Review Paper

Plumbago zeylinica Linn. (Chitrak) - Review as Rasayan (Rejuvenator / Antiaging)

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ABSTRACT

The Ayurved cures the aging and its allied ill-effects by use of Rasayan (Rejuvenating / Antiaging) drugs. Chitrak (Plumbago zeylinica) is an age old Rasayan herb in traditional Ayurved. This herb has been well researched in recent times.

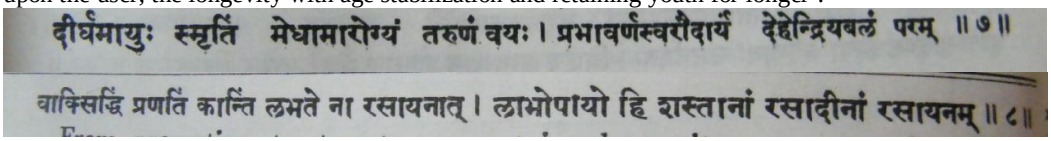
This Review Article researches the classic Ayurvedic Texts about its therapeutic benefits. Then this Review Article reviews the modern research on Chitrak to assess and validate the claims of Ayurvic Texts about its Rasayan benefits. It is evident from the review of the research that some aspects of Rasayan therapeutic benefits have not been focused yet for research like Adaptogen and Aphrodisiac. Similarly the research has just started in the immunomodulation and memory aspects and needs to be undertaken in detail. This comprehensive pharmacological screening will be very useful in future product development also, particularly for the life style diseases and disorders.

Key Words: Plumbago zeylinica, Chitrak, Anti aging, Rasayan, Immunomodulator, Anti cancer.

1. INTRODUCTION

1.1 RASAYAN

The Rasayan branch of Ayurveda deals specifically with and Rasayan herbs and formulations that bestows upon the user, the longevity with age stabilization and retaining youth for longer[1].

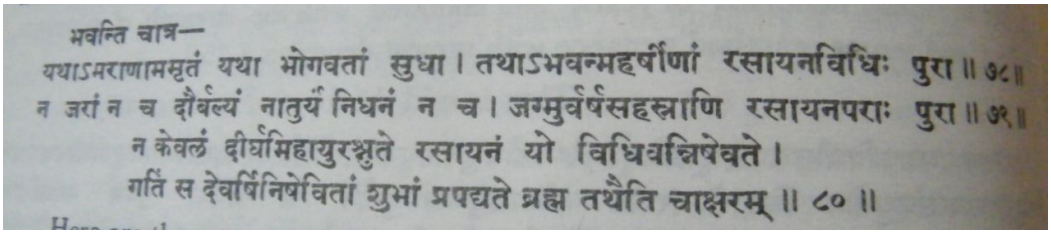

दीर्घमायुः स्मृतिं मेधामारोग्यं तरुणं वयः। प्रभावर्णस्वरौदार्यं देहेन्द्रियबलं परम् ॥ ७ ॥

वाक्सिद्धिं प्रणतिं कान्तिं लभते नरो रसायनात्। लाभोपायो हि शस्तानां रसादीनां रसायनम् ॥ ८ ॥

From the rasayan treatment, one attains longevity, memory, intelligence, freedom from disorders, youthful age, excellence of luster, complexion and voice, oratory, optimum strength of physique and sense organs, respectability and brilliance. It means the attaining the excellent Rasa etc.

These antiaging attributes will also incorporate being Adaptogen, Antioxidant, Anti cancer, Antiaging Aphrodisiac and Immunomodulator.

1.1.1 RASAYAN BENEFITS[2]


भवन्ति चात्र—

यथाऽमराणाममृतं यथा भोगवतां सुधा। तथाऽभवन्महर्षीणां रसायनविधिः पुरा ॥ ७८ ॥

न जरां न च दौर्बल्यं नातुर्यं निधनं न च। जग्मुर्वर्षसहस्राणि रसायनपराः पुरा ॥ ७९ ॥

न केवलं दीर्घमिहायुरश्नुते रसायनं यो विधिवन्निषेवते।

गतिं स देवर्षिनिषेवितां शुभां प्रपद्यते ब्रह्म तथैति चाक्षरम् ॥ ८० ॥

As was the nector to the gods and ambrosia for the serpents so was the Rasayan for the great sages in early times. The persons using Rasayan treatment in early ages lived very long life unaffected by old age, debility, illness untimely death.

One who uses the Rasayan treatment methodically attains not only long life but also the

1.2 Chitrak (Plumbago zeylinica)

Chitrak consists of dried mature root of Plumbago zeylanica Linn. (Fam.Plumbaginaceae), a large perennial sub-scandent shrub, found throughout India in wild state and occasionally cultivated in gardens.

1.2.1 Chitrak flowers: Chitrak (Plumbago zeylinica) is found with three different colored flowers viz. White, red and blue.

A. White flowered Chitrak

English : Lead war
Sanskrit : Agni, Vahni, Krishanu, Huashaa, Dahana, Hutabhuk

1. Bengali : Chita
2. Gujrati : Chitrakmula
3. Hindi : Chira, Chitra
4. Kannada : Chitramula, Vahni, Bilichitramoola
5. Kashmiri : Chitra, Shatranja
6. Malayalam : Vellakeduveli, Thumpokkoduveli
7. Marathi : Chitraka
8. Oriya : Chitamula, Chitoparu
9. Punjabi : Chitra
10. Tamil : Chitramoolam, Kodiveli
11. Telugu : Chitramulam

B. Red flowered Chitrak:

Colored Lead wart
Botanical name- Plumbago rosea Linn
Geographic distribution-Sikkim

Names in other languages

Hindi- Laal Cheeta, Laal chit-ur
Bengali- Laal chitta, Rakto chito
Marathi- Laal chitrak
Kannad- Chitramool
Telugu- Yerra chitramoolam
Therapeutic Effects- It is more effective than P. zeylinica (more so as Rasayan-rejuvenator)

C. Blue flowered chitrak-

Plumbago capensis Thumb
Geographic distribution-Africa
Therapeutic Effects- more effective than P. zeylinica and P. rosea (more so as rasayan)

1.2.2 Chitrak in classical Ayurvedic texts[3,4]

चित्रक

चित्रकमूलं दीपनीय पाचनीय गुदशोथार्शः शूलहराणाम् ।

(च० सू०; 25/40)

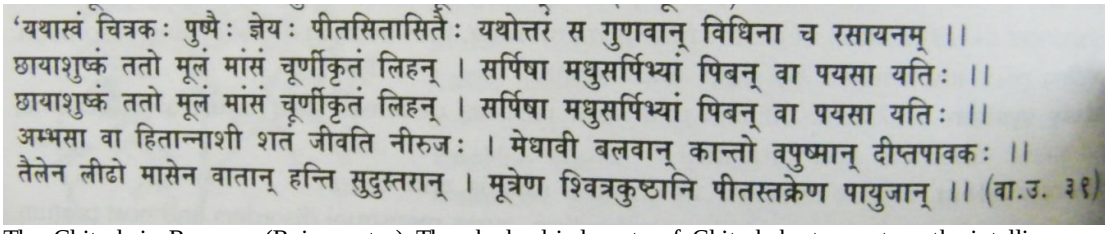
चित्रको दहनो व्यालः पाठीनो दारुणोऽग्निकः ।
ज्योतिष्को वल्लरी वह्नि पाली पाठी कटुः शिखी ।।
कृष्णारुणोऽनलो द्वीपी चित्रभानुश्च पावकः ।
चित्रकोऽग्निसमः पाके कटुकः कफशोफजित् ।
वातोदरार्शो ग्रहणीक्षयपाण्डुविनाशनः ।।

(ध० नि०, शतपुष्पादिवर्ग; 80–81)

चित्रकोऽनलनामा च पाठी व्यालस्तथोषणः ।
चित्रकः कटुकः पाके वह्निकृत्पाचनो लघुः ।।
रूक्षोष्णो ग्रहणीकुष्ठशोथार्शः कृमिकासनुत् ।
वातश्लेष्महरो ग्राही वातार्शः श्लेष्मपित्तहृत् ।।

(भा० प्र०, हरीतक्यादिवर्ग; 70-71)

The other names of Chitrak – Analnama, Paathi, Vyal, Ushan and all names of fire (Agni) Therapeutic uses- It enhances the digestive capacity. It cures leprosy, piles, cough and inflammation.


