Man has used plants for various medicinal purposes from time immemorial. These plant derived medicines were also found to have pronounced therapeutic effects on human body. In today’s world, the interest in medicinal plants is increasing and a number of studies are being carried out focusing on various bioactive principles. The advantage of such plant based medicines is that, they possess a complex mixture of many naturally occurring compounds and mostly act synergistically. Hence, they are better candidates as they pose lesser allergic reactions and smoother action that can be well tolerated for long time administration.

The present investigation mainly dealt with the phytochemical and molecular systematics of four *Aristolochia* species; *A. indica*, *A. tagala*, *A. ringens* and *A. krisagathra*. All the four species are used as medicinal and *A. ringens* because of its nature of flowers is also used as an ornamental. *Aristolochia* L. is an important genus of the family *Aristolochiaceae* consisting species mainly distributed in the tropical and also in the subtropical and temperate areas of both hemispheres. However, proper documentation of the genus is not accomplished in India. The genus is important medicinally and they have a long history of medicinal use in Europe, Asia, Africa and Central America. The members of the genus have also been used in Indian medicine for snakebite, rheumatism, hypertension and in childbirth (birthwort).

Phytochemistry is the branch that deals with the various phytochemicals and such metabolites of plants especially secondary metabolites which have proven to be useful for chemosystematics based on its distribution in plants. The phytochemical studies focused on the essential oil composition and aristolochic acid I quantification.

Essential oils are complex mixtures composed of various components and are having various medicinal activities. The section on essential oil involved the study of essential oil composition of *A. indica*, *A. tagala*, *A. ringens* and *A. krisagathra*. Both the leaves and stem oil of the four species were studied. The influence of the three seasons, pre-monsoon, monsoon and post-monsoon on the oil composition of
the leaf and stem oil was also analyzed. The analysis showed that seasons affect the oil composition. Except for *A. ringens* stem oil, all other oils showed the highest number of identified volatiles in the post-monsoon period. The essential oil of *Aristolochia* can be regarded as caryophyllene type because the sesquiterpene hydrocarbon caryophyllene was present in all the species both in the stem and leaf oil irrespective of seasons. But there were differences in their percentage composition. The major compound showed variation with the three seasons in some cases. In *A. indica*, the major compound in the leaf and stem remained same in all the three seasons. In *A. tagala* leaf oil, the major compound remained the same (bicyclogermacrene) in pre-monsoon and post-monsoon but varied in the monsoon (spathulenol) whereas stem oil showed the same compound in all seasons (D-limonene). Three different compounds dominated in three seasons in *A. ringens* leaf oil whereas stem oil showed similar compound as dominant one in pre-monsoon and post-monsoon with difference in the monsoon period. In *A. krisagathra*, leaf oil was dominated by copaene in pre- and post-monsoon period, whereas delta-cadinene was the major compound in the monsoon. *A. krisagathra* stem oil showed alloaromadendrene as the major compound in the pre- and post-monsoon period and spathulenol in monsoon period. Varying nature of dominant compounds with seasons was evident in some oils and in such oils it was noted that the pre-monsoon and post-monsoon periods maintained stability in the major compound except for leaf oil of *A. ringens*. Only monsoon season showed a difference in the major compound from the other two seasons. In some cases, the compound that was the predominant one in a season was found completely absent in other. The analyses considering the seasons clearly indicated that seasons play a major role in the essential oil composition.

The leaf and stem oils also showed marked differences. The mean of the three seasons for both stem and leaf oil was noted and then the influence of difference in plant parts was studied. In the leaf and stem oils of the same species different compounds showed dominance indicating the influence of the plant part in oil composition. *A. indica* stem oil was dominated by beta-guaiene, leaf oil with caryophyllene; *A. tagala* and *A. ringens* stem oil was dominated by D-limonene and
leaf oils with bicyclogermacrene; *A. krisagathra* stem oil with alloaromadendrene and leaf oil with copaene as the major compounds. *A. tagala* and *A. ringens* stem oils showed a higher content of monoterpenes to sesquiterpenes whereas sesquiterpenes predominated in all other oils.

To have an overall oil composition of four species, the mean of stem and leaf oil was taken. The principal compounds identified were caryophyllene for *A. indica*, D-limonene for *A. tagala* and *A. ringens*, and allaromadendrene for *A. krisagathra*. Sesquiterpenes formed the predominant group in all the four species followed by monoterpenes which corresponds to the previous literatures. On the contrary, diterpenes though reported as another dominant group in *Aristolochia*, it formed only a small percentage in the present study and was absent in *A. indica*.

