Comparative Analysis of Metabolites in Contrasting Chickpea Cultivars

Project Report Submitted to Central University of Punjab

For the award of degree of

M.Sc. in Life Sciences with Specialization in Plant Sciences

In

Department of Plant Sciences

By

Alokesh Ghosh

16mslsps07

Supervisor: Dr. Vinay Kumar

Department of Plant Sciences

School of Basic and Applied Sciences

Central University of Punjab, Bathinda

May, 2018

DECLARATION

I declare that the project entitled "COMPARATIVE ANALYSIS OF METABOLITES IN CONTRASTING CHICKPEA CULTIVARS" has been prepared by me under the guidance of Dr. Vinay Kumar, Assistant Professor, Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously. All the ideas and references have been duly acknowledged.

Alokesh Ghosh
Reg. No.- 16mslsps07
Department of Plant Sciences
Central University of Punjab
Bathinda- 151001
Date- May, 2018

CERTIFICATE

This is to certify that the project report entitled "COMPARATIVE ANALYSIS OF METABOLITES IN CONTRASTING CHICKPEA CULTIVARS", submitted to the faculty of Department of Plant Sciences, Central University of Punjab, Bathinda for the degree of M.Sc., was carried out by Alokesh Ghosh at the Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda under my guidance and no part of this report has been submitted for any other degree or to any other university/ institution.

Dr. Vinay Kumar
Department of Plant Sciences
Central University of Punjab
Bathinda- 151001
Date- May, 2018

ABSTRACT

Title: COMPARATIVE ANALYSIS OF METABOLITES IN CONTRASTING CHICKPEA CULTIVARS.

Name of student: Alokesh Ghosh

Registration number: 16mslsps07

Degree for which submitted: Masters of Science

Name of supervisor: Dr. Vinay Kumar

Name of Department: Department of Plant Sciences

Name of school: School of Basic and Applied Sciences

Key words: Chickpea, seed development, metabolites, phenolics, anthocyanin.

Phenolics are the major class of plant secondary metabolites. Among half of the plant phenolics are the flavonoids. The production of these metabolites induces during stresses and thus helps the plant to cope with the environment. Anthocyanins, a class of flavonoids protects plant from harmful UV rays, attracts the pollinators and seed dispersal, helps in mimicry and also helps in the root nodulation process in legumes. Chickpea is a leguminous and major pulse crop plant, shown in the winter season faces lots of stress. Anthocyanins and other phenolics help the plant to tolerate such stresses. Desi cultivars have more phenolics and anthocyanin as compared to the kabuli one. Flowers have higher concentration of anthocyanin than in leaves. Untargeted metabolomics profiling of the dry powdered mature seed samples of contrasting chickpea cultivars detects primary and secondary metabolites such as fatty acids, phenolics, phelols, terpenes, esters,sugar,etc in the seeds of chickpea.

Alokesh Ghosh **Dr. Vinay Kumar**

(Student) **(Supervisor)**

DEDICATION

This project work is dedicated to my beloved parents, maa and papa and to my loving family.

ACKNOWLEDGEMENTS

It gives me immense pleasure to express my gratitude and deepest appreciation to all those, who has helped me in all fair possible means to complete this research work. With the blessing of the almighty, firstly I would like to express my sincere and utmost gratitude to my esteemed supervisor **Dr. Vinay Kumar** for his scholarly guidance, motivation, support and providing me the opportunity to complete my M.Sc. dissertation work. I would like to thank him from the deepest core for encouraging me and teaching me the right way in solving problems during my research duration. He was always ready for his advice whenever needed and thus making this research work more fruitful.

Taking this opportunity, I would like to thank **Prof. Ashok Dhawan**, who has always been an ideal and inspiration in my whole course duration. My deepest gratitude to **Hon'ble Chancellor, Dr. Sardara Singh Johl, Hon'ble Vice-Chancellor, Prof. R.K. Kohli, Prof. P. Ramarao** (Dean of Academic Affairs) and my teachers **Dr. Sanjeev Thakur**, (HoD of Department of Plant Sciences), **Dr. Krishna Choudhury, Dr. Felix Bast, Dr. Pankaj Bharadwaj** and **Dr. M.S. Dhanya** and to all other esteemed members of the Central University of Punjab for providing me all research facilities to carry out this research work and for their teachings that came handy to draft this dissertation report.I would like to thank my senior PhD scholars, **Arindam Adhikary**, **Sumandeep Juneza**, **Rashmi Saini, Samreet** and **Renu Yadav** for their help and guidance. I am also thankful to my fellow lab mates, **Ajay**, **Upasana** and **Reetu** for their help and support in making this research work successful. I am also thankfull to my dear friends, **Souvik**, **Partha**, **Desoshree** and **Rakesh** and my lovely juniors, **Aparajita**, **Uddalok**, **Anisha** and **Harpreet** for their support and helping hand whenever needed.

My sincere gratitude goes to my **Maa**, **Papa** and **Buda** for their unconditional love and blessings throughout my life. I am thankful to all my **Pisi**, my **cousin brothers and sisters** and my dear **Baptu, Bumba** and **Baba** for their love, wishes and support. Last but not the least I would like to thank all those who have helped me directly or indirectly to complete my research work successfully.

Alokesh Ghosh

TABLE OF CONTENTS

Sl. No	Contents	Page No.
1	List of tables	viii
2	List of figures	ix-x
3	List of appendices	xi
4	List of abbreviations	xii
5	Chapter-1: Introduction	1-3
6	Chapter-2: Review of literature	4-11
7	Chapter-3: Materials and methods	12-16
8	Chapter-4:Results and discussions	17-34
9	Conclusions and future perspective	35-36
10	References	37-44
11	Appendices	45-52

LIST OF TABLES

Table No.	Table Description	Page No.
2.1	Classification of Plant Phenolic Compounds based on carbon skeleton	10
4.1	Developmental changes in pod morphology during maturation stages in chickpea cultivars	21
	Developmental changes in seed coat morphology during maturation stages in chickpea cultivars	22
4.3	Developmental changes in cotyledon morphology during maturation stages in chickpea cultivars	22
4.4	Total harvested seeds with their developmental stages	23
4.5	Total phenolics content of the three chickpea cultivars	25
4.6	Metabolites detected in the chloroform extract of himchana	26
4.7	Metabolites detected in the chloroform extract of ICC4958	27
4.8	Metabolites detected in the chloroform extract of JGK-03	28
4.9	Metabolites detected in the methanol extract of himchana	29
4.10	Metabolites detected in the methanolextract of ICC4958	30
4.11	Metabolites detected in the methanol extract of JGK-03	31
4.12	Metabolites detected in the chloroform extract of all the three cultivars: Himchana, ICC4058 and JGK-03. ‘P’ denotes presence and ‘A’ denotes absence of metabolites	32
4.13	Metabolites detected in the methanol extract of all the three cultivars: Himchana, ICC4058 and JGK-03. ‘P’ denotes presence and ‘A’ denotes absence of metabolites	33
4.14	Common metabolites present in all cultivars in both the extracts	33

LIST OF FIGURES

Figure No.	Figure Description	Page No.
1.1	Three cultivars of Chickpea grown in the field of university campus	3
2.1	Flowers of Chickpea cultivars. (A) & (B) are the desi type and (C) kabuli type.	7
2.2	: Pods of the three cultivars of Chickpea	7
2.3	The development of seed and its various maturation stages in soybean (Adapted from Goldberg et al., 2007)	8
2.4	Biosynthesis pathway of plant secondary metabolites. Designed using ChemDraw13 (Addapted from Taiz and Zeiger. Plant Physiology, 1991).	9
4.1	Comparative anthocyanin content in selected leaf sample of the contrasting Chickpea cultivars. Red colour shows the concentration of himchana. Blue shows the concentration of ICC4958 and green colour shows that of JGK-03. L3, L5 and L7 were the 3rd, 5th and seventh leaf respectively. Error bars represents arithmetic mean ± standard deviation calculated from triplicates.	18
4.2	Leaf sample used for anthocyanin content estimation	19
4.3	Average anthocyanin contents in the leaf samples of the three cultivars of chickpea	19
4.4	The floral organs used for anthocyanin content estimation. (A) Flower and pedicle of himchana. (B) Flower and pedicle of ICC4958 and (C) Flower and pedicle of JGK-03	20
4.5	Anthocyanin contents in flower and pedicle of the Chickpea cultivars grown in the university campus field. Blue colour indicates the flower tissue and the red colour indicate the pedicle tissue. Error bars represents arithmetic mean ± standard deviation calculated from triplicates	20

4.6	Harvested dry seeds of chickpea cultivars: (A) Himchana, (B) ICC4958, and (C) JGK-03	23
4.7	Total phenolics content in Chickpea lines. Blue colour denotes TPC in the 50% Acetone extract and the red colour denotes TPC in 70% methanol extract. Error bars represents arithmetic mean ± standard deviation calculated from triplicates	24

LIST OF APPENDICES

Sl. No.	Description	Page No.
1	Standard curve of Gallic acid	46
2	Chromatogram of Chloroform Extract of Himchana	47
3	Chromatogram of Chloroform Extract of ICC4958	48
4	Chromatogram of Chloroform Extract of JGK-03	49
5	Chromatogram of Chloroform Extract of Himchana	50
6	Chromatogram of Chloroform Extract of ICC4958	51
7	Chromatogram of Chloroform Extract of JGK-03	52

LIST OF ABBREVIATIONS

Sl. No.	Full Form	Abbreviation
1	Ultra-violet	UV
2	Gas chromatography Mass spectroscopy	GC-MS
3	Liquid chromatography Mass spectroscopy	LC-MS
4	Nuclear Magnetic Resonance	NMR
5	Millimeter	mm
6	Milligram	mg
7	Nanometer	nm
8	Microgram	µg
9	Microlitter	µl
10	Gram	gm
11	Early Maturation	EM
12	Mid Maturation	MM
13	Late Maturation	LM

CHAPTER 1

INTRODUCTION

Chickpea is considered to be the third largest cultivated leguminous crop in the world. The growth and production of the chickpea has been greatly affected by several biotic and abiotic stresses. As grown mostly in the dry and winter season, chilling and drought stress are more common that affects chickpea production. To cope up with these stresses, plant produces plenty of metabolites that play major role in the cell integrity, growth, storage of energy, cell signalling, formation of membrane and scaffolding, replenishment of cell and many other functions (Wen et al. 2015 and Khan et al., 2018). Plants also develop certain mechanism to modify their biochemistry and physiology to cope up with several stress factors through several metabolic changes (Khan et al., 2017, Kim et al., 2011, Khan at al., 2018 & Wen at al., 2015). The major morphological and biochemical change can be seen during development of pod, seed, seed coat and cotyledon of chickpea that results in different metabolite production. Metabolites are organic molecules with low molecular weight. They are either the intermediate or the end product of metabolism. Plant metabolites have a vast array of functions from basic growth and development to reproductive success strategies and several biotic and abiotic stress managements. They have been grouped into Primary Metabolites and Secondary Metabolites. Primary metabolites are the hydrophilic molecules and the product of the primary metabolism. Play important role in all biochemical pathways for the normal growth, development and reproduction of plant body (Dias et al. 2015). Primary metabolites include lipids, proteins and carbohydrates. Secondary metabolites are the intermediates and the end products of secondary metabolism. Specific set of organisms undergo secondary metabolism to give rise to secondary metabolites for different important ecological functions. Secondary metabolites acts as insect and herbivore repellent and deterrent, antimicrobial, attracts pollinator insects and birds, seeds and fruits dispersers, attracts root nodule bacteria and protects plants from harmful UV rays and helps to compete with the other growing plants (Allelopathy). Plant secondary metabolites also act as medicine and bioactive compound to several human diseases. Plant Secondary Metabolites have been classified into three groups – Phenolics, Terpenes and Nitrogen containing Secondary Metabolite (Alkaloids).

Whole of these metabolites produced by organism in its entire life cycle is referred as metabolome and their study is called metabolomics

(Desbrosses et al., 2005). Metabolomics study can be conducted in two different ways- Targeted Metabolomics and Untargeted Metabolomics (Bennett et al., 2008 and Tautenhahn et al., 2012). Targeted metabolomics deals with the study of specific metabolites in any biological system. Untargeted metabolomics deals with large data set to know the complete metabolome (Bennett et al., 2008 and Tautenhahn et al., 2012). Metabolomics study is achieved by GC-MS, LC-MS, NMR, etc.

Importance of phenolics and anthocyanin

- **Diverse array of plant and animal interactions**: Includes pollination, repellent of herbivores, fugivores and parasites. (Lev-Yadun S & Gould K.S, 2008).
- **Antimicrobial agent**: Defend against microbes and phytopathogens (Pervaiz et al.2017)
- **Sunscreen for plants**: Protect chloroplast from photoinhibition during high radiation. (Gould et al. 2000).
- **Antioxidant**: Protect plant cells against ultraviolet (UV) radiation and high light intensity, cold temperature & water stress (Pervaiz et al.2017).

Concentration of Anthocyanin increases during drought in chickpea (Makar, 2009). It appears that Chickpea cultivars with higher anthocyanin content are relatively more stable and yield often better.(Pundir et al, 1985). As anthocyanin and phenolics plays an important role in plants, study has been conducted to estimate and compare anthocyanin content and total phenolics content from different tissues of chickpea cultivars (Fig No. 1.1). Metabolites analysis was performed for differential metabolite profiling of the three chickpea cultivars using GC-MS.


.

Fig. No. 1.1 : Three cultivars of Chickpea grown in the field of university campus.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Classification of chickpea (http://www.nipgr.res.in/NGCPCG/Taxonomy.html)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliosida
Order	Fabales
Family	Fabaceae
Sub-family	Faboideae
Genus	Cicer
Species	C. arietinum L.

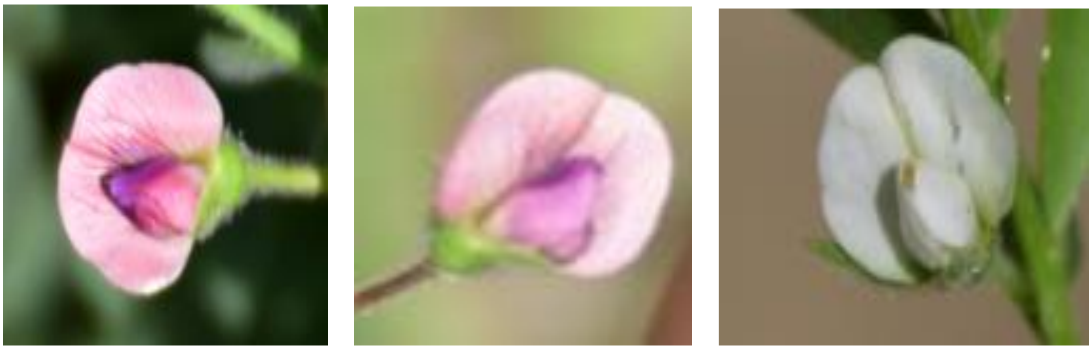
2.2 History and geographical distributions

Cicer arietinum L. is an ancient domesticated crop plant commonly known as Chickpea or Bengal gram is a bushy plant with or without glandular or non-glandular hairs. The two words 'Cicer' and 'arietinum' came into existence from Latin and were derived from the Greek word 'Kikus' which means strength or force and 'Krios' which is the synonym for both the ram and chickpea as chickpea seeds resemblance with the head of the ram(Aries)(Maesen,1987). It is believed that chickpea was originated from the south-eastern part of Turkey and adjoining areas of Syria (Ladizinsky,1975). Cicer areticulatum is considered to be the wild progenitor of the cultivated chickpea on the basis of electrophoresis of the protein from chickpea seeds (Ladizinsky, 1976). There are many postulated origin of Chickpea by different plant taxonomist. According to De Candolle (1883), chickpea was originated from the south of the Cusasus and from the north of Persia. However, Valvilov (1926) postulated two primary origin of chickpea, the Southwest of Asia and the Mediterranean and one secondary origin in Ethiopia. Valvilov (1926) showed that seeds with larger seeds cultivars were abundant in the Mediterranean basin and those with smaller seed cultivars were prominent in the east. The Linguistic data showed that the large seeded chickpea cultivar first arrived in India via Kabul, the capital of Afghan and hence given the name Kabuli in Hindi around 200 years back (Maesen, 1972). The chickpea seeds with dark seed coat and smaller seeds are known as desi or local type. Vander Maesen, 1987 reported that there are 43 species of Chickpea. Nine of which have been annualy cultivated, 33 are perennial and one more is unspecified. Chickpea is the major cultivated pulse crop in India and widely grown in dried regions of India. Main Chickpea producing states are Madhya Pradesh, Uttar Pradesh, Rajasthan, Andhra Pradesh and Maharashtra. Madhya Pradesh contributes around 40% of the total Chickpea production in India around 6 million tons followed by Uttar Pradesh and Rajasthan that contributes 16% and 4% respectively of the total production in India(Tiwari et al. 2016). Chickpea is cultivated in many parts of the world but in different seasons. 33 countries in West Asia , South Asia, Southern Europe, North Africa, East Africa, Australia, North and South America. The showing period of chickpea is different in different regions due to different in photoperiod, temperature, humidity and precipitations. Chickpea is shown in India in winter season.

2.3 Botanical descriptions

Chickpea is herbaceous leguminous plant with height range between 20-100 cm. Some tall cultivars can grow up to 130 cm in length in favourable conditions (Reddy et al.1985). The stem is solid, green, and herbaceous and profusely branched into primary, secondary and tertiary branches (Cubero,1987). Based on the growth habits, five different type of growth habit exhibits in Chickpea. They are erect, semi-erect, spreading, semi-spreading and prostrate (Pundir et al. 1985). The seed germinates at an optimum temperature of 25- 33˚C with hypogeal germination. Plumule developes into erect shoots and the radicle grows positively geotropic to give rise to well-developed root system. The primary root system develops and produces long lateral roots. The first leaf has 2-3 pairs of leaflets with a terminal one. Leaves are compound, unipinnate and shows alternate phyllotaxy. A single rachis contains 11-13 leaflets and is petiolate. Leaflets are 8-17 mm long and 5-14 mm and a terminal leaflet with serrated leaf blade and are pubescent.

Flowers are solitary and papilionaceous borne on the axillary raceme both have pedicle and peduncle of 6-13 mm long. Flowers are pink to purple in the desi type and white in the kabuli type as shown in the fig. no. 2.1. Chickpea have one carpel per flower.



(A) Himchana (B) ICC 4958 (C) JGK-03

Fig No.2.1: Flowers of Chickpea cultivars. (A) & (B) are the desi type and (C) kabuli type.

Pods of chickpea (fig no. 2.2) are inflated, several post–fertilization changes occurs for 5-6 days to give rise to pod. The pods are green, hairy and glandular with three distinct layers of wall - Outer exocarp, middle mesocarp comprising with 6-8 parenchymatous layer and inner endocarp layer. The inner endocarp layer have further two layers – fibrous layer(3-4 layers of fibres) and inner parenchymatous layer with 5-6 layer of parenchyma (Cubero, 1987). Pod may be ovate, rhomboid or oblong in shape with a great variability in size. Each pod consists of two to three seeds. Pod filling is greatly affected by different biotic and abiotic stress.

Fig No.2.2: Pods of the three cultivars of Chickpea.

Chickpea seed consists of thick seed coat with two layers of outer testa and inner integument. The seed coat is thick and green in the young stage and during the maturation process the seed coat gets thinned and its colour fades way to light yellow to dark brown. Seeds may be angular, beaked and wrinkled. The colour of the seed and its weight varies from variety to variety.

Seed matures through different developmental stages that can be visualized morphologically and biochemically. The molecular physiological mechanismof the development of embryo and its genetics and seed storage were well documented in Arabidopsis, legumes and cereals by Sreenivasulu and Wobus, 2013. This study reveals the level of metabolite production during seed maturation. The development of seed and its various maturation stages have been described in soybean by Goldberg et al., 2007 and have been shown in the fig. no.2.3.