'यथास्वं चित्रकः पुष्पैः ज्ञेयः पीतसितासितैः यथोत्तरं स गुणवान् विधिना च रसायनम् ।।
छायाशुष्कं ततो मूलं मांसं चूर्णीकृतं लिहन् । सर्पिषा मधुसर्पिभ्यां पिबन् वा पयसा यतिः ।।
छायाशुष्कं ततो मूलं मांसं चूर्णीकृतं लिहन् । सर्पिषा मधुसर्पिभ्यां पिबन् वा पयसा यतिः ।।
अम्भसा वा हितान्नाशी शत जीवति नीरुजः । मेधावी बलवान् कान्तो वपुष्मान् दीप्तपावकः ।।
तैलेन लीढो मासेन वातान् हन्ति सुदुस्तरान् । मूत्रेण श्वित्रकुष्ठानि पीतस्तक्रेण पायुजान् ।। (वा.उ. ३१)

The Chitrak is Rasayan (Rejuvenator) The shade dried roots of Chitrak bestows strength, intelligence and longevity[5].

DOSE - 1-2 g of the drug in powder form[3]

1.2.3 Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Ranunculales
Family : Plumbaginaceae
Genus : Plumbago
Species :

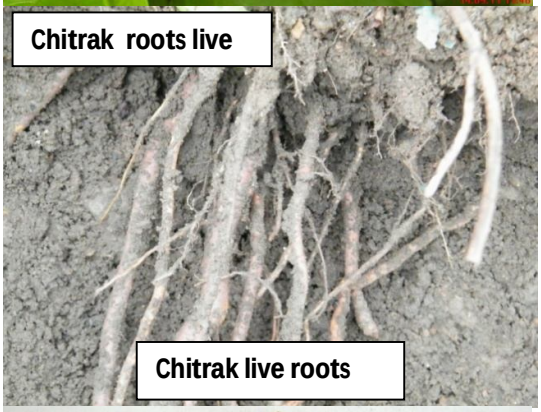
- *Ceratostigma*
- *Limonium*
- *Limonium carolinianum (sea lavender)*
- *Plumbagella*
- *Plumbago* (leadwort)
- *Plumbago capensis* (cape leadwort)
- *Plumbago coerulea*
- *Plumbago europaea*
- *Plumbago rosea*
- *Plumbago pulchella*
- *Plumbago scandens*
- *Plumbago zeylanica* (Ceylon leadwort)
- *Statice*
- *Statice limonium* (English sea lavender).

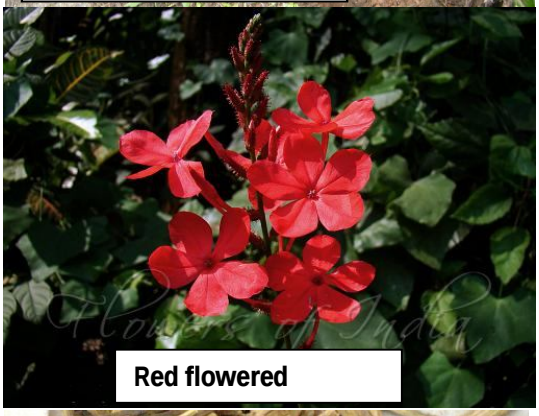
1.3 Plumbago zeylinica roots

The root of *Plumbago zeylanica* (Chitraka, Chitramulamu) collected at the stage of flowering can be pharmaceutically moulded into two main categories of dosage forms by using (A) Fresh root and (B) Dry drug (root).

1.3.1 FRESH ROOTS & DRY ROOTS

The freshly collected root can be converted in the following forms for therapeutic use.





1.3 Names in English

White leadwort, Ceylon leadwort, plumbago India:Chitrak, chitramol

1.3.2 Names in different Foreign languages:

Nepal: Chitu
German: Bleiwurz, Zahnkraut
African vernacular names:
Arabia: Ensain, enkin Chagga: Osuhure Kilongo: mzura Ndebele: matsisa
Swahili: Sanza Tswana: Mosikomabe
Malaysia: Celaka, Celaka bukit, Celaka Putih, Ceraka
Indonesia (in its diff regions): Ceraka (Sumatera); Daun encok, Ki encok (Sunda); Gadong encok, Poksor (Jawa); Kareka (Madura); Bama (Bali); Oporie (Timor);
Philippine: Sagdikit (Tagalog); Bagbag, Talankaw (Iloc)
Tibet: Tsi tra ka (Wylie)

1.6 Botany

Plumbago zeylanica, a rambling subscandent perennial herb or under shrub with green branches, stems somewhat woody, spreading, terate, striate, glabous. Leaves alternate, ovate or oblong, petiole narrow, amplexicaul at the base and often dialted into stipule like auricles. Flowers white, in axillary and terminal elongated spikes, bisexual. Calyx densely covered with stalked, sticky glands. Corolla white, very slender, tubular. Stameus 5, free. Ovary superior, 5-gonous, one celled, ovule one, basal. Roots are light yellow coloured when fresh, reddish brown when dry, found in the form of taugh pieces, straight unbranched or slightly branched with or without secondary roots, with

uniform and smooth texture, strong and characteristic odour with acrid and bitter taste [6]

a) Macroscopic

Roots 30 cm or more in length, 6 mm or more in diameter as also as short stout pieces, including root stocks reddish to deep brown, scars of rootlets present, bark thin and brown, internal structure striated, odour, disagreeable, taste, acrid.

b) Microscopic

Transverse section of root shows outer most tissue of cork consisting of 5 -7 row, of cubical to rectangular dark brown cells, secondary cortex consists of 2-3 rows of thinwalled rectangular, light brown cells, most of the cortex cells contain starch grains, secondary cortex followed by a wide zone of cortex, composed of large polygonal to tangentially elongated parenchymatous cells varying in size and shape, containing starch grains and some cells with yellow contents, fibres scattered singly or in groups of 2-6, phloem a narrow zone of polygonal, thin-walled cells, consisting of usual elements and phloem fibres, similar to cortical zone, phloem fibres usually in groups of 2-5 or more but occasionally occurring singly, lignified with pointed ends and narrow lumen, similar in shape and size to those of secondary cortex, cambium indistinct, xylem light yellow to 39 whitish, vessels radially arranged with pitted thickenings, medullary rays straight, 1-6 seriate, cells radially elongated starch filled with starch grains, stone cells absent.

1.7 Chemical constituents

*The naphthoquinones plumbagin, composed naphthoquinones like plumbagin, 3-biplumbagin, chloroplumbagin, chitranone, elliptone.

*The coumarins seselin, 5-methoxyseselin, suberosin and xanthyletin

*Other compounds were 2,2-dimethyl-5-hydroxy-6-acetylchromene, plumbagin acid, ß-sitosterol, ß-sitosteryl-glucoside, bakuchiol, 12-hydroxyisobakuchiol, saponaretin, isoorientin, isoaffinetin, psorealen.

