3. PHYTOCHEMICAL STUDIES

3.1. Introduction

Most of the pharmaceutical industries are highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world is becoming endangered at an alarming rate because of extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, and urbanisation.

Hence there has been a strong need for proactive understanding in the conservation, cultivation, and sustainable usage of important medicinal plant species for future use (Nalawade and Tsay, 2004). Also significant evidence shows that the supply of plants for traditional medicines fails to satisfy the increasing demand (Cunningham, 1993). An efficient and most suited alternative solution to the problems faced by the phytopharmaceutical industry is the development of in vitro systems for the production of medicinal plants and their extracts. In modern medicine, plants have been used as sources of direct therapeutic agents, as models for new synthetic compounds, and as taxonomic markers for discovery of new compounds. They served as a raw material base for the elaboration of more complex semisynthetic chemical compounds (Akerele, 1992).

For the widespread acceptance of herbal medicines, standardization, quality control of the herbal materials, as well as evaluation of efficacy, safety and quality of the
phytopharmaceutical are indispensable (Huie, 2002). Identification of individual components of complex mixtures of phytochemicals requires the use of several techniques. One of the most popular methods of studying phytochemical composition is GC-MS, which allows the identification of the specific natural compounds found in a plant extracts by comparing their relative retention times and indices and their mass spectra (Yani et al., 2005; Adams, 2007).

3.2 Review

The crude methanol extract and chloroform fraction of the whole plant of *Physalis minima* Linn (Solanaceae) was investigated for anti-inflammatory, analgesic and antipyretic activities in NMRI mice and Wistar rats which showed marked anti-inflammatory and analgesic activities which lead the whole plant of *Physalis minima* Linn could be considered as a potential plant species for bioactivity-guided isolation of natural anti-inflammatory and analgesic agents. (M.A.Khan et al., 2009).

The structure of withaminimin, a new ergostane-type steroid from *Physalis minima*, was established by spectral analysis ($^1$H and $^{13}$C NMR, MS) (Hugo E.Gottlieb et al., 1987).

b-Withaphysalin, Physalin-b, Dihydroxyphysalins-isolated from P.minima is used as plant tonic, diuretic, purgative, in diseases of spleen, dropsy, gout, urinary diseases(SRISTI Innovations,2008).
Antifertility Effects of the Petroleum Ether Extract of Physalis Minima on Female Albino Rats were reported by Sudhakaran et al., 1999.

The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine). Not only, that plant-derived drug offers a stable market world wide, but also plants continue to be an important source for new drugs.

With this informations, an attempt was made to study the different Phytochemical compounds present in leaf, root and fruit seed of Physalis minima through GC-MS analysis.

### 3.3. Materials and methods

**Plant sample preparation**

Fresh, healthy leaves, root and seed of Physalis minima were collected from Tiruchirappalli, shade dried at room temperature ±30º for a period of 10 days and made into powder. (Abdulmoniem et al., 2006; Retnam et al., 2007).
Alcoholic extraction

10 gm powdered plant material (leaf, root, seed) of Physalis minima was soaked in 30 ml of ethanol overnight. It was filtered through whatmann filter paper No.41 along with 2 gm sodium sulphate which has been wetted with absolute alcohol to remove the sediments and traces of water in the filtrate. The filtrates were then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1 ml. The extract contained both polar and non-polar phytochemical components. (Vanitha, 2007).

Analysis of the sample extract

Equipment Details:
Column: Elite-1 (100% Dimethyl poly siloxane), 30m x 0.25mm ID x um df
Equipment: GC Clarus 500 Perkin Elmer
Carrier gas: Helium 1 ml/min
Detector: Mass detector- Turbo mass gold-Perkin Elmer
Software: Turbo mass 5.1.
Sample injected: 2 ul split: 10.1

Oven Temperature settings:
110 deg – 2min hold
Upto 280 deg C at the rate of 5 deg/min – 9 min hold
Injector temp: 250 deg C
Total GC time: 45 min
**MS Programme:**

Library used: NIST Ver.2.0 – Year 2005  
Inlet line temperature: 200 deg C  
Source temperature: 200 deg C  
Electron energy: 70 eV  
Mass scan: (m/z) 45 – 450  
MS Time: 45 min

**Identification of compounds**

The chemical constituents were identified by Gas Chromatography (GC) comparing their Kovats indices with those of authentic standards which are inbuilt in the equipment and self explanatory which are available in the laboratory. Further identification was done by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer Database using NIST libraries and with those published in the literature (Admas, 1989). The percentage of each component is calculated from the relative peak area of each component in the chromatogram.

