CHAPTER 2
LITERATURE REVIEW

2.1 Bauhinia racemosa Lam

2.1.1 Background

Bauhinia racemosa Lam, belonging to the family Caesalpiniaceae is a small deciduous tree used in the indigenous system of medicine [1,2]. Its synonym is Bauhinia vahlii Wight & Arn [3]. It is named as Mountain ebony in English, Kachnal/Kanchanara/Sonpatta in Hindi, Gul-e-anehnal in Urdu and Sona/Sonpatta or Apta in Marathi [4]. It is of religious significance. It is being considered to be as valuable as gold for its medicinal values. Therefore, there is a ritual of exchanging Sonpatta (leaf of gold) leaves during Dussehra in India. It teaches to enjoy the joy of giving [5,6]. It is having antifilarial activity, abortifacient, anti-anxiety, anthelmintic, antimicrobial, antihistaminic, anti-inflammatory, analgesic, and antipyretic effects, antimalarial, anti-oxidant, anticarcinogenic, antitumor, anti-ulcerogenic, hepatoprotective, and various other pharmacological activities. It is traditionally used in for the treatment of various ailments like diarrhoea, dysentery, headache, jaundice, etc.

Flowers are white and appear in axillary or terminal racemes. Flowering takes place in March to June. Fruit is a pod, oblong, compressed, often twisted and dark green. Fruiting takes place through the season. Bark is rough and black. Leaves are orbicular, bifoliate and alternate distichous with entire margins. Their apices are mucronate and bases are chordate (Figure 2.1).

Bauhinia (Caesalpiniaceae) is mostly present in lowland and drier forest types of northwestern South America, extending to Brazil and Argentina, recorded in cerrado [7]. B. racemosa is very common in foothills upto 1000 m in India and Srilanka. It is inhabited in semi-arid region of
Rajasthan in India along with various fodder trees like *Prosopis cineraria*, *Acacia nilotica*, *Albezia lebbek*, *Ailanthes excelsa*, *Azadirachta indica* [8]. It is a tropical dry thorn found in transects in the Nilgiri landscape of Western Ghats in India [9,10].

Mean wood specific gravity (WSG, g cm\(^{-3}\)), height range (m) and mean basal area (BA, m\(^2\) ha\(^{-1}\)), carbon density (CD, kg-C ha\(^{-1}\)), carbon accumulation rate per unit basal area (CA\(_b\), kg-C m\(^{-2}\) yr\(^{-1}\)) and carbon accumulation rate per unit ground area (CA\(_a\), kg-C ha\(^{-1}\) yr\(^{-1}\)) of the tree *B. racemosa* on Hathinala site of tropical dry forest were found to be 0.57, 3.4-4.5, 0.08, 430, 523, 41.8 respectively [11]. Density, basal area (m\(^2\) ha\(^{-1}\)) and importance value index (IVI) of woody species *B. racemosa* in the valley of the Slopka forest were 10±0, 0.06±0.06 and 1.9 respectively. It’s IVI on different aspects of hill slopes (valley, east-facing slope and south-facing slope) in the undisturbed Slopka forest (the average of 30 quadrats of 100 m\(^2\)) were 1.96, 2.99 and 3.18 respectively. Density, basal area (cm\(^2\) 100 m\(^2\)) and IVI in relation to human disturbance in the Sariska Tiger Project (based on 130 quadrats of 100 m\(^2\) each in each of the study site Slopka forest-undisturbed, Kalighati forest-protected and Bharthari forest-partially disturbed) were 0.30, 18.96, 1.63; 0.13, 8.06, 2.92 and 0.07, 1.32, 1.23 respectively [12].

It is found in highly disturbed stand of dry deciduous forest of Western Ghats, India too along with *Albizia amara* and *Pleiospemium alatum* [13]. It is one of the many new seedlings emerging indicating better regeneration potential in the low lantana cover site of Vindhyan tropical dry deciduous forest of India [14]. Its seeds, in Vindhyan hill tract in the Sonebhadra district of Uttar Pradesh, India have the weight of 0.124 g/seed and are relatively shade-intolerant [15]. It is propagated easily from seed and can grow in poor and even very harsh climatic conditions [5].
2.1.2 Traditional Uses

*Bauhinia racemosa* is one of the plants commonly used by Ayurvedic doctors for the prevention and treatment of cancer [4]. Traditionally, mixture of its bark and *Bridelia retusa* is given orally to women to develop sterility in tribal area of southern Rajasthan and act as contraceptive [16,17]. The folk people and the locales of the Shiwalik Himalaya of Uttarakhand utilize it in the form of different products as ethnomedicine, fodder, food, fibre etc. It is commercially exploited by drug dealers and it come in threatened categories. Therefore, there is an urgent need of its conservation for sustainable development [18]. This is one of the plants eaten by the Rhesus monkey (*Macaca mulatta*) and Hanuman langur (*Presbytis entellus*) in Himachal Pradesh [19].

Its stem bark is kept in house as snake repellent. Leaves are chewed by two people and air is blown in patient’s ear, after which a glass of water is given to drink to cure scorpion bite [20]. Pounded bark powder of Kachnal
Tree, in 10 g quantity, or decoction with water in 20 ml quantity, is used twice per day for chronic dysentery and diarrhoea in the tribals of Sonaghati of Sonbhadra district, Uttar Pradesh, India [21,22]. It is used in headache also [22].

