased the reproducibility and resolution by automating different steps and using smaller particle sizes (Tistaert et al., 2011).

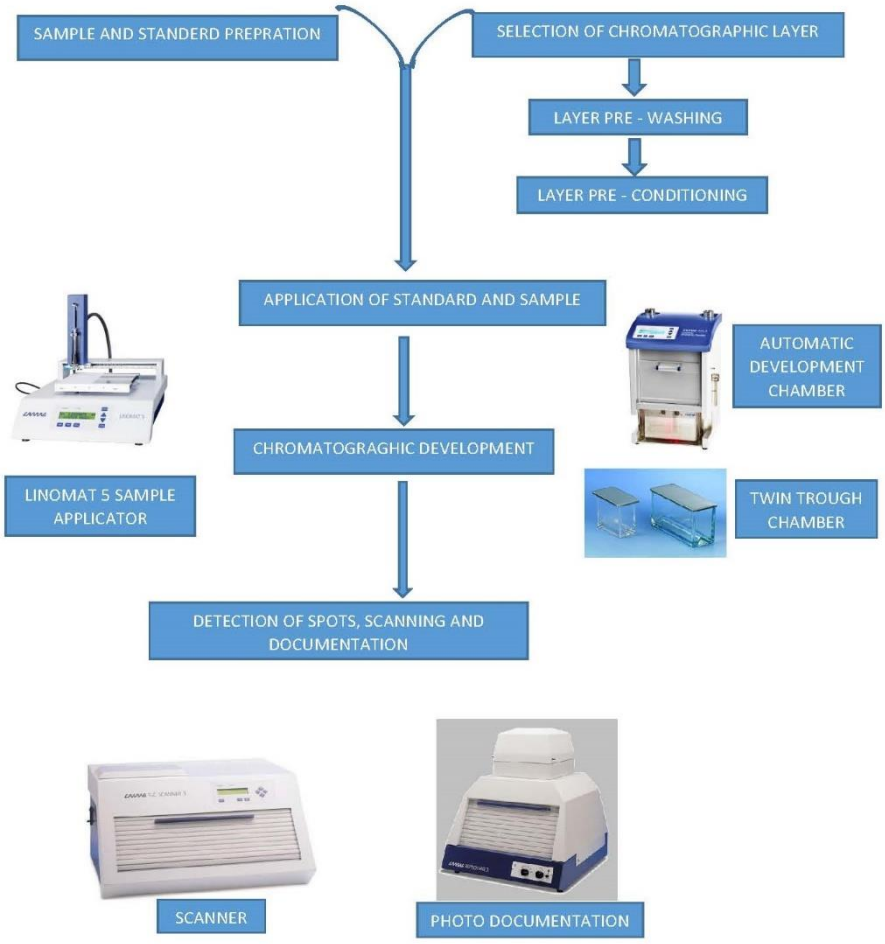


Figure 5.1. High performance thin layer chromatography schematic diagram

5.1.1. Equipments and reagents

The CAMAG HPTLC system consisting of WINCATS software, LINOMAT V automatic sample applicator, and automatic development chamber, scanning densitometer CAMAG scanner 3 and photo documentation apparatus CAMAG reprostar 3 were used. All the solvents were used of analytical grade.100 µl syringe (HAMILTON, Switzerland) was used for sample application on HPTLC plates. Stationary phase used was Aluminium based silica gel plate 60 F254 (Merck, Mumbai) with 20 cm x 10 cm in a particle size of 5-10 µm. Tolune and ethyl acetate, of analytical grade were purchased from Merck (Mumbai, India). Whatman's syringe filter (NYL 0.45 µ) was used for the filtration of samples and standard.

5.1.2. Chromatographic conditions

HPTLC analysis of *Lagenaria siceraria* cultivars was performed with biomarker cucurbitacin E using isocratic technique by external methods. Mobile phase was optimized with petroleum ether and ethyl acetate in a ratio of 6:4 v/v. The temperature was kept at 25°c and mobile phase was developed in a twin trough glass chamber. Standard stock solution was applied consequently in the range of 2-10 µl with 2 µl gradual increment. All total 25 tracks in HPTLC plates were used for standardization including standard and sample solution respectively in a band wise fashion. After development, plates were dried by hand dryer. The Coloured bands were observed at 254 to 366 nm.

5.1.3. Preparation of Standard/Sample Solutions

5.1.3.1. Standard Solution

Cucurbitan E, 1.0 mg/ml

About 1 mg of Cucurbitan E standard was weighed and put in to 2 mL eppendorf tube. The standard was dissolved in methanol and volume was made upto 1 ml with methanol. It was then mixed in vortex mixture and put to ultrasonication bath till the material completely dissolved. It was then filtered through 0.45µ syringe filter and kept for further study.

5.1.3.2. Preparation of sample solution

About 10 mg of *Lagenaria siceraria* extract of each variety was dissolved in 1 ml methanol in two different eppendorf tubes. Then the extract was dissolved in methanol and subjected to ultrasonication till the extracts were completely dissolved. It was then filtered through 0.45µ syringe filter and kept for further study.

5.1.4. Optimization of Mobile Phase

The mobile phase was optimized for the HPTLC analysis. Out of the many mobile phases had been tried and following mobile phase gave the better separation thus optimized mobile phase was

Petroleum ether : Ethyl acetate= 6 : 4

5.1.5. Application of standard and extracts

The external standard calibration curve for Cucurbitan E was prepared with calibration solutions in a concentration range of 200 to 1000 ng/ml. Then 50 µl of standard solution was drawn into CAMAG LINOMAT syringe and put to linomat applicator to give the concentrations of standard required. The same method applied for the extract.

5.1.6. Development

The plate was then dried and developed in a CAMAG twin trough glass chamber with the mobile phase. After development, the plate was dried and scanned in camag TLC scanner 3 at a wavelength of 254 nm

Software used : WINCATS

Analysis mode : Peak area versus concentration of standard and extracts

Calculation : By using following equation by WINCATS.

$$Y = m X + C$$

Where,

X = Concentration of Analyte/ metabolite in ng/ml

Y = Peak area

m = Slope of calibration Curve

C = Intercept.

5.1.7. Method validation for HPTLC study

Validation of the HPTLC method was done as recommended by the International Conference on Harmonisation (ICH) guidelines (ICH, 1996, 2005) and FDA (1994) Guidelines defining the Linearity, Specificity, Limits of Quantification and detection, precision, accuracy and robustness.

5.1.7.1. Specificity

The results for HPTLC standardization profiles were checked in terms of specificity according to the ICH guidelines to minimise errors due to the contamination of the sample. The specificity of the method was determined by analysing the standard and test samples. The purity of peaks was checked using multivariate analysis by comparison of retention times and peak area of standard compound with extract and fractions.

5.1.7.2. Limits of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated by the method based on standard deviation (σ) and the slope (S) of the calibration plot, using the formula LOD=3:1 σ/S and LOQ= 10:1 σ/s (ICH, 1996, 2005 and FDA, 1994); where, σ =standard deviation of the response from a number of blank run and S = slope of calibration plot.

5.1.7.3. Accuracy

Accuracy of the method was determined by percentage recovery of marker in the plant extract and fractions. The method was studied by performing standard addition technique and it is expressed in terms of percentage relative standard deviations (%RSD) from mean recovery of the theoretical concentrations. Prior to injection, the tests were spiked with two different known amounts of standard compounds in doublets. Analyses were done under the sent ambient condition to calculate the overall average recovery. The mean amounts of the markers achieved were taken as real values to calculate the spike recoveries.

5.1.7.4. Precision

The precision of the method was assessed by injecting four replicates at two different concentrations for the reference compound, the extract and fractions. Values were represented as % RSD of intraday and inter-day runs. The mean amount and RSD values were calculated. The intra-day precision of the assay was determined by analysing two concentrations in one day. Also, the intra-day precision was resolute over two successive days by analysing the same concentrations. Injections were done in four replicates to determine the repeatability of the process.

5.1.7.5. Robustness

The robustness of the proposed method was investigated by an analysis of samples under different experimental conditions. The test solutions were analysed with variation of flow rate, mobile phase composition, detection wave length and column temperature and using different columns of same configuration to determine their influence effect on the retention time.

5.1.8. Statistical Analysis

Statistical analysis was performed using the graph pad prism Version 5.0. The results were represented as the mean ± SD.

5.1.9. Results and Discussion

The R_f value of cucurbitacin E was found to be 0.44. The percentage content of cucurbitacin E in cultivers of lagenaria siceraria aqueous and methanolic extract respectively was found to be as mentioned in Table 5.1. This was determined by a calibration curve with the equation of Y = -328.027+3.881*X (correlation coefficient = 0.99833 and standard deviation = ± 4.09%) as shown in fig. where X represents amount of cucurbitacin E and Y represents area under the curve.

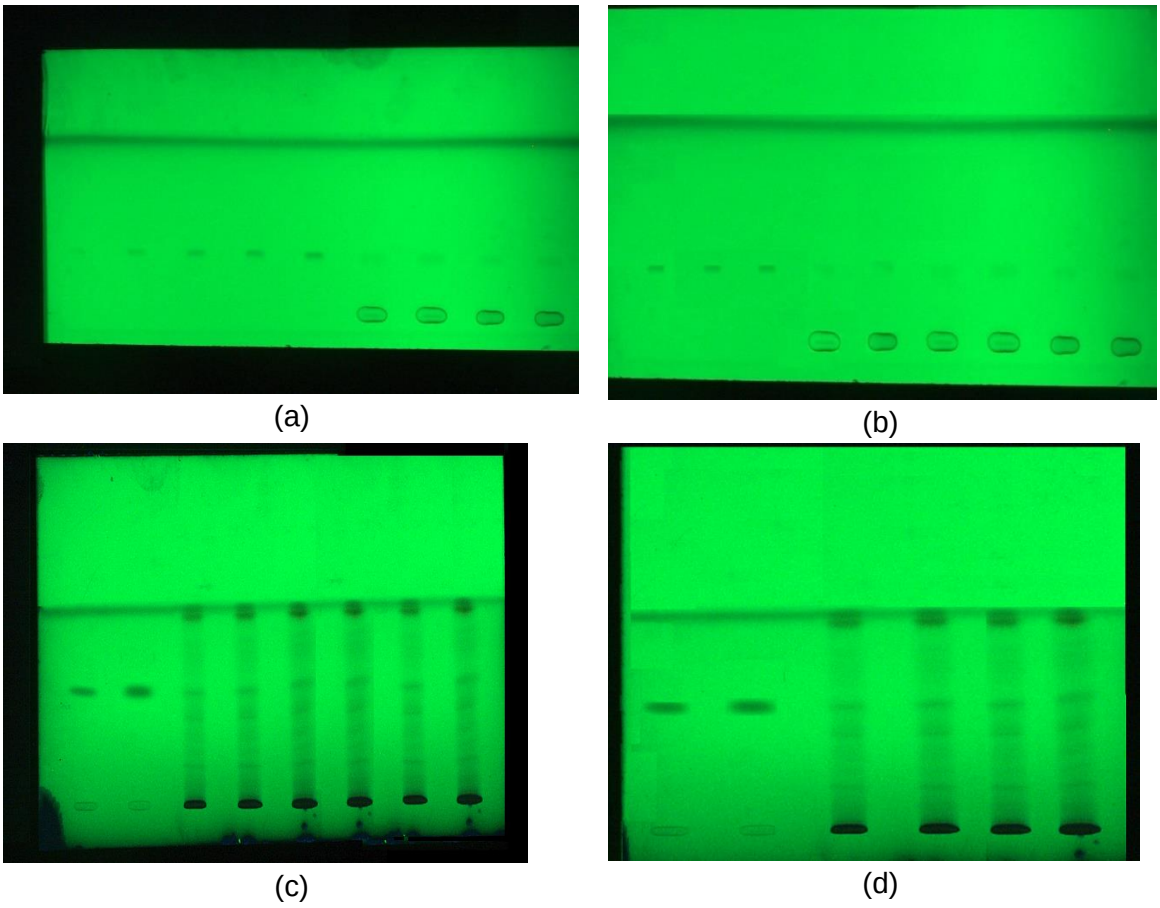


Figure 5.2. Photograph of plates observed at 254nm. (a & b:methanolic extracts; c & d:aqueous extracts)

