Phytochemical analysis and antimicrobial activity of *Aparajita* (*Clitoria ternatea* Linn.) against rice pathogens

A Thesis

Submitted to the

Assam Agricultural University

In partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (Agriculture)

IN

AGRICULTURAL BIOCHEMISTRY

By

Mr. Abhijit Debnath

Regn. No. 78 of 2016

[Roll No. 2016-AMJ-41]

DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL CHEMISTRY
FACULTY OF AGRICULTURE
ASSAM AGRICULTURAL UNIVERSITY
JORHAT-785013 (ASSAM)

JULY, 2018

ASSAM AGRICULTURAL UNIVERSITY
FACULTY OF AGRICULTURE

CERTIFICATE – I

This is to certify that the thesis entitled **"Phytochemical analysis and antimicrobial activity of *Aparajita* (*Clitoria ternatea* Linn.) against rice pathogens."** submitted to the Faculty of Agriculture, Assam Agricultural University in partial fulfilment for the degree of **Master of Science (Agriculture)** in **Biochemistry and Agricultural Chemistry** is a record of research work carried out by **Abhijit Debnath** under my personal supervision and guidance.

All helps received by him have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

(R. Kandali)
Major Advisor
Professor
Department of Biochemistry & Agricultural Chemistry
College of Agriculture
Assam Agricultural University
Jorhat – 785013

Dated : Jorhat

The …………, 2018

CERTIFICATE – II

This is to certify that the thesis entitled **"Phytochemical analysis and antimicrobial activity of *Aparajita* (*Clitoria ternatea* Linn.) against rice pathogens**.**"** submitted by **Abhijit Debnath**, **Roll No. 2016-AMJ-41** to the Assam Agricultural University, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Biochemistry and Agricultural Chemistry** has been examined and approved by the Student's Advisory Committee after viva-voce.

____
Major Advisor

____
Chairman
Board of Examiner

Members of the Advisory Committee:

1. __________________________
(Member, Major Discipline)

2. __________________________
(Member, Minor Discipline)

3. _____________________________
(Member, Supporting Discipline)

____
Professor & Head
Department of Biochemistry &
Agricultural Chemistry
Assam Agricultural University
Jorhat – 785013 (Assam)

____
Director
Post Graduate Studies
Assam Agricultural University
Jorhat- 785013(Assam)

Acknowledgement

The author with immense admiration takes the privilege to express deep sense of gratitude and indebtedness to his major advisor and chairman of advisory committee, Dr. Ranjan Kandali,, Professor, Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat-13 for rendering kind help, inspiring motivation, invaluable guidance and suggestions during the entire period of the research work and in producing the manuscript.

The author acknowledges his appreciation to Dr. T. C. Sarmah, Professor and Head, Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat and he also takes the opportunity to express his deep sense of indebtedness to the members of his advisory committee, Dr. A. M. Baruah, Professor, Department of Biochemistry and Agricultural Chemistry, Dr. P. Dutta, Scientist, Department of Plant Pathology and Mrs. B. Neog, Assistant Professor, Department of Agricultural Statistics, Assam agricultural University, Jorhat for their genuine concern, valuable advice and encouragement throughout the investigation. The Author extends his special gratitude and appreciation to Dr. S. Baishya, Professor, Dr. (Mrs) P. Das, Principal Scientist, Dr. R. Sarmah, Professor, Dr. S. Rathi, Assistant Professor, Department of Biochemistry and Agricultural Chemistry, Jorhat and Dr. P. Sen, Professor, Department of Agricultural Biotechnology, Assam agricultural University, Jorhat for their guidance, motivation and help in completion of the entire period of study and research work.

The author also likes to offer sincere thanks and gratitude to Dr. C. Hazarika, DPGS, Assam Agricultural University, and all the staff members of the directorate for their valuable suggestions and all kinds of helps towards completion of the M. Sc (Agril.) degree programme in Agricultural Biochemistry.

The author sincerely acknowledges his indebtedness to all the faculty members of Department of Biochemistry and Agricultural Chemistry and laboratory, Assam Agricultural University, Jorhat for their benevolent and enthusiastic support and guidance throughout author's study period in this University. The author takes the privilege of extending his hearty gratitude for the laboratory assistants and office staff of the Department of Biochemistry and Agricultural Chemistry and laboratory, Assam Agricultural University, Jorhat for their help and co-operation throughout the study.

The author tenders his deep sense of gratitude to all the members of Nanotechnology laboratory, Department of Plant Pathology and RNA Laboratory, Department of Agricultural Biotechnology, Assam agricultural University, Jorhat.

The author asserts his deep mindful, indebtedness express his warm sense of appreciation and obligation to his beloved parents, dear sister and teachers for their ineffable moral support, silent sacrifice, blessings, unrelenting encouragement and for being a part of strength and mental support without which it could not have been possible for his to bring this mammoth task to completion.

The author wishes to thank whole heartedly to his Seniors Poulomi Das, Pratul Kumar Nandy, Suman Natta, Rajesh Banik, Utpal Roy, Dweepjyoti Sarkar, Pranjal Kaman, and his classmates Kuldeep, Benjamin, Madhusmita, Ilakshi, and Oliva for their togetherness and care during the entire course of study.

The Author bestows his heartiest thanks to Biswajit Bordoloi for creative guidance and taking pain in final preparation of manuscript meticulously within a short period of time.

At last but not the least, the author expresses his sincere thanks to Almighty God, the invisible hand that holds and leads his in every step of life.

Dated, Jorhat

The …… July, 2018 *(The Author)*

ABSTRACT

Clitoria ternatea Linn. commonly known as butterfly pea and *Aparajita* belongs to the family fabaceae. It was originated from tropical Asia and afterwards widely distributed to African countries. Generally it is grown as ornamental plant due to its attractive flower colour. In traditional ayurvedic medicine, it has been used for centuries as a memory enhancer, antistress, anxiolytic, antidepressant, anticonvulsant, tranquillizing and sedative agent. The tribal people of Tripura use leaf and root part of *Aparajita* against urinary tract infections due to its antimicrobial property.

The present investigation was intended to study the phytochemical composition and antimicrobial activity of *Clitoria ternatea* Linn against rice pathogens. The plant materials were collected from Jagduar, Teok, Jorhat, Assam and Matabari, Gomati District, Tripura and authenticated. The morphological data were taken from the mature plant to narrate the botanical information. Leaf, stem and twig samples of this species were analyzed for total alkaloids, total phenolics and total terpenoid content by standard protocol. The antimicrobial activity of the methanolic plant extract has been tested against some rice pathogens by poison food technique and the natural food colorant from the flower of *C. ternatea* L extracted.

From the results of the present investigation it was observed that the difference in plant morphological characteristics in the two samples was not significant and found statistically at par. The variation in moisture content on fresh weight basis between the two samples *viz*. Assam (S_1) and Tripura (S_2) of *Clitoria ternatea* L. was not significant. On the other hand, the highest moisture content on dry weight basis was found in flower of S_1 collected from Assam which was 13.70% while the leaf recorded the lowest 8.82% of moisture. The total phenolics content recorded in the leaf were 1.524g/100g and 1.277g/100g, in stem 0.706g/100g and 0.682g/100g, and in twig 1.110g/100g and 1.209g/100g in S_1 and S_2 sample, respectively. The alkaloid content of the leaf was found to be 1.000g/100g and 0.875g/100g, in stem 0.753g/100g and 0.627g/100g, and in twig 1.627g/100g and 1.253g/100g in S_1 and S_2 sample, respectively. By the qualitative test it was observed that the terpenoid was absent in leaf, stem, and twig in the both Assam (S_1) and Tripura (S_2) sample but was present in flower of both the sample although the variation was not significant. The total terpenoid content of flower was found to be 0.698g/100g and 0.675g/100g in Assam (S_1) and Tripura (S_2) sample respectively. The antimicrobial activity of methanolic plant extracts of *Clitoria ternatea*

L. was evaluated against *Magnaporthe grisea*, *Drechslera oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae* and *Fusarium* sp. Although the methanolic plant extract of *C. ternatea* L could not inhibit the mycelia growth of *Drechslera oryzae* but the extract proved effective in inhibiting the growth of other remaining pathogens *viz Magnaporthe grisea*, *Rhizoctonia solani*, *Sarocladium oryzae* and *Fusarium* sp.. Highest inhibition of growth was recorded in *Rhizoctonia solani*. The natural food colorant has been extracted from *Clitoria ternatea* L. flower. Variation in color has been observed at different pH. The changes in pH were brought about by adding lemon juice and alkali extracted from *vimkol*. To immobilize the color on to edible inert carrier food material, glucose powder was effective in color immobilization.

Further studies will be required for assessing mode of antimicrobial activity of the methanol extract on the plant pathogens, which will open an interesting area to manage various diseases in crop plants under organic cultivation. More studies will be required to find out the efficacy of the plant extract against other pests. The study further suggested that some of the plant extracts possess compounds with bioactivity properties that could be used as active principles or agents in new drugs for the therapy of infectious diseases. The food colour extracted from the flower of *C. ternatea* L. could be an ideal natural colorant for the food industry in future.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I	INTRODUCTION	1-12
II	REVIEW OF LITERATURE	13-29
III	MATERIALS AND METHODS	30-43
IV	EXPERIMENTAL FINDINGS	44-68
V	DISCUSSION	69-84
VI	SUMMARY AND CONCLUSION	85-87
	BIBLIOGRAPHY	88-99
	APPENDIX	I

LIST OF TABLES

Table No.	Title	Page No.
1	Morphological characteristics of *Clitoria ternatea* Linn.	46
2a	Total moisture content of *Clitoria ternatea* Linn. Samples (fresh weight basis)	47
2b	Total moisture content of *Clitoria ternatea* Linn. Samples (dry weight basis)	48
3	Total phenolic content of *Clitoria ternatea* Linn. samples	50
4	Total alkaloid content of *Clitoria ternatea* Linn. samples	51
5	Total terpenoid content of *Clitoria ternatea* Linn. samples	52
6	Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Magnaporthe grisea*	56
7	Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Drechslera oryzae*	57
8	Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Rhizoctonia solani*	58
9	Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Sarocladium oryzae*	59
10	Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Fusarium* sp. (*Gibberella fujikuroi*)	60

LIST OF FIGURES

Figure No.	Title	Page No.
1	Total moisture content in different parts of *Clitoria ternatea* Linn. (g/100g) fresh weight basis	72
2	Total phenolics content in different parts of *Clitoria ternatea* Linn. (g/100g) on dry weight basis	73
3	Total alkaloid content in different parts of *Clitoria ternatea* Linn. (g/100g) on dry weight basis	75
4	Total terpenoid content in flower of *Clitoria ternatea* L. (g/100g) on dry weight basis	76
5	Percent inhibition of mycelial growth of *Magnaporthe grisea*	80
6	Percent inhibition of mycelial growth of *Rhizoctonia solani*	81
7	Percent inhibition of mycelial growth of *Sarocladium oryzae*	82
8	Percent inhibition of mycelial growth of *Fusarium* sp. (*Gibberella fujikuroi*)	83

LIST OF PHOTOS

Plate No.	Title	Page No.
1	Mature plants of Assam and Tripura sample	32
2	Different parts of *Clitoria ternatea* Linn. (Assam collection)	33
3	Different parts of *Clitoria ternatea* Linn. (Tripura collection)	34
4	Powdered sample of different parts of *Clitoria ternatea* Linn. (Assam collection)	35
5	Powdered sample of different parts of *Clitoria ternatea* Linn. (Tripura collection)	36
6	Methanolic plant extract of Assam and Tripura sample	40
7	Qualitative test for terpenoids in different parts of *Clitoria ternatea* Linn. (A) Assam collection, (B) Tripura collection	53
8	(A) *Magnaporthe grisea* growth under control, (B) Growth of *Magnaporthe grisea* inhibited by plant extract made up from Assam sample, (C) Growth of *Magnaporthe grisea* inhibited by plant extract made up from Tripura sample	61
9	(A) *Drechslera oryzae* growth under control, (B) Growth of *Drechslera oryzae* not inhibited by plant extract made up from Assam sample, (C) Growth of *Drechslera oryzae* not inhibited by plant extract made up from Tripura sample	62
10	(A) *Rhizoctonia solani* growth under control, (B) Growth of *Rhizoctonia solani* inhibited by plant extract made up from Assam sample, (C) Growth of *Rhizoctonia solani* inhibited by plant extract made up from Tripura sample	63
11	(A) *Sarocladium oryzae* growth under control, (B) Growth of *Sarocladium oryzae* inhibited by plant extract made up from Assam sample, (C) Growth of *Sarocladium oryzae* inhibited by plant extract made up from Tripura sample	64

12	(A) *Fusarium* sp. (*Gibberella fujikuroi*) growth under control, (B) Growth of *Fusarium* sp. (*Gibberella fujikuroi*) inhibited by plant extract made up from Assam sample, (C) Growth of *Fusarium* sp. (*Gibberella fujikuroi*) inhibited by plant extract made up from Tripura sample	65
13	(A) Edible food colorant extracted from *Clitoria ternatea* Linn., (B) Concentrated food colorant, (C) Food colorant with carrier material glucose.	67
14	Color variation in different pH by natural alkali and acidic solution	68

ABBREVIATIONS AND SYMBOLS USED

%	:	Percentage
µl	:	Micro liter
cm	:	centimetre
PDA	:	Potato dextrose agar
BOD	:	Biological Oxygen Demand
et al.	:	Et alia
rpm	:	Revolutions per minute
Fig.	:	Figure
gm	:	Gram
hr.	:	Hours
UV	:	Ultra violet
M	:	Molar
mg	:	milligram
ml	:	Milli liter
min	:	Minutes
mM	:	Milli molar
ft	:	Feet
nm	:	Nanometer
°C	:	Degree Celsius
mm	:	Millimeter
lbs	:	Pound
CRD	:	Completely Randomize Design
CD	:	Critical difference
S.Ed	*:*	*Standard error of difference*

CHAPTER I

INTRODUCTION

The North East region of India, known for its varied physiography, climate and soil conditions, harbors a mega diversity of flora and fauna holding approximately 50 per cent of the flora of the Indian subcontinent. The region is a part of the Eastern Himalayas, which is known as a ‘Mega Biodiversity’ center and a ‘Hot spot’ in the world for its rich flora and fauna. This region is homeland of people belonging to different ethnic groups with different cultural entities. Majority of them lives in rural areas still maintaining their individual identities with a primitive state of economic life. These people mainly depend upon plant species available in their area for their day to day needs and thus preserve a good knowledge of traditional health care methods.

Clitoria ternatea Linn. is a perennial climber with attractive blue or white flowers. It is locally known as “butterfly pea”, “*Aparajita*” and “*Shankhapuspi*”. It is traditionally used to treat various ailments. The plant is native to south-east Asia and distributed in tropical Asia including India, the Philippines and Madagascar (Anonymous 1998). Roots, seeds and leaves of *C. ternatea* are commonly used in the Ayurvedic system of medicine to treat different diseases. Extracts of this plant have been used as an ingredient in the Ayurvedic 'Medhya Rasayana' as a rejuvenating recipe used for treatment of neurological disorders and are considered to enhance the intellect (Sharma and Dash 1988). *C. ternatea* L. is one of the important species of plant used throughout the world. *C. ternatea* L. is also known as Blue pea, bluebellvine, butterfly pea, cordofan pea, Darwin pea in English. The other vernacular names are *Aparajita* (Hindi, Assamese, Oriya and Bengali); Gokarni (Gujrati and Marathi); Shankhapushapam (Malayalam); Koyal (Punjabi); Kakkanam (Tamil); Girikarnika Balli (Kannada); Girikarnika, Vishnukranta (Sanskrit) (Al-Snafi, 2016; Gupta *et al*., 2010).

C. ternatea L. is grown as an ornamental plant, because of its attractive flower colors (Gomez and Kalamani, 2003). Besides its aesthetic value, *C. ternatea* is also grown as a highly palatable forage legume which is generally preferred by livestock over other legumes. It exhibits excellent regrowth after cutting within short period and produce high yields. It can be grown with all tall grasses for rotational grazing, hay or silage. The climber yields a useful green fodder throughout the year, particularly during dry period and also dry feed. It is grown either alone or with other perennial grasses in Punjab, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Madhya Pradesh, Andhra Pradesh, Tamil Nadu and Karnataka in India. It is also used as a cover crop, green manure and medicinal plant. The tender shoots, leaves, flowers and tender pods are eaten as vegetable in Kerala and in the Philippines. In Malaysia, the leaves are used to impart a green color to food and the flowers to impart a bright blue color to rice cakes (Mukherjee *et al.*, 2008).

1.1 General characteristics and plant description of *Clitoria ternatea* L.

C. ternatea L. is a vigorous, trailing, scrambling or climbing herbaceous perennial vine. This tropical legume forage grows vigorously as a vine by twining with its slender stems which are sub-erect and woody at the base and the supports comes into contact with or as a creeper on the ground. The plants are 3-5m long and leaves are pinnate, bearing 5-7 elliptical, 3-5cm long leaflets. The flowers are solitary or paired attractive than the green foliage. Its spectacular deep blue flowers are 3-5.5cm long and 2-4cm wide, with a pale yellow or entirely white center, and either notched or rounded at the apex. Flowers are axillary, single or paired. Besides the typical royal blue colour, the other flower colours are white, sky blue, blue-violet, bright purple and mauve. Some flowers even appear in double petaled form, such as *C. ternatea* var. *pleniflora* and *C. ternatea* var. *alba plena*. *C. ternatea* blooms extensively year round in warm, humid and sunny regions worldwide. The fruits are flat, linear, sparsely pubescent pods that dehisce violently at maturity and throw 8-10 dark and shiny seeds. There are numerous ecotypes, agro-types and cultivars that differ in flowers and leaflets. Many cultivars have been bred in Latin America, notably in Cuba and Mexico. *C. ternatea* is a high-quality, protein-rich legume, a "tropical alfalfa" often referred to as a protein bank, that can be grown at low cost. Livestock tend to prefer it over other legumes and grasses and it is therefore much valued as a pasture legume. It is also used for cut and carries feeding systems and cut for hay and silage. A nitrogen fixing legume, *C. ternatea* is used as a green manure. It is a valuable cover crop in rubber and coconut plantations. The young pods are edible and used as vegetables in the Philippines. Butterfly pea is used in fences and in trellises as an ornamental for its showy flowers, valuable for dyeing and in ethno-medicine. All parts of *C. ternatea* contain peptides called cliotides that have potent anti-microbial properties against *Escherichia coli* (Nguyen *et al*., 2011).

1.2 Scientific Classification of *C. ternatea* L.:

The scientific classification of *C. ternatea* is as follows:

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Fabales

Family – Fabaceae

Sub Family – Faboideae

Tribe – Cicereae

Genus – Clitoria

Species – ternatea

Binominal Name – *Clitoria ternatea* L.

1.3 Species diversity, distribution and habitat of *C. ternatea* L

Butterfly pea is belonging to the family of fabaceae. The genus name, *Clitoria*, was derived from the Latin word, *clitoris*, that was coined from the ancient Greek word *kleitoris*, meaning *little hill*. There are several species of genus *Clitoria.* These are *C. albiflora, C. bracteata, C. coelestris, C. parviflora, C. pilosula, C. purpurea*, *C. ternatensium*, *C. spectabilis, C. ternatea, Ternatea vulgaris*. Out of these *C. ternatea* and *C. purpurea* are partially domesticated (Gomez and Kalmani, 2003).

The plant had its origin in tropical Asia. Afterwards, it was widely distributed to African countries, such as Chad, Djibouti, Ethiopia, Somalia, Sudan, Sudan, Kenya, Tanzania, Uganda, Burundi, Cameroon, Gabon, Sao Tome, Zaire, Benin, Cote d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Niger, Nigeria, Senegal, Sierra Leone, Togo, Angola, Malawi, Mozambique, Zambia, Zimbabwe and South Africa. In Asia, it was distributed to Madagascar, Saudi Arabia, Yemen, Iran, Iraq, China Taiwan, Bangladesh, Bhutan, India, Nepal, Pakistan, Sri Lanka, Maldives, Cambodia, Laos; Myanmar, Thailand, Vietnam, Indonesia, Malaysia, Philippines and Singapore. This plant was also distributed in North America, (USA and Mexico); in Southern America (Antigua, Barbuda, Aruba, Bahamas, Barbados, Cayman Islands, Cuba, Dominica, Dominican Republic, Guadeloupe, Haiti, Jamaica, Martinique, Montserrat, Netherlands Antilles, Puerto Rico, St. Kitts and Nevis, St. Vincent and Grenadines), Virgin Islands (British), Virgin Islands (U.S.), French Guiana; in Australia, Northwestern Pacific, South Central Pacific, Southwestern Pacific region (Al-Snafi, 2016).

C. ternatea L. is a fast growing summer legume. It is so fast growing that it can cover the soil within no more than 30-40 days after sowing and yield mature pods within 110 to 150 days. It is naturally found in grassland; open woodland, bush, riverine vegetation, and disturbed places. *C. ternatea* grows within 20°N and 24°S, from sea level up to an altitude of 1600-1800m, and in equatorial Africa up to 2000m. *C. ternatea* does better where average temperature is about 19-28°C and where annual rainfall ranges from 700 to 1500mm. However, it tolerates temperatures as low as 15°C and even some frost as it may re-grow from the stems or from the plant base, provided it is already woody when the frosting occurs. It does well under irrigation but has only a low tolerance of flooding or water logging. It has also some drought tolerance and can grow in places where rainfall is as low as 400-500mm. It can survive a 5-6 month drought in the drier tropics. *C. ternatea* can grow on a wide range of soils but is particularly adapted to shallow, heavy clay and sodic soils (pH 5.5-8.9). It thrives in full sunlight but can also grow under light shade in rubber and coconut plantations. *C. ternatea* is an N-fixing legume and can be used as a ley legume and as green manure. It has proved to be useful in re-vegetation of coal mining sites in central Queensland (Australia).

1.4. Chemical composition of *C. ternatea* L.

From the time immemorial, medicinal plants are traditionally used to treat different ailments and the therapeutic property is dependent on presence of a large number of various phytochemical compounds (Bruneton, 1995). The different medicinal plants produce different secondary metabolites such as free radical scavenging phenolic compounds (phenolic acids, flavonoids, quinone’s, coumarins, lignans and tannins), nitrogenous compounds (alkaloids, amines, betalains), vitamins and terpenoids (carotenoids) (Cai *et al.,* 2003). In different phytochemical screening it has been showed that the *C. ternatea* plants contain tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavanoids, flavonol glycosides, proteins, alkaloids, steroids (Manjula *et al*., 2013; Kamilla *et al*.,2009; Madhu, 2013). The fatty acid profile of *C. ternatea* seeds includes palmitic, stearic, oleic, linoleic, and linolenic acid. Seeds also contained cinnamic acid, anthoxanthin glucoside, a highly basic small protein named finotin, water-soluble mucilage, delphinidin 3, 3', 5'-triglucoside and beta-sitosterol (Al-Snafi, 2016; Vianni and Souto, 1971). Some other substances are also present in seeds are *p*-hydroxycinnamic acid, flavonol-3-glycoside, ethyl-α-d- galactopyranoside, adenosine, 3,5,7,4'-tetrahydroxyflavone, 3-rhamnoglucoside, a polypeptide, hexacosanol, β-sitosterol, γ-sitosterol and an anthoxanthin glucoside (Mukherjee *et al*., 2008). The finotin protein present in seeds has the antimicrobial and insecticidal activity (Kelemu *et al.*, 2004). As *Aparajita* is a leguminous plant it contains nodules in roots, which contain higher amount of plant growth substance such as indole acetic acid, kinetin and gibberelic acid. The root nodules contain free amino acids and amides such as glycine, alanine, valine, leucine, α-aminobutyric acid, γ-aminobutyric acid, aspartic acid, glutamic acid, γ-methyleneglutamic acid, arginine, ornithine, and histidine and γ-aminobutyric acid. Some other chemical compounds that have been isolated and identified from roots were pentacyclic triterpenoids, taraxerol and taraxerone. Ternatins are one of the important blue anthocyanins that are found in the petals of *C. ternatea* flower. The leaves contain β-sitosterol, kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-neohesperiodoside, kaempferol- 3-O-rhamnosyl-(1,6)-glucoside, kaempferol-3-O-rhamnosyl-(1,6)-galactoside and kaempferol-3-O-rhamlnosyl-(1,2)-Ochalmnosyl-(1,2)-O-[rhamnosyl-(1,6)]-glucoside. Lactones *viz*. aparajitin and clitorin from leaves were also reported. The leaves also contain essential oil, colourant and mucilage. The mucilage contains anhydrogalacatan, anhydropentosan, methylpentosan and an alkaloid (Mukherjee *et al*., 2008).