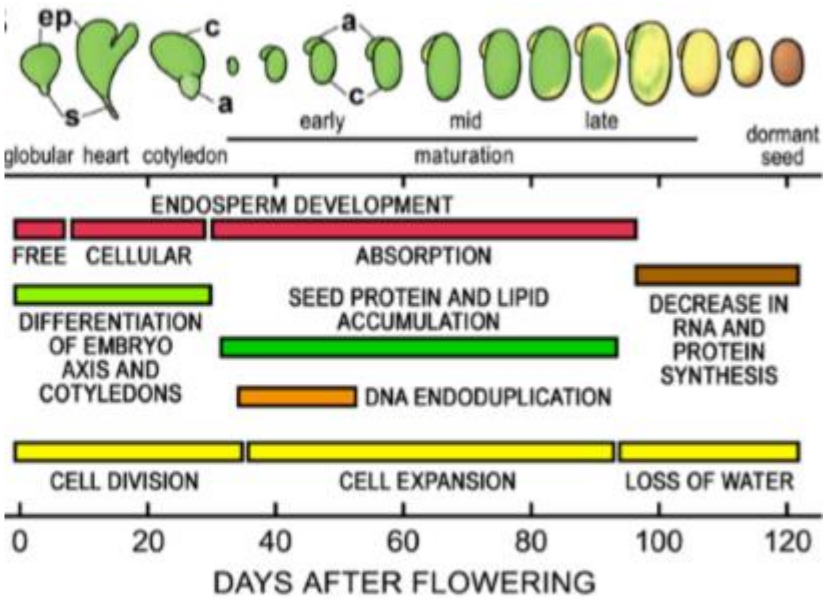


Fig. No. 2.3 The development of seed and its various maturation stages in soybean (Adapted from Goldberg et al., 2007)

2.4 Secondary metabolites in plants

Plant Secondary Metabolites have been classified into three groups – Phenolics, Terpenes and Nitrogen containing secondary metabolite (Alkaloids). The biosynthesis of plant secondary metabolites has been shown in the fig. no. 2.4 that shows that secondary metabolites are formed from the product of primary metabolism. Plant phenolics are the product of shikimik acid pathway and malonic acid pathway. Nitrogen containing secondary products are formed from shikimic acid pathway and terpenes are formed by mevalonic acid pathway and MEP pathway.

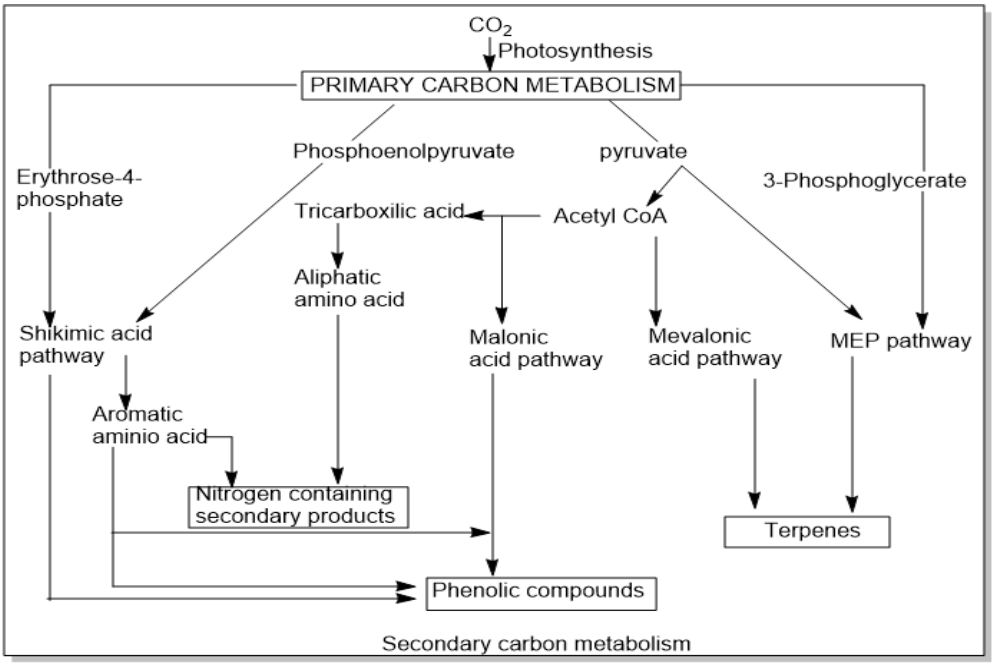


Fig No. 2.4: Biosynthesis pathway of plant secondary metabolites.
Designed using ChemDraw13 (Addapted from Taiz and Zeiger. Plant Physiology, 1991).

Phenolics are plants secondary compounds which have one or more hydroxyl groups bonded to an aromatic benzene ring. Phenolic compounds are grouped into simple phenols and polyphenols that have increased number of phenol units in its molecule. More than 8000 naturally occurring phenolics have been reported in plants (Sulaiman et al., 2013). Plant phenolics acts as antimicrobial, anticarcinogenic, antihypertensive, antioxidant and antimutagenic. They also have the ability to alter the expression of gene. Phenolics are considered to be the largest group of plant secondary metabolites that have antioxidant properties. Phenolics also act as metal chelators, hydrogen donors, radical scavengers due to its high antioxidant property. Phenolics were further has been divided based on its carbon skeleton shown in the table 2.1.

Table 2.1 : Classification of Plant Phenolic Compounds based on carbon skeleton (Cheynier et al., 2013).

Basic skeleton	Type of Phenolics
C_6	Simple Phenols and Benzoquinones
C_6-C_1	Aldehydes & Phenolic acids
C_6-C_2	Phenylacetic acids & Acetophenones,
C_6-C_3	Hydroxycinnamic acids, coumarins, phenylpropanes, and chromones
C_6-C_4	Naphthoquinones
C_6-C_1-C_6	Xanthones
C_6-C_2-C_6	Anthraquinones & Stilbenes
C_6-C_3-C_6	Flavonoids, Isoflavonoids and Neoflavonoids
$(C_6$-C_3-$C_6)_{2,3}$	Bi-, Tri- flavonoids, Proanthocyanidin dimers, trimmers
$(C_6$-$C_3)_2$	Lignans and Neolignans
$(C_6$-$C_3)_n$	Lignins
$(C_6)_n$	Catechol melanins, Phlorotannins
$(C_6$-C_3-$C_6)_n$	Condensed tannins

Flavonoids are the major group of Plant Phenolic compounds that structure consists of two aromatic benzene ring bonded by a 3-Carbon bridge. Flavonoids are found in different tissues of plants as in free-state and as glycosides. Flavonoids are responsible for the coloration of the flower, plant defence against pathogens and herbivores, UV protection and legume nodulation. Moreover, flavonoids also acts as nutraceuticals products as it has anticancer, antiulcer, antiarthritic, anti-angiogenic, anti-allergic, anti-inflammatory, antithrombotic, inhibition of protein kinase and mitrochondrial adhesion inhibition activities (Tabesum et al., 2016). Based on the degree of oxidation of the 3-C bridge, Flavonoids has been further divided into four types, viz. Anthocyanins, Flavones, Flavonols and Isoflavonoids.

Anthocyanins are glycoside member of the flavonoid family. Its structure has a sugar moiety at 3 carbon position and without this sugar moiety they are known as Anthocynidins. Anthocyanin is responsible for the blue, red, purple, yellow pigmentation of flowers, stems and fruits. Anthocyanin attracts the pollinators, helps in seed dispersal, UV protection and during several stresses. The colour of the Anthocyanin depends on the number of methoxy and hydroxyl group in the B ring of Anthocynidin molecule, presence of flanone and flavonol as co-pigments, presence of iron and aluminium as a chelating agents and the vacuole P^H in which Anthocynidin have been stored(Taiz and Zeiger, 1991).

CHAPTER-3

MATERIALS AND METHODS

3.1 Sample Selection

Three cultivars of Chickpea plants were selected for the study. The three cultivars of chickpea include two desi cultivars and one kabuli cultivar.

(A) Desi Cultivar includes- ICC 4958 and Himchana.

(B) Kabuli Cultivar include- JGK-03.

3.1.1 Sample for anthocyanin estimation

The seed of three cultivars of chickpea were sowed in the field area of university campus at same time and samples were harvested at same time from similar healthy plants. The first emerged not fully developed (in length) leaf was considered as the first leaf. 3rd, 5th and 7th leafs were selected for the estimation of anthocyanin contents with reference to the first leaf. Separated flowers and pedicle of the three chickpea cultivars were also selected for comparative study of anthocyanin contents.

3.1.2 Harvesting of seed of different developing stages

Several morphological characteristics have been optimized and considered for the harvesting of seed samples of different developing stages. The first parameter was Pod related changes. Pod is outer covering of seed which undergoes several developmental phases that result in colour change, loss of water content and stiffness. The second parameter was the visible changes in the behaviour of seed coat. Seed coat is outer covering of seed. Young seeds have green and thick seed coat. As they mature, their colours fade away to yellowish brown and their thickness start decreasing. The third parameter was Seed cotyledon characters. Young cotyledons are soft and light yellow to light green in colour. During maturation, it turns greener and then yellow. On the basis of these characters, four stages were selected for harvesting -

A. Early Maturation stage (300-500 mg)

B. Mid maturation Stage (500-700 mg)

C. Late Maturation stage (400-600 mg)

D. Healthy Dry seeds (200-400 mg)

3.1.3 Total phenolics content assay and metabolomics study

Fully dried, mature and healthy seeds of equal size and weight of the above mentioned cultivars were used for TPC analysis and other investigations. The seeds were grinded into fine powder and used for the studies.

3.2 Methods

3.2.1 Estimation of anthocyanin contents

Freshly collected sample were taken and washed with distilled H_2O and dried using tissue paper. Only 100 mg plant sample (leaf, flower and pedicle separately) was weighted. Samples were homogenised in 5ml of 95% methanol: 1N HCl, (9:1). Incubated for 4 hours at 4˚C and centrifuged at 10,000 rpm for 30 min. The reading was taken at 530nm and 657nm (Rabino et al., 1986, Mancinelli et al., 1974, kelefetoglu et al., 2009, Laby et al., 2000, & Yin et al. 2012) using a UV double beam spectrophotometer (UV-1800, Shimadzu, Japan). Anthocyanin content were calculated using the formula $Q_{Anthocyanin} = (A_{530} - 0.25 \times A_{657}) \times (\text{weight of the plant tissue used})^{-1}$. $Q_{Anthocyanin}$ is the corrected value of absorption correlated linearly with the concentration of anthocyanin (Yin et al. 2012). Samples were analysed in biological triplicates.

3.2.2 Harvesting seeds of different maturation stages

The seed of different maturation stage were selected and harvested. Except the dry and mature seeds, the cotyledon and seed coat of all the seed samples were separated and packed with aluminium foil separately and fixed in liquid nitrogen to stop its metabolic activities. All the fixed samples were stored at -80˚C till use for further experiment.

3.2.3 Estimation of total phenolics contents

Dried seeds were grinded into fine powder using electric mixer-grinder. 10 mg of powdered sample was separately extracted in two different solvents. Solvent 1 was 50% acetone and solvent 2 was 70% methanol (Xu et. al., 2007 and Segev et. al., 2010). Sample was shaken at 300 rpm for 3 hours and kept overnight for complete extraction. The sample was then centrifuged for 10 mins at 3000 rpm using Centrifuge 5430R eppendorf and the supernatant was used as sample solution. Total phenolics content was estimated by Folin- Ciocalteu assay

(Singleton et al. 1965, Singleton et al., 1999 and Xu et. al., 2007). Due to the presence of phosphomolybdate and phosphotungstate, Folin-Ciocalteu reagent becomes highly sensitive and form blue coloured complex in basic medium by the reduction of the phenols (Tabasum et al., 2016). Only 50µl of sample solution was dissolved in 3ml of distilled water, 250µl of Folin Ciocalteu reagent and 750µl of 7% Na_2CO_3 in centrifuge tube and vortexed and incubated for 8 minutes at room temperature. To the mixture 950 µl of d.H_20 was added and allowed to incubate for 2 hours (Xu et. al., 2007). The reading was taken at 765nm using d.H_2O as blank by using UV Spectrophotometer (Micro plate reader, Synergy/H1). TPC was calculated as Gallic acid equivalents (mg of GAE/gm sample) using the standard calibration curve ($y = 0.2119x - 0.0327$ & $R^2 = 0.9882$).

3.2.4 Samples analysing using GC-MS.

3.2.4.1 Sample preparation

Seed samples were grinded using electric mixer-grinder to fine powdered sample. 100mg of the sample was separately extracted in to two solvents, 100% chloroform and 100% methanol in conical flasks. The flasks were covered with aluminium foil and left for overnight. The samples were then sonicated for 1 hour at 45˚C using Ultra Sonicator (Ultra Sonicator, Citizen). The solution was filtered using Whatman filter paper 1 for two times. The extract solvents were completely dried using rotor evaporator (Rotot Evaporator, LabIndia) and dissolved in DMSO. The dissolved sample was filtered using filter syringe and the filtrate was taken on GC vials for analysis.

3.2.4.2 GC-MS Analysis

For the analysis of the metabolites GCMS – QP2010 Ultra, Shimadzu, Japan instrument was used. To analyse the samples the temperature of column and injection were set up at 40˚C and 250˚C respectively. The flow control mode was kept in linear velocity where split injection mode split ration was of 5. The column was allowed to flow at 1.24 ml/min with helium gas. Oven temperature program was maintained as - temperature was kept at 40˚C for 3 mins (hold time), the temperature was increased to 220˚C at the rate of 4 fold 5 minutes hold time and the temperature was further increased to 250˚C at the rate of 15 for 5 minutes hold time.

For MS analysis the ion source temperature was kept at 200˚C and interface temperature was kept at 260˚C. Starting mass/charge (m/z) ratio was 50 and ending m/z ratio was 800 i.e. 50-800 m/z. The data were analysed with the chromatograms obtained.

3.2.5 Statistical analysis

All the experiments was performed in three replicates and the results were expressed as arithmetic mean ± standard deviation.

CHAPTER- 4

RESULTS AND DISCUSSIONS

4.1 Comparative analysis of anthocyanin in the contrasting Chickpea cultivars

4.1.1 Himchana has higher anthocyanin content than other cultivars

Relative quantity of anthocyanin was found to be higher in the desi cultivars as compared to the kabuli cultivar. Among the desi cultivars, Himchana yields the highest amount of anthocyanin. Anthocyanin concentration increases from third position leaf to seventh position leaf in all the three cultivars. The comparative data is shown in the fig. no. 4.1.

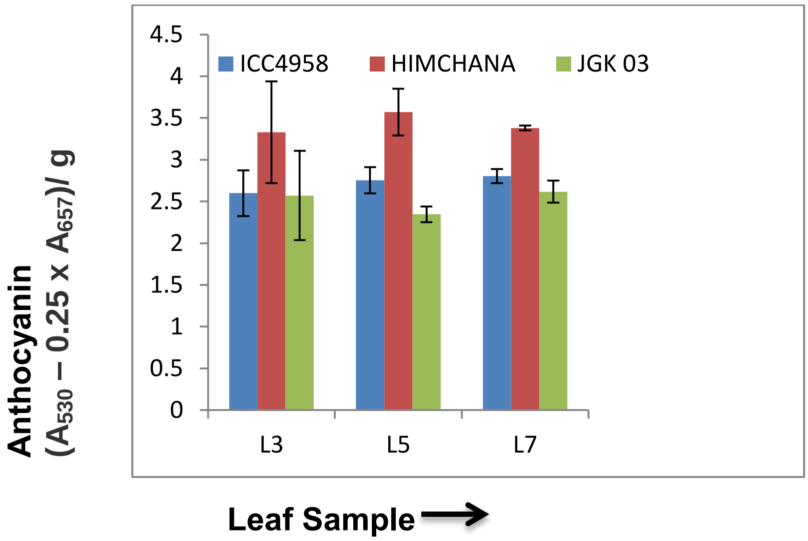


Fig. No. 4.1: Comparative anthocyanin content in selected leaf sample of the contrasting Chickpea cultivars. Red colour shows the concentration of himchana. Blue shows the concentration of ICC4958 and green colour shows that of JGK-03. L3, L5 and L7 were the 3rd, 5th and seventh leaf respectively. Error bars represents arithmetic mean ± standard deviation calculated from triplicates.

The overall anthocyanin content in all the 3rd, 5th and 7th leaf (fig.no. 4.2) in himchana was also found to be higher (3.426389 mg/g F.W.) followed by ICC4958 (2.719445 mg/g F.W) and JGK-03 (2.511 mg/g F.W) respectively as shown in the fig. no. 4.3. Concentration of anthocyanin increases from the young 3rd leaf to 5th leaf and slightly decreases in the 7th leaf. The data so obtained showed that the desi varieties of chickpea are rich in anthocyanin content. Among the desi cultivars, himchana has 1.259 fold higher anthocyanin contents than ICC4958 in the leaf sample. Both Himchana and ICC4958 have 1.36 and 1.08 fold higher anthocyanin contents as compared to the kabuli cultivar, JGK-03 in the leaf sample.

Fig.No. 4.2: Leaf sample used for anthocyanin content estimation.

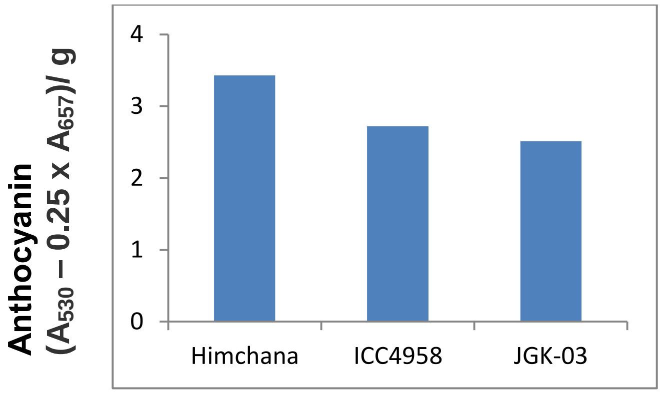


Fig.No. 4.3: Average anthocyanin contents in the leaf samples of the three cultivars of chickpea.

4.1.2 Comparative anthocyanin content in the floral organs of the Chickpea cultivars

4.1.2.1 Morphological study of the flower organs

The difference in colour of the flower and pedicle of the three cultivars of chickpea (fig no.4.4) due to the difference in anthocyanin concentration can be visualised morphologically. As anthocyanins are responsible for the colouration of the flower (Chalker- Scott, 1999 and Hamilton et al., 2001), its concentration was visualized to be higher in Himchana flower followed by ICC4958. JGK-03 flower showed white colouration and thus have very negligible amount of anthocyanin. Moreover, pedicle of ICC4958 has more deep red colour than the pedicle of Himchana which was also due to the difference in anthocyanin concentration.

4.1.2.2 Biochemical study of the floral organ

As in the leaf samples, the amount of anthocyanin was found to be higher in Himchana in both flower and pedicle (fig.no. 4.4) followed by ICC4958 and JGK-03. However, anthocyanin concentration of pedicle was found to be higher than the flower in ICC4958 cultivar. Anthocyanin in JGK-03 was found to be very little and is negligible as shown in the fig no. 4.5.