Roots of P. zeylanica: Two plumbagic acid glucosides, 3′-*O*-β-glucopyranosyl plumbagic acid and 3′-*O*-β-glucopyranosyl plumbagic acid methylester along with five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) were isolated from the roots of *Plumbago zeylanica.* All coumarins were not previously found in this plant. Cytotoxicity of these compounds to various tumor cells lines was evaluated, and plumbagin significantly suppressed growth of Raji, Calu-1, HeLa, and Wish tumor cell lines. Two plumbagic acid glucosides, 3′-*O*-β-glucopyranosyl plumbagic acid and 3′-*O*-β-glucopyranosyl plumbagic acid methylester were isolated from the root of *Plumbago zeylanica*.[85]

Plumbagin :Amongst all these compounds **plumbagin** is the major ingredient (5-hydroxy-2-methyl-1,4-naphthoquinone, (C 11H 8O3),with 1% in the whole plant, but with higher percentages in the **root**, crystallising as slender orange coloured needles, soluble in organic solvents, less soluble in water, volatile with steam.

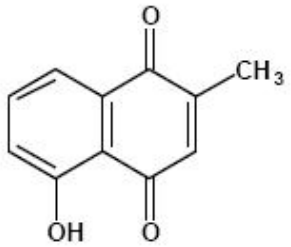
*Chemical Abstracts Service Name: 1,4-Naphthalenedione, 5-hydroxy-2-methyl- (9CI)

*Synonyms and Trade Names: 5-Hydroxy-2-methyl-1,4-naphthoquinone; 2-methyl-juglone; plumbagin; plumbagone

*Structural Class: Bicyclic; naphthoquinone

*Structure, Molecular Formula and Molecular Weight:

C11H8O3 Mol. wt.: 188.18



2 PHARMACOLOGICAL AND CLINICAL PROPERTIES

2.1 Immunomodulation

2.1.1 Modulatory effect of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) on macrophage functions in BALB/c mice. I. Potentiation of macrophage bactericidal activity[7.] The modulatory ability of plumbagin, a natural product from Plumbago zeylanica, was studied on peritoneal macrophages of BALB/c mice. The macrophage functions evaluated were bactericidal activity, hydrogen peroxide and superoxide anion release. The bactericidal capacity of in vivo plumbagin-treated mouse macrophages was estimated against Staphylococcus aureus. In low doses plumbagin exerted a constant increase in bactericidal activity throughout the study period whereas with a high dose a higher response was observed up to six weeks. But in the next two weeks a considerable decline in the bactericidal activity was noticed compared to low dose. Plumbagin was also seen to exert a similar response on oxygen radical release by macrophages in vivo showing a clear correlation between oxygen

radical release and the bactericidal activity. The data indicate that plumbagin augments the macrophage bactericidal activity by potentiating the oxyradical release at low concentration whereas at the higher concentration it has inhibitory activity.

2.1.2 This study screened herbal drug, which consists of a mixture of seven plants, used by the tribal population (in Paderu agency area of Visakhapatnam district of Andhra Pradesh state in India), for the identification of potential immunosuppressive property by testing the aqueous extracts of the plant parts by inducing ovalbumin (OVA) specific IgG antibody responses in a murine system. **The aqueous root extract of Plumbago zeylanica (PZE)** exhibited the significant suppression of OVA-specificIgG antibody response determined by enzyme-linked immunosorbent assay (ELISA). PZE alsosuppressed the anti-OVA antibody response in dose dependent manner. In addition ethyl acetatefraction of PZE eluted from silica gel (PZE-6) was found to exert a significant suppression of OVAstimulated T cell proliferation. Moreover, PZE is potent in exerting the suppressive effect on the downregulation of anti-OVA antibody and T cell responses and in all the three haplotypes of the micestudied, which indicates that the PZE exerted immunosuppression without linking to genetic variation[8].

2.2 Anti oxidant activity

2.2.1 Plumbago zeylanica is a useful Indian medicinal plant. The root of the plant and its constituents are credited with potential therapeutic properties including anti-atherogenic, cardiotonic, hepatoprotective and neuroprotective properties. To examine possible mechanisms of action of P. zeylanica (Chitrak), in relation to its reported beneficial properties, antioxidant effects of the aqueous/alcoholic extracts of root, corresponding to medicinal preparations, and the active ingredient, plumbagin, were studied.

Methods used included: ferric reducing/antioxidant power (FRAP), radical scavenging of 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), lipid peroxidation in rat liver mitochondria induced by different agents, and estimating phenolic and flavonoid content. In FRAP/DPPH assays, boiled ethanolic extracts were the most effective, while in the ABTS assay boiled aqueous extracts were the most efficient. These extracts also significantly inhibited lipid peroxidation induced by cumene hydroperoxide, ascorbate-Fe(2+) and peroxynitrite and contained high amounts of polyphenols and flavonoids.

In conclusion, our studies reveal that extracts of P. zeylanica and its active ingredient plumbagin have significant antioxidant abilities that may possibly explain some of the reported therapeutic effects[9].

2.2.2 The isolation and spectral data for new flavonoid 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one from the roots of *Plumbago zeylanica* were determinedand the antioxidant activity were studied by free radical scavenging and superoxide radical scavenging assays. The plant roots extract reveled significant antioxidant activity as compared to standard flavonoid (quercetin)[10].

2.2.3 Plumbago zeylanica, was tested for its possible in vivo protective effect against cyclophosphamide-induced genotoxicity and oxidative stress in Swiss albino mice. Pretreatment with the alcoholic root extract of Plumbago zeylanica (250 and 500 mg/kg body weight orally for 5 days) significantly reduced the frequency of micronucleated polychromatic erythrocytes (MnPCEs), increased the PCE/NCE (normochromatic erythrocyte) ratio in the bone marrow, and decreased the levels of lipid peroxidation products with concomitant changes in the status of antioxidants. Both doses of Plumbago zeylanica were effective in exerting a protective effect against cyclophosphamide-induced genotoxicity and oxidative stress[11].

2.2.4 Methanolic extracts of *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark), used in Ayurvedic medicines for number of ailments were evaluated for their antioxidant activity by ferric thiocyanate (FTC) assay and compared with thiobarbituric acid (TBA) method. The order of antioxidant potential according to FTC assay was found to be highest in *Plumbago zeylanica* followed by *Holarrhena antidysenterica, Acorus calamus* and *Hemidesmus indicus.* Whereas there is slightly difference in activities as measured by TBA method. The antioxidant activity of medicinal plants was at par with the commercial antioxidant butylated hydroxy toluene (BHT), L-Ascorbic acid and α-tocopherol.

Further, the radical-scavenging activity of the extracts was measured as decolourizing activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) standard solution was recorded maximum for *Hemidesmus indicus* (77.0%) followed by *Plumbago zeylanica* (73.41%), Acorus calamus (20.88%) and Holarrhena antidysenterica (20.06%) extracts at a concentration of 100 µg/ml. Phytochemical analysis revealed the presence of major phytocompounds like alkaloids, glycosides, phenolics and saponins. Moreover, total phenolics concentration equivalents to gallic acid was found in the range of 59.50 to 109.0 mg/g of plant extracts, which correlated with antioxidant activity.

The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines[12].

2.2.5 In this study the antioxidant activity and radical scavenging activity of methanolic extracts of selected plant materials, traditionally used by the tribes of Attapadyregions as folk remedies was evaluated against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. *Cassia occidentalis*, *Clitoria ternatea*, *Trianthema decandra*, *Capparis zeylanica*,*Anisomeles malabarica* and *Plumbago zeylanica* exhibited strong antioxidant activity as compared to other plants. *Trianthema decandra* showed the highest antioxidant activity. Thepresent study reveals that these plants are of therapeutic potential due to their high free radical scavenging activity[13].

