Research Article

Plumbago rosea L. – A Review on Tissue Culture and Pharmacological Research

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ABSTRACT

Plumbago rosea L. (Plumbaginaceae), an important medicinal plant used in codified and non-codified systems of medicine is reviewed with special emphasis on tissue culture and pharmacological studies. The plant is being used for centuries in Ayurvedic medicine and more than 30 herbal formulations were reported for the treatment of various ailments. *P. rosea* is highly amenable to tissue culture and this was established from various published reports by the ease use of nodes, internodes, shoot tips, leaves and roots as explants. Besides, callus, cell suspension, root and hairy root cultures were successfully established for the production of plumbagin. Quinones, polyphenols alkaloids and flavonoids are the major class of phyto-chemicals reported from the plant. Plumbagin a naphthoquinone is the major active compound of the roots which possesses many pharmacological properties in which anticancerous and pro-longevity activities are the most important. So far plumbagin is reported from 15 species and ten leading species were subjected to tissue culture studies *P. rosea* stands 2nd position for plumbagin production. The highest plumbagin enhancement (21 fold) was also achieved in *P. rosea* through immobilization and elicitation of cell suspension cultures.

Keywords: Plumbagin, *Plumbago rosea*, Tissue culture.

INTRODUCTION

Plumbago rosea L. (Plumbaginaceae) syn. *P. indica* L.[1], popularly called Chethikoduveli in Malayalam, Lal Chitrak in Hindi and Rose Leadwort in English is an important medicinal herb distributed in parts of South-East Asia. It is an erect or spreading half woody plant with red flowers, 1.5 m or less in height and traditionally used for treating lead poisoning and an eye disorder called plumbum.[2,3] Though originated in India (Sikkim and Khasi hills) and migrated to neighbouring countries, it is now mostly cultivated in South India, the Phillippines and tropical Africa (Kenya, Tanzania, Zimbabwe, Mozambique and Madagascar) for its medicinal roots.[4,3] Glands on the trichomes of calyx can produce digestive enzymes against certain stimulus and thus *P. rosea* may show carnivorous characters during flowering.[5] An additional function of trichomes is the exclusion of crawling insects from the flowers, which favors flying pollinators for effective cross pollination. Obviously due to over collection and habitat loss which is the order of the day, any natural populations of this species as well as its original intra specific diversity have declined and the species is no more available in most parts of India. It is not surprising therefore, that *P. rosea* is now reported to be rare in parts of India.[6,7,2]

Plumbago rosea is widely used in Ayurveda, Siddha, Unani and Homeopathy and also in uncodified ethnic preparations of the rural folks. Tuberous roots of the plants are reported to have many ethnobotanical uses such as treating oedema, piles, intestinal worms, skin disease, common wart, rheumatism, secondary syphilis, leprosy etc.[8-10,6] Root is a poison and juice of root is a powerful sudorifie. The roots form an ingredient of more than thirty ayurvedic preparations including Herbal extract mixes, Kumaryasawaya, Pippalyasawaya, Yogaraja guggulu, Mahamasha massage oil, Hinguvachadi, Indukanta etc.[7] Root extract is traditionally used as an abortifacient and antifertility medicine[11] while in Myanmar it is also used for leprosy and syphilis. In ayurvedic preparations the roots are used only after purification/curing to reduce toxicity. The root is also acrid, vesicant, alterative, digestive, stimulant, powerful abortifacient and also an oral contraceptive.[8] An ointment made from bruised root mixed with vegetable oil is used as a rubefacient to treat rheumatism and headache. Milky juice of the leaves is applied on the skin for the treatment of scabies, ringworm and hemorrhoids. In Java, the root is used in the veterinary medicine for expelling worms from horses. It is also useful in the early stages of leucoderma and baldness of head.[3] Members of Plumbaginaceae have also been widely investigated for anti-protozoal, anti-malarial activities.[12-14] Besides, different parts of plants have been traditionally used in folk medicines in India, China, and other Asian countries for the treatment of rheumatoid arthritis, dysmenorrhea and cancer. A plant specific active compound present prominently in roots and in traces in other parts forming a potential biomarker is plumbagin, a naphthoquinone responsible for many of the activities of the root drugs.

