ABSTRACT

Traditions are dynamic entities of unchanging knowledge. Traditional medicine is an evolutionary process as communities and individuals continue to discover new techniques that can transform practices. Ethnopharmacology and drug discovery using natural products remain important issues in the current target-rich, lead-poor scenario. Many modern drugs have their origin in ethnopharmacology.

The World Health Organization (WHO) encourages the use of plant-based medicine, especially in developing countries, even if the rationale is to reduce the financial burden on the respective governments. In view of the increasing demand for herbal products in Western countries, well-defined herbal products of proven efficacy and safety have been introduced in the last two decades. In the draft of the National policy on the Indian Systems of Medicine (ISM), priority is being given to research on standardization, pharmacology, toxicology and clinical trials of herbal drugs. Reports on adulteration, contamination with heavy metals, pesticides and microbes in herbal drugs and their effects on health, have necessitated the development of effective identification systems for herbal drugs and their components. Methods that ensure the quality and safety of these products have to be developed in order to ensure the quality and purity of herbal drugs.

Medicinal plants of the wet lands in India are exploited to a very less extent, despite the availability of rich traditional knowledge and also greater possibilities of offering novel bioactive compounds. In recent decades the resurgence of focus is slowly moving towards the wild and aquatic plants of wet land ecosystem to meet the increasing demand for novel herbal drugs. The plants of wet land ecosystems, played an important role in the life of human beings in earlier days, as food, fodder, medicine etc., but with the advancement of agriculture and urbanization, the uses of wet land aquatic herbs are neglected and they are treated as noxious weeds and the wet lands as a menace, aquatic
plants face a great threat of extinction, due to the lack of awareness on their nutritional values in favor of the exotic ones.

*Monochoria vaginalis* and *Monochoria hastata* are emergent aquatic herbs belonging to water-hyacinth family, Pontederiaceae. Even though the plant claims to be medicinally rich in terms of ethno-medicinal practices, considerably very less research has been carried on systematics and as well as for its medicinal and nutritional properties. Hence the present study is an initialization for the first time to explore and compare the detailed Pharmacognostical, Phytochemical and Biological properties of these plants. Identification and standardization of therapeutic elements of these medicinal plants will enhance the health care system and nutritional status of ethnic communities.

WHO has strongly highlighted the need for safety evaluation and quality assurance of traditional medicine. Thus it becomes compulsory that all herbal preparations and raw materials acquired from the wild and cultivated source has to be screened for the presence of heavy metals to guarantee quality, efficacy and safety of herbal preparation. Fresh, entire plants were collected from the marshy lake (*M. hastata*) and from the streams (*M. vaginalis*) of Ambalavayal, Wayanad District, Kerala, India, in the month of December 2012. The plant specimens were authenticated by Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore.

The plant parts were carefully separated and washed in running tap water to clean the foreign particles and cut into small pieces, with a sharp knife to facilitate drying and shade dried in room temperature for about three days, pulverized and passed through 60-mesh sieve and stored dry until use. The air – dried plant parts were extracted with hydro-alcohol (30:70) by cold maceration. Macroscopical characters were observed and the required samples of different organs were fixed in FAA, the material was processed following the conventional method. Serial transverse sections (10-12 µm) were made from the paraffin embedded vegetative organs with the help of rotary microtome. The sections were stained with toluidine blue, further subjected to a series of changes with ethanol-xylene and mounted in Canada balsam. Powder microscopy was carried out by staining the coarse powder with phloroglucinol and concentrated sulphuric acid to identify the lignified tissues. All the anatomical investigations were documented with
photographs of different magnifications obtained from Nikon Photolab 2 light microscope Ash values, extractive values and foaming index were determined according to WHO guidelines and moisture content was determined by drying at 105°C, until a constant weight was achieved.

Preliminary phytochemical screenings for the presence of alkaloids, flavonoids, sterols, polyphenols etc., were carried out according to the standard official methods for all the extracts prepared. Bioactive contents like Total Phenols, flavonoids, sterols, saponins and triterpenoids were carried out using 96 well plate and UV method using standard protocols, Al, Cu, Zn, Ca, Co, Cr, Fe, Ni, Mg and Mn concentrations were determined using Flame atomic absorption spectrophotometry (FAAS) system (AA 7000 AAS, Lab India Instrument Pvt. Ltd). Cd and Pb were determined using Graphite furnace atomic absorption spectrometry (GF-AAS) system equipped with AA700 AAS.

GC-MS analysis for phytoconstituents of hydro-alcoholic (70%) extracts was performed with Bruker GC-MS SCION TQ equipped with a Finnigan Trace DSQ and an electron impact (EI) ion source. Interpretation on mass spectrum of GC-MS was done using the database of in-built libraries like NIST 8. Finger print analysis of the hydro-alcoholic extracts was carried out by HPTLC. HPTLC plates were developed in Automated Multiple Development Chamber (AMD2, Camag) with six steps gradient elution method. Images of plates were captured using a TLC-Visualizer (Camag, Muttenz, Switzerland) with a 12 bit camera (Camag) under UV light 254 nm, 366 nm before derivatization and at 540 nm, after derivatization with anisaldehyde sulphuric acid, aluminium chloride and DPPH. winCATS planar chromatography manager software was used for quantitative evaluation of plates and to transform images into chromatogram.

Hydro-alcoholic extracts of all the parts were dissolved in methanol and analyzed chemically, to determine the presence of different chemical constituents, MHL, MHS, and MHR extracts were selected for column chromatography. Standard procedure of column chromatography was followed for all the selected extracts. Quantification and validation of selected isolated compounds were done by HPTLC, HPLC and LC-MS. Invitro antioxidant activity like DPPH scavenging activity, Hydroxyl free radical scavenging activity and Nitric oxide radical scavenging activity were carried out using 96
well plate method. Hydro-alcoholic extract of leaf of both species against digestive enzymes related to diabetes and their effect on glucose uptake in 3T3L1 adipocytes were investigated.

Pharmacognostical studies were carried out according to WHO guidelines for raw materials, this serves as valuable information for the preparation of herbal monograph of these two species. The anatomical characterization of *M. vaginalis* and *M. hastata*, confirms the botanical identity and can reveal several adaptation natures of the plants to the environment. DNA barcoding was carried out for the authentication of plants, elemental analysis revealed that, both the edible species of *Monochoria* are good source of essential elements like magnesium, calcium, iron and copper etc., and at the same time toxic elements like Cd, As, Hg and Pb were within the permissible limits of regulatory bodies. The study unraveled the presence of different phytoconstituents from the fingerprint analysis of hydro alcoholic extracts by HPTLC and GC-MS.

Biologically potent compounds like quercetin and stigmasterol were isolated from the plants for the first time. The results of antioxidant activity clearly indicate that the hydro alcoholic extracts of all the parts of the selected plants were effective against free radical mediated diseases and also helpful to draw attention for further studies. These plants may be used as a potential source of natural antioxidant and in the development of functional food and raw materials of medicine. Further the ethno-medicinal claims of antidiabetic activity of the leaves were proved scientifically and it provides an important baseline data of the two herbs possessing anti diabetic property, which can be exploited for diabetes prevention and associated metabolic dysfunctions.

The study concludes that, these under exploited plants are medicinally potent with various bioactive constituents. The current findings meet the regulatory requirements of WHO guidelines for raw materials and ICH guidelines for the quantification and validation of selected phytoconstituents, hence for the preparation of monograph of these aquatic macrophytes.