The principal compound analysis grouped *A. tagala* and *A. ringens* together as was evident from the essential oil analysis. Both were having the same principal compounds and shared many similarities. The cluster analysis placed *A. tagala* and *A. ringens* in the same clade. *A. tagala* is more closely associated to *A. indica* and *A. krisagathra* in morphology than to the exotic *A. ringens*. The results were thus indicative of the influence of geography in essential oil composition. The two species, *A. tagala* and *A. ringens* collected from same geographical areas shared many similarities and hence grouped together.

The quantification of aristolochic acid I formed another aspect of phytochemical study. Aristolochic acids are characteristic of the family Aristolochiaceae and are reported to have both beneficial and harmful effects. The *Aristolochia* species used in traditional medicines especially in China was withdrawn later due to the potential health hazards reported. Chinese herbal nephropathy was found associated with the intake of *Aristolochia* species containing aristolochic acids. However, these compounds have also been reported to have therapeutic effects. In India, *Aristolochia* species are used for medicinal purposes especially by the tribals. Aristolochic acids are also important from ecological point of view as they are involved in plant-animal interaction. They act as oviposition stimulant in papilionid group of butterflies and are also involved in chemical defense. These aspects make the study of the compound important.
The quantification of aristolochic acid I was done using HPLC. The highest concentration was found in *A. krisagathra* followed by *A. ringens*. Among the seasons, the post-monsoon period showed a higher concentration in *A. indica*, *A. tagala* and *A. ringens*. In *A. krisagathra* the highest concentration was in the monsoon period. There are no earlier reports or studies on the presence of aristolochic acids in *A. krisagathra*.

The second major section of the study deals with the molecular systematics. Molecular studies are the need of the hour and are being widely used for identification, classification and to infer phylogenetic relationships.

The first part involved barcoding of the four species of *Aristolochia* using the markers *rbcL*, *matK*, *trnH-psbA*, ITS and ITS2. The results were used to determine the reliable marker gene for *Aristolochia*. ITS2 showed higher percentage of variation among the sequences of the four species and the cladogram obtained was in congruence with the morphological observations. It placed *A. indica* and *A. krisagathra* in a single clade as sister taxa and *A. tagala* as their sister clade. *trnH-psbA* showed good variability percentage but the tree obtained placed *A. indica* with *A. tagala* in a single clade and *A. krisagathra* as its sister clade. Morphologically it is found that *A. krisagathra* resembles *A. indica* and may be misidentified due to the similarity by non-taxonists. *rbcL* was found as the weakest one in discriminating the four species showing less variation and more conserved regions. Variation percentage shown by *matK* was also lesser than *trnH-psbA* and ITS2; ITS sequencing did not work in *A. indica*. Previous literatures reported *trnH-psbA* as the ideal barcode for *Aristolochia*. In opposition, though the number of species was limited to make an exact conclusion, the present study showcases ITS2 as a potential candidate barcode for *Aristolochia* species.

Another area of molecular study dealt with the phylogeny of *Aristolochia* species using the core barcodes *rbcL* and *matK*. *Thottea* was placed as the outgroup taxon and was found basal to *Aristolochia* in the phylogenetic study. The phylogenetic tree obtained by *rbcL* did not get strong support from bootstrapping. Moreover, *A. promissa* coming under the subgenera *ParAristolochia* was placed with the Isotrematinae which was found odd to the general classification. *matK*
phylogeny received strong support from bootstrapping and the tree was in correlation with the classification. In the phylogenetic trees, *A. indica* and *A. krisagathra* formed the sister taxons and *A. tagala* the sister clade. *A. ringens* being exotic was placed in an entirely separate clade.

The phytochemical studies did not provide any clues on the host preference of *Pachliopta aristolochiae*. The essential oil analysis showed highest similarity between *A. ringens* and *A. tagala*. Aristolochic acid I was present in *A. ringens* at a concentration next to *A. krisagathra*. Hence, these phytochemical aspects did not clarify on why *Pachliopta aristolochiae* larvae did not feed on *A. ringens*. Thus, *A. ringens* did not show any significant difference with regard to the oil composition and aristolochic acid I from other *Aristolochia* species studied. However, they stood quite different in the molecular studies.

Thus on considering the phytochemistry and molecular studies, it was found that the phenetic relationship based on essential oil did not agree with molecular relationship. Phytochemical systematics using essential oil placed *A. tagala* and *A. ringens* together which were quite distinct in their morphology whereas molecular systematics corresponds to the morphological observation. This is because essential oil is influenced by many factors that include seasons, plant part, geography etc. The information generated from analyzing oil composition can never be neglected as they can provide valuable clues to taxonomists and other fields of biology regarding important bioactive principles. But as observed from the study and as per the earlier reports, its use is limited for systematics, the successful application of which requires the consideration of several other factors.