Conventional propagation of *Plumbago rosea* is done using stem cuttings or root suckers and each plant can produce 60-70 g root biomass in 18 months. It has been reported that the plant is shade-loving and is suitable for intercropping in coconut and rubber plantations.[15] Since the tuberous roots are destructively harvested, wild collections continued for generations resulted in depletion of the resource base.[16] The domestic market of *Plumbago* roots in India was 1,285 tones in 2004-2006

which increased at 10% per annum.[17] Requirement of *Plumbago* roots in Kerala is estimated to be 800 tones annually.[18] The cost of *P. rosea* tuberous roots in Kerala ranges from Rs.60-120/kg and its steady demand in the Ayurvedic industry led to domestication and commercial cultivation of the crop in Kerala.[2] At present this species is cultivated on commercial scale in hilly tracts of Kerala. While plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone), is the major bioactive compound of roots (0.9-1% gdw), the aerial parts contain steroids (sitosterol, stigmasterol and campesterol), quinines (Plumbagin and 6-hydroxy plumbagin) and flavanoides (plumbaginol). Some flavanoids such as plumbaginol- a di-hydroflavanol have also been reported in aerial parts of the plant.[19,20] Flowers of *P. rosea* are ornamental and cultivated in botanical gardens. The flowers contain pigments of 3-rhamnosides viz. pelargonidin, cyanidin, delphinidin, kaempferol together with a mono and di-galloylglucose.[21,22]

The marker compound, plumbagin ($C_{11}H_8O_3$) was first isolated in 1829[23] and a detailed study on its extraction, isolation, crystallization, solubility and chemical reactions is described.[24] Plumbagin was successfully synthesized through chemical process[25] and is a simple molecule with double benzene ring structure (Figure 1). The amount of plumbagin synthesized in plants varies according to growth, age and flowering as well as its locality, conditions of soil and season of the year. Plumbagin content is high in the roots of older plants grown in dry soil[6]. The plant when attacked by a holoparasite, *Cuscuta reflexa* produces large amounts of plumbagin around the haustoria ultimately killing the parasite along with some of its own cells.[26] Plumbagin is a naphthoquinone in yellow needle shaped crystals with a melting point ranging 78-79^{0}C. It is soluble in alcohol, acetone, chloroform, benzene, and acetic acid and slightly soluble in hot water.[27] India owns two process patents for the production of plumbagin.[28,29] There are 91 patents involving plumbagin from the United States patent office and 34 patents registered in European patent office (Source: Chemspider.com).

As a root drug, root extracts of *P. rosea* and two other *Plumbago* spp. in the coastal and eastern regions of Kenya display wide spectrum of activities.[30] *In vitro* tests showed presence of one or more anti mutagens. The ethanol extract of the leaves showed activity against herpes simplex virus type 1 (HSV-1).[31,3] Root extract is also effective against filarial parasite in cattle and is reported to be used as a macrofilaricide.[32] Additionally, pure plumbagin has many pharmacological properties of which anticancerous[33,34] and antimicrobial activities are most important.[35,36,31] However, *E.coli* is resistant to plumbagin.[37] Plumbagin extends lifespan at low doses in *Caenorhabditis elegans*[38] and inhibits tumour angiogenesis.[39]

Plumbagin is also toxic as it generates superoxide anion reactive oxygen species that can damage various biomolecules.[40] It is a powerful irritant in small doses and stimulates the central nervous system. Large doses may cause death from respiratory failure and paralysis. Plumbagin in low doses inhibit cell mitosis and in higher concentrations exhibited radiomimetic, nucleotoxic and cytotoxic effects.[41] It arrests cell cycle and induces apoptosis through reactive oxygen species in human melanoma A375.S2 cells.[42] Plumbagin is successfully labeled with ^{125}I using chloramine–T method. ^{125}I exhibited significant uptake in tumors of Swiss albino mice bearing fibrosarcoma indicating the potential of iodinated plumbagin in tumor therapy.[43] Plumbagin has also shown anti-implantation and abortifacient activities in rats. Clinical studies revealed that topical application of plumbagin isolated from *P. rosea* is useful in patients with common wart.[10] At low doses, plumbagin has significant tumour inhibitory effects against *Ehrlich ascites* carcinoma in mice.[31,3] Plumbagin possess selectivity towards cancer cells and does not damage normal epithelial, lung and cervical cells which is a desirable attribute.[44-47] The pharmacokinetics of plumbagin is carried out in rats and oral bio-availability of the drug has also been studied to satisfactory extent.[48]

Plumbago rosea is a medicinal plant mainly cultivated for its tuberous roots in South India and has become rare in wild in several parts of India.[6,7,2] This review is aimed for highlighting the recent progress in conservation and sustainable utilization of this important medicinal plant.

Flowering plant of *Plumbago rosea* L.