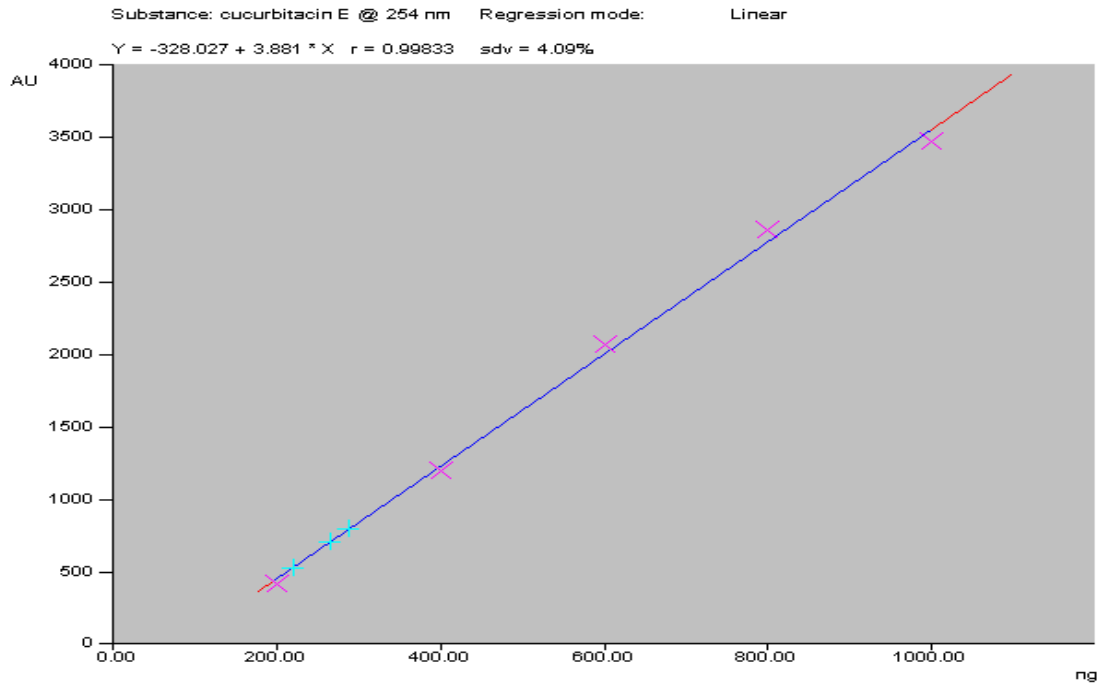


Figure 5.3. Calibration curve for Cucurbitacin E

Chromatograms of standard and extracts

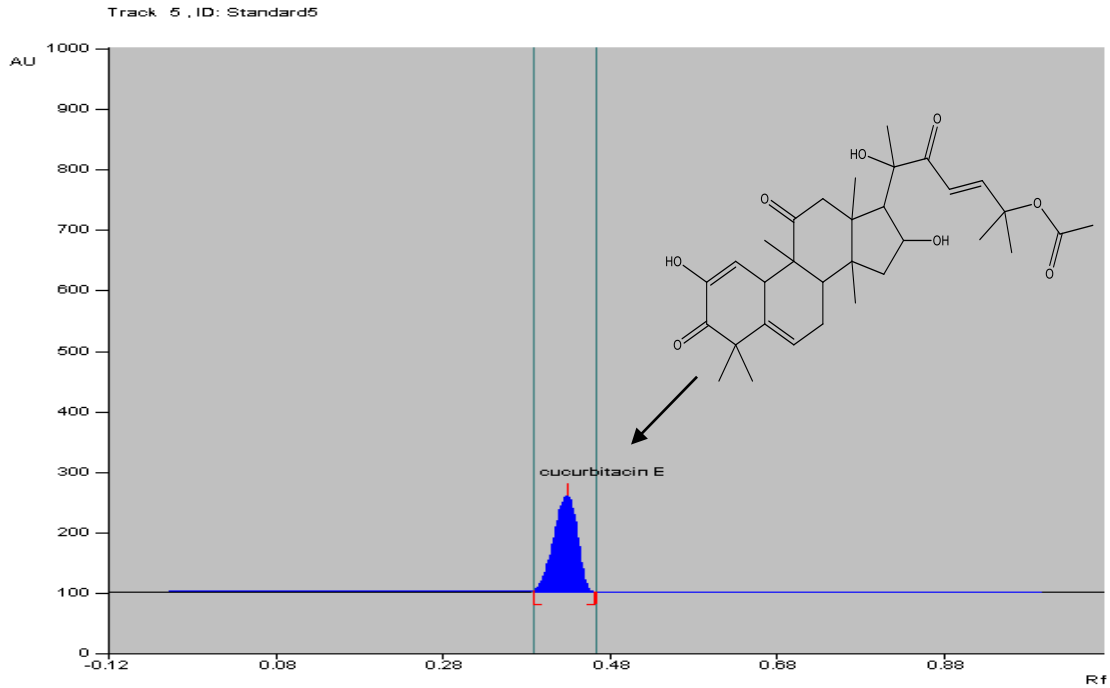


Figure 5.4. Chromatogram of Cucurbitacin E

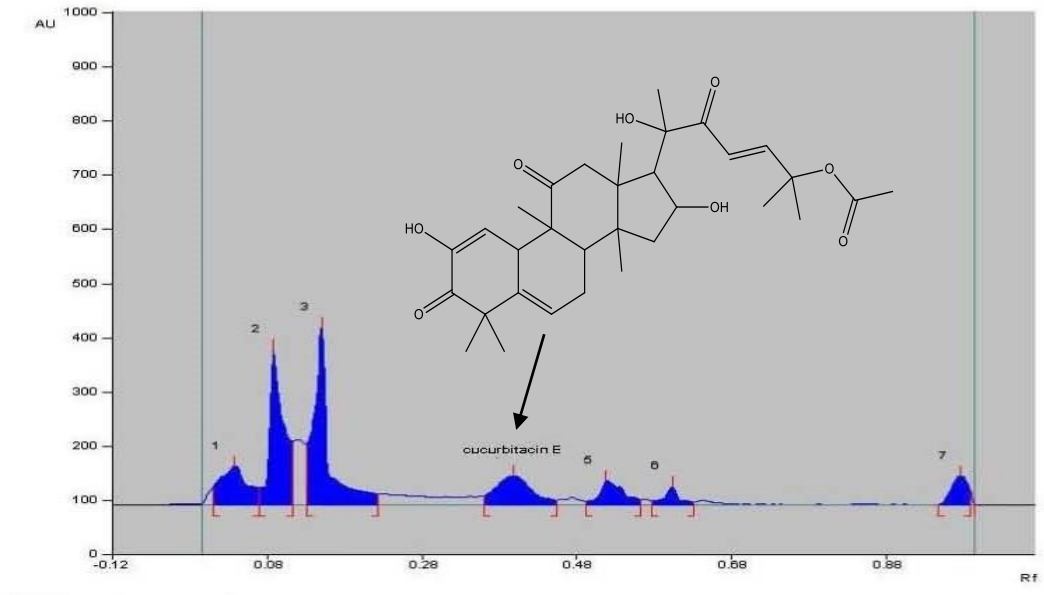


Figure 5.5 Chromatogram of Round Gourd aqueous extract

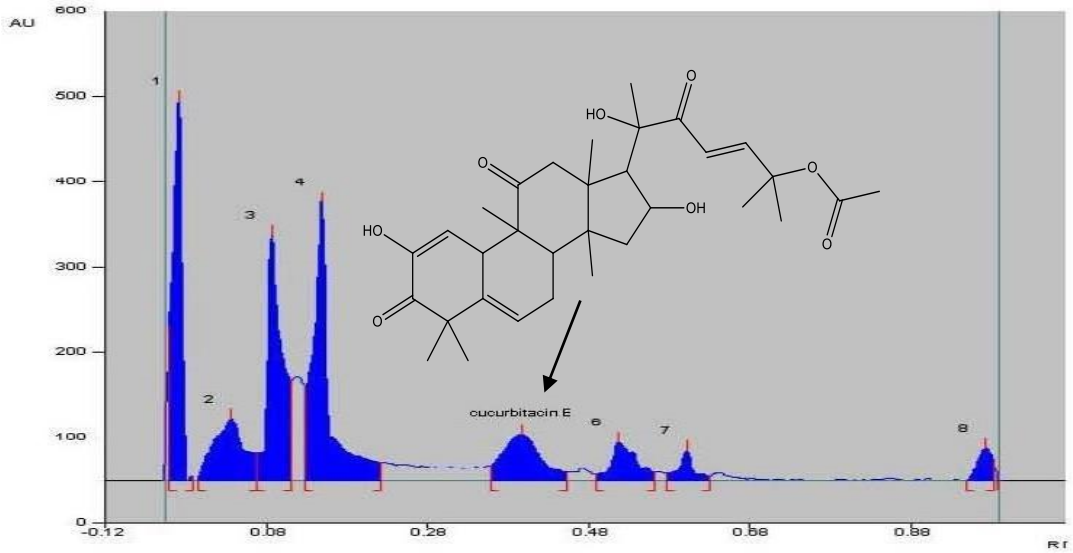


Figure 5.6. Chromatogram of Kettle Gourd aqueous extract

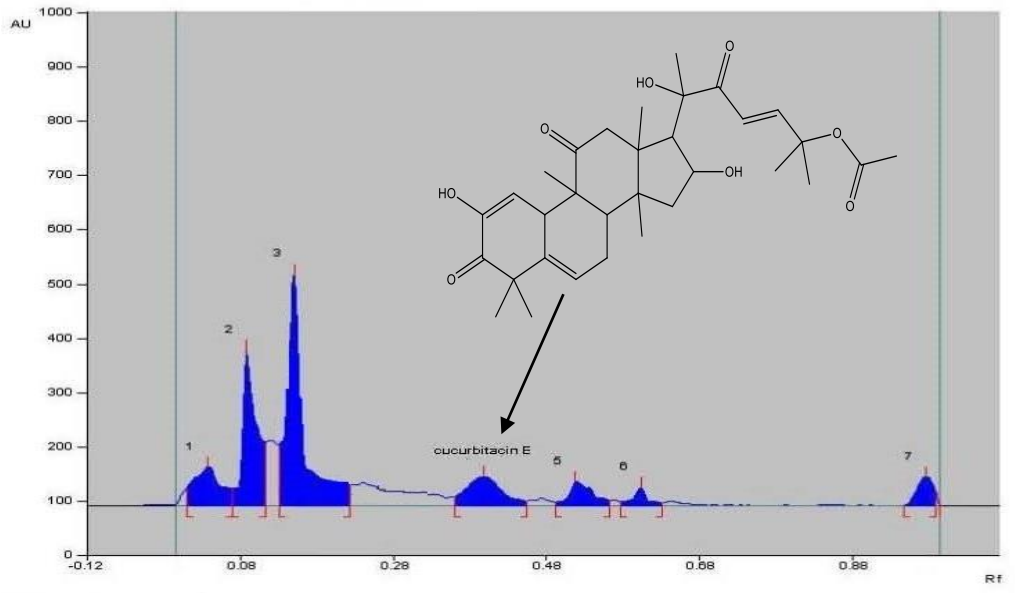


Figure 5.7. Chromatogram of Long Sausage Gourd aqueous extract

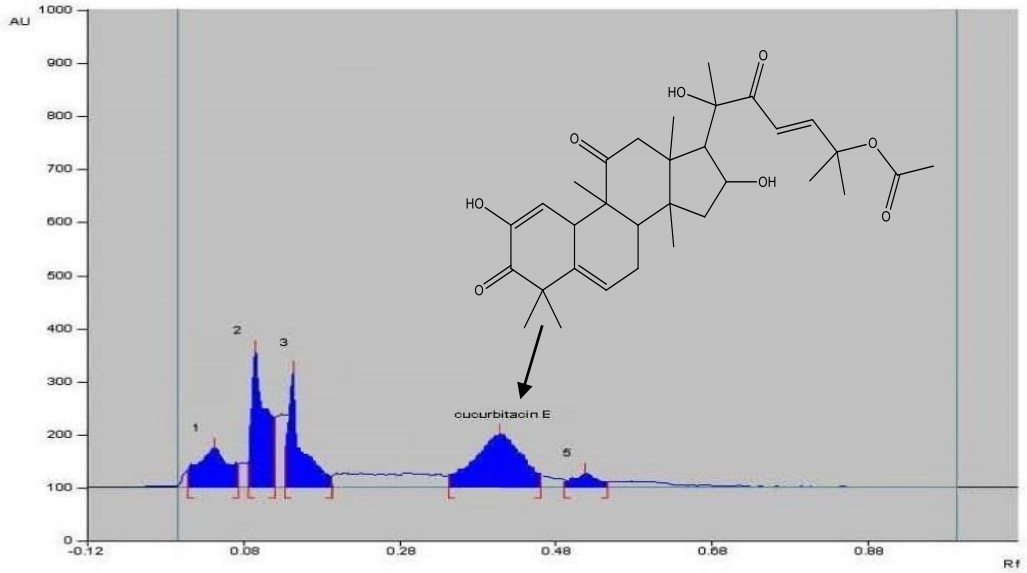


Figure 5.8. Chromatogram of Indian Hybrid aqueous extract

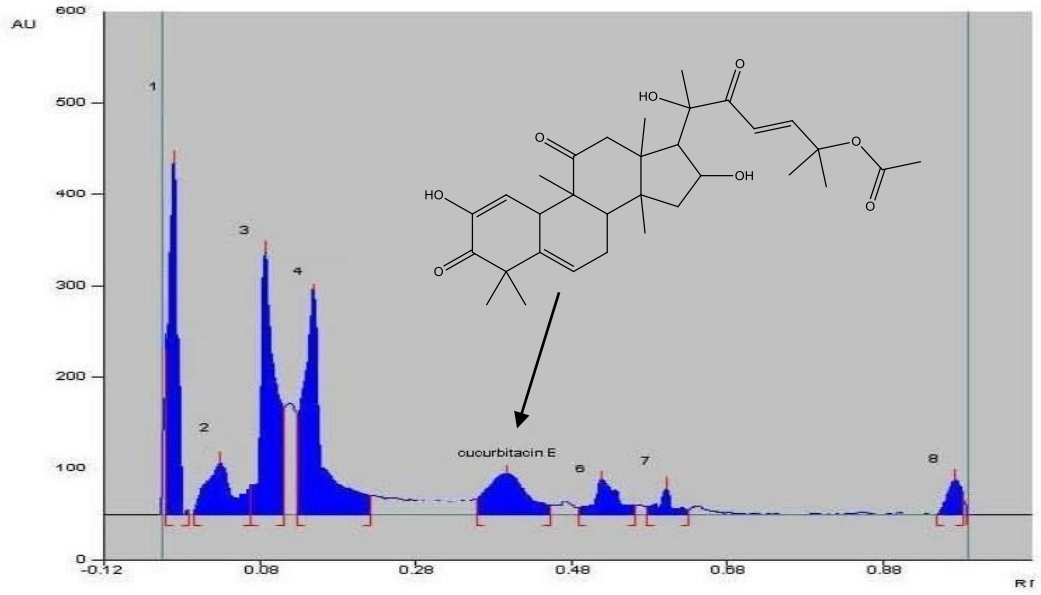


Figure 5.9. Chromatogram of Green Bell Hybrid aqueous extract

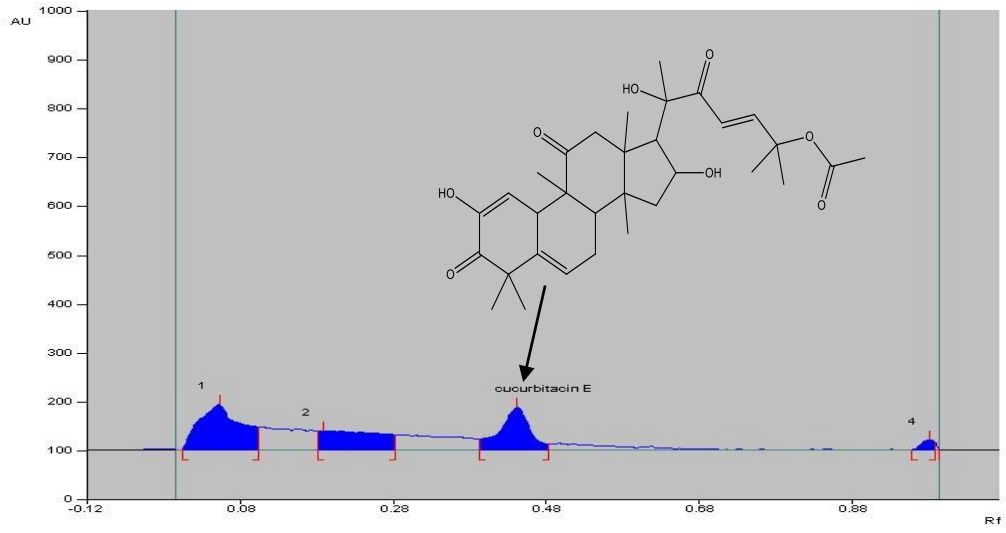


Figure 5.10. Chromatogram of Round Gourd methanolic extract

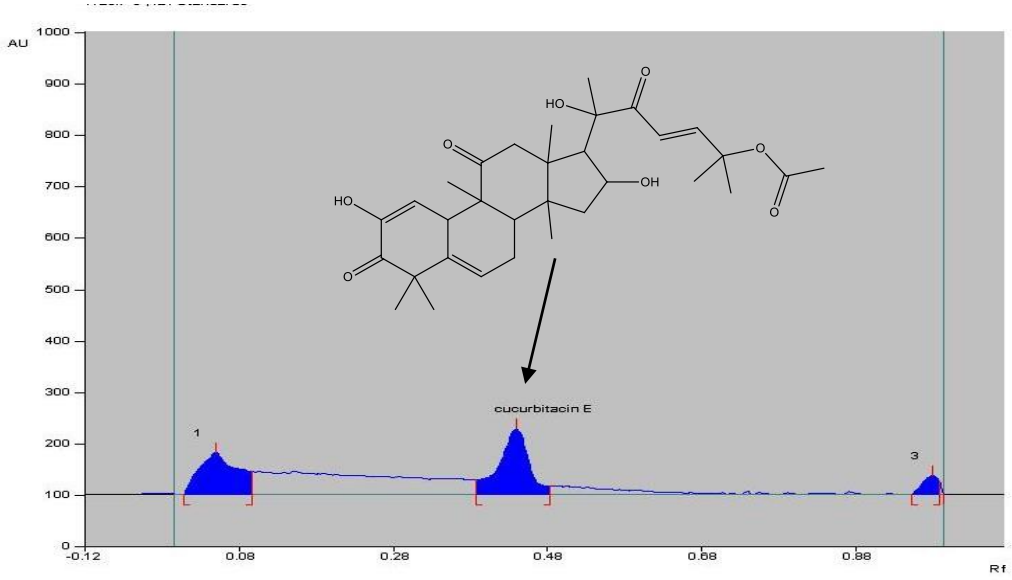


Figure 5.11. Chromatogram of Kettle Gourd methanolic extract

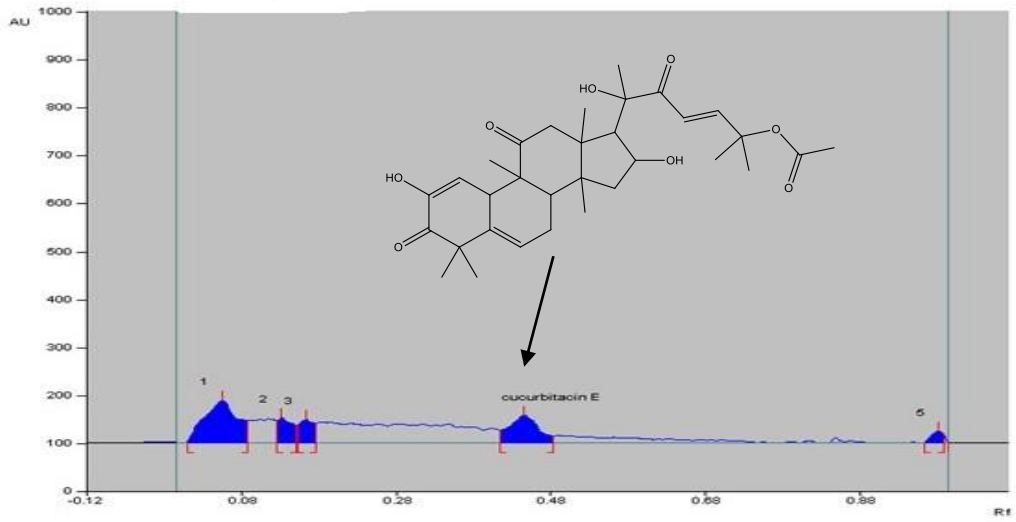


Figure 5.12. Chromatogram of Long Sausage Gourd methanolic extract

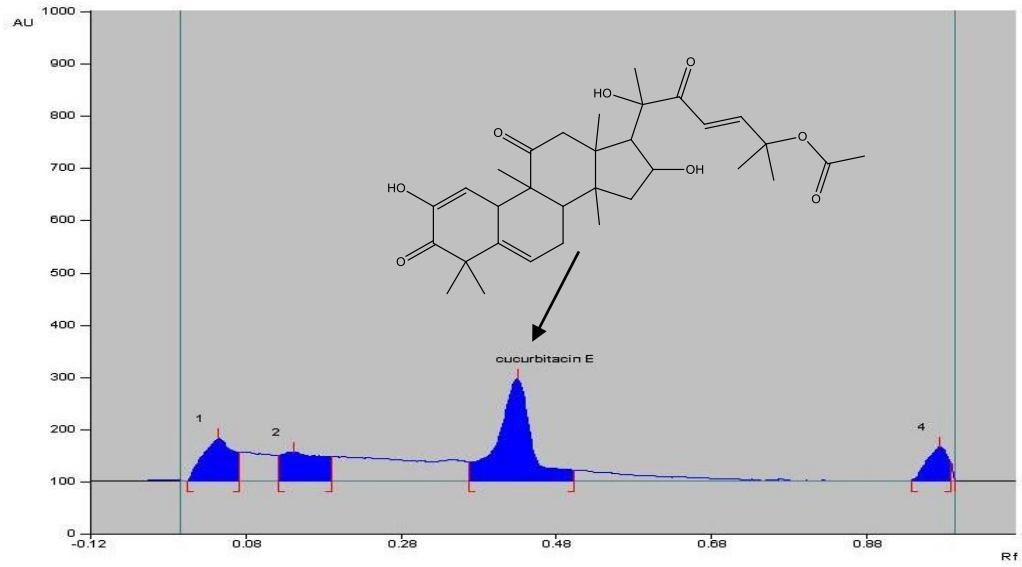


Figure 5.13. Chromatogram of Indian Hybrid methanolic extract

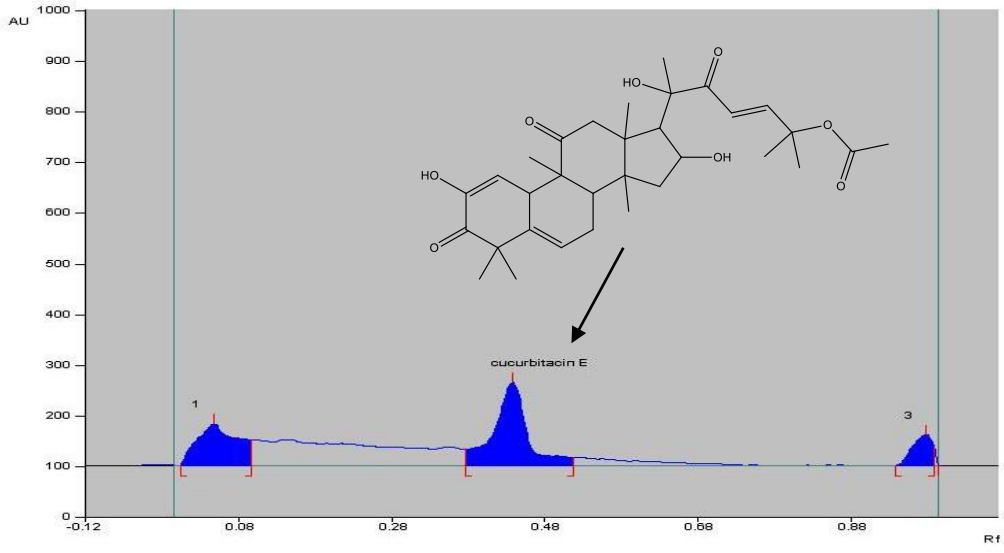


Figure 5.14. Chromatogram of Green Bell Hybrid methonolic extract

Table 5.1. Amount of cucurbitacin E present in extract of cultivars of *Lagenaria siceraria* was found to be:

S No.	Name of The Cultivars	% Yield of Extract(%w/w)		% Cucurbitacin E content Found (%w/w)	
		Aqueous	**Methanolic**	**Aqueous**	**Methanolic**
1	Round gourd	3.83	2.7844	0.13	0.041
2	Kettle gourd	5.881	1.1522	0.10	0.032
3	Long sausage gourd	7.249	1.7543	0.18	0.063
4	Green bell hybrid	4.08	2.372	0.23	0.080
5	Indian hybrid	4.201	1.943	0.38	0.094

5.1.10. Conclusion

In the above study the individual plant cultivars of *Lagenaria siceraria* were standardized through HPTLC method with their respective marker cucurbitacin E. It is concluded that this method of estimation of cucurbitacin E through this method is accurate, precise and specific with good reproducibility. This method is quite acceptable for commercial use The Rf values of the marker cucurbitacin E was found to be 0.44 respectively and the amount of this markers present in the respective plant cultivars extracts (aqueous and methanolic) were Round gourd (0.13, 0.041), Kettle gourd (0.10, 0.032), Long sausage gourd (0.18, 0.063), Green bell hybrid (0.23, 0.080), Indian hybrid (0.38, 0.094) percent (%) w/w. The indian hybrid showed presence of maximum amount of cucurbitacin E while in other cultivars, was found in order, green bell hybrid > long sausage > gourd round gourd > kettle gourd.