1.5. Traditional medicinal use of *C. ternatea* L.

The history of the use of medicinal plants for the alleviation of diseases by human beings has its origin in primitive times. Already institutionalized to a large extent globally, alternative medicine forms a link between folk medicine and modern Western medicine. Many of the healing concepts date back to well before the advent of modern medicine. Utilization of natural products as pharmacological tools could lead to a number of new major therapeutically active metabolites. Natural products are the sources of most active ingredients to produce medicine in western countries. It has been widely accepted and applied in the drug discovery. From the ancient period of time many drug molecules were obtained from the bio-prospecting of medicinal plants (Mukherjee *et al*., 2009). Apart from human use, many plant species are used in animal husbandry as the primary source of healthcare in India. The reliance on medicinal plants is also due to the cultural preference of different communities. Medicinal plants have strong acceptance in religious activities of the native communities of India, who worship plants in the form of gods, goddesses and minor deities. *Clitoria ternatea* (*Aparajita*), *Origanum vulgare* (Oregano), *Saussurea obvallata* (snow lotus or Brahma Kamal), *Ocimum sanctum* (Holy Basil or Tulsi), *Cedrus deodara* (Cedarwood or Deodar cedar), *Cynodon dactylon* (Bermudagrass or Dubori), *Aegle marmelos* (Bael fruit), *Juniperus communis* (Common juniper), *Nardostachys grandiflora* (Jatamansi or Indian Spikenard), *Zanthoxylum armatum* (Winged prickly-ash or Darmar), *Ficus benghalensis* (Indian Banyan tree) and *Ficus religiosa* (Bodhi tree) are some of the examples of plants highly used for medicinal as well as for religious purposes by the Hindus in Northern India (Kala *et al*., 2006). If a balanced conservation and cultivation of medicinal, aromatic and dye plants can be promoted, the effort will protect human health; help to treat domestic animals to alleviate rural poverty, to address gender imbalances and to improve local economy. India has an ancient heritage of traditional medicine. Indian traditional medicine is mainly based on various systems, including Ayurveda, Siddha and Unani (ASU), with the emerging interest of the world in adopting and studying traditional systems and in exploiting their potential from different healthcare perspectives. Ayurveda is one of the oldest traditional systems of medicine accepted in world (Jaiswal and Williams, 2017). The medicinal system of Ayurveda, evolved in India with a rationale logical foundation and it has survived as a distinct entity from remote antiquity to the present day. The origin of Ayurveda is attributed to Atharva Veda where made several diseases with their treatments are mentioned. Later, from the 6th Century BC to 7th Century AD, there was systematic development of the science and it is called Samhita period. By this time, a number of classical works were produced by several authors and during this period there is evidence of organized medical care (Narayanaswamy, 1981). The Government of India has initiated several attempts to explore the possibility of evaluating these systems for their therapeutic potential as originally practiced, as well as to help generate data to put them in national healthcare programs. The Ministry of Health and Family Welfare, Government of India, has undertaken various initiatives for the development and preservation of these aspects of cultural heritage. The Department of AYUSH (Ayurveda, Yoga, Siddha, Unani and Homeopathy) regulates education and research in these systems. The National Medicinal Plant Board (NMPB), which deals with conservation and research issues in botanicals, is working to address issues in these areas (Mukherjee *et al*., 2009).

C. ternatea L. Is a very well known Ayurvedic medicine that has been used for different purposes. In Ayurvedic science the different parts of *C. ternatea* L. have been widely used as a brain tonic, promoting memory and intelligence as already mentioned. According to 'Medhya Rasayana' there are different medicinal plants which has been used for drug development and *Clitoria ternatea*, *Celastrus panniculatas*, *Acorus calamus*, *Centella asiatica* and *Withania somanifera* are one of the Ayurvedic 'medha' drugs. With the advancement of Ayurvedic tradition and its scientific exploration, several classes of plant species have been studied in order to evaluate their therapeutic potentials and to isolate the lead compounds. *Clitoria ternatea* has witnessed a pharmacological and toxicological evaluation of these claims pointing to some important therapeutic benefits of this traditional drug which has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders and considered to strengthen a person's intellect (Mukherjee *et al.*, 2008).

Clitoria ternatea have an important impact on Indian traditional medicinal science. The juice of whole plant and seeds are used to treat stomatitis, piles, and sterility in females, hematemesis, psychosis, epilepsy, leucorrhea, insomnia and polyurea. The root extracts are bitter, refrigerant, laxative, intellect-promoting, diuretic, anthelmintic, tonic and they are useful in dementia, hemicrania, burning sensations, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy and as a cathartic (Mohan *et al.*, 2014). The root, stem and flower are used against snake bite and scorpion sting (Morris, 1999). The root and seed is used as a 'tonic of the nerves', alternative and laxative (Mukherjee *et al.*, 2008). The flower extract has been used in insect bites and skin disease (Agrawal *et al*., 2007). It has been shown that it has number of pharmacological activities such as possessing anxiolytic, antidepressant, anticonvulsant, anti-stress, sedative, antipyretic, anti-inflammatory, analgesic, anthelmintic and anti-microbial activities. The extract of *C. ternatea* L. has been shown to improve learning ability, enhance memory, and increase apical and basal dendritic branches, and increase acetylcholine content and acetylcholinesterase activity in rats. Due to its use in treating different diseases, *Clitoria ternatea* L. has gained a lot of importance in medicinal industries (Mohan *et al.*, 2014).

In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquillizing and sedative agent. The tribal people of Tripura used leaf and root part of

Aparajita against urinary tract infections, burning sensation in urinary tract, lack of urination, frequent urination (Hossan *et al*., 2010). In Chinese medicine it was used traditionally in an attempt to treat sexual ailments, like infertility and gonorrhoea to control menstrual discharge and also as an aphrodisiac. In animal tests, the methanolic extract of *Aparajita* roots demonstrated nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. The active constituents include tannins, resins, starch, taraxerol and taraxerone. Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *Clitoria ternatea* extract. All these peptides belong to the cyclotide family and activity studies show that cleotides display potent antimicrobial activity against *E. coli*, *K. pneumonia* and *P. aeruginosa*. These peptides have potential to be leading molecules for the development of novel antimicrobial and anti cancer agents.

1.6. Insecticidal activity of *C. ternatea* L.

An insecticide is a substance used by humans to gain some advantage in the struggle with various insects that are considered pests. However, the benefits of insecticide use are partly offset by important damages that may result. There are numerous cases of people being poisoned by accidental exposures to toxic insecticides. Moreover, ecological damage may be caused by the use of insecticides, sometimes resulting in the deaths of large numbers of wildlife. Humans have been using insecticides since a long ago. The Egyptians used unspecified chemicals to combat fleas in their homes about 3,500 years ago, and arsenic has been used as an insecticide in China for at least 2,900 years. Today of course, insecticide use is much more prevalent. During the 1990s, more than 300 insecticides were available, in hundreds of different formulations and commercial products. Almost all insecticides are chemicals. Some are natural biochemicals extracted from plants, while others are inorganic chemicals based on toxic metals or compounds of arsenic. However, most modern insecticides are organic chemicals that have been synthesized by chemists. The costs of developing a new insecticide and testing it for its usefulness, toxicology, and environmental effects are huge, equivalent to at least $20-30 million. However, if an insecticide effective against an important pest is discovered, the profits are also potentially huge. Many plant species produce substances that protect them by killing or repelling the insects that feed on them. It is possible to create effective, natural insecticides from these substances to protect crops. Natural pesticides have many advantages over synthetic ones and may be more cost-effective as a whole, considering the environmental cost of chemical alternatives. Natural pesticides are biodegradable, barely leave residues in the soil and are less likely to harm humans or animals. In addition, they are cheaper and more accessible in less developed countries. The finotin protein that is present in the seeds has insecticidal activity (Kelemu *et al*., 2004). *C. ternatea* extract mixture caused oviposition and feeding deterrence as well as direct toxicity to *Helicoverpa* spp. (Mensah *et al*., 2014). Innovate Ag, a research and development company based in Wee Waa, New South Wales, has secured the intellectual property from its research into the insecticidal and repellent qualities of the butterfly pea against *Helicoverpa* spp., green mirids, green vegetable bugs and whiteflies. The name of the insecticide is SERO-X and first time it has been applied in cotton. Different parts of *C. ternatea* plants shows the most promising mosquito larvicidal activity against three major mosquito vectors *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* (Mathew *et al*., 2009). Leaf and seed extracts of *C ternatea* have some allelochemicals with inhibitory effect on germination and growth of the Mexican poppy (*Argemone mexicana L.*) (Namkeleja *et al*., 2013).

1.7. Antimicrobial and antibacterial activity of *C. ternatea* L.

The silver nanoparticle that has been synthesized by using whole plant extracts of *Apartajita* have the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* (Malabadi *et al*., 2012). The different parts of plant extracts have antimicrobial and antibacterial activity against some Gram-positive and Gram-negative bacteria, yeasts, and molds: Gram-positive bacteria include *Bacillus cereus*, *B. subtilis*, *B. thuringiensis*, *Staphylococcus aureus*, *Streptococcus faecalis*; Gram-negative bacteria include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterobacter aerogens*, *Proteus mirabilis*, *Herbaspirillum* spp.; yeasts include *Candida albicans* and *Saccharomyces cerevisiae*; molds include *Rhizopus* spp., *Aspergilus niger*, *Penicillium* spp. (Kamilla *et al*., 2009; Nigam and Shrivastava, 2013; Darsini and Shamshad, 2015). The seed protein (finotin) has broad and potent inhibitory effect on the growth of various important fungal pathogens of plants, namely *Rhizoctonia solani, Fusarium solani, Colletotrichum lindemuthianum, Lasiodiplodia theobromae, Pyricularia grisea, Bipolaris oryzae and Colletotrichum gloeosporioides.* It also inhibits the common bean bacterial blight pathogen *Xanthomonas axonopodis* pv. *Phaseoli*. The finotin has powerful inhibitory properties against the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus* (Kelemu *et al*., 2004).

1.8. Natural food colorant

Man has always been interested in colors. The art of dyeing is as old as human civilization. In Europe, it was practiced during the Bronze Age. The earliest written record of the use of natural dyes in China comes from year 2600 BC. In Indian subcontinent, dyeing was known as early as in the Indus Valley period (2500 BC) and has been substantiated by findings of colored garments of cloth and traces of madder dye in the ruins of Mohenjodaro and Harappa civilization (3500 BC) (Chattopadhyay *et al*., 2008).

Color is one of the main features of any kind of food items as it enhances the appearance and acceptability of food. During processing and handling the color of food is lost, to make the food commodity attractive to the consumers, synthetic or natural colors are added. There are several types of artificial food colorants are present in the market but biocolorants are now gaining popularity and considerable significance due to consumer awareness because synthetic colorants cause severe health problems. Moreover, biocolorants are prepared from plant origin. The main food biocolorants are anthocyanin, carotenoids, flavanoids, anthocyanidins, chlorophyll, betalain and crocin, which are extracted from plants (Rymbai *et al.*, 2011). The anthocyanin is the largest group of water soluble pigment in the plant kingdom. These are commonly found in flowers and the fruits of many plants. Most of the red, purple, and blue-colored flowers contained anthocyanins. In present day diet, increasing in the dependence on processed foods has become deficient in anthocyanins. The addition of natural anthocyanin extracts, to give colour to processed foodstuffs, could be regarded in this context as maintaining current levels. It is found in plants have a wide range of usage. The different colored pigments extracted from flowers, fruits, and vegetables are traditionally used as dye and food colorant. Apart from natural colorants, some of the anthocyanin rich flowers and fruits have been traditionally used as medicine to treat various diseases. The plant anthocyanins have been widely studied for their medicinal values. Anthocyanins possess antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects, as well as prevention of cardiovascular diseases (Khoo *et al.*, 2017). Among the flavonoids, the anthocyanins are the main colorant molecules, derivatives of pelargonidin provide the basis for orange-red hues, derivatives of cyanidin for red hues and derivatives of delphinidin for lilac to blue hues.

Most naturally occurring anthocyanins occur as a glycoside containing one of several aglycone cores. A major aglycone core of Butterfly Pea petals had been identified as kaempferol. Due to the attractive flower color and edibility nature of *Clitoria ternatea* Linn. is frequently used in Thailand and Malaysia to color rice and other food delicacies (Taif *et al*., 2017). Due to the anti-oxidant activity this flower also been used as butterfly pea tea. As per international guidelines there are two authorities for food colors in the legal framework, European Union (EU) legal frame work and United States (US) legal frame work. Both in the EU and the US only approved colors can be used in foods. As per both the guidelines anthocyanins are approved as food colors (Lehto *et al*., 2017).

In North-East India rice is the major cultivated crop. This crop is affected by a number of disease and pests. Rice blast caused by *Magnaporthe grisea* and rice brown leaf spot caused by *Drechslera oryzae* are two prominent disease of rice apart from many other diseases caused by different pathogens. Generally blast and brown spot diseases are manage by chemicals. Chemical fungicides provide significant benefits by suppressing the diseases that invade agricultural crops. Consequently, there is an increasing public pressure discover alternatives for crop protection. Bio-pesticides offer several advantages including complete biodegradability and water solubility over chemical and synthesized pesticides. In recent times Government of India put emphasis in organic cultivation in North – East India. Organic farming is not a new to farming community of the North – East. The farmers have retained traditional practices and have shown an inclination towards organic farming that is being harnessed for the development of region with ecological benefits. Several forms of organic farming are being successfully practiced in diverse climate particularly in rainfed, tribal, mountains and hill areas of the region. The region provides considerable scope and opportunity for organic farming due to least utilization of chemical inputs. With the sizable acreage under organic cultivation, the North East has tremendous potential to grow crops organically and emerge as a major producer of organic products. Biochemical characterization and profiling the various secondary metabolites present in *C. ternatea* (*Aparajita*) can help to make the bioactive agents to be commercialized and used in pharmaceutical industry. Thus, with these fundamental considerations, to quantitative evaluation of the major secondary phytochemical groups of this plant species from the region, a comprehensive research programme has been formulated with the following objective:

- To analyze the content of some secondary metabolites in different parts of *Clitorea ternatea* Linn.
- To determine the efficacy of plant extract of *C. ternatea* against pathogens causing rice blast and rice brown spot.

CHAPTER II

REVIEW OF LITERATURE

2.1 Phytochemical composition of *Clitoria ternatea* L.

Clitoria ternatea L. is rich in secondary metabolites as indicated by its therapeutic potential against different ailments. The plant has many pharmacological properties including antioxidant, hypolipidemic, anticancer, antiinflammatory, analgesic, antipyretic, antidiabetic, antimicrobial, gastro-intestinal antiparasitic, insecticidal and many other pharmacological effects (Al-Snafi, 2016). The preliminary phytochemical screening showed that *Clitoria ternatea* L. contains alkaloids, phenols, triterpenoids, flavanoids, tannins, phlobatannin, carbohydrates, saponins, flavonol glycosides, proteins, antharaquinone, anthocyanins, cardiac glycosides, volatile oils and steroids.

Reshmi and Reghunath (2011) worked on six selected high yielding accessions (MP-73, MP-74, MP-76, MP-81, MP-83 and MP-85) of *Aparajita* (*Clitoria ternatea* L.) and these were evaluated for pod yield and alkaloid content. They observed that pod and seed yield was more under open condition and the percent seed alkaloid content did not vary significantly between the shaded and open conditions. The crude alkaloid content varied between 0.25% to 0.43%.

Kumar *et al*. (2017) carried out the qualitative phytochemical analysis for carbohydrates, starch, chlorophyll, alkaloids, total phenolics, total flavonoids, tannins, steroids of three varieties of *Aparajita*, *Clitoria ternatea* L. var. *ternatea* Hook. F. (white), *Clitoria ternatea* L. var. *ternatea* Hook. F. (Blue) and *Clitoria ternatea* L. var. *Pleniflora* Fantz. (Blue var).

Joshi *et al.* (1981) examined the chemical composition of seeds *of Clitoria ternatea* Linn. They revealed the presence of palmitic, stearic, oleic, linoleic and linolenic acids in. seed oil. Protein constituted 38.4% and consisted of 18 amino acids and the essential amino acid patterns are lysine, histidine, threonine, phenylalanine, tyrosine, valine, methionine, cystine, leucine and isoleucine.

Neelamma *et al.* (2016) investigated the phyto-constituents present in root extracts of *C. ternatea* L. Preliminary phytochemical studies confirmed the presence of carbohydrates, proteins, alkaloids, glycosides, resins, steroids, saponins, phenols and flavonoids.

Shekhawat and Vijayvergia (2010) comparatively studied the different metabolites *viz*. protein, lipid, starch, phenol, and carbohydrates in different plant parts of *Clitoria ternatea* L. Low amounts of sugar of about 102mg/g dry weight and protein of about 21.0mg/g dry weight were observed in root of *C. ternatea*. The phenol content in leaf of *C. ternatea* was observed to be 18.0mg/g dry weight.

Khatoon *et al*. (2015) have done the comparative pharmacognostical studies of blue and white flower varieties of *Aparajita* (*Clitoria ternatea* L.). Their main focus was on macroscopy, qualitative and quantitative microscopy, physicochemical parameters, quantitative estimation of phenolics, flavonoid, TLC profiling with phenolics (caffeic and ferulic acid) and terpenoid markers (lupeol and β- sitostrol). They found the white and blue corolla in macroscopical studies. In microscopical studies they got more starch grains in root, variations in pericyclic fibers, xylem vessels and pith in stem. They also found the presence of steroid, triterpenoids, flavonoids, alkaloids, carbohydrate, glycosides, tannins and saponins in both the varieties by qualitative screening. The total phenolic content varied from 0.23% to 0.58% and the total flavonoid content varied from 0.10% to 0.15%. While in TLC fingerprinting profiling both blue and white varieties showed the similarities in the presence of lupeol, β-sitosterol, caffeic acid and ferulic acid.

Kavitha and Premalakshmi (2013) analysed the ethanolic extract of *Aparajita* (*Clitoria ternatea* L.) leaves for active chemical ingredients by both qualitatively and quantitatively. They carried out the preliminary screening for alkaloids, flavonoids, free amino acids, glycosides, oils, phenols, proteins, reducing sugars, saponins, steroids, tannins and terpenoids. They reported the absence of oils, saponins and terpenoids. The quantitative estimation of ethanolic extract of *C. ternatea* found rich amounts of nutrients such as vitamin C of about 176.03±1.19mg AAE/g, total proteins of about 3110±8.02mg/g and total carbohydrate of 118.83±0.47mg/g. The total phenol (245.14±6.97mg TAE/g) was relatively high compared to tannins (78.75±2.09mg TAE/g) and flavonoids (20.48±0.96mg RE/g).

Ponnusamy *et al*. (2013) analysed the alkaloid profile of *Clitoria ternatea* Linn by High Performance Thin Layer Chromatography (HPTLC). The methanolic extract of stem, leaves, and seeds of *C. ternatea* showed the presence of 26 different types of alkaloids with Rf values ranging from 0.02 to 0.93. In general more degree of alkaloids diversity was observed in seeds compared to leaves and stems. They also reported that out of 10 different alkaloids of seeds seven wit Rf values 0.15, 0.23, 0.41, 0.52, 0.62, 0.67 and 0.79 were unique, which are not present in the vegetative parts of the plant.

Anarthe *et al*. (2017) have done the *in vitro* and *in vivo* screening to evaluate immunomodulatory activity of *Clitoria ternatea* L. methanolic extract. The methanolic extract of *Aparajita* at the dose of 100, 200 and 400mg/kg bd. wt. along with the antigen (sheep red blood cells) showed significant increase in the production of circulating antibody titre and the number of plaque forming cells (PFC) in response to sheep red blood cells in the spleen. The methanolic extract showed significant ($p<0.01$) increase in the delayed type hypersensitivity response facilitated by footpad thickness response, significantly ameliorated haematological parameters (WBC, RBC and Hb) and also restored the myelosuppressive effects induced by Azathioprine. It showed increase in the levels of lymphocytes and rosettes formation when results were compared with standard as levamisole. This extract also showed significant immunomodulatory activity at the dose of 100, 200 and 400mg/kg bd. wt. but amongst them 100 mg/kg bd. wt. was found to be potent.

Kaisoon *et al*. (2011) worked on 12 edible flowers of Thailand. In Thailand the flower of *Clitoria ternatea* Linn. was one of the important vegetable. They had screened that the edible flowers was a potential source of bioactive components with high antioxidant properties that may be of interest to consumers and public health workers.

Vats (2014) have carried out a comperative studiy on *Clitorea ternatea* L. and *Origanum vulgare* L. He worked on phytochemical and anti oxidant activity of both the species. The total phenolic content in *Clitoria ternatea* and *Origanum vulgare* was found to be 204±1.2 and 247.91±0.09µg GAE/mg of extract, respectively. The total flavonoid content was found to be less than the total phenolic content. TFC was estimated to be 80µg/mg of extract in *Clitoria ternatea* and 108µg/mg of extract in *Origanum vulgare.* The DPPH scavenging activity revealed that *Clitoria ternatea* had better activity than *Origanum vulgare* with IC_{50} value 480±1.5µg/ml and

595±0.08µg/ml, respectively. The ferric reduction activity was observed to be more in *Clitoria ternatea* (1600µM/l) as compared to *Origanum vulgare* (1310µM/l).

Manjula *et al*. (2013) reported the presence of various phytochemicals like alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols. They had done the quantitative estimation of total flavonoids, saponins and phenols which has provided information regarding the medicinal potential of the plant. The root extracts were quantitatively analyzed for secondary metabolites like flavonoids, saponins, and phenols. The shoot, flower and seed extracts were quantitatively analyzed for total flavonoids. A high percentage of flavonoids were observed in the aqueous extract of roots (110±0.13mg/100 gm) and a lower percentage in shoots, flowers and seeds. Optimum level of Phenols (45±0.13mg/100 gm) and Saponins (2.0±0.6mg/100 gm) was reported to be present in the roots.

Rabeta and Nabil (2013) studied the total phenolic compounds and scavenging activity in *Clitoria ternatea* Linn. and *Vitex negundo* Linn. Their study was done to assess the total phenolic compounds (TPC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity in the flowers and leaves of *Clitoria ternatea* Linn. and *Vitex negundo* Linn. by using methanol and water extraction. Their study confirmed that *Vitex negundo* Linn. contains higher amount of TPC compared to *Clitoria ternatea*. In *Vitex negundo* Linn., the TPC in methanol and water extract ranged from 83.3 to 97.3mg GAE/g sample and 18.8 to 25.8mg GAE/g, respectively, while TPC obtained were 61.7 – 64.8mg GAE/g sample and 18.5- 20.7mg GAE/g samples for methanol and water extraction of *Clitoria ternatea*, respectively. The methanol extract of *Vitex negundo* Linn. showed higher DPPH scavenging activity compared with *Clitoria ternatea*. In contrast, DPPH scavenging activity for water extract showed the opposite result. The type of solvent used to extract the plant material and concentration of extracts used showed significance difference ($P<0.05$) on the amount of DPPH scavenged by the plant extract.

Madhu (2013) worked on phytochemical evalution and antioxidant activity of *in vitro* grown plants of *Clitoria ternatea* L. via multiple shoot induction of nodes through callogenesis and organogenesis with the help of Murashige and Skoog's medium supplemented with 6-benzylamino purine, 2, 4-dichlorophenoxy acetic acid and α-napthelene acetic acid. The preliminary phytochemical screening showed the presence of alkaloids, flavonoids, saponins, carbohydrates, steroids and phenol in products of *Clitoria ternatea* L grown *in vitro*. The ethanolic extract of *in vitro* grown *Clitoria ternatea* significantly inhibited the DPPH free radicals at the concentration ranging from 25-600μg/ml. It showed highest inhibition 67% at 600μg/ml equal to *in vivo* grown plant 70% at 600μg/ml.

