

1. INTRODUCTION

Senna obtusifolia belongs to the genus *Senna* Mill. of tribe Cassieae, subtribe Cassiinae, family Leguminosae or Fabaceae and subfamily Caesalpinioideae. The genus *Senna*, one of the most diverse genera with around 350 species of shrubs, sub shrubs, trees and herbs are found in continents of North and South America, Africa, Australia, Pacific islands and Asia (Irwin and Barneby 1982; Marazzi et al. 2006). However, major diversity of *Senna* is concentrated mostly in diverse seasonal and topographical climates. Most *Senna* species occurs in the American continent along with some others in the tropical Africa, Australia and Madagascar. On the other hand, predominant occurrences of a few species of *Senna* is observed in the Asia-Pacific region (Randell and Barlow, 1998).

The genus *Senna* was previously considered as *Cassia* by Linnaeus in his “Species Plantarum” (1753). Phillip Miller (1974) in his fourth edition of “The Gardeners Dictionary” segregated *Senna* from *Cassia*. Initially till 1982, *Senna*, *Cassia* and *Chamaecrista* were not segregated by many authors following Linnaeus and broadly included in *Cassia sensu lato*. Thereafter, the genus *Cassia* L. was raised to Cassiinae subtribe level by Irwin and Barneby (1981, 1982). The later comprised of three subgenera - *Cassia* L. sensu stricto, *Senna* P. Mill. and *Chamaecrista* Moench.

S. obtusifolia (L.) H.S. Irwin and Barneby, a species under the genus *Senna* Mill., is a widely distributed annual herb or an undershrub that occurs in the wild throughout the Indian tropics as well as in other tropical and subtropical countries like Sri Lanka, West China, Africa, Europe, America, Australia, New Zealand, Japan etc. Though the species is presumed to be originated in America, it occurs in a wide geographical area in the

neo-tropical and pan tropical regions. *S. obtusifolia* extends its habitat in almost all conditions including dry and humid forests, roadside pastures, rock outcrops, etc. (Acharya et al., 2011). The species is extensively found in cultivated fields, grasslands, waste lands, settlement areas, and in some disturbed areas. The species is quite prevalent throughout the whole Indian subcontinent as well as in different parts of West Bengal.

The species is an annual foetid herb and grows in the rainy season as a weed on wasteland with small and beautiful yellow flowers at its axils. The plant is 0.5-2.0 meters tall, with deep tap root, nearly hairless stems, stipules present around 5-10 millimeters long, pinnate compound leaves, obovate, oblong and base more or less oblique, leaflets in 3 pairs opposite, rounded at the top with a notch at the mid of minutely acute apex, rachis grooved, one stipel or gland is present between the lowest pairs of leaflets, petiolules found and 2.5 milimetres in length, bracts present, 4-8 mm long, sepals five, pale green, petals five, 12-15 mm long, pale yellow, stamens 10 of which 7 are fertile, and the upper three stamens are staminoidal, ovary slightly glabrous, fruit pod, subglabrous to glabrous, obliquely separate with 30-50 rhombohedral seeds in each pod. Plants are mainly propagated by the seeds.

Long back in 725 AD Chen Zangchi introduced the use of *Cassia* in Chinese pharmacopoeia - Bencao shiyi which is a supplement for the pharmaceutical natural histories (Deshpande, 2008). The plant is otherwise identified as a medicinal one by the name “Chakramarda” or “Dadrughnah” in Sanskrit, “Dadmari” in Ayurveda, “Panwar” in Unani, “Charota” or “Chakvad” in Hindi, “Jui Ming Zi” in Chinese, “Ketsumeishi” in Japanese, “Chakunda” in Bengali and also Sicklepod, Foetid cassia, Sickle Senna, Coffee Pod, Chinese Senna, Java Bean, Tovar is extensively and long used as