2.3 Anti Cancer

2.3.1 Two plumbagic acid glucosides, 3'-O-beta-glucopyranosyl plumbagic acid and 3'-O-beta-glucopyranosyl plumbagic acid methylester along with five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) were isolated from the roots of Plumbago zeylanica. All coumarins were not previously found in this plant. Cytotoxicity of these compounds to various tumor cells lines was evaluated, and plumbagin significantly suppressed growth of Raji, Calu-1, HeLa, and Wish tumor cell lines[14].

2.3.2 Plumbagin, derived from the medicinal plant Plumbago zeylanica, modulates cellular proliferation, carcinogenesis, and radioresistance, all known to be regulated by the activation of the transcription factor NF-kappaB, suggesting plumbagin might affect the NF-kappaB activation pathway. We found that plumbagin inhibited NF-kappaB activation induced by TNF, and other carcinogens and inflammatory stimuli (e.g. phorbol 12-myristate 13-acetate, H2O2, cigarette smoke condensate, interleukin-1beta, lipopolysaccharide, and okadaic acid). Plumbagin also suppressed the constitutive NF-kappaB activation in certain tumor cells. The suppression of NF-kappaB activation correlated with sequential inhibition of the tumor necrosis factor (TNF)-induced activation of IkappaBalpha kinase, IkappaBalpha phosphorylation, IkappaBalpha degradation, p65 phosphorylation, p65 nuclear translocation, and the NF-kappaB-dependent reporter gene expression activated by TNF, TNFR1, TRAF2, NIK, IKK-beta, and the p65 subunit of NF-kappaB. Plumbagin also suppressed the direct binding of nuclear p65 and recombinant p65 to the DNA, and this binding was reversed by dithiothreitol both in vitro and in vivo. However, plumbagin did not inhibit p65 binding to DNA when cells were transfected with the p65 plasmid containing cysteine 38 mutated to serine. Plumbagin down-regulated the expression of NF-kappaB-regulated anti-apoptotic (IAP1, IAP2, Bcl-2, Bcl-xL, cFLIP, Bfl-1/A1, and survivin), proliferative (cyclin D1 and COX-2), and angiogenic (matrix metalloproteinase-9 and vascular endothelial growth factor) gene products. This led to potentiation of apoptosis induced by TNF and paclitaxel and inhibited cell invasion. Overall, our results indicate that plumbagin is a potent inhibitor of the NF-kappaB activation pathway that leads to suppression of NF-kappaB-regulated gene products. This may explain its cell growth modulatory, anticarcinogenic, and radiosensitizing effects previously described[15].

2.3.3 In this study we further revealed the mitochondrial pathway involved in plumbagin-induced apoptosis. We also found that the generation of ROS was a critical mediator in plumbagin-induced apoptosis, which would be abrogated completely by antioxidant, NAC. The anticancer effect of plumbagin was investigated in vivo using NB4 tumor xenograft in NOD/SCID mice. The incidence of formation, growth characteristics, body weight and volume of tumors were observed. The histopathologic examination of tumors and organs were made. The results showed that intraperitoneal injection of plumbagin (2mg/kg body weight) daily for 3 weeks resulted to a 64.49% reduction of tumor volume compared with the control. Furthermore, there was no overt manifestation of toxicity such as weight loss, tissue damage and behavior change which appeared in Doxorubicin-treated mice (1mg/kg thrice a week). These results indicate that plumbagin has potential as a novel therapeutic agent for myeloid leukemia[16].

2.3.4 For the evaluation of cytotoxicity, the crude dichloromethane extract was subjected to silica gel column chromatography and 120 fractions were collected. Their structures were elucidated with the help of spectroscopic techniques. High performance liquid chromatography (HPLC) was performed to determine the purity of gugultetrol-18-ferrulate in crude extract and the structure of betasitosterol and gugultetrol-18-ferrulate was identified using nuclear magnetic resonance spectroscopy analysis (1H and 13C NMR), Infra red and mass spectroscopy. The lethal concentration (LC50) value was observed for crude extract, betasitosterol, gugultetrol-18-ferrulate and it was found to be 90, 75 and 65 ppm, respectively[17].

2.3.5 Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men. Hormone-refractory invasive PCa is the end stage and accounts for the majority of PCa patient deaths. We present here that plumbagin (PL), a quinoid

constituent isolated from the root of the medicinal plant Plumbago zeylanica L., may be a potential novel agent in the control of hormone-refractory PCa. Specific observations are the findings that PL inhibited PCa cell invasion and selectively induced apoptosis in PCa cells but not in immortalized nontumorigenic prostate epithelial RWPE-1 cells. In addition, i.p. administration of PL (2 mg/kg body weight), beginning 3 days after ectopic implantation of hormone-refractory DU145 PCa cells, delayed tumor growth by 3 weeks and reduced both tumor weight and volume by 90%. Discontinuation of PL treatment in PL-treated mice for as long as 4 weeks did not result in progression of tumor growth. PL, at concentrations as low as 5 micromol/L, inhibited in both cultured PCa cells and DU145 xenografts (a) the expression of protein kinase Cepsilon (PKCepsilon), phosphatidylinositol 3-kinase, phosphorylated AKT, phosphorylated Janus-activated kinase-2, and phosphorylated signal transducer and activator of transcription 3 (Stat3); (b) the DNA-binding activity of transcription factors activator protein-1, nuclear factor-kappaB, and Stat3; and (c) Bcl-xL, cdc25A, and cyclooxygenase-2 expression. The results indicate for the first time, using both in vitro and in vivo preclinical models, that PL inhibits the growth and invasion of PCa. PL inhibits multiple molecular targets including PKCepsilon, a predictive biomarker of PCa aggressiveness. PL may be a novel agent for therapy of hormone-refractory PCa.[18].

2.3.6 The purpose of this investigation was to identify the changes in the rate of glycolysis and gluconeogenesis in tumour-bearing rats and the effects of treatment with Plumbagin. The levels of certain glycolytic enzymes, namely, hexokinase; phosphoglucoisomerase; and aldolase levels increased ($p < 0.001$) in hepatoma bearing rats, whereas they decreased in Plumbagin administered rats to near normal levels. Certain gluconeogenic enzymes, namely, glucose-6-phosphatase and fructose-1,6-diphosphatase decreased ($p < 0.001$) in tumour hosts, whereas Plumbagin administration increased the gluconeogenic enzyme levels in the treated animals. These investigations indicate the molecular basis of the different biological behaviour of 3MeDAB induced hepatoma and the anticarcinogenic property of Plumbagin against hepatoma studied in rats[19].

2.3.7. Using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay, 3-(4,5-B-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for cell growth inhibition, histone/DNA ELISA, homogeneous caspase-3/7 assay for apoptosis as well as alkaline comet assay for DNA single-strand breaks detection in this report, we confirm that plumbagin causes effective cell growth inhibition, induces apoptosis and generates single-strand breaks in cancer cells. Incubation of cancer cells with scavengers of ROS and neocuproine inhibited the cytotoxic action of plumbagin proving that generation of ROS and Cu(I) are the critical mediators in plumbagin-induced cell growth inhibition. This study is the first to investigate the copper-mediated anticancer mechanism of plumbagin in human cancer cells and these properties of plumbagin could be further explored for the development of anticancer agents with higher therapeutic indices, especially for skin cancer[20].

