Kerala, being a state with rich herbal resources, explorations and technological advancements will provide remedies for our increasing needs of care and cure. But with the increasing demand for herbal medicines, commercial establishments also have proliferated. Hence a greater need for proper standardization has arisen. Quality maintenance of herbal drug is possible, only if care is taken right from the collection of raw materials to their processing and preparation of rational combinations.

The ability of a herbal medicine to affect body systems depends on its chemical constituent. The attributed chemical effectiveness is achieved only when the exact plant is used. The primary and important step in any medicinal system is to identify the exact plant.

The present investigation entitled “Phytochemical studies and biological activities of selected members of family Asclepiadaceae” is a comprehensive evaluation of the selected medicinal plants. It incorporates identification and authentication of the selected plants, study of the pollinial morphology, physicochemical and chromatographic analysis, phytochemical estimation, antioxidant property and antibacterial activities. The specimens selected are Asclepias curassavica L., Calotropis gigantea (L.) R.Br., Gymnema sylvestre (Retz.) R.Br., Holostemma ada-kodien Schult., Pergularia daemia (Forssk.) Chiov., Tylophora indica (Burm.f.) Merr. and Wattakaka volubilis (L.f.) Stapf.

Each species was given the morphological characters, common names, habitat, botanical name and distribution which aid in the
identification procedure. Physico chemical constants like ash value are useful in the standardisation of crude drugs. Ash values of the leaf extracts and stem extracts can give an idea of the mineral content in the plants. It is used to determine the quality and purity of the crude drug. The highest quantity of ash was observed in the *Tylophora indica* (Burm.f.) Merr. leaf (13.08 ± 0.24%) and stem (9.82 ± 0.09%) extract and the lowest value was observed in *Gymnema sylvestre* (Retz.) R.Br. leaf (7.09 ± 0.08%) and stem (4.32 ± 0.03%) extract. Compared to the stems, the leaves showed higher ash value. According to Indian Pharmacopoeia, the ash value of *Gymnema sylvestre* (Retz.) R.Br. is 12%. For all other plants, the standard ash value is not reported. Hence the ash values determined can be taken as a reference standard for future studies to determine the quality of the crude drug.

The ICP-AES detection of selected minerals such as sodium, potassium, calcium, iron, magnesium and zinc were conducted. The calcium and potassium content were more in all the seven leaf and stem samples under study and the percentage of iron, magnesium, sodium and zinc were low. The Na/K ratio is significant, where a value of less than 1 is recommended for controlling high blood pressure. The Na/K ratio ranges from 0.058 to 0.536 in all the samples studied which seems to be ideal, and therapeutically good for hypertension. Similarly the Ca/Mg ratio can vary from 4:1 to 1:1. In all the leaf samples the Ca/Mg ratio was within the ideal range except *Wattakaka volubilis* (L.f.) Stapf. But in the case of stem the ratio exceeded the upper limit in *Asclepias curassavica* L., *Gymnema sylvestre* (Retz.) R.Br., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. If these plants have to be used as one of the ingredients in Ayurvedic formulations, it has to be supplemented with the deficient mineral to avoid adverse side effects.
Water soluble extractive value gives the crude estimate of the water soluble extractable matter present in the sample. Similarly ethanol soluble extractive value gives the crude estimate of the ethanol soluble extractable matter present in the sample. From the extractive values it was observed that the water soluble extractive values were always higher than alcohol soluble extractive values. This indicated that polar compounds were in large amount. From the extractive values, it was observed that *Pergularia daemia* (Forssk.) Chiov. leaf showed the highest quantity of water soluble extractive value (25.18%) and *Gymnema sylvestre* (Retz.) R.Br. leaf showed the highest amount of alcohol soluble extractive value (12.81%). The leaf extractive value was always higher than the stem extractive value which showed that the contents were more in the leaves than in stem.

Qualitative phytochemical screening confirmed the presence of flavonoids, tannins, sterols, phenols and terpenoids in all the selected genera. Saponins were present in *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma adakodien* Schult., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. Its concentration was high in *Gymnema sylvestre* (Retz.) R.Br. and *Wattakaka volubilis* (L.f.) Stapf. Alkaloids were observed in *Gymnema sylvestre* (Retz.) R.Br., *Tylophora indica* (Burm.f.) Merr. and *Pergularia daemia* (Forssk.) Chiov. The curative properties of these medicinal plants are due to the presence of these secondary metabolites.