Whole plant juices either wild or cultivated, administered with vegetable oil orally is used in leucorrhoea by 5% people of Sariska region of Rajasthan [23]. For the treatment of jaundice, water extracts of its bark, leaves and roots are taken two times daily after meal for 2-4 weeks in the region of Jalgaon, Dhuleand, Nandurbar districts of Maharashtra and also the stem bark of *Woodfordia fructicosa* (L.) Kurz ground with its bark and *Oroxylum indicum* are taken as poultice administered in two spoonfuls banana fruit twice a day for 5-7 days in Adilabad district of Andhra Pradesh [24]. Leaves of *B. racemosa*, *Aloe vera* and *P. murex* crushed together and mixed with water given to animals three times a day can relief food poisoning in cattle [25].

2.1.3 Phytochemistry

Phytochemical screening of the plant leaves reveals the presence of carbohydrates [26-29], alkaloids [26-29], steroids [26,28,29], glycosides [28,30], tannins [26-30], saponins [27,28,30], phenolic compounds [27,28,30], flavonoids [26,27,28,30,31], protein [27,30], oil and fats [27,28]. Bioassay guided fractionation of ethanolic extract of the leaves led to the isolation of galactolipid and catechin class of the compounds (A-G) (Figure 2.2) from the most active *n*-butanol fraction [31]. The screening of the heartwood reveals the presence of polycyclic phenolics, racemosol and pacharin [31-33]. The screening of the stem bark reveals the presence of triterpenoids and sterols [31]. Phytochemical screening of the seed reveals the presence of palmitic and stearic acids [34]. It also contains phenolics, flavonoids, saponins, glycosides, tannins and proteins [30].
De-\textit{O}-methylracemosol (\textbf{Figure 2.3}) was obtained from the column chromatographic separation of a benzene extract of the roots, recrystallized from benzene as brown crystals [35]. Racemosol and de-\textit{O}-methylracemosol were synthesized by Sae-Lao et al (2006) [36]. The key steps involved were the lateral lithiation reaction of \textit{O}-methyl toluate and the pyran formation via a tandem demethylation-cyclization reaction. The structure of pacharin, previously isolated from the heartwood of \textit{B. racemosa} Lamk has been established as 1,7-dihydroxy-3-methoxy-2-methyl-dibenzo(2,3-6,7)oxepin by a study of its chemical and spectroscopic properties, including X-ray analysis [33,37]. Resveratrol (trans-3,5,4’-trihydroxystilbene), a phytoalexin is obtained from several plants and also from \textit{B. racemosa} [38].

Crude fibre, fat and carbohydrate contents were found to be 1.91%, 15.53% and 36.12% respectively in \textit{B. racemosa} seeds. Its saponification and acid values were found to be 343.72, 5.64 [39]. Crude proteins, crude lipids, ash and nitrogen free extractives constitute 19.84%, 9.52%, 3.31%, and 60.65%, respectively in Ayyanarkoil Forest germplasm of the tribal pulse, \textit{B. racemosa} Lamk; whereas, in Mundanthurai Wildlife Sanctuary germplasm they constitute 19.31%, 8.94%, 3.81% and 61.30% respectively. The caloric values were found to be 407.64 KCal (Ayyanarkoil Forest germplasm) and 402.90 KCal (Mundanthurai Wildlife Sanctuary germplasm). Essential amino acids like isoleucine, tyrosine, phenylalanine and lysine were found to be high in the seed proteins of both the germplasms. The fatty acids, palmitic, oleic and linoleic acids were found to be relatively higher in the seed lipids of both the germplasms. Both the germplasms seemed to be a rich source of calcium, potassium, magnesium, zinc, manganese and iron. Antinutritional substances like total free phenols, tannins, L-DOPA and phytohaemagglutinating activity were also investigated [40].
Dahikar et al. (2011) showed that petroleum ether extract of B. racemosa (PEEBR) had only alkaloids and steroids occurring in high concentration [26]. The chloroform extract (CEBR) and ethyl acetate extract (EAEBR) of B. racemosa had similar photochemical constituents, alkaloids and steroids in higher concentration, carbohydrates, flavonoids, glycosides and saponins. Methanol extract of B. racemosa (MEBR) had alkaloids, steroids, flavonoids, saponins and tannins, but no protein. Kumar et al. (2010) found the presence of protein, oil and fats, phenolic compounds, flavonoids, alkaloids, saponins, tannins and carbohydrates as major phytochemical groups in crude MEBR and aqueous extract of B. racemosa (AEBR) leaf [27]. Manohar et al. (2011) found the presence of carbohydrates, glycosides, alkaloids, phytosterols, saponins, tannins, phenolics, flavanoids, fixed oils and fats as phytochemicals in crude MEBR and AEBR leaf [28]. Anjaneyulu et al. (1986) reported a new tetracyclic phenol, racemosol from the hexane extract (HEBR) of the heart-wood of B. racemosa Lamk [32]. Racemosol was crystallized as deep red prisms from chloroform. It is soluble in alkali giving yellow colour which reverts to the red colour on acidification. Racemosol diacetate and racemosol dimethylether was synthesized by him. Prakash and Khosa (1976) reported octacosane, β-amyrin and β-sitosterol in PEEBR stem bark from column chromatographic resolution over Brockmann alumina [41]. Elution of the column with petroleum ether (60°-80°) furnished octacosane, with benzene β-amyrin and with benzene:chloroform (3:1) β-sitosterol.
Figure 2.2: Isolated phytoconstituents (A-H) from the leaves of Bauhinia racemosa [Galactolipids: (2S)-1,2-di-O-linolenoyl-3-O-α-galactopyranosyl(1→6)-O-β-galactopyranosyl glycerol (A), (2S)-1-O-
linolenoyl-2-O-palmitoyl-3-O-α-galactopyranosyl(1→6)-O-β-galactopyranosyl glycerol (B), (2S)-1-O-oleoyl-2-O-palmitoyl-3-O-α-galactopyranosyl(1→6)-O-β-galactopyranosyl glycerol (C); Catechins: (−)-epiafzelechin (D), (−)-epicatechin (F), (−)-catechin (G), protocatechuic acid (E); and Long chain fatty acid alcohol: octacosanol (H). 