Morphology of the plant

Plumbago rosea L. (family-Plumbaginaceae) is a perennial herb or small shrub grown up to 1-1.5 m tall. Stem is erect, trailing or climbing, simple or branched from the base (Figure 1). The leaves are alternate, simple, entire and stipules absent. The petiole is short and the auricles are absent. The leaf blade is narrowly ovate to elliptical-ovate in shape measuring 5 cm-15 cm x 2 cm-8 cm. The base rounded to obtuse in shape, apex acute, papery. The inflorescence is an elongated spike or raceme, many-flowered, measuring 10-30 cm long, glabrous. The bracts ovate in shape measuring 2–3 mm long, apex acuminate. The peduncle measures 2–10 cm long. The flowers are bisexual, regular and pentamery. The pedicel measures 0-1 mm long. The calyx is tubular in shape measuring 8-9 mm long, glandular and red in colour. The corolla tube measures 2.5-4.5 cm long. The lobes obovate in shape measuring 1.5-3 cm in diameter, apex rounded,

mucronate, purple to red in colour; stamens free, exserted; ovary superior, ellipsoid-ovoid, 1-celled, style filiform, stigma lobes 5, seeds absent.[2,3]

Conventional propagation practices

Plumbago rosea is propagated vegetatively by stem cuttings or root tubers. Menon and Nybe[49] attempted on increasing root production in *P. rosea* and successfully achieved enhanced root production by dipping the stem cuttings in auxins. This shade loving plants suitability for intercropping in coconut and rubber plantations were also studied.[15] Detailed studies on various agro-techniques including propagation, planting, land preparation, manuring, after-care, crop protection, harvest and post harvest handling have been published by various workers.[50,2]

Medicinal properties

Many ethnobotanical uses of tuberous roots of *P. rosea* such as an abortifacient and treating oedema, piles, intestinal worms, skin disease, common wart, rheumatism, secondary syphilis, leprosy was reported. The roots form an ingredient of many ayurvedic preparations.[6-11] It was reported that in ayurvedic preparations the roots are used only after reducing the toxicity through purification/curing and several traditional purification methods have been reported.[9,51,52] A monograph on *P. rosea* has been published from Sri Lanka describing its traditional usage, pharmacognosy, phytochemistry and pharmacology.[53] The root has many other properties and is also useful in the early stages of leucoderma and baldness of head.[3]

Tissue culture studies on *P. rosea*

The first report on *in vitro* studies on *Plumbago rosea* was the production of callus, roots, shoots and inflorescence/flower using internodal explants by Nitsch and Nitsch[54] in 1967 and established the synergistic effect of adenine with various cytokinins in bud formation of *P. rosea* using internodal segments.[55] Floral induction using 2-chloroethanephosphonic acid was also reported in *in vitro* shoots.[56] Studies on *in vitro* regeneration of *P. rosea* have suggested the need for the development of a package for production of quality planting material on sustainable basis to support cultivation.[57,58] Chetia and Handique[7] substantiated the work of Nitsch, *et al*[55] in that addition of adenine sulphate promoted high frequency shoot multiplication. Reports are available on regeneration of plants without callusing on leaf explants and regeneration of plants *via.* somatic embryogenesis.[59,60] *In vitro* regeneration of plants through indirect organogenesis and their performance under field cultivation leading to early harvest of tuberous roots has been reported earlier.[61] The effect of mannitol on slow growth of *in vitro* cultivated plants was successfully reported for *in vitro* conservation of *P. rosea*[62]. Komaraiah[63], reported the use of acetyl salicylic acid for the induction of somatic embryogenesis in cell suspension and callus cultures. Later a complete package for culture initiation up to cultivation and production leading to consistent production and supply of planting materials was developed through direct organogenesis.[64-66] Regeneration of plants on *in vitro* derived aseptic leaf explants was also reported.[67] Regeneration of microshoots on the leaf explants of field grown plants without callus phase was reported[68] and a high frequency regeneration of plants on tuberous roots of the field grown plants was also reported.[69]

Plumbagin production through callus cultures was first reported in *P. zeylanica.*[70] The use of cell cultures for plumbagin production was established in *P. rosea.*[71,72] Influence of plant hormones and selection of stable cell lines for accumulation of plumbagin was reported by Komaraiah.[73] Different strategies such as optimization of media components, cell line development by single cloning, elicitation, permeabilisation, immobilization, *in situ* extraction of products and cultivation of cells in modified bioreactors were reported for the production of plumbagin in *P. rosea* with 20-25 times increase in plumbagin production.[71,74,75] Studies show that plumbagin production is growth associated and maximum production is obtained in the logarithmic phase of root growth.[75]