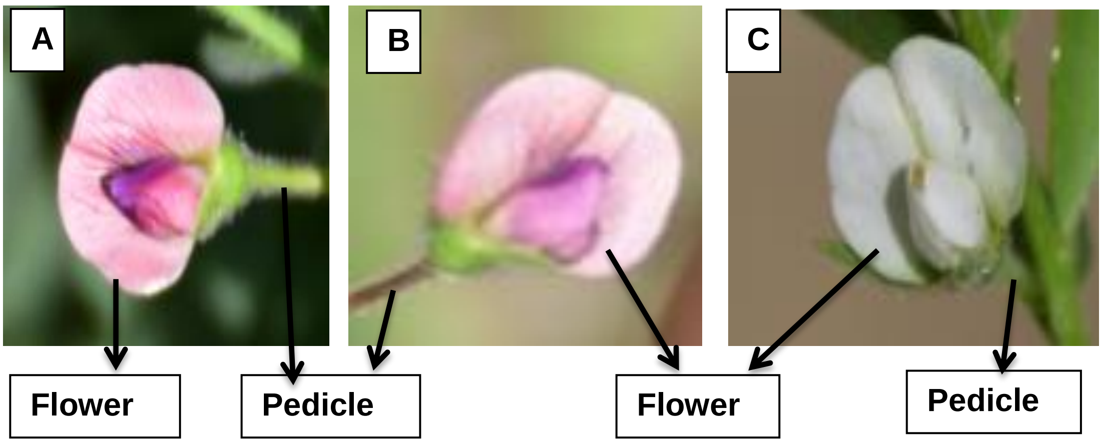


Fig. No. 4.4- The floral organs used for anthocyanin content estimation. (A) Flower and pedicle of himchana. (B) Flower and pedicle of ICC4958 and (C) Flower and pedicle of JGK-03.

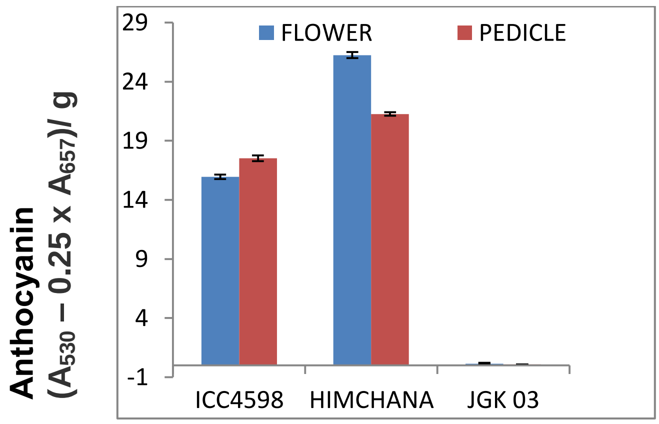


Fig. No. 4.5- Anthocyanin contents in flower and pedicle of the Chickpea cultivars grown in the university campus field. Blue colour indicates the flower tissue and the red colour indicate the pedicle tissue. Error bars represents arithmetic mean ± standard deviation calculated from triplicates.

4.2 Changes in characters of seed of different maturation stages

Seed maturation and development is a characteristic change that seed undergoes and categorized in three main events, embryogenesis, filling of nutrients and desiccation. Embryogenesis involved in developmental changes like globular stage, heart shaped, torpedo stage and cotyledon stage. Nutrient filling includes the maturation phases like early maturation (EM), mid maturation (MM) and late maturation (LM). Desiccation includes the dry and mature seed. The young seed are present at the tip of the branch as chickpea shows raceme inflorescence.

Table 4.1 Developmental changes in pod morphology during maturation stages in chickpea cultivars

Stages → Pod characters ↓	Cotyledon	Early Maturation	Mid Maturation	Late Maturation	Dry & Mature
Pod wall	Highly hydrated	Well hydrated	Hydrated	Less hydrated	Papery
Colour	Deep green	Deep- slightly light green	Light green	Pale green	Brown
Pod stiffness	Soft	Tip stiff	Whole pod stiff	Highly stiff	Loose

In the cotyledon stage the colour of the pod was deep green and its wall was highly hydrated and soft and located at the terminal end of the twig. Seed coat colour was dark green, seed very small, hydrated and whitish in colour. In the early maturation stage, the pod was flappy, stiff at tip, smooth, well hydrated and slightly light green. Seed coat colour was also intense green, seed was soft and its size got increased slightly. Whole pod became stiff, light green in the MM stage. The pod size got increased due to the seed nutrient filling. Pod turns slightly light green in colour but cotyledons turned greener. The pod in the LM stage the pod became less hydrated, pale green and stiff because of the increased seed size. Seed coat turned pale and got dehydrated and thickness got reduced. Seed coat was difficult to separate in this stage and size of the seed got decreased and colour of the seed became yellow. In the mature dry stage the pod became dry and brown, seed coat became scally and reddish brown. Seed turns dry and yellow and hard. The changes observed in the pod, seed coat and cotyledon has been shown in the form of table 4.1, 4.2 and 4.3.

Table 4.2 Developmental changes in seed coat morphology during maturation stages in chickpea cultivars

Stages→ Seed coat characters↓	Cotyledon	Early Maturation	Mid Maturation	Late Maturation	Dry & Mature
Thickness	Very thick & hydrated	Slightly less thick and hydrated	Thin and little less hydrated	Papery soft and little less hydrated	Light and scally
Colour	Deep green	Deep-slightly light green	Light green	Pale green	Dark-brown
Stiffness	Very soft	Slightly stiff	Stiff	Hard	Very hard

Table 4.3 Developmental changes in cotyledon morphology during maturation stages in chickpea cultivars

Stages→ Cotyledon characters↓	Cotyledon	Early Maturation	Mid Maturation	Late Maturation	Dry & Mature
Size	Very small	Small	Large, maximum nutrient filling	Large, start to dehydrate	Small and dry
Colour	Whitish	Green	Dark green	Light green-yellow	yellow

The developmental changes observed in the seed coat of different maturation stages in this study can be closely related to the developmental changes documented by Goldberg et al., 1994 and Wagmaister et al., 2007. The colour of the seed coat turned brown from green. This may be because of the oxidation of secondary metabolites like proanthocyanidins. As the seed matures, seed coat size got increased to provide the surface area for the increasing cotyledon due to nutrient filling.

The seeds of different developmental stages were harvested (EM, MM & LM) for making of seasonal biological replicates (table 4.4 and fig no. 4.6) till further study. Dry seeds so harvested will be used for planting in the next season. The seeds harvested of different maturation stage can be used to study the accumulation of different metabolites in the maturation process.

Table 4.4 Total harvested seeds with their developmental stages

Maturation Stage	No. of seed of Himchana	No. of seed of ICC4958	No. of seed of JGK-03
EM	120	120	120
MM	120	120	120
LM	120	108	108

Fig. No. 4.6: Harvested dry seeds of chickpea cultivars: (A) Himchana, (B) ICC4958, and (C) JGK-03

4.3 Comparative analysis of the total phenolics content in the contrasting Chickpea cultivars

The experiment was performed using two different extracting solvent for total phenolics content. The two solvent used were 50% acetone and 70% methanol. ICC4958 showed the highest amount of phenolics followed by Himchana and JGK-03. Total phenolics content in Himchana, ICC4958 and JGK-03 in 50% acetone extract were 29.8 µg/mg of D.W., 31.21 µg/mg of D.W. and 13.68 µg/mg of D.W. of the sample respectively. The TPC in 70% methanol extract of Himchana, ICC4958 and JGK-03 were 15.29 µg/mg of D.W., 13.688 µg/mg of D.W. and 11.769 µg/mg of D.W. of the sample respectively.

The desi cultivars showed higher amount of phenolics than the kabuli one also documented by Singh et al., 1982. Himchana has the highest concentration of anthocyanin among the three cultivars but surprisingly TPC was found to be higher in ICC4958. As anthocyanin also belongs to the group phenolics, the variation in results may be because of the up and down regulation of the biosynthesis of anthocyanin and other phenolics.

50% Acetone showed better solubility of the phenolics than 70% methanol as studied by Singh et al., 2016, Xu et al., 2007 and Segev et al.,2010. The comparison has been shown in fig. 4.7 and table 4.5.

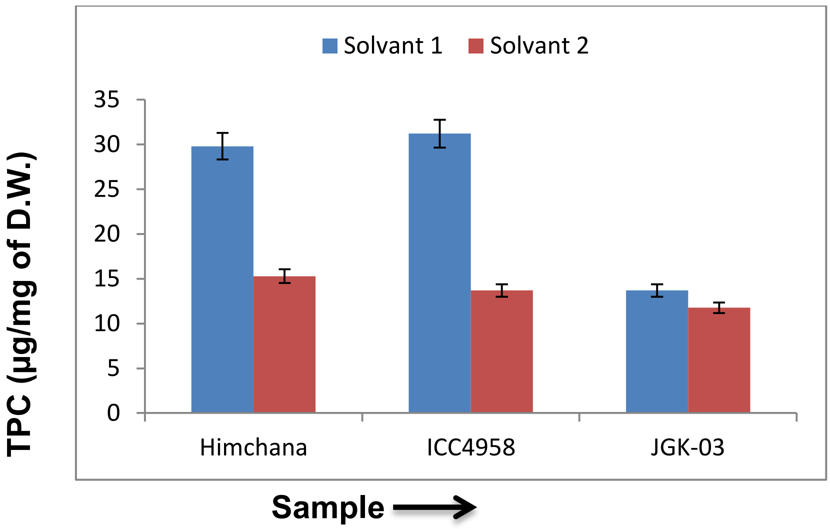


Fig. 4.7 - Total phenolics content in Chickpea lines. Blue colour denotes TPC in the 50% Acetone extract and the red colour denotes TPC in 70% methanol extract. Error bars represents arithmetic mean ± standard deviation calculated from triplicates.

Table 4.5: Total phenolics content of the three chickpea cultivars.

Sample	TPC in 50% acetone (µg/mg of D.W.)	TPC in 70% methanol (µg/mg of D.W.)
Himchana	29.8	15.29
ICC4958	31.21	13.688
JGK-03	13.68	11.769

The physical and chemical nature of the samples, property of solvent, solvent-sample ratio, time of extraction and temperature were the few parameters that effects the solubility of sample in an solvent (Naczk et al., 2006, Dai et al., 2010 and Singh et al., 2016). Solubility of the sample compounds is directly proportional to the polarity of the solvents. Polar solvents are being used for the phenolics extraction from plant matrix. For phenolics study 50% acetone was regarded as best extracting solvent because efficiency of the extracting solvent got increased by the mixture of organic solution and water by dissolving the hydrophilic as well as lipophilic compounds in it (Naczk et al., 2006, Dai et al., 2010 and Singh et al., 2016)**.**

4.4 Metabolite analysis in the chloroform extract of the samples by GC-MS

4.4.1 Metabolites analysis in the chloroform extract of himchana

In the chloroform extract of himchana a total of 24 metabolites were detected. Among those 8 were fatty acids, 11 were alkanes, 2 were acidic compounds, and one compound each of ketone, phenol and alcohol. The tabulated data has been shown in the table 4.6.

Table 4.6: Metabolites detected in the chloroform extract of himchana

Sl. No.	Compound Name	Category	Retention Time	Area(%)
1.	Cyclopropane	Alkane	20.624	2.19
2.	Dodecane	Alkane	20.953	1.22
3.	3-hexadecane	Alkane	27.859	6.22
4.	Tetradecane	Alkane	28.134	1.98
5.	Phenol	Phenol	31.717	9.45
6.	E-14-Hexadecenal	Fatty Acids	34.322	9.89
7.	Hexadecane	Alkane	34.549	2.26
8.	E-15-Heptadecenal	Fatty Acid	40.127	11.23
9.	Heptadecane	Alkane	40.317	2.11
10.	Dibutyl phthalate	Pthalic Acid	44.330	0.21
11.	Pentadecanoic acid	Fatty Acid	44.628	2.21
12.	1-Heneicosanol	Fatty Acids	45.392	9.66
13.	Hepatadecane	Alkane	45.547	1.63
14.	9-octadecanoic acid	Fatty Acids	48.807	1.44
15.	1-Heneicosanol	Fatty Acids	50.469	7.93
16.	Nonadecane	Alkane	50.635	2.65
17.	Benzenemethanol	Alcohol	50.775	0.25
18.	Cyclohexanone	Ketone	54.030	4.51
19.	13-methyltetradec-9-enoic acid	Fatty Acids	54.358	1.52
20.	Octadecanoic acid	Fatty Acids	54.464	1.24
21.	Heptadecyl trifluoroacetate	Alkane	55.821	5.62
22.	Eicosane	Alkane	55.928	1.08
23.	Trifluroacetic acid	Acid	58.118	3.06
24.	Cyclopropane	Alkane	58.208	1.14

4.4.2 Metabolites analysis in the chloroform extract of ICC4958

In the chloroform extract of ICC4958, a total of 31 compounds were detected. Among those 8 were fatty acids, 13 were alkanes, 4 were alkene and one compound each of phenol, pthalic acid, alkaloid, carbohydrate, ester and acetate. The tabulated data is shown in the table 4.7

Table 4.7: Metabolites detected in the chloroform extract of ICC4958

Sl. No.	Compound name	Category	Retention time	Area (%)
1.	2-Dodecene	Alkene	12.678	0.15
2.	3-tetradecene	Alkene	20.627	2.13
3.	Dodecane	Lipids	20.958	128
4.	2-octadecane	Alkane	27.867	6.26
5.	Dodcane	Alkane	28.139	2.12
6.	Phenol	Phenol	31.720	9.67
7.	5-Eicosene	Alkene	34.333	9.96
8.	Hexadecane	Alkane	34.553	2.47
9.	Tridecane	Alkane	34.692	0.19
10.	E-15-Heptaadecenal	Fatty Acids	40.142	11.34
11.	Hexadecane	Alkane	40.323	2.33
12.	Eicosane	Alkane	42.983	0.10
13.	7,9-Di-teri-butyl-1-oxaspiro(4,5)decane	Alkane	43.070	0.29
14.	Dibutyl phthalate	Phthalic Acid	44.332	0.32
15.	Pentadecanoic acid	Fatty Acid	44.654	3.31
16.	2-Hexadecene	Alkene	45.242	0.32
17.	E-15-Heptadecenal	Fatty Acid	45.405	10.99
18.	Heptadecane	Alkane	45.553	2.06
19.	Undecane	Alkane	45.733	0.17
20.	11,14-Eicosadienoic acid	Fatty Acid	48.809	0.77
21.	2-Hexadecane	Alkane	50.308	0.25
22.	Heptadecane	Alkane	50.644	1.59
23.	Palmidrol	Alkaloid	54.200	2.26
24.	Hexadecanoic acid	Fatty Acid	54.373	0.94
25.	Octadecanoic acid	Fatty Acid	54.458	0.89
26.	2,3-anhydro-d-mannosan	Carbohydrate	54.550	0.07
27.	Heptadecyl trifluroacetate	Ester	55.827	5.83
28.	Heneicosane	Fatty Acid	55.932	1.08
29.	Bicyclo[3,1,1]heptane-3-ol	Alkane	56.102	0.12
30.	1,3,5-trisilacyclohexane	Alkane	57.446	0.59
31.	Octacosyl trifluroacetate	Acetate	58.115	1.04

4.4.3 Metabolites analysis in the chloroform extract of JGK-03

In the chloroform extract ofJGK-03, a total of 28 metabolites were detected. Among those 7 were fatty acids, 14 were alkanes, 2 were alkene and one compound each of phenol, ester, ketone, alcohol and triterpene. The tabulated data is shown in the table 4.8

Table 4.8: Metabolites detected in the chloroform extract of JGK-03

Sl. No.	Compound name	Category	Retention time	Area (%)
1.	Cyclopropane	Alkane	12.680	0.36
2.	Decane	Alkane	13.042	0.13
3.	Cyclopropane	Alkane	20.629	2.63
4.	Dodecane	Alkane	20.958	1.49
5.	5-Octadecane	Alkane	27.865	5.93
6.	Tetradecane	Alkane	28.139	1.86
7.	Phenol	Phenol	31.713	8.81
8.	5-Eicosene	Alkene	34.325	9.10
9.	Hexadecane	Alkane	34.552	2.14
10.	2-bromo dodecane	Alkane	37.505	0.18
11.	E-15-Heptadecenal	Fatty Acids	40134	10.17
12.	Heptadecane	Alkane	40.320	2.16
13.	7,9-Di-tri-butyl-1-oxaspiro(4,5) decane	Alkane	43.073	0.26
14.	Dibutyl phthalate	Ester	44.333	0.38
15.	n-Hexadecanoic acid	Fatty Acid	44.649	4.33
16.	E-15-Heptadecenal	Fatty Acid	45.396	9.96
17.	Heptadecane	Alkane	45.550	1.84
18.	Eicosane	Alkane	47.992	0.18
19.	1,E—11,Z-13-Octadecatriene	Alkene	48.812	1.49
20.	Octadecanoic acid	Fatty Acids	49.611	0.64
21.	1-Heneicosanol	Fatty Acids	50.475	7.65
22.	Eicosane	Alkane	50.642	1.42
23.	Palmidrol	Fatty Acid	54.205	2.39
24.	1-Heneicosanol	Fatty Acid	55.825	5.74
25.	Nonadecane	Alkane	56.437	2.64
26.	1-propanone	Ketone	56.959	0.52
27.	Cyclohexanol	Alcohol	57.447	0.66
28.	Squalene	Triterpene	48.694	1.52

4.5 Metabolites analysis in the methanol extract of the sample by GC-MS

4.5.1 Metabolites analysis in the methanol extract of himchana

In the methanol extract of himchana, a total of 24 compounds were detected. Among those 15 were fatty acids, 3 were alkanes, 2 were vitamin E and one compound each of phenol, ketone, acid and alkene. The tabulated data is shown in the table 4.9.

Table 4.9: Metabolites detected in the methanol extract of himchana

Sl. No	Compound name	Category	Retention time	Area (%)
1.	Cyclopropane	Alkane	20.624	0.49
2.	9-octadecane	Alkane	27.858	1.89
3.	Phenol	Phenol	31.730	1.04
4.	9-octadecane	Alkane	34.315	1.87
5.	E-15-heptadecenal	Fatty Acid	40.123	2.55
6.	Hexadecanoic acid	Fatty Acid	43.619	5.80
7.	n-hexadecanoic acid	Fatty Acid	44.611	1.60
8.	E-15-heptadecenal	Fatty Acid	45.386	2.27
9.	9,12-octadecadienoic acid	Fatty Acid	47.780	34.55
10.	10-octadecanoic acid	Fatty Acid	47.940	14.51
11.	10-octadecanoic acid	Fatty Acid	48.069	0.94
12.	Heptadecanoic acid	Fatty Acid	48.599	0.66
13.	9-octadecanoic acid	Fatty Acid	48.843	2.14
14.	Trichloroacetic acid	Fatty Acid	50.467	1.60
15.	2-(2-bromomethyl) cyclohexanone	Ketone	54.038	3.04
16.	Beta-tocopherol	Vitamin E	54.213	5.30
17.	Beta-tocopherol	Vitamin E	54.353	3.04
18.	Methyl16-hydroxy-hexadecanoate	Fatty Acid	54.466	1.02
19.	5-eicosene	Alkene	55.819	3.21
20.	3-cyclopentylpropionic acid	Acid	56.967	1.21
21.	Octanoic acid	Fatty Acid	57.095	1.17
22.	Isopropyl linoleate	Fatty Acid	57.311	158
23.	Hexadecanoic acid	Fatty Acid	57.455	2.50
24.	Hexadecanoic acid	Fatty Acid	58.321	2.50

4.5.2 Metabolites analysis in the methanol of ICC4958.

In the methanol extract of ICC4958, a total of 28 compounds were detected. Among them 18 were fatty acids, 2 were alkenes, 2 were vitamin E, 2 were acids, 2 were ketones and one compound each of phenol and steroid. The tabulated data is shown in the table 4.10.