5.2. RP-HPLC Standardization plant Extracts (aqueous and methanolic)

HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed-phase (RP) columns are the most popular columns used in the analytical separation of herbal medicines (Liang et al., 2004). Though optimal separation for this kind of technique requires many parameters like mobile phase composition, pH of mobile phase, pump pressure, column chemistry, particle size of stationary phase, temperature etc., still HPLC is an easy to operate, fully automatable technique with high resolution, selectivity and sensitivity (Tistaert et al., 2011). The efficiency of these columns is highly dependent on their particle size (currently 3 – 5 µm). Smaller particle sizes drastically increase the backpressure: when the particle size is halved, the pressure quadruples.

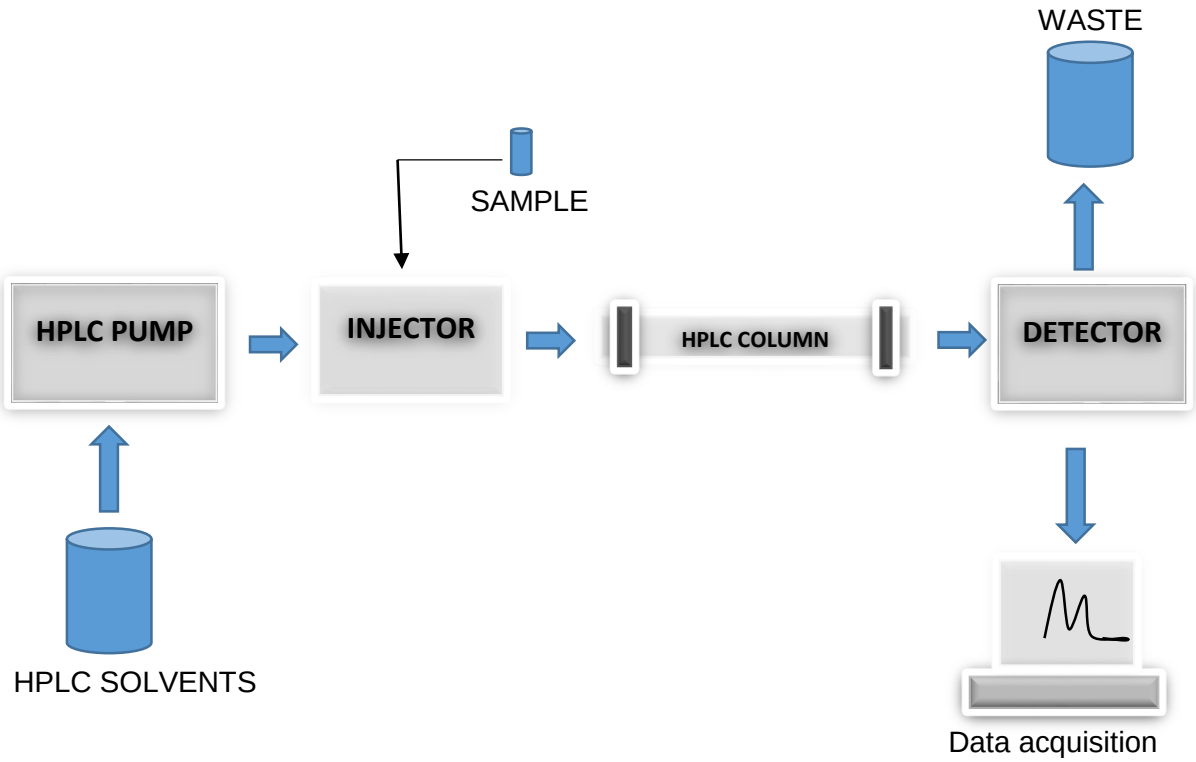


Figure 5.15. High performance liquid chromatography schematic diagram

However, HPLC requires rather expensive machinery and often uses large volumes of environmentally unfriendly liquids, which are the main drawbacks of this technique along with the undetected co-elution of compounds and the vulnerability of conventional (silica-based) columns to relatively basic (pH > 9) and acidic (pH < 2) mobile phases and high temperatures.

RP-HPLC method has been employed to standardize and identify the chemical components expected to be present in the selected plants. This is done to ensure the

quality, efficacy and safety of the herbal drugs present in those plants. RP-HPLC provides a very reliable way of determining the purity and percentage content of the active biomarker in the plant extracts. In this chapter the standardization and quantification of the five cultivers of plant *Lagenaria siceraria* extract (both methanolic and aqueous) have been described by using cucurbitacin E as biomarker.

5.2.1. Equipments and reagents

All the solvents used for chromatography were of HPLC grade. Acetonitrile (HPLC grade), glacial acetic acid (HPLC grade) were obtained from Merck (Mumbai, India). Membrane filters (0.45 µM pore size) was purchased from Millipore. Milli-Q water was used for all the analysis. Cucurbitacin E (percentage purity ≥ 95%) was procured from Sigma Aldrich and other analytical grade chemicals and reagents used for HPLC were purchased locally. For the analysis Waters-600 HPLC (USA) along with 600 controller, TM-600 pump, Inline Degasser AF and 2489 UV/Visible Detector was employed. Orion 2 Star pH Benchtop pH meter from Thermo Scientific (USA) was used to optimize theph of mobile phase.

5.2.2. Preparation of sample solution

About 10.0 mg of liophyllized extracts of *Lagenaria siceraria* cultivars was taken in 2 ml of eppendorf tube each. 1.0 ml of acetonitrile was added with it and mixed in vortex mixture and put to ultra-sonication bath till the material completely dissolved. It was then filtered through 0.45 µ syringe filter and kept for further study.

5.2.3. Preparation of standard solution

About 1.0 mg of Cucurbitacin E (Sigma Aldrich) standard was weighed and put in to 1 mL eppendorf tube. 1.0 ml of acetonitrile was added with it and mixed in vortex mixture and put to ultra-sonication bath till the material completely dissolved. Finally the volume was made up to 1 ml with acetonitrile and mixed in vortex mixture for 10 min. It was then filtered through 0.45 µ syringe filter and kept for further study.

5.2.4. Chromatographic conditions

Standardization of *Lagenaria siceraria* cultivers extracts was performed by HPLC (Waters-600) using Cucurbitacin E as standard. Waters Spherisorb (Ireland) C18 column (250 × 4.6 mm, 5 µm particle size) was used as stationary phase. Acetonitrile was used to prepare extract and standard, they were filtered through a Whatman's NYL 0.45µm syringe filter before injection. The mobile phase was optimized with acetonitrile: water (in 1% glacial acetic acid) at the ratio of 70:30, (v/v), at a flow rate of 1 ml/min. the optimum λmax was selected at 254nm. The concentration of cucurbitacin E in extracts was determined by preparation of calibration curve ranges from 10– 100 µg/mL.

5.2.5. Application of standard and extracts

The external standard calibration curve for Cucurbitacin E was prepared with calibration solutions in a concentration range of 100-100 µg/ml. 20µl of standard solution was injected in the rheodyne loop. The same method applied for the extract.

5.2.6. Development

The flow was monitored at wavelenth of 254 nm.

Software used	:	Empower2
Analysis mode	:	Peak area versus concentration of standard and extracts
Calculation	:	By using following equation

$$Y = m X + C$$

Where,

X = Concentration of Analyte/ metabolite in ng/ml
Y = Peak area
m = Slope of calibration Curve
C = Intercept.

5.2.7. Method validation for RP-HPLC study

Validation of the HPLC method was done as recommended by the International Conference on Harmonisation (ICH) guidelines (ICH, 1996, 2005) and FDA (1994) Guidelines defining the Linearity, Specificity, Limits of Quantification and detection, precision, accuracy and robustness.

5.2.7.1. Specificity

The results which were getting from standardization were checked in terms of specificity according to the ICH guidelines to minimize errors due to the contamination of the sample. The specificity of the method was determined by analyzing the standard and test samples. The purity of peaks was checked using multivariate analysis by comparison of retention times and peak area of standard compound with extract and fractions.

5.2.7.2. Limits of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were calculated by the method based on standard deviation (σ) and the slope (S) of the calibration plot, using the formula LOD=3:1 σ/S and LOQ= 10:1 σ/s (ICH, 1996, 2005 and FDA, 1994); where, σ =standard deviation of the response from a number of blank run and S = slope of calibration plot.

5.2.7.3. Accuracy

Accuracy of the method was determined by percentage recovery of marker in the plant extract and fractions. The method was studied by performing standard addition technique and it is expressed in terms of percentage relative standard deviations (%RSD) from mean recovery of the theoretical concentrations. Prior to injection, the tests were spiked with three different known amounts of standard compounds in triplicates. Analyses were done under the ambient condition to calculate the overall average recovery. The mean amounts of the markers achieved were taken as real values to calculate the spike recoveries.

5.2.7.4. Precision

The precision of the method was assessed by injecting six replicates at three different concentrations for the reference compound, the extract and fractions. Values were represented as % RSD of intraday and inter-day runs. The mean amount and RSD values were calculated. The intra-day precision of the assay was determined by analysing three concentrations in one day. Also, the intra-day precision was resolute over three successive days by analysing the same concentrations. Injections were done in six replicates to determine the repeatability of the process.

5.2.7.5. Robustness

The robustness of the proposed method was investigated by an analysis of samples under different experimental conditions. The test solutions were analysed with variation of flow rate, mobile phase composition, detection wave length and column temperature and using different columns of same configuration to determine their influence effect on the retention time.

5.2.8. Statistical analysis

Statistical analysis was performed using the graph pad prism Version 5.0. The results were represented as the mean ± SD

5.2.9. Results and discussion

5.2.9.1. Preparation of calibration curve of standard cucurbitacin E

The calibration curve was found to be linear in the concentration range of 1-100 µg/ml. The correlation co-efficient were found from the calibration curve as >0.99, which confirms that the data is closer to the line of best fit. The regression equation was found to be Y=19111X-54747.

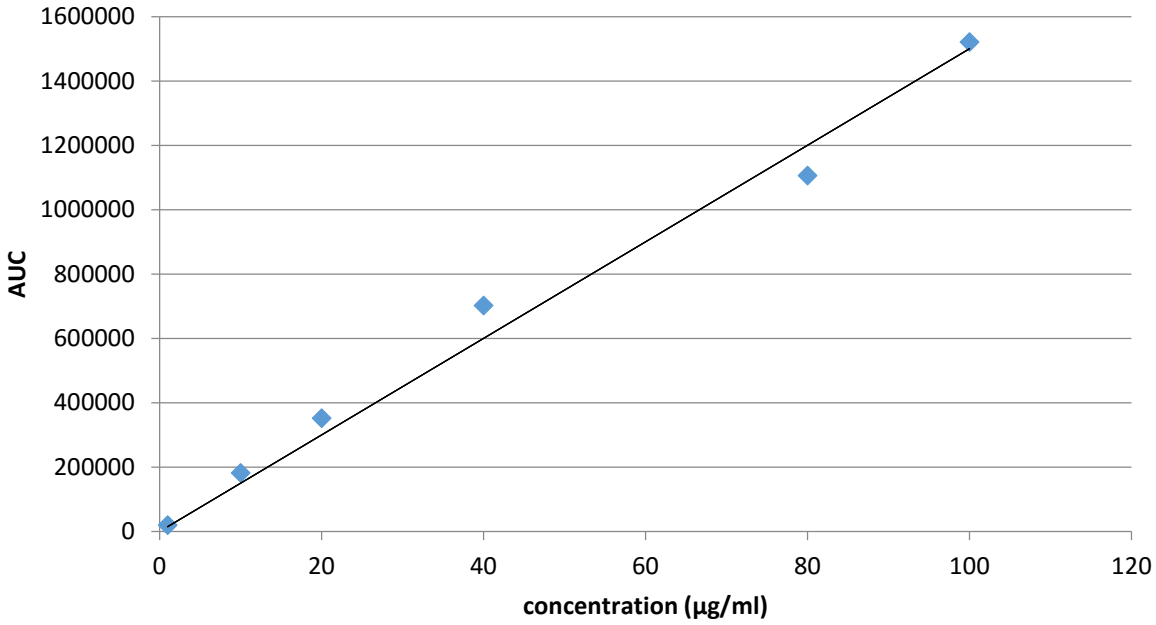


Figure 5.16. Calibration curve of standard Cucurbitacin E

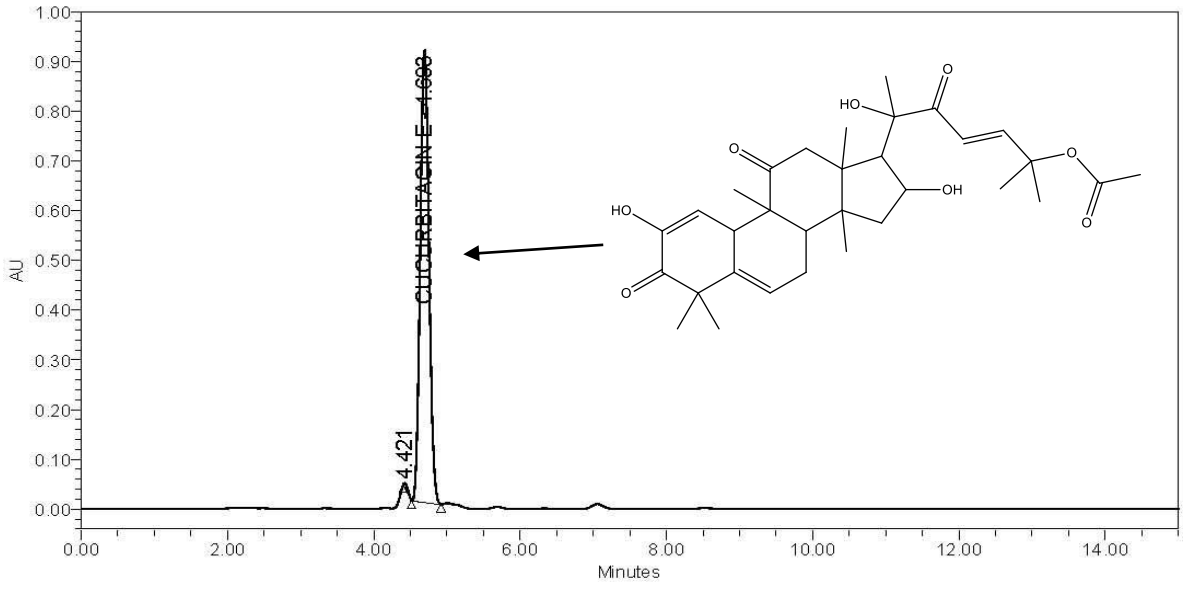


Figure 5.17. - RP-HPLC chromatogram of cucurbitacin E standard

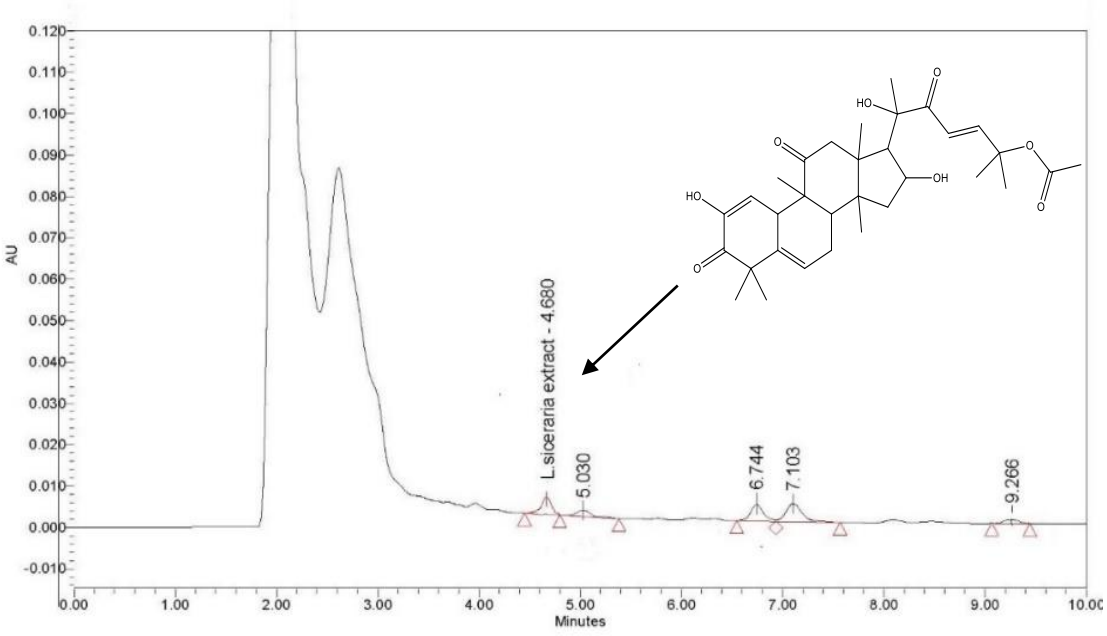


Figure 5.18. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Round Gourd)