Role of carbon source (sucrose) was well demonstrated for plumbagin production in the cell cultures of *Drosophyllum.*[76] Bioproduction of plumbagin was reported through multiple shoot culture of *Drosera*, an insectivorous plant.[77] Attempts were done for enhancing plumbagin production in *in vitro* cultures *via*. elicitor, somatic embryogenesis and immobilization of cells. The first report in this line was in *Drosophyllum lusitanicum*[78] which increased plumbagin production was achieved by adding chitin as elicitor. Later, 21 fold increases in plumbagin production was achieved by coupling immobilization and elicitation techniques. Komaraiah[74] reported the use of elicitors such as yeast extract and chitosan for the enhanced production of plumbagin in cell cultures of *P. rosea*. It was also demonstrated that plumbagin synthesis was found more in embryogenic cell suspension cultures than non- embryogenic cultures of *P. rosea.*[63]

Elicitors such as yeast-extract and chitosan enhanced the synthesis of plumbagin in cell suspension cultures.[74] Plumbagin accumulation in acetyl salicylic acid induced embryogenic suspension culture was thrice higher than the non embryogenic cells in suspension cultures.[63] Perusal of literature illustrated list of medicinal plants screened for plumbagin and methods employed for *in vitro* production of the compound (Table 1 and 2).

The first report on establishing adventitious root cultures of *P. rosea* for the production of plumbagin was by Satheeshkumar.[90] Later, establishment of root cultures using young leaf segments was also reported.[82] Induction and establishment of transformed hairy root culture of *P. rosea* using different strains of *A. rhizogenes* (ATCC 15834, A4 and LBA 9402) for the production of plumbagin

has been reported.[83,91] In addition influence of exogenous phytohormones on growth and plumbagin accumulation in hairy root cultures has been investigated and established the significant role of gibberellic acid on highest growth of roots and yield of plumbagin with NAA.[89]

Table 1: List of plants used for tissue culture leading to plumbagin production

Plants	Type of culture system/Source	Plumbagin % (g DW)	Reference
Dionaea binata	*In vitro* plants	1.4	Hook[79]
Dionaea capensis	*In vitro* plants	0.5	
Dionaea muscipula	*In vitro* plants Cell suspension	5.3 2.59	
Drosera capensis	*In vitro* plants	0.0048	Crouch[80]
Drosera intermedia	*In vitro* plants	0.274	Grevenstuk and Gonçalves[81]
Drosera natalensis	*In vitro* plants	0.025	Crouch[80]
Drosera rotundifolia	*In vitro* plants	0.6	Hook[79]
Drosophyllum lusitanicum	Cell Suspension cultures	0.08 f.w	Nahalka[76]
Plumbago rosea	Callus cultures	0.005	Satheeshkumar and Seeni[72]
	Root cultures	0.020	Pharkphoom[82]
	Callus cultures	0.02-0.035 f.w	Komaraiah[73]
	Roots of tissue culture derived plants established in the field	1.5	Satheeshkumar and Seeni[61]
	Hairy root cultures	0.069	Gangopadhya[83]
Plumbago zeylanica (*P. scandens*)	Callus cultures	0.0001-0.003 fw	Heble,[70]

Table 2: List of plants subjected to enhanced production of plumbagin under *in vitro*

Plant	Culture System	Methodology	Agent	Increase of plumbagin (in fold)	Total plumbagin (% Dry weight)	References
Drosera burmani	Whole plant	Elicitation	Yeast extract	3.5 (roots)	0.88	Putalun[84]
Drosera indica	Whole plant	Elicitation	Yeast extract	5.4	0.269	Thaweesak[85]
Drosophyllum lusitanicum	Cell suspension	Elicitation	Chitin	1st report	Not reported	Nahalka[78]
Nepenthes Khasiana	Root	Elicitation	Chitin	1.6	2.17	Raj[86]
Plumbago rosea	Cell	Elicitation	Chitosan	6.7	2.887	Komaraiah[72]
P. rosea	Cell	Elicitation, immobilization and *in situ* adsorption	Chitosan	21	9.213	Komaraiah[73]
P. rosea	Cell Suspension	Somatic embryogenesis	Acetyl salicylic acid	3		Komaraiah[63]
P. rosea	Callus	Elicitation	Copper Sulphate	2		Karadi[87]
P. rosea	Hairy root	Elicitation	Methyl Jasmonate	5	4.6	Martin[88]
P. rosea	Hairy root	Elicitation	Chitosan+ Methyl Jasmonate	2.2	2.856	Gangopadhya[89]