Deka (2015) detected and estimated some phytochemicals of *Clitoria ternatea* Linn. roots which were necessary for the treatment of gynecological disorders. This study detected phytochemicals such as flavonoids, alkaloids, polyphenols and saponins by thin layer chromatography. Polyphenols were highest to be found in this experiment whereas saponins were the lowest.

Deka *et al.* (2013) worked on various plant parts (stem, flower, leaves, seeds and root) of *Clitoria ternatea* L. for estimation of proximate constituents. Total ash, crude protein, total lipid, crude fiber, soluble carbohydrates, insoluble and soluble minerals were found during the investigation.

Deka *et al*. (2013) had also done the biochemical estimation of primary metabolites and mineral composition of *Clitoria ternatea* Linn. roots. It has been reported that the plant had anti -diarrheal, anti histamic, anti-diabetic, cholinergic activity etc. Traditionally the root has been used for the treatment of leucorrhoea, diarrhea, urinary problems, diuretic, impotency, infertility stomach trouble etc. They have estimated primary metabolites such as total ash, crude protein, crude fiber, total lipids and carbohydrates along with the mineral composition of the root of the plant. Carbohydrate among all the primary metabolites have been found in the highest amount and magnesium was highest among all minerals under the study.

Kazuma *et al*. (2003) worked on malonylated flavonol glycosides from the petals of *Clitoria ternatea* Linn. They isolated three flavonol glycosides, kaempferol 3-O-(2´´- O – α – rhamnosyl - 6´´ - O - malonyl) – β - glucoside, quercetin 3 – O - (2´´- O – α – rhamnosyl - 6´´- O - malonyl) –β - glucoside, and myricetin 3-O-(2´´,6´´- di – O – α - rhamnosyl)- β -glucoside were isolated from the petals of *Clitoria ternatea* Linn.

Swain *et al.* (2012) worked on independent transformation of root somaclones (rhizoclones) of *Aparajita* (*Clitoria ternatea* L.) were established using explant co-cultivation with *Agrobacterium rhizogenes*. The major compound isolated and purified from the transformed root extracts was identified as the pentacyclic triterpenoid compound taraxerol using IR, H-NMR, and C-NMR spectroscopy. The taraxerol yield in cultured hairy roots, as quantified by HPTLC analysis, was up to 4-

fold on dry weight basis compared to that in natural roots. Scanning of bands from cultured transformed roots and natural roots gave super imposable spectra with standard taraxerol, suggesting a remarkable homology in composition. To date, this is the first report claiming production of the cancer therapeutic phytochemical taraxerol in genetically transformed root cultures as a viable alternative to in vivo roots of naturally occurring plant species.

Yeotkar and Malode (2014) studied the phytochemical diversity among the different variants of *Clitoria ternatea* L. and *C. Biflora* found in Amravati region. They had studied on three variants of *C. ternatea* L. (white petalloid, blue petalloid and double petalloid flower) and *C. biflora* (wild). They did the quantitative analysis of vitamins (B_2, C) and minerals (Na, K, Ca). As per the phytochemical analysis though the plants look same morphologically the investigated variants had great diversity in point of view of vitamins and minerals contents.

Kumar *et al.* (2016) worked on leaf extract of *Aparajita* (*Clitoria ternatea* L.) by different solvents, phytochemical screening, separation and characterization of bioactive compound. The leaves were extracted with various solvent such as petroleum ether, hexane, n-butanol, butyl acetate, chloroform, ethyl acetate, acetone, methanol, ethanol, acetic acid and water by successive hot continuous soxhlet method. The phytochemical investigation showed the presence of carbohydrates, glycosides, flavonoids, tannins, saponins, steroids, volatile oils, fats and oils. In TLC profiling all the extracts showed the impressive results that directed towards the presence of number of phytoconstituents. The extracts of *Clitoria ternatea* L. leaves FTIR analysis proved the presence of oximes, alcohols, phenols, alkanes, carboxylic acids, aldehydes, nitrogen compounds, amides, amines, and alkyl halides compounds.

Ponnuswamy and Wesely (2011) comparatively studied the primary metabolites (protein, lipids, starch, and carbohydrates) in different plant parts (seed, leaf, and stem) of *Clitoria ternatea* Linn. The highest amount of nutrients observed were soluble sugar 40.92mg/100g in stem, protein 13.96mg/100g in seed, carbohydrates 36.24mg/100g in stem, total Ash 9.95mg/100g in leaf and lipid 12.3mg/100g in seed respectively.

Kamkaen and Wilkinson (2009) studied the antioxidant activity of *Clitoria ternatea* flower petal extracts. The aqueous extracts were found to have stronger antioxidant activity as measured by DPPH scavenging activity than ethanol extracts with IC_{50} values of 1mg/ml and 4mg/ml, respectively.

Jain and Shukla (2011) worked on pharmacognostic evaluation and phytochemical properties on stem of *Clitoria ternatea* Linn. The transverse section of butterfly pea stems shows cork, cortex, pericyclic fibers, xylem parenchyma, xylem fibers, and medullary rays. Quantitative pharmacognostic analysis of the powder of the stem revealed moisture content 5.92±0.23%, total ash 3.76±0.32%, acid- insoluble ash 11±0.09%, alcohol extractive value of 9.84±0.19% and water extractive value 24.8±0.22%.

Jain *et al*. (2010) worked on *in vitro* evaluation of *Clitoria ternatea* Linn. stem extract for antioxidant property. They investigated preliminary phytochemical analysis *viz*. phytosterols, phenolic compound, flavonoids and carbohydrates. The methanolic extract showed the maximum free radical scavenging capacity as compared to the acetone extract.

Sarumathy *et al.* (2011) worked on phytoconstituents, nephro-protective and antioxidant activities of *Aparajita* (*Clitoria ternatea* Linn.) Phytoconstituents like Varidiflorene, Pterocarpin, Homopterocarpin, Isoparvifuran, Hexadecanoic acid, ethyl ester, Myo-Inositol, 4-C-methyl-, 1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-, Propane, 1,1-diethoxy - were identified from ethanol extract of *Clitoria ternatea* Linn. by using a gas chromatograph-mass spectrograph (GC MS).

2.2 Antimicrobial activity of *C.ternatea* L. (*Aparajita*)

Yadava and Verma (2003) worked on novel flavonol glycoside that has been isolated from the roots of *Clitoria ternatea* Linn. The flavonol glycoside 3,5,4'-trihydroxyflavonol -5-O-α -L-xylopyranosyl - (1→3)- O – β – D - galactopyranosyl-(1→6) – O – β – D - glucopyranoside, the biologically active compound showed the antibacterial and antifungal activity against some bacterial and fungal micro organism such as *Escherichia coli*, *Salmonela typhymurium*, *Staphyloccus aureus, Bacillus coagulans, Bacillus subtilis, Penicillium digitatum, Rhizopus oligosporus*, *Aspergillus fumigatus, Penicillum notatum, Fusarium oxysporum*. All the micro organisms showed the zone of inhibition against flavonol glycoside, out of that *Bacillus subtilis*, *Salmonela typhymurium* showed the better antibacterial activity and *Penicillium digitatum*, *Fusarium oxysporum* showed the better anti fungal activity.

Chakraborty *et al*. (2017) worked on phytochemical screening, biochemical evaluation and anti microbial activity of *Clitoria ternatea* Linn. plant extracts. The phytochemical analysis revealed the presence of flavonoids, carbohydrates, phenols, saponins, tannins, quinines, terpenoids and oxalate components in leaves and seed extracts of methanol. The aqueous and methanol extracts of seeds alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acids, proteins, terpenoids were present. The antibacterial study against *E.coli* and *B.subtlis*, showed the zone of inhibition which was more in case of methanol extracts. In antifungal analysis, the extracts showed equal effects against *A. niger* whereas the methanol extracts of seeds were little more effective against in case of *P. chrysogenum*.

Wulansari and Armayanti (2018) tested the effectiveness of flower extract to inhibit the growth of *Salmonella typhi* bacteria that causes typhoid fever. The concentration of flower extract at concentration 20%, 40% and 60% inhibited the growth of *Salmonella typhi* were 2.97±0.39, 4.90±0.40 and 7.78±0.5mm. The higher extract of concentration exhibited stronger antibacterial characteristics in inhibiting bacterial growth.

Darsini and Shamshad (2015) investigated the antibacterial and anti fungal activities of ethanol, methanol, hexane and aqueous extract of *Clitoria ternatea* L. The plant extract were tested against *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteriae* and a fungal pathogen called *Candida albicans.* Methanol extract was found to give a strong antimicrobial effect when compared to the other extracts. They also analysied the phytochemicals of the plant extract and concluded that tannins, flavonoids, alkaloids, steroids and terpenoids were present while the anthraquinones and saponins were found to be absent in plant material.

Nigam and Sahu (2014) studied the antibacterial effect of methanolic, petroleum ether, and chloroform extract of leaf and stem of *Aparajita* (*Clitoria ternatea*). These extracts were tested against *Bacillus subtilis, Staphylococcus aureus; Gram negative bacteria: Escherichia coli, Pseudomonas aeroginosa*. The leaf and stem extracts exhibited anti-microbial activities with zones of inhibition ranging from 1.2 – 2mm in leaves and 0.5 – 1.5mm in stem with methanol and petroleum ether extract in disk diffusion and in well diffusion method. *Bacillus subtilis* showed the maximum zone of inhibition (2.5mm) in stems and leaves in methanol extract. The qualitative phytochemical study of these plant extracts confirmed the presence of various primary and secondary metabolites such as carbohydrates, proteins, reducing sugars, tannins, flavanoids, glycosides, and resins.

Valivittan and Isaac (2016) studied the phytochemical, antioxidant, antibacterial and antifungal activities of *Aparajita* (*Clitoria ternatea* Linn.) flower. They have evaluated antioxidants and free radical scavenging activities with the help of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The qualitative phytochemical study of *Aparajita* flower confirmed the presence of tannins, saponins, flavonoids, proteins, steroids and anthroquinones. Evaluation of antibacterial activity was then followed using 5 microorganisms (*E.coli*, *Staphylococcus aureus*, *Bacillus spp*., *Salmonella typhi* and *Pseudomonas aeruginosa*). The Assay was performed by agar disc diffusion method. Then Antifungal activity assay was carried out using *Candida albicans, Aspergillus niger, Rhizopus spp.* and *Trichoderma viride*.

Anand *et al*. (2011) investigated the antibacterial properties of *Clitoria ternatea* L. The petroleum ether, ethyl acetate and methanol extracts from the leaves were tested against *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumonia, Proteus vulgaris* and *Salmonella typhi* by agar disc and well diffusion methods. The results showed promising antibacterial activity against the tested microbial pathogens. Among these, methanol extract was found to possess a more potent inhibitory effect when compared to the other extracts *viz*. Petroleum ether and Ethyl acetate. The results of this study validate the use of methanol extract of this species in ethnomedicine, favouring the isolation of antibacterial agents from the leaf extracts of *Clitoria ternatea* Linn.

Kelemu *et al*. (2004) purified a highly basic small protein from seeds of *C. ternatea* to homogeneity by using ultrafiltration with Centricon-3 membrane tubes and preparative granulated bed isoelectric focusing (IEF). The seed protein, designated as ‘finotin’, had broad and potent inhibitory effect on the growth of various important fungal pathogens of plants, namely *Rhizoctonia solani*, *Fusarium solani*, *Colletotrichum lindemuthianum*, *Lasiodiplodia theobromae*, *Pyricularia grisea*, *Bipolaris oryzae* and *Colletotrichum gloeosporioides*. It also inhibited the common bean bacterial blight pathogen *Xanthomonas axonopodis* pv. *phaseoli*. Moreover, finotin had powerful inhibitory properties against the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus*.

Mathew *et al*. (2009) studied the larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* L. extracts against three mosquito vector species *Aedes aegypti*, Culex *quinquefasciatus* and *Anopheles stephensi*. The methanol extracts of *C. ternatea* leaves, roots, flowers, and seeds were tested. The seed extract was effective against the larvae of all the three species with LC_{50} values 65.2, 154.5, and 54.4ppm, for *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus,* respectively. Among the three plant species studied *C. ternatea* was showing the most promising mosquito larvicidal activity.

Nigam and Shrivastava (2013) reported the anti microbial activity of *Clitoria ternatea* Linn. leaf against the *Staphylococcus aureus*. The methanolic extract of leaves inhibited the growth of Gram positive bacteria *Staphylococcus aureus*. They also confirmed the presence of various phytochemicals like saponins, terpenoids, steroids and tannins.

Mhaskar *et al*. (2010) studied on callus induction and antimicrobial activity of seed and callus extracts of *Clitoria ternatea* L. The aqueous extracts of both seed and callus were evaluated for the antimicrobial activity against selected pathogenic fungi and bacteria using the agar well diffusion technique. Six different bacterial strains *viz*. *Bacillus subtilis*, *Escherichia coli*, *Micrococcus flavus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhii* and four different fungal strains namely *Aspergillus flavus*, *Aspergillus ochraceous*, *Rhizopus oryzae* and *Aspergillus niger* were used. The seed extract of *Clitoria ternatea* showed the maximum zone of inhibition (22±0.5 mm) against *Escherichia coli* and minimum (14±1.0 mm) with *Micrococcus flavus*. The callus extract showed maximum zone of inhibition (16±2.0 mm) against *almonella typhi*, the minimum was against *Escherichia coli* and *Staphylococcus aureus* (12±1.0mm and 12±0.9mm, respectively). The seed extract of *C. ternatea* showed strong antifungal activity against all the tested fungi but the callus extract exhibited marginal antifungal activity.

Packiam *et al*. (2016) worked on the comparative studies of phytoconstituents, antibacterial and pesticidal activities of blue and white varieties of *Clitoria ternatea* Linn. The methanolic extract of *Aparajita* was used for the qualitative analysis of some secondary metabolites *viz.* terpenoids, phlobatannins, tannins, flavonoids, and phenols. The anti bacterial activity of methanolic extract against *Eschericia coli* and *Pseudomonas aeruginosa* was examined by well diffusion method and the pesticidal activity was tested against *Sitophilus oryzae*. The results showed that both the flowers have antibacterial activity against both the micro organisms. But the inhibition percentage is more in *E. coli* than the *P. aeruginosa.* The pesticidal activity was observed as 100% mortality against *Sitophilus oryzae*.

Kamilla *et al*. (2009) studied the *in vitro* antimicrobial activity of the methanol extract of leaf, stems, flower, seed and root of *Clitoria ternatea* L by the agar diffusion and broth dilution methods. They used 12 bacterial species, 2 yeast species, and 3 filamentous fungal species. Gram positive bacteria included *Bacillus cereus*, *Bacillus subtilis*, *Bacillus thuringiensis, Staphylococcus aureus*, *Streptococcus faecalis*; Gram negative bacteria included *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi, Enterobacter aerogens, Proteus mirabilis, Herbaspirillum* spp.; Yeasts included *Candida albicans* and *Saccharomyces cerevisiae*; Molds included *Rhizopus* spp.*, Aspergilus niger, Penicillium* spp., in their study. The leaf and root extracts were found to be most effective against all of the tested organisms ($P<0.05$). The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal activity (MFC) values of *Clitoria ternatea* extracts ranged from 0.3mg/ml to 100.00mg/ml. The extracts were also screened for tannin, phlobatannin, flavonoid, antharaquinone, alkaloid, saponin, cardiac glycosides, volatile oils, steroids and terpenoids. The anthraquinone and saponin were absent in all the plant parts investigated.

Phaune *et al.* (2013) evaluated the antimicromial activity of *Clitoria ternatea* Linn. (*Aparajita*) flower against the *Staphylococcus aureus* and observed significant antimicrobial activity. They also reported that the flower extract of *Aparajita* can be used as an indicator in various acid base titrations.

Uma *et al.* (2009) investigated *in vitro* antimicrobial activity of various extracts of *Clitorea ternatea* Linn. flower. The antimicrobial activity was tested by disc diffusion method and minimum inhibitory concentration by two-fold serial dilution method. Aqueous, methanol and chloroform extracts exhibited activity against *E.coli*, *Salmonella typhimurium*, *Klesiella pneumoniae* and *Pseudomonas aureginosa*.

Taur *et al.* (2009) investigated the phytochemical properties and evaluated the clonidine induced catalepsy in mice by the seed extracts of *Clitoria ternatea* Linn. (*Aparajita*)**.** They observed that ethanol and benzene extract at dose 75 and 100 mg/kg, *i.e*. showed significant inhibition of clonodine induced catalepsy as compare to control group. Clonidine induced catalepsy by releasing histamine in brain so the study confirmed that *Clitoria ternatea* Linn. seeds were having antihistaminic potential.

Das and Chatterjee (2014a) worked on antifungal activities of the leaf extract of *Aparajita* (*Clitoria ternatea* Linn.) against *Fusarium oxysporum ciceri* which infect pea seeds. The infestation reduces the activity of some enzymes *viz*. amylase, protease catalase, peroxidase, superoxide bismutase and dehydrogenase in pea (*Pisum sativum* L.) seeds. Administration of 50% ethanolic extract of *C. ternatea* restored the enzyme activity levels to normal.

Das and Chatterjee (2014b) worked on brine shrimp cytotoxicity of 50% aqueous ethanolic leaf extract of *Clitoria ternatea* L. The experimental results revealed that 0.28-0.38% concentration of the extract possessed cytotoxic potentiality against brine shrimp.

Das *et al*. (2014) investigated the biological control of *Fusarium oxysporum* in *Pisum sativum* seeds.Different solvent extract viz. petroleum ether, chloroform and 50% ethanolic leaf extract of *Aparajita* (*Clitoria ternatea* Linn.) tested against *Fusarium oxysporum* for its antifungal activity by agar cup method. The 50% ethanolic extract of leaf showed the antifungal activity against *Fusarium oxysporum.*

Gowd *et al.* (2012) evaluated the antimicrobial activity of three medicinal plants *viz*. *Terminalia chebula* Retz., *Clitoria ternatea* Linn., and *Wedelia chinensis* (Osbeck.) Merr. against three pathogenic microorganisms in the oral cavity *viz*. *Streptococcus mutans*, *Lactobacillus casei*, and *Staphylococcus aureus*. 5%, 10%, 25%, and 50% aqueous extracts from the fruits of *Terminalia chebula*, flowers of *Clitoria ternatea*, and leaves of *Wedelia chinensis.* were used The result showed that the diameter of zone of inhibition increased with increase in concentration of extract and the antimicrobial efficacy of the aqueous extracts of the three plants was observed in the increasing order *Wedelia chinensis* < *Clitoria ternatea* < *Terminalia chebula*.

In another experiment *Clitoria ternatea* showed antimictrobial activity against seven strains of urinary pathogens including methicilin resistant *Staphylococcus aureus*. These result showed that crude methanolic plant extracts had wide range of antibacterial activity against urinary tract infection pathogen particularly methicilin resistant *Staphylococcus aureus* (Balasundaram *et al*., 2011).

Antibacterial activity of *Clitoria ternatea* was also assessed by synthesizing silver nanoparticles using whole plant extracts by using disc diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. These silver nanoparticles showed very good antibacterial activity against these deadly pathogens (Malabadi *et al.*, 2012).

Parimaladevi *et al.* (2003) worked on anti-inflammatory, analgesic and antipyretic properties of root extract of *Clitoria ternatea* Linn. The methanolic extract when given to rats by oral route inhibited both the rat paw oedema caused by carrageenin and vascular permeability induced by acetic acid in rats. Moreover, the extract exhibited a significant inhibition in yeast-induced pyrexia in rats. In the acetic acid-induced writhing response, the extract markedly reduced the number of writhings at doses of 200 and 400mg/kg in mice.

Parimaladevi *et al.* (2004) also evaluated the antipyretic potentiality of *Clitoria ternatea* L. extract in rats. The methanolic extract of *Clitoria ternatea* L. root of the blue flowered variety was evaluated for its anti-pyretic potential on normal body temperature and yeast-induced pyrexia in albino rats. Yeast suspension (10 ml/kg body wt.) increased rectal temperature after 19 hours of subcutaneous injection. The extract, at doses of 200, 300 and 400 mg/kg body wt., p.o., produced significant reduction in normal body temperature and yeast-provoked elevated temperature in a dose-dependent manner. The effect extended up to 5 hours after the drug administration. The anti-pyretic effect of the extract was comparable to that of paracetamol (150mg/kg body wt., p.o.), a standard anti-pyretic agent.

Daisy and Rajathi (2009) investigated the therapeutic effects of the aqueous extract of *Clitoria ternatea* Linn. (*Aparajita*) leaves and flowers on alloxan-induced diabetes in rats. The aqueous extracts of *Clitoria ternatea* L. leaves and flowers significantly ($P<0.05$) reduced serum glucose, glycosylated hemoglobin and the activities of gluconeogenic enzyme, glucose-6- phosphatase, but increased serum insulin, liver and skeletal muscle glycogen and the activity of the glycolytic enzyme, glucokinase. For all the biochemical tests performed, the leaf extract-treated rat showed essentially the same profile as those treated with the flower extract.

Rao *et al.* (2017) worked on antimicrobial activity of aqueous and ethanolic extract of *C. ternatea* roots against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*. Both the ethanolic and aqueous root extracts showed zone of inhibition against *E. coli*, *P. aeruginosa*, multidrug resistant strain of *K. pneumonia*. *C. albicans* growth was inhibited by only the alcoholic extract.

Nahar *et al.* (2010) evaluated the possible anthelmintic effects of crude fresh juice of leaves of *Clitoria ternatea* Linn. using adult earth worm *Pheretima posthuma*. Three concentrations (25, 50 and 100mg/ml) of juice were studied for the determination of time of paralysis and death of the earth worms. The result revealed that fresh juice significantly showed paralysis and also caused death of worms especially at higher concentration of 100mg/ml, as compared to standard reference albendazole.

Radha *et al.* (2014) worked on phytochemical screening and antibacterial activity of *Clitoria ternatea* Linn. against *Proteus mirabilis* from urinary tract infected patients. 63 urine samples were collected from Urinary tract infected patients; microscopic observation and biochemical characterization were done to identify the presence of bacteria. Different solvent extracts viz. acetone, isopropyl alcohol and petroleum ether were used for phytochemical screening and anti bacterial activity. The leaf extract showed better antibacterial activity against isolated *Proteus mirabilis* from urinary tract infected patients.

2.3 Scientific validation of ethnomedicinal uses of *C. ternatea* L.

Salhan *et al.* (2011) investigated the *in-vitro* comparative anthelmintic activity of aqueous and ethanolic leaf extracts of *Clitoria ternatea* Linn. using *Eisenia foetida* at different concentrations. The study involved the determination of time of paralysis and time of death of the worms. At a concentration of 100mg/ml both the ethanolic and the aqueous extracts showed very significant bioactivity as compared to the standard drug levamisole (0.55mg/ml). The time of paralysis and death time was observed as 18±1.57 and 53.33±0.33 with aqueous extract and 12.33±0.80 and 32.33±0.71 with ethanolic extracts, respectively.

Upwar *et al.* (2010) evaluated the anti-diarrhoeal activity of the root of *Clitoria ternatea* Linn. The alcoholic extract of *Clitoria ternatea* Linn. root was investigated for its anti-diarrhoeal property in Wister albino rats to substantiate Ayurvedic claim. Alcoholic extract of the root of this plant, at graded doses (100, 200 and 400 mg/kg body weight) was investigated for anti-diarrhoeal activity in term of reduction in the rate of defecation and consistency of faeces in castor oil induced diarrhoea. The results are compared with the standard drug loperamide (3 mg/kg body weight). Extract produced profound decrease in intestinal transit (4.45 - 34.60%) also significantly inhibited castor oil induced enteropooling comparable to that of intraperitoneal injection of standard drug atropine sulphate at dose 5 mg/kg body weight. They conclude that alcoholic extract of *Clitoria ternatea* Linn. root possess significant anti-diarrhoeal activity.