ayurvedic, traditional, unani, and conventionally used in Chinese medicines for centuries (Yen et al., 1998; Naeem and Khan, 2006; Jain and Patil, 2010). The plant has long been used in different folklore practices, ethnomedicine, along with traditional ayurvedic and herbal medicines in the Asian countries. It is a major component in ‘chakramardha tailam’, ‘pamari taila’, and ‘dadrughani vati’, which are still in use as an ayurvedic formulation in India, majorly in rural and tribal areas (Bhalerao et al., 2013). The fermented leaves of *S. obtusifolia* are known as ‘kawal’. It is considered as substitute of meat due to its high-quality protein content and is consumed by the fur community of Sudan (Dirar et al., 1985). Traditionally, ‘kawal’ is applied in treating Jaundice (El Ghazali, 1987). Thus, the ethnomedicinal, folklore and the traditional values of this species along with its immense potential in modern medicinal practices have attracted major interest from researchers which has helped in the development of new paths of research. Due to its immense ethnopharmacological significance the plant is gradually gaining much interest for medicinal use in recent times.

The plant is endowed with scores of medicinal properties of which its use as a laxative as well as tonic holds much reputation. It is also used to alleviate “heat” of liver for the virtue of its cold and moist nature. It was effective in hypercholesterolemia and hypertension treatments (Li et al., 2004; Dave and Ledwani, 2012). In traditional Chinese practice, Juemingzi is widely accepted for the teary and red eyes treatment, conjunctivitis, dizziness, headache and improves visual acuity (Guo et al., 1998). The plant is claimed to be enriched with anti-inflammatory, hepatoprotective, neuroprotective, antidiabetic, antibacterial, antioxidant, estrogenic and anti-estrogenic properties (Andrade-cetto and Heinrich, 2005; Kim et al., 2008, 2009; Busmann et al., 2010; Lin et al., 2015; Liu et al., 2015; Yi et al., 2016; Seo et al., 2017). The plant is also known to have an ethnomedicinal use as an antidote against snake and scorpion

bites and also to combat malarial vectors (Bagavan and Rahuman, 2011; Diarra et al., 2015; Upasani et al., 2017). The plant extract is used as remedy for skin problems, and diseases like rheumatic and osteoarthritis (Kamaraj et al., 2011). Sicklepod herbs are effective for treating neurogenetic disease like Parkinson's. (Ju et al., 2010; Ittiyavirah and Hameed, 2014). Anti-cancer potential of the plant has been reported from the recent works (Hsu and Chung, 2012; Du et al., 2013).

S. obtusifolia is now exported from India to the western countries for its unique galactomannan ratio (Harry-o' Kuru et al., 2009) recommended as hydrocolloid additive in pet foods as poultry feed (Talpada et al., 1980; Katoch and Bhowmik, 1983; Bayerlein et al., 1989). The seed of this species is comprised of 1-3% anthraquinones, 5-7% fats, 14-19% proteins, and 66-69% carbohydrates (Crawford et al., 1990; Abbott et al., 1998; Harry-o' Kuru et al., 2009). About 41% of the seed is extractable (Abbott et al., 1998). Sicklepod flour is of high demand in the international markets. The seeds contain various phenolics, proteins and galactomannans. The plant is also used in petroleum, textile and paper industries. Seed gum isolated from the species acts as natural binding agent in pharmaceutical industries for making tablets (Kundlik et al., 2012). These natural excipients have advantages over the synthetic gums and are accepted globally due to its bio-acceptance, biocompatibility, minimum side effects, low cost, renewable nature, patient tolerance and public acceptance (Kulkarni et al., 2002; Pawar et al., 2004; Kumar et al., 2012).

Cultivation of *S. obtusifolia* is now encouraged in India in the districts of Chhattisgarh, Gujarat and Maharashtra and are exported in large scale to the western countries like America, Mexico and other European countries. Sicklepod perform well in crop rotation along with peanut-cotton-corn (Johnson et al., 1994). Tissue culture and hairy

root culture techniques are well established in the plant for mass production of the active compounds (Zaprometov, 1989; Muranaka, 2010; Quraishi, 2011).