2.3.8 In India, natural preparations derived from the plants are widely use for the treatments of various diseases. Hence, it becomes necessary to assess the modulating action of the plant extract when associated with other substances. Potassium canrenoate (PC) is a synthetic steroid and is used in the treatment of hypertension. It is not only a genotoxic agent, but also a tumor-initiating agent. In the present study, the effect of various doses (i.e., 5, 10, 20, and 30 µM) of PC were studied for their genotoxic effects in the presence of S9 mix in cultured human lymphocytes, using mitotic index, chromosomal aberrations, sister chromatid exchanges, and replication index as parameters. PC was found to be genotoxic at 20 and 30 µM. Treatment of 30 µM of PC was given along with different doses of Plumbago zeylanica extract (i.e., 107.5, 212.5, 315, and 417 µg/mL) of the culture medium. A dose-dependent decrease in the genotoxic effects of PC was observed. The result suggested that the plant extract per se does not have genotoxic potential, but can modulate the genotoxicity of PC in cultured human lymphocytes[21].

2.3.9 Present study aims at a preliminary phytochemical screening and anticancer evaluation of *plumbago zeylanica* Linn. against Ehrlich Ascites Carcinoma in animal model. Results indicates that ethanolic extract of *plumbago zeylanica* Linn. possess significant anticancer activity and also reduce elevated level of lipid peroxidation due to higher content of terpenoids and flavonoids. Thus ethanolic extract of *plumbago zeylanica* Linn. could have vast therapeutic application against cancer[22]

2.3.10 This study was aimed to investigate the effects of plumbagin on the proliferation, cell cycle and apoptosis of APL cell line NB4 Cells. Cell inhibitory rates were detected by MTT colorimetric assay; morphologic changes were observed under light microscope and transmission electron microscope; apoptosis-inducing effects were determined by DNA gel electrophoresis, annexin V/PI double-stained and PI single-stained flow cytometry. The results demonstrated that 2-15 micromol/L plumbagin inhibited the proliferation of NB4 cells in a dose-dependent manner. The

morphologic changes of cell apoptosis, such as chromsome condensation and apoptotic body formation, were observed by light microscope and transmission electron microscope. Cell cycle analysis showed that NB4 cells were blocked in G2/M phase of cell cycle. And plumbagin induced annexin V+/PI- cell increase and DNA fragmentation. There was a correlation between cell apoptosis rates and the concentrations of plumbagin in dose-dependent manner ($P < 0.05$. The present study shows that plumbagin can inhibit cell proliferation, block cell cycle and induce apoptosis of APL cell line NB4 cells[23].

2.3.11 The bioassay-guided fractionation of the dichloromethane extract of aerial parts of Plumbago zeylanica led to the isolation of beta-sitosterol, beta-sitosteryl-3beta-glucopyranoside, beta-sitosteryl-3beta-glucopyranoside-6'-O-palmitate (1), lupenone, lupeol acetate, plumbagin and trilinolein. Compound 1 showed cytotoxic activity against MCF7 and Bowes cancer cell lines (IC50 113 microM and 152 microM, respectively), beta-sitosterol inhibited Bowes cell growth (IC50 36.5 microM) and plumbagin was cytotoxic against MCF7 and Bowes cells (IC50 1.28 microM and 1.39 microM, respectively)[24].

2.4 ANTI ALLERGY

2.4.1 The antiallergic properties of the 70% ethanol extract from Plumbago zeylanica stems (EPZ) were investigated in the present study. The extract (500, 1000 mg/kg, p.o.) dose-dependently inhibited systemic anaphylactic shock induced by compound 48/80 in mice, reduced homologous passive cutaneous anaphylaxis and skin reactions induced by histamine or serotonin in rats, significant differences were observed at the dose of 1000 mg/kg. In vitro, EPZ (5, 20, 50 microg/ml) concentration-dependently reduced histamine release from rat peritoneal mast cells caused by compound 48/80 and antigen. EPZ (50 microg/ml) markedly increased intracellular cAMP content of rat mast cells. These findings demonstrate that EPZ inhibits mast cell-dependent immediate allergic reactions, which is probably mediated by reducing the release of mediators such as histamine from mast cells via elevating intracellular cAMP level and weakening the inflammatory action of mediators[25].

2.5 ANTI-CHOLESTEROL

2.5.1 Plumbagin (2-methyl-5-hydroxy, 1:4 naphthoquinone) isolated from the roots of Plumbago zeylanica when administered to hyperlipidaemic rabbits, reduced serum cholesterol and LDL-Chol. by 53 to 86 percent and 61 to 91 percent respectively. It lowered cholesterol/phospholipid ratio by 45.8 percent and elevates the decreased HDL-Chol significantly. Further, Plumbagin treatment prevented the accumulation of cholesterol and triglycerides in liver and aorta and regressed atheromatous plaques of thoracic and abdominal aorta. Plumbagin treated hyperlipidaemic subjects excreted more fecal cholesterol and phospholipids. In conclusion- Plumbagin feeding brings about a definite regression of atheroma and prevents the accumulation of cholesterol and triglycerides in liver and aorta[26].

2.5.2 Effect of ethanolic extract (50% v/v) of Plumbago zeylanica (root) alone and combined withvitamin E (an antioxidant)was studied in experimentally induced hyperlipidaemic rabbits. There was significant reduction in serum total cholesterol, LDL cholesterol and triglyceride levels.Marked reduction was observed with the formulation of P.zeylanica and vitamin E. The totalcholesterol/HDL and LDL/HDL cholesterol ratios were found significantly decreased ($P<0.05$) .P.zeylanica showed good margin of safety as determined by acute toxicity studies in albinorats and albino rabbits, as well as by the absence of adverse effects on haematological and biochemical parameters in albino rabbits upto 60 days of administration[27].

2.6 ANTI-INFLAMMATOTY ACTIVITY

2.6.1. In order to validate these ethnobotanical practices, the anti-inflammatory and antinociceptive activities of various leaf extracts (petroleum ether (60-80 degrees), chloroform, acetone, ethanol, and aqueous) were studied using in vivo experimental models at two dose levels (200 and 400 mg/kg, p.o.). Anti-inflammatory activity was tested using the carrageenan induced rat hind paw edema method while analgesic activity was studied using the hot plate and formalin induced models. Diclofenac (100 mg/ kg) was used as the reference standard in both anti-inflammatory and analgesic models and morphine (10 mg/ kg, i.p.) was used as the reference standard in the formalin induced analgesic model. The acetone extract significantly ($p < 0.01$) reduced inflammation in the rats when compared to the control group. As for the analgesia effect, the acetone and petroleum ether extracts significantly ($p < 0.01$) decreased the pain stimulus only in the later phase of the formalin test, suggesting that the drug could be peripherally acting. Bioassay-guided fractionation of the acetone extract led to the isolation and identification of plumbagin. Structure elucidation of plumbagin confirmed it as 5-hydroxy-2-methyl-1,4-naphthoquinone, a naphthaquinone derivative, through spectral techniques[28].

2.6.2. In the current study, we investigated and characterized the anti-inflammatory and analgesic effects of PL orally administrated in a range of dosages from 5 to 20 mg/kg. We also examined the role of nuclear factor κB (NF-κB) and

proinflammatory cytokines and mediators in this effect. The results showed that PL significantly and dose-dependently suppressed the paw edema of rats induced by carrageenan and various proinflammatory mediators, including histamine, serotonin, bradykinin, and prostaglandin E2. PL reduced the number of writhing episodes of mice induced by the intraperitoneal injection of acetic acid, but it did not reduce the writhing episode numbers induced by MgSO4 in mice or prolong the tail-flick reaction time of rats to noxious thermal pain. Mechanistic studies showed that PL effectively decreased the production of the proinflammatory cytokines interleukin 1β, interleukin 6, and tumor necrosis factor α. It also inhibited the expression of the proinflammatory mediators inducible nitric-oxide synthase and cyclooxygenase 2, whereas it did not inhibit the expression of cyclooxygenase 1. Further studies demonstrated that PL suppressed inhibitor of κBα phosphorylation and degradation, thus inhibiting the phosphorylation of the p65 subunit of NF-κB. This study suggests that PL has a potential to be developed into an anti-inflammatory agent for treating inflammatory diseases[29].