Figure 2.3: Other isolated phytoconstituents from Bauhinia racemosa [pacharin (A), racemosol (B), de-O-methylracemosol (C), resveratrol (D), octacosane (E), β-amyрин (F), β-sitosterol (G)].

2.1.4 Pharmacology

Bauhinia racemosa is having antifilarial activity [31], abortifacient [42], anti-anxiety [43], anthelmintic [44], antimicrobial [26,27,28,29,45,46], antihistaminic [47], anti-inflammatory [48], analgesic [48-50], and antipyretic effects [48,51], antimalarial [52], anti-oxidant [46,53,54,55], anticarcinogenic [56], antitumor [54], anti-ulcerogenic [57,58], hepatoprotective [55,59], and various other pharmacological activities [60].
It does not induce any toxic effects [61]. Its seed mucilage is used as a binder for pharmaceutical dosage forms. It was found to be useful for the preparation of uncoated tablet dosage form in 8% w/w binder concentration [62].

2.1.4.1 Anti-anxiety activity

The extract MEBR, administered orally (po) in two different doses of 150 mg/kg and 300 mg/kg body weight (b wt) in adult male Swiss albino mice, was able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze. It also increased the time spent by mice in the illuminated side of the light-dark test, showed significant increase in nose poking and decrease locomotion in hole board test, as well as caused significant reduction in freezing (immobility with rigid body posture) time in comparison with control animals. These effects were comparable to that of the diazepam (2.0 mg/kg b wt, po) indicating that MEBR is an effective anxiolytic agent [43].

2.1.4.2 Antifilarial activity

The n-butanol fraction of ethanolic extract of B. racemosa (EEBR) showed promising adulticidal (IC$_{50}$ 5.46 mg/mL) and microfilaricidal (IC$_{50}$ 4.89 mg/ml) activities with Minimum Inhibitory Concentration (MIC) of 15.6 mg/ml. Among the active galactolipids, 1 emerged as the lead molecule which was active on both forms of lymphatic filarial parasite, Brugia malayi. It was found to be better than the standard drug ivermectin and diethylcarbamazine (DEC) in terms of dose and efficacy [31].

2.1.4.3 Anthelmintic activity

AEBR, EEBR and PEEBR whole plant of B. racemosa have anthelmintic activity. The crude EEBR significantly demonstrated paralysis and also caused death of earthworm (Pheretima posthuma) in dose dependent manner
(50, 75 and 100 mg/ml) in *in vitro* study, while AEBR and PEEBR showed weak anthelmintic effect compared to albendazole [44].

### 2.1.4.4 Antihistaminic activity

EEBR leaves inhibited clonidine-induced catalepsy in male Swiss albino mice at a dose of 50 mg/kg *b wt*, administered intraperitoneally (*ip*). But, there was no effect on haloperidol-induced catalepsy suggesting that the inhibition is through an antihistaminic action and that there is no role of dopamine. It has role in the treatment of asthma [47].

### 2.1.4.5 Anti-inflammatory, analgesic and antipyretic effects

MEBR possesses potent anti-inflammatory, analgesic and antipyretic activities. In acute phase of inflammation, a maximum inhibition of 44.9, 43.2, 44.8 and 45.9% (*p*<0.001) was noted at the dose of 200 mg/kg *b wt* of Wistar albino rats after 3 h of treatment with MEBR in carrageenan, dextran, histamine and serotonin-induced paw oedema, respectively. Administration of MEBR (200 mg/kg *b wt*) and indomethacin (10 mg/kg *b wt*) significantly (*p*<0.05) decreased the formation of granuloma tissue induced by cotton pellet method at a rate of 50.4 and 56.2%, respectively. The extract also inhibited peritoneal leukocyte migration in mice. The MEBR also produced significant (*p*<0.01) analgesic activity in mice in the models, acetic acid-induced writhing and hotplate tests. Further, the MEBR potentiated the morphine- and aspirin-induced analgesic action. Treatment with MEBR showed a significant (*p*<0.01) dose-dependent reduction in yeast-induced hyperpyrexia in rats [48].

AEBR stem bark at the dose of 200 mg/kg *b wt* produced significant analgesic activity by ‘Tail Immersion Method’ in Wistar rats whereas 100mg/kg dose did not produce significant results when compared with control receiving only normal saline (*p*<0.01). EEBR produced significant results at both the doses (*p*<0.01) [49].
EEBR stem bark shows marked antipyretic activity in a dose dependent manner in healthy Wistar albino rats with 50% sex ratio where the pyrexia was induced by injecting a suspension of 15% of brewer’s yeast and 2% gum acacia in normal saline subcutaneously below the nape of neck in the volume of 1 ml/100 gm of animal weight. The temperature was brought back to normal after 4 hrs of post administration of extracts. AEER in a dose of 200 mg/kg \( b \text{ wt} \) and EEBR in the doses of 100 mg/kg and 200 mg/kg \( b \text{ wt} \) was found to have significant effect and was found significant at 5% level of significance [51].

