A quantitative study on the selected phytochemicals of six indigenous medicinal plants of Mahe, U.T. of Puducherry, India

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Abstract
A quantitative study was conducted to estimate the chlorophyll, carotenoids, lycopene, protein, and percentage of H₂O₂ scavenging capacity of six selected medicinal plants of Mahe region. The plants selected were Cardiospermum halicacabum of family Sapindaceae, Desmodium triflorum of Fabaceae, Piper longum of Piperaceae, Scoparia dulcis of Scrophulariaceae, Tabernaemontana divaricata of Apocynaceae and Thespesia populnea of Malvaceae. The study showed that Thespesia populnea showed maximum chlorophyll content per gram of the leaf, Piper longum showed the minimum of the same. Tabernaemontana divaricata showed the maximum of carotenoids and Piper longum showed the minimum. Thespesia populnea showed the maximum of lycopene and Desmodium triflorum showed the minimum. Percentage of H₂O₂ scavenging capacity was maximum in Piper longum and minimum in Desmodium triflorum. Total protein content was maximum in Scoparia dulcis and minimum in Desmodium triflorum. The present study is a useful prelude for further investigations in this line.

Keywords: Chlorophylls, Carotenoids, Lycopene, H₂O₂ scavenging capacity, Indigenous medicinal plants, Mahe

1. Introduction
Plants and animals, the two inseparable sides of the biological spectrum, inter depend each other for their co-existence. In this association, plants have got upper hand because of autotrophy and on which the animals depend on. Human civilization was enriched by the constant association with the plants in the form of food, shelter, clothes and medicines. In ancient times, the only ways to cure diseases was by using plant and animal biodiversity. Plants are naturally gifted as they have the potential to heal life threatening diseases [1]. Nature has provided a complete store house of remedies to cure all the ailments of mankind. Use of plants as a source of medicine has been inherited from the beginning of human civilization itself. Plants have become the basis for the development of medicines, i.e., they are the natural blue prints for the development of new drugs and also it is a phyto medicine for the treatment of diseases [2]. Enormous quantum of research works have produced huge amount of data across the world which depict their therapeutic potentials and also their protective effects against various diseases. The knowledge of various phytochemicals has provided the basis for several traditional medicine systems like Ayurveda, Unani, Folk, and Chinese. Due to the rapid increase in the rate of advancement of illness and their diagnosis, the drug discovery from active constituents of plants has more importance. Phytochemicals occur naturally in medicinal plants in their leaves, stems, roots, fruits or seeds that have defence mechanism and protection from various diseases. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as primary as well as secondary metabolites [3, 4]. Chlorophyll, protein and common sugars include primary constituents and secondary products include alkaloids, flavonoids, terpenoids, saponins, tannins, glycosides etc. In recent years, chemical analysis and biological assays have begun to play an important role in ethno botanical studies [5]. Such analyses have led to the discovery of novel bioactive phytochemicals. The qualitative and quantitative estimation of the phytochemical constituents of medicinal plant is considered to be an important step in medicinal plant research [6]. The drugs contained in medicinal plants are called active principles [7, 8] and plants contain a variety of active principles. Most of the studies related with the indigenous uses of medicinal plants are qualitative. Quantitative estimations of chlorophyll, carotenoids, lycopene, H₂O₂ scavenging effects, proteins etc. of six major medicinal plants which are used in traditional medicines of post-natal care of Mahe region are studied in this research which has got much acceptance in the field of...
local medicines. Chlorophyll has many health benefits due to its structural similarity to haemoglobin of human blood and its good chelating ability. It has anti mutagenic and anti-carcinogenic properties. H₂O₂ like free radicals are called pre-oxidants that they attack macromolecules including protein, DNA and lipid causing cellular and tissue damage. Lycopene is an effective component to alleviate or prevent the complications of diabetes[9].