Verma *et al.* (2013) investigated the pancreatic regeneration potential of diferent fractions of the ethanol extract of *Clitoria ternatea* Linn. The antidiabetic and antihyperlipidemic potential were evaluated in streptozotocin-induced diabetic rats and correlated with its *in vivo* and *in vitro* antioxidant activity. The polyphenolic, flavonoid and flavanone contents were assessed and correlated with its antidiabetic activity. The most significant pancreatic regeneration activity, antidiabetic and antihyperlipidemic activity was shown by ethanol extract and butanol soluble fraction at a dose level of 200 mg/kg.

Chavan *et al.* (2014) screened the leaves of *Clitoria ternatea* Linn for its phytochemical and pharmacological especially analgesic properties using hotPhoto and tail immersion method with mice. The analgesic study of *Clitoria ternatea* Linn. leaves showed that the petroleum ether extract of the leaves had significant activity compared to pentazocin which was used as a standard.

Saxena and Saxena (2013) worked on *Clitoria ternatea* Linn. leaf extract prepared in methanol, water, chloroform and petroleum ether. The anti ulcer activity of the different extracts showed good results as compared to standard drug. The methanol extract is very much active in protecting the animal against ulcer at the high dose. Chloroform extracts was least effective.

Evaluation of anti inflammatory, analgesic, and phytochemical screening of *C. ternatea* L. was done by Shyamkumar and Ishwar (2012). The petrolioum ether extract was safe even at the dose of 2000 mg/kg body weight by acute activity. By the phytochemical studies they found taraxerol in the plant.

Bhatia *et al*. (2014) evaluated the analgesic and anti-inflammatory activity of different extracts of *Clitoria ternatea* Linn. using carrageenan-induced paw edema and tail flick method in rats respectively at various dose levels. The ethanolic extract (400 mg/kg) showed maximum inhibition (23.80%) at 6^{th} hr and the early onset of action was found at 3^{rd} hour with only petroleum ether extract (400 mg/kg) showed significant effect ($p<0.001$) compared to positive control group. Petroleum ether extracts (100 mg/kg, 200 mg/kg and 400 mg/kg) showed statisticaly significant inhibition from 4^{th} to 6^{th} hr. In analgesic activity, both ethanol and petroleum ether extract showed the same type of effect but ethanol treated extract exhibited long lasting effect up to 2 hr. The findings of the study provided justification of the use of the plant in the treatment of inflammatory conditions.

Deka and Kalita (2011) investigated the preliminary phytochemical analysis and acute oral toxicity of the C ternatea *L.* (*Aparajita*) plant. The preliminary phytochemical showed the presence of protein, carbohydrates, glycosides, resins, saponin, flavonoid, alkaloids, steroids and phenol. The acute oral toxicity study showed no mortality up to a dose of 3000 mg per kg body weight.

The evaluation of antimitotic and antiproliferative activity of extracts of *C. ternatea* has been done by Phulbandhe *et al.*(2015). The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drug with antimitotic activity. The anti-proliferation activity was evaluated using Yeast as a model system.

The *in vitro* activity of antidiabetic, antioxidant and anti inflammatory has been done by Suganya *et al*. (2014). Preliminary phytochemical screening of ethanolic extract of leaves and flower revealed the presence of various bioactive components like alkaloids, flavonoids, steroids, glycosides, phenol, saponin, terpenoids and tannin in both leaves and flowers. Anthroquinone is absent in both the parts studied. The *in vitro* antidiabetic potential of plant extract was confirmed through non enzymatic glycation, glucose uptake by yeast cells and amylase inhibition methods. Anti-inflammatory activity was also confirmed.

Generally the sunscreen resources mainly obtained from plants in form of natural substances which are ability to absorb ultraviolet ray in the UVA region. Patil *et al.* (2016) evaluated UV absorption ability of flowers from *Clitoria ternata* Linn. *Canna indica* Linn., as an anti-solar agent. The result indicated the ability of extracts to absorb UV radiation and hence proved its UV protection ability. All extract showed a prominent absorbance at 200 – 240nm. The little absorbance was noted at the range of 310 – 340nm of hydro alcoholic extract of *Clitoria ternata* Linn.

2.4 *C. ternatea* L. (*Aparajita*) – as a good source of colour dye

Deka *et al*. (2014) worked on some natural dyes of Assam. They took six sources of natural dyes *viz.*, *Tectona grandis* (teak), *Nyctanthes arbor-tristis* (night-flowering jasmine), *Lawsonia inermis* (henna), *Tagetes patula* (marigold), *Clitoria* *ternatea* (*Aparajita*), *Curcuma longa* (turmeric) and Phutuki (*Melastoma malabathricum* L). Alum (Potassium aluminium sulphate) copper sulphate, vinegar and ammonia were used as mordants. Experimental results indicated that varieties of colours can be produced from a single plant depending on the types of mordant used. The treated fabrics showed excellent colour fastness properties. The treated samples did not exhibit any colour fading and maintained the original texture.

Taif *et al*. (2017) extracted the dye from the dried flower of *Aparajita*. The traditional boiling method was applied for the extraction of natural colour. The extracted colour studied for its dyeing ability on silk fabric samples by using pre-mordanting, simultaneous mordanting, and post-mordanting dyeing procedures. The test results showed that all fabrics samples produced a different range of colors where the most brilliant colours were registered with pre-mordanting method.

CHAPTER III

MATERIALS AND METHODS

3.1 Location of research works and time period

The present research work entitled "Phytochemical analysis and antimicrobial activity of *Aparajita* (*Clitoria ternatea* Linn.) against rice pathogens" was carried out in the laboratory of Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat. The experimental site was located at 94°10´ E longitude and 26°44´ N latitude and at an altitude of 91 meters above mean sea level. Being situated in the monsoon subtropical zone, the climate of Jorhat was characterized by hot and humid summer, dry and cool winter, high rainfall of above 2000mm per annum and high average humidity of around 85-90%. The maximum temperature rises above 35°C during the month of July and August while the minimum temperature falls to around 7°C during December and January. The experiment was conducted during the period starting from August, 2016 to June, 2018.

3.2 Cleaning and sterilization of glassware

All the glassware were cleaned and sterilized before use. The glassware were washed thoroughly with tap water followed by distilled water which were then dried in hot air oven. The glassware were wrapped with aluminium foil to prevent contamination during cooling and kept for use. The glassware were sterilized at 160°C for 2hrs in a hot air oven.

3.3 Collection of samples

Plant sample of *Aparajita* (*Clitoria ternatea* Linn.) was collected from Teok, Jorhat district, Assam and from Matabari village, Matabari block, Gomati district, Tripura and authenticated. The samples were cleaned and brought to the laboratory in dry zip polyethene bag (Photo 1-3).

3.4 Study of morphological characters

Morphological study is a prerequisite for proper identification and systematic analysis of plants. The plants were raised in pots of size 2ft x 2ft x 2ft. The plants were under constant observation for studying morphological characters.

Morphological characters of the selected plants were measured and recorded as per the procedure described below:

- **Plant height:** Height of the plant was measured in centimeter (m) from the base of the stem near ground level to the top most twig and measured in m.
- **Leaf length:** It is the length of the leaf blade from the base to the tip of the leaf. It was measured in cm.
- **Leaf breadth:** It is the width of a leaf at the middle position of the leaf blade in cm.
- **Pod length:** It was measured in cm.
- **No. of seeds per pod:** The total number of seeds present in a pod.
- **Flower length:** It is the length of the flower blade from the base to the tip of the flower. It was measured in cm.
- **Flower breadth:** It is the width of a flower at the middle position of the flower blade in cm.

3.5 Determination of moisture:

Moisture content was determined by following the method of AOAC (1970). For this approximately 10g of sample is weighed in aluminium moisture boxes and dried in an oven at 100°C (±2°C) for 16 hours. Cooled in desiccators and weighed. The experiment was conducted in triplicate and the mean was recorded.

Calculation:

$$\text{Moisture content (g/100g sample)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

3.6 Preparation of sample

The *Aparajita* (*Clitoria ternatea* Linn.) leaves, twigs and stems were separated and dried in an oven at 40^{0}C for 2 hours. Each of these plant parts were ground into fine powdered form in laboratory grinder machine and kept separately in dry polythene bags. The powdered samples were analysed for phytochemical present. The whole plants were collected, dried and ground into fine powdered form for anti microbial activity (Photo 4, 5).

Assam collection

Tripura collection

Photo 1. Mature plants of Assam and Tripura sample

Leaf length

Leaf breadth

Pod and Seeds

Flower

Photo 2. Different parts of *Clitoria ternatea* Linn. (Assam collection)

Leaf length

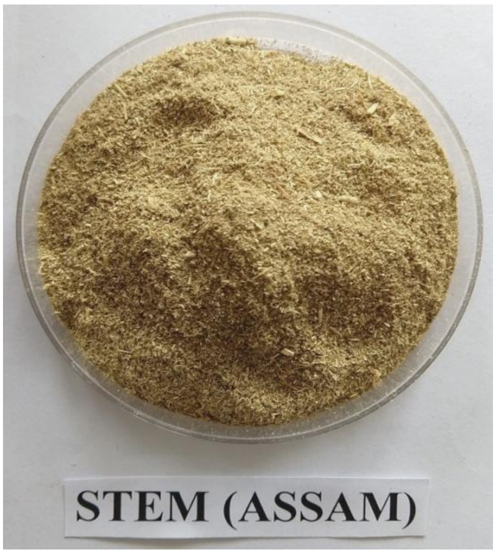
Leaf breadth

Pod and Seeds

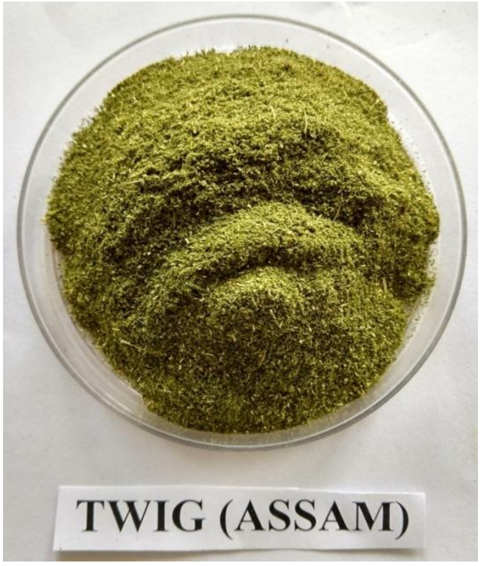
Flower

Photo 3. Different parts of *Clitoria ternatea* Linn. (Tripura collection)

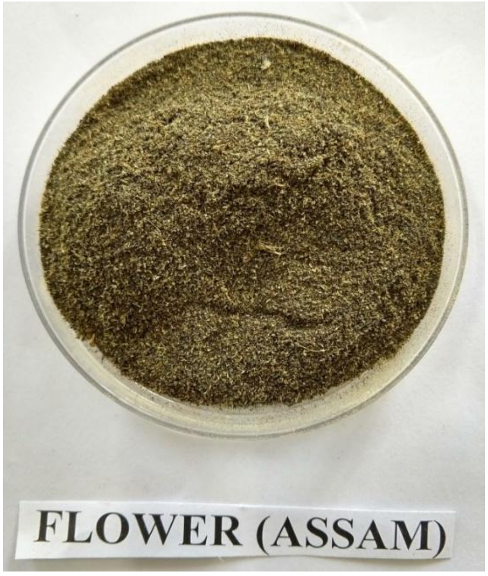
Leaf



Stem



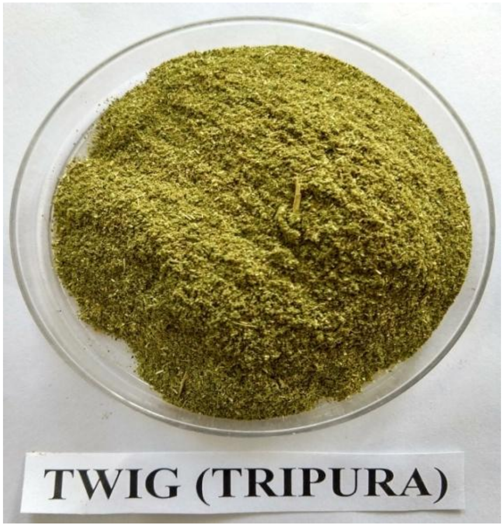
Twig



Flower

Photo 4. Powdered sample of different parts of *Clitoria ternatea* Linn. (Assam collection)

Leaf **Stem**



Twig **Flower**

Photo 5. Powdered sample of different parts of *Clitoria ternatea* Linn. (Tripura collection)

3.7 Estimation of total alkaloid from leaf, twig and stem extract

The total alkaloid content was estimated by the method of Harborne (1973). For this, 5 g of the sample was extracted with 200 ml of 10% acetic acid in ethanol and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the preparation was complete. The whole solution was allowed to the extract until the precipitated was collected and washed with dilute ammonium hydroxide and then filtered through Whatman no. 40 filter paper. The residue was the alkaloid, which was dried and weighed.

3.8 Estimation of total phenolics from leaf, twig and stem extract of *C. ternatea* L.

Total phenolics were determined using the Folin–Ciocalteau reagent as reported by Singleton and Rossi (1965) with modifications as described by Kaur and Kapoor (2002). Essentially Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000rpm for 15min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5ml of distilled water. One-hundred microlitres of this extract was diluted to 3ml with water and 0.5ml of Folin–Ciocalteau reagent was added. After 3min, 2ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650nm in a spectrometer after 60min using catechol as a standard. The results were expressed as mg catechol/100g of fresh weight material**.**

3.9 Estimation of total terpenoids from leaf, twig, stem and flower extract of *C. ternatea* L.

The total terpenoid content was estimated by the method of Chakraborty *et al*. (2017). 10g of two powdered sample was dissolved in 100ml of 60% methanol solution. The flasks were kept in room temperature in rotary shaker at 100rpm for 72hrs. Then the extracts were filtered separately by using Whatman No.1 filter paper and stored refrigerator at 4°C for further use.

The qualitative test for terpenoids has been done by Salkowski test. For this, 1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid and observed immediately for formation of a reddish brown precipitate which indicated presence of terpenoids.

For the quantitative estimation of total terpenoids, 1ml of plant extract was treated with 3ml of chloroform. The sample mixture was thoroughly vortexed and left for 3mins. Then 200ul of conc. H_2SO_4 was added and mixture was incubated for 1.5-2hrs in dark condition and during incubation a reddish brown precipitation was formed. Then carefully and gently all the supernatant of the reaction mixture was decanted without disturbing the precipitation. 3ml of 95% (v/v) methanol was added and vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance reading was taken at 538nm using UV visible spectrophotometer. The total terpenoid content was calculated was calibration curve of linalool and the results were expressed as linolool equivalent.

3.10 Determination of antimicrobial activity of plant extract of *Clitoria ternatea* Linn.

3.10.1 Preparation of PDA media

The requirements were as follows:

1. PDA (39.9g, readymade)
2. Distilled water (1000ml)
3. Beaker
4. Measuring cylinder
5. Conical flasks
6. Non absorbent cotton
7. Brown paper
8. Rubber band

The procedure was as follows:

1. 39.9g of PDA powder was suspended in 1000 ml distilled water.
2. Heated to boiling to dissolve the medium completely.
3. Dispensed about 100ml of medium into each of the ten 250ml conical flasks.

4. Plugged the mouth of the flasks with non-absorbent cotton.
5. Sterilized the medium by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
6. Mixed well before dispensing.
7. Photos were prepared of the media containing 15-20ml in each test tube.

3.10.2 Test microorganism

The antimicrobial activity of plant extract of *Clitoria ternatea* Linn. had been tested against five plant pathogenic fungus *viz*. *Magnaporthe grisea*, *Drechslera oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae* and *Fusarium* sp.(*Gibberella fujikuroi*). Out of these *Magnaporthe grisea* (MTCC 1477) were collected from Institute of Microbial Technology (IMTECH), Chandigarh (appendix I). The remaining four cultures *viz. Drechslera oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae* and *Fusarium* sp. were collected from the culture bank maintained under Nanotechnology Laboratory, Department of plant of Plant Pathology, AAU, Jorhat.

3.10.3 Maintainance of culture

Pure culture of all the collected fungus was maintained on PDA medium by periodic transfer in fresh medium. A loopful of inoculum from cultured tubes were transferred to PDA Photos, incubated in the BOD incubator (Orbitek) at 28±1°C and maintained as pure culture. After complete sporulation, for subsequent studies the fungus was sub cultured and stored in refrigerator 4°C.

3.10.4 Preparation of plant extract

The dried, powdered plant sample was extracted with methanol using maceration techniques. Ten grams of powdered flowers were extracted with 100ml of solvent with occasional shaking for 3 days at room temperature. The extracts were filtered, concentrated and dried at 50°C and stored at 4°C for further analysis (Uma *et al*., 2009) (Photo 6).

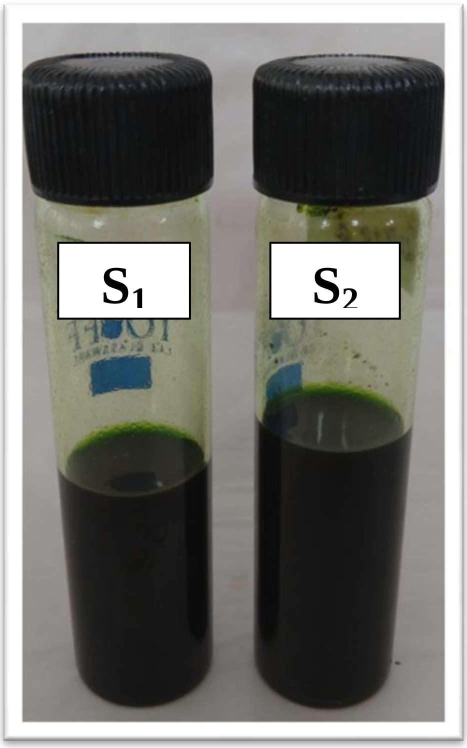


Photo 6. Methanolic plant extract of Assam (S_1) and Tripura (S_2) sample

3.10.5 Treatment combinations

For the determination of antimicrobial activity of plant extract of *Clitoria ternatea* Linn. the treatment combinations were –

1. T_0 (Control) = PDA + microorganism
2. T_1 = PDA + microorganism + Plant extract (1mg/ml)
3. T_2 = PDA + microorganism + Plant extract (2mg/ml)
4. T_3 = PDA + microorganism + Plant extract (3mg/ml

3.10.6 *In vitro* study of efficacy plant extract against pathogens

The antimicrobial activity of *Clitoria ternatea* Linn. extract against phyto pathogens were evaluated under *in vitro* conditions by poisoned food technique (Kim *et al*., 2012).

In poisoned food technique, PDA medium was poisoned at the desired concentration and poured into sterilized petriPhotos and were allowed to solidify. After the solidification of the media, mycelia disc of the fungi were cut out using a sterilized cork borer which was placed at the centre and a control was taken with a normal PDA medium. All the Photos were incubated at 28±1°C in a BOD incubator (REICO) till full growth was observed in the control. After incubation of fungi on different culture containing plant extracts, the radial growth of fungal mycelium was recorded. Mycelia growth inhibition was calculated when growth of mycelia in the control Photo reached the edge of the petri dish. The following formula was used for calculation of growth inhibition (%).

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

Where, C = colony diameter (cm) of the control

T = colony diameter (cm) of the test Photo.

3.11 Natural colour extraction from *Clitoria ternatea* Linn. flower

3.11.1 Optimization experiment

Natural colour (dye) was prepared by using water extraction. The purpose of using water extraction is to make sure that the colour is safe from the chemical and harmful substance. Fresh blue *Clitoria ternatea* L. flowers were collected. These flowers were first cleaned and dried in oven at low temperature (40^0C) in order to remove all the moisture content in the flower. Then, the flower was cut and grinded into small pieces, about 1mm, to increase the surface area. A range of temperatures (50, 60, 70 ^{0}C) , time (10, 20, 30, 40 minutes) and solid: liquid ratios (1:5, 1:10, 1:20, 1:30, 1:40) were tested and optimization of parameters were done based on optimum colour yield. The optimization experiment showed 60^{0}C temperature, 20 minute time and 1:20 ratio of solid : liquid was optimum condition for better colour yield.

3.11.2 Colour extraction

Therefore, the blue natural colour (dye) was extracted by applying ratio 1:20 corresponding to the ratio of 1 g of raw material to 20ml of distilled water at 60^{0}C for 20 minutes and filtered through muslin cloth to remove the flower debris. The filtrate containing some of flower debris was then centrifuged at 4^{0}C, 10,000rpm for 15 minutes (Farrell, 2012). Finally, filtration of the supernatant was done by using stainless steel inlet filter of pore size 2 micrometer. The resulting aqueous colour extract was further concentrated on a hot water bath and was used for the further experiment.

3.11.3 Immobilization of colour

To immobilize the color on to edible inert carrier food material such as rice powder and glucose powder was tried. Glucose powder was proved effective. So glucose powder was added as a carrier material to the concentrated aqueous colour extract (dye) at 1: 3 ratios to make it a viscous solution. Incubated at 4°C for overnight, the viscous solution will turn solid as aqueous colour will be absorbed and immobilized on glucose. Then, this was further powdered by mortar and pestle and stored it in air tight container for further use.

3.11.4 Colour variation through pH change using edible material

The colour variation through pH change was observed by using aqueous extracts of *Citrus reticulata* L. (*Kazinemu*) fruit and *Musa bulbisiana* L. (*vimkol*) peel. Dry *vimkol* peels were burnt and aqueous extract was prepared which was filtered through muslin cloth. *Kazinemu was* squeezed and aqueous extract was prepared which was filtered through muslin cloth. Both the extracts were used to change the pH of the colour extract in a range of pH 3-12 and the resultant colour variation was observed.

3.12 Statistical analysis

The data obtained from laboratory experiments were analyzed statistically. The experiment was laid out in Complete Randomized design (CRD). All analysis was performed in triplicate and the average has been reported. The data were analysed by one way analysis of variance (ANOVA). The standard error of the mean difference (S. Ed. $\pm$) was calculated. The treatment means were compared among themselves by calculating critical difference (C.D. at $P<0.05$) as per method of Panse and Sukhatme (1978). The analysis of variance (ANOVA) was done with treatments and replications. The critical differences were calculated by the formula:

$$CD = t_{0.05,\ \text{error d.f.}} \times S.Ed$$

$$\text{Where, } S.Ed = \sqrt{\frac{2\ EMS}{r}}$$

The difference of treatments means were tested by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

CHAPTER IV

EXPERIMENTAL FINDINGS

Clitoria ternatea Linn (*Aparajita*) is an important medicinal plant available in North East India which is still unexplored and underutilized. It contains several interesting bioactive constituents and possesses health promoting properties. Phytochemical characterization and profiling of the various secondary metabolites present in the different samples can help to search for bioactive agents to be commercialized and used in pharmaceutical industry. The present investigation was conducted with an aim to determine phytochemical constituents, specifically the total phenolics, total alkaloids and total terpenoids from leaf, stem and twig extracts, and to evaluate the antimicrobial activity of plant extracts against some fungal pathogens of rice. The results of the investigations are presented below.

4.1 Plant morphological characteristics of *C. ternatea* L.

The plant morphological characteristics of *Clitoria ternatea* L. were analyzed in samples collected from Assam (S_1) and Tripura (S_2) and has been given in table 1. The plant height at maturity was 3.51m and 3.68m in the sample S_1 and S_2 respectively. Similarly, the leaf length was found to be 10.83cm and 10.43cm in the sample S_1 and S_2 respectively. The leaf breadth was recorded to be 7.97cm in S_1 and 8.53cm in S_2. The sample S_1 recorded an average pod of 10.87cm and while S_2 recorded a pod length of 11.03cm. The average no. of seed per pod was lower in S_1 which recorded 7.33 as compared to 9.00 in the S_2. The flower length was recorded to be 4.63cm and 4.47cm and the flower breadth was observed to be 3.46cm and 3.20cm in S_1 and S_2 respectively. The differences in plant morphological characteristics in the two samples were found statistically non significant.

