Chapter 1

INTRODUCTION
Hybanthus enneaspermus (L) F. Muell is a medicinal herb of the family Violaceae and is popularly known as ‘pink ladies slipper’ in English and ‘padmacarini’ in Sanskrit. The genus Hybanthus consists of about 150 species that are widely distributed in the tropics and the subtropics (Airy-Shaw, 1973). In India, H. enneaspermus is found in the warmer parts of the Deccan peninsula. Generally, it grows mixed with weeds like Ammania baccifera L., Oldenlandia alata J. Koenig ex Roxb., Heliotropium bracteatum R. Br. and Lindernia oppositifolia (Retz.) Mukerjee.

Hybanthus enneaspermus is widely used in Ayurveda, Siddha and other folk medicine systems. The plant has been reported to cure urinary calculi, strangury, painful dysentery, vomiting, burning sensation, wandering of mind, urethral discharge, blood trouble, asthma, epilepsy and cough and also for giving tone to the breasts (Kirtikar and Basu, 1991). It has aphrodisiac, demulcent, tonic and diuretic properties and is used against urinary infections, diarrhea, leucorrhoea, dysuria, inflammation and sterility (Tripathy et al., 2009). In Siddha system of medicine, the species is used in the preparation of ‘Orithalthamarai choornam’ which is used against gonorrheal diseases of women, leucorrhoea, to increase sexual power and to improve the quality of semen. The plant is commonly used by various tribes of Kerala, Tamil Nadu and Andhra Pradesh for their health care practices and as a vegetable.

The botanical identity of ‘padmacarini’ is controversial. Some physicians consider it as Nervilia aragoana or Habenaria grandiflora. However, Kirtikar and Basu (1918), Nadkarni (1954) and Chopra et al. (1956) have described ‘padmacarini’ as H. enneaspermus. The plant is under threat in its natural habitat (Prakash et al., 1999; Sonappanavar and
Jayaraj, 2011). The non-availability and controversy in botanical identity may lead to adulteration and decrease the efficacy of the raw drug. Moreover, it is difficult to identify *H. enneaspermus* from its co-existing weeds in the absence of flowers because of the common morphology. Therefore, a detailed authentication of the drug is quite essential for both pharmaceutical companies as well as public health care sector and to ensure reproducible quality of herbal medicine. Even though, Retnam and De Britto (2007a) have studied some aspects of its pharmacognostic characterization, no detailed study is available. The present study describes a more efficient standardization protocol including chemical and biological tools for the authentication of drug.

*Hybanthus enneaspermus* has been reported to contain aurantiamide acetate, isoaborinol, β-sitosterol and triterpene (Prakash, 1999; Retnam and De Britto, 2003). The plant also contains cyclotides - short peptides with biological activities (Simonson et al., 2005). Previous pharmacological studies reveal that the plant has cardioprotective (Radhika et al., 2011), antifungal (Napoleon et al., 2011), hypoglycemic (Awobajo and Olatunji-Bello, 2010), antiarthritic (Tripathy et al., 2009), antibacterial (Sahoo et al., 2006), antiplasmodial (Weniger et al., 2004), anticonvulsant and free radical scavenging (Hemalatha et al., 2003) activities. However, *H. enneaspermus*, is reported as a rare medicinal plant under threat in the natural habitat due to over exploitation, overgrazing, seasonal habitat, sporadic distribution and poor germination of seeds (Prakash et al., 1999; Sonapanavar and Jayaraj, 2011). Thus, development of mass propagation techniques and alternate methods for the production of raw drugs/metabolites should definitely improve the threat status of the plant.
Considering the prospects of the multifarious properties of the herb, further scientific studies are necessary to exploit the unknown potential. Hence, the current study was aimed to undertake a probing investigation into the antioxidant and anticancer potential of *H. enneaspermus* in detail. Even though, some preliminary studies on antioxidant activity of *H. enneaspermus* are available, an exhaustive account on free radical scavenging activities has not yet been presented. A study to compare the antioxidant potential and chemical profile of field grown and *in vitro* samples of *H. enneaspermus* will explore the possibilities of an alternate source of crude drug without disturbing the rare natural flora.

Hence, the current study is aimed initially to authenticate the plant using various pharmacognostic tools, secondly, to standardise *in vitro* techniques for rapid multiplication and production of biomass and finally, to evaluate the unexplored bioactivities such as antioxidant competence and anticancer property of *Hybanthus enneaspermus*.

**OBJECTIVES**

1. Authentication of *Hybanthus enneaspermus* by evaluating pharmacognostic parameters such as macroscopic and microscopic features, physicochemical, chemical and biological parameters.

2. Standardisation of *in vitro* protocol for mass propagation and biomass production.

3. Evaluation of bioactivities such as antioxidant and anticancer properties of *H. enneaspermus*.

4. Comparative analysis of phytochemical and bioactive properties of field grown plants, *in vitro* plants, and biomass developed *in vitro*. 