1. INTRODUCTION

Since ancient times, people are dependent on plants not only as a source of food but also to fulfill their medicinal needs. Human beings were reported to know the medicinal properties of plants from around 5000 years ago (Sofowora, 1996). According to WHO, about 80% of world population still rely on traditional medicines for their primary health care needs (Farnsworth *et al*., 1985). The use of different plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005), where low-income people of small isolated villages and native communities depend on folk medicine for the treatment of common diseases (Rojas *et al.*, 2006). It has become the most affordable and easily accessible way of treatment in the primary health care system of poor communities especially in remote areas. People in the remote villages are mostly deprived of modern medical facilities because of the difficulty in reaching the nearest healthcare centre. Thus they are highly dependent on traditional medicine for treatment of common ailments in their day-to-day life. This has led to more faith in traditional medicine by the people residing in villages. Despite of alongside co-existence of modern medicine, certain factors such as cost, accessibility along with some historical as well as cultural perspectives are responsible for maintaining the popularity of herbal medicines (Vishwakarma *et al*., 2013). This is not only used as primary health care in rural areas of developing countries but is equally popular in developed countries too (Selvaraj *et al*., 2009).

Ethnomedicine has contributed immensely in modern system of medicine as these are used by the pharmaceutical industry in finding new and effective therapeutic agents in the field of medicine (Cox and Balick, 1994). Medicinal plants are natural wealth which can provide new products and bioactive compounds for drug development (Gangwar *et al*., 2010). Approximately 25% of prescribed drugs and 11% of drugs listed to be essential by WHO are obtained from plants. Similarly, numerous synthetic drugs are also derived from precursor compounds originating from plants (Rates, 2001). Ethno-botanical studies are often significant in finding locally important plant species which could be used for the discovery of new plant based drugs (Cotton, 1996). Numerous plant-originated drugs found in clinical medicine today were recorded to be derived from traditional medicine (Li-Weber, 2009). Morphine was the first

pharmacologically active compound that was isolated in pure form from a plant although its structure was later elucidated after 1923 (Sneader, 2005). In 19th century, numerous alkaloids were isolated from plants which were used as drugs. Some of these are atropine (*Atropa belladonna*), cocaine (*Erythroxylum coca*), caffeine (*Coffea arabica*), ephedrine (*Ephedra species*), morphine and codeine (*Papaver somniferum*), quinine (*Cinchona cordifolia* Mutis ex Humb.) etc. Some other well known drugs derived from plants are taxol from *Taxus brevifolia* (Wani and Horwitz, 2014); curcumin from *Curcuma longa* L. (Fu and Lin, 2015); artemisinin from *Artemesia annua* L. (Zhao *et al.*, 2012) etc. Fabricant and Farnsworth (2001) studied the correlation between the medicinal plants used in traditional medicine and medicines used in modern healthcare discovered from those plants. He revealed that 88 single chemical entities isolated from 72 medicinal plants have been introduced into modern therapy, many of which have the same or a similar therapeutic purpose as their original ethnomedicinal uses. These plant-derived compounds such as atropine (anticholinergic), codeine (cough suppressant), colchicine (antigout), ephedrine (bronchodilator), morphine (analgesic), physostigmine (cholinesterase inhibitor) etc are still widely used as single-agent or combination formulations in prescription drugs (Sneader, 1996).

The extensive demand of medicinal plants either for research or for commercial purposes might lead to the random collection of plants. Plant materials are likely to be at high risk of adulteration. It may get adulterated with inorganic substances, or with some other plants parts which might be toxic sometimes or may not meet the quality of the drug with its bioactivity. Thus quality assessment by standardization of any herbal formulation or plant materials to be used is of vital importance in order to justify their acceptance in modern system of medicine (Kokare *et al.*, 2014). Standardization is a code of conduct which ensures the correct substance at correct amount for a desired therapeutic activity with the guarantee of quality, safety, and efficacy (Nikam *et al.*, 2012; Ekka *et al.*, 2008). For basic study, pharmacognostic study serves as a useful tool with some simple methods such as physicochemical analysis with powder microscopy (to identify plants in dried form), fluorescence tests etc. Chromatographic methods such as TLC, HPTLC, GC-MS etc can also authenticate a plant sample by the identification of active phytoconstituents and observing/comparing their R_f values. The word

'standardization' covers the entire field of study from the beginning with the cultivation of medicinal plant to its clinical application (Sahil *et al*., 2011). It also focuses all aspects of research of medicinal plants from its traditional medicinal use, utilization for curing a particular disease, isolation and identification of active constituents for safety and efficacy of a formulation along with clinical evaluation (Chanda, 2014).