2.6.3. This work involves the study of anti inflammatory and cytotoxic effects of *Plumbago zeylanica*. The root of *P. zeylanica* extracted with methanol was used for determining the anti inflammatory effects. The methanolic extracts at 300 and 500 mg/kg produced 31.03 and 60.3% inhibition of acute inflammation, respectively, in Carrageenin induced raw paw oedema confirming that *P. zeylanica* roots are effective against acute inflammation. The use of *Plumbago* species as an effective anti inflammatory agent and its cytotoxic effects have been ascertained and proved[30].

2.6.4 The haemagglutination, free radical scavenging and membrane stabilizing activities of combined ethyl acetate/diethylether extract of root of Plumbago zeylanica were investigated. The combined fraction (ethyl acetate/diethylether) was chromatographed on silica gel column using hexane/ethyl acetate gradient elution (100:0 – 0:100 v/v) to afford seven fractions (A-F) based on Rf values and phytochemical evaluation tests. The fractions were assayed for phytochemical constituents, total phenol contents, free radical scavenging and membrane stabilizing activities and toxicity using haemaglutination assay method. The phytoconstituents include anthraquinones, flavonoids and phenolic acids. The phenolic contents in the fractions ranged between 0.27 ± 0.04 and 0.93 ± 0.28 mg/g tannic acid equivalent (TAE). The fractions exhibited strong and appreciable free radical scavenging and membrane stabilizing activities and agglutinated red blood cells, which implied toxicity. These activities compared favourably with standard anti-inflammatory and anti-oxidant drugs. The results revealed that while some of the fractions haemaglutinated red blood cells, others provided effective antioxidant and anti-inflammatory activities[31].

2.6.5 Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone), a quinone isolated from the roots of Plumbago zeylanica was recently reported to suppress the activation of NF-kappaB in tumor cells. NF-kappaB, a ubiquitous transcription factor, plays a central role in regulating diverse processes in leukocytes like cellular proliferation, expression of immunoregulatory genes and apoptosis during innate and adaptive immune responses. Consequently, plumbagin might affect the biological functions of leukocytes participating in various immune responses. The present report describes novel immunomodulatory effects of plumbagin. Plumbagin inhibited T cell proliferation in response to polyclonal mitogen Concanavalin A (Con A) by blocking cell cycle progression. It also suppressed expression of early and late activation markers CD69 and CD25 respectively, in activated T cells. At these immunosuppressive doses (up to 5 microM), plumbagin did not reduce the viability of lymphocytes. Further, the inhibition of T cell proliferation by plumbagin was accompanied by a decrease in the levels of Con A induced IL-2, IL-4, IL-6 and IFN-gamma cytokines. Similar immunosuppressive effects of plumbagin on cytokine levels were seen in vivo. To characterize the mechanism of inhibitory action of plumbagin, the mitogen induced IkappaB-alpha degradation and nuclear translocation of NF-kappaB was studied in lymphocytes. Plumbagin completely inhibited Con A induced IkappaB-alpha degradation and NF-kappaB activation. Further, plumbagin prevented Graft Versus Host Disease-induced mortality in mice. To our knowledge this is the first report showing the immunomodulatory effects of plumbagin in lymphocytes via modulation of NF-kappaB activation[32].

2.7 MEMORY

2.7.1 The present study is to investigate the effect of Plumbago zeylanica roots on learning and memory of mice. The exteroceptive behaviour model (Elevated plus maze and Passive avoidance paradigm) and interoceptivebehaviour model i.e. scopolamine induced amnesia were employed to evaluate the effect of Plumbagozeylanica roots on learning and memory of mice. The Chloroform extract of Plumbago zeylanica (100, 200 and400 mg/kg. p.o.) was administered for 10 successive days in separate group of animals. The Plumbagozeylanica at dose 200mg/kg. has shown promising memory enhancing effect in mice. Furthermore, the extractsignificantly reversed the amnesia induced by scopolamine (0.4mg/kg i.p.). The reversal of scopolamineinduced amnesia may

be due to facilitation of cholinergic transmission in mice brain. Antioxidant,hypolipidaemic and anti-atherosclerotic properties of P. zeylanica may be contributing favourably to memory enhancing effect[33].

2.8 LIVER PROTECTIVE

2.8.1 Comparing with Ara-Amp, the effects of the water-soluble extracts from the single herb of the formula for Ganduqing on HBeAg and HBsAg expression in 2.2.15 cells were studied. The results showed that the extracts of Serissa serissoides (DC) Druce, Hibiseus mutabilis Linn, Paedeuia scangens (Lour) Merr var tomentosa (BL) Hand-Mazz, Plumbago zeylanica L, Garcinia oblougifolia Champ and Begpnia edulia Levl had marked inhibition effects on HBeAg and HBsAg which expressed by 2.2.15 cells[34].

2.9 CNS STIMULATION

2.9.1 The effects of a 50% ethanol extract of the root of Plumbago zeylanica (P. zeylanica) were investigated on locomotor behaviour and central dopaminergic activity in rats. The effects on the ambulatory behaviour were assessed along with the levels of dopamine (DA) and its metabolite homovanillic acid (HVA) in the striatum after a single oral dose (100, 200 and 300 mg/kg body weight) of the extract. The extract significantly increased the spontaneous motility in animals. The ambulatory and rotatory behaviour in the treated groups were higher than in the control group ($p < 0.05$). There were marked differences in the ambulatory behaviour between 100 and 300 mg/kg, indicating that the responses were stimulatory and dose-dependent. The stereotypic behaviour which is characteristic of a dopamine agonist showed biphasic effects. However, there was no significant difference between the groups ($p > 0.05$). The results showed that the extract of the root of P. zeylanica specifically enhanced the spontaneous ambulatory activity without inducing stereotypic behaviour. The neurochemical estimations revealed elevated levels of DA and HVA in striatum compared with the control rats ($p < 0.01$). The levels were higher for the 100 mg/kg treated group than the other groups. The levels declined by increasing the dosage of the extract to 200 mg/kg and 300 mg/kg, however, these levels remained higher than the control group. The relationship between motor activity and levels of dopamine are not parallel. These behavioural and biochemical results indicated stimulatory properties of the extract of the root of P. zeylanica, which may be mediated by dopaminergic mechanisms in the rat brain[35].

2.10 HYPERGLYCEMIA

2.10.1 The effects of the ethanol extract of the root of Plumbago zeylanica on key enzymes of glycolysis and other biochemical parameters were studied in the rat. The results show that thigh muscle hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase activities were significantly reduced ($p < 0.05$) by 12.07%, 51.02%, 24.32% and 25.16% respectively in rats treated with the ethanol extract of Plumbago zeylanica when compared with the controls. Serum pyruvate and lactate were significantly lowered in the experimental rats by 23.64% and 46.29%, respectively. The difference between the supernatant protein means was not statistically different ($p > 0.05$) suggesting the preservation of protein synthesis in the muscle of the extract-treated rats. The reduction in the activities of the key enzymes of glycolysis and its end-products suggests a reduction in flux across the glycolytic pathway in the extract-treated rats. This may be a result of impaired delivery to, and utilization of, glucose by the peripheral tissue, thus substantiating the reported hyperglycaemia in the extract-treated rats[36].