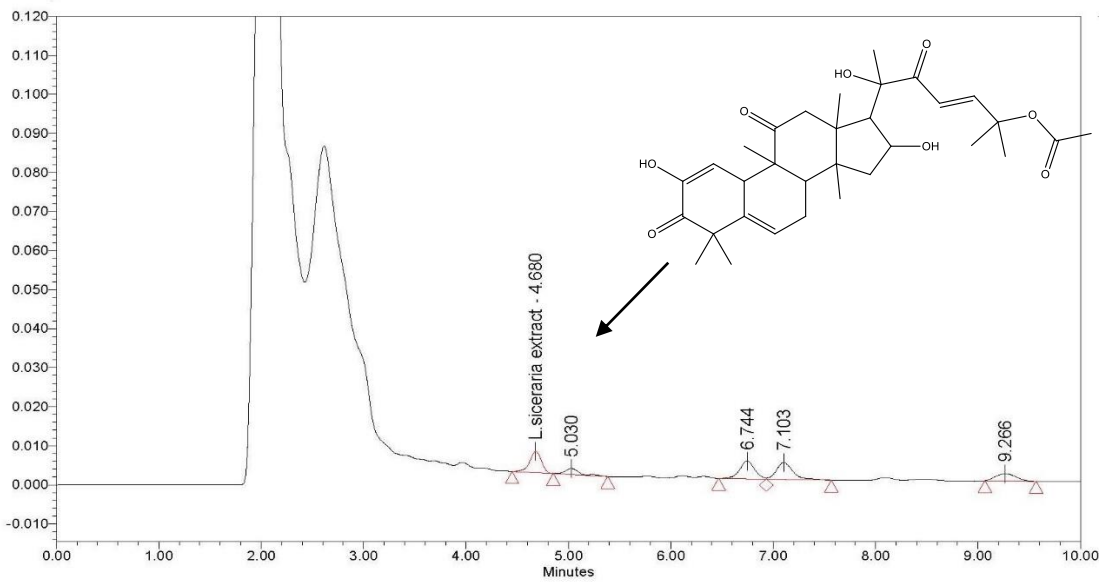


Figure 5.19. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Kettle Gourd)

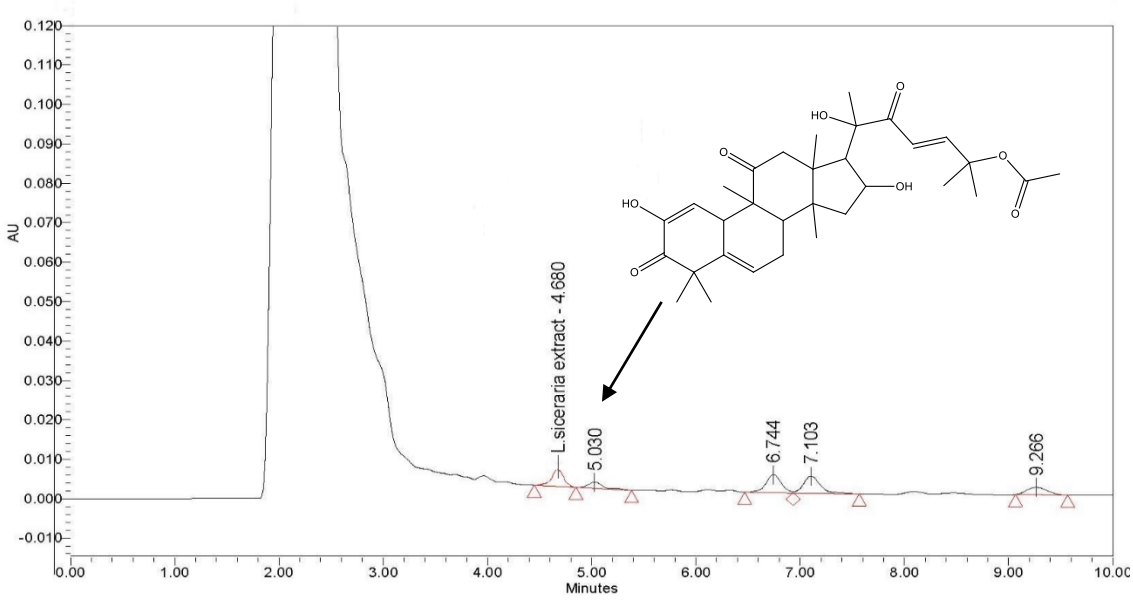


Figure 5.20. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Long Sausage Gourd)

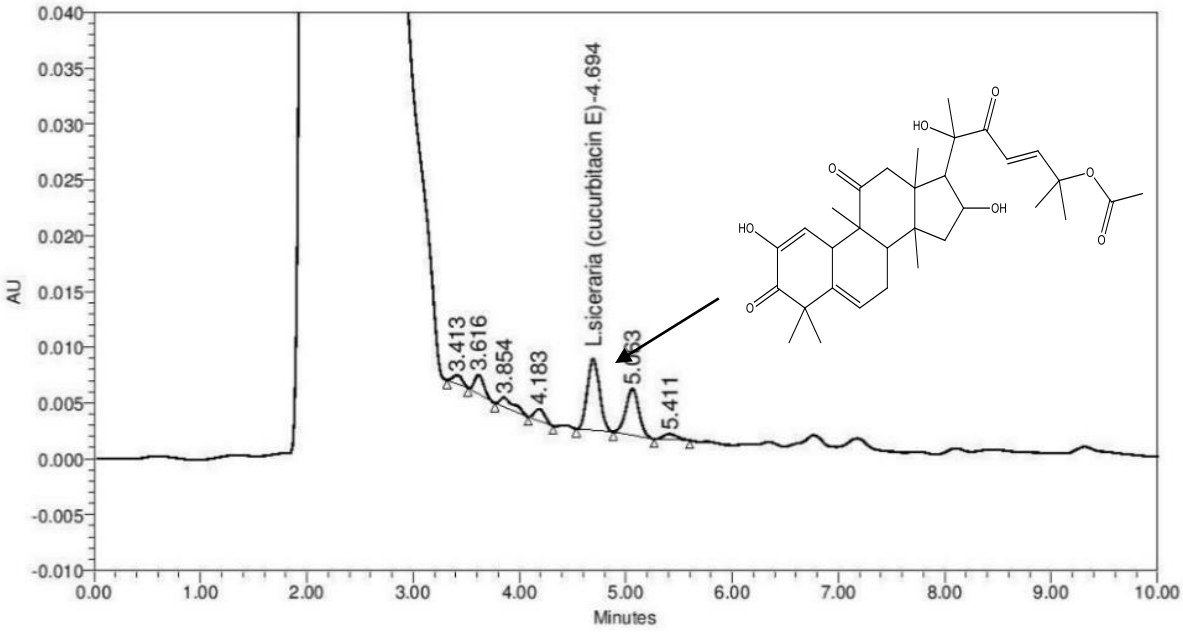


Figure 5.21. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Green Bell Hybrid)

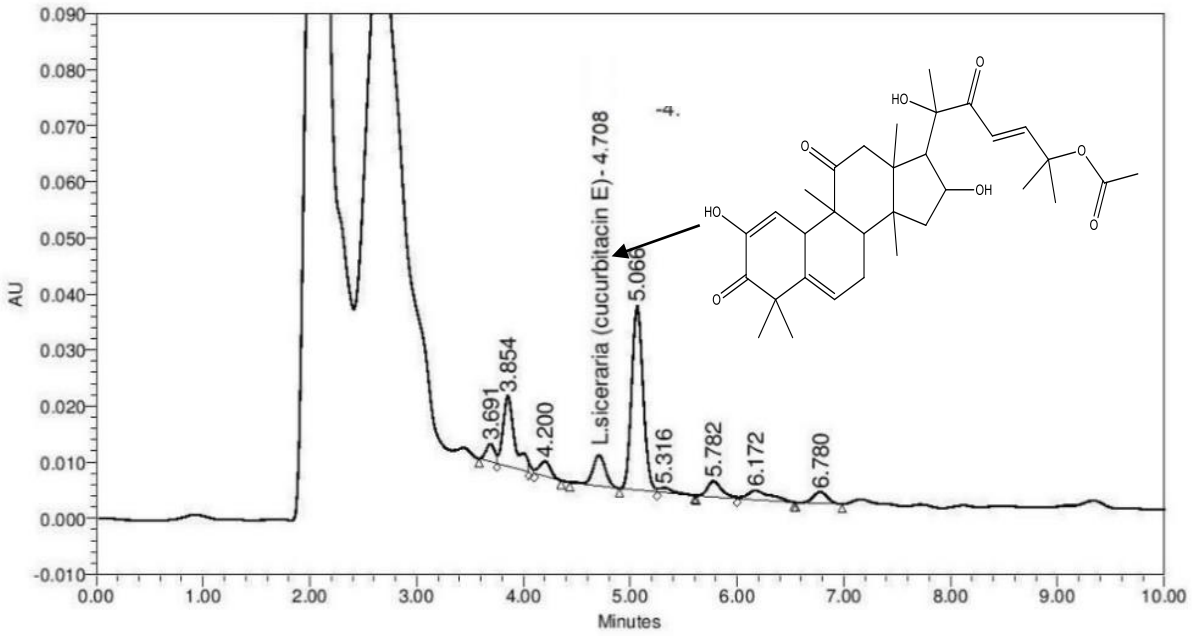


Figure 5.22. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Indian Hybrid)

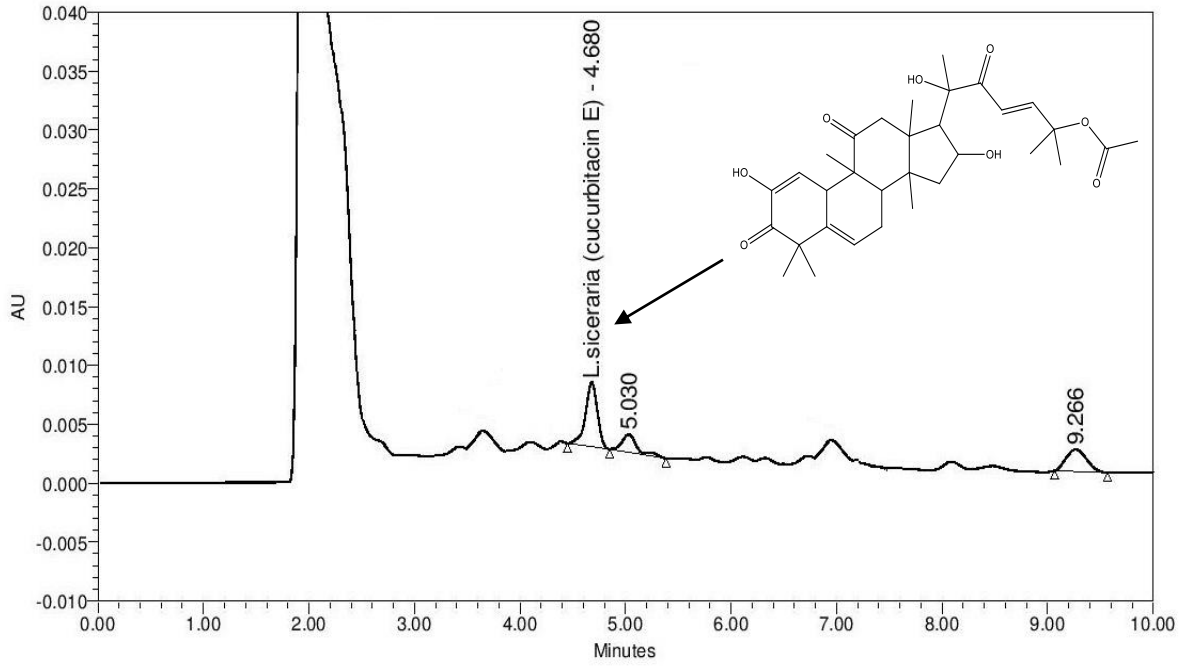


Figure 5.23 - RP-HPLC chromatogram of Lagenaria siceraria (Molina) Standl (Round Gourd methanolic extract)

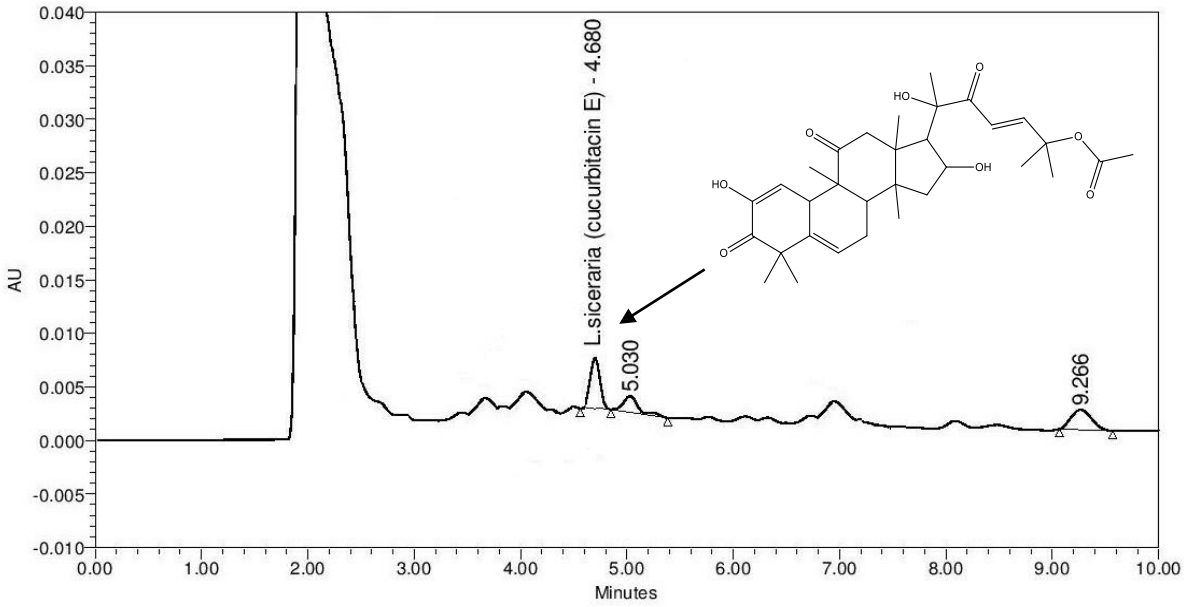


Figure 5.24. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Kettle Gourd methanolic extract)

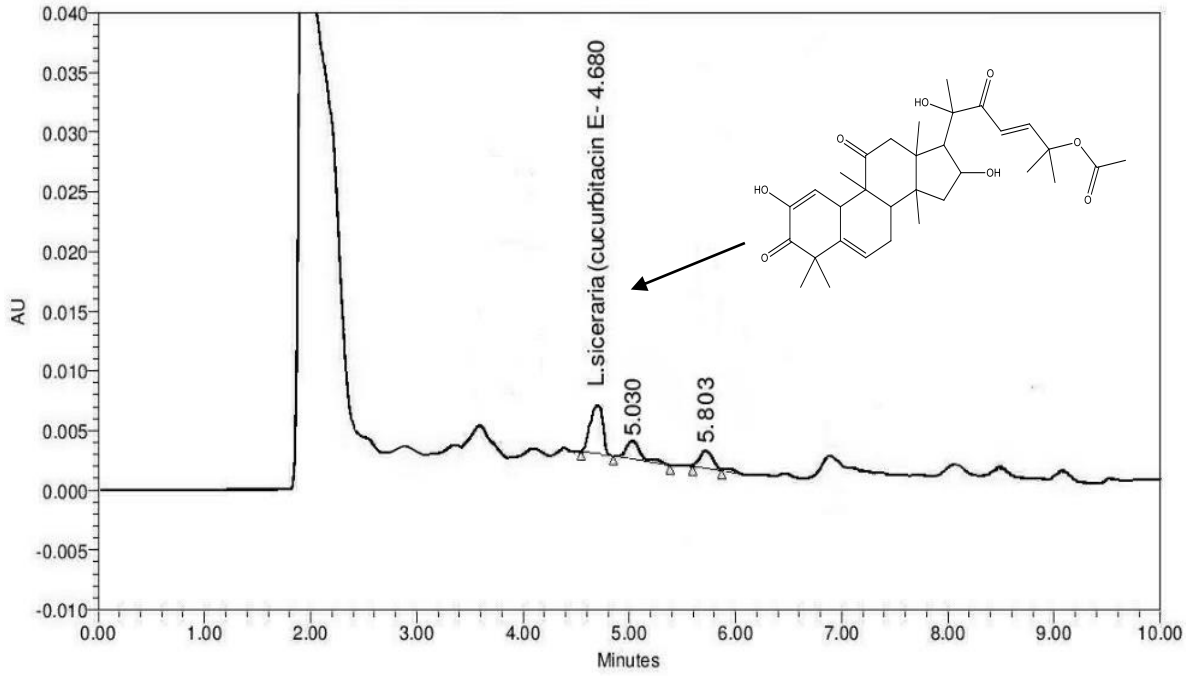


Figure 5.25. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Long Sausage Gourd methanolic extract)

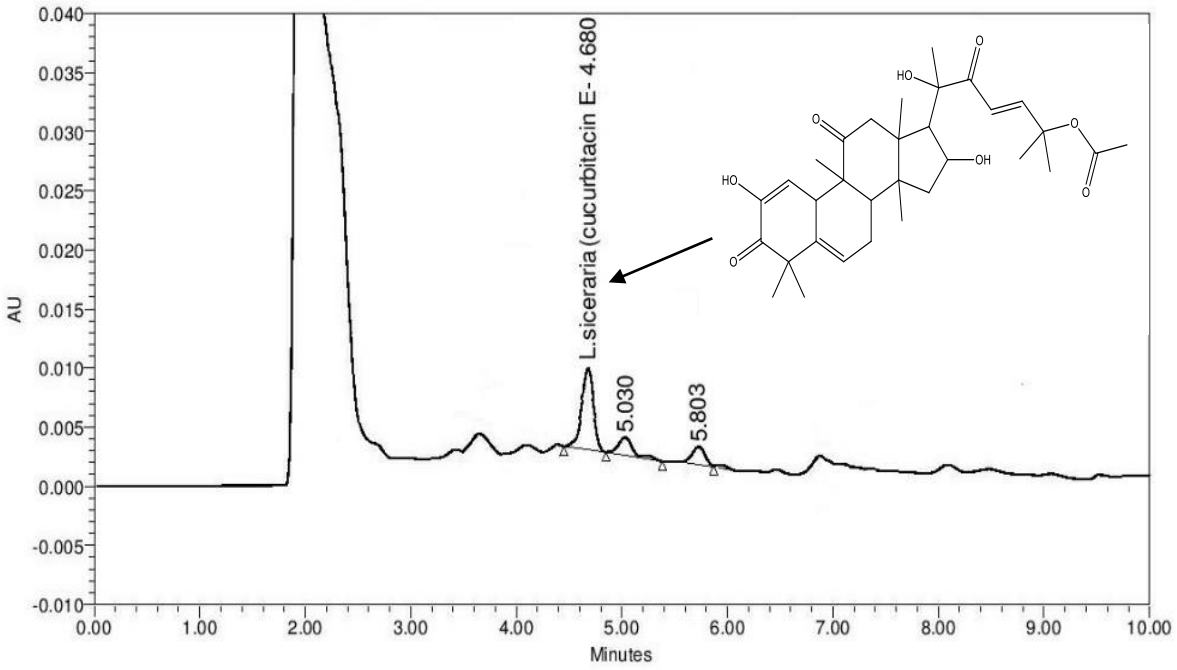


Figure 5.26. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Green Bell Hybrid methanolic extract)

