1. INTRODUCTION

Biodiversity and ecosystem diversity are reflected in the cultural and religious diversity of ethnic-communities all over the world. The socio-cultural as well as aesthetic values attached to species and landscapes are reflected in many traditional knowledge systems and ethno-medicinal practices, and they are evolved, based on a close understanding of biodiversity. Traditional Knowledge associated with biodiversity is a living body of functional knowledge system that is developed, sustained, refined and passed on from generation to generation of people through continuous interaction, observation and experimentation with their surroundings. This can be visualized in a wide variety of contexts such as agricultural knowledge, scientific knowledge, technical knowledge, ecological knowledge, medicinal knowledge and biodiversity-related knowledge (WIPO, 2014). Traditional medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants, other plant materials or combinations thereof as active ingredients (WHO, 2013a). Since traditional knowledge is getting eroded rapidly because of the changing lifestyles of the people, there is an urgent need to systematically document such valuable information for the welfare and betterment of posterity (Lalitha et al., 2011). Most of the therapeutic drugs which are being used today are the outcome of the studies on traditional knowledge. Hence, the systematic documentation of traditional knowledge related to ethnobotanically important plants deserves
utmost importance, and it appears this is particularly true with fungus infected plants.

1.1 Systematic documentation

Systematic documentation of traditional knowledge has been found to be one of the approaches for conservation and utilization of biological resources in a sustainable manner. It is intended to document, preserve, and protect treasures of knowledge still retained by various indigenous groups in the world. But this functional knowledge system is rapidly dwindling due to the influence of modern life styles, reduction in number of traditional healers and the lack of interest of younger generations to carry on with the tradition. Hence there is an urgent need and necessity to systematically document this vast treasure of knowledge prior to its extinction.

Presently, documentation pertaining to the fungus infected plants are fragmentary and there exists a huge lacuna in this regard. Moreover, with the advent of modernization, most of these functional knowledge systems are facing threat of extinction. In this context, scientific scrutiny and validation of the documented knowledge has become inevitable (Singh and Dubey, 2012; Henrique et al., 2014). In the last few decades, the main strategy of drug development based on traditional medicine is of a single target, single compound, based on a super reductionism that involves mostly tests of compounds at the molecular level. However, of late, it is recognised that this approach is not suitable for studies on traditional medicines. We are now witnessing the entry of a new informational paradigm in the scientific validation
of natural products. A more thoughtful approach by incorporating modern tools like reverse pharmacology, systems biology, proteomics, metabolomic sciences seems much more suited to confirm efficacy and to obtain information that might lead to understanding the mode of action. A Systems Approach or the science of wholeness is the unique philosophy of Ayurveda. When multiple cell types and diverse pathways contribute to the disease, a single molecule may not be effective in modulation of multiple targets and such conditions require combination therapy (Vaidya et al., 2001). Since an array of secondary metabolites are being triggered in fungus infected medicinal plants, comparable holistic approaches are inevitable for the pharmacological investigation of fungus infected medicinal plants.

1.2 Plant fungus association

All plants in natural ecosystems are thought to be symbiotic with mycorrhizal and/or endophytic fungi (Rusty et al., 2008), and the very existence of the fungi infected plants has been known for over hundred years. Fungi occupies a prominent position in the biological world because of their variety, economic and environmental importance. The group is particularly explored for various medicaments all over the world both in the classical and modern systems. The association between fungi and plants represents a unique balanced multi-partite ecological relationship, which along with other abiotic components of nature forms a complex interplay of the components. The fungus infected plants, especially the ethnomedicinally important plants have been considered to be unique, and are viewed as an outstanding source of bioactive natural products.
In fact, the fungi that live inside the tissues of living plants are an under explored area of research. Fungus infection on plants induces the production of an array of secondary metabolites, and these have proved to be a golden mine to isolate new and novel molecules, useful for the treatment of various ailments. Once the properties of the secondary metabolites are revealed, it would replace conventional molecules and ultimately enhance the health industry.

Fungal infection modifies the chemical profile of the host and the fungi acts as biotransformer or biocatalyst. During this event, it converts one or more compounds into other forms which may be either useful or harmful. The symbiotic association of plant and fungi seems to be extended from the level of simple contact to a highly sophisticated and complex genomic level interaction. It is observed that some genes are expressed only in the fungus infected plants (Johnson et al., 2003). This long term genomic relationship results in the co-evolution of fungi with their host plants (Germaine et al., 2004).

The fungal flora found on plants exert their influence on the host plant’s physiology. The host plants overcome the adverse effects of the infection by producing phytoalexins, and forms a complex system with a different class of secondary metabolites. The utility of such metabolites produced by plants infected with different fungi has been proved time and again. The systemic nature of these results in the translocation to different parts of the host (Singh and Dubey, 2012). The association equips the plants to tolerate stressful conditions viz., drought, heavy metal tolerance, herbivory, temperature, salinity (Clement et al., 2001). This property has been extensively used to develop
modern phytoremediation techniques by artificially inoculating the plants with fungal spores (Hrishikesh et al., 2010).

During microbial association in plants they are often organized and embedded within an extracellular polymeric substance matrix, termed as biofilms. Infections by biofilms are difficult to defend by plants because these structures are particularly resistant to most of the secondary metabolites produced by the plants. One of the regulatory mechanisms suggested to play a significant role coordinating biofilm formation for many species is intercellular signalling or quorum sensing (Patricia and Arturo, 2012). This is effected by producing small signalling molecules such as hormones by microorganisms which in turn binds with the target genes and activates certain metabolic processes such as bioluminescence, biofilm development, efflux pump expression, toxin production, sporulation, swarming, antibiotic biosynthesis, plasmid conjugal transfer and the production of virulence determinants in animal, fish and plant pathogens etc. (Miller et al., 2001; Williams et al., 2007). In Fungi, this is particularly accomplished by the production of farnesol like substances.