### 2.1.4.6 Antimalarial activity

Racemosol and de-\( O \)-methylracemosol exhibited cytotoxicity against KB cell line (EC\(_{50}\) at 15.0 \( \mu \text{g/ml} \) and 5.6 \( \mu \text{g/ml} \), respectively) and BC cell line (EC\(_{50}\) at 6.1 \( \mu \text{g/ml} \) and 3.6 \( \mu \text{g/ml} \), respectively) exhibiting moderate antimalarial activity against parasite \( \text{Plasmodium falciparum} \) (EC\(_{50}\) at 0.9 \( \mu \text{g/ml} \) and 2 \( \mu \text{g/ml} \), respectively) [52].

### 2.1.4.7 Antimicrobial activity

In an attempt to develop a new pharmaceutical drug from natural origin for prevention of enteric infection, antibacterial activity of various extract of the leaves was carried out by disc diffusion method against certain enteric bacterial pathogens such as \( \text{E. coli, S. aureus, Klebsiella pneumonia, Enterobacter aerogenes, P. aeruginosa, Salmonella typhimurium, S. typhi, Staphylococcus epidermidis} \) and \( \text{Proteus vulgaris} \) and found that MEBR had wide range of antibacterial activity against enteric bacterial pathogens than PEEBR, where as EAEBR was of slightly higher antibacterial activity than CEBR [26].

MEBR leaves showed significantly higher inhibitory effect compared to AEER on tested organisms. The MEBR showed a broad spectrum of antimicrobial activity in agar well diffusion method as it inhibited Gram
negative bacteria (*E. coli, Micrococcus luteus* and *P. aeruginosa*), Gram positive bacteria (*Bacillus subtilis*) on Mueller hinton agar and fungi (*Candida albicans* and *A. niger*) on Sabouraud dextrose agar. Both the extracts showed maximum relative percentage inhibition against *A. niger*. MIC values determined by modified agar well diffusion method for MEBR varied from 1.5-25 mg/ml [27].

The antimicrobial studies of AEBR and MEBR leaves were carried out by cup-plate agar diffusion method against bacterial pathogens *B. subtilies, S. typhi, S. aureus* (zone of inhibition of MEBR: 6 mm, 6 mm, 7 mm respectively and that of AEBR: 4 mm, 4 mm, 5 mm respectively). It concluded that MEBR was having good antimicrobial activity [28]. MEBR bark had wide range of antimicrobial activity against enteric bacterial pathogens such as *E. coli, S. aureus, B. subtilis, P. aeruginosa* and fungi *A. niger and C. albicans* than EEBR, where as EEBR had slightly higher antibacterial activity than AEBR in agar well diffusion method [29].

As compared to HEBR, the MEBR extracts of all the examined sixteen plants of the family Caesalpiniaaceae, collected around Karachi, Pakistan, showed stronger growth inhibitions against both bacteria and fungi. HEBR showed antibacterial activity against *Corynebacterium diptheriae; Escherichia coli; Salmonella typhi; Staphylococcus aureus; Staphylococcus pyogenes* while MEBR against *Bacillus cereus; Shigella sonii; Pseudomonas aeruginosa; S. typhi; Shigella boydii; S. aureus; S. pyogenes*. HEBR also showed antifungal activity against *Trichophyton longifuses; Pseudallescheria boydii; Aspergillus niger; Microsporum canis* while MEBR against *T. longifuses; P. boydii; M. canis; Trichophyton mentagrophytes; Trichophyton simii; Fusarium solani; Macrophomina phaseolina; Trichophyton schoenleinii* [43].
The anti-oxidant activity of the MEBR bark increased in a concentration dependent manner. About 50, 100, 250 and 500 µg/ml MEBR inhibited the peroxidation of a linoleic acid emulsion by 62.43, 67.21, 71.04 and 76.83%, respectively. Similarly, the effect of MEBR on reducing power increased in a concentration-dependent manner. The IC\(_{50}\) values were found to be 152.29 µg/ml, 78.34 µg/ml and more than 1000 µg/ml for DPPH radical-scavenging, nitric oxide-radical scavenging and hydroxyl radical-scavenging assays, respectively. MEBR scavenged also the superoxide generated by the PMS/NADH-NBT system. Total phenolic content was also determined and 64.7 µg pyrocatechol phenol equivalent was detected in MEBR (1 mg). MEBR showed broad-spectrum antimicrobial activity determined by disc diffusion against all tested microorganisms; four Gram positive bacteria \textit{S. aureus}, \textit{Streptococcus pneumonia}, \textit{M. luteus}, \textit{S. epidermidis}, five Gram negative bacteria \textit{P. aeruginosa}, \textit{E. coli}, \textit{S. typhi}, \textit{Shigella dysenteriae}, \textit{Vibrio cholerae} and four fungal species \textit{C. albicans}, \textit{A. niger}, \textit{Aspergillus flavus}, and \textit{Alternaria solani}. The results obtained indicate that MEBR can be a potential source of natural antioxidant and antimicrobial agents [46].

