INTRODUCTION
Ever since ancient times, the drugs derived from plant kingdom are used to alleviate or to
cure human diseases. The indigenous system of medicine is gradually gaining popularity
mainly because of less or no toxic or side effect of herbal drugs. Simultaneously, there is a
growing interest among researchers for traditional remedies used by ancient tribes and old
civilizations. It is believed that investigation of such ancient drugs on scientific line with
modern scientific appliances and methodologies may unravel a vast of number of effective
remedies for the treatment of diseases and alleviation of human suffering.

It is estimated that about 80% of world population residing in the rural areas of the
developing and under developing countries greatly rely on medicinal plants and its extracts
since it is the only affordable and accessible source of primary health care especially in the
absence of access to modern medical facilities (WHO 2002). In the 20th century there was a
renaissance and rejuvenation in the traditional system of medicine, thereby increasing the use
of plant based medicine both in and out of the country.

The inventory of medicinal plants, their availability and uses started as early as 3,500 BC or
even prior to that in Indian, Egyptian and Chinese civilization. It is estimated that about
2,50,000 to 5,00,000 plant species present on earth possesses medicinal properties and are
used for curative of number of disorders. In most part of Asia, traditional medicinal plants are
part of a culture and it is passed through the generations (Nurliani 2004), and hence
promoting the use of medicinal plant for health purposes.

Plant based drugs of the Indian system of medicine such as Ayurveda, Unani, Siddha and
Homoeopathy are in great demand, and supply base of 90 per cent herbal raw drugs is largely
from wild sources. The growth of the plants and their secondary metabolite contents (the
main component giving medicinal property in plants) are greatly influenced by the
environment where it grows. However, for majority of the medicinal plants there are no

Induced Chemical Mutagenesis (Ethyl methane sulphonate and Diethyl sulphate) In *Andrographis paniculata* (Burm. F.)
Nees (Family: Acanthaceae)
information available about the most favourable temperature, humidity and soil condition responsible for growth of and for procuring maximum yield of secondary metabolites.

_Brunchi paniculata_ (Burm.f.) Ness (Family: Acanthaceae; Synonyms: _Brunchis paniculata_ Wall ex. Ness-DMPRD, 1990, _Justica latebrossa_ Russ., _J paniculata_ Burm.f., _J stricta_ Lam. Ex Steud- Hooker 1885, Anonymous 1998); commonly known as Kalmegh (other common names: Kalafath, Kan-jang, Alui, Charita, Cherota, Chiraita, Cheretta, Kariyat, Green chiretta, Halviva, Kreat, Sinta, Rice bitters, Sambilata, Sambiloto, Andrographidis- Kraut-Hsu 1986, Chang 1986, Kapoor 1990, Maunwongyathi 1994, Ruengrungsri and Tantiwat 1994, Farnsworth 1998), is an annual herb (Kuppusamy and Murugan 2008) as well as reported to be a perennial shrub (Hanchanlerd et al. 1994). The species is native to India and Sri Lanka, and widely found as well as cultivated in tropical and subtropical Asia, south-east Asia and India (Chang 1986, Kapoor 1990). It is also reported from different phytogeographical and edaphic zones of China, America, West Indies and Christmas Island in Indian Ocean (Lattoo et al. 2006).

_A. paniculata_ is prominent in 26 Ayurvedic formulations as evidenced from Indian Pharmacopoeia; while, in Traditional Chinese Medicine it is an important “cold property” herb used to release body heat in fever (Bobbarala et al. 2009). The species is well explored therapeutically and effectively used as imunostimulant (Puri et al. 1993) and for asthma, gonorrhoea, piles (Rao 1914), dysentery and dyspepsia (Bhalla et al. 1982), blood purification (Vohora 1985), influenza (Dey 1986), gastric complaints and diarrhoea (Gupta et al. 1990), pharyngitonsillitis (Thamlikitkul et al. 1991), fever (Ahmad and Asmawi 1993), loss of scalp hair (Home et al. 1992), snake bite (Gupta and Srivastava 1994), myocardial ischemia (Guo et al. 1995), common cold (Melchior et al. 1996), diabetes (Zhang and Tan 2000), respiratory tract infections (Coon and Ernst 2004), jaundice (Tomar et al. 1983) among others.