Approximately 22,000 bioactive secondary metabolites from microorganisms had been described in published works and about 8,600 of these are of fungal origin. The plants infected by fungi also provide an array of bioactive secondary metabolites with unique structures which includes alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthenes, chinones, phenols, isocoumarins, benzopyranones,
cytochalasins, enniatines and others. Most of the secondary metabolites are very good natural antioxidants having a wide array of medicinal properties like hepatoprotection, anti-inflammation, immunomodulation, anticarcinogenicity, anti-infertility etc.

1.3 Reproductive disorders

There has been a rapid increase in the reproductive ailments all over the world, and the dependency on traditional medicaments for curing reproductive ailments has seen an upsurge. Reproductive disorders have been considered as a disease of reproductive system causing failure of achieving a clinical pregnancy. This includes polycystic ovarian syndrome, primary ovarian failure or premature ovarian failure, endometriosis, genital TB, fibroids, genetic problems in females, and varicocele, idiopathic semen disorders, testicular failure, cryptorchidism, obstruction etc. in males. Infertility is a critical component of reproductive health, and has often been neglected in these efforts. The inability to have children affects men and women across the globe. It can lead to distress and depression, as well as discrimination and ostracism. According to a recent report (Maya et al., 2012), 48.5 million couples are unable to have a child, of which 19.2 million couples are unable to have a first child, and 29.3 million couples are unable to have an additional child. Infertility among females is a growing crisis and is an area which needs attention. Diverse factors such as age, change in lifestyle, life style stress, obesity, family history, working status, region and religion, infection, late marriage, postponement of child bearing for more than one year, partner living abroad, smoking and consumption, chemicals, pollutants,
radiations, drugs and hormone imbalance are known to induce female infertility (WHO, 2013b). Among the various infertility conditions in females, Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrine disorder of unknown etiology affecting five to ten percent of women of reproductive age as well as premenopausal women. It is characterized by hyperandrogenism, ovulatory dysfunction, anovulation, menstrual disorder, amenorrhea, poly cystic ovaries (PCO) and infertility (Ehrmann, 2005). PCO is characterized by an increased number of small antral follicles with arrested development and a hypertrophied theca cell layer (Jonard et al., 2004).

Reliable pharmacological control of human fertility has resulted in far-reaching changes in the lives of individuals and communities where it is available. Many of the improvements in maternal and infant morbidity and mortality are due to pharmacological interventions, where they are within the reach of the people. Increasing specificity of treatments for various disorders and discomforts of reproductive system function has increased the quality of life of many women, and reduced health and economic costs. Treatment of infertility shows increasing success and is a growing industry which often now relies on specific pharmacological intervention. A wide range of herbal medicine are used to regulate the menstrual cycle, enhance fertility, and as either abortifacient or anti-abortifacient. Research findings in course of time have become basic leads for chemical, pharmacological, clinical and biochemical investigations, which ultimately gave birth to drug discovery.
Olea dioica Roxb., belonging to the family ‘Oleaceae’ is a tree species commonly found distributed in the semi-evergreen and moist deciduous forests in diverse geographical niches of India. The plant is locally known as ‘Edana’. Previously, the plant was known for its sporadic use as a medicinal plant. Often, during the winter season the plant is seen to be infected with the rust fungus Zaghouania oleae (E.J. Butler) Cummins, belonging to the family Pucciniaceae (Cummins, 1960). The infection of Z. oleae is restricted to leaves and tender shoots, causes blister on leaves, hypertrophy, littling, thickening and unusual elongation of the infected shoot (Hosagaudar, 1988). These hypertrophied tender twigs are being cooked and eaten by Chenchu tribal ladies in Andhra Pradesh to beget children (unpublished report, Hosagaudar). Preliminary phytochemical screening of the ethanolic extracts of the infected leaves substantiated the same (Vimalkumar et al., 2014).

In this background, the topic entitled “Systematic documentation of traditional knowledge on fungus infected ethnomedicinal plants with special reference to the scientific validation of anti-infertility property of Olea dioica Roxb., infected with Zaghouania oleae (E.J. Butler) Cummins” has been selected.

The major objectives of the study are mainly two fold:
(i) To systematically document traditional knowledge related to fungus infected ethnomedicinal plants and their uses in traditional medicine.
(ii) To scientifically validate anti-infertility property of *Olea dioica* Roxb., infected with the fungus *Zaghouania oleae* (E.J. Butler) Cummins in comparison with non-infected plants.

A multidisciplinary approach with the integration of some advanced methodologies and techniques has been adopted to achieve the objectives, the major lines of work envisaged are as under:

A. Systematic Documentation of Traditional Knowledge:

(i) Documentation of traditional knowledge related to fungus infected ethnobotanically important plants.

(ii) Data analysis of documented information for scientific validation.

B. Reproductive pharmacological study:

(i) Estrous cycle studies of the female rats administered with *O dioica* infected with *Z. oleae* and non-infected plants.

(ii) Pharmacological study on *O. dioica* infected with *Z. oleae* and non-infected plants to evaluate its anti-infertility property by letrozole induced polycystic ovarian syndrome rat models.

(iii) Evaluation of the reproductive indices of the female rat administered with both the infected and non-infected plant extracts.

C. Preliminary phytochemical studies:

(i) Comparative preliminary phytochemical analysis of *O. dioica* infected with *Z. oleae* and non-infected plants.