In the present investigation an attempt was made by quantitative estimation to study some of the phytochemicals of selected medicinal plants such as Cardiospermum halicacabum of family sapindaceae which is one of the ten sacred plants (Dasa pushpa) and can be used for the treatment of rheumatism, stiffness of the limb, chicken pox, pulmonary affection, as anthelmintic, also is used with food in order to get colour, taste and its therapeutic properties, Desmodium triflorum of fabaceae which is effective against human pathogenic micro-organisms and the reduction of SGPT (Serum Glutamic-Pyruvic Transaminase), Piper longum of piperaceae which is used as a remedy for respiratory and digestive disorders, paralytic and arthritic disorders, acts as stimulants and to reduce cholesterol, Scoparia dulcis of scrophulariaceae against diabetes, bronchitis, kidney stone, snake bite, stomach problems and also as analgesic and anti- pyretic agents, Tabernaemontana divaricata of apocynaceae which has anti-cancerous properties, can enhance the cholinergic activity in both peripheral and central nervous system which has therapeutic benefits against Alzheimer’s, against skin diseases, against eye sore and also as analgesic and astringent and Thespesia populnea of malvaceae which is used against scabies, psoriasis, as liver tonic and to increase blood count.

Different colours associated with higher plants (green leaves in the spring & summer, yellow or red leaves in the fall, orange colour of carrots etc.) are due to the presence of pigment molecules such as chlorophylls, carotenoids etc. Many works have thrown light on the pigment composition in medicinal plants. The studies on chlorophyll and carotenoids have been investigated in leaves of Sesbania rostrata, S. exaltata and S. sespan where S. exaltata comprises of highest amount of both the pigments[10]. Studies on Ocimum sanctum, Azadirachta indica, Murraya koenigii, and Mentha arvensis indicated that the highest chlorophyll content is in Azadirachta indica followed by Murraya koenigii[11]. Works on Cuminum resulted in the presence of proteins, carotenoids, β carotenes etc. [12,13]. The colour of red tomatoes (Solanum lycopersicum L.) is mostly from the carotenoid pigment lycopene which has protective effects against diabetes, cardiovascular problems and some cancers. Highly nutritional carotenoid compounds are seen in wheat grains [14]. Citrus is a complex source of carotenoids with the largest number of carotenones found in any fruit [15]. Carotenoids are potent antioxidants and free radical scavengers. They can modulate pathogenesis of cancers and coronary heart disease [16]. The therapeutic properties of Moringa is well explained by its phytochemical compounds like β carotene, protein etc. [17-19]. Studies on Mimosa pudica indicate that they are rich in proteins [20, 21]. Jatropha curcas is a rich source of protein which is a valuable energy source [22]. It is indicated that H₂O₂ protects the tissues from damage and also indicates that β carotene is an excellent scavenger of singlet oxygen [23]. Studies on Kalanchoe pinnata showed that they comprise lycopenes, β carotenes etc. and have got very powerful H₂O₂ Scavenging effects [24].

2. Materials and methods
The fresh weights of the sample were taken for the quantitative estimations. i.e. quantitative estimation of chlorophyll, carotenoids, lycopene, hydrogen peroxide scavenging effects, and protein. The chlorophyll content in various plants was estimated by the method of Witham et al. [22]. Chlorophyll was extracted in 80% acetone and the absorbance was measured at 645 nm and 663 nm. The amount of chlorophyll was calculated using the absorption coefficient. The ability of the leaf extract to scavenge Hydrogen peroxide was assessed by the method of Ruch et al. [24]. Lycopene was estimated by the method described by Zakaria et al. [27]. Carotenoids were estimated by Tan and Soderstrom method [28]. The protein content in various plants was estimated by Bradford method [29].