**3.4. Results and Discussion**

The chemical components identified, the percentage of each constituents and their retention times, nature of the components and their activities of the ethanolic extracts of leaf, root and fruit of Physalis minima are summarized in the Tables 3.1., 3.2., 3.3. The spectrum of some compounds are presented in Plates 3.1.a., 3.1.b., 3.1.c.
The major compounds identified from the ethanolic extract of leaf are Glycerin (5.08%), Propane, 1,1,3-triethoxy- (2.92%), 1,6-Anhydro-á-D-glucopyranose (levoglucosan) (2.79%), Undecanoic acid (3.07%), (1R,3R,4R,5R)-(−)-Quinic acid [Synonyms: Quinic acid] (18.06%), Undecanal, 2-methyl-(2.41%), n-Hexadecanoic acid(3.12%), Phytol(27.34%), Vitamin E (11.10%), Campesterol(4.16%), Cholest-5-en-3-ol, 24-propylidene-, (3á)- (7.16%), Fucosterol(2.01%).

The other compounds were represented by less than 2% peak area. The major compounds identified from the ethanolic extract of leaf are presented in Table 3.1. The GC-MS chromatogram of the same is presented in Plate 3.c.

The chromatogram of ethanolic extract of root extract contained the major compounds, Glycerin(4.02%), Hexanediamide, N,N'-di-benzoyloxy- (41.02%), n-Hexadecanoic acid(12.19%), 11,14-Eicosadienoic acid, methyl ester(4.46%), Campesterol (11.93%), Cholesta-22,24-dien-5-ol, 4,4-dimethyl-(6.96%), Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4á)- (2.19%).

The major compounds identified from the ethanolic extract of root are presented in Table 3.2.

The GC-MS chromatogram of the same is presented in Plate 3.b.

The major compounds identified from ethanolic extract of fruit are Propane, 1,1,3-triethoxy- (28.02%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(4.84%), 4H-Pyran-
4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(9.56%), Dodecanoic acid (9.78%), 1,2,3,5-Cyclohexanetetrol, (1\(\alpha\),2\(\alpha\),3\(\alpha\),5\(\alpha\))-(41.98%), 2-Propenoic acid, 2-(dimethylamino)ethyl ester (5.82%). The major compounds identified from the ethanolic extract of fruit are presented in Table 3.3. The GC-MS chromatogram of the same is presented in Plate 3.c.

6\(\beta\)-ethoxy compound as a constituent of \(P.\) minima was isolated by Masao Kawai et al., (1996). Similar ethoxy compounds Propane, 1,1,3-triethoxy- (2.96%) from leaf extract and Propane, 1,1,3-triethoxy-(28.02%) from fruit extract of the same plant have been identified through GC-MS study.

A new 13,14-seco-16,24-cyclosteroid, physalin L, has been isolated, along with known compounds, physalin B, epoxyphysalin B and physalin D from \(Physalis\) minima. (G. Sen, H. D. Pathak, 1995). Campesterol (4.16%) from leaf extract, Campesterol (11.93%) from root extract and Cholest-5-en-3-ol, 24-propylidene-, (3\(\alpha\))-(7.16%) from leaf extract, Cholesta-22, 24-dien-5-ol, 4,4-dimethyl-(6.96%) from root extract which are steroid compounds are reported from this study.

