INTRODUCTION

From the time immemorial, plants have been widely used as curative agents in traditional medicines for variety of ailments. World Health Organization has reported approximately 21,000 plant species used around the world for medicinal purpose. In India about 2,500 plants species belongs to more than 1000 genera are being used in the indigenous system of medicine (Srivastava et al., 1995). India is tenth among the plants rich countries of world and fourth among the Asian countries. Northeast India including Assam is represented by about 130 different tribes out of total 427 of India having their own traditional practices. Many herbal remedies individually or in combination have been recommended for the cure of different diseases in traditional medicinal practices by the ethnic communities of Northeast India.

_Aristolochia_ (Aristolochiaceae) is an important genus widely used in traditional medicine (Che et al., 1984). During the past two decades, this genus has attracted much interest and has been the subject of numerous chemical and pharmacological studies. It is a rich source of aristolochic acids, which are unique to this genus, and of terpenoids (Das et al., 2010).

The family Aristolochiaceae comprises of 8 genera and 450-600 species distributed in tropical and subtropical regions. The members of the genus _Aristolochia_ are mostly distributed in tropical, subtropical, and mediterranean regions of the world (Sarma and Tanti, 2015; Neinhuis et al., 2005; Wanke et al., 2006, 2007). In India, eight species of _Aristolochia_ has so far been reported and five species of _Aristolochia_ viz., _A. tagala_ Chamisso, _A. cathcartii_ Hooker.F, _A. indica_ Linn., _A. saccata_ Wall. and _A. platanifolia_ Duchart Vern. are available in Assam (Kanjilal and Bor, 1940; Baruah et al., 2012). The species of _Aristolochia_ are shrubby or perennial herbs, usually climbing.

The genus _Aristolochia_ consists of a large number of species are cultivated as ornamentals (Wu et al., 2002) and popularly used as sources of abortifacient, emmenagogue, sedative, analgesic, anticancer, anti-inflammatory, anti-feedant, muscle relaxant, antihistaminic, and antiallergic drugs, for intestinal worms, in the treatment of cholera, stomach ache, abdominal pain, rheumatism, malaria, wounds and skin diseases, and also useful in treatment of different types of poisonous bites and stings (Che et al.,
The genus is a source of aristolochic acid which has been evaluated in China for the treatment of wounds and infectious diseases; it was found to be useful for promoting wound healing in ulcers, burns and scalds. The seeds are tasteless, useful in inflammation, biliousness, dry cough, joints pain, dysphoea of children, purgative. The plant is good for snake bite. The juice of fresh leaves is useful in the group of children, by inducing vomiting without causing any depression (Kubmarawa et al., 2007; Lajide et al., 1993; Lemos et al., 1993; Lopes et al., 1990; Nok 2001; Shafi et al., 2002; Terada et al., 1987; Yu et al., 2007). The plants also are used as an abortifacient in different parts of India. The root, stem and leaves are recommended for the treatment of snake bite (Charaka, vaghbhata, bapat, ainslie, rheede, Roberts) and scorpion- stings (Charaka).

The genetic diversity of medicinal plants in the world is becoming endangered at an alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines. Further, extensive destruction of the plant rich habitat as a result of forest degradation, agricultural encroachment, urbanization, etc., are also some important factors (Sarma and Tanti, 2015). Hence, there is a strong need for proactive understanding in the conservation, cultivation and sustainable usage of important medicinal plant species for future use. Considering the potentiality of Aristolochia sp., the present study has been emphasized to characterize few selected species of Aristolochia available in Assam.

Karyotype analysis can be used for many purposes, such as to study chromosomal aberrations, cellular functions, taxonomic relationships, and to gather information about past evolutionary events. An examination of karyotypic differences is often of great value in understanding the nature of plant variations, particularly from the level of population to genus. Karyotypes also show differences in absolute chromosome size, indicating changes in nuclear DNA in evolution (Govindarajan and Subramanian, 1983). Karyotype is a morphological aspect of the chromosome complement of an organism or species as seen at mitotic metaphase. The number, size and shape of chromosomes constitute the karyotype. The karyotype is an important biological attribute which provides individuality and a cyto-taxonomic status of species. Karyotypes are useful as they permit rapid recognition of any aberrations either in chromosome number or morphology or both. Karyotype can also help in establishing evolutionary relationship between different species (Berr et al., 2006).
Here, karyomorphological analysis has been conducted for four species of *Aristolochia* available in Assam as adequate karyomorphological information are not available for these species.