2.11 ANTI VIRAL (SKIN) ACTIVITY

2.11.1 Acokanthera schimperi (Apocynaceae), Euclea schimperi (Ebenaceae), Inula confertiflora (Asteraceae), Melilotus elegans (Leguminosae), and Plumbago zeylanica (Plumbaginaceae), are some of the medicinal plants used in Ethiopia for treatment of various skin disorders. In this study, the antiviral activities of the 80% methanolic extracts of these plants have been examined against coxsackievirus B3 (CVB3), influenza A virus and herpes simplex virus type1 Kupka (HSV-1) using cytopathic effect (CPE) inhibitory assays in HeLa, MDCK, and GMK cells, respectively. In parallel, the cytotoxicity was quantified using a crystal violet uptake assay. The antiviral activity of the most active compound was confirmed with plaque reduction assays. The results revealed that the extracts of Acokanthera schimperi and Euclea schimperi showed antiviral activity against all three tested viruses albeit with unequal efficacy. Whereas the Acokanthera schimperi extract exhibited the strongest activity against CVB3, the extract of Euclea schimperi inhibited influenzavirus A replication most effectively. A weak anti-influenzavirus A activity was also exhibited by the other plant extracts tested. In addition, CVB3 was inhibited by the extracts of Plumbago zeylanica and HSV-1 by Inula confertiflora. Thus, the extracts of these plants, particularly those of Acokanthera schimperi, Euclea schimperi and Inula confertiflora which showed activity against CVB3 and HSV-1 support their traditional use in the treatment of skin diseases of viral origin[37].

2.12 ANTI MALERIAL

2.12.1 Plants traditionally used in India to treat fever or malaria were examined in vitro for antiplasmodial properties against Plasmodium

falciparum. Of 80 analysed ethanol extracts, from 47 species, significant effects were found for 31 of the extracts. These represent 23 different species from 20 families. Of the active species 20 were tested against P. falciparum for the first time. The following five species seems to be of special interest for further antimalarial studies, Casearia elliptica, Holarrhena pubescens, Pongamia pinnata, Soymida febrifuga, and Plumbago zeylanica[38].

2.13 ANTI BACTERIAL

2.13.1 Alcoholic crude extracts and some fractions from 15 traditionally used Indian medicinal plants were investigated for their ability to inhibit the growth of extended spectrum beta-lactamases (ESbetaL)-producing multidrug-resistant enteric bacteria. The test bacteria Eschrichia coli and Shigella were resistant to 16-23 antibiotics with intermediate or resistance to beta-lactams (minimum inhibitory concentration (MIC) value range 16-1024 microg/ml). The crude plant extracts demonstrated zone of inhibition in the range of 11-29 mm against one or more test bacteria. On the basis of promising activity, 12 plants were selected to determine their efficacy in terms of MIC, which ranged from 0.64 mg/ml to 10.24 mg/ml. The extracts of Acorus calamus, Hemidesmus indicus, Holarrhena antidysenterica and Plumbago zeylanica demonstrated relatively high activity as compared to other plant extracts and were fractionated into acetone, ethyl acetate and methanol. Acetone fraction in most of the cases exhibited higher potency (low MIC value) as compared to ethyl acetate and methanol fraction. However, in Plumbago zeylanica, ethyl acetate fraction was most active. Synergistic interactions among crude extracts were demonstrated in the 12 different combinations against ESbetaL-producing E. coli (ESbetaL-02). Certain combinations exhibited significant synergy with enlargement of combined inhibition zone size by 5 mm. Interaction of crude extracts with five antibiotics (Tetracycline, ciprofloxacin, nalidixic acid, chloramphenicol and streptomycin) demonstrated synergistic interaction with tetracycline and ciprofloxacin by 10 and 3 plant extracts respectively. Phytochemical analysis and thin layer chromatography (TLC) bioautography of crude extracts showed the presence of alkaloids, phenols and flavonoids as active phytoconstituents. Most active fractions of four plants were subjected to Infrared spectroscopy and the major groups of compounds were detected. The plant extracts were further tested for their in vitro haemolytic activity to sheep erythrocytes and demonstrated no haemolysis at recommended doses. Further activity-guided fractionation of active fractions is needed to isolate and characterize the active principle in order to establish the mode of action against the ESbetaL-producing multidrug-resistant enteric bacteria and the mechanism of synergy[39].

2.13.2 Anti-methicillin-resistant Staphylococcus aureus (MRSA) activity of ethanolic extracts of four medicinal plants namely Acorus calamus (rhizome) Hemidesmus indicus (stem), Holarrhena antidysenterica (bark), and Plumbago zeylanica (root), were detected with inhibition zone size ranged from 11 to 44 mm and minimum inhibitory concentration (MIC) varied from 0.32 to 3.25 mg/mL. Further, ethyl acetate, acetone and methanol fractions of above plants demonstrated antibacterial activity. The potency of these fractions based on zone of inhibition and MIC value was relatively higher in P. zeylanica (ethylacetate fraction), followed by acetone fractions of H. indicus, A. calamus, and H. antidysenterica. Time kill assay with most promising fractions of these plant extracts, demonstrated concentration-dependent killing of MRSA within 9-12 h of incubation. Interestingly, synergistic interaction among alcoholic extracts and some fractions of above four plants was evident against MRSA. Further, synergistic interaction of these extracts was detected with one or more antibiotics tested (tetracycline, chloramphenicol, ciprofloxacin, cefuroxime and ceftidizime). The findings also validate the traditional uses of above plants against infectious diseases. Phytochemical studies demonstrated flavonoids and phenols as major active constituents. Further investigations are needed to characterize the active principle and its interaction mechanism with antibiotics[40].

2.13.3 The synergistic activity of antimycobacterial constituents from Saudi plants was evaluated in combination with isonicotinic acid hydrazide (INH) against four atypical organisms, namely, Mycobacterium intracellulare, M. smegmatis, M. xenopei and M. chelonei. The potency of INH was increased four-fold, using an in vitro checkerboard method, against each mycobacteria when tested with a subtoxic concentration of the totarol, isolated from J. procera. The MIC values of totarol, ferulenol (from Ferula communis) and plumbagin (from Plumbago zeylanica) were thus lowered from 1.25–2.5 to 0.15–0.3 µg/mL due to synergism with INH. When tested against the resistant strain of M. tuberculosis H37Rv, plumbagin and 7β-hydroxyabieta-8,13-dien-11,12-dione exhibited inhibitory activity at <12.5 µg/mL, while others were inactive at this concentration[41].

2.13.4 This work assesses the antibacterial activity of plumbagin (5-hydroxy-2-methylnaphthalene-1,4-dione) and ofmethanol, chloroform and aqueous extracts of Plumbago zeylanica L. root against various pathogenic bacteria,and the

minimum inhibitory concentrations (MICs). Plumbagin and chloroform extracts of Plumbago zeylanicaL. root showed antibacterial activity against Escherichia coli, Salmonella typhi and Staphylococcus aureus. Inhibition against Klebsiella pneumoniae, Serratia marcescens and Bacillus subtilis was moderate, and lower against Proteus vulgaris and Pseudomonas aeruginosa. The methanolic extract exhibited moderate activity andthe aqueous extract weak activity against the bacterial strains as assessed by disc diffusion assays. The bioactivecompound plumbagin and extract of Plumbago zeylanica root show a wide spectrum of antibacterial activity.The compound shows promise as a new drug for various bacterial infectious diseases[42].

2.13.5 Alcoholic extract of *Plumbago zeylanica* (root) was tested against multidrug-resistant clinical isolates of bacteria (*Salmonella paratyphi*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae* and a R-plasmid-harbouring standard strain, *E.coli* x^+). The extract exhibited strong antibacterial activity against all test bacteria irrespective of their antibiotic resistance behaviour. Phytochemical analysis of crude extract revealed the presence of flavonoids, saponins and naphthoquinone[43].