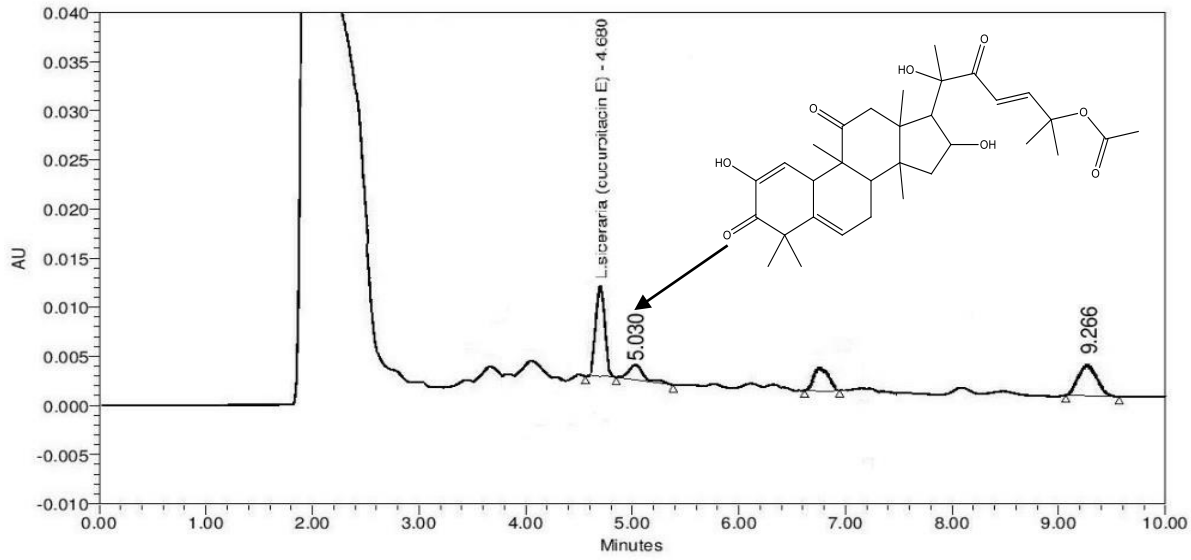


Figure 5.27. - RP-HPLC chromatogram of *Lagenaria siceraria* (Molina) Standl (Indian Hybrid methanolic extract)

5.2.9.2. Quantification of Cucurbitacin E in Lagenaria siceraria extracts

The content of cucurbitacin E found in different cultivars of *Lagenaria siceraria* (Molina) Standl fruits has been represented in Table 5.2. The chromatograms of the standard and of different cultivars have been shown in Figures 17 to 29. The study showed that the cultivar named Indian hybrid cultivar has the maximum amount of cucurbitacin E

Table 5.2.: Percentage yield and cucurbitacin E content in five different cultivars of *Lagenaria siceraria* (Molina) Standl Fruit

S No.	Name of the cultivars	% Yield of extract(w/w)		% Cucurbitacin E content found (w/w)	
		Aqueous	Methanolic	Aqueous	Methanolic
1	Round gourd	3.83	2.7844	2.05	0.68
2	Kettle gourd	5.881	1.1522	2.31	0.84
3	Long sausage gourd	7.249	1.7543	2.84	0.96
4	Green bell hybrid	4.08	2.372	3.23	1.62
5	Indian hybrid	4.201	1.943	5.23	1.92

5.2.9.3. Comparative chromatogram of extracts of five cultivars of Lagenara siceraria

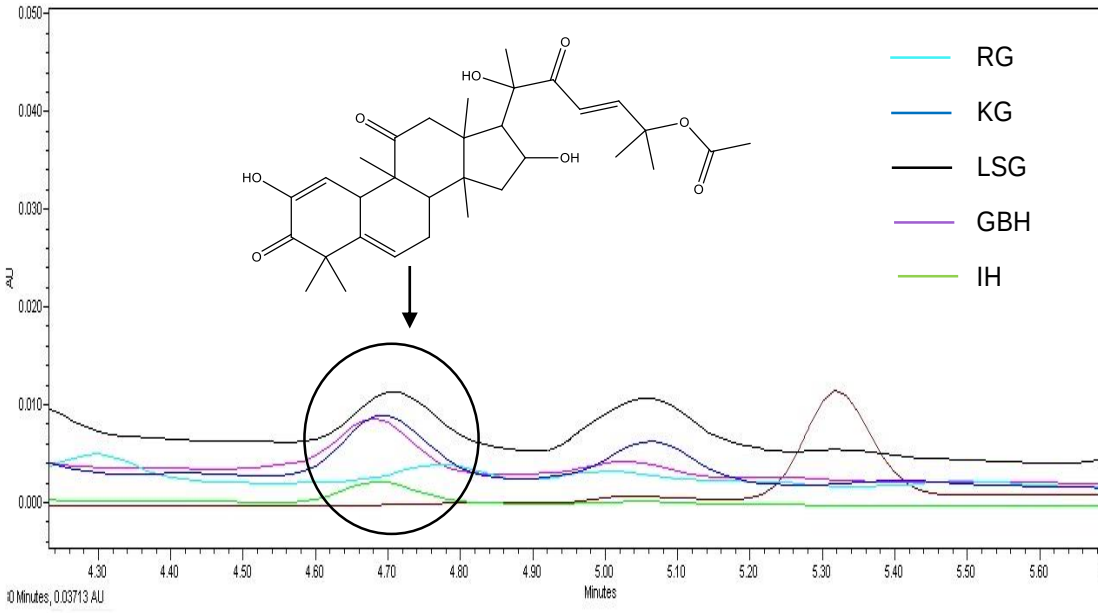


Figure 5.28. Comparative chromatogram of methanolic extracts of five varieties of *Lagenara siceraria*. (RG: Round Gourd; KG: Kettle Gourd; LSG: Long Sausage Gourd; GBH: Green Bell Hybrid; IH: Indian Hybrid.)

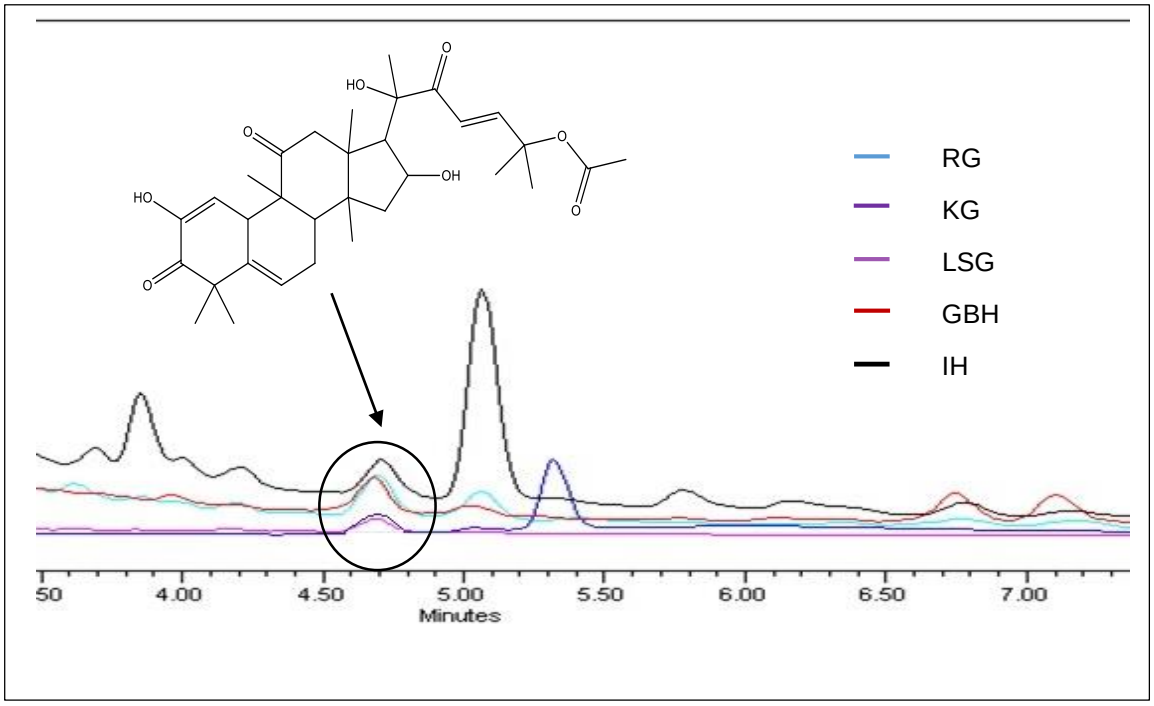


Figure 5.29. Comparative chromatogram of lyophilized extracts of five varieties of *Lagenara siceraria.* (RG: Round Gourd; KG: Kettle Gourd; LSG: Long Sausage Gourd; GBH: Green Bell Hybrid; IH: Indian Hybrid.)

5.2.10. Conclusion

The above comparative study revealed the difference in phytoconstituents present in different cultivars of *Lagenaria siceraria*. The Indian hybrid showed maximum amount of cucurbitacin E presence while in other cultivars, was found in order, green bell hybrid > long sausage > gourd round gourd > kettle gourd. The developed HPLC method is very accurate, precise and reproducible for quantification of cucurbitacin E with a narrow linear range. It will also be able to give insight on the cucurbitacin E content in food stuff. The study will further necessary for quality control of *L. siceraria* which has been used to manage several lifestyle related disorder. This method is quite acceptable for commercial use. In this method we have calculated that the percentage content of cucurbitacin E in extracts both methanolic and aqueous.

5. 3. Atomic Absorption Spectroscopy analysis for detection of heavy metals and minerals in plant extracts.

Thus, quantification of metals in plants, especially medicinal herbs, is part of quality control, which has been established by their purity, safety and efficacy (Mukherjee, 2002). Many curative effects of herbal formulations used in the treatment and cure are due to presence of very minute quantities of trace elements. These elements are iron (Fe), copper (Cu), cobalt, nickel (Ni), zinc (Zn), magnesium, manganese (Mn), molybdenum, chromium (Cr), vanadium, lithium, selenium, fluorine (F) and iodine (I) (Shirin et al., 2010) Other heavy metals normally present in plants like lead (Pb), cadmium (Cd) and mercury (Hg) are toxic at very lower concentration (Lobet et al., 2003). World Health organization (WHO, 1989) declares the maximum permissible levels in food and drug materials for arsenic (As), Cd and Pb as amount to 1.0, 0.3 and 10 mg/kg, respectively, (Nema et al., 2012). The limit of individual metals by Government of India was prescribed and followed (Ahmmed et al., 2016).

In the present study, five cultivars of plant *Lagenaria siceraria* i.e. Round Gourd, Kettle Gourd, Long Sausage Gourd, Green Bell Hybrid and Indian Hybrid were selected to determine the trace (Cu, Cr, Mn, Fe and Co) and heavy (As, Pb and Hg) metals through atomic absorption spectrometry and thereby to assure the safety and therapeutic application of these plants.

5.3.1. Instrumentation

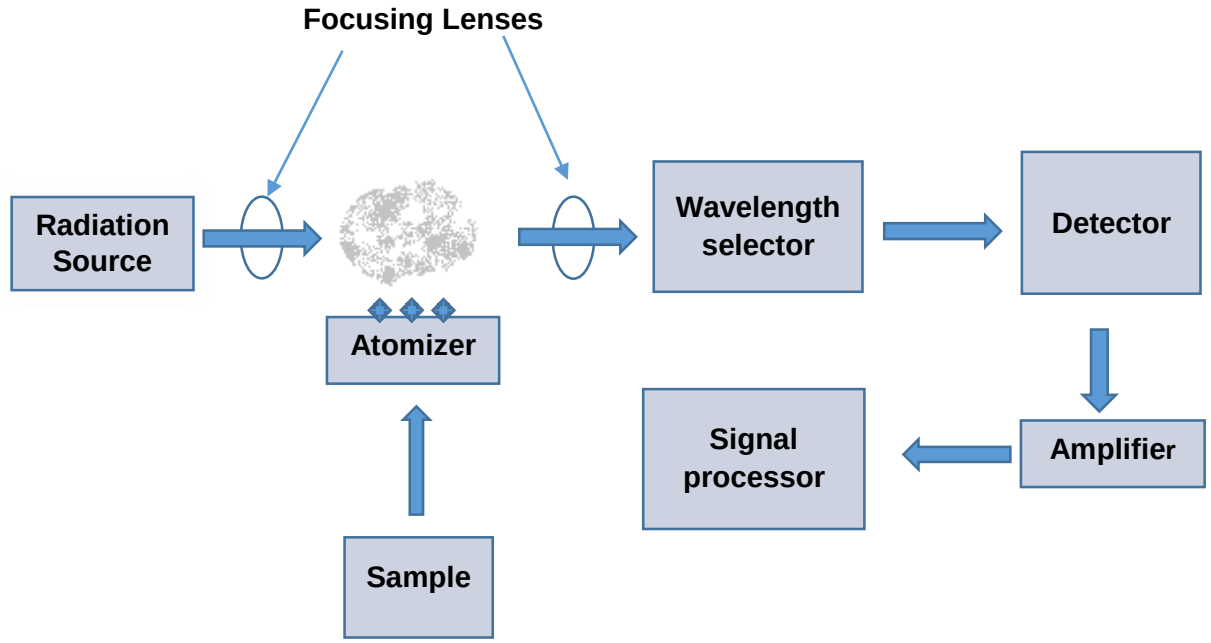


Figure 5.30. Schematic diagram of Atomic Absorption Spectroscopy

An atomic absorption spectrometer consists of the following elements Source: Single-element or multi-element hollow cathode tubes generally are employed as sources in

atomic absorption. Collision of these atoms with an inert gas such as argon induces excitation of the metal atoms and subsequent emission of characteristic radiation.

Burner: The quality of the burner, the type of fuel and the ratio of fuel to oxidize are the important factors which affect the result of analysis by an atomic absorption instrument.

Monochromator: The monochromator is used to pass the resonance line and filter out other.

Phototube and Amplifier: They mainly detect and amplifythe signal. Following factors are affecting the atomic absorption spectrometer.

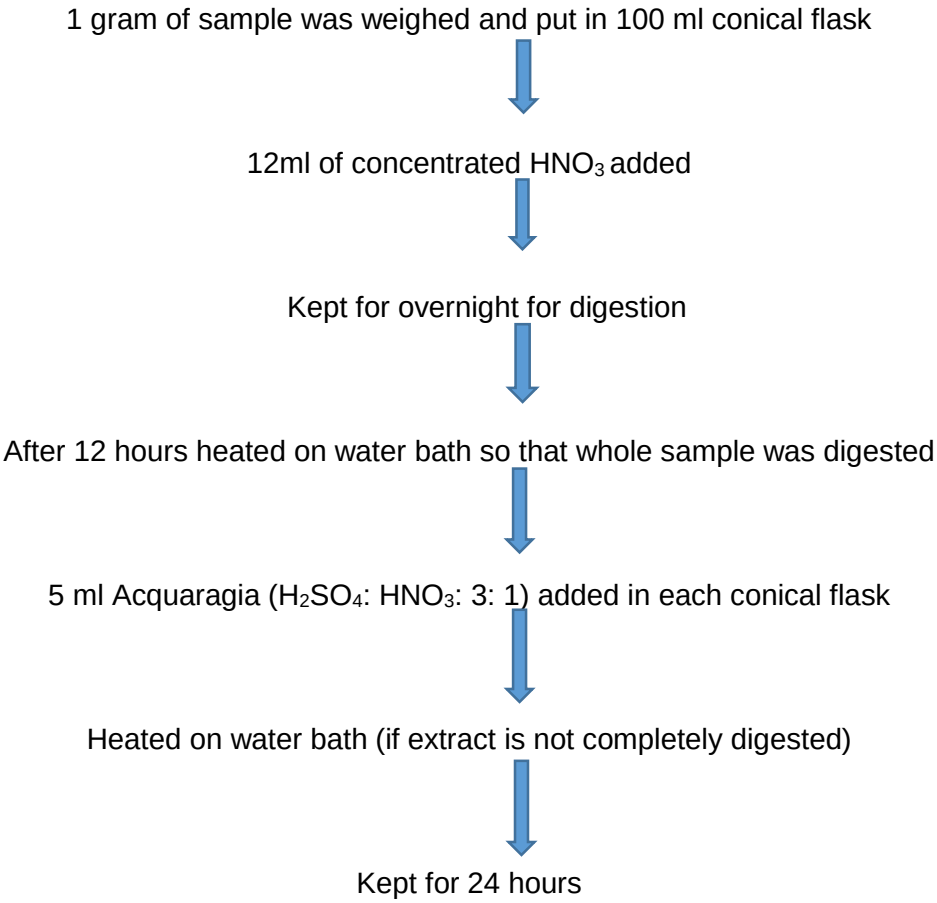
The atomic absorption measurements were performed using Thermofisher AA 303 atomic absorption spectrometer (Nashik, Maharashtra, India) with hollow cathode lamp as light source. For the analysis of all the metals, oxy-acetylene flame was used. Hydride generator with sodium borohydride and concentrated HCl were used to convert arsenic and mercury into their volatile hydride forms. The vaporized hydride present in the system was transferred to the optical cell using peristaltic pump. Cold vapor analysis was applied to determine the mercury content. Standard solutions of the tested metals/elements were used for the determination of corresponding elements. The instrumental configuration and experimental condition for proper analysis of heavy metals like arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), cupper (Cu), zinc (Zn) and mercury (Hg) are shown in Table 5.1.

Table 5.3. Instrumental condition for trace and heavy metal analysis by Atomic Absorption Spectrometry

Elements **AAS specification**	**Copper**	**Chromium**	**Iron**	**Cadmium**	**Arsenic**	**Lead**	**Mercury**
Wavelength	324.8	357.9	248.3	228.80	193.7	217.0	253.7
Current (mA)	5.0	5.0	9.0	7.0	12.0	9.0	3.0
Flame	AA	AA	AA	AA	AA	AA	-
Fuel (L/min)	3.05	2.90	2.99	5.53	2.40	2.90	7.66
Slit width(nm)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Working range (ppm)	1-5	2-8	2-10	0.5-2.0	0.04-0.1	2-10	1-0.2
Read time (sec)	3	3	3	3	3	3	3
Wash time (sec)	15	15	15	15	15	15	15

AA - Air acetylene flame

5.3.2. Sample Preparation



5.3.3. Results

The concentration of trace metals and heavy metals of the five different cultivers of plant *Lagenaria siceraria* were determined by AAS. The concentration of different heavy metals are given in table 5.4. All heavy metals determined are found to be within the limits prescribed as per WHO specifecations.