*Induced Chemical Mutagenesis (Ethyl methane sulphonate and Diethyl sulphate) In _Andrographis paniculata_ (Burm. F.) Nees (Family: Acanthaceae)*
The species also possesses antiulcerogenic (Vishwanathan et al. 1981), antityphoid (Anonymous 1985), antisnake venom (Selvanayagam et al. 1994), antiplatelet aggregation (Amroyan et al. 1999), anti HIV (Calabrese et al. 2000), antimalarial (Dua et al. 2000), antifertility (Akbarsha and Murugan 2000), anti-inflammatory (Shen et al. 2002) and antihyperglycemic (Rao 2006) properties. Bioeffectivity of the species against phytopathogens (bacteria - *Erwinia caratovora*, *Pseudomonas marginales*, *Pseudomonas syringae*, *Pseudomonas aeruginosa* and *Xanthomonas compestris*; fungi - *Acremonium strictum*, *Alternaria alternata*, *Aspergillus flavus*, *Bipolaris bicolor*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium oxysporum*, *Pencillium expansum*, *Rhizoctonia solani*, *Tiarospora phaseolina* and *Ustilago maydis*) was also noted from methanolic (95%), chloroform (80%) and hexane (65%) extracts (Bobbarala et al. 2009).

Bright et al. (2001) reported that the species also possesses insecticidal activity against cowpea weevil (*Callosobruchus chinensis* L.) during post harvest storage of cowpea (*Vigna unguiculata* L. Walp.) in terms of adult mortality, total egg output and emergence of F1 adults. The chemical constituents of *A. paniculata* might be responsible for the mortality of the pest along with reduction in egg laying and adult emergence. Zaridah et al. (2001) reported that aqueous extract of dried leaves of *A. paniculata* possess antifilarial activity when tested in vitro against adult worms of subperiodic *Brugia malayi*. Uawonggul et al. (2006) demonstrated that extract of the species may be used as an antidote against scorpion venom (*Heterometrus laoticus*), and the activity was measured from relative lysis of fibroblast cells.

Kuppusamy and Murugan (2008) reported mosquitocidal effect of *A. paniculata* (ethanolic extract of whole plant) against the malarial vector *Anopheles stephensi* Liston (Diptera: culicidae). The ethanolic extract of the species was found to possess larvicidal, pupicidal, adulticidal and ovicidal activities and these were attributed to the toxic impact of...
andrographolide, 14-deoxy-11,12-didehydroandrographolides, andrographin and homoandrographolide, 19-β-D glucosides, flavonoids and related compounds either singly or jointly. Elango et al. (2010) revealed that leaf hexane extracts of *A. paniculata* is a potent repellent, ovicidal and oviposition deterrent against *Culex tritaeniorhynchus* (Japanese encephalitis vector).

The primary medicinal component of *A. paniculata* is andrographolide (diterpene lactone) which is bitter in taste and colorless crystalline in appearance. Analysis of the whole plant (dry basis) yields andrographolides – C_{20}H_{30}O_{5}, mp 230 - 239°C, 0.6% (Gorter 1911); 14-deoxy-11-o xoandrographolide, C_{20}H_{28}O_{5}, mp 100°C, 0.12% (Saraswat et al. 1995); 14-deoxy-11, 12-didehydroandrographolide – andrographolide D, C_{20}H_{30}O_{4}, mp 203 - 204°C, 0.06% (Visen et al. 1993); 14-deoxyandrographolide, C_{20}H_{30}O_{4}, mp 175°C, 0.02% and a non-bitter constituent, neoandrographolide – C_{26}H_{40}O_{8}, mp 167 - 168°C, 0.005% (Chao and Lin 2010). The leaves contain the maximum amount of andrographolide (1.0% to 2.39%), while the seeds contain the lowest (Sharma et al. 1992). The leaves also possess diterpenoids (bitter principles) viz. deoxyandrographolide, 19β-D-glucoside and neo-andrographolide. The roots of the species contains apigenin – 7,4’-di-o-methyl ether, andrographolide and a new natural flavones, 5-hydroxy 7,8,2’,3’-tetramethoxy flavones (C_{19}H_{18}O_{7}, mp 150 - 151°C, yield – 0.006%). They also contain monohydroxy trimethyl flavones, andrographin (C_{18}H_{16}O_{6}, mp 190 - 191°C) and a dihydroxy-di-methoxy flavone, panicolin (C_{17}H_{4}O_{6}, mp 263 - 264°C) apart from the presence of α-sitosterol (Saxena et al. 1998).

