CHAPTER 1

INTRODUCTION

1.1 Bauhinia racemosa Lam

*Bauhinia racemosa* Lam (BR) belonging to the family Caesalpiniiaceae is a small deciduous tree used in the indigenous system of medicine and is very common in foothills upto 1000 m in India and Srilanka [1,2]. It is commonly named as Mountain ebony (English), Kachnal/Kanchanara/Sonpatta (Hindi), Gul-e-anehnal (Urdu) and Sona/Sonpatta or Apta (Marathi) [3]. The various parts of the plant viz., stem bark, leaves and roots are practiced in various indigenous systems of medicine and popular among the various ethnic groups in India for the cure of variety of ailments [4,5]. The bark of BR is reported to have antifilarial, abortifacient, anthelmintic, analgesic, antipyretic, antimalarial, anti-ulcerogenic, hepatoprotective and various other pharmacological activities [6-13]. Previously reported phytochemical constituents from its bark are octacosane, β-amyrin, β-sitosterol, triterpenoids and sterols [6,14].

1.2 Cordia dichotoma Linn

*Cordia dichotoma* Linn (CD) belonging to the family Boraginaceae is a small to moderate-sized deciduous tree with a short bole and spreading crown widely distributed in India and Srilanka [15]. It is commonly named as Indian cherry (English), Lasura/Bhokar/Borla (Hindi), Vadgundo/Gunda (Gujarati). The various parts of the plant viz., stem bark and leaves are practiced in various indigenous systems of medicine viz., Ayurveda and Unani and popular among the various ethnic groups in India for the cure of variety of ailments as an astringent, anthelmentic, diuretic, demulcent, anti-diabetic and expectorant. Its leaves are traditionally used for the treatment of jaundice at Dandakaranya area, Andhra Pradesh in India. It is reported to have antioxidant, juvenomimetic, antifertility, anti-inflammatory and various other
pharmacological activities [16-18]. Carotenoids are mainly present in their leaves which have potent antioxidant activity [19].

1.3 Standardization of the crude drugs
Natural remedies from medicinal plants are found to be safe and effective. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [20]. Standardization of the plant materials is the need of the day. Monographs on these plant materials contained in several pharmacopoeias describe only the physicochemical parameters [21]. World Health Organization (WHO) has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [22]. High Performance Thin-Layer Chromatography (HPTLC) is a valuable tool for reliable identification, authentification and standardization of herbal drugs. It can provide chromatographic fingerprints that can be visualized and stored as electronic images [23]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to Indian Traditional Medicine and Chinese Traditional Herbal Medicine. Maintaining quality standards of the herbal drugs is the need of today due to their increasing demands.

1.4 Anticancer activity of the extracts
Cancer is one of the leading causes of death [24]. Chemotherapy is an important option for the management of cancer in the clinical settings apart from the utility of surgical operations and irradiation. Medicinal plants are one of the major sources of chemotherapy drugs in modern as well as traditional medicine throughout the world [25]. Phytochemicals or extracts from them have positive effects against cancer, compared with chemotherapy or recent hormonal treatments [26]. Phenolics like coumarins, flavonoids, stilbenes, tannins, lignans and lignins are among the most widely occurring
secondary metabolites in the medicinal plants. These phenolics are known to have anticancer activity on various cancer cell lines and induce apoptosis [27].

At present MTT (Methyl-Thiazolyl-Tetrazolium) assay is widely used for assessment of cell viability and proliferation studies [28]. A yellowish aqueous solution of MTT, on reduction in the cytoplasm by dehydrogenases and reducing agents like NADH, yields a lipid soluble purple colored MTT formazan [29]. It is currently thought that the amount of formazan is directly proportional to the number of viable cells [30].

The cells in which the DNA or other components are irreversibly damaged by various causes under normal conditions undergo programmed cell death called as apoptosis. These cells undergo serial structural and molecular changes during the process of apoptosis characterized by plasma membrane blebbing, chromatin compaction, DNA fragmentation, cell shrinkage and collapse of the cell into small intact fragments called as apoptotic bodies [31]. DAPI (4’,6-diamidino-2-phenylindole), a DNA-specific dye that displays a blue fluorescence, can pass through intact and living cell membrane but apoptosis increases cell membrane permeability and its uptake leaving a stronger blue fluorescence [32].

The disruption of active mitochondria is a distinctive feature of the early stages of apoptosis. It includes changes in the mitochondrial membrane potential (MMP) and redox potential [33]. A dual-emission potential-sensitive fluoro-probe JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide) is a mitochondrion-selective dye. In normal cells, due to high MMP (polarized mitochondria), the dye concentrates in the mitochondrial matrix and it forms red fluorescent aggregates (J-aggregates). Any event that dissipates the MMP (depolarized mitochondria) prevents the accumulation of the JC-1 dye in the mitochondria and thus, the dye is
dispersed throughout the entire cell leading to a shift from red fluorescence (J-aggregates, \( \lambda_{\text{max}} \approx 590 \text{ nm} \)) to green fluorescence (JC-1 monomers, \( \lambda_{\text{max}} \approx 529 \text{ nm} \)) \[34\]. A decrease in red/green ratio is indicative of apoptosis.

Many stimuli such as anticancer drugs prompt cells to produce reactive oxygen species (ROS) \[35,36\]. These ROSs induce apoptosis \[37\]. DCFH-DA (2,7-dichlorodihydrofluorescein diacetate) can pass cell membranes and is cleaved by intracellular esterases to DCFH and thereby trapped within the cells. A variety of ROSs oxidize DCFH to the fluorescent DCF (2,7-dichlorofluorescein) resulting in fluorescence \[38\].

Phenolics and carotenoids are mainly present in the leaves of \textit{Cordia dichotoma} which have potent antioxidant activity and can show anticancer activity too \[27,39\].

1.5 \textbf{Antioxidant activity of the extracts}

Oxidative stress initiated by ROS such as nitric oxide (NO), superoxide anion radical (\( O_2^{-} \)), peroxo radical (\( \text{ROO}^{\cdot} \)) and hydroxy radical (\( \text{\cdot} \text{OH} \)) has a major role in the pathogenesis of several diseases such as liver cirrhosis, atherosclerosis including cancer \[40\]. Deficient DNA-repair or DNA damage by it is reported to have a prognostic or etiological role in cancer, the disease which is now one of the leading causes of death \[24,41\]. The phytochemicals such as flavonoids, alkaloids and terpenes have received major attention in recent years due to their various pharmacological properties including cancer chemopreventive and cytotoxic effects. Phenolics and flavonoids are among the most widely occurring phytochemicals having antioxidant activity. They scavenge the ROS due to their antioxidant property and prevent carcinogenesis. They are known to have anticarcinogenic or anticancer properties and induce programmed cell death called as apoptosis in various cancer cell lines due to their pro-oxidant property \[42-44\].
The present study was thus designed to set standard phytochemical and analytical profiles of the BR bark and CD leaves. Phenolic contents, flavonoid contents, anticancer and antioxidant activities of methanolic extracts of Bauhinia racemosa bark (MEBR) and Cordia dichotoma leaves (MECD) were evaluated too.
References