Table 4.10: Metabolites detected in the methanol extract of ICC4958

Sl. No.	Compound name	Category	Retention Time	Area (%)
1.	3-tetradecene	Alkene	20.585	0.19
2.	5-octadecene	Alkene	27.792	0.81
3.	Phenol	Phenol	31.667	0.49
4.	E-14-hexadecenal	Fatty Acids	34.250	1.61
5.	8-Pentadecanone	Ketone	36.600	0.20
6.	E-15-heptadecenal	Fatty Acids	40.058	1.65
7.	8-octadecanone	Ketone	42.200	0.39
8.	Hexadecanoic acid	Fatty Acids	43.533	8.68
9.	Pentadecanoic acid	Fatty Acids	44.550	0.60
10.	E-15-heptaadecenal	Fatty Acids	45.325	1.10
11.	9,12-octadecadienoic acid	Fatty Acids	47.683	46.25
12.	10-octadecaenoic acid	Fatty Acids	47.867	27.42
13.	13-octadecaenoic acid	Fatty Acids	48.025	1.27
14.	Methyl stearate	Fatty Acids	48.533	1.03
15.	9,17-octadecadienal	Fatty Acids	48.692	1.02
16.	Trifluroacetic acid	Acid	50.392	0.73
17.	Methyl-9-eicosenoate	Fatty Acids	53.792	0.22
18.	Gamma tocopherol	Vitamin E	54.225	3.88
19.	Gamma tocopherol	Vitamin E	54.362	4.31
20.	Methyl18-methylnonadecanoate	Fatty Acids	54.457	2.50
21.	Thero-4-hydroxy1-homoarginine lactate	Fatty Acids	54.575	0.07
22.	1-heptaecosanol	Fatty Acids	55.823	0.34
23.	3-cyclopentayl propanoic acid	Acid	56.964	1.26
24.	9,12-octadecadienoyl chloride	Fatty Acids	57.308	0.38
25.	9,12-octadecadienoyl chloride	Fatty Acids	58.319	0.84
26.	Hexadecanoic acid	Fatty Acids	58.108	0.08
27.	Campesterol	Steroid	58.775	0.37
28.	Heptadecanoic acid	Fatty Acids	58.775	0.37

4.5.3 Metabolites analysis in the methanol of JGK-03

In the methanol extract of JGK-03, a total of 29 compounds were detected. Among them 16 were fatty acids, 3 were alkanes, 2 were vitamin E, 4 acidic compounds and one compound each of phenol, ketone, alcohol and alkaloid. The tabulated data is shown in the table 4.11

Table 4.11: Metabolites detected in the methanol extract of JGK-03

Sl. No.	Compound name	Category	Retention time	Area (%)
1.	5-tetradecane	Alkane	20.620	0.34
2.	5-octadecane	Alkane	27.851	1.00
3.	Phenol	Phenol	31.718	0.75
4.	E-14-Hexadecenal	Fatty Acids	34.312	2.50
5.	8-octodecanone	Ketone	36..636	0.45
6.	E-14-hexadecenal	Fatty Acids	40.119	2.83
7.	Pentadecanoic acid	Fatty Acids	42.250	0.60
8.	n-Hexadecanoic acid	Fatty Acids	43.615	6.87
9.	E-15-heptadecenal	Fatty Acids	44.611	1.40
10.	9,12-octadecadienoic acid	Fatty Acids	45.558	0.15
11.	10-octadecaenoic acid	Fatty Acids	47.792	37.68
12.	10-octadecaenioc acid	Fatty Acids	47.946	13.66
13.	Methyl sterarate	Fatty Acids	48.074	0.86
14.	9,12-octadecanoic acid	Fatty Acids	45.599	0.88
15.	Methyl stereate	Fatty Acids	48.813	2.03
16.	9,12-octadecadienoic acid	Fatty Acids	48.949	1.70
17.	OctanamideE-15-Heptadecanal	Fatty Acids	50.463	0.77
18.	1,7-dioxaspiro[5,5]undec-2-en	Fatty Acids	54.035	2.33
19.	Delta-tocoferol	Vitamin E	54.207	5.52
20.	Delta-tocoferol	Vitamin E	54.352	4.54
21.	Cyclopentylpropanoic acid	Acid	54.467	0.75
22.	Heptadecyl trifluroacetate	Acid	55.817	0.58
23.	3-cyclopentylpropanoic acid	Acid	56.963	2.64
24.	Fumaric acid	Acid	57.094	0.93
25.	Isopropayl linoleate	Fatty Acids	57.311	0.09
26.	Cyclohexanol	Alcohol	57.446	0.82
27.	Hexacosylheptafluorobutyrate	Alkane	58.103	1.29
28.	Hexadecanoic acid	Fatty Acids	58.322	323
29.	Aspidofractinine	Alkaloid	58.772	0.92

4.6 Common metabolites present in all the three cultivars in chloroform extract.

A total of 17 metabolites are present in common in either two or all the three cultivars of chickpea. Among them 9 metabolites were detected in all the three cultivars of chickpea in the chloroform extract. The tabulated data is shown in the table 4.12

Table 4.12: Metabolites detected in the chloroform extract of all the three cultivars: Himchana, ICC4058 and JGK-03. 'P' denotes presence and 'A' denotes absence of metabolites

Sl. No	Compound	Himchana	ICC4958	JGK-03
1.	Cyclopropane	Present	Absent	Present
2.	Dodecane	Present	Present	Present
3.	Tetradecane	Present	Absent	Present
4.	Phenole	Present	Present	Present
5.	Hexadecane	Present	Present	Present
6.	e-15-heptadecenal	Present	Present	Present
7.	Heptadecane	Present	Present	Present
8.	Dibutyl phthalate	Present	Present	Present
9.	Pentadecanoic acid	Present	Present	Absent
10.	1-heneicosanol	Present	Absent	Present
11.	Nonadecane	Present	Absent	Present
12.	Octadecanoic acid	Present	Present	Present
13.	Heptadecyltrifluroacetate	Present	Present	Absent
14.	Eicosane	Present	Present	Present
15.	5-eicosene	Absent	Present	Present
16.	7,9-di-tri butyl-1-oxaspiro(4,5) decane	Absent	Present	Present
17.	Palmidrol	Absent	Present	Present

4.7 Common metabolites present in all the three cultivars in the methanol extract.

A total of 12 metabolites are present in common in either two or all the three cultivars of chickpea in the methanol extract. Among them 6 metabolites were detected in all the three cultivars of chickpea in the methanol extract. The tabulated data is shown in the table 4.13.

Table 4.13: Metabolites detected in the methanol extract of all the three cultivars: Himchana, ICC4058 and JGK-03. 'P' denotes presence and 'A' denotes absence of metabolites

Sl. No.	Compound	Himchana	ICC4958	JGK-03
1.	Phenol	Present	Present	Present
2.	14-hexadecenal	Absent	Present	Present
3.	E-15-heptadecenal	Present	Present	Present
4.	n-hexadecanoic acid	Present	Present	Present
5.	Pentadecanoic acid	Absent	Present	Present
6.	9,12-octadecadieonic acid	Present	Present	Present
7.	8-octadecanone	Absent	Present	Present
8.	10-octadecanoic acid	Present	Present	Present
9.	Methylstearate	Absent	Present	Present
10.	3-cyclopentylpropionic acid	Present	Present	Present
11.	Heptadecanoic acid	Present	Absent	Absent
12.	Isopropyllinoleate	Present	Present	Present

4.8 Common metabolites present in all the three cultivars in both extraction solvent

A total of 3 metabolites were detected in both the solvent extract in all the three chickpea cultivars. Out of the three, one metabolite i.e. pentadecanoic acid was detected only in ICC4958 in the two extract solvent but in Himchana, it was detected only in the chloroform extract and in JGK-03 it was detected only in the methanol extract. The tabulated data is shown in the table 4.14

Table 4.14: Common metabolites present in all cultivars in both the extracts

Sl. No.	Compound	Himchana	ICC4958	JGK-03
1.	Phenol	Present	present	Present
2.	E-15-heptadecenal	Present	present	Present
3.	Pentadecanoic acid	Absent in methanol extract, present in chloroform extract	present	Present in methanol extract, absent in chloroform extract

Out of 24 metabolites in the chloroform extract of Himchana, the major compounds were E-15-Heptadecinal, a fatty acid with an area percentage 11.23%, phenol (area% = 9.45%), E-14-Hexadecenal (area% = 9.89%) and 1-Heneicosanol (area% = 9.66%). Out of 31 metabolites in the chloroform extract of ICC4958, the major compounds were E-15-Heptadecinal, a fatty acid with an area percentage of 11.34% and 10.99%, phenol (area%=9.67%) and 5-Eicosene (area%=9.96%). Out of 28 metabolites in the chloroform extract of JGK-03, the major compounds detected were E-15-Heptadecinal (area%=9.96% and 10.17%), 5-Eicosene (area%= 9.10%) and phenol (area%=8.81%).

In the methanol extract of Himchana, a total of 24 metabolites were detected. Out of them, the major compounds were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 34.55 and 14.51 respectively. In the methanol extract of ICC4958, a total of 28 metabolites were detected. Out of them, the major compounds were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 46.25 and 27.42 respectively. In the methanol extract of JGK-03, a total of 29 metabolites were detected. Out of them, the major compounds detected were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 37.68 and 13.66 respectively.

A total of 9 metabolites were commonly detected in the chloroform extract in all the three cultivars of chickpea and 8 metabolites were commonly detected only in the two cultivars in the same extract. In the methanol extract, 6 metabolites were commonly present in all the three cultivars and 6 metabolites were commonly present in only of the two cultivars. Phenol and E-15-heptadecenal were commonly detected in all the three cultivars of chickpea in both the extract solvents and Pentadecanoic Acid was absent in methanol extract but present in chloroform extract of Himchana, present in both the extracts of ICC4958 and present in methanol extract but absent in chloroform extract of JGK-03.

The two solvent used for the extraction were of different polarity. Methanol being highly polar and chloroform with less polarity showed significance difference in metabolites extraction. Polar compounds dissolved more in the polar solvents, whereas the less polar one got dissolved in the non-polar solvent.

CONCLUSIONS

&

FUTURE PERSPECTIVES

Conclusions

Chickpea is considered to be the third largest cultivated leguminous crop in the world. The yield of chickpea gets affected by several biotic and abiotic stresses like drought, cold, heat, salinity, etc. plant undergoes many developmental changes in its physiology and biochemistry to up cope with the environment. During the development of seed, the pod, seed coat as well as the cotyledons of the chickpea undergoes several morphological and biochemical changes. Seed filling is high in the mid maturation stage. Chickpea is rich in phenolics and differs in concentration from genotype to genotype. The desi type chickpea cultivar is richer in phenolics content as compared to the kabuli type cultivar. The anthocyanin content was found to be higher in the desi variety Himchana followed by another desi variety ICC4958 and Kabuli Variety, JGK-03. TPC was also found to be higher in the desi variety, ICC4958 followed by himchana and JGK-03. Among the two Solvent used for TPC determination, solvent 1 i.e. 50% Acetone was found to be the best as compared with solvent 2 i.e. 70% Methanol. Different metabolites like Carbohydrate, Fatty Acids, Alcohol, acids, Phenols, Terpenes, Alkaloid, Vitamin E, Steroid, Alkanes, Alkenes, Ketones, Aldehydes, amino acid and Ester of Phthalic Acid has been detected in the Chickpea cultivars by GC-MS analysis. Out of them, 17 & 12 metabolites were found to be common in either two or all the three cultivars in chloroform and methanol extract sample respectively. Out of these, 3 metabolites were detected in both the extract in all the three cultivars.

Future perspective

As the desi cultivars of chickpea are rich in phenolics, its biological activity as an antioxidant can be performed using various lines of cancer cells. Metabolomics study with both targeted and non-targeted approach of the seed sample of different maturation stage will definitely reveal the metabolism processes and metabolic pathways. Further, transcriptomics study may be done to know the up and down regulations of the genes responsible for the metabolites production.

REFERENCES

Aranha, B. C., Hoffmann, J. F., Barbieri, R. L., Rombaldi, C. V., & Chaves, F. C. (2017). Untargeted Metabolomic Analysis of Capsicum spp. by GC–MS. Phytochemical Analysis, 28(5), 439-447.

Burton J, [1984].Legume inoculants and their use. A pocket manual jointly prepared by Nitrogen Fixation for Tropical Agricultural Legumes (NifTAL) Project, USA and FAO.FAO, Rome (Italy). Land and Water Development Div. FAO, Rome (Italy).

C. T., & Balachandran, I. (2012). Total phenolics and total flavonoids in selected Indian medicinal plants. Indian journal of pharmaceutical sciences, 74 (3), 258.

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. Photochemistry and photobiology, 70(1), 1-9.

Chen, X.-Q., Wang, X.-B., Guan, R.-F., Tu, J., Gong, Z.-H., Zheng, N., . . . Ying, M.-M. (2013). Blood anticoagulation and antiplatelet activity of green tea (−)-epigallocatechin (EGC) in mice. Food & function, 4(10), 1521-1525.

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiology and Biochemistry, 72, 1-20.

Cubero, J. I. (1987). Morphology of chickpea. The chickpea, 35-66.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15**(**10), 7313-7352.

Daniel Anthony Dias, Camilla Beate Hill, Nirupama Samanmalie Jayasinghe, Judith Atieno, Tim Sutton, Ute Roessner,(2015),” Quantitative profiling of polar primary metabolites of two chickpea Cultivars with contrasting responses to salinity”. Journal of Chromatography B, **1000**, 1–13.

De Candolle, A. (1883). Origine des plantes cultivées (Vol. 43). G. Baillière et cie.

Desbrosses, G., Steinhauser, D., Kopka, J., & Udvardi, M. (2005). Metabolome analysis using GC-MS. In Lotus japonicus Handbook (pp. 165-174). Springer, Dordrecht.

Edirisinghe, I., Banaszewski, K., Cappozzo, J., McCarthy, D., & Burton-Freeman, B. M. (2011). Effect of black currant anthocyanins on the activation of endothelial nitric oxide synthase (eNOS) in vitro in human endothelial cells. Journal of Agricultural and Food Chemistry, 59(16), 8616-8624.

Frei, B., & Higdon, J. V. (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. The Journal of nutrition, 133(10), 3275S-3284S.

Ghasemzadeh, A., & Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of medicinal plants research, 5(31), 6697-6703.

Goldberg, R. B., De Paiva, G., & Yadegari, R. (1994). Plant embryogenesis: zygote to seed. Science, 266(5185), 605-614.

Gould et al. (2000), “Functional role of anthocyanins in the leaves of Quintinia serrata A. Cunn”. Journal of Exp. Botany, Vol.51,No.347, pp. 1107-1115.

Gould et al. (2009), “*Anthocyanins”*,Springer Science.

Hamilton, W. D., & Brown, S. P. (2001). Autumn tree colours as a handicap signal. Proceedings of the Royal Society of London B: Biological Sciences, 268(1475), 1489-1493.

Heijnen, C., Haenen, G., Van Acker, F., Van der Vijgh, W., & Bast, A. (2001). Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups. Toxicology in vitro, 15(1), 3-6.

Hidalgo, M., Martin-Santamaria, S., Recio, I., Sanchez-Moreno, C., Pascual-Teresa, B., Rimbach, G., & Pascual-Teresa, S. (2012). Potential anti-inflammatory, anti-adhesive, anti/estrogenic, and angiotensin-converting enzyme inhibitory activities of anthocyanins and their gut metabolites. Genes & nutrition, 7(2), 295.

Kalefetoğlu Macar, T., & Ekmekçi, Y. (2009). Alterations in photochemical and physiological activities of chickpea (Cicer arietinum L.) cultivars under drought stress. Journal of agronomy and crop science, 195(5), 335-346.

Khan, N., Ali, S., Shahid, M. A., & Kharabian-Masouleh, A. (2017). Advances in detection of stress tolerance in plants through metabolomics approaches. Plant Omics, 10(3), 153.

Khan, N., Bano, A., Rahman, M. A., Rathinasabapathi, B., & Babar, M. A. (2018). UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (Cicer arietinum) metabolome following long-term drought stress. Plant, cell & environment.

Kim,H.K., & Verpoorte, R. (2010). Sample preparation for plant metabolomics. Phytochemical Analysis, 21(1), 4-13.

Kim, H. K., Choi,Y.H., & Verpoorte, R. (2011). NMR-based plant metabolomics: where do we stand, where do we go?. Trends in biotechnology, 29(6), 267-275.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal, 2013.

Laby et al. (2000), "The Arabidopsis sugar-insensitive mutant sis4 and sis5 are defective in abseicic acid synthesis and response. The Plant Journal 23(J), 585-596.

Ladizinsky, G. (1975). A new Cicer from Turkey. Notes Roy. Bot. Gard. Edinburgh, 34(2), 201-202.

Ladizinsky, G., & Adler, A. (1976). Genetic relationships among the annual species of Cicer L. Theoretical and Applied Genetics, 48(4), 197-203.

Landi M., Tattini M., Gould K. S. (2015). "Multiple functional roles of anthocyanins in plant-environment interactions." Environ. Exp. Bot. **119**, 4–17. 10.1016

Le, B. H., Wagmaister, J. A., Kawashima, T., Bui, A. Q., Harada, J. J., & Goldberg, R. B. (2007). Using genomics to study legume seed development. Plant physiology, 144(2), 562-574.

Le, B. H., Wagmaister, J. A., Kawashima, T., Bui, A. Q., Harada, J. J., & Goldberg, R. B. (2007). Using genomics to study legume seed development. Plant physiology, 144(2), 562-574.

Lee, S. G., Kim, B., Yang, Y., Pham, T. X., Park, Y.-K., Manatou, J., Lee, J.-Y. (2014). Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF-κB independent of NRF2-mediated mechanism. The Journal of nutritional biochemistry, 25(4), 404-411.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., ... & Chen, H. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules, 21(10), 1374.

Lotito, S. B., & Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radical Biology and Medicine, 41(12), 1727-1746.

Lotito, S. B., Zhang, W.-J., Yang, C. S., Crozier, A., & Frei, B. (2011). Metabolic conversion of dietary flavonoids alters their anti-inflammatory and antioxidant properties. Free Radical Biology and Medicine, 51(2), 454-463.

Lu, W., Bennett, B. D., & Rabinowitz, J. D. (2008). Analytical strategies for LC–MS-based targeted metabolomics. Journal of Chromatography B, 871(2), 236-242.

Mancinelli, A. L., Tai, P. K. K., & Susinno, R. (1974). Photocontrol of anthocyanin synthesis: phytochrome, chlorophyll and anthocyanin synthesis. Photochemistry and Photobiology, 20(1), 71-79.

Mira, L., Tereza Fernandez, M., Santos, M., Rocha, R., Helena Florêncio, M., & Jennings, K. R. (2002). Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free radical research, 36(11), 1199-1208.

Müller, A., Düchting, P., & Weiler, E. W. (2002). A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to Arabidopsis thaliana. Planta, 216(1), 44-56.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of pharmaceutical and biomedical analysis, 41(5), 1523-1542.

Özeker, E. (1999). Phenolic compounds and their importance. Anadolu Ege *Tarımsal Araştırm*a Enstitüsü Dergisi, 9(2).

Pervaiz et al.(2017). Naturally Occurring Anthocyanin, Structure, Functions and Biosynthetic Pathway in Fruit Plants. J Plant Biochem Physiol.5:2.

Pundir, R. P. S., Rao, N. K., & van den Maesen, L. J. G. (1985). Distribution of qualitative traits in the world germplasm of chickpea (Cicer arietinum L.). Euphytica, 34(3), 697-703.

Rabino, I., & Mancinelli, A. L. (1986). Light, temperature, and anthocyanin production. Plant Physiology, 81(3), 922-924.

Ramalingam, A., Kudapa, H., Pazhamala, L. T., Weckwerth, W., & Varshney, R. K. (2015). Proteomics and metabolomics: two emerging areas for legume improvement. Frontiers in plant science, 6, 1116.

Reddy, M. V., Singh, K. B., Saxena, M. C., & Malhotra, R. S. (1985). Tallest chickpeas?. International chickpea newsletter.