Densitometric methods using HPTLC had been used to separate the different phenols and flavonoids. In the phenolic profile among all the leaf extracts *Tylophora indica* (Burm.f.) Merr. had maximum number of peaks (seventeen), which represents highest number of phenolic compounds. The lowest number of phenolic compounds (nine) were seen
in *Gymnema sylvestre* (Retz.) R.Br. In the case of stem extracts, *Tylophora indica* (Burm.f.) Merr. showed the maximum number of peaks (ten). From the HPTLC fingerprint profile of flavonoids in the leaf extract the highest number of flavonoid compounds were observed in *Pergularia daemia* (Forssk.) Chiov. (ten). HPTLC fingerprint profiles prepared for all the samples were unique and specific for each extract. These fingerprint profiles can be used for the authentication and identification of the source plant even if it is in the dried and powdered form. It can also be used to detect the adulteration and to determine the quality and purity of the drug obtained from these plants. The chromatogram clearly revealed the presence of similar compounds in the seven plants.

HPTLC analysis had been conducted to study the presence of biologically active compounds like β-sitosterol, lupeol and quercetin in the plants. The presence of β-sitosterol was found in *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. leaf and stem extracts. Lupeol was detected in *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. leaf and stem extracts. Quercetin was found to be present only in *Holostemma ada-kodien* Schult. leaf and stem extracts. These can be used as marker compounds for the proper identification of the drug.

HPLC analysis of amino acids showed that the plants under study are rich sources of essential and non-essential amino acids except the essential amino acids lysine and methionine. Amino acid valine was absent only in *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br.
and *Tylophora indica* (Burm.f.) Merr. Among the seven plants *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov. and *Wattakaka volubilis* (L.f.) Stapf. were traditionally used in the treatment of diabetes. Evidences suggest that amino acids play an important role in the prevention of diabetes and diabetes associated complications. The results of amino acid analysis from the present study add scientific validity to the traditional use of these four plants in the treatment of diabetes. Glutamic and aspartic acid are the amino acids required for the proper functioning of brain. In the present study, aspartic acid was found to be present in all the seven plants in comparatively larger quantity and its quantity was exceptionally high in plants viz., *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br. and *Tylophora indica* (Burm.f.) Merr. These plants can be used as a good source of aspartic acid. Glutamic acid was present in *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov. and *Wattakaka volubilis* (L.f.) Stapf.

Quantitative estimation of polyphenolic compounds revealed that the highest total polyphenolic and flavonoid content was recorded in *Pergularia daemia* (Forssk.) Chiov. followed by *Tylophora indica* (Burm.f.) Merr. The lowest total polyphenolic and flavonoid content was found in *Calotropis gigantea* (L.) R.Br.

There was marked increase in the quantity of flavonoids and phenols in the wild plants when compared to the cultivated plants. Most phenolic compounds have been considered to be involved in the chemical defence mechanism of plants against plant pathogens, herbivores, soil salinity and water scarcity. This could be the reason behind the comparative increase of phytoconstituents in wild plants.
On quantification of phenol and flavonoids during dry and rainy season, it was found that there was notable increase in the content during dry season. The total flavonoid content also showed marked increase (P<0.01) in *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov. and *Wattakaka volubilis* (L.f.) Stapf. collected during dry season. The polyphenol content showed significant increase (P<0.01) in *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. collected during dry season when compared to that of rainy season.

In phenolic content obtained from mature and young plants significant increase was observed in the mature plants of *Calotropis gigantea* (L.) R.Br. and *Wattakaka volubilis* (L.f.) Stapf. (P<0.01). From the statistical analysis of flavonoid content variation a major difference at 1% level of significance was observed in the mature plants of *Calotropis gigantea* (L.) R.Br. and *Holostemma ada-kodien* Schult.