### 2.1.4.8 Anti-oxidant activity

AEBR leaves showed good anti-oxidant activity in different systems of assays. IC\(_{50}\) values found were 1739, 536, 216 and 797 µg/ml for hydroxyl radical-scavenging, superoxide radical-scavenging, DPPH radical-scavenging and lipid peroxidation preventive activities, respectively. Total antioxidant capacity was found to be 16.5 and 58.1 gallic acid equivalent, GAE and amino acid equivalent, AAE (µg/mg plant material) respectively. Phenol and flavonoid content was found to be 150 GAE and 13 Catechin Equivalent (µg/mg plant material) respectively [53].
2.1.4.9  *Antitumor and anticarcinogenic activity*

Administration of MEBR bark was able to suppress neoplastic nodule development/hepatocellular lesion formation in *N*-nitrosodiethylamine induced hepatocarcinogenesis in male Wistar albino rats. The extract treatment increased the level of antioxidants vitamin C, vitamin E, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), and dramatically decreased lipid peroxidation levels indicated by low level of malondialdehyde (MDA). MEBR also produced a protective effect by decreasing the levels of serum enzymes (SGOT, SGPT and ALP) and bilirubin, and increasing the protein and uric acid levels. It suggested that MEBR exert chemopreventive effects by suppressing nodule development and decreasing lipid peroxidation and enhancing the levels of antioxidants in NDEA carcinogenesis by reducing the formation of free radicals [56].

The MEBR bark (at the doses of 50, 100, and 200 mg/kg bw) showed decrease in transplantable murine tumor volume, packed cell volume and viable cell count, and increase in nonviable cell count and mean survival time thereby increasing life span of Ehrlich Ascites Carcinoma (EAC) tumor bearing mice. Hematological profile (hemoglobin content, red blood cell and white blood cell counts) reverted to more or less normal levels in extract treated mice. The level of lipid peroxidation was decreased and that of GSH, SOD and CAT was increased. Thus, MEBR bark exhibits antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice. Gupta M et al proposed that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, etc, present in MEBR bark are responsible for its potent antitumor activity [54].
2.1.4.10 Anti-ulcerogenic activity

The effects of MEBR (flower buds) on the volume of gastric juice secreted, acid output, peptic activity, mucin activity and curative ratio were recorded in aspirin-induced gastric ulcers in Sprague-Dawley rats. It showed significant decrease in ulcer index [57]. AEBR dried fruit powder (200 mg/kg b wt) and EEBR (100 mg/kg and 200 mg/kg b wt) produced antiulcer activity in Wistar albino rats when the treated rats were administered paracetamol at a dose rate of 200 mg/kg b wt orally after one hour [58]. After 24 h, the number of ulcers, ulcer score, percent incidence, ulcer index and healing index were recorded as anti-ulcer parameters.

2.1.4.11 Hepatoprotective activity

The MEBR bark at the doses of 50, 100 and 200 mg/kg and silymarin at 25 mg/kg b wt produced significant ($p<0.05$) hepatoprotective effect by decreasing the activity of serum enzymes (serum transaminases; SGOT, SGPT and ALP), bilirubin and lipid peroxidation and significantly increasing the levels of GSH, SOD, CAT and protein in a dose dependant manner in different groups of Wistar albino rats administered with paracetamol (500 mg/kg, po once in a day for 7 days) and carbon tetrachloride (30% CCl$_4$, 1 ml/kg b wt in liquid paraffin 3 doses ip at 72 h interval). MEBR also showed antioxidant effects on FeCl$_2$-ascorbate induced lipid peroxidation in rat liver homogenate and on superoxide scavenging activity. From these results, it was suggested that MEBR could protect the liver cells from paracetamol and CCl$_4$-induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from paracetamol or CCl$_4$ [55].

*B. racemosa* stem bark was found effective as hepatoprotective, through *in vivo* and histopathological studies of paraffin sections of Male Wistar albino rat’s liver and biochemical parameters. The animals treated
with EAEBR exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. The hepatoprotective effect of EAEBR was comparable to that of silymarin, a standard hepatoprotective agent [59].

2.1.4.12 Toxicological effects

MEBR bark administered ip at the doses of 100 and 200 mg/kg b wt twice a week for thirteen weeks to Swiss albino mice did not induce any toxic effects. Adverse effect was noted at the dose of 400 mg/kg b wt. Its administration at the dose of 400 mg/kg b wt significantly elevated the levels of bilirubin, serum enzymes (SGOT, SGPT and ALP) and altered the level of urea, uric acid, creatinine, cholesterol, glucose and hematological parameters (hemoglobin content, total count of RBC and WBC, differential count of leucocytes such as neutrophil, lymphocyte, monocyte, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count) [61].

2.1.5 Marketed Formulation

Masanumas2 is a tablet formulation for the second month of pregnancy. Each coated tablet contains Ashmantak (Bauhinia racemosa) bark extract, Black Sesame (Sesumum indicum) seed extract, Manjishtha (Rubia cordifolia) stem extract and Shatavari (Asparagus recemosus) root extract 60 mg each. Each pack contains 120 tabs. Dose: 2 tablets with lunch and 2 tablets with dinner. Indications: Habitual abortions, repeated abortions, high value pregnancies, high risk pregnancies [63].

The extensive literature survey revealed B. racemosa to be a valuable and important medicinal plant used for the treatment of several ailments like headache, fever, skin and blood diseases, jaundice, chronic dysentery, diarrhoea, and leucorrhoea, infection of malaria, boil, glandular swelling, tumors and cancer. It is also used to cure scorpion bite, to relief food
poisoning in cattle and as contraceptive by women. Pharmacological studies carried out on the fresh plant materials, crude extracts and isolated active principles of *B. racemosa* provide a support for its numerous traditional uses. Recent studies have been focused on evaluating the abortifacient activity, anthelmintic, anti-anxiety, antimicrobial, antifilarial, anticarcinogenic, anti-inflammatory, analgesic and antipyretic effects, hepatoprotective, antitumor, anti-ulcerogenic, antihistaminic and antimalarial activities. Most of the mentioned pharmacological studies were aimed on validating its traditional uses. The different parts of *B. racemosa* have been employed for the treatment of various ailments. Most of the pharmacological studies that have been carried out on *B. racemosa* were conducted using uncharacterized crude extracts. Thus, it is difficult to reproduce the results of these studies and point the bioactive principles. Hence, there is a need of phytochemical standardization and bioactivity-guided identification of bioactive metabolites. Phytochemical research carried out on *B. racemosa* had led to the isolation of some active principles. However, the vast traditional use and proven pharmacological activities of *B. racemosa* indicates that an immense scope still exists for its phytochemical exploration. The outcome of such phytochemical study may further expand its existing therapeutic potential. The outcome of the future research will provide a convincing support for the future clinical use of *B. racemosa* in modern medicine.
2.2 *Cordia dichotoma* Linn