Segev, A., Badani, H., Galili, L., Hovav, R., Kapulnik, Y., Shomer, I., & Galili, S. (2011). Total phenolic content and antioxidant activity of Chickpea (Cicer arietinum L.) as affected by soaking and cooking conditions. Food and Nutrition Sciences, 2(07), 724.

Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M., & Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (Cicer arietinum L.). Journal of food science, 75(2).

Singh, J., Singh, V., Shukla, S., & Rai, A. K. (2016). Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents. J Nutr Food Sci, 6(565), 2.

Singh, U., Kherdekar, M. S., & Jambunathan, R. (1982). Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars. The levels of amylase inhibitors, levels of oligosaccharides and in vitro starch digestibility. Journal of Food Science, 47(2), 510-512.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology (Vol. **299**, pp. 152-178). Academic press.

Spencer, J. P., Rice-Evans, C., & Williams, R. J. (2003). Modulation of pro-survival Akt/protein kinase B and ERK1/2 signaling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. Journal of Biological Chemistry, 278(37), 34783-34793.

Sulaiman, G. M., Hussien, N. N., Marzoog, T. R., & Awad, H. A. (2013). Phenolic content, antioxidant, antimicrobial and cytotoxic activities of ethanolic extract of Salix alba. Am J Biochem Biotechnol, 9(1), 41-46.

TABASUM, S., KHARE, S., & JAIN, K. (2016). Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of Abrus precatorius l. seeds. Asian J Pharm Clin Res, 9(2), 371-374.

Taiz, L., & Zeiger, E. (1991). Plant Physiology The Benjamin. Cummings Redwood City, 565.

Tautenhahn, R., Cho, K., Uritboonthai, W., Zhu, Z., Patti, G. J., & Siuzdak, G. (2012). An accelerated workflow for untargeted metabolomics using the METLIN database. Nature biotechnology, 30(9), 826.

Tiwari, S., Lata, C., Chauhan, P. S., & Nautiyal, C. S. (2016). Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in Cicer arietinum L. during drought stress and recovery. Plant Physiology and Biochemistry, 99, 108-117.

Van der Maesen, L. J. G. (1972). Cicer L., a monograph of the genus, with special reference to the chickpea (Cicer arietinum L.), its ecology and cultivation (Doctoral dissertation, Veenman).

Van der Maesen, L. J. G. (1987). Origin, history and taxonomy of chickpea. In The chickpea (pp. 11-34).

Vavilov, N. I. (1926). Studies on the origin of cultivated plants.

Wang, D., Wei, X., Yan, X., Jin, T., & Ling, W. (2010). Protocatechuic acid, a metabolite of anthocyanins, inhibits monocyte adhesion and reduces atherosclerosis in apolipoprotein E-deficient mice. Journal of Agricultural and Food Chemistry, 58(24), 12722-12728.

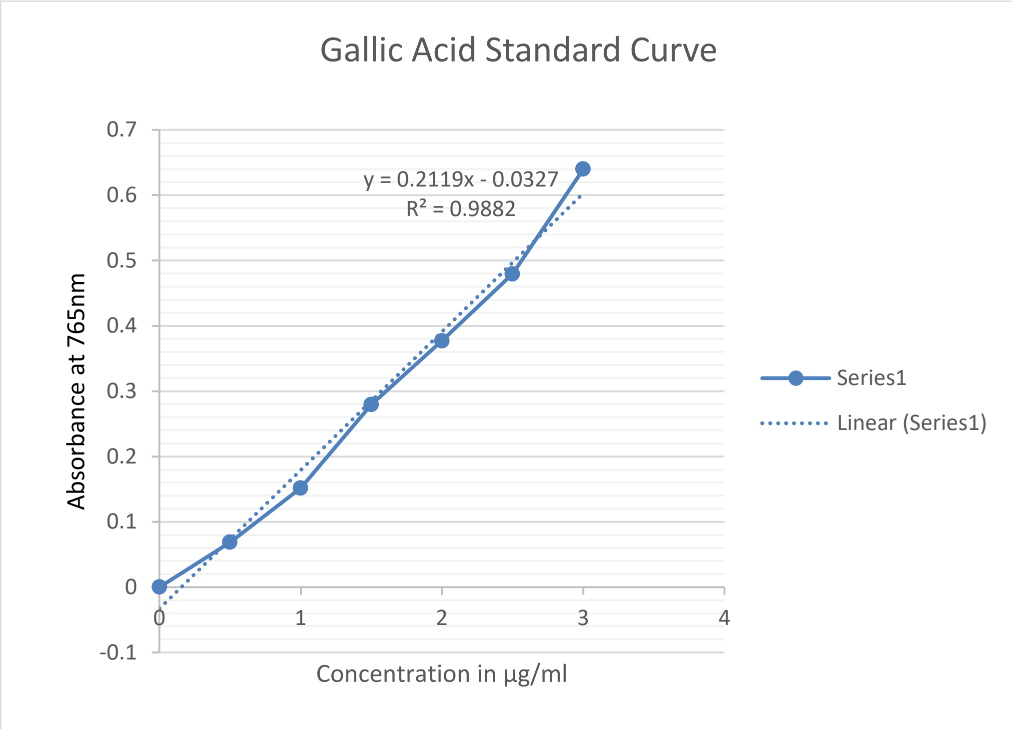
Wen, W., Li, K., Alseekh, S., Omranian, N., Zhao, L., Zhou, Y & Florian, A. (2015). Genetic determinants of the network of primary metabolism and their relationships to plant performance in a maize recombinant inbred line population. The Plant Cell, 27(7), 1839-1856

Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of food science, 72(2).

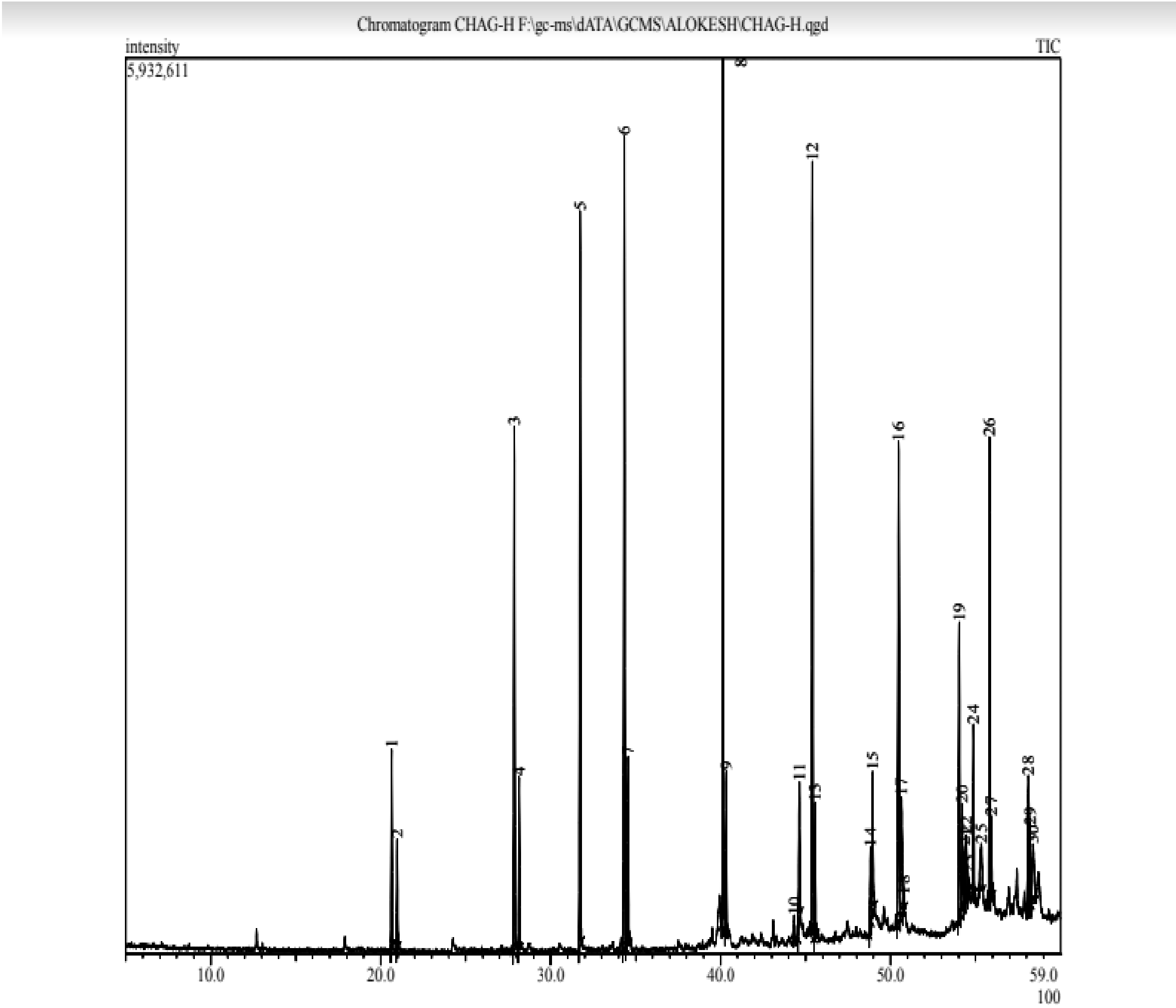
Yin, R., Messner, B., Faus-Kessler, T., Hoffmann, T., Schwab, W., Hajirezaei, M. R. & Schäffner, A. R. (2012). Feedback inhibition of the general phenylpropanoid and flavonol biosynthetic pathways upon a compromised flavonol-3-O-glycosylation. Journal of experimental botany, 63(7), 2465-2478.

APPENDICES

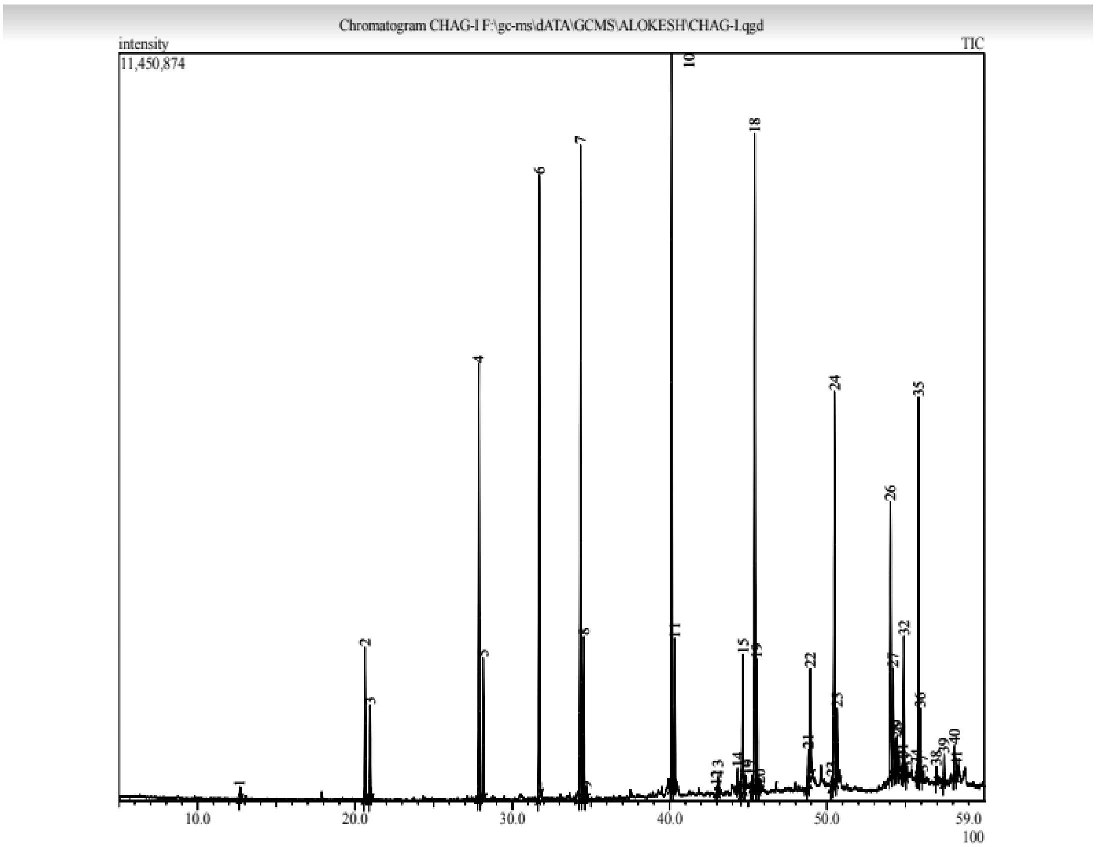
Standard curve of Gallic acid



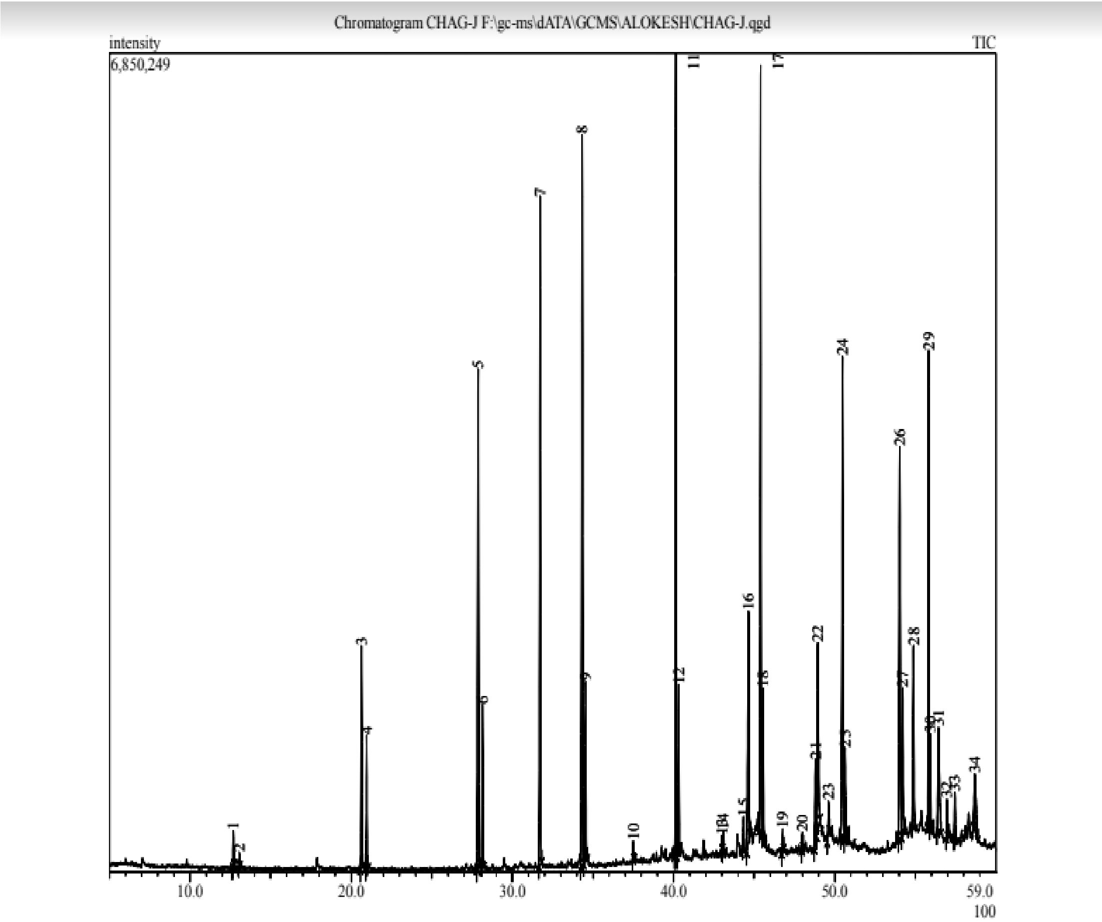
Chromatogram of Chloroform Extract of Himchana



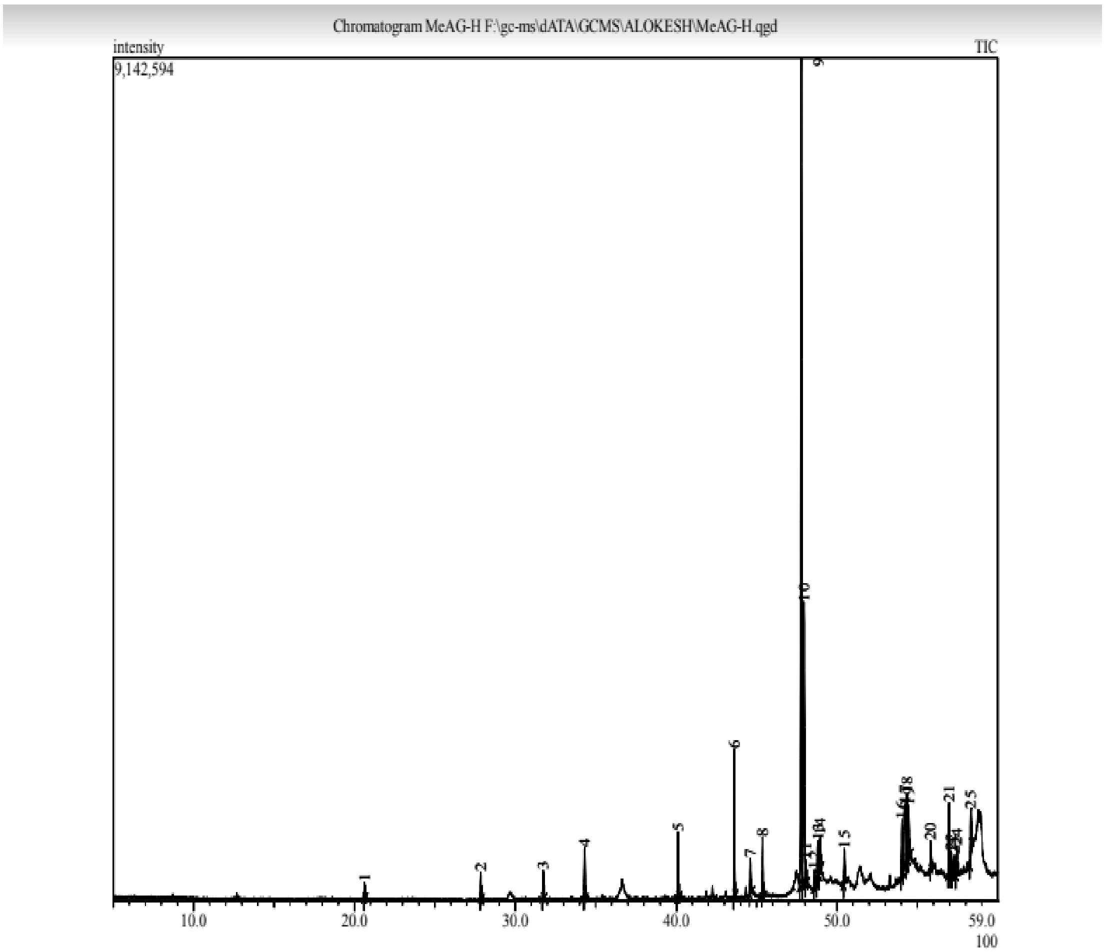
Chromatogram of Chloroform Extract of ICC4958