These data indicate that leaves from wild plants collected during dry season showed higher polyphenol and flavonoid content. Significant variation was not observed in the flavonoid and polyphenol content between the young and mature plants. Hence during the preparation of an Ayurvedic formulation, the time of harvest, the habitat and the age of the plant determine its quality. In the case of plants used for medicinal purposes, all these factors must be considered, besides the post harvesting managements.

The results of anti microbial studies revealed that methanolic and ethanolic extracts are showing higher anti microbial activity against both gram positive and gram negative bacteria. The flavonoids extracted from
the plants showed comparatively higher bactericidal activity. From the determination of MIC value, the flavonoids of *Asclepias curassavica* L. and *Tylophora indica* (Burm.f.) Merr. were found to possess more bactericidal activity on *Staphylococcus aureus* when compared to the other plants. The test organisms are resistant to the water and hydroalcoholic extracts except that of *Gymnema sylvestre* (Retz.) R.Br. and *Wattakaka volubilis* (L.f.) Stapf. The water and hydro alcoholic extracts of these plants are rich in saponins. So saponins may be responsible for the bactericidal activity in these plants.

Highest free radical scavenging activity (antioxidant activity) was found in *Pergularia daemia* (Forssk.) Chiov. and the lowest activity was found in *Calotropis gigantea* (L.) R.Br. In the present study, it was observed that the plant which had the highest polyphenolic content was found to possess the best antioxidant activity and the one which had the lowest polyphenolic content, was found to possess the least antioxidant activity. There was direct correlation between polyphenol content and antioxidant activity (correlation coefficient $R^2 = 0.6557$). The P value obtained was 0.027 which is statistically significant at 5% level.

It was observed that the plant which had the highest flavonoid content was found to possess the best antioxidant activity and the one which had the lowest flavonoid content, was also found to possess the least antioxidant activity. The P value obtained was 0.004 which is less than 0.01. So the correlation ($R^2 = 0.8337$) is very significant at 1% level. The results obtained in the present study indicate that the leaves of these medicinal plants have great importance as therapeutic agents in preventing oxidative stress related degenerative diseases. Such antioxidants could replace synthetic toxic antioxidants.
Cluster analysis was conducted on the basis of data obtained from HPTLC studies of phenol, flavonoid, and amino acid profile and dendrograms were prepared. The results obtained by cluster analysis in the three distributions were not uniform. The clusters developed do not tally with any of the already existing classifications. For conclusive results more genetically controlled parameters should be included.

The shape, size, orientation of pollinia, pollinial attachment to caudicle are the valuable characters studied for the analysis of diversification of pollinia. The pollinia, caudicle and corpusculam showed great variation in form and structure. The pollinial orientation has been used as a diagnostic character for the systematic studies in the family Asclepiadaceae. Based on the pollinial characters, the species diversification was established in this study. These studies conclude that the pollinia of different genera vary in morphology. It is an important morphological character which is taxonomically significant. Hence based on pollinial studies an artificial key has been provided for the taxa under study.

All the parameters studied in the present work, would help to identify and authenticate the plants at source and to check the purity and quality of the plant drug. The fingerprint profile would help to investigate the substitutes and adulterants. The pollinial characters can be taken as a tool for taxonomic classification and correct identification. The present investigation reveals that collection of the plants for therapeutic use should be done on scientific basis. Parameters such as the time of harvest, the habitat and the age of the plant also determine the quality of the crude drug used in the preparation of Ayurvedic formulations. Phytochemical investigation and the study of the antioxidant activity have provided scientific validity for the ethno pharmacological uses of these plants in the treatment and prevention of various diseases and disorders.
amino acid composition and the presence of biologically active compounds like β-sitosterol, lupeol and quercetin in the different members of the family also play a vital role in the biological potency of the drug. The flavonoid compounds in the plants are also responsible for its antibacterial and antioxidant activity. As there is not much pharmacognostical work on these traditionally valued drugs, the present work can also therefore be used as one of the tools for standardization of crude drug, to identify and decide the authenticity of the drug in herbal industry or trade.

In conclusion, the results obtained from our screening confirm the therapeutic potency of these medicinal plants. Extracts analyzed thus provide a rationale for their use in traditional medicine. These results also form a good basis for further pharmacological studies and conservation of wild varieties for the future benefit of mankind.