Physalindicanols, New Biogenetic Precursors of C28-Steroidal Lactones were isolated from Physalis minima var. indica (Sinha SC et al.1987). Campesterol (\(C_{28}H_{48}O\))(4.16%) from leaf extract and Cholesta-8,24-dien-3-ol, 4-methyl-, (3\(\alpha\),4\(\alpha\))-(\(C_{28}H_{46}O\)) (2.19%) from root extract are similar molecular weight compounds identified in the present study.
5-Methoxy-6,7-methylenedioxyflavone was isolated from *Physalis minima* together with the known compound, 5,6,7-trimethoxyflavone (*Ng Ang Sera, 1988*). 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(9.56%) which is a flavonoid compound is identified from the fruit extract of *Physalis minima* L.

For the widespread acceptance of herbal medicines, standardization, quality control of the herbal materials, evaluation of efficacy, safety and quality of the phytopharmaceutical are indispensable (Huie, 2002).

Vitamin-E is related with hypoglycaemic activity (*Sivan et al., 1996*). This compound was rich leaf extract. Phytol, the precursor for vitamin E is also distributed in the leaf extracts.

**Dodecanoic acid, ethyl ester** (C14H28O2)

Synonyms: Ethyl laurate; Lauric acid; ethyl ester; Ethyl dodecanoate
This compound was reported with antibacterial, antioxidant, antiviral, hypercholesterolemic and candidicide activity (*Mitova et al., 2003*).

**Tetradecanoic acid** (C14H28O2)

Synonyms: Myristic acid; n- Tetradecanoic acid; n-Tetraepcoic acid; Neo-Fat14; Crod acid; 1-Tridecanecarboxylic acid; n-Tetradecan-1-oic acid
The compound was reported to be a lubricant and nematicide (Zheng et al., 1992) and also showed antibacterial activity against Gram-positive and Gram-negative bacteria (Salman et al., 2006; Yayli et al., 2006).

Hexadecane (C16H34)

Synonyms: n-Cetane; n-Hexadecane; Cetane Antibacterial activity of this compound was reported by Slantchev et al. (2002) and Yayli et al. (2006).

Oleic acid (C18H34O2)

Synonyms: (9E)-octadec-9-enoic acid; (9E)-Octadecenoic acid; (9Z)-octadec-9-enoate; (9Z)-octadec-9-enoic acid; (9Z)-Octadecenoic acid; (E)-Oleic acid; (Z)-2-(Methylolyleylamino) ethanesulphonic acid; (Z),(Z)-Octadec-9-enoic acid; delta.9-cis-Oleic acid; 9, 10-Octadecenoic Acid; 9-CIS-Octadecenoic acid; 9-elaidic acid; 9-Octadecenoic acid

The compound possesses antimicrobial (Novak et al., 1961), hypercholesterolemic (Natali et al., 2007), dermatitigenic (Newmark, 1997), anti inflammatory and anti tumor activity (Kimura, 2002; Yunfeng et al., 2007).

1,6-Anhydro-a-D-glucopyranose (C6H10O5) (2.79 %)

Synonyms: Levoglucosan; 1,6-Anhydro-β-D-glucose; Leucoglucosan; Anhydro -d- mannosan; 1,6-Anhydro -β -D-glucose; 1,6-Anhydro- β-glucopyranose; β-D-Glucopyranose; 1,6-anhydro-; β-D- Glucopyranose, 1,6- anhydro-; 1,6- Anhydro -β-D- gluco- pyranose; 1,6- Anhydro; β-d-talopyranose; 1,6-Anhydro-β; D-glucopyranose.
Kinase, high and lower photochemical, anthropogenic, fungal, hydrolyzing and biological activity (Luyen et al., 2007; Natalie Kehrwald et al., 2010; Hoffmann et al., 2010; Zhang et al., 2010; Waghmare et al., 2010).

**n-Hexadecanoic acid** (C16H32O2) (3.12%):

Synonyms: n-Hexadecanoic acid; Palmitic acid; Pentadecanecarboxylic acid; 1-Pentadecanecarboxylic acid; Cetylic acid; Hexadecylic acid; Hydrofol; Hexadecanoic acid (palmitic acid); Hexadecanoic (palmitic) acid; Palmitic acid (hexadecanoic acid).