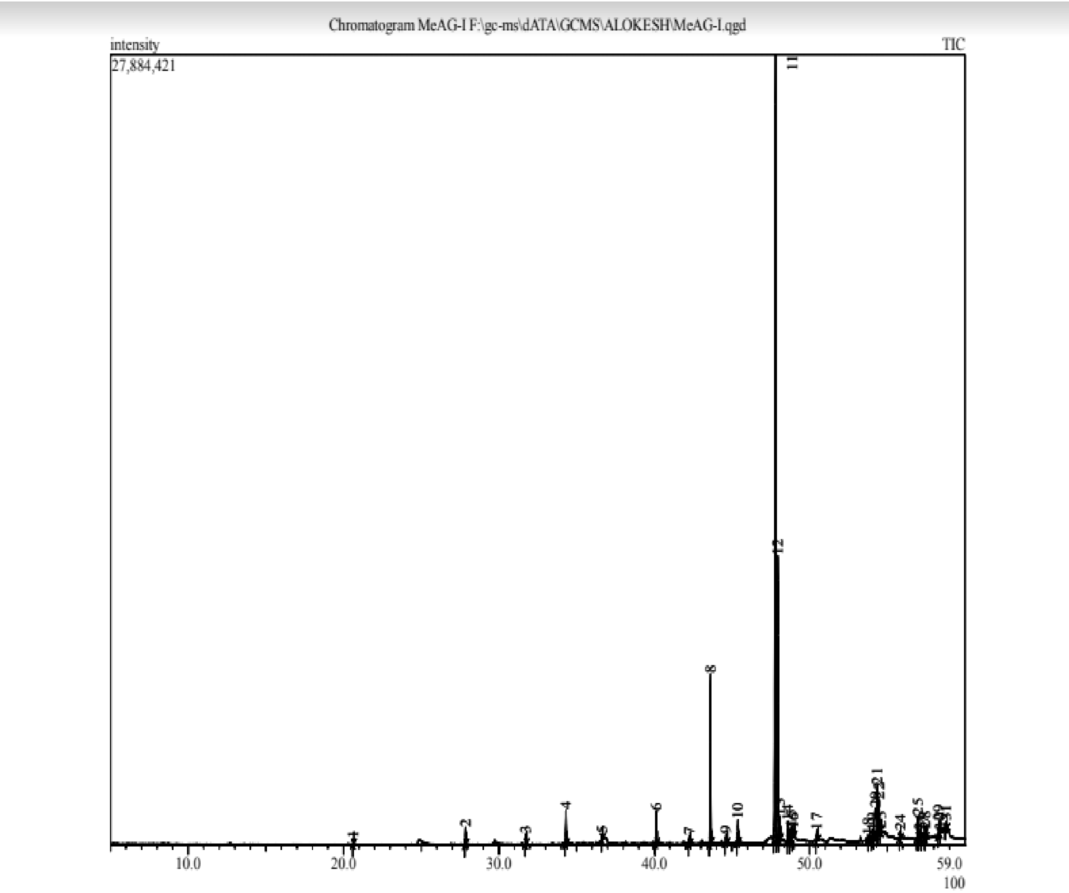
Chromatogram of Chloroform extract of JGK-03



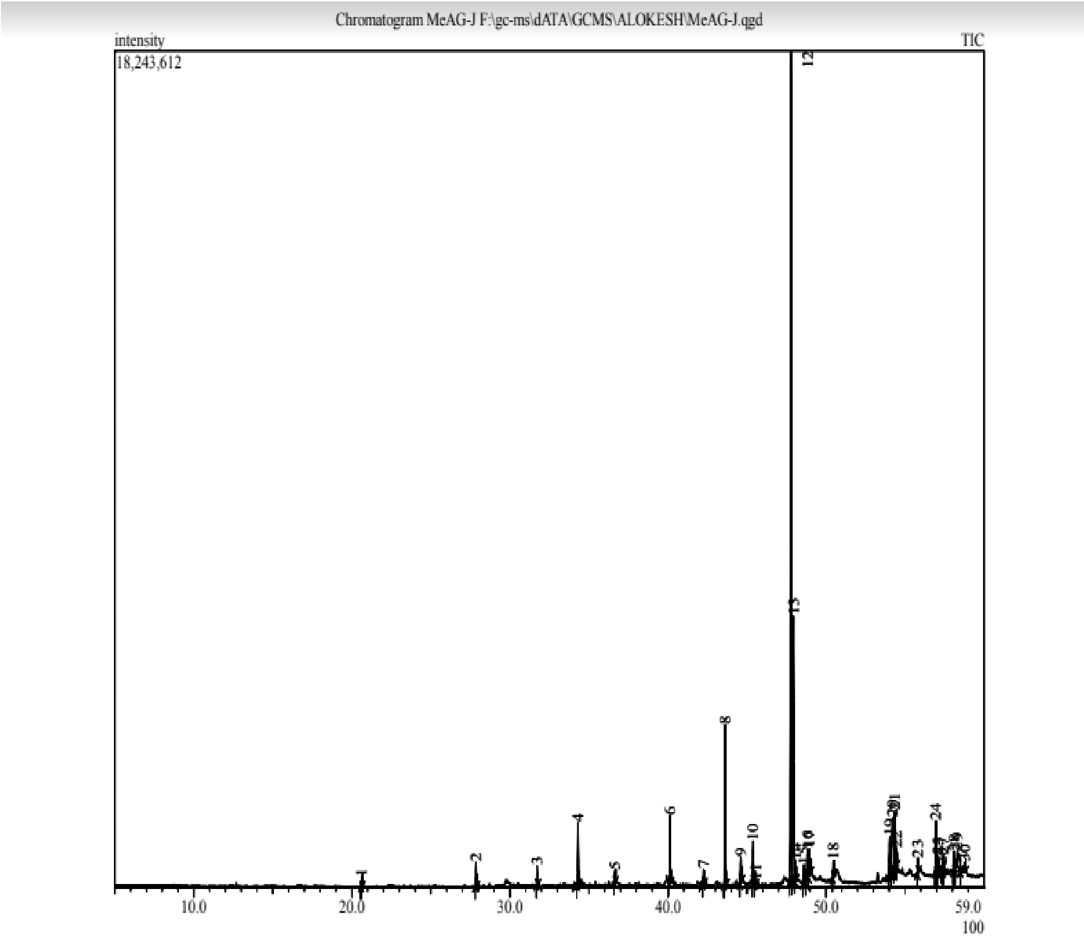
Chromatogram of Methanol Extract of Himchana



Chromatogram of Methanol Extract of ICC4958



Chromatogram of methanol extract of JGK-03



URKUND

Urkund Analysis Result

Analysed Document: Final report.docx (D39704049)
Submitted: 6/1/2018 6:52:00 AM
Submitted By: felix.bast@gmail.com
Significance: 1 %

Sources included in the report:

http://oar.icrisat.org/884/1/63640.pdf
http://www.nipgr.res.in/NGCPCG/Taxonomy.html

Instances where selected sources appear:

2

CHAPTER 1 INTRODUCTION

Chickpea is considered to be the third largest cultivated leguminous crop in the world. The growth and production of the chickpea has been greatly affected by several biotic and abiotic stresses. As grown mostly in the dry and winter season, chilling and drought stress are more common that affects chickpea production. To cope up with these stresses, plant produces plenty of metabolites that play major role in the cell integrity, growth, storage of energy, cell signalling, formation of membrane and scaffolding, replenishment of cell and many other functions (Wen et al. 2015 and Khan et al., 2018). Plants also develop certain mechanism to modify their biochemistry and physiology to cope up with several stress factors through several metabolic changes (Khan et al., 2017, Kim et al., 2011, Khan at al., 2018 & Wen at al., 2015). The major morphological and biochemical change can be seen during development of pod, seed, seed coat and cotyledon of chickpea that results in different metabolite production. Metabolites are organic molecules with low molecular weight. They are either the intermediate or the end product of metabolism. Plant metabolites have a vast array of functions from basic growth and development to reproductive success strategies and several biotic and abiotic stress managements. They have been grouped into Primary Metabolites and Secondary Metabolites. Primary metabolites are the hydrophilic molecules and the product of the primary metabolism. Play important role in all biochemical pathways for the normal growth, development and reproduction of plant body (Dias et al. 2015). Primary metabolites include lipids, proteins and carbohydrates. Secondary metabolites are the intermediates and the end products of secondary metabolism. Specific set of organisms undergo secondary metabolism to give rise to secondary metabolites for different important ecological functions. Secondary metabolites acts as insect and herbivore repellent and deterrent, antimicrobial, attracts pollinator insects and birds, seeds and fruits dispersers, attracts root nodule bacteria and protects plants from harmful UV rays and helps to compete with the other growing plants (Allelopathy). Plant secondary metabolites also act as medicine and bioactive compound to several human diseases. Plant Secondary Metabolites have been classified into three groups – Phenolics, Terpenes and Nitrogen containing Secondary Metabolite (Alkaloids).

Whole of these metabolites produced by organism in its entire life cycle is referred as metabolome and their study is called metabolomics (Desbrosses et al., 2005). Metabolomics study can be conducted in two different ways- Targeted Metabolomics and Untargeted Metabolomics (Bennett et al., 2008 and Tautenhahn et al., 2012). Targeted metabolomics deals with the study of specific metabolites in any biological system. Untargeted metabolomics deals with large data set to know the complete metabolome (Bennett et al., 2008 and Tautenhahn et al., 2012). Metabolomics study is achieved by GC-MS, LC-MS, NMR, etc. Importance of phenolics and anthocyanin • Diverse array of plant and animal interactions: Includes pollination, repellent of herbivores, fugivores and parasites. (Lev-Yadun S & Gould K.S, 2008). • Antimicrobial agent: Defend against microbes and phytopathogens (Pervaiz et al.2017) • Sunscreen for plants: Protect chloroplast from photoinhibition during high radiation. (Gould et al. 2000). • Antioxidant: Protect plant cells against ultraviolet (UV) radiation and high light intensity, cold temperature & water stress (Pervaiz et al.2017). • Concentration of Anthocyanin increases during drought in chickpea. (Makar, 2009) •

0: http://oar.icrisat.org/884/1/63640.pdf 100%

It appears that Chickpea cultivars with higher anthocyanin content are relatively more stable and yield

often better.(Pundir et al, 1985). As anthocyanin and phenolics plays an important role in plants study has been conducted to estimate and compare anthocyanin content and total phenolics content from different tissues of Chickpea cultivars (Fig No. 1.1). Metabolites analysis was performed for differential metabolite profiling of the three chickpea cultivars using GC-MS. .

Fig. No. 1.1 : Three cultivars of Chickpea grown in the field of university campus.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Classification of chickpea (http://www.nipgr.res.in/NGCPCG/Taxonomy.html)

0: http://www.nipgr.res.in/NGCPCG/Taxonomy.html 72%

Kingdom Plantae Division Magnoliophyta Class Magnoliosida Order Fabales Family Fabaceae Sub-family Faboideae Genus Cicer Species C. arietinum

L.

2.2 History and geographical distributions Cicer arietinum L. is an ancient domesticated crop plant commonly known as Chickpea or Bengal gram is a bushy plant with or without glandular or non-glandular hairs. The two words 'Cicer' and 'arietinum' came into existence from Latin and were derived from the Greek word 'Kikus' which means strength or force and 'Krios' which is the synonym for both the ram and chickpea as chickpea seeds resemblance with the head of the ram(Aries)(Maesen,1987). It is believed that chickpea was originated from the south-eastern part of Turkey and adjoining areas of Syria (Ladizinsky,1975). Cicer areticulatum is considered to be the wild progenitor of the cultivated chickpea on the basis of electrophoresis of the protein from chickpea seeds (Ladizinsky, 1976). There are many postulated origin of Chickpea by different plant taxonomist. According to De Candolle (1883), chickpea was originated from the south of the Cusasus and from the north of Persia. However, Valvilov (1926) postulated two primary origin of chickpea, the Southwest of Asia and the Mediterranean and one secondary origin in Ethiopia. Valvilov (1926) showed that seeds with larger seeds cultivars were abundant in the Mediterranean basin and those with smaller seed cultivars were prominent in the east. The Linguistic data showed that the large seeded chickpea cultivar first arrived in India via Kabul, the capital of Afghan and hence given the name Kabuli in Hindi around 200 years back (Maesen, 1972). The chickpea seeds with dark seed coat and smaller seeds are known as desi or local type. Vander Maesen, 1987 reported that there are 43 species of Chickpea. Nine of which have been annualy cultivated, 33 are perennial and one more is unspecified. Chickpea is the major cultivated pulse crop in India and widely grown in dried regions of India. Main Chickpea producing states are Madhya

Pradesh, Uttar Pradesh, Rajasthan, Andhra Pradesh and Maharashtra. Madhya Pradesh contributes around 40% of the total Chickpea production in India around 6 million tons followed by Uttar Pradesh and Rajasthan that contributes 16% and 4% respectively of the total production in India(Tiwari et al. 2016). Chickpea is cultivated in many parts of the world but in different seasons. 33 countries in West Asia , South Asia, Southern Europe, North Africa, East Africa, Australia, North and South America. The showing period of chickpea is different in different regions due to different in photoperiod, temperature, humidity and precipitations. Chickpea is shown in India in winter season.

2.3 Botanical descriptions Chickpea is herbaceous leguminous plant with height range between 20-100 cm. Some tall cultivars can grow up to 130 cm in length in favourable conditions (Reddy et al.1985). The stem is solid, green, and herbaceous and profusely branched into primary, secondary and tertiary branches (Cubero,1987). Based on the growth habits, five different type of growth habit exhibits in Chickpea. They are erect, semi-erect, spreading, semi-spreading and prostrate (Pundir et al. 1985). The seed germinates at an optimum temperature of 25- 33˚C with hypogeal germination. Plumule developes into erect shoots and the radicle grows positively geotropic to give rise to well-developed root system. The primary root system develops and produces long lateral roots. The first leaf has 2-3 pairs of leaflets with a terminal one. Leaves are compound, unipinnate and shows alternate phyllotaxy. A single rachis contains 11-13 leaflets and is petiolate. Leaflets are 8-17 mm long and 5-14 mm and a terminal leaflet with serrated leaf blade and are pubescent. Flowers are solitary and papilionaceous borne on the axillary raceme both have pedicle and peduncle of 6-13 mm long. Flowers are pink to purple in the desi type and white in the kabuli type as shown in the figure 2. Chickpea have one carpel per flower.

(A) Himchana (B) ICC 4958 (C) JGK-03 Fig No.2.1: Flowers of Chickpea cultivars. (A) & (B) are the desi type and (C) kabuli type. Pods of chickpea are inflated, several post–fertilization changes occurs for 5-6 days to give rise to pod. The pods are green, hairy and glandular with three distinct layers of wall - Outer exocarp, middle mesocarp comprising with 6-8 parenchymatous layer and inner endocarp layer. The inner endocarp layer have further two layers – fibrous layer(3-4 layers of fibres) and inner parenchymatous layer with 5-6 layer of parenchyma (Cubero, 1987). Pod may be ovate, rhomboid or oblong in shape with a great variability in size. Each pod consists of two to three seeds. Pod filling is greatly affected by different biotic and abiotic stress.

Fig No.2.3: Pods of the three cultivars of Chickpea. Chickpea seed consists of thick seed coat with two layers of outer testa and inner integument. The seed coat is thick and green in the young stage and during the maturation process the seed coat gets thinned and its colour fades way to light yellow to dark brown. Seeds may be angular, beaked and wrinkled. The colour of the seed and its weight varies from variety to variety. Seed matures through different developmental stages that can be visualized morphologically and biochemically. The molecular physiological mechanismof the development of embryo and its genetics and seed storage were well documented in Arabidopsis, legumes and cereals by Sreenivasulu and Wobus, 2013. This study reveals the level of metabolite production during seed maturation.

The development of seed and its various maturation stages have been described in soybean by Goldberg et al., 2007 and have been shown in the fig. no.2.4.

Fig. No. 2.4 The development of seed and its various maturation stages in soybean.(Adapted from Goldberg et al., 2007) 2.4 Secondary metabolites in plants Plant Secondary Metabolites have been classified into three groups – Phenolics, Terpenes and Nitrogen containing secondary metabolite (Alkaloids). The biosynthesis of plant secondary metabolites has been shown in the fig. no. 2.5 that shows that secondary metabolites are formed from the product of primary metabolism. Plant phenolics are the product of shikimik acid pathway and malonic acid pathway. Nitrogen containing secondary products are formed from shikimic acid pathway and terpenes are formed by mevalonic acid pathway and MEP pathway.

Fig No. 2.5: Biosynthesis pathway of plant secondary metabolites. Designed using ChemDraw13 (Addapted from Taiz and Zeiger. Plant Physiology, 1991).

Phenolics are plants secondary compounds which have one or more hydroxyl groups bonded to an aromatic benzene ring. Phenolic compounds are grouped into simple phenols and polyphenols that have increased number of phenol units in its molecule. More than 8000 naturally occurring phenolics have been reported in plants (Sulaiman et al., 2013). Plant phenolics acts as antimicrobial, anticarcinogenic, antihypertensive, antioxidant and antimutagenic. They also have the ability to alter the expression of gene. Phenolics are considered to be the largest group of plant secondary metabolites that have antioxidant properties. Phenolics also act as metal chelators, hydrogen donors, radical scavengers due to its high antioxidant property. Phenolics were further has been divided based on its carbon skeleton shown in the table 2.1.

Table 2.1 : Classification of Plant Phenolic Compounds based on carbon skeleton (Cheynier et al., 2013). Basic skeleton Type of Phenolics C6 Simple Phenols and Benzoquinones C6-C1 Aldehydes & Phenolic acids C6-C2 Phenylacetic acids & Acetophenones, C6-C3 Hydroxycinnamic acids, coumarins, phenylpropanes, and chromones C6-C4 Naphthoquinones C6-C1-C6 Xanthones C6-C2-C6 Anthraquinones & Stilbenes C6-C3-C6 Flavonoids, Isoflavonoids and Neoflavonoids (C6-C3-C6)2,3 Bi-, Tri- flavonoids, Proanthocyanidin dimers, trimmers (C6-C3)2 Lignans and Neolignans (C6-C3)n Lignins (C6)n Catechol melanins, Phlorotannins (C6-C3-C6)n Condensed tannins

Flavonoids are the major group of Plant Phenolic compounds that structure consists of two aromatic benzene ring bonded by a 3-Carbon bridge. Flavonoids are found in different tissues of plants as in free-state and as glycosides. Flavonoids are responsible for the coloration of the flower, plant defence against pathogens and herbivores, UV protection and legume nodulation. Moreover, flavonoids also acts as nutraceuticals products as it has anticancer, antiulcer, antiarthritic, anti-angiogenic, anti-allergic, anti-inflammatory, antithrombotic, inhibition of protein kinase and mitrochondrial adhesion inhibition activities (Tabesum et al., 2016). Based on the degree of oxidation of the 3-C bridge, Flavonoids has been further divided into four types, viz. Anthocyanins, Flavones, Flavonols and Isoflavonoids. Anthocyanins are glycoside member of the flavonoid family. Its structure has a sugar moiety at 3 carbon position and without this sugar moiety they are known as Anthocynidins. Anthocyanin is responsible for the blue, red, purple, yellow pigmentation of flowers, stems and fruits. Anthocyanin attracts the pollinators, helps in seed dispersal, UV protection and during several stresses. The colour of the Anthocyanin depends on the number of methoxy and hydroxyl group in the B ring of Anthocynidin molecule, presence of flanone and flavonol as co-pigments, presence of iron and aluminium as a chelating agents and the vacuole PH in which Anthocynidin have been stored(Taiz and Zeiger, 1991).

CHAPTER-3 MATERIALS AND METHODS

3.1 Sample Selection Three cultivars of Chickpea plants were selected for the study. The three cultivars of chickpea include two desi cultivars and one kabuli cultivar. (A) Desi Cultivar includes- ICC 4958 and Himchana. (B) Kabuli Cultivar include- JGK-03.

3.1.1 Sample for anthocyanin estimation The seed of three cultivars of chickpea were sowed in the field area of university campus at same time and samples were harvested at same time from similar healthy plants. The first emerged not fully developed (in length) leaf was considered as the first leaf. 3rd, 5th and 7th leafs were selected for the estimation of anthocyanin contents with reference to the first leaf. Separated flowers and pedicle of the three chickpea cultivars were also selected for comparative study of anthocyanin contents.