3. Results and Discussion
The total chlorophyll content per gram of leaf extracted and quantified in the case of the selected six medicinal plants showed variations. It was 1.53 mg/g in Cardiospermum halicacabum. Vivek et al. have estimated the chlorophyll content in Cardiospermum as 2.703 mg/g [30]. This disparity in the content could be attributed to the seasonal changes and edaphic characters that promoted the development of chlorophyll in former. Chlorophyll content was 1.54 mg/g in Desmodium triflorum and there is a report by Misra et al. in which 3.5 mg/g of chlorophyll content in dry leaf mass of the same [31]. Piper longum showed a chlorophyll content of 0.97 mg/g and a reduced chlorophyll content has been reported by Gogoi et al. in this plant [32]. Chlorophyll content was estimated as 1.80 mg/g in leaves of Scoparia dulcis. Mishra et al. have reported 1.35 mg/g of chlorophyll in this plant [33]. The chlorophyll content was 3.36 mg/g in Tabernaemontana divaricata and this quantity was found to be much higher than (0.9mg/g) that was estimated by Amulya et al. [34]. The chlorophyll content of the fresh leaf was 3.95 mg/g in Thespesia populnea. It was reported by Das et al. that Thespesia leaf chlorophyll content was only 1.66 mg/g in a study based on mangrove associates [35]. The present study showed maximum chlorophyll content in Thespesia and minimum in Cardiospermum. The total content of Carotenoids in leaf extract was 0.74 µg/ml in Cardiospermum halicacabum, 1.06 µg/ml in Desmodium triflorum, 0.10 µg/ml in Piper longum, 0.84 µg/ml in Scoparia dulcis, 1.9 µg/ml in Tabernaemontana divaricata and 0.48 µg/ml in Thespesia populnea. Veera et al. have reported 0.24 mg/g and 0.095 mg/g of carotenoids in the leaves of Desmodium and Piper longum respectively [36]. Nambiar et al. have reported 0.054 mg/g carotenoids in leaves of Scoparia plant [37]. The present study showed maximum carotenoid content in Scoparia and minimum in Piper longum.

The Lycopene content was 1.40 mg/g in Cardiospermum halicacabum, 0.30 mg/g in Desmodium triflorum, 0.66 mg/g in Piper longum, 1.36 mg/g in Scoparia dulcis, 1.90 mg/g in Tabernaemontana divaricata and 2.36 mg/g in Thespesia populnea. In present study, maximum amount of Lycopene was shown by Thespesia leaves and minimum by Desmodium leaves. The protein content was 0.20 mg/g in Cardiospermum halicacabum, 0.18 mg/g in Desmodium triflorum, 0.24 mg/g in Piper longum, 0.44 mg/g in Scoparia dulcis, 0.28 mg/g in Tabernaemontana divaricata and 0.26 mg/g in Thespesia populnea. Maximum protein content was shown by Scoparia and Minimum by Desmodium. Other plant species showed protein contents in moderate levels. The Hydrogen peroxide scavenging percentage was 0.58 in Cardiospermum halicacabum, 0.19 in Desmodium triflorum, 0.89 in Piper

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longum, 0.76 in Scoparia dulcis, 0.37 in Tabernaemontana divaricata and 0.63 in Thespesia populnea. Piper showed higher Hydrogen peroxide scavenging efficiency than all other plants whereas Desmodium showed minimum.

4. Conclusion
A quantitative study was conducted to estimate the chlorophyll, carotenoids, lycopene, protein, and percentage of H<sub>2</sub>O<sub>2</sub> scavenging capacity of six selected medicinal plants of Mahe region. The plants selected were Cardiospermum halicacabum of family Sapindaceae, Desmodium triflorum of Fabaceae, Piper longum of Piperaceae, Scoparia dulcis of Scrophulariaceae, Tabernaemontana divaricata of Apocynaceae, and Thespesia populnea of Malvaceae. The study showed that Thespesia populnea showed maximum chlorophyll content per gram of the leaf, Piper longum showed the minimum of the same. Tabernaemontana divaricata showed the maximum of carotenoids and Piper longum showed the minimum. Thespesia populnea showed the maximum of lycopene and Desmodium triflorum showed the minimum. Percentage of H<sub>2</sub>O<sub>2</sub> scavenging capacity was maximum in Piper longum and minimum in Desmodium triflorum. Total protein content was maximum in Scoparia dulcis and minimum in Desmodium triflorum. The present study is a useful prelude for further investigations in this line.

5. Acknowledgment
Authors are thankful to D.S.T.E. Puducherry for the financial assistance in the form of a Minor Project. Deep gratitude is expressed to Principal and staff members of Department of Botany of Mahatma Gandhi Govt. Arts College, Mahe, for their whole hearted support for the successful completion of the Project.

6. References
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