A single herbal formulation may contain many beneficial phytochemical constituents, such as alkaloids, flavonoids, terpenoids etc which are responsible for desired pharmacological effect (Parasuraman, 2014). These are secondary metabolites produced by plant which are mainly used for defense purposes as well as for colour and odour. In addition to this, the positive health effects of plants for managing disease related with oxidative stress is credited to these bioactive substances which often exhibited a wide range of biological and pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anti-diabetic and anti-carcinogenic activities (Rasineni *et al*., 2008). It has been reported that phytochemicals protect the cell constituents against destructive oxidative damage, inhibition of hydrolytic and oxidative enzymes including lipid peroxidation thus reducing the risk of various degenerative diseases caused by oxidative stress (Patel *et al*., 2010). Most of the distinguished drugs used in modern medicines are produced indirectly from medicinal plants (Gangwar *et al*., 2010) and about 90% of raw materials come from wild sources. The ethnopharmacological approach is helpful in exploring wild under-utilized plants which are traditionally used for the treatment of different ailments, and till now, which are not being recognized in modern medicine.

Recently there has been an increasing interest in the therapeutic potentials of plants as antioxidants for reducing free radical mediated tissue injury. These free radicals are either produced by physiological or biochemical processes or by pollution, exposure to UV radiation etc. Free radicals are highly reactive molecules capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage (Shivaprasad, 2005). Main class of free radicals generated in living organism was derived from oxygen, such as superoxide, hydroxyl ion, etc and are called reactive oxygen species (ROS) (Fang *et al*., 2002; Valko *et al*., 2007). Another major radical are radical nitrogen species (RNS) such as nitric oxide

(NO) and nitrogen dioxide (NO_2). The damage or decrease in function of lipid, proteins, DNA etc in biological processes by ROS and RNS is called an oxidative stress or nitrosative stress. Organisms produce antioxidant molecules to protect cells from the oxidative stress by scavenging the free radicals (Ayoola *et al*., 2008). At a low concentration in the body, antioxidants could protect the cells and its content like proteins, lipids, carbohydrates, and DNA significantly (Gupta *et al*., 2006). However in higher concentration, supplementary antioxidant should be taken to protect the body. Although several synthetic antioxidants such as butylated hydroxytoluene (BHT) are commercially available, but are quite unsafe and their toxicity is a matter of concern due to which there is a trend to substitute them with naturally occurring antioxidants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). Moreover, they also find their use as nutraceutical and phytoceuticals components as they have significant impact on the status of human health and disease prevention (Haque *et al.*, 2004). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006).

India has been recognized by herbal practitioners for over 3000 medicinal plants having therapeutic potentials (Dubey *et al*., 2004). In rural India, 70% of the population is dependent on the traditional system of medicine. Medicinal plants have met the healthcare need for a million of the ethnic and indigenous people living in the rural sectors of India. According to a study conducted by the Ministry of Environments and Forest, Government of India, tribal communities in India are utilizing over 1,0000 wild plants for primary health care (Unial *et al*., 2011; Pushpangadan, 2002). The Indian Himalayan Region (IHR) has been the highest source of medicine for the people of this region including people living in the other parts of India. The pharmaceutical sector in India is using 280 medicinal plants, of which 175 are found in the IHR (Dhar *et al*., 2000). In India around 16000 species of higher plants are found and out of these 7500 species are used for medicinal and health care purpose by different ethnic communities (Arora, 1987). North East India harbours many precious medicinal plants and most of

the communities living in this region rely on ethno-medicine for their health care practices. The local tribal people utilize different parts of plants to deal with common illness. The study on traditional health care practices of the North Eastern tribes revealed that the same plant is used by different communities to heal different diseases (Bhuyan, 2015). In India the importance of 2,416 plants for ethno-medicinal purposes has been recorded; among which about 1,963 plants are used by different tribal societies of North East India alone (Sajem and Gosai, 2006).