3.1.2 Harvesting of seed of different developing stages Several morphological characteristics have been optimized and considered for the harvesting of seed samples of different developing stages. The first parameter was Pod related changes. Pod is outer covering of seed which undergoes several developmental phases that result in colour change, loss of water content and stiffness. The second parameter was the visible changes in the behaviour of seed coat. Seed coat is outer covering of seed. Young seeds have green and thick seed coat. As they mature, their colours fade away to yellowish brown and their thickness start decreasing. The third parameter was Seed cotyledon characters. Young cotyledons are soft and light yellow to light green in colour. During maturation, it turns greener and then yellow. On the basis of these characters, four stages were selected for harvesting - A. Early Maturation stage (300-500 mg) B. Mid maturation Stage (500-700 mg) C. Late Maturation stage (400-600 mg) D. Healthy Dry seeds (200-400 mg)

3.1.3 Total phenolics content assay and metabolomics study Fully dried, mature and healthy seeds of equal size and weight of the above mentioned cultivars were used for TPC analysis and other investigations. The seeds were grinded into fine powder and used for the studies.

3.2 Methods 3.2.1 Estimation of anthocyanin contents Freshly collected sample were taken and washed with distilled H_2O and dried using tissue paper. Only 100 mg plant sample (leaf, flower and pedicle separately) was weighted. Samples were homogenised in 5ml of 95% methanol: 1N HCl, (9:1). Incubated for 4 hours at 4˚C and centrifuged at 10,000 rpm for 30 min. The reading was taken at 530nm and 657nm (Rabino et al., 1986, Mancinelli et al., 1974, kelefetoglu et al., 2009, Laby et al., 2000, & Yin et al. 2012) using a UV double beam spectrophotometer (UV-1800, Shimadzu, Japan). Anthocyanin content were calculated using the formula QAnthocyanin= (A530 – 0.25 x A657) x (weight of the plant tissue used) -1.

QAnthocyanin is the corrected value of absorption correlated linearly with the concentration of anthocyanin (Yin et al. 2012). Samples were analysed in biological triplicates.

3.2.2 Harvesting seeds of different maturation stages

The seed of different maturation stage were selected and harvested. Except the dry and mature seeds, the cotyledon and seed coat of all the seed samples were separated and packed with aluminium foil separately and fixed in liquid nitrogen to stop its metabolic activities. All the fixed samples were stored at -80˚C till use for further experiment.

3.2.3 Estimation of total phenolics contents Dried seeds were grinded into fine powder using electric mixer-grinder. 10 mg of powdered sample was separately extracted in two different solvents. Solvent 1 was 50% acetone and solvent 2 was 70% methanol (Xu et. al., 2007 and Segev et. al., 2010). Sample was shaken at 300 rpm for 3 hours and kept overnight for complete extraction. The sample was then centrifuged for 10 mins at 3000 rpm using Centrifuge 5430R eppendorf and the supernatant was used as sample solution. Total phenolics content was estimated by Folin- Ciocalteu assay (Singleton et al. 1965, Singleton et al., 1999 and Xu et. al., 2007). Due to the presence of phosphomolybdate and phosphotungstate, Folin-Ciocalteu reagent becomes highly sensitive and form blue coloured complex in basic medium by the reduction of the phenols (Tabasum et al., 2016). Only 50μl of sample solution was dissolved in 3ml of distilled water, 250μl of Folin Ciocalteu reagent and 750μl of 7% Na2CO3 in centrifuge tube and vortexed and incubated for 8 minutes at room temperature. To the mixture 950 μl of d.H20 was added and allowed to incubate for 2 hours (Xu et. al., 2007). The reading was taken at 765nm using d.H2O as blank by using UV Spectrophotometer (Micro plate reader, Synergy/H1). TPC was calculated as Gallic acid equivalents (mg of GAE/gm sample) using the standard calibration curve (y = 0.2119x - 0.0327 & R^2 = 0.9882).

3.2.4 Samples analysing using GC-MS. 3.2.4.1 Sample preparation Seed samples were grinded using electric mixer-grinder to fine powdered sample. 100mg of the sample was separately extracted in to two solvents, 100% chloroform and 100% methanol in conical flasks. The flasks were covered with aluminium foil and left for overnight. The samples were then sonicated for 1 hour at 45˚C using Ultra Sonicator (Ultra Sonicator, Citizen). The solution was filtered using Whatman filter paper 1 for two times. The extract solvents were completely dried using rotor evaporator (Rotot Evaporator, LabIndia) and dissolved in DMSO. The dissolved sample was filtered using filter syringe and the filtrate was taken on GC vials for analysis.

3.1.4.2 GC-MS Analysis For the analysis of the metabolites GCMS – QP2010 Ultra, Shimadzu, Japan instrument was used. To analyse the samples the temperature of column and injection were set up at 40˚C and 250˚C respectively. The flow control mode was kept in linear velocity where split injection mode split ration was of 5. The column was allowed to flow at 1.24 ml/ min with helium gas. Oven temperature program was maintained as - temperature was kept at 40˚C for 3 mins (hold time), the temperature was increased to 220˚C at the rate of 4 fold 5 minutes hold time and the temperature was further increased to 250˚C at the rate of 15 for 5 minutes hold time. For MS analysis the ion source temperature was kept at 200˚C and interface temperature was kept at 260˚C. Starting mass/charge (m/z) ratio was 50 and ending m/z ratio was 800 i.e. 50-800 m/z. The data were analysed with the chromatograms obtained.

3.2.5 Statistical analysis All the experiments was performed in three replicates and the results were expressed as arithmetic mean ± standard deviation.

CHAPTER- 4 RESULTS AND DISCUSSIONS

4.1 Comparative analysis of anthocyanin in the contrasting Chickpea cultivars 4.1.1 Himchana has higher anthocyanin content than other cultivars Relative quantity of anthocyanin was found to be higher in the desi cultivars as compared to the kabuli cultivar. Among the desi cultivars, Himchana yields the highest amount of anthocyanin. Anthocyanin concentration increases from third position leaf to seventh position leaf in all the three cultivars. The comparative data is shown in the fig. no. 4.1.

Anthocyanin (A530 – 0.25 x A657)/ g

Leaf Sample

ICC4958 0.27414640249326683 0.15827849927685481 8.3229101480992232E-2 0.27414640249326683 0.15827849927685481 8.3229101480992232E-2 L3 L5 L7 2.6 2.7541666666666664 2.8041666666666671 HIMCHANA 0.6101144018406186 0.27960165116346042 2.8867513459481443E-2 0.6101144018406186 0.27960165116346042 2.8867513459481443E-2 L3 L5 L7 3.3291666666666671 3.5708333333333333 3.3791666666666669 JGK 03 0.53536397276370029 9.464847243000396E-2 0.13129959380490597 0.53536397276370029 9.464847243000396E-2 0.13129959380490597 L3 L5 L7 2.5708333333333333 2.3458333333333332 2.6166666666666671

Fig. No. 4.1: Comparative anthocyanin content in selected leaf sample of the contrasting Chickpea cultivars. Red colour shows the concentration of himchana. Blue shows the concentration of ICC4958 and green colour shows that of JGK-03. L3, L5 and L7 were the 3rd, 5th and seventh leaf respectively. Error bars represents arithmetic mean ± standard deviation calculated from triplicates. The overall anthocyanin content in all the 3rd, 5th and 7th leaf (fig.no. 4.2) in himchana was also found to be higher (3.426389 mg/g F.W.) followed by ICC4958 (2.719445 mg/g F.W) and JGK-03 (2.511 mg/g F.W) respectively as shown in the fig. no. 4.3. Concentration of anthocyanin increases from the young 3rd leaf to 5th leaf and slightly decreases in the 7th leaf. The data so obtained showed that the desi varieties of chickpea are rich in anthocyanin content. Among the desi cultivars, himchana has 1.259 fold higher anthocyanin contents than ICC4958 in the leaf sample. Both Himchana and ICC4958 have 1.36 and 1.08 fold higher anthocyanin contents as compared to the kabuli cultivar, JGK-03 in the leaf sample.

Fig.No. 4.2: Leaf sample used for anthocyanin content estimation.

Anthocyanin (A530 – 0.25 x A657)/ g Average amount Himchana ICC4958 JGK-03 3.4263889999999999 2.7194449999999999 2.5110000000000001

Fig.No. 4.3: Average anthocyanin contents in the leaf samples of the three cultivars of chickpea. 4.1.2 Comparative anthocyanin content in the floral organs of the Chickpea cultivars 4.1.2.1 Morphological study of the flower organs The difference in colour of the flower and pedicle of the three cultivars of chickpea (fig no.4.4) due to the difference in anthocyanin concentration can be visualised morphologically. As anthocyanins are responsible for the colouration of the flower (Chalker- Scott, 1999 and Hamilton et al., 2001), its concentration was visualized to be higher in Himchana flower followed by ICC4958. JGK-03 flower showed white colouration and thus have very negligible amount of anthocyanin. Moreover, pedicle of ICC4958 has more deep red colour than the pedicle of Himchana which was also due to the difference in anthocyanin concentration. 4.1.2.1 Biochemical study of the floral organ As in the leaf samples, the amount of anthocyanin was found to be higher in Himchana in both flower and pedicle (fig.no. 4.4) followed by ICC4958 and JGK-03. However, anthocyanin concentration of pedicle was found to be higher than the flower in ICC4958 cultivar. Anthocyanin in JGK-03 was found to be very little and is negligible as shown in the fig no. 4.5.

B

Flower

A

Flower

Pedicle

Pedicle

C

Fig. No. 4.4- The floral organs used for anthocyanin content estimation. (A) Flower and pedicle of himchana. (B) Flower and pedicle of ICC4958 and (C) Flower and pedicle of JGK-03.

Anthocyanin (A530 – 0.25 x A657)/ g

FLOWER 0.18929694486000878 0.25041632002194519 7.9385662013573513E-2 0.18929694486000878 0.25041632002194519 7.9385662013573513E-2 ICC4598 HIMCHANA JGK 03 15.945833333333331 26.241666666666664 0.13333333333333339 PEDICLE 0.24968730444297585 0.14415703243338737 2.1650635094610977E-2 0.24968730444297585 0.14415703243338737 2.1650635094610977E-2 ICC4598 HIMCHANA JGK 03 17.524999999999999 21.262499999999999 7.5000000000000067E-2

Fig. No. 4.5- Anthocyanin contents in flower and pedicle of the Chickpea cultivars grown in the university campus field. Blue colour indicates the flower tissue and the red colour indicate the pedicle tissue. Error bars represents arithmetic mean ± standard deviation calculated from triplicates. 4.2 Changes in characters of seed of different maturation stages Seed maturation and development is a characteristic change that seed undergoes and categorized in three main events, embryogenesis, filling of nutrients and desiccation. Embryogenesis involved in developmental changes like globular stage, heart shaped, torpedo stage and cotyledon stage. Nutrient filling includes the maturation phases like early maturation (EM), mid maturation (MM) and late maturation (LM). Desiccation includes the dry and mature seed. The young seed are present at the tip of the branch as chickpea shows raceme inflorescence.

Table 4.1 Developmental changes in pod morphology during maturation stages in chickpea cultivars

Stages

Pod characters Cotyledon Early Maturation Mid Maturation Late Maturation Dry & Mature Pod wall Highly hydrated Well hydrated Hydrated

Less hydrated Papery

Colour Deep green Deep- slightly light green Light green Pale green Brown

Pod stiffness Soft Tip stiff Whole pod stiff Highly stiff Loose

In the cotyledon stage the colour of the pod was deep green and its wall was highly hydrated and soft and located at the terminal end of the twig. Seed coat colour was dark green, seed very small, hydrated and whitish in colour. In the early maturation stage, the pod was flappy, stiff at tip, smooth, well hydrated and slightly light green. Seed coat colour was also intense green, seed was soft and its size got increased slightly. Whole pod became stiff, light green in the MM stage. The pod size got increased due to the seed nutrient filling. Pod turns slightly light green in colour but cotyledons turned greener. The pod in the LM stage the pod became less hydrated, pale green and stiff because of the increased seed size. Seed coat turned pale and got dehydrated and thickness got reduced. Seed coat was difficult to separate in this stage and size of the seed got decreased and colour of the seed became yellow. In the mature dry stage the pod became dry and brown, seed coat became scally and reddish brown. Seed turns dry and yellow and hard.

Table 4.2 Developmental changes in seed coat morphology during maturation stages in chickpea cultivars

Stages

Seed coat characters Cotyledon Early Maturation Mid Maturation Late Maturation Dry & Mature Thickness Very thick & hydrated Slightly less thick and hydrated Thin and little less hydrated

Papery soft and little less hydrated Light and scally Colour Deep green Deep- slightly light green Light green Pale green Dark-brown

Stiffness Very soft Slightly stiff Stiff Hard Very hard

Table 4.3 Developmental changes in cotyledon morphology during maturation stages in chickpea cultivars

Stages

Cotyledon characters Cotyledon Early Maturation Mid Maturation Late Maturation Dry & Mature Size Very small Small Large, maximum nutrient filling Large, start to dehydrate Small and dry

Colour whitish Green Dark green Light green- yellow yellow

The developmental changes observed in the seed coat of different maturation stages in this study can be closely related to the developmental changes documented by Goldberg et al., 1994 and Wagmaister et al., 2007. The colour of the seed coat turned brown from green. This may be because of the oxidation of secondary metabolites like proanthocyanidins. As the seed matures, seed coat size got increased to provide the surface area for the increasing cotyledon due to nutrient filling. The seeds of different developmental stages were harvested (EM, MM & LM) for making of seasonal biological replicates till further study. Dry seeds so harvested will be used for planting in the next season. The seeds harvested of different maturation stage can be used to study the accumulation of different metabolites in the maturation process.

Table 4.3 Total harvested seeds with their developmental stages Maturation Stage No. of seed of Himchana No. of seed of ICC4958 No. of seed of JGK-03 EM 120 120 120 MM 120 120 120 LM 120 108 108

C

B

A

Fig. No. Harvested dry seeds of chickpea cultivars: (A) Himchana, (B) ICC4958, and (C) JGK-03

4.2 Comparative analysis of the total phenolics content in the contrasting Chickpea cultivars The experiment was performed using two different extracting solvent for total phenolics content. The two solvent used were 50% acetone and 70% methanol. ICC4958 showed the highest amount of phenolics followed by Himchana and JGK-03. Total phenolics content in Himchana, ICC4958 and JGK-03 in 50% acetone extract were 29.8 µg/mg of D.W., 31.21 µg/mg of D.W. and 13.68 µg/mg of D.W. of the sample respectively. The TPC in 70% methanol extract of Himchana, ICC4958 and JGK-03 were 15.29 µg/mg of D.W., 13.688 µg/mg of D.W. and 11.769 µg/mg of D.W. of the sample respectively. The desi cultivars showed higher amount of phenolics than the kabuli one also documented by Singh et al., 1982. Himchana has the highest concentration of anthocyanin among the three cultivars but surprisingly TPC was found to be higher in ICC4958. As anthocyanin also belongs to the group phenolics, the variation in results may be because of the up and down regulation of the biosynthesis of anthocyanin and other phenolics. 50% Acetone showed better solubility of the phenolics than 70% methanol as studied by Singh et al., 2016, Xu et al., 2007 and Segev et al.,2010. The comparison has been shown in fig. 4.3 and table 4.1.

Sample

TPC (µg/mg of D.W.) Solvant 1 Himchana ICC4958 JGK-03 29.797074091552616 31.212836243511092 13.68884694038068 Solvant 2 Himchana ICC4958 JGK-03 15.293377379266948 13.68884694038068 11.76970268994809

Fig. 4.1- Total phenolics content in Chickpea lines. Blue colour denotes TPC in the 50% Acetone extract and the red colour denotes TPC in 70% methanol extract. Error bars represents arithmetic mean ± standard deviation calculated from triplicates. Table 4.1: Total phenolics content of the three chickpea cultivars. Sample TPC in 50% acetone (µg/mg of D.W.) TPC in 70% methanol (µg/mg of D.W.) Himchana 29.8 15.29 ICC4958 31.21 13.688 JGK-03 13.68 11.769

The physical and chemical nature of the samples, property of solvent, solvent-sample ratio, time of extraction and temperature were the few parameters that effects the solubility of sample in an solvent (Naczk et al., 2006, Dai et al., 2010 and Singh et al., 2016). Solubility of the sample compounds is directly proportional to the polarity of the solvents. Polar solvents are being used for the phenolics extraction from plant matrix. For phenolics study 50% acetone was regarded as best extracting solvent because efficiency of the extracting solvent got increased by the mixture of organic solution and water by dissolving the hydrophilic as well as lipophilic compounds in it (Naczk et al., 2006, Dai et al., 2010 and Singh et al., 2016). 4.3 Metabolite analysis in the chloroform extract of the samples by GC-MS 4.3.1 Metabolites analysis in the chloroform extract of himchana In the chloroform extract of himchana a total of 24 metabolites were detected. Among those 8 were fatty acids, 11 were alkanes, 2 were acidic compounds, and one compound each of ketone, phenol and alcohol. The tabulated data has been shown in the table 4.2.