2.2.1 Background

*Cordia dichotoma* Linn belonging to family Boraginaceae is a 3-4 m tall tree with 2-5 cm long petiole, 6-13×4-9 cm leaf blade ovate to elliptic, sparsely pubescent or glabrous, margin usually subundulate to undulate dentate which grows in Sri Lanka, India and other warmer countries (Figure 2.4) [64]. It is commonly known as Indian cherry in English and Lasora in Hindi. Its medicinal properties is known since long time and traditionally used to cure several ailments. Its fruits are used as expectorant, astringent, cooling, emollient, purgative and anthelmintic [65]. Anti-inflammatory, analgesic, hepatoprotective and several other activities have also been reported from the plant [66,67]. Instant Joshanda Granules, polyherbal formulations, are extensively used by the people in India for the treatment of cough, sore throat, catarrh, common cold, respiratory distress, catarrh, flu like ailments, fevers of which *Cordia dichotoma* is the chief ingredient [68].

![Figure 2.4: Parts of Cordia dichotoma. (A) Whole plant; (B) twig with leaves and fruits; and (C) twig with flowers.](image)

2.2.2 Traditional Uses

Leaf paste of *Cordia dichotoma* is given to the animal with water for the treatment of diarrhea. Its leaves and fruits are given to the animal suffering from leucorrhoea [69]. The fruit of the plant is used as purgative, diuretic, antihelminthic, useful in dry cough, wound purification, for cure of mouth
ulcer, jaundice and increase male potency [70,71]. Leaves are used for headaches and ulcers including decoction for sore throat [72]. Bark is used in the treatment of ulcerative colitis and colic pain [70]. 100 g powder of stem bark is taken with a glass of water to cure menstrual disorders by Korku tribe of Amravati district of Maharashtra, India [73]. It is used as abortifacient in traditional/folkloric medicine [74]. The pickled fruit is eaten as an appetizer in certain areas of Taiwan. The indigestible seeds and sticky pulp of the fruit can form a phytobezoar if ingested excessively [75].

2.2.3 Phytochemistry

The dry powdered seeds of *Cordia dichotoma* were found to contain glycosides, alkaloids, carbohydrates, tannins and saponins [76]. Alkaloids, flavonoids, amino acids and proteins are present in the fruits of *Cordia dichotoma* [70,71]. Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of the ethanolic extract of leaves demonstrated the presence of common phytocompounds phenols, tannins and glycosides as major active constituents [77]. The antinutritional factors such as phytic acid (0.2±0.1%), tannin (1.386±0.0152%) and oxalic acid (2.133±0.2081%) were found to be present in the powdered wild edible fruits of the plant. Saponin was absent [78]. *Cordia dichotoma* bark is identified as botanical source of *Shleshmataka* in Ayurvedic pharmacopoeia [79]. Apigenin was isolated by column chromatography from methanol fraction of crude methanol extract of *C. dichotoma* bark [80]. Arabinoglucan was isolated from the fruits of *Cordia dichotoma* [81]. The phytoconstituents arabinoglucan, D-glucose and L-arabinose are present in fruits; linolinic acid in seed; and quercetin in leaves [82]. The structure and properties of the newly identified natural cellulose fabrics from the branches of the *Cordia dichotoma* indicated that they could be appropriate for blending and processing by biodegradable polymers to make green composites [83]. The
mucilage isolated from the aqueous filtrate of macerated seeds, whose pulp was previously removed, possess binding properties comparable to the starch. An increase in mucilage concentration led to decrease in friability and increase in disintegration time of the tablets [84].

2.2.4 Pharmacology

2.2.4.1 Analgesic, antibacterial and cytotoxic activities

The crude ethanol extract of leaves of *Cordia dichotoma* produced significant writhing inhibition in acetic acid induced writhing in mice at the oral dose of 500 mg/kg *b wt* (*p*<0.001) which was comparable to that of the standard drug diclofenac sodium at the dose of 25 mg/kg *b wt*. The extract showed significant zone of inhibition against both Gram negative and Gram positive bacteria *Streptococcus aureus, Streptococcus pyogenes, Vibrio cholerae, Streptococcus epidermis, Hafnia* and *Escherichia coli* in disc diffusion study which is comparable to that of Kanamycin (30 µg/ml). The extract also showed potent toxicity against the brine shrimp *Artemia salina* (*LC*$_{50}$: 20 µg/ml and *LC*$_{90}$: 180 µg/ml) in brine shrimp lethality bioassay [66].