*A. paniculata* is hermaphrodite, self compatible and a habitual inbreeder. Both stigma and anthers are in intimate proximity showing synchronization of anther dehiscence and stigma receptivity respectively thereby providing autonomous selfing in the species (Lattoo et al. 2006). Self pollination offers little scope of genetic variations and therefore improvement in the species through conventional breeding (exploring the existing germplasm(s)) techniques.
is rather difficult. It is of utmost importance to raise superior ‘plant types’ in the species (exploring the existing germplasms(s)) keeping in mind the objective of enhancing the bioactive compound(s).

Gustafsson (1947) advocated that mutation approach is superior to other methods for crop improvement specially in cases where the required amount of variation could be produced rapidly and economically with least investment of land and labour. Mutation provides an opportunity to create hitherto unknown alleles so that the plant breeder does not remain handicapped due to limited allelic variation at one or more gene loci of interest (Gupta 1998). Fried (1969) suggested that induced mutagenesis is important in creating variability in the breeding population to improve yield and related traits. Apart from having a great relevance in modern plant breeding, induced mutation is used for reconstruction of plant ideotypes, upgradation of protein and getting transgressive variants (Swaminathan 1972). Brock (1977) suggested that induced mutations may be exploited by means of selection or recombination and selection or with other methods of manipulating genetic variation.

In mutation breeding, the most important aspects are induction, identification, isolation and utilization of drastic changes of the phenotype brought about by mutational events among the genes (Scossiroli 1965). Mutability of genes varies in response to mutagens (Hagberg et al. 1963) but rate of mutation per locus does not appear to vary substantially in eukaryotes; although, the number of loci that can be mutated to give a particular mutant phenotype can vary widely (Brock 1977). Experiments performed in induced mutagenesis in different plant species suggested that the effect induced varies with the varying mutagens and with variation in mutagen doses administered. Thus, selecting of a mutagen and its optimum dose for a genotype is an important step in any mutation breeding programme (Siddiqui and Khan 1999).
Since the discovery of chemical mutagens by Oehlkers (1943), Auerbach (1943), Rapoport (1948) and other subsequent workers, a tremendous upsurge of interest was directed towards mutation research relating to problem of primary effects and degree of mutability induced by different mutagens (Hagberg et al. 1963). Chemical mutagens were able to induce such effects which were not at all realized in radiation experiments (Fahmy and Fahmy 1957). Attractive mutagenic potentiality, relative ease of application and comparatively low cost of the chemical mutagens, generated significant interest in using them for the artificial induction of mutation.

Kharkwal (2012) reported that mutation breeding possibly contributed the most to the global agriculture and provided more than 3000 mutant varieties with enhanced production and productivity in about 175 plant species. The author reported that China (741 varieties), India (343), Japan (233), USA (128), Vietnam (50), France (42), Pakistan (42), Bangladesh (40), Bulgaria (38), Canada (37), Italy (36), Brazil (36), Spain (35), U.K. (34), Poland (30) and Portugal (30) were the countries that successfully developed and released large number of mutant varieties in different agricultural crops, thereby suggesting the significant contribution of the methodology. Induced mutagenesis also provided food and nutritional security in many countries of the world.

With the view to create desirable genetic variations in quick span of time by widening the existing gene pool, the methodology of induced chemical mutagenesis (ethyl methane sulphonate-EMS and diethyl sulphate-dES) was adopted and the mutagen doses were administered in *Andrographis paniculata* (Burm.f.) Ness (Family: Acanthaceae). The present investigation deals with the following aspects: 1) morphological, anatomical, palynological, cytological and biochemical aspects of the germplasm under investigation; 2) assessment of mutagenic sensitivity of the species and determination of mutagenic efficiency and effectiveness of the mutagens; 3) screening of morphological mutants from $M_2$ mutagenized
population; 4) segregation analysis of the macromutants at M$_3$; 5) cytogenetical analysis of
the true breeding M$_4$ mutants in comparison to control; 6) statistical assessment of
quantitative traits of M$_4$ mutants in relation to control; 7) quantitative analysis of total
andrographolide content as well as andrographolide content estimated by HPTLC method in
control as well as in M$_4$ mutants; 8) quantitative and qualitative (SDS-PAGE) estimation of
seed protein in M$_4$ plant types and 9) genetic assessment of the M$_4$ plant types (M$_4$ harvested
seeds were used) using RAPD marker.

The present objective of the work is to develop important genetic resources (plant type
mutants with enhanced seed/leaf/bioactive compounds and with marker trait(s) for efficient
breeding) in *A. paniculata*, which previously did not exist in natural population.