4.2. Total moisture content

A non significant variation was observed in the total moisture content on fresh weight basis of the different parts of *Clitoria ternatea* Linn. samples. The leaf, stem, twig and flower of sample S_1 contained a moisture content of 72.90, 38.76, 65.24 and 78.04% respectively where as sample S_2 had 71.96, 40.06, 68.61 and 77.00% moisture in leaf, stem, twig and flower, respectively. The total moisture content on fresh weight basis in the different parts of the samples of *Clitoria ternatea* L. are presented in the table 2a. In case of dry weight basis a significant variation was observed in the total moisture content of the leaf and twig of *Clitoria ternatea* Linn. samples of S_1 and $S_{2.}$ On the other hand, non significant variations were observed in the total moisture content in stem and flower of *Clitoria ternatea* Linn. samples of S_1 and S_2. The leaf, stem, twig and flower of sample S_1 contained a moisture content of 8.82, 9.76, 11.38, and 13.7% respectively where as sample S_2 had 8.02, 9.78, 10.82, and 13.08% moisture in leaf, stem, twig and flower, respectively. The total moisture content on dry weight basis in the different parts of the samples of *Clitoria ternatea* L. are presented in the table 2b.

Table 1. Morphological characteristics of *Clitoria ternatea* Linn.

(Results are average of triplicates)

Sample	**Plant height (m)**	**Leaf length (cm)**	**Leaf breadth (cm)**	**Pod length (cm)**	**No. of seeds per pod**	**Flower length (cm)**	**Flower breadth (cm)**
Assam (S_1)	3.51	10.83	7.97	10.87	7.30	4.63	3.46
Tripura (S_2)	3.68	10.43	8.53	11.03	9.00	4.47	3.20
CD $_{(0.05)}$	NS	NS	NS	NS	NS	NS	NS
SE(d)	0.200	1.092	0.663	0.219	0.471	0.149	0.211
SE(m)	0.141	0.772	0.448	0.309	0.667	0.105	0.149

NS= Non significant

Table 2a. Total moisture content of *Clitoria ternatea* Linn. Samples

(Results are the average of triplicates)

Sample	**Total moisture content (g/100g) (fresh weight basis)**			
	Leaf	**Stem**	**Twig**	**Flower**
Assam (S_1)	72.90	38.76	65.24	78.04
Tripura (S_2)	71.96	40.06	68.61	77.00
CD $_{(0.05)}$	NS	NS	NS	NS
SE(d)	0.357	0.768	1.322	1.226
SE(m)	0.253	0.543	0.935	0.867

NS= Non significant

Table 2b. Total moisture content of *Clitoria ternatea* Linn. Samples

(Results are the average of triplicates)

Sample	**Total moisture content (g/100g) (dry weight basis)**			
	Leaf	**Stem**	**Twig**	**Flower**
Assam (S_1)	8.82	9.76	11.38	13.70
Tripura (S_2)	8.02	9.78	10.82	13.08
CD $_{(0.05)}$	0.131	NS	0.106	NS
SE(d)	0.028	0.057	0.023	0.406
SE(m)	0.020	0.040	0.016	0.287

NS= Non significant

4.3. Total phenolic content

Significant variation was observed in the total phenolic content of the different parts of *Clitoria ternatea* Linn samples. The phenolic content in the leaf and the stem of the sample S_1 were 1.524g/100g and 0.706g/100g, respectively, which was higher than in sample S_2 whereas the twig of the sample S_2 had higher total phenolic content of 1.209g/100g as compared to 1.110 g/100g recorded in S_2. The total phenolic content in the different parts of the samples of *Clitoria ternatea* are presented in the table 3.

4.4. Total alkaloid content

Non significant variation was observed in the total alkaloid content in leaf extract of *C. ternatea* L. of samples S_1 and S_2 in which the later recorded the minimum. On the other hand, significant variations was observed in the total alkaloid content in stem and twig of *Clitoria ternatea* Linn samples of S_1 and S_2. The leaf, stem, and twig from the sample S_1 collected from Assam exhibited higher amount of total alkaloid content which were 1.000g/100g, 0.753g/100g and 1.627g/100g respectively. The total alkaloid content in the different parts of the samples of *Clitoria ternatea* are presented in the table 4.

4.5. Total terpenoid content

No significant variation has been observed in the total terpenoid content of the two samples of *Clitoria ternatea* Linn. The leaf, stem and twig extracts did not show positive result in qualitative test for terpenoid but positive result was obtained for flower extract (photo 7). When estimated quantitatively, flowers of sample S_1 collected from Assam had higher total terpenoid content of 0.698 g/100g than the sample S_2 which had total terpenoid content of 0.675g/100g. The total terpenoid content in the different parts of the samples of *Clitoria ternatea* are presented in the table 5.

Table 3. Total phenolic content of *Clitoria ternatea* Linn. samples

(Results are the average of triplicates)

Sample	**Total phenolic content (g/100g) dry weight basis (Catechol equivalent)**		
	Leaf	**Stem**	**Twig**
Assam (S_1)	1.524 ± 0.024	0.706 ± 0.002	1.110 ± 0.010
Tripura (S_2)	1.277 ± 0.009	0.682 ± 0.008	1.209 ± 0.008
CD $_{(0.05)}$	0.074	0.024	0.037
SE(d)	0.026	0.008	0.013
SE(m)	0.018	0.006	0.009

Table 4. Total alkaloid content of *Clitoria ternatea* Linn. samples

(Results are the average of triplicates)

Sample	**Total alkaloid content (g/100g) (Dry weight basis)**		
	Leaf	**Stem**	**Twig**
Assam (S_1)	1.000	0.753	1.627
Tripura (S_2)	0.875	0.627	1.253
$CD_{(0.05)}$	NS	0.108	0.142
SE(d)	0.125	0.038	0.050
SE(m)	0.088	0.027	0.035

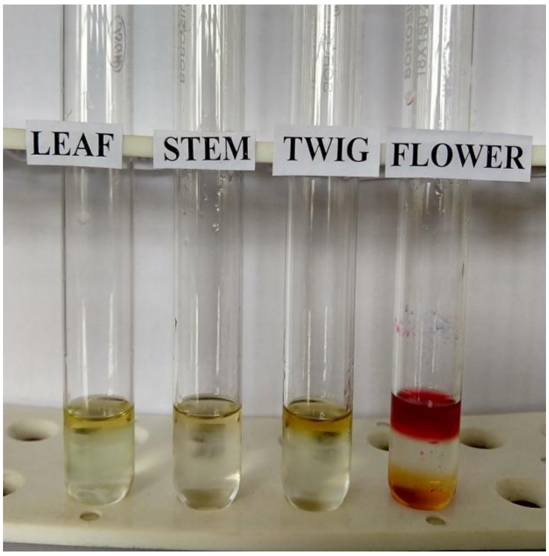
NS= Non significant

Table 5. Total terpenoid content of *Clitoria ternatea* Linn. samples (Results are the average of triplicates, expressed in linalool equivalent)

Sample	Total terpenoid content (g/100g) (dry weight basis)			
	Leaf	**Stem**	**Twig**	**Flower**
Assam (S_1)	ND	ND	ND	0.698 ± 0.007
Tripura (S_2)	ND	ND	ND	0.675 ± 0.070
CD $_{(0.05)}$	-	-	-	NS
SE(d)	-	-	-	0.050
SE(m)	-	-	-	0.070

ND= Not detected NS= Non significant

(A)



(B)

Photo 7. Qualitative test for terpenoids in different parts of *Clitoria ternatea* Linn. (A) Assam collection, (B) Tripura collection

4.6. Anti microbial activity

4.6.1 Antimicrobial activity against *Magnaporthe grisea*

The antimicrobial activity of methanolic extract of whole plant of *Clitoria ternatea* Linn. was evaluated against *Magnaporthe grisea* causing rice blast disease. The results are shown in table 6 and photo 8. Significant difference was observed in between the treatments in their antimicrobial activity as shown by mycelial growth inhibition at 5% level of significance. The mycelial growth inhibition percentages were expressed in angular transformation value and are given in parentheses in the table. A high mycelial growth inhibition percent of 41.15 was recorded in S_1 with treatment with 3mg/ml concentration (T_3). This treatment also recorded highest mycelial growth inhibition of 43.58 per cent in sample S_2.collected from Tripura.

4.6.2 Antimicrobial activity against *Drechslera oryzae*

The antimicrobial activity of methanolic extract of whole plant of *Clitoria ternatea* L. was also tested against *Drechslera oryzae* causing rice brown spot and the results are presented in table 7 and photo 9. The methanolic plant extract of both the samples *viz.* S_1 and S_2 could not inhibit the mycelial growth of *Drechslera oryzae* in all the concentrations.

4.6.3 Antimicrobial activity against *Rhizictonia solani*

The antimicrobial activity of methanolic extract of *Clitoria ternatea* L. was further evaluated against *Rhizictonia solani* causing sheath blight of rice and are presented in table 8 and photo 10. Significant variations were observed in between the treatments in both the samples at 5% level of significance. The highest mycelial growth inhibition percentage as expressed in angular transformed value was observed in the treatment T_3 with a concentration of 3mg/ml in both the samples *viz.* S_1 and S_2 which were 60.99 and 57.80, respectively.

4.6.4 Antimicrobial activity against *Sarocladium oryzae*

The antimicrobial activity of methanolic extract of whole plant of *Clitoria ternatea* Linn. against *Sarocladium oryzae* causing sheath rot disease of rice are described in table 9 and Photo 11. Significant difference was observed in between the treatments at 5% level of significance. The highest mycelia growth inhibition percentage has been observed in the treatment T_3 with concentration of 3mg/ml in both the samples. Among the two samples S_1 collected from Assam recorded a high of 48.56 while S_2 collected from Tripura showed a high of 47.86 percent as expressed in angular transformed value.

4.6.5 Antimicrobial activity against *Fusarium* sp.

The data on antimicrobial activity of methanolic extract of whole plant of *Clitoria ternatea* Linn. against *Fusarium* sp. (*Gibberella fujikuroi*) causing bakanae disease of rice seedling are presented in table 10 and photo 12. Significant difference was observed in between the treatments at 5% level of significance. The mycelial growth inhibition percentage as expressed in angular transformed value was observed highest in the treatment T_3 with concentration of 3mg/ml. Sample S_1 recorded as high as 48.04 while sample S_2 showed a highest of 46.28 per cent.

Table 6. Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Magnaporthe grisea* (Results are the average of triplicates)

Treatments	Mycelial growth inhibition %	
	Assam (S_1)	Tripura (S_2)
T_1	11.14 (19.48)^c	10.51 (18.78)^c
T_2	25.79 (30.52)^b	25.80 (30.50)^b
T_3	43.3 (41.15)^a	47.53 (43.58)^a
Control	0.00^d	0.00^d
CD $_{(0.05)}$	1.557	2.687
SE(d)	0.624	1.077
SE(m)	0.441	0.762

NB: Figures in parentheses indicate the angular transformation of per cent inhibition of mycelial growth.

Table 7. Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Drechslera oryzae* (Results are the average of triplicates)

Treatments	Mycelial growth inhibition %	
	Assam	**Tripura**
T_1	0.00	0.00
T_2	0.00	0.00
T_3	0.00	0.00
Control	0.00	0.00

Table 8. Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Rhizoctonia solani* (Results are the average of triplicates)

Treatments	Mycelial growth inhibition %	
	Assam	**Tripura**
T_1	25.41 (30.24)^{c}	35.44 (36.53)^{c}
T_2	53.10 (46.78)^{b}	49.1 (44.49)^{b}
T_3	76.47 (60.99)^{a}	71.61 (57.80)^{a}
Control	0.00^{d}	0.00^{d}
CD $_{(0.05)}$	2.972	4.737
SE(d)	1.192	1.899
SE(m)	0.843	1.343

Table 9. Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Sarocladium oryzae* (Results are the average of triplicates)

Treatments	Mycelial growth inhibition %	
	Assam	Tripura
T_1	13.65 (21.65)[c]	13.33 (21.41)[c]
T_2	32.47 (34.72)[b]	30.70 (33.64)[b]
T_3	56.15 (48.56)[a]	54.94 (47.86)[a]
Control	0.00[d]	0.00[d]
$CD_{(0.05)}$	5.092	1.380
SE(d)	2.041	0.553
SE(m)	1.443	0.391

Table 10. Antimicrobial activity of *Clitoria ternatea* Linn. plant extracts against *Fusarium* sp. (*Gibberella fujikuroi*) (Results are the average of triplicates)

Treatments	Mycelial growth inhibition %	
	Assam	**Tripura**
T_1	22.13 (24.40)^c	17.18 (24.78)^c
T_2	28.71 (32.37)^b	35.61 (36.60)^b
T_3	55.30 (48.04)^a	52.24 (46.28)^a
Control	0.00^d	0.00^d
$CD_{(0.05)}$	3.437	5.027
SE(d)	1.378	2.015
SE(m)	0.974	1.425

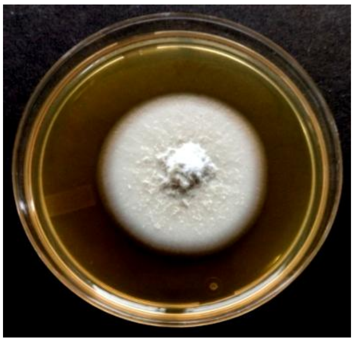
(A)

Control

(B)

T_1

T_2

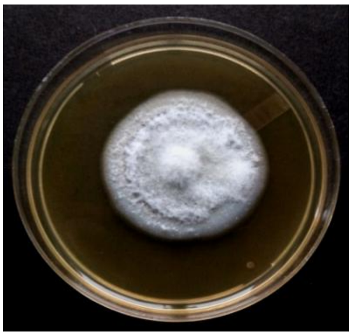


T_3

(C)

T_1

T_2



T_3

Photo 8. (A) *Magnaporthe grisea* growth under control, (B) Growth of *Magnaporthe grisea* inhibited by plant extract made up from Assam sample, (C) Growth of *Magnaporthe grisea* inhibited by plant extract made up from Tripura sample.

(A)

Control

(B)

T_1 **T_2** **T_3**

(C)

T_1 **T_2** **T_3**

Photo 9. (A) *Drechslera oryzae* growth under control, (B) Growth of *Drechslera oryzae* not inhibited by plant extract made up from Assam sample, (C) Growth of *Drechslera oryzae* not inhibited by plant extract made up from Tripura sample.

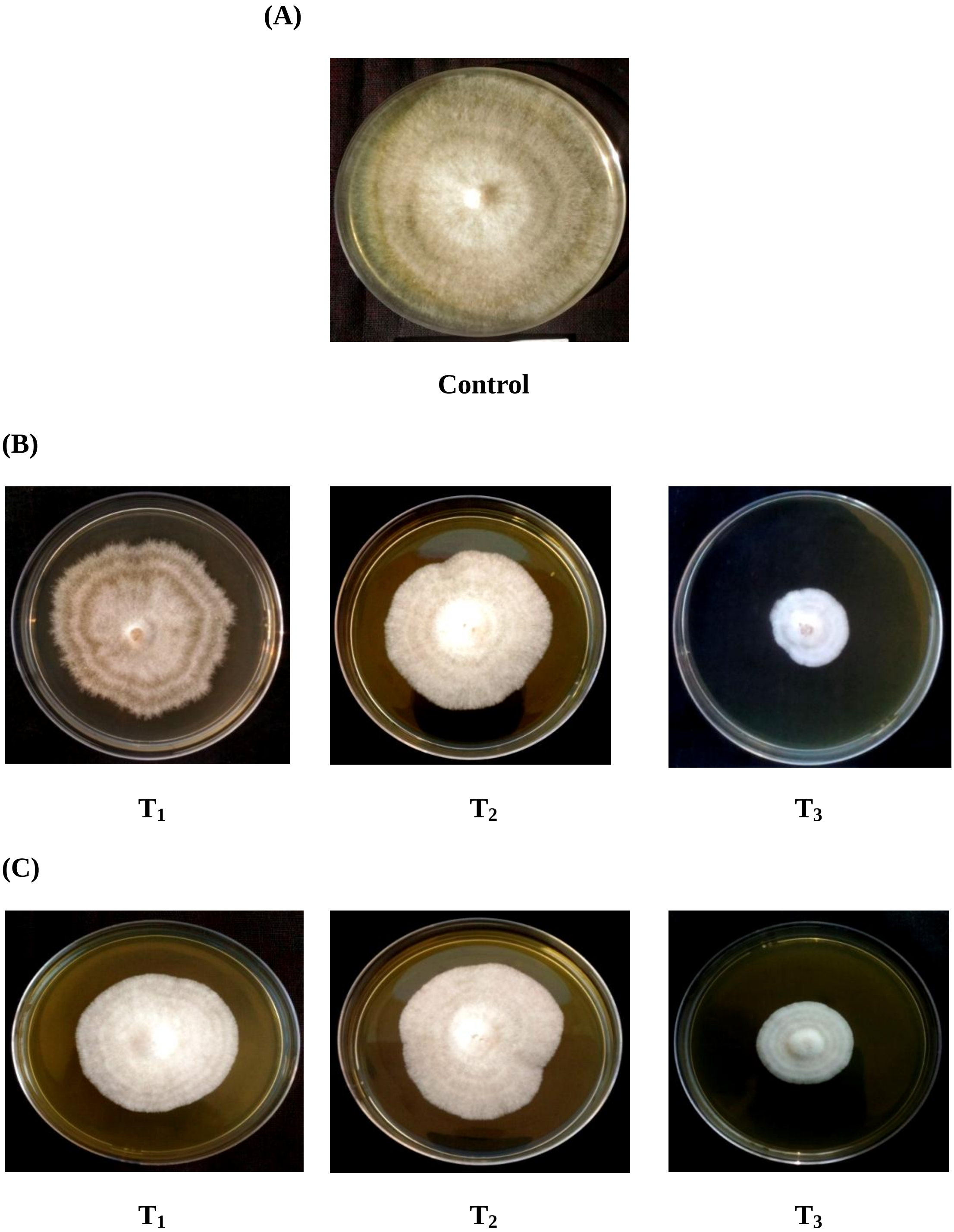
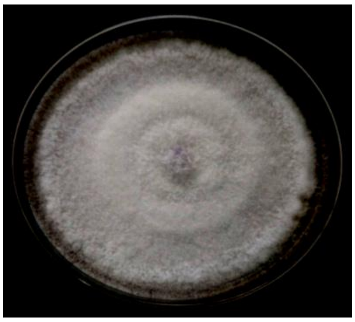


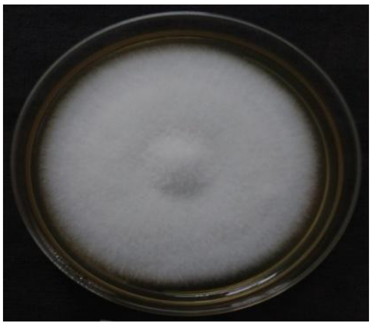
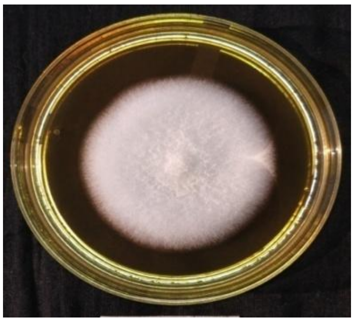
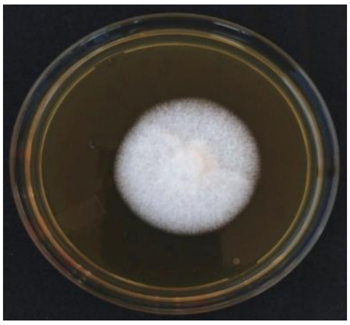
Photo 10. (A) *Rhizoctonia solani* growth under control, (B) Growth of *Rhizoctonia solani* inhibited by plant extract made up from Assam sample, (C) Growth of *Rhizoctonia solani* inhibited by plant extract made up from Tripura sample.

(A)



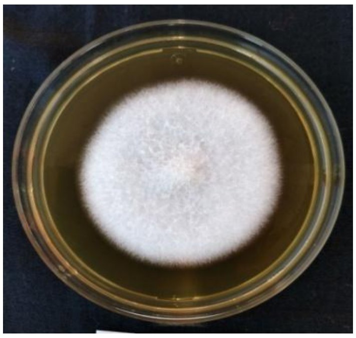
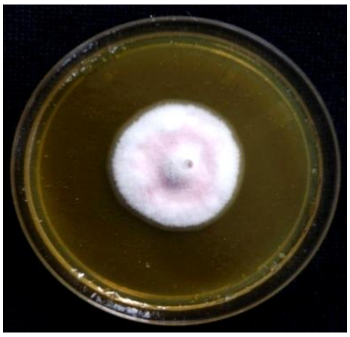
Control

(B)

T_1 **T_2** **T_3**

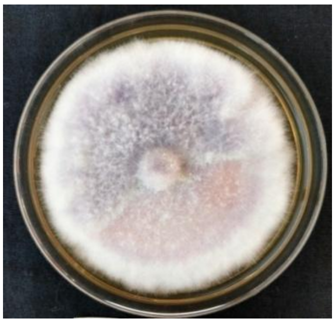
(C)

T_1 **T_2** **T_3**

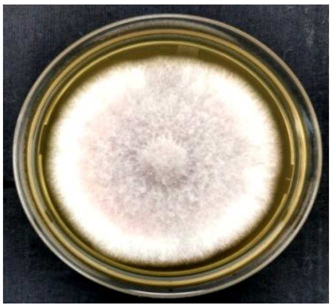
Photo 11. (A) *Sarocladium oryzae* growth under control, (B) Growth of *Sarocladium oryzae* inhibited by plant extract made up from Assam sample, (C) Growth of *Sarocladium oryzae* inhibited by plant extract made up from Tripura sample.

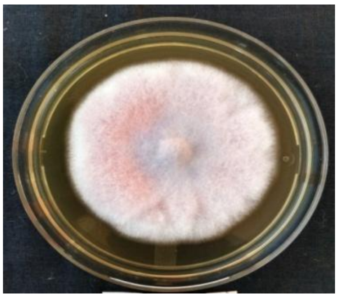
(A)

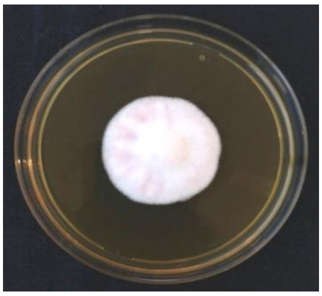


Control

(B)

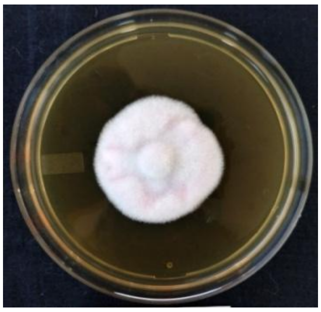






T_1 T_2 T_3

(C)



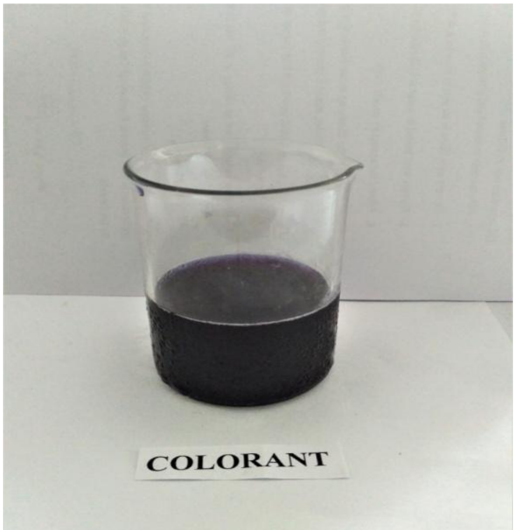
T_1 T_2 T_3

Photo 12. (A) *Fusarium* sp. (*Gibberella fujikuroi*) growth under control, (B) Growth of *Fusarium* sp. (*Gibberella fujikuroi*) inhibited by plant extract made up from Assam sample, (C) Growth of *Fusarium* sp. (*Gibberella fujikuroi*) inhibited by plant extract made up from Tripura sample.