2.2.4.2 Anthelmentic activity

Five concentrations (10, 25, 50, 75 and 100 mg/ml) of ethanolic and aqueous extracts prepared from pulp obtained after separation of seeds from fruits of *Cordia dichotoma* by Soxhlet extraction were studied for anthelmentic activity by using *Eudrilus euginiae* earthworms. Both ethanolic and aqueous extracts showed paralysis and death of worms in concentration dependent manner. Aqueous extract showed significant activity than ethanolic extract. The extracts also showed the presence of alkaloid, glycosides, saponins, flavonoid, triterpenoids, proteins, aminoacids and carbohydrates in preliminary phytochemical investigations [65].
2.2.4.3 Antibacterial activity

Antibacterial activity of alcoholic and aqueous extracts of thirty four medicinal plants including *Cordia dichotoma* were screened for potential antibacterial activity against six bacterial strains belonging to Enterobacteriaceae, viz. *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhimurium, Proteus mirabilis* and *Proteus vulgaris* by the agar well diffusion and disc diffusion methods. The ethanol extracts were more active than aqueous extracts for all the plants studied. Aqueous extract of *Cordia dichotoma* inhibited only to *Proteus mirabilis*, while ethanolic extract inhibited to *Klebsiella pneumonia* and *Proteus mirabilis* [85].

Antibacterial activity of *Cordia dichotoma* leaves extract studied by two different methods i.e. well diffusion and disc diffusion methods against procured strain of *E. coli* revealed that it possess antibacterial activity against *E. coli*. The activity of extract is due to the flavonoid active constituent. Phytochemical analysis showed positive result for tannins, protein and flavonoids [86].

Antibacterial activity of petroleum ether, chloroform, methanol and aqueous extracts of *Cordia dichotoma* ripened fruits against urinary tract pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* and *Staphylococcus aureus* using disc diffusion method was evaluated. Results of the study showed that the methanol extract exhibited better antibacterial activity against the bacterial strains as compared to other extracts [87].

2.2.4.4 Antioxidant activity or free radical scavenging activity

The methanolic extract of seeds and leaves of *Cordia dichotoma* demonstrated positive antioxidant activity in a concentration dependent manner during investigation of their free radical scavenging potential using in
vitro models viz. 1,1, diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide model. The IC\textsubscript{50} values for leaves were found 51.83 µg/ml and 55.73 µg/ml for DPPH and H\textsubscript{2}O\textsubscript{2} models respectively. The IC\textsubscript{50} values for seeds were found 57.22 µg/ml and 48.28 µg/ml for DPPH and H\textsubscript{2}O\textsubscript{2} models respectively. In both the models, the methanolic extract of the leaves exhibited low IC\textsubscript{50} values as compared to the seeds’ extracts. Thus, this activity was more pronounced in leaves as compared to seeds [88].

During nitroblue tetrazolium chloride (NBT) superoxide radical scavenging assay, maximum antioxidant activity of ethanol and acetone extracts of the fruits of the plant Cordia dichotoma was noticed to be 54.5 and 55.5% respectively. The inhibitory potential expressed in IC\textsubscript{50} values of acetone and ethanol extracts of C. dichotoma were found to be 131.0 and 149.0 µg/ml respectively [89].

The activity of peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT) was determined in the fruits. The SOD and CAT activities were increased in mature fruits than ripened fruits, whereas POX activity was found to be more in ripened fruits as compared to mature fruits [90].

C. dichotoma contains a considerable amount of phenols. The IC\textsubscript{50} value was found to be 28 µg/ml for the methanolic extract and 36 for butanolic extract in DPPH scavenging assay. The study revealed that the bark has significant radical scavenging activity [79].

2.2.4.5 Antidiabetic activity

Marles and Farnsworth (1995) provided information on more than 1200 species of plants reported to have been used to treat diabetes and/or investigated for antidiabetic activity, with a detailed review of representative plants and some of great diversity of plant constituents with hypoglycemic activity [91]. The aerial parts of Cordia dichotoma administered orally were devoid of normal antidiabetic activity.
The dose of 500 and 1000 mg/kg of aqueous leaves extract administered orally to alloxan induced and normoglycemic Wistar rats showed a significant ($p<0.5$) decrease in blood glucose levels after 4, 8 and 24 h. The dose of 1000 mg/kg of the extract in normoglycemic rats significantly ($p<0.05$) decreased the blood glucose levels at 8 and 24 h [92].

The methanol extract of fruits of *Cordia dichotoma* reduced the blood glucose level in glucose loaded animal and alloxan induced diabetic animal models when compared to diabetic control group and exerted significant hypoglycemic and antidiabetic activities compared to standard drug metformin. The extract also reduced the rate of body weight loss in normal and alloxan induced diabetic animals [93].

### 2.2.4.6 Antimicrobial activity

Alcohol is found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane [94].

Ethanolic extract of the leaves showed antimicrobial activity only against *S. aureus* (inhibition zone: 21-30 mm) and *S. dysenteriae* (inhibition zone: 10-20 mm) in the study antimicrobial activity against certain drug-resistant bacteria *Salmonella paratyphi*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Shigella dysenteriae* and a yeast *Candida albicans* of clinical origin. No correlation was observed between susceptibility of test strains with the extract and antibiotic resistance behavior of the microbial strains [77].

The acetone, chloroform, hexane, aqueous and ethanol extracts of the *C. dichotoma* leaves and the chloroform, aqueous and acetone extracts of the *C. dichotoma* stem bark did not show any antimicrobial activity against tested strains of bacteria and fungi determined by macrobroth dilution method on the concentration of 25 mg/ml to 0.10 mg/ml of the extracts. Only the hexane and ethanol extracts were active against only certain fungal strains [95].
The acetone and ethanol extracts of *Cordia dichotoma* fruits exhibited highest antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. Acetone extract showed maximum inhibitory zone of 19.1 mm whereas no inhibitory effect was observed for the aqueous extract [89].

