Introduction

Medicinal plant constitutes a very important bioresource in India because it has one of the richest plant based ethnomedical traditions in the world. India has often been referred to as 'Medicinal Garden of the World' (Sharma *et al.,* 2008). Herbal Medicines from plants play a major role in curing many diseases compare to allopathic medicine. The World Health Organization estimated that, 80% of the population of developing countries relies on medicinal plants. In modern medicine, about 25% of drugs are isolated from the plants (Ncube *et al.,* 2008). The potentiality of medicinal property of plant materials result from the secondary metabolites present in the plants such as alkaloids, glycosides, steroids, tannins, phenols, flavonoids, terpenoids etc. Every secondary metabolite plays a major role in curing various diseases as they possess various properties such as antioxidant, antimicrobial, antiparasitic, anticancer, anti-inflammatory, antipyretic etc. (Jain *et al.,* 2019).

Currently, there has been an increased interest globally to identify the antioxidant compounds from plants with no side effects (Seitz and Stickel, 2006). Drug discovery from medicinal plants has played an important role in the treatment of cancer. Recently advances in cancer research and significant development in the field of synthetic drugs, there is a global need for the discovery of new medicines of natural origin which are less toxic with fewer side effects and are most potent. In the last half century the plant secondary metabolites and their derivatives have been used to cure cancer (Butler, 2004). Plant with significant amount of antioxidant property is use to prevent oxidative stress which leads to cancer.

Some medicinal plants although highly desirable as herbal source of drug cannot be procured in sufficient quantity to meet the demand because the plants are not cultivable or slow growing. Demand for medicinal plants is

increasing due to growing recognition of natural products being non-toxic, having no side effects. Furthermore increasing rely on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from the plants as well as from traditional medicine (Vieira and Skorupa, 1993; Verpoorte, 2000).

Many medicinal plants are propagated on large scale under commercial sector required for pharmaceutical industry. Some of the commercially important medicinal plants are *Rauvolfia serpentina,* yield a compound reserpine which is used to treat hypertension; Aloe vera is used as anti allergic, antiageing and antihistaminic properties; *Withania somnifera* having anti-inflammatory, antiarthritic and anticancer properties. Plants such as *Bacopa monnieri, Centella asiatica, Catharanthus roseus, Aegle marmelos* and *Juniperus communis* etc were propagated in largle scale inorder to meet the requirement of pharmaceutical industries (Chaturvedi *et al.,* 2007). In other hand, the production of crude drugs subject to variation of active principle due to genetic variability and time, season and geographical regions of collection of medicinal plants which will affect the quality of herbal drug.

Phytochemical analysis has always been an essential part of drug discovery process for finding new compounds with therapeutic efficacy which is used for the treatment of various diseases. The modern techniques such as HPLC, HPTLC, GCMS, OPLC, UV spectroscopy, IR spectroscopy, Mass spectroscopy, Nuclear magnetic resonance spectroscopy, X-Ray Crystallography etc were used in the detection and quantification of phytoconstituents. As the phytoconstituents are present in very low quantities in plants, these techniques are highly useful for identification and isolation of phytoconstituents (Banu and Cathrine, 2015; Gupta, 2017). WHO (1998) recommends that medicinal plants that form the raw materials for the finished products may be checked for the presence of heavy meals. The chemical constituents in plants, including metal ions are particularly responsible for

medicinal and nutritional properties as well as toxicity. There is an inherent health risk associated with many of these medicinal plants due to the presence of heavy metals (Bhagyashree and Sanjib, 2016).

Plant tissue culture offers an effective method for alleviating the problems facing the pharmaceutical industries (Katiyar *et al.,* 2012). This technique became major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites (Oseni *et al.,* 2018). Micropropagation is generally used for the production and multiplication of rare and endangered medicinal plants. Advances in the area of tissue culture for the production of secondary metabolites by callus culture have made it possible for the increased yield of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, and flavonoids (Ramachandra and Ravishankar, 2002).

The plants belong to the families Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Rutaceae, Piperaceae and Sapotaceae contain rich medicinal properties (Dhar *et al.,* 2002). *Eupatorium* is one of the important genus belongs to the family Asteraceae, is a flowering plant containing around 1200 species. Most of the species are herbaceous perennials and few are shrubs. Among that three different species namely *E. glandulosum, E. odoratum* and *E. triplinerve* have been selected for the present study.

E. glandulosum is a perennial shrub, native of Mexico but has naturalized in many countries. The leaves are used as astringent, thermogenic and stimulant in folklore medicine of India (Kritikar and Basu, 1987). The leaves of this plant are used traditionally by the tribes to cure jaundice, ulcer, stimulant, tooth ache and gum infection (Dahanukar *et al.,* 2000; Bantawa and Rai, 2009: Saha *et al.,* 2011; Desingh *et al.,* 2014).

The plant *Eupatorium odoratum* is a fast growing perennial shrub, native of Central and South America, have spread throughout the tropical and

subtropical areas of the world. In traditional medicine, young leaf juice is used to treat wounds. The boiled roots of the plant are used for urinary retention. The tribal people used the leaf extract to cure skin diseases, poison bites, wounds, burns, cough, diabetes, diarrhea, fever, inflammation and rheumatism. It is mixed with other plants for oral consumption in terms of decoction in the primary health care and external application (Irobi, 1997; Amatya and Tuladhar, 2011).

E. triplinerve commonly called as Ayapana is an ornamental shrub, distributed in tropical regions. In tribal medicine, they are used to cure fever with convulsions, ulcer, haemorrhage, burning sensations, indigestion, pneumonia, cough, mucus and tingling sensations in the body (Hossan *et al.*, 2009).

Thus to consider the medicinal value of the selected plants, the research was undertaken with the following objectives

Objectives

- To screen the leaves of *E. glandulosum, E. odoratum and E. triplinerve* for their pharmacognostical, phytochemical, antimicrobial and antioxidant properties.
- To analyse the genetic variation of selected plants by RAPD technique.
- To study the anticancer property of the selected plants against human colon carcinoma cell line under *in vitro* condition.
- To standardize tissue culture techniques for mass propagation.
- To screen the *in vitro* grown plant materials for secondary metabolites.