4.7. Natural food colorant

Natural food colorant was extracted from *Clitoria ternatea* Linn. flower. The optimization experiment showed 60^0C temperature, 20 minute time and 1:20 ratio of solid : liquid was optimum condition for better colour yield. To immobilize the color on to edible inert carrier food material such as rice powder and glucose powder was tried. Glucose powder was proved effective (Photo 13). The colour variation through pH change was observed by using aqueous extracts of *Citrus reticulata* L (*Kazinemu*) fruit and *Musa bulbisiana* L (*vimkol*) peel. The *Kazinemu* extract had pH of about 3.19 while the *kolakhar* had pH of about 12.10. Both the extracts were used to observe the change in colour of the flower extract with change in pH in a range of pH 3-12 and the resultant colour variation is shown in photo 14.

(A)



Edible food colorant

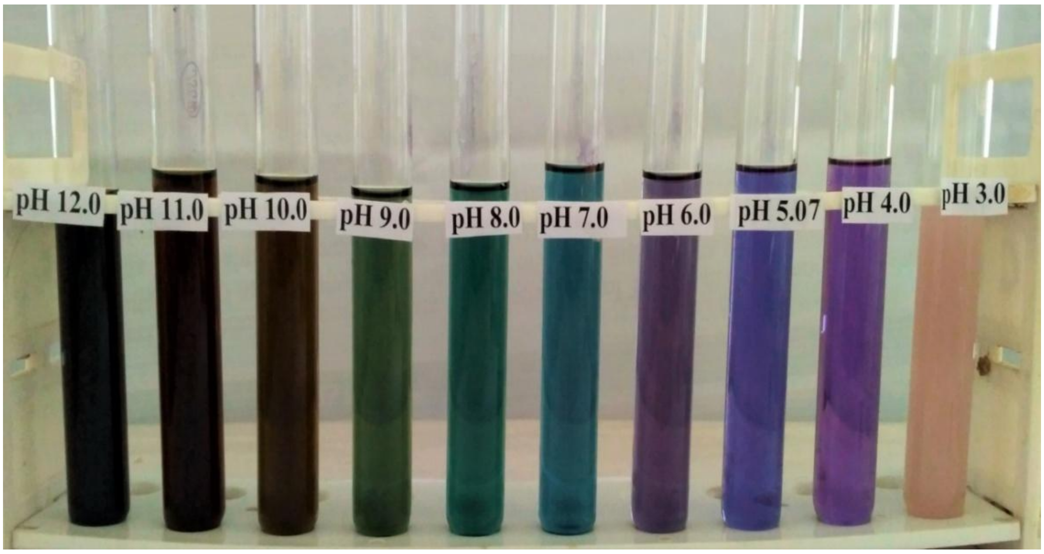
(B)

Concentrated food colorant

(C)

Food colorant with carrier material glucose

Photo 13. (A) Edible food colorant extracted from *Clitoria ternatea* Linn., (B) Concentrated food colorant, (C) Food colorant with carrier material glucose.



Color variation in different pH

Photo 14. Color variation in different pH by natural alkali and acidic solution.

CHAPTER V

DISCUSSION

The medicinal plant species *Clitoria ternatea* Linn. has been used for many centuries by the local people of South East Asia especially in India, Thailand, and Malaysia to treat many different ailments and some regard it as spiritual plant. Though this plant has so much potentiality, still it is considered underutilized specially in the North-Eastern India. Previous analysis has shown that the different parts of *C. ternatea* contained carbohydrate, protein, amino acids, alkaloid flavonoid, phenol, tannin, steroid, tannin and terpenoid (Nigam and Shrivastava, 2013). The present investigation was aimed to determine the total composition of the phytochemicals like phenolics, alkaloids and terpenoids along with the antimicrobial activity of methanolic extracts of *Clitoria ternatea* Linn. The findings of the present investigation are discussed in this chapter.

5.1 Plant morphological characteristics of *C. ternatea* L.

The plant morphological characteristics of *Clitoria ternatea* L. were analyzed in samples collected from Assam (S_1) and Tripura (S_2). The plant height at maturity was 3.51m and 3.68m in the sample S_1 and S_2 respectively. Similarly, the leaf length was found to be 10.83cm and 10.43cm in the sample S_1 and S_2 respectively while the leaf breadth was found to be 7.97cm in S_1 and 8.53cm in S_2. The differences in plant morphological characteristics in the two samples was not significant and found statistically at par.

5.2. Moisture content of *Clitoria ternatea* Linn.

In the present investigation, the highest moisture content was found in flower of the sample S_1 collected from Assam which was 78.04% while the stem recorded the lowest of 38.76% on fresh weight basis (figure 1). The sample S_2 collected from Tripura had intermediate moisture content ranging from 40.06 in stem to 77.00% in flower. The variation in moisture content between the two samples *viz*. S_1 and S_2 of *Clitoria ternatea* L. was not significant. On the other hand, the highest moisture content on dry weight basis was found in flower of S_1 collected from Assam which was 13.70% while the leaf recorded the lowest 8.82% of moisture. The total moisture content of S_2

sample had intermediate moisture content ranging from 8.02% in leaf to 13.08% in flower. The moisture content is an important factor influencing water potential in and out of cell which in turn affects membrane transport, synthesis and storage of phytochemicals. Our findings were in agreement with the results of the moisture content reported by Deshmukh and Jadhav (2014). According to them, the moisture content of *Clitoria ternatea* Linn. leaf contained 74.51% of moisture on fresh weight basis. Kavitha and Premalakshmi (2013) reported that 11.52% of moisture on dry weight basis present in *C. ternatea* leaf sample. Taur *et al*. (2010) also recorded 12.5% moisture content in dried powered sample of *C. ternatea* leaf. Jain and Shukla (2011) reported 5.92% of moisture content in *C. ternatea* stem on dry weight basis.

5.3. Total phenolics content of *Clitoria ternatea* Linn.

Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases like cancer. On the last few years, the identification and development of phenolic compounds or extracts from different plants has become a major area of research related to health and disease prevention (Dai and Mamper, 2010). Phenolic compounds are well-known phytochemicals found more or less in almost all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule (Khoddami *et al.,* 2013). Plant phenolics might be classified in two classes: those that were synthesized during the normal development of plant tissues and those that were synthesized by plants in response to physical injury, infection or other stress (Beckman, 2000). Plant polyphenols imparted effective resistance against the development of cancers, cardio vascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Graf *et al.,* 2005). In the current investigation, the total phenolic content in the leaf and the stem of sample S_1 collected from Assam was found to be 1.524g /100g and 0.706g /100g as catechol equivalent (CE), respectively which was higher than S_2. On the other hand, the total phenolic content in the sample S_2 collected from Tripura contained 1.209g CE/100g in the twig which was higher than S_1(figure 2).

The findings were in agreement with the results obtained by Madhu (2013) who reported that, the ethanolic leaf extract of *Clitoria ternatea* L. contained 1.950g/100g to 2.550g/100g of total phenolic as Gallic Acid Equivalent (GAE). Similar results were also reported by Rabeta and Nabil (2013). They obtained the total phenolics in aqueous *Clitoria ternatea* L. plant in the range of 1.85g GAE/100g to 2.58g GAE/100g. On the other hand, Neelamma *et al*. (2016) reported that the methanolic extract of *Clitoria ternatea* L. plant contained 0.973g GAE/100g of total phenolics.

5.4. Alkaloid content of *Clitoria ternatea* Linn.

Alkaloids formed one of the largest group of plant secondary metabolites, being present in several pharmacologically active plant families. Alkaloids were comprised of neuroactive molecules and many of these biomolecules were already in use including caffeine, nicotine, as well as life-saving medicines such as emetine used to fight oral intoxication and the antitumorals like vincristine and vinblastine (Matsuura and Fett-Neto, 2017). Owing to their potent biological activity, many of the approximately 12,000 known alkaloids have been exploited as pharmaceuticals, stimulants, narcotics, and poisons. Alkaloids were also reported to play a defensive role against herbivores and pathogens (Ziegler and Facchini, 2008).

In the present investigation the leaf, stem and twig of the sample S_1 collected from Assam exhibited significantly higher total alkaloid content which were 1.000g/100g, 0.753 g/100g and 1.627g/100g respectively (figure 3). The total alkaloid content of S_2 sample revealed an amount of 0.875, 0.627 and 1.253 g/100g in leaf, stem and twig respectively. Reshmi and Raghunath (2011) reported 0.25 to 0.43 per cent alkaloid content on dry weight basis in seeds of *Clitoria ternatea* L. Earlier, Nigam and Shrivastava (2013) disclosed the presence of alkaloids in methanol and ethyl acetate extract of *Clitoria ternatea* Linn. leaves determined by Mayer's, Dragendroff's and Wagner's tests. Qualitative tests were also performed by Taur *et al*. (2010) who reported the presence of alkaloids in the aqueous methanol, ethyl acetate and benzene extract of *C. ternatea* leaf. Moreover Khatoon *et al*. (2015) also reported the presence of alkaloids in *Clitoria ternatea* Linn. leaf. The methanolic extract of stem, leaves and seeds of Clitoria ternatea showed the presence of 26 different types of alkaloids with 21 different Rf values with range 0.02 to 0.93 (Ponnusamy *et al.* 2013).

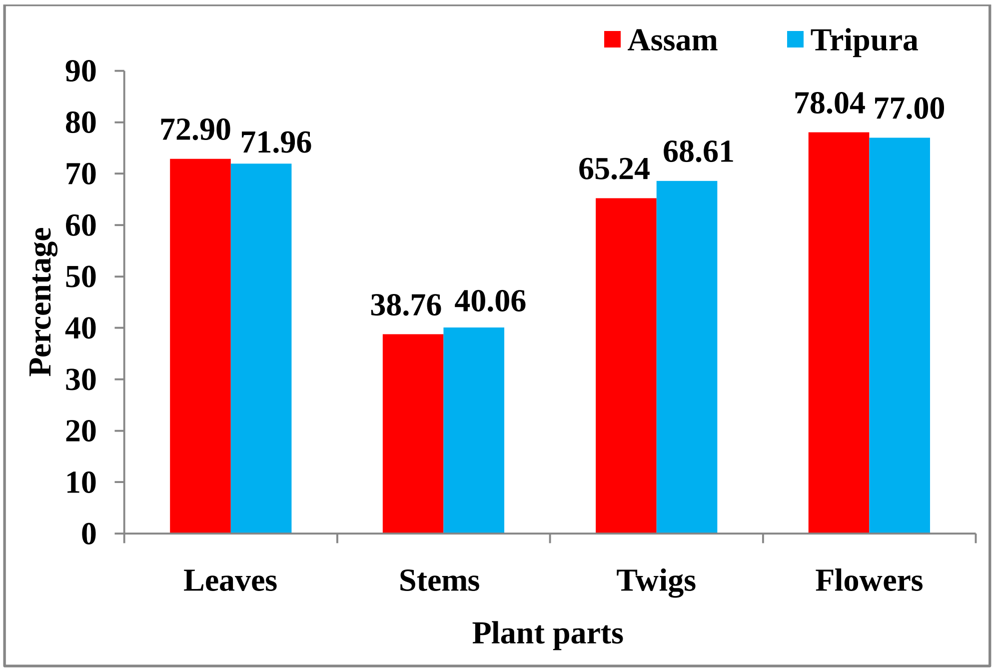


Fig. 1. Total moisture content in different parts of *Clitoria ternatea* Linn. (g/100g) fresh weight basis

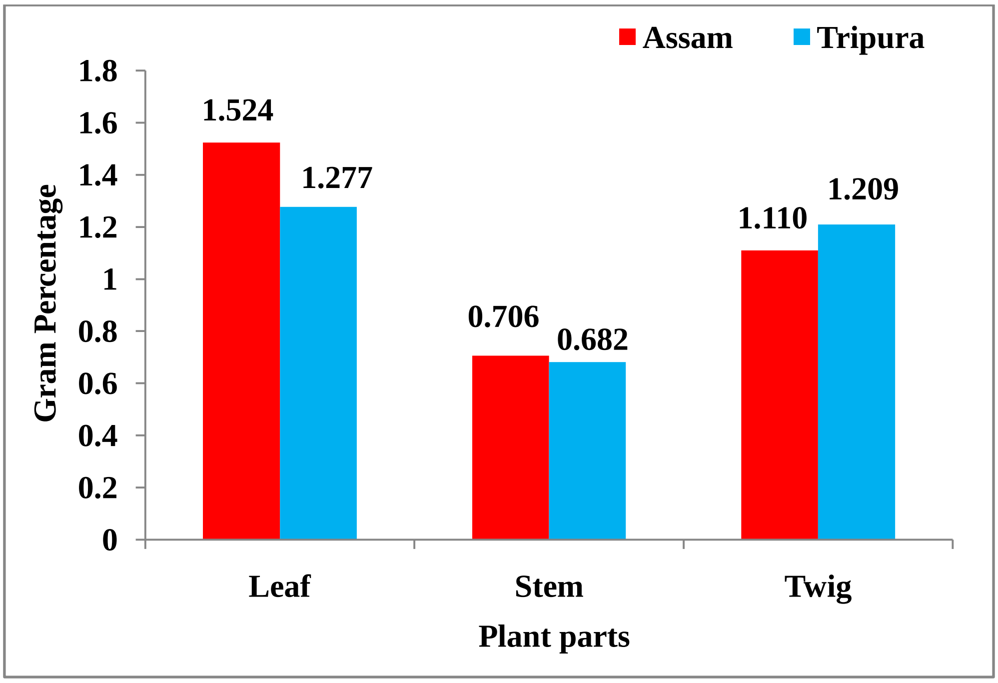


Fig. 2. Total Phenolics Content in different parts of *Clitoria ternatea* Linn. (g/100g) on dry weight basis

5.5. Terpenoids content of *Clitoria ternatea* Linn.

Terpenoids constituted one of the most diverse groups of phytochemicals produced by the plants. Plants synthesized terpenoid metabolites for a variety of basic functions in growth and development but used the majority of terpenoids for more specialized chemical interactions and protection in the abiotic and biotic environment, especially in plant defense (Tholl, 2015). Terpenes were the most numerous and structurally diverse natural plant products. The isoprene (2 methyl 1, 3 buta-di-ene) unit, the building block of terpenes, is a five-carbon molecule. The single isoprene unit represents the most basic class of terpenes, the hemiterpenes. Based on number of isoprene units, the terpenes are known as monoterpene (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), tetraterpenes and polyterpenes while terpenoids contained varying number of carbon atoms.(Zwenger and Basu, 2008).

During the present investigation, terpenoids were not detected in leaf, stem and the twig extract in either of the two samples by qualitative test. But, the flower extract tested positive (photo 7). The flower extract of the sample S_1 collected from Assam showed higher total terpenoid content of 0.698g/100g whereas S_2 from the Tripura recorded 0.675g/100g (figure 4). The results were in concomitant with earlier observation of Darsini and Shamshad (2013) who reported the absence of terpenoids in *Clitoria ternatea* leaf and stem but found their presence in flower. Kavitha and Premalakshmi (2013) also reported the absence of terpenoids in *Clitoria ternatea* leaf.

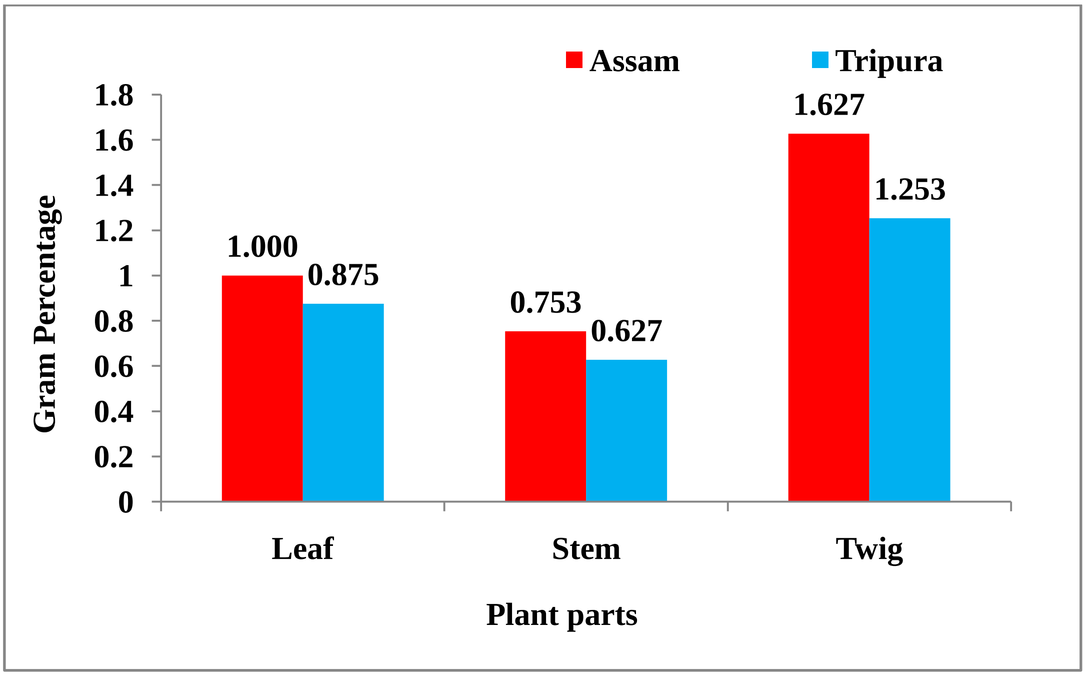


Fig. 3. Total Alkaloid Content in different parts of *Clitoria ternatea* Linn. (g/100g) on dry weight basis

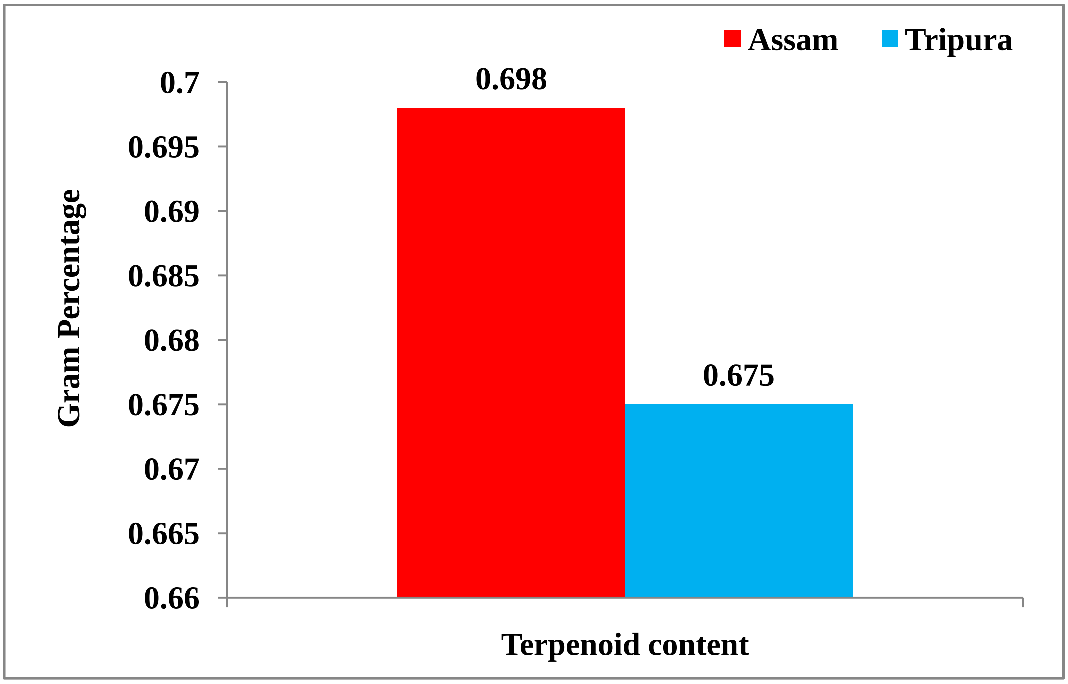


Fig. 4. Total Terpenoid Content in flower of *Clitoria ternatea* L. (g/100g) on dry weight basis

5.6. Antimicrobial activity of *Clitoria ternatea* Linn.

For the treatment of diseases, many compounds are employed to kill micro-organisms or prevent their growth and are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms. In the present days, the adverse effect of chemical fungicides becomes a burning topic. People were trying to reduce the use of chemical fungicides by introducing new approaches to control the diseases as the concept of organic farming has been becoming more and more popular. By using plant products or plant derived products might be a new approach of disease control.

The plant contains biochemicals which are produced during the metabolic processes of the cells such as amino acids, sugars, glycosides, organic acids, enzymes, alkaloids, nucleotides and flavonones and inorganic ions. Generally these substances are ideal nutrients for microbes and help in their growth, but a number of inhibitory compounds are also contained in the exudates. Plant produces thousands of naturally synthesized compounds many of which are toxic to the pathogens. These compounds synthesized and stored in vacuoles, lysosomes, heartwood and periderm. Alternatively less toxic precursors of these antimicrobial compounds stored in vacuoles. In general the antimicrobial substances are unsaturated lactones, cyanogenic glycosides, sulphur containing compounds, phenols, phenolic glycosides and saponins. These inhibitory and antimicrobial substances directly affect the microorganisms and function as antagonists of the pathogens.

The presence of secondary metabolites confers the plant with growth inhibitory properties against the pathogen. Out of these metabolites alkaloids, phenolics, and terpenoids are the most important ones which have the antimicrobial properties against the pathogens. Alkaloids are natural products that contain heterocyclic nitrogen atoms and are basic in character. Most of the alkaloids are well defined crystalline substances which unite with acids to form salts. In plants the alkaloids exist in free state or as salts. Due to the bittery taste, alkaloids are significant for the protection and survival of plant because they ensure the resistance of plants against micro-organisms due to their antibacterial and antifungal action, against insects and herbivores as feeding deterrents and also against other plants by means of alleopathy. One of the mechanism of action of highly aromatic planner quaternary alkaloids is attributed to their ability to intercalate with DNA. Phenolics are well known as antifungal, antibacterial and antiviral properties produced by the plants. The phenolic compounds are responsible for first step of defense which is known as basal resistance by destroying the pathogen cell wall. Phenolics performed as antimicrobial compounds that include simple phenols, phenolic acids, and flavonols which inhibit the growth of fungi, helps to limit pathogen attachment, invasion and infection. The cell wall which act as a major line of defense against fungal pathogens contains cellulose, lignin, and a heterogeneous polymer composed of phenolic compound that gives the cell rigidity. The phenolic compounds present in the plant tissue act as deterrents to pathogens. Phytoalexins, the low molecular weight compounds were isoflavonoids with antibiotic and antifungal properties that disrupt the pathogen metabolism and cellular structure (Kumar *et al*., 2014). Another important secondary metabolite called terpenoids which is structurally a most diverse group. They have their role of direct and indirect defense of plant. The phytoalexins functions in plant's direct defense or by signals in indirect defense responses which involves herbivores and their natural enemies. The volatile oil present in the plants had direct toxic effect to the pathogens (Cheng *et al*. 2007). If the pathogen is capable of suppressing basal defense then plants may respond another line of defense, the hypersensitive reaction (HR). The HR is characterized by delivered plant cell suicide at the site of infection. The HR may limit pathogen interference by sacrificing a few cells in order to save the rest of the plant. Once the HR has been triggered the plant tissues may become highly resistant to a wide range of pathogens for extending period this phenomenon is known as systemic acquired resistance (SAR).