Petroleum ether, benzene, chloroform, acetone, methanol and aqueous extracts of the plant were investigated for the antimicrobial efficacy by modified Kirby-Bauer disc diffusion method against the clinical isolates of oral cancer cases like the fungal pathogens *Aspergillus fumigates*, *Candida albicans* and bacterial pathogens *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*. Only *S. aureus* was inhibited significantly by the petroleum ether and acetone extracts (inhibition zone: 10 and 11.33 mm respectively; MIC: 250 and 125 µg/ml respectively) while only *S. epidermidis* by aqueous extract (inhibition zone: 10.33 mm; MIC: 125 µg/ml). Even, other isolates were not inhibited by the remaining extracts [96].

The methanol and butanol extracts of the bark showed remarkable zone of inhibition of bacterial growth and fungal growth comparable with that of standards drugs against the organisms tested *Escherichia coli*, *Pseudomonas aeruginosa*, *St. pyogenes*, *Staphylococcus aureus*, *Aspergillus niger*, *A.clavatus*, and *Candida albicans*. The activity of extracts increased linearly with increase in extract concentration [97].

### 2.2.4.7 Antiulcer activity

Ethyl acetate, butanol and butanone fractions of ethanolic fruit extract significantly decreased the volume of gastric secretion, free acidity, total acidity and ulcer index with respect to control during the study of anti-ulcer effect of fruit extracts (300 mg/kg *b wt*) in Wistar rats using pyloric ligation, aspirin and indomethacin induced ulcer models [98].
The aqueous extract of fresh ripened fruits was found to be more effective than alcoholic extract as compared to ranitidine in aspirin induced gastric ulcer model ($p<0.001$) as well as in pylorus ligation model in rats [99].

2.2.4.8 **Anti-inflammatory activity**

The ethanol and aqueous extracts of *Cordia dichotoma* seeds at a dose of 250 mg/kg and 500 mg/kg orally showed significant activity compared with the control and diclofenac sodium (10 mg/kg as standard) in dextran-induced paw oedema and carrageenan-induced paw oedema models in rats on different phases of acute inflammation [76].

Apigenin (5 mg/kg, *po*) showed significant healing and reduction in inflammatory enzymes myeloperoxidase (from 360±0.2 U/ml due to acetic acid induction to normal 222±22.5 U/ml due to treatment) and malondialdehyde (from 9.98±1.5 nmol/ml to 2.11±1.5 nmol/ml) when screened for ulcerative colitis induced by intrarectal administration of 150 µl, 5% acetic acid ($pH$ 2.5) 3 cm from the anal margin. It was concluded that apigenin from *C. dichotoma* bark may be responsible for the treatment of ulcerative colitis [80].

The methanol fraction of methanolic extract of *C. dichotoma* bark showed effective treatment of ulcerative colitis. It showed good healing and lower pathological scores in treated animals. It significantly reduced MDA and MPO levels in tissue and blood. It showed antioxidant potential and contains a high level of phenolics [100].

2.2.4.9 **Anti-cerebrovascular insufficiency activity**

Long term cerebral hypoperfusion in rats resulted in propensity towards listlessness and anxiety (elevated plus maze test and open field paradigm) accompanied by deficits in memory and learning (Morri’s water maze test) and tendency towards depression (Porsolt’s swim test). Gliosis, cellular edema, astrocytosis and inflammatory changes were observed in forebrain.
Treatment by *Cordia dichotoma* (250 mg/kg *po* for 28 days) alleviated these cognitive, behavioral and histopathological changes suggesting that *Cordia dichotoma* may be useful in cerebrovascular insufficiency conditions [101].

### 2.2.4.10 Angiotensin-converting enzyme inhibitory activity

The ethanolic extract of bark showed high ability to inhibit the angiotensin converting enzyme [102].

### 2.2.4.11 Diuretic activity

Petroleum ether, solvent ether and butanol fractions of alcoholic extract of the fruits 300 mg/kg of body weight were tested for diuretic activity in rats for total urine volume, urine concentration of Na\(^+\) and K\(^+\) and showed increase in cation excretion and urine volume [103].

### 2.2.4.12 Hepatoprotective activity

The methanolic extract of the leaves (300 mg/kg) significantly reduced the ALT (*p*<0.001), AST (*p*<0.001) and TBR levels (*p*<0.01) and at 500 mg/kg extract dose significantly reduced the AST ALT (*p*<0.001), (*p*<0.001), TBR (*p*<0.01) and lipid peroxide levels (*p*<0.05) in male Wistar rats with liver damage by carbon tetrachloride treatment [67].

### 2.2.4.13 Antifertility activity

The reversible nature of the developed phytopharmaceutical was studied by performing pharmacological analyses followed by chronic toxicity studies. The biochemical and histological estimations detected the reversible contraceptive potential of the drug after withdrawal. The observations also suggested that the developed phyto-pharmaceutical has potential antifertility activity with safety aspects [104].

### 2.2.4.14 Wound healing activity

Petroleum ether, solvent ether, ethyl acetate, butanol and butanone successive fractions of ethanolic extract of *Cordia dichotoma* fruits showed significant
(\(p<0.001\)) wound healing activity on excision, incision and dead space wound models on Wistar albino rats [105].

2.2.5 Marketed Formulation

Instant Joshanda Granules, polyherbal formulations, are extensively used by the people in India for the treatment of cough, sore throat, catarrh, common cold, respiratory distress, catarrh, flu like ailments, fevers of which *Cordia dichotoma* is the chief ingredient [68].
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