Larvicidal activity, antibacterial, antifungal, antioxidant activity, hypocholesterolemic nematicide, pesticide, anti androgenic flavor and hemolytic activity (Okwu and Ighodaro, 2009; Liu et al., 2009; Manilal et al., 2009; Praveenkumar et al., 2010).

**Phytol** (C20H40O) (27.34%):


Propane, 1,1,3-triethoxy- shows a peak value of (2.96%) from leaf extract and The same compound shows a peak value of (28.02%) from fruit extract of the same plant.

2-Furancarboxaldehyde,5-(hydroxymethyl)-[5-Hydroxymethylfurfural] shows a peak value of(0.65%) from root extract and the same shows a peak value of (4.84%)from fruit extract.

Dodecanoic acid shows the peak value of(9.78%) from the fruit extract and the same compound has a peak value of (0.49) from the root extract.

Glycerin from the leaf extract shows a peak value of(5.08%) while that from the root shows a peak value of(4.02%).

The unequal amounts of same compound in different parts of the same plant may reason the usage of particular parts of the plants in different healing therapy.
The phytochemical screening indicated the presence of compounds important chemical compounds such as Phytol, Vitamin E, Oleic Acid, Campesterol, n-Hexadecanoic acid which are reported to have various therapeutic uses. To conclude, the obtained result could form a good basis for selection of plant species for further investigation in the potential discovery of new valuable bioactive compounds. Presence of antimicrobial and anticancer compounds in all the three leaves, root and fruit, based on the compounds identified from GC-MS analysis, initiated to carry out antimicrobial and anticancer studies in this plant.
3.5. Ethnobotanical uses compared with Activity of the compounds identified through GC-MS.

Medicinal plants that are native to India and their use in various traditional system of medicine are induced awe-inspiring. The ethnobotany of ubiquitous plants provide a rich resource for natural drug research and development.

Heyne reports that in Java the root of Physalis minima L. is used as a vermifuge, and an extract of it, for fever. Dalgado states that a decoction of the roots is prescribed for diabetes.

Ridley says that a poultice of the leaves, smeared with oil and heated, is applied to ulcers. He adds that a decoction of the leaves with Plantago major is given in gonorrhoea. Its value, he indicates, lies in its being a diuretic.

According to Nadkarni the fruit is considered alternative, diuretic, and aperient, being useful in dropsy, urinary diseases, and gout. He reports that the fruit is said to infuse vigor in a worn-out system and to offset premature decay. Dymock tells that in the Konkan the plant is made into a paste with rice water and applied to restore flaccid breasts. Kirtikar and Basu state that the fruit is used for gonorrhoea in the Punjab. Burkill and Haniff assert that the Malays use it as a poultice for headache and intestinal pains. (Vanila et al., 2005).
A synthesis of ethnomedical uses and modern biological knowledge has been done in order to substantiate or verify the traditional uses in 40 medicinal plants used by women in hamlets in and around Anaikatty hills of Coimbatore District, Tamil Nadu, India (Bhanumathi et al., 1999), 51 plants by women in hamlets or villages of Jawhar and Mokhada talukas in Thane district, Maharasthra, India (Bhanumathi et al., 2000a), Thirty-four species of plants used by local women in hamlets of Banjar taluka, Kulu district, Himachal Pradesh, India, (Bhanumathi et al., 2000b)

Women in these areas possess a rich knowledge of medicinal plants and still continue the medical tradition of using plants as medicine for themselves, their families and others around them. Information on their chemistry and biology from the scientific literature for each plant collected has been incorporated with traditional knowledge to explain or substantiate traditional medicinal uses.

A similar attempt has been made with the activity of the compounds as reported in Dr.Duke’s Phytochemical and Ethnobotanical Databases which are screened during GC-MS analysis of root, leaves and fruit of Physalis minima to explain or substantiate or justify the traditional medicinal uses. The results are tabulated in table 3.4