In the present investigation the antimicrobial activity of methanolic extract of *Clitoria ternatea* were examined by poisoned food technique. The different test organisms used in the experiment were *Magnaporthe grisea*, *Drechslera oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae*, and *Fusarium* sp. The methanolic extract of the whole plant of *C. ternatea* could inhibit the mycelial growth of all the fungal species except *Drechslera oryzae* (figure 5-8). The sample S_1 collected from Assam and S_2 from Tripura at a concentration of 3mg/ml (T_3) inhibited the mycelial growth of *Rhizoctonia solani* as much as 60.99 and 57.80 percent respectively followed by *Sarocladium oryzae* (48.56 and 47.86 per cent), *Fusarium* sp. (48.08 and 46.28 per cent) and *Magnaporthe grisesa* (41.15 and 43.58 per cent) respectively. The inhibition of mycelial growth by the methanolic extract can provide a lead to isolation of bioactive antifungal compounds from this plant species. Yadava and Verma (2003) reported that the petroleum ether seed extract of *Clitoria ternatea* L showed high antifungal activity with a zone of inhibition (13.5 mm) against *Fusarium oxysporum.*

5.7. *Clitoria ternatea* Linn. flower as a natural food colorant

Coloring of food or adding color to food has been done since long ago for making the food attractive. By this the color of food is enhanced and also variation of color is imparted in same food. There are two types of colorant, synthetic and natural. Synthetic colorants are commercially available as powders, granules or solutions. These synthetic colorants are chemically synthesized and also have toxic effects. But the colorants synthesized from natural sources especially from the edible plant parts are safe for human consumption. In plant the color forms due to the presence of flavonoids specially anthocyanins. It is a water soluble vacuolar pigment. The anthocyanin are synthesized from polypropanoid pathway. Generally the anthocyanins are present in different parts of plant such as leaves, stems, fruits and flowers. Depending on pH the pigments may be blue, red or purple. The stability of color is influenced by factors such as structure and concentration, pH, temperature, light, oxygen, solvents, enzymes present, other flavonoids, proteins and metallic ions (Rodriguez-Amaya, 2016). Natural food colorants, apart from being safe, also posses human health promoting properties. The natural food colorants increase the consumer preference because of its organic nature, variety and flavors (Rymbai *et al*, 2011).

In the present investigation the natural food colorant has been isolated from *Aparajita* (*Clitoria ternatea* Linn.) flower. Natural colour (dye) was prepared by using water extraction method. The purpose of using water extraction is to make sure that the colour retains its edible character and is safe from the chemical and harmful substance. The optimization experiment showed that the colour extraction conditions of 60^0C temperature, 20 minute time and 1:20 ratio of solid : liquid were optimum condition for better colour yield. To immobilize the color on to edible inert carrier, food material such as rice powder and glucose powder were tried where glucose powder proved effective. The colour variation through pH change was observed by using aqueous extracts of *Citrus reticulata* L. (*Kazinemu*) fruit and *Musa bulbisiana* L. (*vimkol*) peel which were edible in character. Both the extracts were used to change the pH of the colour extract in a range of pH 3-12 and the resultant colour varied from light pink (pH 3.0) to dark yellow (pH 12.0) (Photo 14)

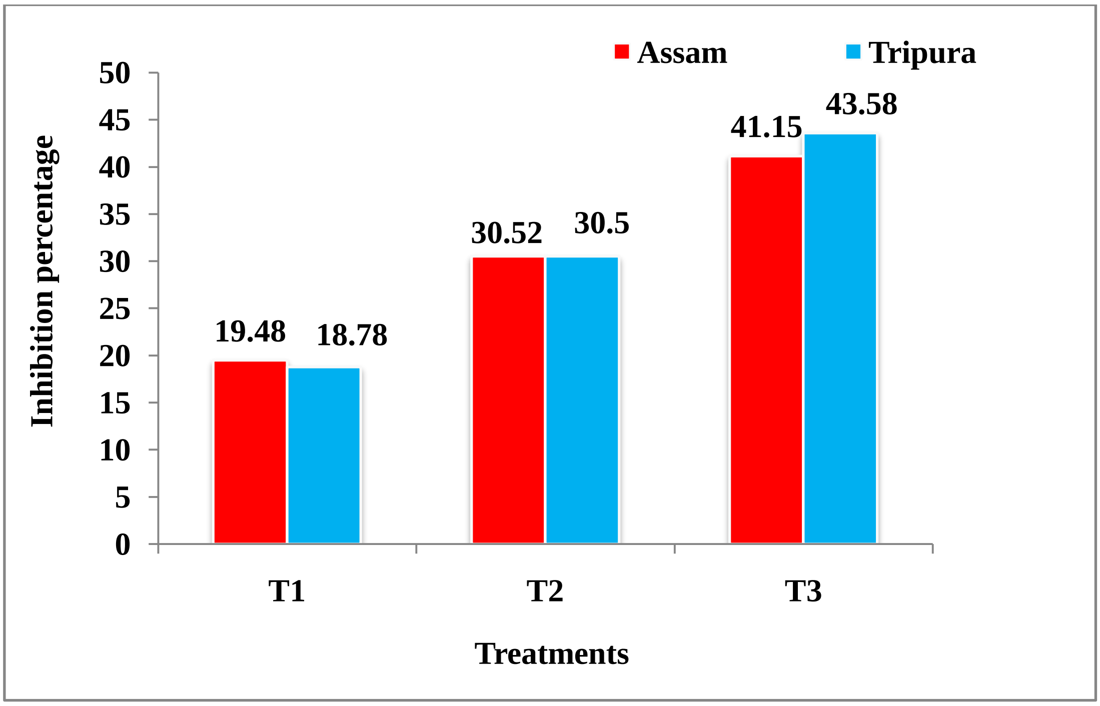


Fig. 5. Percent inhibition of mycelial growth of *Magnaporthe grisea*

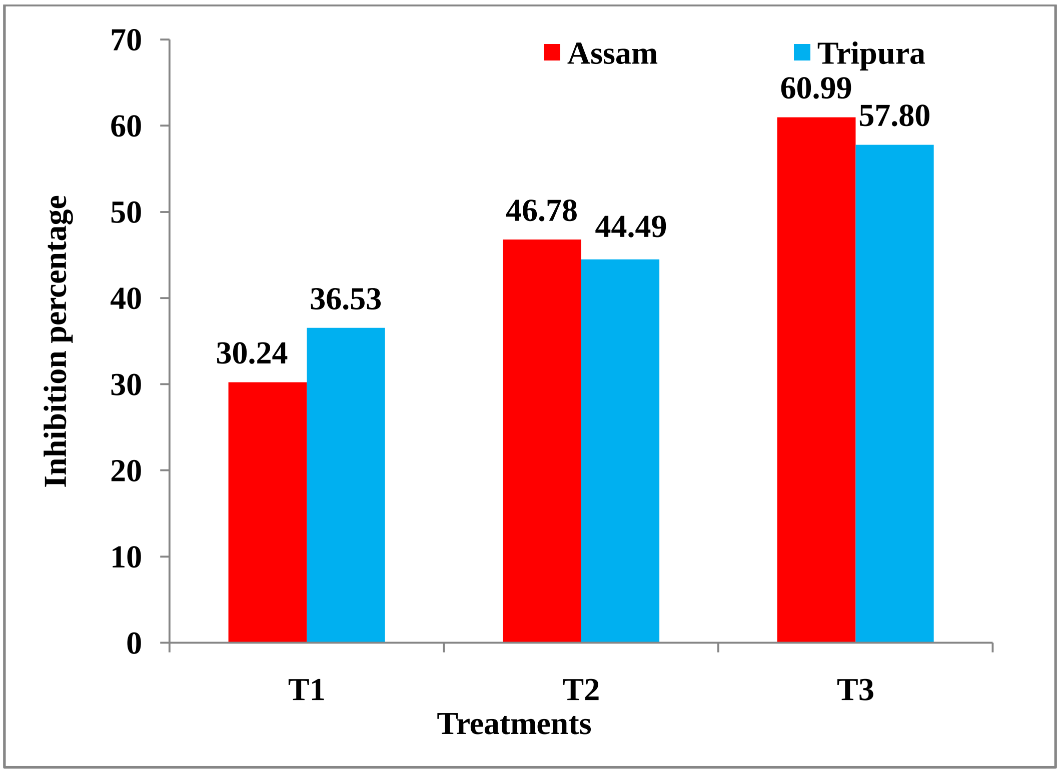


Fig. 6. Percent inhibition of mycelial growth of *Rhizoctonia solani*

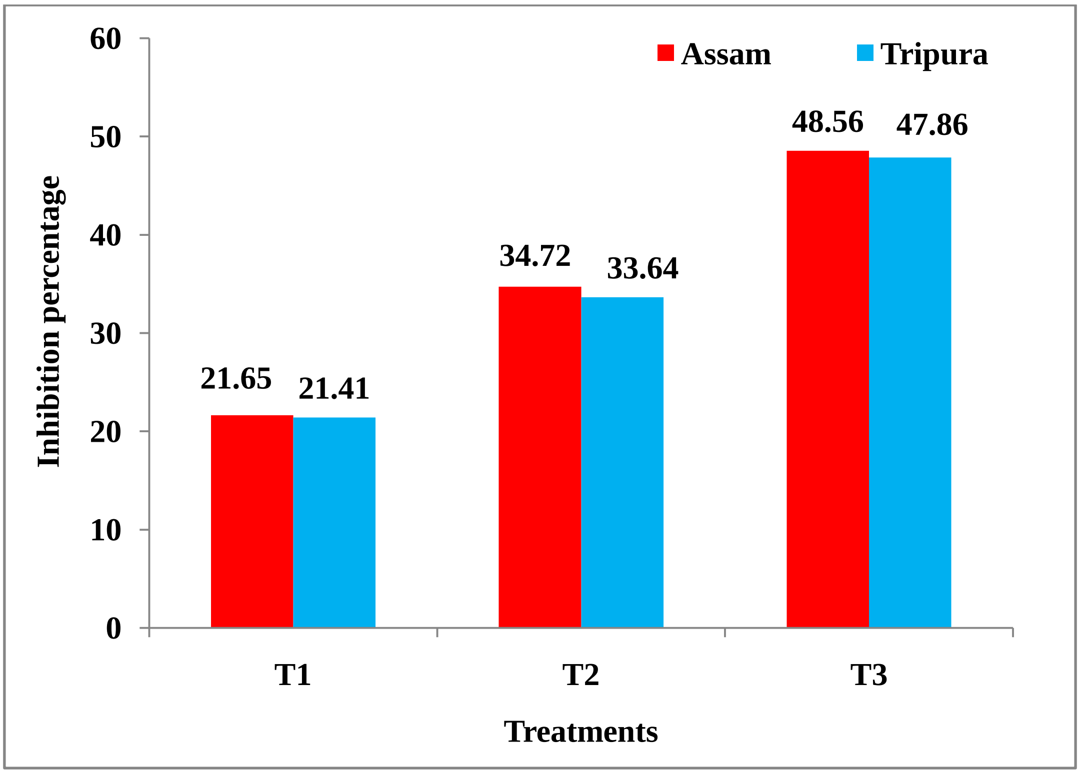


Fig. 7. Percent inhibition of mycelial growth of *Sarocladium oryzae*

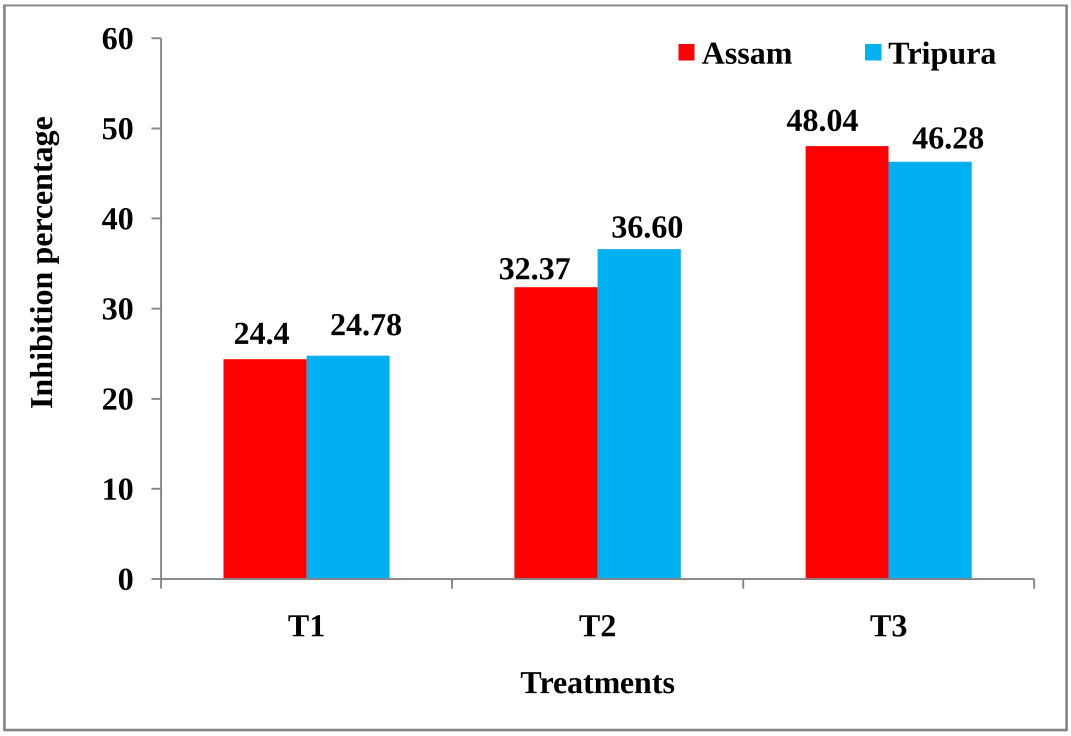


Fig. 8. Percent inhibition of mycelial growth of *Fusarium* sp. (*Gibberella fujikuroi*)

Goodwin and Mercer (2003) reported that three most common anthocyanins *viz*. pelargonidin, delphinidine and cyanidin differ only in the number of hydroxyl groups in their structure. Because of their ionic character, both the intensity and shade of colour of anthocyanidins vary with changes in pH. In acid solution (methanol-HCl) the colours very from orange-red (pelargonidin, abs. max. 520nm) through magneta (cyanidin; abs. max. 535nm) to mauve (delphinidin; abs. max. 545nm). The differences depend on the number of hydroxyl groups in its structure.

Mukherjee *et al.* (2008) reported that the tender parts of *Clitoria ternatea* L were eaten as vegetables in Kerela and Philippines. Ezzudin and Rabeta (2018) observed that the flower of this tree has violate, deep-blue and white colouration and it is usually used as a natural colourant in food preparation especially in the local culinary scene such as for the preparation of *nasi kerabu* and *kuih tekan*. Moreover, this plant could act as a food source for the livestock due to the taste and nutritious value it has. The blue flower of *C. ternatea* L. (*Aparajita*) contained anthocyanin.

Earlier different researchers isolated ternatins A3, B4, B3, B2, C1, C2, C3, C4, C5, and D2, D3 and preternatins A3 and C4 from *C. ternatea* flowers (Terahara and Masahiro,1996; Terahara,1998). Srivastava and Pande (`1977) identified three closely related anthocyanins namlely malvidin-3-β-glucoside, delphinidin-3-β-glucoside and 3-methyl derivative of delphinidin-3-β-glucoside.

The results of the present study confirmed the ethnomedicinal usage and pharmacological importance of the medicinally important plant *Clitoria ternatea* L. The study further suggested that some of the plant extracts possess compounds with bioactivity properties that could be used as active principles or agents in new drugs for the therapy of infectious diseases. The food colour extracted from the flower of *C. ternatea* L. could be an ideal natural colourant for the food industry in future.

CHAPTER VI

SUMMARY AND CONCLUSION

The present investigation was intended to study as well as to quantify the phytochemical composition, to extract the natural colourant from flower and to evaluate the antimicrobial property of *Clitoria ternatea* L. which is an underutilized plant species found in North East India. The plant materials were collected from Jagduar, Teok, Jorhat district, Assam and Matabari, Gomati district, Tripura and authenticated. The plants were also raised from seeds in pots of size 2ft x 2ft x 2ft in the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat and kept under continuous observation throughout the experimental period. The morphological data were taken from the mature plant to narrate the botanical information. Leaf, stem, twig and flower of the two samples were analyzed for total phenolics, total alkaloids and total terpenoids. The natural colour from the flower was extracted and the antimicrobial activity of the methanolic plant extract was determined.

The difference in plant morphological characteristics in the two samples was not significant and found statistically at par. The variation in moisture content on fresh weight basis between the two samples *viz*. S_1 and S_2 of *Clitoria ternatea* L. was not significant. On the other hand, the highest moisture content on dry weight basis was found in flower of S_1 collected from Assam which was 13.70% while the leaf recorded the lowest 8.82% of moisture.

From the results of the present investigation it was observed that the total phenolics content in the leaf obtained which were 1.524g/100g and 1.277g/100g, in stem 0.706g/100g and 0.682g/100g, and in twig 1.110g/100g and 1.209g/100g in S_1 and S_2 sample, respectively.

The alkaloid content of the leaf was found to be 1.000g/100g and 0.875g/100g, in stem 0.753g/100g and 0.627g/100g, and in twig 1.627g/100g and 1.253g/100g in S_1 and S_2 sample, respectively.

By the qualitative test it was observed that the terpenoid was found to be absent in leaf, stem, and twig in the both Assam (S_1) and Tripura (S_2) sample but was present in flower of both the sample and the variation was non significant. The total terpenoid content of flower was found to be 0.698g/100g and 0.675g/100g in Assam (S_1) and Tripura (S_2) sample respectively.

The antimicrobial activity of the methanolic extract of whole plant of Assam (S_1) and Tripura (S_2) sample of *Clitoria ternatea* L. was evaluated against five different rice pathogens, *viz*., *Magnaporthe grisea*, *Drechslera oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae*, and *Fusarium sp.* (*Gibberella fujikuroi*). The methanol extract of both the plant sample showed potential antimicrobial activity against *Magnaporthe grisea*, *Rhizoctonia solani*, *Sarocladium oryzae*, and *Fusarium sp.* (*Gibberella fujikuroi*). But both the plant sample extract did not show any antimicrobial activity in terms of mycelial growth inhibition against *Drechslera oryzae.*

The natural food colorant has been extracted from *Clitoria ternatea* L. flower. Variation in color has been observed at different pH. The changes in pH were brought about by adding lemon juice and alkali extracted from *vimkol*. To immobilize the color on to edible inert carrier food material such as rice powder and glucose powder, it was proved that glucose powder was effective in color immobilization.

From the above experimental results it can be concluded that-

- The leaf contained higher phenolic content as compared to stem and twig in both the sample of *Clitoria ternatea* Linn.
- The alkaloid content was higher in twig part as compared to other parts of the plant.
- The terpenoid content was found to be absent in leaf, stem, twig and present in flower in both the sample.
- The methanolic plant extract of *Clitoria ternatea* L. showed the potential antimicrobial activity against *M. grisea*, *R.solani*, *S.oryzae* and *Fusarium* sp. which implies its potential novel lead in future for use in organic farming.
- The methanolic plant extract of *Clitoria ternatea* L. did not show any antimicrobial activity against *D. oryzae.*
- The natural food colorant was extracted from flower of *Clitoria ternatea* L. Variation in color was observed due to change in pH brought about by adding lemon juice and alkali extracted from *vimkol.*

From the view point of the above experimental results it can be concluded that owing to different geographical, ecological and soil conditions the phytochemical composition of the plant may vary from one another. The North East region has its own unique combination of living species, habitats and ecosystems, which together make up its diversity rich resource. Medicinal plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), terpenoids (including carotenoids). Epidemiological studies has shown that many of these compounds posses anti-inflammatory, anticarcinogenic, antiatherosclerotic, antitumor, antimutagenic, antimicrobial activities to a greater or lesser extent and different parts of *Clitoria ternatea* L. plant has exhibited many of the above mentioned properties during the present investigation.

Future prospect

The vast medicinal plant resources of North-East India have not been fully identified, inventoried and characterized. *Clitoria ternatea* L. is rich in many bioactive compounds. It is of utmost important that these should be characterized and evaluated keeping in mind the upcoming scientific approaches, which may lead to the outcome of some new bioformulation that can be used to manufacture new biopesticides, as a substitute of chemical fungicides and thereby reducing the adverse effect of synthetic fungicides in the environment. Further studies are required for analysis of mode of action of antimicrobial activity of the methanolic plant extract on the plant pathogens of different crops, which will open an interesting area to manage various diseases in crop plants under organic cultivation.

Acknowledgement

The author thanks the concerned authority of MTCC, IMTECH, Chandigarh for the supply of the fungal culture. The author acknowledges the help received from the Dept. of Plant Pathology, Dept. of Agril. Biotechnology and Dept. of Agril. Statistics in carrying out the research work.

BIBLIOGRAPHY

A.O.A.C (1970). *Official Methods of Analysis.* 10th Ed. Association of Analytical Chemists, Washington D.C.

Agrawal, P.; Deshmukh, S.; Ali, A.; Patil, S.; Magdum, C.S.; Mohite, S.K. and Nandgude, T.D. (2007).Wild flowers as medicine. *International Journal of Green Pharmacy (IJGP)*. **1**(1):12-13.

Al-Snafi, A.E. (2016). Pharmacological importance of *Clitoria ternatea* – A review. *IOSR Journal of Pharmacy*. **6**(3): 68-83.

Anand, S.P.; Doss, A. and Nandagopalan, V. (2011). Antibacterial studies on leaves of *Clitoria ternatea* Linn.-A high potential medicinal plant. *Int. J. Appl. Bio. Pharm. Technol.* **2**(3): 453-456.

Anarthe, S.J.; Spoorthisudha, P.; Swetha, M. and Raju, M.G. (2017). *In vitro* and *in vivo* screening of *Clitoria ternatea* (Linn.) for Immunomodulatory activity. *Int. J. Chem. Tech. Res.* **10**(1): 116-125.

Anonymous, (1998). Wealth of India, Raw material, *CSIR*, New Delhi.

Balasundaram, A.; Kumari, P.R.; John, G. and Selvakumar, B.N. (2011). Antimicrobial activity of the leaf extracts of two medicinal plants against MRSA (Methicilin Resistant *Staphylococcus aureus*) from human urinary tract pathogens. *Res. J. Microbiol.* **6**(7): 625-631.

Beckman, C.H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? *Physiological and Molecular Plant Pathology*. **57**(3): 101-110.

Bhatia, M.; Chahal, J. and Gupta, S. (2014). Analgesic and anti-inflammatory activities of *Clitoria ternatea* Linn. Leaves extract on rat model. *Int. J. of Pharma. Sci. and Res.* **5**(2): 600-606.

Bruneton, J. (1995). Pharmacognosy, Phytochemistry, Medicinal Plants. *Lavoisier Publishing Inc, C.,* Paris.

Cai, Y.; Sun, M. and Corke, H. (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry*. **51**(8): 2288-2294.

Chakraborty, S.; Sahoo, S.; Bhagat, A. and Dixit, S. (2017). Studies on antimicrobial activity, phytochemical screening tests, biochemical evaluation of *Clitorea ternatea* Linn. plant extracts. *International Journal of Research – Granthaalayah*. **5**(10): 197-208.

Chattopadhyay, P.; Chatterjee, S. and Sen, S.K. (2008). Biotechnological potential of natural food grade biocolorants. *African Journal of Biotechnology*. **7**(17): 2972-2985.

Chavan, S.S.; Jadhav, R.S.; Kharat, D.; Mankar, S.D. and Godge, R.K. (2015). Evaluation of Analgesic Activity and Phytochemical Screening of *Clitoria ternatea* Linn. *British Journal of Pharmaceutical Research*. **6**(4): 255-260.

Cheng, A.X.; Lou, Y.G.; Mao, Y.B.; Lu, S.; Wang, L.J. and Chen, X.Y. (2007). Plant terpenoids: biosynthesis and ecological functions. *Journal of Integrative Plant Biology*. **49**(2): 179-186.

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

Daisy, P. and Rajathi, M. (2009). Hypoglycemic effects of *Clitoria ternatea* Linn. (Fabaceae) in alloxan-induced diabetes in rats. *Tropical Journal of Pharmaceutical Research*. **8**(5): 393-398.

Darsini, I.P. and Shamshad, S. (2015). Antimicrobial Activity and Phytochemical Evaluation of *Clitoria Ternatea. International Journal of Science and Research*. **4**(5): 823-825.

Das, N. and Chatterjee, P. (2014a). Antifungal effect of *Clitoria ternatea* L. leaf extract on seeds of *Pisum sativum* L. in relation to the activities of some enzymes. *Int. J. Res. Ayu. Pharm.* **5**(1)**:** 99-101.