India has a rich heritage of herbal medicines amongst the countries of South East Asia. Huge quantities of plant materials and extracts are imported for the manufacture of ayurvedic, unani and homeopathic medicines. Making health care and medical facilities available to the people now a major concern for a large number of countries. Due to toxic and adverse effect of synthetic and chemical drugs, the modern medicines are being prepared from plant based materials. Religious cultural faith, weak economy and lack of modern healthcare practices in the remote villages are still dependent on various plants and parts to prepare their traditional medicines.

Plants are rich sources of pharmaceutically important compounds, but there is a need to synthesis these compounds within laboratory conditions. Micropropagation is an important technology since many secondary plant metabolites can’t be synthesised chemically. Many plant species are undiscovered and their medicinal properties unknown; and even the medicinal remedies past down from generations are being lost. Further research and conservation of all plant species including medicinal plants is needed to preserve nature’s natural drugs. Advances in plant tissue culture will enable rapid multiplication and sustainable use of medicinal plants for future generations.

Micropropagation insures a good regular supply of medicinal plants, using minimum space and time. The advantages of *in vitro* micropropagation of medicinal plant are: (i) higher rate of multiplication, (ii) environment can be controlled or altered to meet specific needs of the plant, (iii) plant available all year round (independent of regional or seasonal variation), (iv) identification and production of clones with desired characteristics, (v) production of secondary metabolites, (vi) new and improved genetically engineered plant can be produced, (vii) conservation of threatened plant species and (viii) preservation of genetic material by cryopreservation.

For further research into the biochemical compositions and potential medicinal values of *Aristolochia* sp., an efficient *in-vitro* regeneration system for the production of plants is required because - naturally grown plants are exposed to various abiotic as well
as biotic stresses which can affect the medicinal values of the harvested tissues. In addition, *in-vitro* propagation methods offer powerful tools for germplasm conservation and mass multiplication of the threatened plant species. Due to the collection of large amount of plant materials, also the underground organs, with subsequent eradication of the natural populations, the natural habitat are tremendously decreasing. Thus, there is an urgent need for domestication of these plants. The application of *in-vitro* propagation techniques might offer the possibility of producing large number of uniform plants for further field cultures. Over-exploitation of the natural population for medicinal use and the lack of systematic efforts for cultivation call the need for *in-vitro* culture.

Since the root of the plant is more valuable, people up-rooting these plants for drug from the wild populations unscientifically. This results the plant to face the risk of depletion. Therefore, developing the effective reproducing micropropagation protocol for *in-vitro* propagating is very much essential to make available of the plant materials throughout the years for pharmaco-based industries and also for their conservation.

This is the technique of growing plant cells, tissues and organs in an artificially prepared nutrient medium static or liquid, under aseptic conditions. The principal advantages of using tissue culture techniques for economically important plants from a limited number of stock plants and at a much quicker rate; rapid multiplication of plants which do not give seeds or have recalcitrant seeds; preservation of threatened and rare species of plants for biodiversity conservation; production of plants with changed genotype (haploids, hybrids *etc.*); production of uniform clones from highly heterogeneous plants; production of disease free plant materials etc.

Phylogenetic placement of the medicinal plants is an important task for their further exploitation. Differences in genetic level may not express in the phenotype because mostly gene expression is regulated by environmental parameters. Finding out the genetic relatedness among closely related medicinal plants might be helpful for bioprospection. DNA marker based analyses is the best option so far to find out their genetic relatedness and phylogenetic placement in molecular level.

The commonly used PCR-based DNA marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites (Staub *et al.*, 1996; Gupta and
Varshney, 2000). The major limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism. ISSR-PCR is a technique that overcomes most of these limitations (Zietkiewicz *et al.*, 1994; Gupta *et al.*, 1994; Wu *et al.*, 1994; Meyer *et al.*, 1993). It is rapidly being used by the research community in various fields of plant improvement (Godwin, 1997). The technique is useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of plant species. In this method SSRs are used as primers to amplify mainly the inter-SSR regions. SSRs or microsatellites are short tandem repeats (STRs) or variable number of tandem repeats (VNTRs) of 1–4 bases of DNA ubiquitously present in eukaryote genomes (Tautz and Renz, 1984). They are dispersed throughout the genome and vary in the number of repeat units. Moreover, ISSR markers offer other advantages in the detection of somaclonal variation, notably a high degree of sensitivity, reproducibility, and the dominant representation of polymorphic genetic alleles.

In view of medicinal and economic importance of *Aristolochia* (Aristolochiaceae), in this study, the main aim is to characterize the chromosomes and to develop a suitable *in-vitro* propagation protocol of few selected species available in Assam. On this basis, following objectives of the study were proposed:

1. Karyomorphological study of some species of *Aristolochia* Linn.

2. Standardization of in-vitro propagation protocols of some species of *Aristolochia* Linn.