2.13.6 A total of 82 Indian medicinal plants traditionally used in medicines were subjected to preliminary antibacterial screening against several pathogenic and opportunistic microorganisms. Aqueous, hexane and alcoholic extracts of each plant were tested for their antibacterial activity using agar well diffusion method at sample concentration of 200 mg/ml. The results indicated that out of 82 plants, 56 exhibited antibacterial activity against one or more test pathogens. Interestingly, extracts of five plants showed strong and broad spectrum activity as compared to rest of 51 plant extracts which demonstrated moderate activity. On the whole the alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts. Among various extracts, only alcoholic extracts of Emblica officinalis, Terminalia chebula, Terminalia belerica, Plumbago zeylanica and Holarrhena antidysenterica were found to show potentially interesting activity against test bacteria. These active crude alcoholic extracts were also assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity[44].

2.13.7 Plumbagin, a compound derived from the roots of Plumbago zeylanica (Chitramool) was studied for its effect on the development of antibiotic resistance using antibiotic sensitive strains of Escherichia coli and Staphylococcus aureus. A delayed growth was seen when these organisms were inoculated into the antibiotic (streptomycin/rifampicin) medium, due to development of resistance in some of the cells. However, the growth was completely prevented when the bacteria were grown in the medium containing antibiotic and plumbagin together, and this was attributed to prevention of development of antibiotic resistant cells[45].

2.14 BLOOD COAUGULATION

2.14.1 Plumbago zeylanica (PZ) is extensively used in Indian systems of medicine for its medicinal properties. The structure of its active principle is similar to that of vitamin K. Its effect on blood coagulation profile after chronic administration has not been reported so far. The PZ extract (2 mg/kg body weight) and napthoquinone (2 mg/kg body weight) given to individual groups were screened for its effect on bleeding time (BT), clotting time (CT), prothrombin time (PT), platelet count and platelet adhesion in albino rats after 1-day, 15-day and 31-day treatment. There was no change in the platelet count in the treated groups when compared to the control levels. But the platelet adhesion was significantly decreased after PZ and also napthaquinone-treated animals in both with and without blood volume reduction after 15th as well as 31st day. Since the napthoquinone-treated group also showed similar response the changes observed after PZ treatment may be due to this component. Even at a lower dosage level (2 mg/kg body weight), the chronic PZ administration prolongs the bleeding time by altering platelet adhesiveness and the coagulation[46].

2.15 OTHER THERAPEUTIC USES

2.15.1 In Ethopia the powdered bark, root or leaves is used to treat gonorrhea, syphilis, and tuberculosis. The Zambians make use of the roots boiled in milk as a remedy for inflammation of the mouth, throat and chest. In Himachal Pradesh the native use a paste of the roots to induce drainage of abscesses. In Indonesia it is used as an antirheumatic remedy by local application over the affected site. However, it has to be used with great caution because of its vesicant effects on the skin[47-50].

2.15.2 SKIN DISEASES

Decoction of the roots is used for scabies. It is widely used to treat various skin affections including leprosy, ringworms, dermatitis, acne, sores and ulcers. Most of the time the roots are the part that is being used for skin problems. However, care must be taken as it can also cause skin irritation and vesicular eruptions. Dried and pulverized roots is a remedy for parasitic skin infestation. Paste of the root in vinegar, milk and water is used to treat influenza and balckwater fever in Africa[51-56].

2.15.3 ANTIFERTILITY ACTIVITY

The acetone and ethanol extracts of leaves of *P.*

zeylanica showed effective interruption in oestrous cycle of rats. There was prolonged dioestrous stage of the oestrous cycle corresponding to a temporary inhibition of ovulation. This antiovulatory activity is reversible upon discontinuation of the treatment[57].

2.15.4 GYNAECOLOGICAL AND OBSTETRICAL DISEASES

The Malays provide decoction of the plant on the third day post-partum. The leaves are eaten as a vegetable as a measure of delaying menstruation. Illicit induction of abortion is done by giving a decoction of the roots; a practice which should be frowned upon by the society. In Africa the pulped roots or aerial parts are inserted into the vagina to induce abortion; a dangerous practice which could result in death[58-63].

2.15.5 GENITO-URINARY DISEASES

In Indonesia the leaves is applied over the pubic region to help ease dysuria. The barks, roots or leaves are used to treat gonorrhea and syphilis[64-65].

2.15.6 GASTROINTESTINAL DISEASES

The Chinese believes that *P. zeylanica* has the ability to increase digestive heat with fire-like power. In Nepals it is being used to treat dyspepsia, diarrhea and haemorrhoids. In Ayurveda, the plant is recommended for removing worms, stimulating appetite, absorbing excess fluids from the intestines, destroying toxins and as a digestive. In Mauritius and Rodrigues Islands, the inhabitants recognized the roots as a remedy for diarhhoea and dyspepsia. Amongst the worms that succumbs to it is the hookworm. In Ghana the roots are applied as an enema for the treatment of haemorrhoids[66-74]

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2.15.7 HEADACHE, ANTIPERIODIC AND SUDORIFIC ACTIVITY

Extracts of the roots is used to treat hypertension in a Malay community. A paste of the roots is applied behind the ear to help relieve headache. Another way of relieving headache is by applying the root paste in the palate. Tincture of the root bark is an antiperiodic and a sudorific. The root cooked with meat in a soup is considered an aphrodisiac amongst the Zimbabwe people[75-80].

3 TOXICOLOGICAL STUDIES

3.1 Genotoxicity activity: Plumbagin, the most active compound in extracts of the roots of *P. zeylanica* was found to induce micronuclei at all doses studied (4mg/kg, 8mg/kg and 16mg/kg) and proved to be toxic to bone marrow cells of Swiss albino mice. Glutathione S-transferase (GST) activity was significantly inhibited by higher doses of plumbagin (8 mg and 16 mg/kg b.w.). While plumbagin by itself has genotoxic activity, the same investigator (Sivakumar et al) found that the alcoholic extract of the root of *P. zeylanica* in total proved to protect their Swiss albino mice from the genotoxic effects of cyclophosphamide. The extract instead significantly reduced the frequency of micronucleated polychromatic erythrocytes, increased the PCE/NCE ratio in the bone marrow, and decreased the levels of lipid peroxidation products with concomitant changes in the status of antioxidant. While plumbagin per se could cause genotoxicity, Demma et al proved that when in low dose (non-DNA damaging dose) in combination with NQNO or catechol, it could significantly reduce the catechol-induce DNA damage. This proves tha the non-DNA damaging concentration of plumbagin diminished the DNA damage induced by catechol by an antioxidative action[81-83].

3.2 Toxicity study on plant extract of Plumbago zeylanica used in Ethiopian traditional medicine Toxicity studies done by Teshome K et al [84] This study showed that the primary irritant index to be 2.00 in rabbits; sensitization test in mice showed it to be non-sensitizer in the dose range of 4 – 10 mg/ml; acute dermal toxicity test on rats did not produce any overt signs of toxicity except for a weight gain difference between the test and control groups of female rats. Repeated dose toxicity test was associated with increased relative testes weight as well as higher values for Blood Urea Nitrogen and K^+, an observation not supported by histopathological analyses.

4 CONCLUSION

It is evident from the review of the research that some aspects of Rasayan therapeutic benefits have not been focused yet for research like Adaptogen and Aphrodisiac. Similarly the research has just started in the immunomodulation and memory aspects and needs to be undertaken in detail. This comprehensive pharmacological screening will be very useful in future product development also, particularly for the life style diseases and disorders.

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