Das, N. and Chatterjee, P. (2014b). Evaluation of brine shrimp cytotoxicity of 50% aqueous ethanolic leaf extract of *Clitoria ternatea* L. *Asian. J. Pharm. Clin. Res.*, **7**(1): 118-120.

Das, N.; Ghosh, A. and Chatterjee, P. (2014). Anti fungal effect of *Clitoria ternatea* L. leaf extract on seeds of *Psivium sativum* L. in relation to some biochemical parameters. *Int J. Res. Ayu. Pharm.* **5**(4): 536-539.

Deka, B.; Deka, P.; Borgohain, R. and Neog, M. (2014). Exploration of plant derived natural dyes in Assam. *Asian Journal of Home Science*. **9**(1): 17-20.

Deka, M. (2015). Detection and estimation of phytochemicals necessary for the treatment of gynecological disorders from *Clitoria ternatea* L. root. *Journal of Applied and Fundamental Sciences*. **1**(1): 107-112.

Deka, M. and Kalita, J. C. (2011). Priliminary phytochemical analysis and acute oral toxicity study of *Clitoria ternatea* Linn. roots in albino mice. *International research journal of pharmacy*. **2**(12): 139-140.

Deka, M.; Medhi, A.K. and Kalita, J.C. (2013). Biochemical estimation of primary metabolites and mineral composition of *Clitoria ternatea* Linn. roots. *The Bioscan*, **8**(2): 713-713.

Deka, M.; Medhi, A.K.; Kalita, J.C.; Sarma, K.K. and Deka, L. (2013). Proximate Analysis of Primary Metabolites in Different Parts of *Clitoria ternatea* L. A Comparative Study. *International Archive of Applied Sciences and Technology*. **4**(3): 62-67.

Deshmukh, S. and Jadhav, V. (2014). Bromatological and mineral assessment of *Clitoria ternatea* Linn. Leaves. *Energy (KJ)*. **459**(9.20): 489-24.

Devi, B.P.; Boominathan, R. and Mandal, S.C. (2003). Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. *Fitoterapia*. **74**(4): 345-349.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*. **11**(1): 1-42.

Ezzudin, M.R. & Rabeta, M.S. (2018). A potential of Telang tree (*Clitoria ternatea*) in human health. *Food Research*, **2**(5): 415-420.

Farrell, M.J. (2012). “Sustainable Cotton Dyeing. Advanced Materials Research”, Doctor of Philosophy thesis, North Carolina State University, Raleigh, North Carolina.

Gomez, S.M. and Kalamani, A. (2003). Butterfly pea (*Clitoria ternatea*): A nutritive multipurpose forage legume for the tropics–an overview. *Pakistan Journal of Nutrition*, **2**(6): 374-379.

Goodwin, T. W., & Mercer, E. I. (2003). *Introduction to plant biochemistry*. New Delhi : CBS Publishers and Distributors.

Gowd, P.M.J.S.; Kumar M.M.G.; Shankar S.A.J.; Sujatha, B. and Sreedevi E. (2012). Evaluation of three medicinal plants for anti-microbial activity. *AYU*. **33**(3):423-428.

Graf, B.A., Milbury, P.E. and Blumberg, J.B. (2005). Flavonols, flavones, flavanones, and human health: epidemiological evidence. *Journal of medicinal food*. **8**(3): 281-290.

Gupta, G.K.; Chahal, J. and Bhatia, M. (2010). *Clitoria ternatea* (L.): Old and new aspects. *J Pharm Res*. **3**(11): 2610-2614.

Harborne, J.B. (1973). Phytochemical methods, London. *Chapman and hall*. Ltd. pp. 49-188.

Hossan, S.; Agarwala, B.; Sarwar, S.; Karim, M.; Jahan, R. and Rahmatullah, M. (2010). Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. *Ethnobotany Research and Applications*. **8**: 61-74.

Jain, R.A., and Shukla, S.H. (2011). Pharmacognostic Evaluation and Phytochemical Studies on Stem of *Clitoria ternatea* Linn. *Pharmacognosy Journal*. **3**(24): 62-66.

Jain, R.A.; Shukla, S.H. and Saluja, A.K. (2010). *In-vitro* evaluation of *Clitoria ternatea* stem extract for antioxidant property. *Int. J. Pharm. Sci. Res.* **1**(12): 84-94.

Jaiswal, Y.S. and Williams, L.L. (2017). A glimpse of Ayurveda–The forgotten history and principles of Indian traditional medicine. *Journal of traditional and complementary medicine*. **7**(1): 50-53.

Joshi, S.S.; Shrivastava, R.K. and Shrivastava, D.K. (1981). Chemical examination of *Clitoria ternatea* seeds. *Journal of the American Oil Chemists' Society*. **58**(6): 714.

Kaisoon, O.; Siriamornpun, S.; Weerapreeyakul, N.; and Meeso, N. (2011). Phenolic compounds and antioxidant activities of edible flowers from Thailand. *Journal of functional foods*. **3**(2): 88-99.

Kala, C.P.; Dhyani, P.P. and Sajwan, B.S. (2006). Developing the medicinal plants sector in northern India: challenges and opportunities. *Journal of ethnobiology and ethnomedicine*. **2**(1): 32.

Kamilla, L.; Mnsor, S. M.; Ramanathan, S. and Sasidharan, S. (2009). Antimicrobial activity of *Clitoria ternatea* (L.) extracts. *Pharmacologyonline*. **1:** 731-738.

Kamkaen, N. and Wilkinson, J.M. (2009). The antioxidant activity of *Clitoria ternatea* flower petal extracts and eye gel. *Phytotherapy research*. **23**(11): 1624-1625.

Kaur, C., and Kapoor, H.C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, **37**(2): 153-161.

Kavitha, R. and Premalakshmi, V. (2013). Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. *Int. J. Pharm. Bio. Sci.* **4**(4): 236-242.

Kazuma, K.; Noda, N. and Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternatea. Phytochemistry*. **62**(2): 229-237.

Kelemu, S.; Cardona, C. and Segura, G. (2004). Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. *Plant Physiology and Biochemistry*. **42**(11): 867-873.

Khatoon, S.; Irshad, S.; Rawat, A.K.S. and Misra, P.K. (2015). Comparative Pharmacognostical Studies of Blue and White Flower Varieties of *Clitoria ternatea* L. *J. Pharmacogn. Nat. Prod.* **1**(109): 37-39.

Khoddami, A.; Wilkes, M.A. and Roberts, T.H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*. **18**(2): 2328-2375.

Khoo, H.E.; Azlan, A.; Tang, S.T. and Lim, S.M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and nutrition research*. **61**(1).

Kim, S.W.; Jung, J.H.; Lamsal, K.; Kim, Y.S.; Min, J.S. & Lee, Y.S. (2012). Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. *Mycobiology*, **40**(1): 53-58.

Kumar, A.; Singh, A.K. and Singh, S. (2016). Extraction of phytochemical screening, separation and characterization of bioactive compounds from leaves extracts of *Clitoria ternatea* Linn. (*Aparajita*). *Int. J. Res. Ayurveda Pharm.* **7**(5): 70-78.

Kumar, L.M.K.M.; Kalariya, A.K.; Bishi, S.K. and Mann, A. (2014). Plant Phenolics: Important Bio-Weapon against Pathogens and Insect Herbivores. *Popular Kheti.* 2(3): 149-152.

Kumar, R.T., Kumar R.S. and Anju VS (2017). Phytochemical and antibacterial activities of crude leaf and root extracts of *Clitoria ternatea* varieties (Fabaceae). *Journal of Pharmacognosy and Phytochemistry*. **6**(6): 1104-1108.

Lehto, S.; Buchweitz, M.; Klimm, A.; Straßburger, R.; Bechtold, C. and Ulberth, F. (2017). Comparison of food colour regulations in the EU and the US: a review of current provisions. *Food Additives and Contaminants.* **Part A, 34**(3): 335-355.

Madhu, K. (2013). Phytochemical screening and antioxidant activity of *in vitro* grown plants *Clitoria ternatea* L, using DPPH assay. *Asian J. Pharm. Clin. Res.* **6**(2): 38-42.

Malabadi, R.B.; Mulgund, G.S.; Meti, N.T.; Nataraja, K. and Kumar, S.V. (2012). Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoria ternatea*. *Research in Pharmacy*. **2**(4): 10-21.

Manjula, P.; Mohan, C.; Sreekanth, D.; Keerthi, B. and Prathibha Devi, B. (2013). Phytochemical analysis of *Clitoria ternatea* Linn. a valuable medicinal plant. *The Journal of Indian Botanical Society*. **92**(3-4): 173-178.

Mathew, N.; Anitha, M.G.; Bala, T.S.L.; Sivakumar, S.M.; Narmadha, R. and Kalyanasundaram, M. (2009). Larvicidal activity of *Saraca indica*, *Nyctanthes arbortristis*, and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitology research*. **104**(5): 1017-1025.

Matsuura, H.N. and Fett-Neto, A.G. (2017). Plant alkaloids: main features, toxicity, and mechanisms of action. *In plant toxins*.pp. 243-261. Springer, Dordrecht.

Mensah, R.; Leach, D.; Young, A.; Watts, N. and Glennie, P. (2015). Development of *Clitoria ternatea* as a biopesticide for cotton pest management: assessment of product effect on *Helicoverpa* spp. and their natural enemies. *Entomologia Experimentalis et Applicata*. **154**(2): 131-145.

Mhaskar, A.V.; Prakash, K.; Vishwakarma, K.S. and Maheshwari, V.L. (2010). Callus Induction and Antimicrobial Activity of Seed and Callus Extracts of *Clitoria ternatea* L. *Current Trends in Biotechnology and Pharmacy*. **4**(1): 561-567.

Mohan, C.; Manjula, P.; Kumar, B.K., Naresh, B.; Ramadevi, B. and Devi, B.P. (2014). *In vitro* propagation of *Clitoria ternatea* L., a valuable medicinal plant. *Trends in Biosciences*, **7**(18): 2698-2701.

Morris, J.B. (1999). Legume genetic resources with novel "value added" industrial and pharmaceutical use. *Perspectives on new crops and new uses*. pp. 196-201.

Mukherjee, P.K.; Kumar, V.; Kumar, N.S. and Heinrich, M. (2008). The Ayurvedic medicine *Clitoria ternatea*—from traditional use to scientific assessment. *Journal of ethnopharmacology*, **120**(3): 291-301.

Mukherjee, P.K.; Sahoo, A.K.; Narayanan, N.; Kumar, N.S. and Ponnusankar, S. (2009). Lead finding from medicinal plants with hepatoprotective potentials. *Expert opinion on drug discovery*. **4**(5): 545-576.

Mukherjee, P.K.; Venkatesh, P. and Ponnusankar, S. (2010). Ethnopharmacology and integrative medicine–let the history tell the future. *Journal of Ayurveda and integrative medicine*. **1**(2): 100-109.

Nahar, K.; Rahman, M.A.; Parvin, M.N. and Sarwar, S. (2010). Evaluation of Anthelmintic Activity of Aqueous Leaf Extract of *Clitoria ternatea* Linn. *Stamford Journal of Pharmaceutical Sciences*. **3**(1): 46-48.

Namkeleja, H.S.; Tarimo, M.T. and Ndakidemi, P.A. (2013). Allelopathic Effect of Aqueous Extract of *Argemone mexicana* L. on Germination and Growth of *Brachiaria dictyoneura* L. and *Clitoria ternatea* L. *American Journal of Plant Sciences*. **4**(11): 2138-2147.

Narayanaswamy, V. (1981). Origin and development of ayurveda:(a brief history). *Ancient science of life*. **1**(1): 1-7.

Neelamma, G.; Vanitha, B.; Sai Suresh Nehru; Rajesh Kumar and Durai Swamy, B. (2016). Phytochemical screening and estimation of total phenols total flavonoids and evaluations *in vitro* anti oxident and anti inflammatory activities of various extracts of *Clitoria ternatea* root. *International journal of current research*. **8**(11): 42354-42358

Nguyen, G.K.T.; Zhang, S.; Nguyen, N.T.K.; Nguyen, P.Q.T.; Chiu, M.S.; Hardjojo, A. and Tam, J.P. (2011). Discovery and characterization of novel cyclotides originated from chimeric precursors consisting of albumin-1 chain and cyclotide domains in the Fabaceae family. *Journal of Biological Chemistry*. **286**(27): 24275–24287.

Nigam, S. and Sahu, A. (2014). Estimation of antimicrobial and phytochemical activity of *Clitoria ternatea* stem and leaf extracts against some selected bacterial strain. *International Journal of Research and Development in Pharmacy and Life Sciences.* **3**(5): 1194-1199.

Nigam, S. and Shrivastava, P.N. (2013) Phytochemical screening and antimicrobial activity of *Clitoria ternatea* leaves against *Staphylococcus aureus*. *Int. J. Pharm. Bio. Sci.* **4**(3): (B) 963 – 968.

Packiam, K.K.; Thiruvenkatasamy, P.; Vellingiri, V.; Rasiappan, S.; Veeraiyan, S.; Manogaran, S.; Sundramurthi, P.M.K. and Dhakshinamoorthy, M. (2016). Comparative Studies on The Phytoconstituents, Antibacterial and Pesticidal Activities of Blue and White Varieties of *Clitoria ternatea* Linn. *Acta Biomedica Scientia.* **3**(4): 213-218.

Pahune, B.; Niranjane, K.; Danao, K.; Bodhe, M. and Rokade, V. (2013). Antimicrobial activity of *Clitoria ternatea* L. flower extract and use as a natural indicator in acid base titration. *J. Natl. Prod.* **3**(2): 48-51.

Panse, V.G., and Sukhatme, P.V. (1978). Statistical methods for agricultural workers. *Statistical methods for agricultural workers.*, (Ed. 3).

Parimaladevi, B.; Boominathan, R. and Mandal, S.C. (2003). Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. *Fitoterapia*. **74**(4): 345-349.

Parimaladevi, B.; Boominathan, R. and Mandal, S.C. (2004). Evaluation of antipyretic potential of *Clitoria ternatea* L. extract in rats. *Phytomedicine*. **11**(4): 323-326.

Patil, S.B.; Naikawade, N.S.; Magdum, C.S., and Sathe, S.S. (2016). Sun protective activity of *Clitoria ternata* linn, *Canna indica* linn flower. *Mintage Journal of Pharmaceutical and Medical Sciences*. **5**(1): 18-19.

Phulbandhe, J.V.; Deo, S.; Inam, F. and Bhoyar, P.K. (2015). To evaluate antimitotic and antiproliferative activity of extracts of C. *ternatea. World Journal of Pharmaceutical Research.* **4**(8): 2171-2182.

Ponnusamy, S.; Gnanaraj, W.E. and Antonisamy, J.M. (2013). Alkaloids Profile of *Clitoria ternatea* Linn by High Performance Thin Layer Chromatography (HPTLC). *Indo American Journal of Pharmaceutical Research*. **3**(3): 2592-2599.

Ponnuswamy, S. and Wesely, J.D.E.G. (2011). Comparative study of primary metabolites in different plant parts of *Clitoria ternatea* Linn. *J. Chem. Pharm. Res.* **3**(4): 614-617.

Rabeta, M.S. and An Nabil, Z. (2013). Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* Linn. *International Food Research Journal*. **20**(1): 495-500.

Radha, K.V.; Arun, T and Pidugu, S. (2014). Antibacterial activity and phytochemical screening of *Clitoria ternatea* Linn. against *proteus mirabilis* from urinary tract infected patients. *World Journal of Pharmaceutical Research.* **3**(3): 4351- 4366.

Rao, A.S.; Shobha, K.L.; Almeida, P. and Rai, K.S. (2017). *In vitro* antimicrobial activity of root extract of *Clitoria ternatea. Asian Journal of Pharmaceutical and Clinical Research*. **10**(11): 52-54.

Reshmi, C.R.; and Reghunath, B.R. (2011). Yield and alkaloid content of *Clitoria ternatea* L. as influenced by shaded and open conditions. *Asian Journal of Horticulture*. **6**(2): 445-448.

Rodriguez-Amaya, D.B. (2018). Natural food pigments and colorants. In *Bioactive Molecules in Food* (pp. 1-35). Springer, Cham.

Rymbai, H., Sharma, R. R., and Srivastav, M. (2011). Bio-colorants and its implications in health and food industry – a review. *International Journal of Pharm Tech Research*. **3**(4): 2228-2244.

Salhan, M.; Kumar, B.; Tiwari, P.; Sharma, P.; Sandhar, H.K. and Gautam, M. (2011). Comparative anthelmintic activity of aqueous and ethanolic leaf extracts of *Clitoria ternatea*. *Int. J. Drug Dev. Res.* **3**(1): 62-69.

Sarumathy, K.; Dhana Rajan, M.S.; Vijay, T. and Jayakanthi, J. (2011). Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *Clitoria ternatea*. *Journal of Applied Pharmaceutical Science*. **1**(5): 164-172.

Saxena, A. and Saxena,V. (2015). Study of different extracts of *Clitoria Ternatea* leaves protection against ulcer. *International Journal of Science and Research*. **4**(5): 651-653.

Sharma RK and Dash B. (1988). Charaka Samhitha; **3**: 46.

Shekhawat, N. and Vijayvergia, R. (2010). Comparative study of primary metabolites in different plant parts of *Clitoria ternatea* (L.), *Guazuma ulmifolia* (Lam.) and *Madhuca indica* (Gmel.). *Journal of chemical and pharmaceutical research*. **2**(2): 168-171.

Shyamkumar and Ishwar, B. (2012). Anti-inflammatory, analgesic, and phytochemical studies of *Clitoria ternatea* Linn. flower extract. *Int. Res. J. Pharm.* **3**(3): 208-210.

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

Srivastava, B.K. and Pande, C.S. (1977). Anthocyanins from the flowers of *Clitoria ternatea, Planta medica*, **32**(6): 138-140.

Subramanian, M.S. and Prathyusha, P. (2011). Pharmaco-phytochemical characterization of *Clitoria ternatea* Linn. *International Journal of Pharm. Tech. Research*. **3**(1): 606-612.

Suganya G.; Sampath Kumar P.; Dheeba B. and Sivakumar R. (2014). *In vitro* antidiabetic, antioxidant and anti-inflammatory activity of *Clitoria ternatea* L. *Int. J. Pharm. Pharm. Sci.* **6**(7): 342-347.

Swain, S.S.; Rout, K.K. and Chand, P.K. (2012). Production of triterpenoid anti-cancer compound taraxerol in *Agrobacterium*-transformed root cultures of butterfly pea (*Clitoria ternatea* L.). *Applied biochemistry and biotechnology*. **168**(3): 487-503.

Taif, B.; Som, S.H.M. and Yusof, U.Z.M. (2017). Extraction of natural dyes from *Clitoria ternatea* flower. *MATTER: International Journal of Science and Technology*. **3**(1): 81-90.

Taur, D.J.; Patil, R.Y. and Khalate, A.H. (2009). Phytochemical investigation and evaluation of *Clitoria ternatea* seeds extracts on clonidine induced catalepsy in mice. *Pharmacologyonline*. **3**: 215-220.

Taur, D.J.; Taware, S.B.; Patil, R.N.; Patil, R.Y. and Kharya, M.D. (2010). Pharmacognostical and preliminary phytochemical evaluation of *Clitoria ternatea* leaves. *Pharmacognosy Journal*. **2**(9): 260-265.

Terahara, N.; Oda, M.; Matsui, T.; Osajima, Y.; Saito, N.; Toki, K. and Honda, T. (1996). Five new anthocyanins, ternatins A3, B4, B3, B2, and D2, from *Clitoria ternatea* flowers. *Journal of natural products*. **59**(2): 139-144.

Terahara, N.; Toki, K.; Saito, N.; Honda, T.; Matsui, T. and Osajima, Y. (1998). Eight New Anthocyanins, Ternatins C1− C5 and D3 and Preternatins A3 and C4 from Young *Clitoria ternatea* Flowers. *Journal of natural products*. **61**(11): 1361-1367.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In *Biotechnology of isoprenoids*. pp. 63-106. Springer, Cham.

Uma, B.; Prabhakar, K. and Rajendran, S. (2009). Phytochemical analysis and antimicrobial activity of *Clitorea ternatea* Linn. against extended spectrum beta lactamase producing enteric and urinary pathogens. *Asian Journal of Pharmaceutical and Clinical Research*. **2**(4): 94-96.

Upwar, N.; Patel, R.; Waseem, N. and Mahobia, N.K. (2010). Evaluation of antidiarrhoeal activity of the root of *Clitoria ternatea* Linn. *Int. J. Pharm. Sci. Rev. Res.* **5**(1): 131-134.

Valivittan, and Isaac, C. (2016). Evalution of Phytochemical and Antimicrobial Activity of Flowers of *Clitoria ternatea. International Journal of Computer Science and Wireless Security*. **3**(2):141- 145.

Vats S. (2014). Antioxidant activity of *Clitorea ternatea* L. and *Origanum vulgare* L.: A comparative analysis. *Researcher*. **6**(11): 56-58.

Verma, P.R.; Itankar, P.R., and Arora, S.K. (2013). Evaluation of antidiabetic antihyperlipidemic and pancreatic regeneration, potential of aerial parts of *Clitoria ternatea*. *Revista Brasileira de Farmacognosia*. **23**(5): 819-829.

Vianni, R.; and Souto, S.M. (1971). Oil content and fatty acid composition of *Clitoria ternatea* seeds. *Arquivos da Universidade Federal Rural do Rio de Janeiro*. **1**: 47-50.

Wulansari. N.T. and Armayanti, L.Y. (2018). The effectiveness of *Clitoria ternatea* flower extract to inhibit the growth of *Salmonella typhi* bacteria that causes typhoid fever infection. *International Journal of Development Research*. **8**(2): 19090-19093.

Yadava, R.N., and Verma, V. (2003). Antimicrobial activity of a novel flavonol glycoside isolated from the roots of *Clitoria ternatea* Linn. *Asian Journal of Chemistry*. **15**(2): 842-846.

Yeotkar, S.D. and Malode, S.N. (2014). Phytochemical diversity among the different variants of climber *Clitoria ternatea* L. and *C. biflora* found in Amravati region. *International Journal of Research in Biosciences Agriculture and Technology*. Special issue. pp. 152-157.

Ziegler, J. and Facchini, P.J. (2008). Alkaloid biosynthesis: metabolism and trafficking. *Annu. Rev. Plant Biol.* **59**: 735-769.

Zwenger, S. and Basu, C. (2008). Plant terpenoids: applications and future potentials. *Biotechnology and Molecular Biolosgy Reviews*. **3**(1): 1-7.

APPENDIX I

Microbial Type Culture Collection and Gene Bank (MTCC)
CSIR-Institute of Microbial Technology, Sector-39A
Chandigarh – 160036, India

INVOICE

CSIR-IMTECH- GSTIN : 04AAATC2716R2ZM
PAN : AAATC2716R

Invoice details:
Invoice No: MTCC/SUP.1/201802151316/13847/3256
Service Type: Cultures of Microorganisms

ABHIJIT DEBNATH (Schools / Colleges / Universities)

Customer No:	13847	Order Date:	February, 15, 2018
Address:	ASSAM AGRICULTURE UNIVERSITY, Jorhat - 785013, Assam, India	Payment Mode:	Online
Email:	abhijit.debnath14@gmail.com	Amount (INR):	2,440
Phone:	8807919873	Invoice Date	March, 04, 2018
Department:	DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL CHEMISTRY	Organization:	ASSAM AGRICULTURAL UNIVERSITY
Designation:	Research scholor/student/contractual	GSTIN No.:	NA

Cultures of Microorganisms All Amounts are in INR

S. No.	MTCC NO	Genus/Species	HSN code	Qty	Amount	Total
1	1477	Pyricularia oryzae	30029030	1	2,000	2,000
					Sub Total (INR):	2,000.00
					IGST or CGST/SGST (12.00%) :	240.00
					Total with IGST :	2240.00
					Shipping by courier (INR):	200.00
					Total Amount (INR):	2440.00

S. No.	MTCC ID	Genus	Species	Incubation	Growth Media No.
1	1477	Pyricularia	oryzae	7 days	135