Table 5.4. Metal content of selected plant extract (Aqueous) detected by Atomic Absorption Spectrometry (in ppm)

Plant Cultivars Used	Copper (mean ±SEM)	Chromium (mean ±SEM)	Cadmium (mean ±SEM)	Lead (mean ±SEM)	Arsenic (mean ±SEM)	Mercury (mean ±SEM)
Round Gourd	10.27±0.54	0.97±0.12	0.011±0.002	0.38±0.01	0.00	0.010±0.001
Kettle Gourd	6.56±0.24	1.93±0.20	0.010±0.001	0.48±0.02	0.00	0.013±0.001
Long Sausage Gourd	7.28±0.09	0.84±0.05	0.011±0.001	0.28±0.04	0.00	0.010±0.001
Green Bell Hybrid	8.85±0.46	1.36±0.11	0.011±0.003	0.07±0.03	0.00	0.012±0.001
Indian Hybrid	5.98±0.98	1.26±0.18	0.013±0.003	0.08±0.03	0.00	0.011±0.001

5.3.4. Conclusion

The concentration of trace metals and heavy metals of the five different cultivers of plant *Lagenaria siceraria* were determined by AAS. The quantitative determinations were carried out using standard calibration curve obtained by the standard solution of metals having optimal detectable concentration ranges. The concentration of the metals obtained in plant material was expressed in terms of parts per million. The levels of heavy metals quantified in all the plant samples were found within prescribed limits.

Chapter – 6

Lipoprotein lipase assay of plant extract

6. 1. Introduction

6. 2. Plant cultivers assayed

6. 3. In vitro assay of plant cultivers– methodlogy

6. 4. Results

6. 5. Conclusion

6.1. Introduction

Lipid metabolism is elegantly balanced to maintain homeostasis. When the balance is lost, obesity or hyperlipidemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs. Therefore, the control of lipid metabolism by drugs could be used to prevent or treat these diseases. A growing number of enzymes involved in lipid metabolic pathways are being identified and characterized; as such they represent a rich pool of potential therapeutic targets for obesity and other metabolic disorders (Birari et al. 2007). Pancreatic lipase plays a key role for triglyceride absorption in the small intestine. This enzyme is secreted from the pancreas and hydrolyzes triglycerides into glycerol and fatty acids (Lee et al., 2001).Lipases or acylglycerol acylhydrolases (EC 3.1.1.3) are esterases hydrolyzing esters of glycerol with long-chain aliphatic acids. They are enzymes found widely in nature. Lipases of different origin show different affinities for tri-, di- and monoglycerides, they may also hydrolyze esters of other aliphatic alcohols with different specificity for the acyl chain length (Maurich et al., 2003). In organic medium, lipases also catalyze esterification, transes-terification and interesterification reactions. These enzymes are applied in several industrial processes including the synthesisand degradation of engineering thermoplastics, production of pharmaceuticals, agrochemicals, cosmetics, flavors and fragrances,emulsifiers, structured lipids, pretreatment of lipid-rich waste waters, biodiesel synthesis by transesterification of triglycerides with short-chain alcohols and concentrated fatty acids by hydrolysis of oils and fats (Hasan et al., 2006).The hydrolysis of oils and fats from several sources for the production of concentrated free fatty acids and glycerol is relevant in the industrial processing of natural oils and fats.(Santos et al., 2013.).

Bearing this, the suppression of triacylglycerol absorption by inhibiting lipase is a major approach to avert obesity. In the past years this has led to the usage of Orlistat as an agent for obesity managment. Orlistat inhibits triacylglycerol absorption by the inhibition of pancreatic lipase, and its long-term administration results in weight loss, suggesting its efficacy for the treatment of obesity. It is however associated with side effects such as decreased vitamin D absorption and myopathy, indicating that alternatives to its usage should be researched (Goncalves et al., 2010).

6.2. Plant varieties used for in – vitro assay

- *Round Gourd* - A slightly smaller size gourd than the bushel, with a more rounded shape. Green weight is about 20 lbs, this is the perfect size gourd for novelty baskets and decorations (Chimonyo et al., 2013).

- *Long sausage gourd* - Fruits are cylindrical can be harvested at small size as well as full size stages for cooking and eating. White soft flesh is very tender and delicious, suitable for stir-fry and soup cooking. Plants are very vigorous and

should be well watered in warm summer. Plants can be grown along supports or on the ground (Chimonyo et al., 2013).

- *Green bell hybrid* - These are annuals, vegetables, vines and climbers having height of 6- 12 in (15-30 cm). Green Bell is a new high quality hybrid variety developed by Kaoshung Horticulature Research Center in Taiwan. Fruits with green skin and white stripes, in bell shape. Plants grow very well in subtropical climates. Plants are vigorous and tolerant to disease attacks. Very productive and easy to grow (Essien et al., 2013).

- *Kettle gourd* - Annuals, vines and climbers, having height of almost 6-12 in (15-30 cm) of fruit and 10-12 ft. (3-3.6 m) of plant. The foliage is herbaceous, blue-green, velvet/fuzzy-textured (Shivraj et al., 2005)

- *Indian hybrid* - This hybrid variety is a vigorous plant with prolific bearing habit, producing fruits continuously for a long time. Relatively short cylindrical fruits with 25-30 cm. In length and 300-350 grams in weight are very tender and delicious. Light green skin with white flesh fruits with slow seed maturity. Plants grow vigorously in warm climates, starting to set fruits in 45 days after the transplanting. (Shivraj et al., 2005).

6.3. Lipoprotein lipase assay of five cultivers of Lagenaria siceraria

6.3.1. Methodlogy

Lipase activity was evaluated by the hydrolysis of p-nitrophenyl caprylate (pNPC) spectrophotometrically recorded at 405 nm and at pH 7.0 in a thermostated microplate reader at 37˚C according to previous work. The concentrations of pNPC and PLwere 250 and 2.5µM, respectively. In each well of a 96-well microplate phosphate buffer, pNPC and respective test/std./blank fraction were added. After an automatic shaking step to ensure homogenization the enzyme was added, and the measurement process began. IC50 were determined through nonlinear regression of the plots of percentage relative avtivity= f ([concentration]). The experiments were performed in phosphate buffer (50 mM, pH 7.0) to simulate the conditions that occur in the duodenum after neutralization of gastric fluids. An experiment in which all solvents were incubated with PL or pNPC separately was conducted to determine if aggregation influenced the measurement at 405 nm corresponding to the enzymatic activity.
The absorbance was measured at 405nm using a UV–visible spectrophotometer (spectramax). Orlistat was used as a positive control. The percentage relative activity of enzyme was calculated as per following formula:

Percentage Relative Activity = rate with inhibitor / rate without inhibitor * 100

Table 6 1. Representation of the procedure applied in enzymatic assay

Name of Sample	DMSO (µl)	Phosphate buffer (µl)	Substrate (µl)	Enzyme (µl)	Orlistat (µl)	Test sample (µl)	Vortex and incubate at 37°C for 10 min	Total volume (µl)
Blank	50	50 + 50		50				200
Negative control	50	50	50	50				200
Orlistat		50	50	50	50			200
Test samples		50	50	50		50		200

6.3.1. Statistical Analysis

The IC_{50} value (50% inhibitory concentration) was calculated by nonlinear regression using software GraphPad Prism version 5.0. The concentration-response curve was obtained by plotting the percentage relative activity versus concentration. The differences within and between groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multi comparison Dennett test compared with the positive control.

6.4. Result

The present study revealed the affectivity of selected plant extracts compared to the standard orlistat. The results of enzymatic assay performed and the IC_{50} value calculated as shown in Table 6.2.

Table 6.2. IC50 value of different cultivers by lipoprotein lipase inhibition assay

S. No.	Plant Cultivers	IC_{50} Values (µg/ml)	
		Aqueous	**Methanolic**
1	Long Sausage Gourd	358.692 ± 15.958	403.838 ± 25.355
2	Kettle Gourd	421.193 ± 20.050	557.985 ± 25.629
3	Round Gourd	188.028 ± 10.53	626.648 ± 26.024
4	Indian Hybrid	153.952 ± 7.997	548.519 ± 30.857
5	Green Bell Hybrid	338.245 ± 26.059	661.551 ± 19.281
6	Cucurbitacin E	522.227 ± 40.170	
7	Orlistat	103.643 ± 4.835	

The efficacy of the lyophilised Indian Hybrid, lyophilised Round Gourd and methanolic Long Sausage Gourd against the lipoprotein lipase were found to be comparable to that of the standard. These extracts showed an IC_{50} value (153.9529 ± 7.997, 188.028 ± 10.53, 403.8386 ± 25.355) comparable to that of orlistat (103.6431 ± 4.835) where as others showed activity but significantly at higher concentration. The dose dependent interaction curve also revealed less difference between the interactions for Indian hybrid as compared to that shown by orlistat. The statistical analysis was done by one way ANOVA (Analysis of variance) followed by Dennett comparisons test.

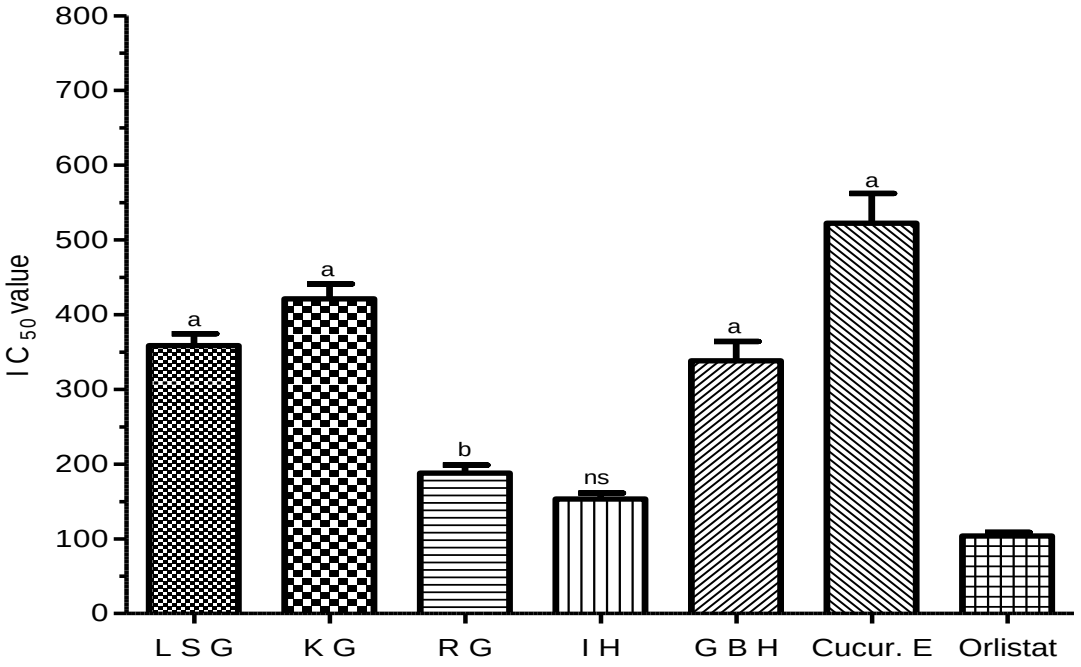


Figure 6.1. The IC_{50} value of aqueous extract of five cultivars of *Lagenaria siceraria* on lipoprotein lipase. All the components and biomarkers showed significantly ($p < 0.001$) less inhibition compared with positive inhibitor Orlistat except round gourd (b) and Indian hybrid (ns). Values were expressed as mean ± SD (n = 3); a = $p < 0.001$; b = $p < 0.01$; ns = not significant. (RG: Round Gourd; KG: Kettle Gourd; LSG: Long Sausage Gourd; GBH: Green Bell Hybrid; IH: Indian Hybrid.)

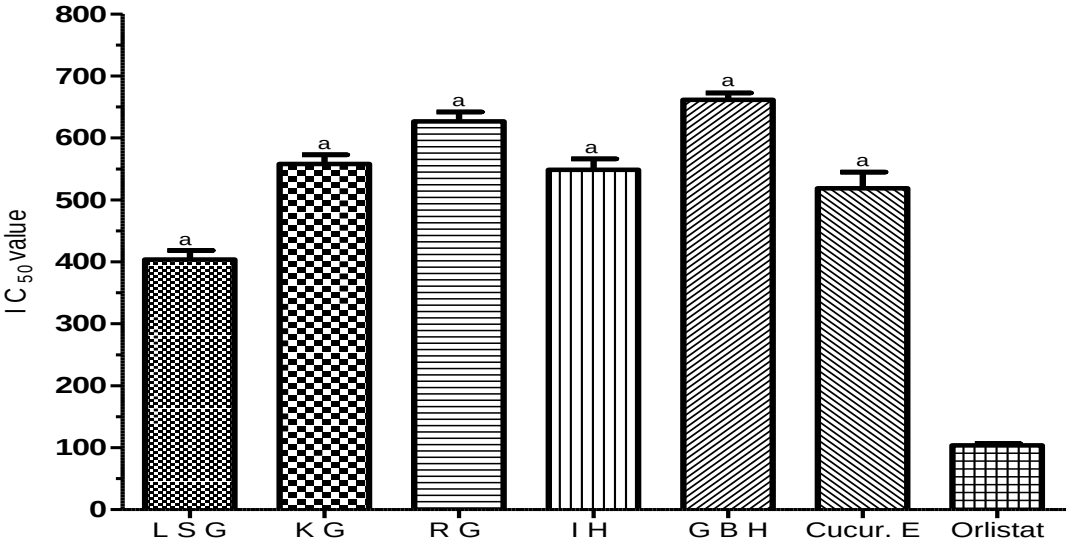


Figure 6.2. The I C $_{50}$ value of methanolic extract of five cultivars of *Lagenaria siceraria* on lipoprotein lipase. All the components and biomarkers showed significantly ($p < 0.001$) less inhibition compared with positive inhibitor Orlistat. Values were expressed as mean ± SD ($n = 3$); a = $p < 0.001$; b = $p < 0.01$; ns = not significant. (RG: Round Gourd; KG: Kettle Gourd; LSG: Long Sausage Gourd; GBH: Green Bell Hybrid; IH: Indian Hybrid.)

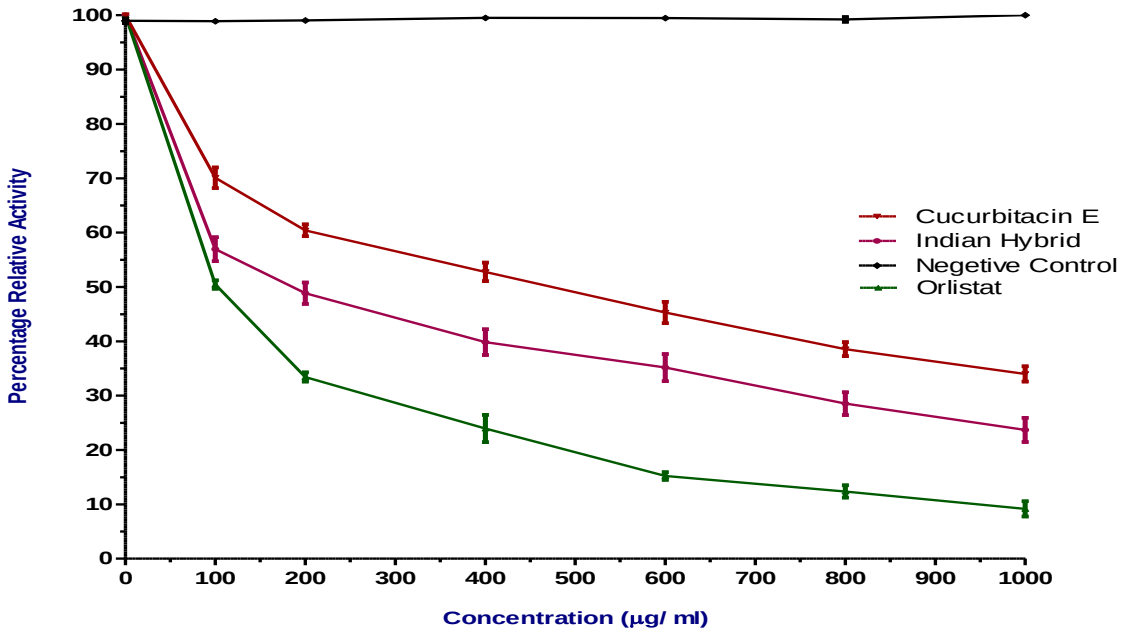


Figure 6.3. Dose response curve between % relative activity of enzyme and concentration for aqueous extract of Indian hybrid of *Lagenaria siceraria,* cucurbitacin E and orlistat.

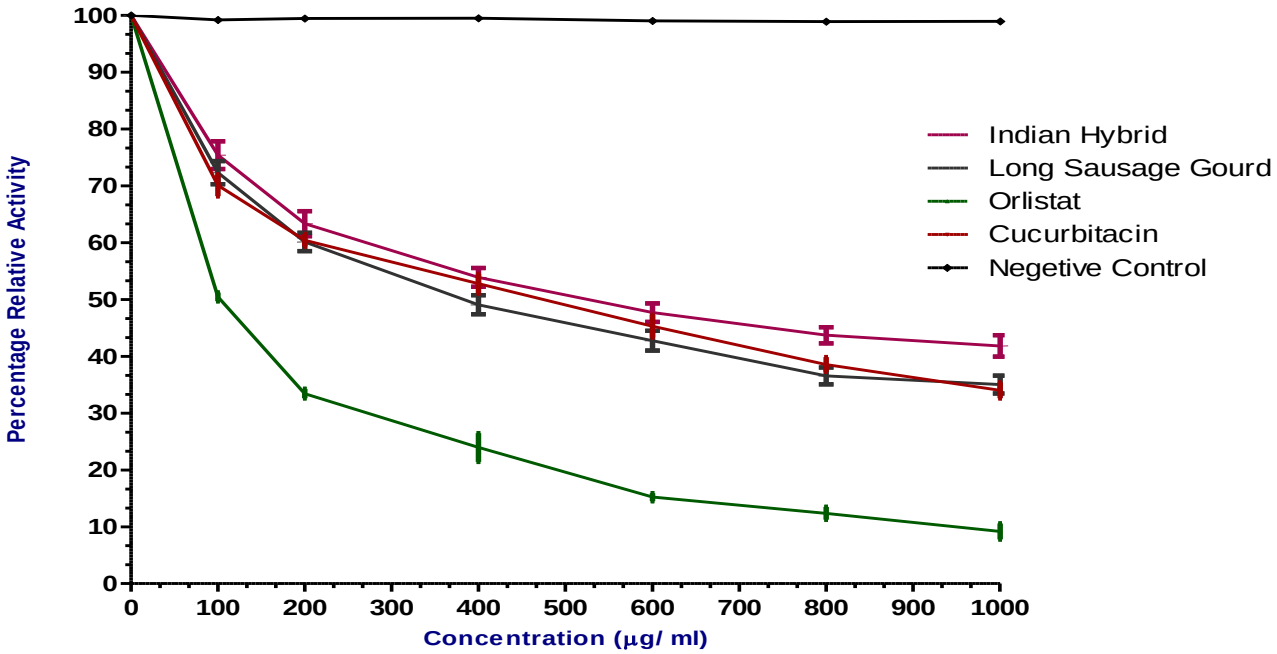


Figure 6.4. Dose response curve between % relative activity of enzyme and concentration for methanolic extract of Indian hybrid, long sausage gourd of *Lagenaria siceraria,* cucurbitacin E and orlistat.