Sikkim is one of the North-Eastern states of India situated in the eastern Himalayas with 80% of its geographical area under forest with over 4500 species of flowering plants consisting of many medicinally important plants (Rai and Sundriyal, 1997). It is identified as one of the biodiversity hotspots of Eastern Himalayas. Himalayan region is estimated to constitute over 10,000 species of aromatic and medicinal plants, 490 species of which are present in Sikkim (Lachungpa, 2009). Precipitation throughout winter and summer season has contributed to the lush green vegetation of this region and it has a rich diversity of flora and fauna. The immensely rich flora of Sikkim has a number of raw drugs described in Ayurvedic texts. Among 420 plants used by the tribal people to cure various diseases in Sikkim Himalayan region, only a few have been exploited commercially (Panda and Misra 2010). The present local inhabitants of Sikkim use numerous herbal remedies for treatment of various ailments which have remained unexplored (Gurung, 2002). Over the ages, Sikkim has developed rich cultural practices of folk medicines. Folk medicine is known as the treatment of diseases outside clinical medicine by plants or simple remedies based on experience and knowledge transferred from generation to generation (Sherpa *et al*., 2015). It is an unofficial health practice in Sikkim existing traditionally and has been learned verbally or through demonstration and observation. Plant is a major constituent of folk medicines. A particular plant used by a practitioner is usually selected after countless hits and trials of treatments to achieve its medicinal value. This as a result has led to numerous advantageous medicinal plants to be treasured as medicine. Extensive studies and systematic documentation of traditional knowledge have globally been recognized as high priority especially in the past couple of decades

as a tool to explore newer novel properties and highlighted pharmaceutical potential of various species (Badola and Pradhan 2013).

Though the folk medicine has strong impact on the people of rural areas but modernization is gradually affecting this field with the introduction of modern medicine even in rural areas. The folk medicinal practices are slowly declining with fear of extinction in near future. There are other factors also that are responsible of this declination such as threats to valuable medicinal plants by increasing commercialization of medicinal plants leading to their deterioration and lack of knowledge of traditional healers for the conservation of such plants. Due to increasing national and international demand, medicinal plants are facing continuous exploitation from their natural habitat. The uncontrolled exploitation along with several other factors like destruction of habitat, overgrazing, forest fire, tourism development, expansion of agriculture, urbanization etc. are leading to deterioration of important plant habitats and selective eradication of commercially more valuable plants (Badoni and Badoni, 2000). The traditional herbal knowledge is passed from generation to generation in the verbal form by herbal practitioners. However, since cultural systems are dynamic, the skills are fragile and easily forgettable as most of the indigenous knowledge transfer in the country is based on oral transmission (Koleva *et al*., 2002). Today majority of world's population is running behind the herbal medication system because of their efficacy, safety and lesser side effects. Higher study as well as advanced research on the traditional medicines is the demand for today. Proper documentation of this knowledge is extremely essential before its complete depletion from our regions.

Some ample amount of surveys on ethnomedicinal uses of plants have been done in Sikkim by various researchers. Most of them have focused on some ethnic groups such as Lepchas, Sherpas, Limboos etc (Pradhan and Badola 2008; Jha *et al*., 2016; Dwivedi, 2016). After critically passing through existing literature review, some lacunas were traced regarding the study of ethnomedicinal plants of Sikkim. Firstly, most of the studies are mainly focused on the documentation of traditional uses of medicinal plants while lacking the study on phytochemical evaluation and pharmacological importance of these plants with scientific validation. Secondly, there was not a single report on standardization of herbal formulation used by the

practitioners. Thirdly, most of the areas or villages of Sikkim were documented by various authors during survey but scanty reports were available from the West district of Sikkim despite having numerous tribal communities residing and practicing the traditional herbal healing system in this region for long period. Sikkim is comprised of four districts (East, West, North and South). Most of the surveys were done in North and East Sikkim with few areas of South. As discussed above, it is highly necessary to standardize any herbal formulation before giving a human trial for the safety and authentication. Evaluation of pharmacological activity and phytochemical content are equally important for scientific validation for the use of these plants in traditional system. The documentation of medicinal plants used in traditional system can also be useful for spreading awareness among the villagers especially the herbal practitioners considering their importance for conservation of valuable medicinal plants. This study might introduce some new plants with possibly new novel compounds for the discovery of new drugs. Therefore an attempt was made on collecting comprehensive data from the villages of West Sikkim particularly and carried out the phytochemical analysis present in them followed by judging their pharmacological potential and purification of the best performing herbal formulation for the identification of the bioactive phytoconstituents present in them.

The main objectives of this research work are:

1. Ethnomedicinal survey of West Sikkim along with recognition of formula and process of traditional phytotherapy
2. Screening of antioxidants of ethnomedicine and their herbal counterparts
3. Quantitative profiling and optimization of different free-radical scavenging potential under various solvent extraction systems
4. Evaluation of bioactive phytochemicals of ethnomedicine
5. Evaluation of antibacterial activity of ethnomedicine
6. Determination of other *in vitro* and *in vivo* pharmacological properties of ethnomedicine
7. Pharmacognostic characterisation of powdered plant samples
8. Measuring alteration of bioactivity of phytomedicine through process variation

9. Isolation and purification of bioactive substances for their effective use in nutraceutical industry