Table 4.2: Metabolites detected in the chloroform extract of himchana Sl. No. Compound Name Category Retention Time Area(%) 1. Cyclopropane Alkane 20.624 2.19 2. Dodecane Alkane 20.953 1.22 3. 3-hexadecane Alkane 27.859 6.22 4. Tetradecane Alkane 28.134 1.98 5. Phenol Phenol 31.717 9.45 6. E-14-Hexadecenal Fatty Acids 34.322 9.89 7. Hexadecane Alkane 34.549 2.26 8. E-15-Heptadecenal Fatty Acid 40.127 11.23 9. Heptadecane Alkane 40.317 2.11 10. Dibutyl phthalate Pthalic Acid 44.330 0.21 11. Pentadecanoic acid Fatty Acid 44.628 2.21 12. 1-Heneicosanol Fatty Acids 45.392 9.66 13. Hepatadecane Alkane 45.547 1.63 14. 9-octadecanoic acid Fatty Acids 48.807 1.44 15. 1-Heneicosanol Fatty Acids 50.469 7.93 16. Nonadecane Alkane 50.635 2.65 17. Benzenemethanol Alcohol 50.775 0.25 18. Cyclohexanone Ketone 54.030 4.51 19. 13-methyltetradec-9-enoic acid Fatty Acids 54.358 1.52 20. Octadecanoic acid Fatty Acids 54.464 1.24 21. Heptadecyl trifluoroacetate Alkane 55.821 5.62 22. Eicosane Alkane 55.928 1.08 23. Trifluroacetic acid Acid 58.118 3.06 24. Cyclopropane Alkane 58.208 1.14

4.3.2 Metabolites analysis in the chloroform extract of ICC4958 In the chloroform extract of ICC4958, a total of 31 compounds were detected. Among those 8 were fatty acids, 13 were alkanes, 4 were alkene and one compound each of phenol, pthalic acid, alkaloid, carbohydrate, ester and acetate. The tabulated data is shown in the table 4.3

Table 4.3: Metabolites detected in the chloroform extract of ICC4958 Sl. No. Compound name Category Retention time Area (%) 1. 2-Dodecene Alkene 12.678 0.15 2. 3-tetradecene Alkene

20.627 2.13 3. Dodecane Lipids 20.958 128 4. 2-octadecane Alkane 27.867 6.26 5. Dodcane Alkane 28.139 2.12 6. Phenol Phenol 31.720 9.67 7. 5-Eicosene Alkene 34.333 9.96 8. Hexadecane Alkane 34.553 2.47 9. Tridecane Alkane 34.692 0.19 10. E-15-Heptaadecenal Fatty Acids 40.142 11.34 11. Hexadecane Alkane 40.323 2.33 12. Eicosane Alkane 42.983 0.10 13. 7,9-Di-teri-butyl-1-oxaspiro(4,5)decane Alkane 43.070 0.29 14. Dibutyl phthalate Phthalic Acid 44.332 0.32 15. Pentadecanoic acid Fatty Acid 44.654 3.31 16. 2-Hexadecene Alkene 45.242 0.32 17. E-15-Heptadecenal Fatty Acid 45.405 10.99 18. Heptadecane Alkane 45.553 2.06 19. Undecane Alkane 45.733 0.17 20. 11,14-Eicosadienoic acid Fatty Acid 48.809 0.77 21. 2-Hexadecane Alkane 50.308 0.25 22. Heptadecane Alkane 50.644 1.59 23. Palmidrol Alkaloid 54.200 2.26 24. Hexadecanoic acid Fatty Acid 54.373 0.94 25. Octadecanoic acid Fatty Acid 54.458 0.89 26. 2,3-anhydro-d-mannosan Carbohydrate 54.550 0.07 27. Heptadecyl trifluroacetate Ester 55.827 5.83 28. Heneicosane Fatty Acid 55.932 1.08 29. Bicyclo[3,1,1] heptane-3-ol Alkane 56.102 0.12 30. 1,3,5-trisilacyclohexane Alkane 57.446 0.59 31. Octacosyl trifluroacetate Acetate 58.115 1.04

4.3.3 Metabolites analysis in the chloroform extract of JGK-03 In the chloroform extract ofJGK-03, a total of 28 metabolites were detected. Among those 7 were fatty acids, 14 were alkanes, 2 were alkene and one compound each of phenol, ester, ketone, alcohol and triterpene. The tabulated data is shown in the table 4.4

Table 4.4: Metabolites detected in the chloroform extract of JGK-03 Sl. No. Compound name Category Retention time Area (%) 1. Cyclopropane Alkane 12.680 0.36 2. Decane Alkane 13.042 0.13 3. Cyclopropane Alkane 20.629 2.63 4. Dodecane Alkane 20.958 1.49 5. 5-Octadecane Alkane 27.865 5.93 6. Tetradecane Alkane 28.139 1.86 7. Phenol Phenol 31.713 8.81 8. 5-Eicosene Alkene 34.325 9.10 9. Hexadecane Alkane 34.552 2.14 10. 2-bromo dodecane Alkane 37.505 0.18 11. E-15-Heptadecenal Fatty Acids 40134 10.17 12. Heptadecane Alkane 40.320 2.16 13. 7,9-Di-tri-butyl-1-oxaspiro(4,5) decane Alkane 43.073 0.26 14. Dibutyl phthalate Ester 44.333 0.38 15. n-Hexadecanoic acid Fatty Acid 44.649 4.33 16. E-15-Heptadecenal Fatty Acid 45.396 9.96 17. Heptadecane Alkane 45.550 1.84 18. Eicosane Alkane 47.992 0.18 19. 1,E—11,Z-13-Octadecatriene Alkene 48.812 1.49 20. Octadecanoic acid Fatty Acids 49.611 0.64 21. 1-Heneicosanol Fatty Acids 50.475 7.65 22. Eicosane Alkane 50.642 1.42 23. Palmidrol Fatty Acid 54.205 2.39 24. 1-Heneicosanol Fatty Acid 55.825 5.74 25. Nonadecane Alkane 56.437 2.64 26. 1-propanone Ketone 56.959 0.52 27. Cyclohexanol Alcohol 57.447 0.66 28. Squalene Triterpene 48.694 1.52

4.4 Metabolites analysis in the methanol extract of the sample by GC-MS 4.4.1 Metabolites analysis in the methanol extract of himchana In the methanol extract of himchana, a total of 24 compounds were detected. Among those 15 were fatty acids, 3 were alkanes, 2 were vitamin E and one compound each of phenol, ketone, acid and alkene. The tabulated data is shown in the table 4.5. Table 4.5: Metabolites detected in the methanol extract of himchana Sl. No Compound name Category Retention time Area (%) 1. Cyclopropane Alkane 20.624 0.49 2. 9-octadecane Alkane 27.858 1.89 3. Phenol Phenol 31.730 1.04 4. 9-octadecane Alkane 34.315 1.87 5. E-15-heptadecenal Fatty Acid 40.123 2.55 6. Hexadecanoic acid Fatty Acid 43.619 5.80 7. n-hexadecanoic acid Fatty Acid 44.611 1.60 8. E-15-heptadecenal Fatty Acid 45.386 2.27 9. 9,12-octadecadienoic acid Fatty Acid 47.780 34.55 10. 10-octadecanoic acid Fatty Acid 47.940 14.51

11. 10-octadecanoic acid Fatty Acid 48.069 0.94 12. Heptadecanoic acid Fatty Acid 48.599 0.66 13. 9-octadecanoic acid Fatty Acid 48.843 2.14 14. Trichloroacetic acid Fatty Acid 50.467 1.60 15. 2-(2-bromomethyl) cyclohexanone Ketone 54.038 3.04 16. Beta-tocopherol Vitamin E 54.213 5.30 17. Beta-tocopherol Vitamin E 54.353 3.04 18. Methyl16-hydroxy-hexadecanoate Fatty Acid 54.466 1.02 19. 5-eicosene Alkene 55.819 3.21 20. 3-cyclopentylpropionic acid Acid 56.967 1.21 21. Octanoic acid Fatty Acid 57.095 1.17 22. Isopropyl linoleate Fatty Acid 57.311 158 23. Hexadecanoic acid Fatty Acid 57.455 2.50 24. Hexadecanoic acid Fatty Acid 58.321 2.50 4.4.2 Metabolites analysis in the methanol of ICC4958. In the methanol extract of ICC4958, a total of 28 compounds were detected. Among them 18 were fatty acids, 2 were alkenes, 2 were vitamin E, 2 were acids, 2 were ketones and one compound each of phenol and steroid. The tabulated data is shown in the table 4.6 Table 4.6: Metabolites detected in the methanol extract of ICC4958 Sl. No. Compound name Category Retention Time Area (%) 1. 3-tetradecene Alkene 20.585 0.19 2. 5-octadecene Alkene 27.792 0.81 3. Phenol Phenol 31.667 0.49 4. E-14-hexadecenal Fatty Acids 34.250 1.61 5. 8-Pentadecanone Ketone 36.600 0.20 6. E-15-heptadecenal Fatty Acids 40.058 1.65 7. 8-octadecanone Ketone 42.200 0.39 8. Hexadecanoic acid Fatty Acids 43.533 8.68 9. Pentadecanoic acid Fatty Acids 44.550 0.60 10. E-15-heptaadecenal Fatty Acids 45.325 1.10 11. 9,12-octadecadienoic acid Fatty Acids 47.683 46.25 12. 10-octadecaenoic acid Fatty Acids 47.867 27.42 13. 13-octadecaenoic acid Fatty Acids 48.025 1.27 14. Methyl stearate Fatty Acids 48.533 1.03 15. 9,17-octadecadienal Fatty Acids 48.692 1.02 16. Trifluroacetic acid Acid 50.392 0.73 17. Methyl-9-eicosenoate Fatty Acids 53.792 0.22 18. Gamma tocopherol Vitamin E 54.225 3.88 19. Gamma tocopherol Vitamin E 54.362 4.31 20. Methyl18-methylnonadecanoate Fatty Acids 54.457 2.50 21. Thero-4-hydroxy1-homoarginine lactate Fatty Acids 54.575 0.07 22. 1-heptaecosanol Fatty Acids 55.823 0.34 23. 3-cyclopentayl propanoic acid Acid 56.964 1.26 24. 9,12-octadecadienoyl chloride Fatty Acids 57.308 0.38 25. 9,12-octadecadienoyl chloride Fatty Acids 58.319 0.84 26. Hexadecanoic acid Fatty Acids 58.108 0.08 27. Campesterol Steroid 58.775 0.37 28. Heptadecanoic acid Fatty Acids 58.775 0.37 4.4.3 Metabolites analysis in the methanol of JGK-03 In the methanol extract of JGK-03, a total of 29 compounds were detected. Among them 16 were fatty acids, 3 were alkanes, 2 were vitamin E, 4 acidic compounds and one compound each of phenol, ketone, alcohol and alkaloid. The tabulated data is shown in the table 4.7 Table 4.7: Metabolites detected in the methanol extract of JGK-03 Sl. No. Compound name Category Retention time Area (%) 1. 5-tetradecane Alkane 20.620 0.34 2. 5-octadecane Alkane 27.851 1.00 3. Phenol Phenol 31.718 0.75 4. E-14-Hexadecenal Fatty Acids 34.312 2.50 5. 8-octodecanone Ketone 36..636 0.45 6. E-14-hexadecenal Fatty Acids 40.119 2.83 7. Pentadecanoic acid Fatty Acids 42.250 0.60 8. n-Hexadecanoic acid Fatty Acids 43.615 6.87 9. E-15-heptadecenal Fatty Acids 44.611 1.40 10. 9,12-octadecadienoic acid Fatty Acids 45.558 0.15 11. 10-octadecaenoic acid Fatty Acids 47.792 37.68 12. 10-octadecaenioc acid Fatty Acids 47.946 13.66 13. Methyl sterarate Fatty Acids 48.074 0.86 14. 9,12-octadecanoic acid Fatty Acids 45.599 0.88 15. Methyl stereate Fatty Acids 48.813 2.03 16. 9,12-octadecadienoic acid Fatty Acids 48.949 1.70 17. OctanamideE-15-Heptadecanal Fatty Acids 50.463 0.77 18. 1,7-dioxaspiro[5,5]undec-2-en Fatty Acids 54.035 2.33 19. Delta-tocoferol Vitamin E 54.207 5.52 20. Delta-tocoferol Vitamin E 54.352 4.54 21. Cyclopentylpropanoic acid Acid 54.467 0.75 22. Heptadecyl trifluroacetate Acid 55.817 0.58 23. 3-cyclopentylpropanoic acid Acid 56.963 2.64 24. Fumaric acid Acid 57.094 0.93 25. Isopropayl linoleate Fatty Acids 57.311 0.09 26. Cyclohexanol Alcohol 57.446 0.82 27.

Hexacosylheptafluorobutyrate Alkane 58.103 1.29 28. Hexadecanoic acid Fatty Acids 58.322 323 29. Aspidofractinine Alkaloid 58.772 0.92

4.5 Common metabolites present in all the three cultivars in chloroform extract. A total of 17 metabolites are present in common in either two or all the three cultivars of chickpea. Among them 9 metabolites were detected in all the three cultivars of chickpea in the chloroform extract. The tabulated data is shown in the table 4.8 Table 4.8: Metabolites detected in the chloroform extract of all the three cultivars: Himchana, ICC4058 and JGK-03. 'P' denotes presence and 'A' denotes absence of metabolites Sl. No Compound Himchana ICC4958 JGK-03 1. Cyclopropane Present Absent Present 2. Dodecane Present Present Present 3. Tetradecane Present Absent Present 4. Phenole Present Present Present 5. Hexadecane Present Present Present 6. e-15-heptadecenal Present Present Present 7. Heptadecane Present Present Present 8. Dibutyl phthalate Present Present Present 9. Pentadecanoic acid Present Present Absent 10. 1-heneicosanol Present Absent Present 11. Nonadecane Present Absent Present 12. Octadecanoic acid Present Present Present 13. Heptadecyltrifluroacetate Present Present Absent 14. Eicosane Present Present Present 15. 5-eicosene Absent Present Present 16. 7,9-di-tri butyl-1-oxaspiro(4,5) decane Absent Present Present 17. Palmidrol Absent Present Present

4.6 Common metabolites present in all the three cultivars in the methanol extract. A total of 12 metabolites are present in common in either two or all the three cultivars of chickpea in the methanol extract. Among them 6 metabolites were detected in all the three cultivars of chickpea in the methanol extract. The tabulated data is shown in the table 4.9. Table 4.9: Metabolites detected in the methanol extract of all the three cultivars: Himchana, ICC4058 and JGK-03. 'P' denotes presence and 'A' denotes absence of metabolites Sl. No. Compound Himchana ICC4958 JGK-03 1. Phenol Present Present Present 2. 14-hexadecenal Absent Present Present 3. E-15-heptadecenal Present Present Present 4. n-hexadecanoic acid Present Present Present 5. Pentadecanoic acid Absent Present Present 6. 9,12-octadecadieonic acid Present Present Present 7. 8-octadecanone Absent Present Present 8. 10-octadecanoic acid Present Present Present 9. Methylstearate Absent Present Present 10. 3-cyclopentylpropionic acid Present Present Present 11. Heptadecanoic acid Present Absent Absent 12. Isopropyllinoleate Present Present Present

4.7 Common metabolites present in all the three cultivars in both extraction solvent A total of 3 metabolites were detected in both the solvent extract in all the three chickpea cultivars. Out of the three, one metabolite i.e. pentadecanoic acid was detected only in ICC4958 in the two extract solvent but in Himchana, it was detected only in the chloroform extract and in JGK-03 it was detected only in the methanol extract. The tabulated data is shown in the table 4.10

Table 4.10: Common metabolites present in all cultivars in both the extracts Sl. No. Compound Himchana ICC4958 JGK-03 1. Phenol present present Present 2. E-15-heptadecenal present present Present 3. Pentadecanoic acid Absent in methanol extract, present in chloroform extract present Present in methanol extract, absent in chloroform extract Out of 24 metabolites in the chloroform extract of Himchana, the major compounds were E-15-Heptadecinal, a fatty acid with an area percentage 11.23%, phenol (area% = 9.45%), E-14-Hexadecenal (area% = 9.89%) and 1-Heneicosanol (area% = 9.66%). Out of 31 metabolites in

the chloroform extract of ICC4958, the major compounds were E-15-Heptadecinal, a fatty acid with an area percentage of 11.34% and 10.99%, phenol (area%=9.67%) and 5-Eicosene (area% =9.96%). Out of 28 metabolites in the chloroform extract of JGK-03, the major compounds detected were E-15-Heptadecinal (area%=9.96% and 10.17%), 5-Eicosene (area%= 9.10%) and phenol (area%=8.81%). In the methanol extract of Himchana, a total of 24 metabolites were detected. Out of them, the major compounds were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 34.55 and 14.51 respectively. In the methanol extract of ICC4958, a total of 28 metabolites were detected. Out of them, the major compounds were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 46.25 and 27.42 respectively. In the methanol extract of JGK-03, a total of 29 metabolites were detected. Out of them, the major compounds detected were 9,12-Octadecadienoic acid and 10-Octadecanoic acid, both were fatty acids with area percentage of 37.68 and 13.66 respectively. A total of 9 metabolites were commonly detected in the chloroform extract in all the three cultivars of chickpea and 8 metabolites were commonly detected only in the two cultivars in the same extract. In the methanol extract, 6 metabolites were commonly present in all the three cultivars and 6 metabolites were commonly present in only of the two cultivars. Phenol and E-15-heptadecenal were commonly detected in all the three cultivars of chickpea in both the extract solvents and Pentadecanoic Acid was absent in methanol extract but present in chloroform extract of Himchana, present in both the extracts of ICC4958 and present in methanol extract but absent in chloroform extract of JGK-03. The two solvent used for the extraction were of different polarity. Methanol being highly polar and chloroform with less polarity showed significance difference in metabolites extraction. Polar compounds dissolved more in the polar solvents, whereas the less polar one got dissolved in the non-polar solvent.

CONCLUSIONS & FUTURE PERSPECTIVES

Conclusions Chickpea is considered to be the third largest cultivated leguminous crop in the world. The yield of chickpea gets affected by several biotic and abiotic stresses like drought, cold, heat, salinity, etc. plant undergoes many developmental changes in its physiology and biochemistry to up cope with the environment. During the development of seed, the pod, seed coat as well as the cotyledons of the chickpea undergoes several morphological and biochemical changes. Seed filling is high in the mid maturation stage. Chickpea is rich in phenolics and differs in concentration from genotype to genotype. The desi type chickpea cultivar is richer in phenolics content as compared to the kabuli type cultivar. The anthocyanin content was found to be higher in the desi variety Himchana followed by another desi variety ICC4958 and Kabuli Variety, JGK-03. TPC was also found to be higher in the desi variety, ICC4958 followed by himchana and JGK-03. Among the two Solvent used for TPC determination, solvent 1 i.e. 50% Acetone was found to be the best as compared with solvent 2 i.e. 70% Methanol. Different metabolites like Carbohydrate, Fatty Acids, Alcohol, acids, Phenols, Terpenes, Alkaloid, Vitamin E, Steroid, Alkanes, Alkenes, Ketones, Aldehydes, amino acid and Ester of Phthalic Acid has been detected in the Chickpea cultivars by GC-MS analysis. Out of them, 17 & 12 metabolites were found to be common in either two or all the three cultivars in chloroform and methanol extract sample respectively. Out of these, 3 metabolites were detected in both the extract in all the three cultivars.

Future perspective As the desi cultivars of chickpea are rich in phenolics, its biological activity as an antioxidant can be performed using various lines of cancer cells. Metabolomics study with both targeted and non-targeted approach of the seed sample of different maturation stage will definitely reveal the metabolism processes and metabolic pathways. Further, transcriptomics study may be done to know the up and down regulations of the genes responsible for the metabolites production.

Average amount Himchana ICC4958 JGK-03 3.4263889999999999 2.7194449999999999 2.5110000000000001

Solvant 1 Himchana ICC4958 JGK-03 29.797074091552616 31.212836243511092 13.68884694038068 Solvant 2 Himchana ICC4958 JGK-03 15.293377379266948 13.68884694038068 11.76970268994809

ICC4958 0.27414640249326683 0.15827849927685481 8.3229101480992232E-2 0.27414640249326683 0.15827849927685481 8.3229101480992232E-2 L3 L5 L7 2.6 2.7541666666666664 2.8041666666666671 HIMCHANA 0.6101144018406186 0.27960165116346042 2.8867513459481443E-2 0.6101144018406186 0.27960165116346042 2.8867513459481443E-2 L3 L5 L7 3.3291666666666671 3.5708333333333333 3.3791666666666669 JGK 03 0.53536397276370029 9.464847243000396E-2 0.13129959380490597 0.53536397276370029 9.464847243000396E-2 0.13129959380490597 L3 L5 L7 2.5708333333333333 2.3458333333333332 2.6166666666666671

FLOWER 0.18929694486000878 0.25041632002194519 7.9385662013573513E-2 0.18929694486000878 0.25041632002194519 7.9385662013573513E-2 ICC4598 HIMCHANA JGK 03 15.945833333333331 26.241666666666664 0.13333333333333339 PEDICLE 0.24968730444297585 0.14415703243338737 2.1650635094610977E-2 0.24968730444297585 0.14415703243338737 2.1650635094610977E-2 ICC4598 HIMCHANA JGK 03 17.524999999999999 21.262499999999999 7.5000000000000067E-2

[Metadata removed]

Hit and source - focused comparison, Side by Side:

Left side: As student entered the text in the submitted document.
Right side: As the text appears in the source.

Instances from: http://oar.icrisat.org/884/1/63640.pdf

0: http://oar.icrisat.org/884/1/63640.pdf 100%	0: http://oar.icrisat.org/884/1/63640.pdf 100%
It appears that Chickpea cultivars with higher anthocyanin content are relatively more stable and yield	It appears that chickpea cultivars with higher anthocyanin content are relatively more stable and yield

Instances from: http://www.nipgr.res.in/NGCPCG/Taxonomy.html

1: http://www.nipgr.res.in/NGCPCG/Taxonomy.html 72%	1: http://www.nipgr.res.in/NGCPCG/Taxonomy.html 72%
Kingdom Plantae Division Magnoliophyta Class Magnoliosida Order Fabales Family Fabaceae Sub-family Faboideae Genus Cicer Species C. arietinum	Kingdom: Plantae Division: Magnoliophyta Class: Magnoliopsida Order: Fabales Family: Fabaceae (Leguminosae) Subfamily: Faboideae(Papilionaceae) Genus: Cicer Species:C. arietinum