6.5. Conclusion

Lipoprotein lipase interaction potential of the individual plant cultivars used along with the phyto markers present, was studied and it was concluded that the interaction potential of all were very less to moderate with respect to the positive markers i.e. Orlistat except the round gourd and Indian hybrid aqueous extracts. Both varieties are showing IC_{50} values near to that of positive inhibitor taken. The dose dependent interaction curve also revealed less difference between the interactions for Indian hybrid as compared to that shown by orlistat. This renders the chances for *Lagenaria siceraria* Indian hybrid variety to be a natural analogue of lipoprotein lipase inhibitor. While other verities showed the activity in order of Round Gourd > Green Bell Hybrid > Long Sausage Gourd > Kettle Gourd, among aqueous extracts and Long Sausage Gourd > Kettle Gourd > Round Gourd > Green Bell Hybrid in case of methanolic extracts. The cucurbitacin E showed interaction potential very less to moderate with respect to the positive inhibitor that may reveal the presence of some another phytoconstituents or some synergism responsible for anti hyperlipidemic effect of plant extract.

Chapter – 7

In - vivo antihyperlipidemic study of plant extract

7.1. Introduction

7.2. Methodology

7.3. Results

7.4. Conclusion

7.1. Introduction

The World Health Organization (WHO) reports that high cholesterol contributes to 56% of cases of coronary heart disease (CHD) worldwide and causes about 4.4 million deaths each year. During the past years, several studies and clinical trials have revealed the adverse effects of high blood lipid levels on the progression of atherosclerosis and consequently the development of cardiovascular disease (Chanmee et al., 2013). Condiments, medicinal plants, fruits used in day-to-day preparation of food in Indian kitchens have been identified as hypolipidaemic in Ayurveda (Mohale et al., 2008). Hyperlipidaemia is a condition which characterized by abnormal elevation of lipid such as (triglyceride and cholesterol) and lipoproteins such as LDL, VLDL, etc. levels in the blood. Lipid metabolism is elegantly balanced to maintain homeostasis. When the balance is lost, obesity or hyperlipidaemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs. Therefore, the control of lipid metabolism by drugs could be used to prevent or treat these diseases (Birari et al., 2007).

There are various research works as well as ethnopharmacological evidences regarding its use as anti-obesity and antihyperlipiidaemic. *Lagenaria siceraria* (Molina) Standl belonging to Cucurbitaceae family is a large, pubescent, climbing, or trailing herb cultivated throughout India and the tropical regions of the world. In the present study aqueous extract of the fruits of Indian hybrid cultivar of *Lagenaria siceraria* was used to determine the hypolipedaemic activity of extract in rat model and for comparison a well-known standard atorvastatin (10 mg/kg) was used. The rats were fed these extract and standard by oral route (oral gavage) showed significant antihyperlipidaemic activity at a dose of 200 and 10 mg/kg. The study was carried out for 30 days in which rats were grouped in four groups (n = 4). The methodology for experimentation and estimation was done accordingly.

7.2. Methodology

7.2.1. Animals

Male Albino rats of Wistar strain weighing between 120 and 150 g were used in the study. The animals were housed under standard environmental conditions (temperature 22±2 ◦C; humidity (60±4%) with a 12 h light/dark cycle at the school of natural products. The animals were having free access to water ad libitum. All animal experiments were approved by the Animal Ethical Committee of the Institute, (ref no.: AEC/PHARM/1601/08/2016) and all procedures were conducted according to the “Guide for the Care and Use of Laboratory Animals”.

7.2.2. Preparation of normal and high-fat diet

The both normal and high-fat diets composition are as follows: the normal diet contained whole wheat (67.5 g), yellow corn (62.5 g), barley (37.5 g), anik spray (37.5 g), bone meal (2.5 g), calcium chloride (2.5 g), salt (2.5 g), oil (37.5 g) and 1 tablet of Vitamin B12. The high-fat diet were prepared by mixing calculated amounts of whole wheat (50.0 g), yellow corn (50.0 g), barley (25.0 g), anik spray (37.5 g), bone meal (2.5 g), calcium chloride (2.5 g), salt (2.5 g), oil (25.0 g), butter (25.0 g), 1 tablet of Vitamin B12 and cholesterol (200mg/kg/day). Twelve grams of diet of above composition was supplied to each animal every day (Verma et al., 2012)

7.2.3. Antihyperlipidaemic activity in high-fat diet-induced hyperlipidaemic rats

The Albino Wister rats, with prior approval from the institutional animal ethical committee, weighing between 120 - 150 g were purchased from the Indian Institute of Chemical Biology. Animals were housed in polypropylene cages at a temperature of 25 ± 2˚C with a 12 h dark and light cycle and fed a standard pellet diet and water ad libitum. All rats were fed normal pellet diet for five days to acclimatize the laboratory conditions. The CPCSEA guidelines were followed throughout the experiment. Atorvastatin standard was bought from Sigma Aldrich for comparison. TC, HDL and LDL and VLDL were calculated by the help of commercial kits available in lab. Animals were divided into groups with n = 4/group: group I was the control group fed normal pellet diet for 30 experimental days. Group II received a high fat diet (HFN) for 25 experimental days. The composition of high fat diet is shown in Table I. Group III was a test extract group and rats in this group were fed a high fat diet for 25 days and fed along with the dose of *Lagenaria siceraria* extract. Group IV was fed a high fat diet for 30 days and received once daily administration of atorvastatin, 10 mg/kg p.o. with the high fat diet. Doses were administered in the morning using a Ryle's intragastric tube. Rats in all groups were fasted overnight before collection of blood. Each rat was anesthetized with chloroform. From the retro-orbital plexus, blood was withdrawn by capillary puncture and then centrifuged. The separated serum was analysed for lipid profiles using the diagnostic kits. Statistical analysis All results expressed as the means ± S.D. Statistical significance between groups were determined by a *t test* using graph pad prism 5.0 software where $p < 0.05$ was considered significant.

7.2.4. Estimation of serum lipid profile

Blood lipid profile (TC, HDL cholesterol, LDL cholesterol and TG) was analysed initially on day 0, and finally after 15 and 30 days of normal diet and drug treatment in both control and experimental groups. Serum TC, TG and HDL cholesterol were estimated using commercially available kits VLDL cholesterol was calculated by formula as follows:

LDL (mg/dl) = TC − (HDL + VLDL)

7.2.5. Evaluation of weight gain

During the experimental period, the food consumed and weight gained by rats was recorded on 0th, 15th and 30th day of LSFE treatment.

7.2.6. Estimation of biochemical parameters

The biochemical parameters were determined on 0th, 15th and 30th day of LSFE treatment in hyperlipidaemic rats. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT) were estimated according to the method described by Reitman and Frankel (1957); serum alkaline phosphatase (AP) was estimated according to the method described by Kind and King (1954) using commercial kit; urea was estimated calorimetrically using commercial kit.

7.2.7. Statistical analysis

Experimental values are means ± S.D. of the number of experiments indicated in the legends. Data were evaluated for statistical significance with unpaired two-tailed student *t-test.* A P value of 0.05 or less was considered as statistically significant. The analyses were performed with the Graph-Pad prism 5.0 software (Graphpad Software Inc.).

7.3. Results

7.3.1. Effect of LSFE on serum lipid profile

The rats when fed high-fat diet showed marked hyperlipidaemia. For the whole group, there was a significant ($P < 0.001$) increase in TC (180.4 ± 27.76 to 295.45 ± 19.88mg/dl), LDL cholesterol (71.27 ± 6.03 to 181.43 ± 6.91 mg/dl), VLDL cholesterol (92.7 ± 26.91to 98.31 ± 12.27 mg/dl) and TG (216.75 ± 13.93 to 260.8 ± 8.73 mg/dl). These rats were then divided into a control group and experimental groups each containing 4 rats. Changes in various lipid values of both control and experimental groups are shown in Table9. 1. By the replacement of high-fat diet with normal diet and by continuation of treatment up to 30th day, the lipid levels were significantly reduced. At the 30th day, most significant ($P < 0.001$) reduction in lipid levels was found in the LSFE treated (200 mg/kg; p.o.) groups as compared to the rats fed with high-fat diet, at the 0th day were: TC 246.075 ±17.28 mg/dl vs. 208.2 ± 10.64 mg/dl, LDL cholesterol 197.67 ± 6.30 mg/dl vs. 139.57 ± 6.82mg/dl, TG 225.1 ± 6.05 mg/dl vs. 184.8 ± 8.59 mg/dl, VLDL cholesterol 128.18 ± 16.38 mg/dl vs. 68.47 ± 7.51 mg/dl ($P < 0.001$). Atorvastatin (10mg/kg; p.o.) markedly exerted the most significant ($P < 0.0001$) effects as: TC 238.7 ± 26.29 mg/dl vs. 136.32 ± 17.85 mg/dl, LDL cholesterol 111.05 ± 7.14mg/dl vs. 75.97 ± 6.24 mg/dl, TG 231.8 ± 13.42 mg/dl vs. 134.4 ± 7.84 mg/dl, VLDL cholesterol 127.608 ± 25.52 vs. 60.31 ± 12.49 (Table 7. 1.).

7.3.2. Effect of LSFE on weight gain and bile acids

The rats when fed high-fat diet showed marked increase in gain in weight from 10.25 ± 0.88 to 38.50±2.88 g. At the 30th day, most significant ($P < 0.001$) reduction in weight gain was evidenced in the LSFE-treated (200mg/kg; p.o.) groups as compared to the rats fed with high-fat diet at the 0th day. The weight gain effect evidenced in Atorvastatin (10mg/kg; p.o.) treated rats was: 22.72±2.50 g.

Table 7.1. Effect of Indian Hybrid Aqueous Extract on Serum Lipid Profile in Hyperlipidaemia – Induced Rats. TC – serum cholesterol; TG – triglycerides; LDL – low density protein; VLDL – very low density protein; HFN – high fat normal; HFE – high fat extract; ATV – atorvastatin. Values are expressed in milligrams per 100 ml as means ± SD ($p < 0.05$, significant; $p < 0.001$, highly significant) number of observations are 4. (Values are statistically significant at a = $P < 0.0001$ and b = $P < 0.001$, c = $P < 0.01$, d = $P < 0.05$, ns = not significant)

Lipid Profile	**control**	**Treatment Group (mg/kg;p.o.)**	**Serum Lipid Level (mg/dl) on Day(S)**		
			0th Day	**15th Day**	**30th Day**
TC	180.4 ± 27.76	HFN (0)	229.02 ± 18.97^{c}	272.4 ± 16.12^{b}	295.45 ± 19.88^{c}
		HFE (200)	246.075 ±17.28^{a}	232.7 ± 12.36^{d}	208.2 ± 10.64^{c}
		ATV (10)	238.7 ± 26.29^{a}	164.3 ± 7.29^{a}	136.32 ± 17.85^{a}
TG	216.75 ± 13.93	HFN (0)	266.5 ± 6.80^{b}	248 ± 10.96^{c}	260.8 ± 8.73^{ns}
		HFE (200)	225.1 ± 6.05^{a}	208 ± 3.45^{c}	184.8 ± 8.59^{c}
		ATV (10)	231.8 ± 13.42^{a}	186.4 ± 5.11^{a}	134.4 ± 7.84^{a}
HDL	16.425 ±2.34	HFN (0)	15.82 ± 2.42^{ns}	17.92 ± 2.23^{ns}	15.7 ± 1.05^{ns}
		HFE (200)	0.22 ± 0.029^{ns}	0.18 ± 0.009^{ns}	0.15 ± 0.02^{ns}
		ATV (10)	0.04 ± 0.012^{ns}	0.035 ± 0.01^{ns}	0.03 ± 0.02^{ns}
LDL	71.27 ± 6.03	HFN (0)	196.375 ± 10.41^{a}	173.39 ± 7.56^{c}	181.43 ± 6.91^{d}
		HFE (200)	197.67 ± 6.30^{a}	160.80 ± 9.59^{b}	139.57 ± 6.82^{c}
		ATV (10)	191.05^{a} ± 7.14	132.62 ± 5.79^{a}	75.97 ± 6.24^{a}
VLDL	92.7 ± 26.91	HFN (0)	96.82 ± 27.09^{ns}	91.08 ± 9.99^{ns}	98.31 ± 12.27^{ns}
		HFE (200)	128.18 ± 16.38^{b}	81.71 ± 7.07^{b}	68.47 ± 7.51^{c}
		ATV (10)	127.608 ± 25.52^{b}	71.64 ± 3.87^{a}	60.31 ± 12.49^{c}

7.3.3. Effect of LSFE on biochemical parameters

The LSFE (200mg/kg/day) as well as Atorvastatin (10mg/kg) administered orally at the doses did not alter significantly ($P > 0.05$) the levels of blood glucose and alkaline phosphatase (results not shown). However, after high fat diet feeding, SGOT levels (units/ml) increased substantially from 46.75 ± 12.28 to 54.25 ± 12.42 (units/ml) ($P < 0.001$). Interestingly, these levels were decreased from 40.25 ± 5.73 to 30.25 ± 5.31units/ml in the LSFE treated groups ($P < 0.001$).

Similarly, the levels of SGPT have been substantially increased from 39.21 ± 4.22to 51.27 ± 3.59 units/ml and significantly ($P < 0.001$) decreased to 38.42±3.26 units/ml by the administration of LSFE in the experimental groups. Atorvastatin also showed less significant ($P < 0.05$) reduction of SGOT levels from 34.25 ±4.64 to 14.75 ± 6.89 and significant reduction of SGPT levels ($P < 0.001$) from 56.35 ± 6.37 to 47.1 ± 5.56 (Table 7. 2.).

Table 7.2. Effect of Indian Hybrid aqueous extract on weight gain and biochemical parameters in hyperlipidemia – Induced Rats. TC – serum cholesterol; TG – triglycerides; LDL – low density protein; VLDL – very low density protein; HFN – high fat normal; HFE – high fat extract; ATV – atorvastatin. Values are expressed in milligrams per 100 ml as means ± SD (n = 4). (Values are statistically significant at a = $P < 0.0001$ and b = $P < 0.001$, c = $P < 0.01$, d = $P < 0.05$, ns = not significant)

Parameter(s)	**control**	**Treatment Group (mg/kg;p.o.)**	**0th Day**	**15th Day**	**30th Day**
Weight gain (g)	139.25 ± 10.9	HFN (0)	135.75 ± 4.34^{ns}	155.75 ± 8.30^{c}	177.75 ± 6.07^{b}
		HFE (200)	146.25 ± 4.78^{ns}	167.0 ± 2.44^{d}	156.25 ± 11.08^{ns}
		ATV (10)	161 ± 8.406^{d}	159.5 ± 10.21^{ns}	139.25 ± 11.87^{c}
SGOT (units/ml)	42.5 ± 10.34	HFN (0)	46.75 ± 12.28^{ns}	49.25 ± 10.34^{ns}	54.25 ± 12.42^{d}
		HFE (200)	40.25 ± 5.73^{ns}	36 ±4.69^{ns}	30.25 ± 5.31^{d}
		ATV (10)	34.25 ±4.64^{d}	29.5 ± 6.24^{ns}	24.75 ± 6.89^{d}
SGPT (units/ml)	40.75 ± 8.38	HFN (0)	39.21 ± 4.22^{ns}	45.6 ± 3.42^{d}	51.27 ± 3.59^{d}
		HFE (200)	44.4 ± 6.20^{ns}	41.05 ± 4.85^{ns}	36.2 ± 5.23^{d}
		ATV (10)	56.35 ± 6.37^{c}	52.2 ± 6.73^{ns}	47.1 ± 5.56^{d}
Urea (mg/dl)	31.5 ± 3.69	HFN (0)	39.45 ± 4.18^{ns}	40.96 ± 2.98^{ns}	39.75 ± 3.3^{ns}
		HFE (200)	28.65 ± 4.37^{ns}	25.62 ± 2.22^{ns}	21.65 ± 2.92^{ns}
		ATV (10)	33.75 ± 4.57^{ns}	23.25 ± 3.3^{d}	17.5 ± 3.41^{c}

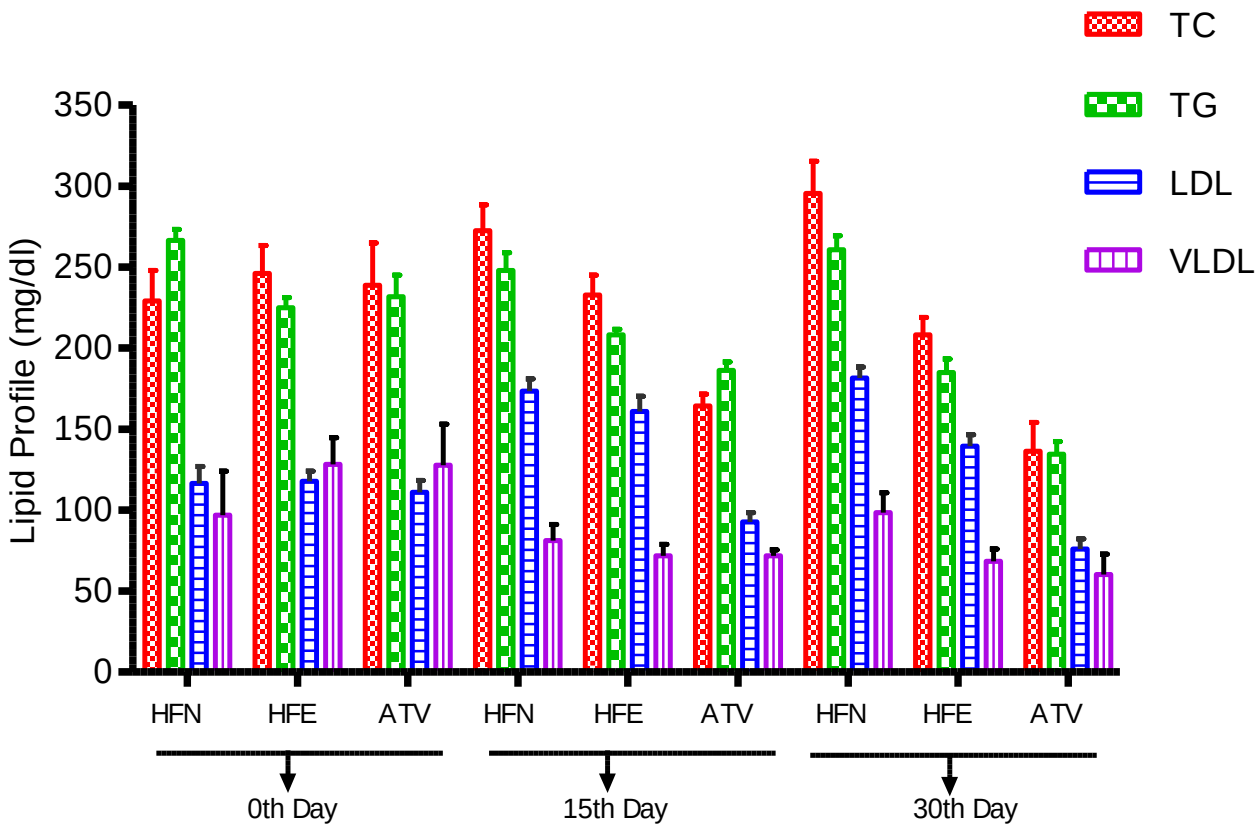


Figure 7.1. Effects of *Lagenaria siceraria* Indian hybrid fresh fruit's aqueous extract on lipid profile of normocholesterolemic rats. TC – serum cholesterol; TG – triglycerides; LDL – low density protein; VLDL – very low density protein; HFN – high fat normal; HFE – high fat extract; ATV – atorvastatin. Values are expressed in milligrams per 100 ml as means ± SD ($p < 0.05$, significant; $p < 0.001$, highly significant) number of observations are 4.