The medicinal and pharmacological properties are attributed to the plant for the presence of significant bioactive phytochemicals in roots, seeds, leaves extracts, young stems, flowers, and green pods. These active biochemicals are of different natures, like, anthraquinones, anthraquinone glycosides, sennosides, naphthopyrones, anthrones, dianthrones, aglycones, lactones, isotoralactones, cassialactones, xanthonones, polyketides, sterols and triterpenoides (Crawford et al., 1990; Harry-o' Kuru et al., 2009; Sob et al., 2008; Dave and Ledwani, 2012). Among the bioactive phytochemicals of this species, anthraquinones are the most important ones in respect of pharmaceutical utilities (Sob et al., 2010).

The natural occurrence of anthraquinones makes the species worthwhile for study due to the wide application of those chemicals. Anthraquinones are synthesized in plants by chorismate/o-succinylbenzoic acid and the polyketide pathways (Liu et al., 2015; Shukla et al., 2017). They are used in dye industry as redox mediators as well as in pharmaceutical industry. Anthraquinones typically occurring in the glycosidic forms are phytochemicals with structurally related to the anthracene (Dave and Ledwani, 2012). Rhein (1,8-dihydroxyanthraquinone-3-carboxylic-acid), the simplest form of important anthraquinones, is found in free state as well as glucoside quite prevalent in *Cassia* and *Senna* including *S. obtusifolia*. Due to the immense medicinal properties in hosts of medicinal fields, the study of rhein gained its momentum in the research community.

Rhein, the preliminary compound of diacerein, used for the treatment of inflammation and osteoarthritis (Mehta and Laddha et al., 2009). Due to recent awareness about the sustainable development and environmental issues of using synthetic products these natural and renewable resources throw a keen interest in research work making it commercially and economically important.

The study of intra-specific diversity occurs at genetic and phenotypic levels (Harper, 1977; Burdon, 1980). Phenetic analyses of eighteen species of *Cassia* sensu lato in Thailand was done by Boonkerd et al. (2005). Phenological diversity of various species are observed by several researchers to reveal the patterns of growth, initiation of flowering and fruiting (Dierig., 2006; Duraisamy and Subramaniam, 2010; Kushwaha et al., 2011; Dinis et al., 2011). Several abiotic factors like temperature, rainfall, changes in day-length and several other climatic factors may affect intraspecific diversity of the species. Intraspecific and interspecific morphological diversity of various species are also a subject of interest among different scientists (Holman and Playford, 2000; Jeruto et al., 2017). Morphological analysis of eight species of *Senna* Mill. was done by Soladoye et al. (2010b). Variations in different morphometric traits for generations can also reveal different intraspecific diversity among the species. Interspecific variations and population diversity among the different species for chromosomal, mitochondrial, nuclear, 4C DNA contents were studied by different ecologists (Pyke et al., 1995; Petit et al., 2005). The difference in active biomolecules synthesized by plant species can focus on the diversity in one hand and helps in identification and demarcation of the better one in terms of the productivity of the interested natural secondary metabolites with the aid of HPLC, ESI MS studies (Landim et al., 2013). Moreover, various molecular biological techniques like RFLP, RAPD, ISSR, SCoT AFLP, etc., are the most trending and modern methods for determining

inter and intra species genetic diversity (Zabeau and Vos, 1993; Rajora and Dancik, 1995; Meudt and Clarke, 2007; Kim et al., 2013; Ramachandran et al., 2013; Mao et al., 2017, 2018).

The species under study is focused with an undefined number of individuals forming an isolated community which may show subtle difference with respect to the other community of the same species, placed distantly, both of which may be called as different provenances. The present investigation is targeted in exploring the diversity, if any, amongst the plants of *S. obtusifolia* collected from various provenances of West Bengal and other parts of India, in respect of their phenology, phenotype, chromosomal account, genotype and the productivity of anthraquinones, specifically the Rhein.

Diversity for any number of traits in combination may prove to be unique and characteristic for respective communities and so, would help identify a provenance. Besides this, if any one or two or so traits are found to be associated with the amount of rhein produced, an easily identifiable externally expressed trait may be quite effective in indicating the amount of rhein present in that, the determination of which could, otherwise, entail an elaborative and expensive measure, involved in it.