7.4. Conclusion

Effects of *Lagenaria siceraria* Indian hybrid fresh fruit's aqueous extract on lipid profile of normocholesterolemic rats was observed for 30 days. The results of the study reveal that the LSFE (200mg/kg/day; p.o.) when administered initially to the hyperlipidemic rats causes a sharper and more significant decrease in the serum TC and LDL cholesterol and TG level. The rats receiving high fat diet along with the extract group showed a decrease in lipid profile, weight gain profile and other biochemical parameters. These all showed the effectiveness of extract in respect to lipid lowering potential. For further comparison a well-known marketed drug atorvastatin was used showing a hypoglycemic effect and compared to the effects showed by extract. The exact mechanism by which

the LSFE reduced the serum cholesterol is not clear yet, further study is required. Several studies reveal that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases (Harrison et al., 2003). Most of the antihyperlipidemic drugs are causing significant reduction in both TC and HDL cholesterol levels (Wilson, 1990). In the present study, significant decrease of cholesterol in the LSFE treated groups is manifested in all the lipoprotein fractions. High levels of TC and most importantly, LDL cholesterol are the predictors of atherosclerosis (Temme et al., 2002). LSFE significantly ($P < 0.01$) reduced both the TC and LDL cholesterol. Recent studies also show that triglycerides are directly or indirectly related to coronary heart diseases (Bainton et al., 1992). In the present study, LSFE markedly decreased the triglycerides level. This is an important advantage in treatment of British men where triglyceride and LDL cholesterol are the predictors of ischemic heart diseases (Bainton et al., 1992). Additionally, the biochemical parameters studied did not show any of the adverse effect of LSFE on experimental animals. Liver enzymes such as SGOT, SGPT and AP are considered to be biochemical markers for assessing liver function. Hepatotoxicity is evidenced by an elevation of the serum marker enzymes. LSFE treatment reduced these liver enzyme levels significantly ($P < 0.001$) in experimental animals showing that LSFE have hepatoprotective action. During the experimentation, albino rats did not show any mortality or any other adverse effects when the rats fed orally with LSFE at the dose of 200mg/kg/day. It is indicating that the LSFE have a good margin of safety. The results concluded that the LS fruits have a definite antihyperlipidemic and hence cardioprotective and antiatherosclerotic potential, there is also a valid scientific basis for consuming it in the treatment of coronary artery diseases in India. Hence, the present study helps to support the traditionally claimed cardioprotective and cardiotonic activity of LS fruits.

Chapter – 8

Discussion and conclusion

8.1. Discussion

8.2. Conclusion

8.1. Discussion

Lagenaria siceraria is traditionally used for the treatment of different diseases such as fever, pain, ulcer, cough, and asthma. A number of active chemical compounds like oleanolic acid, β- sitosterol, campesterol, kaempferol and isoquercitrin isolated from the plant were proved to be potent antioxidant and are useful to overcome various therapeutic complications arising from reactive oxygen species. The pharmacological actions attributed to *Lagenaria siceraria* in Ayurvedic texts have been validated by scientific researches, and the result indicate potent antioxidant activity, diuretic activity, antihyperglycemic activity, anticancer activity, analgesic activity and antidepressant activity exhibited from major component of the plant. Further evaluation on active principles and therapeutic efficacy needs to be carried out on *Lagenaria siceraria* in order to explore the hidden bioactive compound and their clinical application to a new extent.

The five cultivars of *Lagenaria siceraria* i.e. Round Gourd, Long Sausage Gourd, Indian Hybrid, Green Bell Hybrid, Kettle Gourd, were standardized through RP-HPLC and HPTLC method with the respective marker i.e cucurbitacin E. The chromatographic profiling of the plant cultivars was also performed in this regards, leading to give a data about the percentage of the marker compound present in every cultivars and showing its potency. The estimation of cucurbitacin E through this method is accurate, precise and specific with good reproducibility. This method is quite acceptable for commercial use. The data obtained are very useful in differentiating between the cultivars of *Lagenaria siceraria,* having different chemical composition of phytoconstituents composition differentiating them from each other. On the basis of HPLC, HPTLC, it can be concluded that the lyophilized extract of Indian hybrid variety shows the maximum marker content thus can be considered as most potent variety. This also draws our attention towards the safety of the plant extract as the cucurbitacins are highly potent compounds and a small hike in concentration can be toxic. So the cultivar with high cucurbitacin E content must be identified and the toxic level must be estimated. This was done by *in vitro - in vivo* experimentation. Many curative effects of herbal formulations used in the treatment and cure are due to presence of very minute quantities of trace elements. These elements are iron (Fe), copper (Cu), cobalt, nickel (Ni), zinc (Zn), magnesium, manganese (Mn), molybdenum, chromium (Cr), vanadium, lithium, selenium, fluorine (F) and iodine (I) (Shirin et al., 2010) Other heavy metals normally present in plants like lead (Pb), cadmium (Cd) and mercury (Hg) are toxic at very lower concentration. These heavy metals concentration above the normal limits prescribed by government is dangerous. Cu had been proved to be a cause of liver damage. Zn may produces adverse nutrient interactions with Cu. Zn retards the immune function and reduces level of high-density lipoproteins in body. Hg causes neurological disorders and has toxic effect on the kidney. Pb is commonly known to cause renal tumours, reduction in cognitive development, and increase in blood pressure and cardiovascular disease in adults. Cd results in kidney

dysfunction, osteomalacia and reproductive deficiencies. Thus The heavy metal and other trace element analysis of all the selected plant cultivars was also done with the help of atomic absorption spectrometry (AAS) which denoted that heavy metals and other trace element concentration were within the WHO guidelines limit.

The lipoprotein lipase enzyme inhibition assay revealed that the individual plant cultivars and formulations showed very less interaction except Indian hybrid aqueous extract which indicated relatively similar result as that of standard orlistat. The IC_{50} value suggested that the Indian hybrid lyophilized extract, round gourd lyophilised extract, and long sausage guard methanolic extracts are showing good potency as compared to that of orlistat. The marker compound cucurbitacin E showed a relatively less activity as compared to the extracts revelling the idea of presence of other phytoconstituents or synergism in extract resulting in better antihyperlipidemic effects. The dose response profiling was also done to show the therapeutic effects of the varieties of plant with respect to positive control for all the extracts as well as marker compound.in this further, *in -vivo* study was done on rat model in which Effects of *Lagenaria siceraria* Indian hybrid fresh fruit's aqueous extract on lipid profile of normocholesterolemic rats was observed for 30 days. LSFE (200mg/kg/day; p.o.) when administered initially to the hyperlipidaemic rats' causes a sharper and more significant decrease in the serum TC and LDL cholesterol and TG level. Studies revealed that decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases (Harrison et al., 2003). Most antihyperlipidaemic drugs are causes significant reduction in both HDL and TC cholesterol levels (Wilson, 1990). High levels of TC and most importantly, LDL cholesterol are the predictors of atherosclerosis (Temme et al., 2002). Recent studies also show that triglycerides are directly or indirectly related to coronary heart diseases (Bainton et al., 1992). The rats receiving high fat diet along with the extract group showed a decrease in lipid profile, weight gain profile and other biochemical parameters. These all showed the effectiveness of extract in respect to lipid lowering potential. For further comparison a well-known marketed drug atorvastatin was used. The extract showed hypoglycemic effect almost similar to the effects showed by atorvastatin. The results concluded that the plant cultivar have an antihyperlipidaemic and thus cardioprotective and antiatherosclerotic action. Hence, the present study supports the traditional claims of *Lagenaria siceraria* to be cardioprotective and cardiotonic.

There are many lifestyle related disorders emerging. In such conditions if we include *Lagenaria siceraria* in our daily routine diet in form of vegetables or any marketed neutraceuticals, will prove to be a prophylactic measure to prevent from various ailments. The above study explained its anti-obesity as well as antihyperlipidemic activity, and can be an alternative to synthetic drug with more safety margins and less toxicity. The study provides new insights in the quality and safety evaluation of the natural medicinal plant *Lagenaria siceraria* and its cultivars. *In vitro – in vivo* correlation is an effective approach to establish

Lagenaria siceraria as potential antihyperlipidemic and anti-obesity agent from natural products. Further study is needed (bioactivity guided isolation and identification) to identify major phytoconstitiuents responsible for the enzyme inhibition. The mechanistic study of enzyme interactions could be developed and this will enrich scientific validation of natural medicinal plants having strong root in the traditional medicinal culture of our country.

8.2. Conclusion

This explanatory study on different cultivars of *Lagenaria siceraria* e. g. round gourd, Indian Hybrid, green bell hybrid, kettle gourd, long sausage gourd, showed that all the cultivars contains cucurbitacin E. whereas the content of cucurbitacin E was more in Indian Hybrid variety. The lipoprotein lipase inhibition property of aqueous extract of Indian Hybrid was found to be better than any other cultivars. While other verities showed the activity in order of Round Gourd > Green Bell Hybrid > Long Sausage Gourd > Kettle Gourd, among aqueous extracts and Long Sausage Gourd > Kettle Gourd > Round Gourd > Green Bell Hybrid in case of methanolic extracts. In *in –vivo* antihyperlipidemic rat model study the Indian Hybrid extract showed significant lipid lowering activity. The activity of extract was found to be very significant as compared to a marketed positive standard orlistat. Thus this study may help in future for development of any drug or functional food against hyperlipidemia and obesity**.**

Chapter – 9

Summary

9. Summary

Quality evaluation of herbal and traditional medicine is becoming very much important in modern days as usage of medicinal plants gained a huge popularity worldwide. With that standardization is necessary to ensure the safety and efficacy through quality control measures. Unfortunately, herbals are not free from synergistic as well as antagonistic effects and risk associated which is very much uncertain because of the complexity of the natural products and thus becomes one of the major concern. Chemo-profiling and standardization of herbals are done through potential, cost-effective, simple and highly selective tools that can ensure both quality and batch-to-batch reproducibility of the products. *Lagenaria siceraria*, a food plant, almost 25 cultivars are present of different shapes and sizes throughout the world without a scientific differentiation having different compositions of metabolites, shows enormous potential as a medicine in our traditional, ethnobotanical and Ayurveda, but proper documentation and validation is very much necessary to not only assure the quality, authenticity and efficacy but also to come up with good safety profiling to evaluate the risk associated with beyond pharmacological and toxicological effects comparatively among these cultivars. In this thesis work five cultivars of plant *Lagenaria siceraria* were selected and studied individually and then evaluated comparatively. Evaluation of the effects of these plant cultivars through marker profiling by HPTLC, HPLC methods as well as lipoprotein lipase enzyme interaction potential study was performed. The results obtained were useful for an initial assessment of selected plant cultivars for selection of most therapeutically active and to draw comparative relationship between them. Apart from that another approach of this thesis was to perform *in – vivo* antihyperlipidemic study in order to evaluate the therapeutic efficacy and draw *in vitro - in vivo* correlation.

Chapter 1 deals with brief introductory note based on literature reports on all aspects of plant as well as five cultivars of plant *Lagenaria siceraria* covered in this thesis. In this chapter the importance of Lagenaria as a form of natural medicine was discussed. *Lagenaria siceraria* (molina) standley (family: cucurbitaceae) is commonly known as Bottle gourd, an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans *Lagenaria siceraria* is official in Ayurvedic pharmacopoeia of India. *L. Siceraria* fruits are traditionally used as a nutritive agent having cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons and scorpion stings, alternative purgative, and cooling effects. It cures pain, ulcers, and fever and is used for pectoral cough, asthma and other bronchial disorders. *L. Siceraria* leaf contains hypolipidemic properties that can be used to decrease the cholesterol level and thus helpful in case of all elevated steroid level related diseases. Chapter 2 describes hyperlipidemia and its evaluation.

Chapter 3 described the aims and scope of the work and the brief plan of the work.in

In Chapter 4 the collection, different extraction and qualitative estimation of the selected plant cultivars is mentioned. The aqueous and methanolic extraction method gave

adequate amount of extracts e.g. Round Gourd(3.83, 2.7844), Long Sausage Gourd(7.249, 1.7543), Indian Hybrid(4.201, 1.943), Green Bell Hybrid(4.08, 2.372), Kettle Gourd(5.881, 1.1522). Moreover the phytochemical study showed the presence of different phytoconstituent like alkaloids, glycosides, phytosterols, flavoniods, tannins and saponins in the selected plants.

In chapter 5 the standardization of plant cultivars were made with respective markers through HPTLC HPLC and AAS method. The HPTLC method, showed the presence of cucurbitacin E and was found to be: Round gourd (0.13, 0.041), Kettle gourd (0.10, 0.032), Long sausage gourd (0.18, 0.063), Green bell hybrid (0.23, 0.080), Indian hybrid (0.38, 0.094) percentage (%) w/w, and With a R_f value 0.44, respectively. This method standardized the extracts with their markers and thus ensures quality and authenticity of the plant extracts. Another highly accurate and precise chromatographic method RP-HPLC was developed to ensure the standardization of plant cultivars and along with this the chromatographic profiling were also discussed. The plant cultivars were standardized with their respective marker i.e. cucurbitacin E and the concentration of these molecules were found to be Round gourd (2.05, 0.68), Kettle gourd (2.31, 0.84), Long sausage gourd (2.84, 0.96), Green bell hybrid (3.23, 1.62), Indian hybrid (5.23, 1.92) percentage (%) w/w respectively in aqueous and methanolic extracts. The chromatographic profiling of extracts of the plant cultivars gave rise to many major picks For Round Gourd, Long Sausage Gourd, Indian Hybrid, Green Bell Hybrid, Kettle Gourd in a certain mobile phase condition and wavelength. The heavy metal and mineral concentration of the different cultivars of plant *Lagenaria siceraria,* through Atomic absorption spectrometry study was also done. The quantitative determination were carried out using standard calibration curve obtained by standard solution of metals having detectable concentration ranges. The concentration of metals obtained in plant extracts were expressed in parts per million. The results of this study depicted that the plant materials were not contain two prominent toxic metals i.e. lead (Pb) and cadmium (Cd) and the other two toxic elements mercury (Hg) and arsenic (As) were well in limit as fixed by WHO. Although the minerals like copper (Cu) and iron (Fe) were present in sufficient quantity in those plants. However further study in this regards is recommended.

The lipoprotein lIpase enzyme interaction profiling of the selected plant extracts of the five cultivars has been described in Chapter 6. The inhibition assay of all showed very less to moderate potency with respect to the positive markers i.e. Orlistat except the round gourd and Indian hybrid aqueous extracts. Both varieties are showing IC_{50} values (Round Gourd - 188.028 ± 10.53 µg/ml; Indian Hybrid - 153.9529 ± 7.997 µg/ml) near to that of positive inhibitor (103.6431 ± 4.835 µg/ml) taken. The dose dependent interaction curve also revealed less difference between the interactions for Indian hybrid as compared to that shown by orlistat. This renders the chances for *Lagenaria siceraria* Indian hybrid variety to be a natural analogue of synthetic lipoprotein lipase inhibitors with more safety margin. The cucurbitacin E showed interaction potential very less to moderate with

respect to the positive inhibitor that may reveal the presence of some another phytoconstituents or some synergism responsible for lipoprotein lipase inhibition of plant extract.

In Chapter 7 the *in -vivo* antihyperlipidemic experiment was performed on rat model for the most potent extract i. e. Indian hybrid aqueous extract, to devise an *in –vitro - in -vivo* correlation is described. In chapter 8, the effects of *Lagenaria siceraria* Indian hybrid fresh fruit's aqueous extract on lipid profile of normocholesterolemic rats was observed for 30 days. The results of the study reveal that the LSFE (200mg/kg/day; p.o.) when administered initially to the hyperlipidemic rats causes a sharper and more significant decrease in the serum TC and LDL cholesterol and TG level. The rats receiving high fat diet along with the extract group showed a decrease in lipid profile, weight gain profile and other biochemical parameters. These all showed the effectiveness of extract in respect to lipid lowering potential. For further comparison a well-known marketed drug atorvastatin was used showing a hypoglycemic effect and compared to the effects showed by extract. The exact mechanism by which the LSFE reduced the serum cholesterol is not clear yet, further study is required.

Chapter – 10

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