Table 3: Phytochemicals isolated from different parts of *Plumbago rosea*

Plant part	Chemical group	Name of compounds	
Root	quinones	plumbagin, roseanone (binaphthaquinone) Dinda[92], 6-hydroxy plumbagin, droserone, elliptinone (Dinda[36]), plumbagic acid lactone (Dinda[93]).	
	polyphenols	flavonoides	flavonyl methyl ethers–ayanin and azaleatin (Dinda[93]) arachidyl alcohol (Kurian and Sankar[2]), myricetin-3,3′,5′,7-tetra methyl ether, ampelopsin-3′,4′,5′,7-tetramethylether (Ariyanathan [94])
		carboxylic acids	plumbagic acid , roseanoic acid (Ariyanathan[94])
	alkaloids	α-naphthylamine (Kurian and Sankar[2])	
	aliphatic acids	myricyl palmitate, palmitic acid (Dinda[93])	
	steroids	β-sitosterol (Kurian and Sankar[2])	
Aerial part	steroids	campesterol, beta-sitosterol, stigmasterol (Dinda and Chel[19]; Kurian and Sankar[2])	
	quinones	plumbagin, 6-hydroxyplumbagin (Dinda and Chel[19]; Kurian and Sankar[2])	
	flavonoids	plumbaginol (dihydroflavanol) (Dinda[20] ; Kurian and Sankar[2])	
Flower	pigments	pelargonidin, cyanidin, delphinidin, kaempferol (Harborne[21])	
	carbohydrates	mono and di-galloylglucose (Harborne[22])	

Table 4: A list of plants containing plumbagin

Name	Family	Part	% of plumbagin (g.D W)	Reference
Aristea ecklonii	Iridaceae	Rhizome Leaves	Not quantitated	Kumar[98]
Ceratostigma willmottianum	Plumbaginaceae	Rhizome Stem Leaves	1.1(g.fw) 0.02 0.03	Shcherbanovskii and Lukes[99]
Diospyros olen	Diospyraceae	Bark	Not quantitated	Evans[96]
Drosophyllum capensis	Droseraceae	Whole plant	0.0004 (g.fw)	Crouch[80]
Drosera burmannii	Droseraceae	Whole plant	Not quantitated	Madhavan[100]
Drosophyllum lusitanicum	Droseraceae	Whole plant	0.242	Grevenstuk[101]
Drosophyllum natalensis	Droseraceae	Roots Whole plant	0.112 0.223	Crouch[102]
Nephenthes khasiana	Nepenthaceae	Roots	1.3	Raj[86]
Nephenthes thorelii	Nepenthaceae	Roots	0.092	Likhitwitayawuid[103]
Nephenthes ventricosa	Nepenthaceae	Leaves	0.51	Shin[104]
Plumbago angustifolia/ P. europea	Plumbaginaceae	Roots	1.5-2.5 (2-3 year old)	Nahalka[76]
P. capensis / P. auriculata	Plumbaginaceae	Roots Roots	0.429 0.15	Dorni[14], Itoigawa[105]
Plumbago rosea/ P.indica	Plumbaginaceae	Roots Roots	0.9-1.0 0.569	Dinda[36] Dorni[14]
Plumbago zeylanica/ P. scandens	Plumbaginaceae	Roots Roots Roots	0.26 1.34 0.247	De-Paiva[31] Hsieh[106] Dorni[14]
Sparaxis tricolor	Iridaceae	Not reported	Notquantitated	Harborne[107]

Phytochemical constituents

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), is the major bioactive compound (0.9-1% g.dw) present in the roots of *P. rosea* (Fig 2). Other naphthoquinones reported are 6-hydroxyure plumbagin, roseanone (binaphthoquinone), droserone, elliptinone and plumbagic acid lactone.[92,36,93] Polyphenols such as flavanoids (ayanin, azaleatin, arachidyl alcohol, myricetin-3,3′,5′,7-tetra methyl ether, ampelopsin-3′,4′,5′,7-tetramethyl ether) and carboxylic acid (plumbagic acid, roseanoic acid) were also isolated from the roots.[93,2,94] Alkaloids (α-naphthylamine), benzenoids (m-dinitrobenzene), aliphatic acids (myricyl palmitate, palmitic acid), steroids (β-sitosterol and its glycoside) are the other compounds present in the roots. The phytochemicals isolated from different parts of *P. rosea* are summarized (Table 3).

The aerial parts contain steroids (sitosterol, stigmasterol and campesterol), quinones (plumbagin and 6-hydroxy plumbagin) and flavanoids (plumbaginol). Some flavanoids such as plumbaginol (di-hydroflavanol) was also reported in aerial parts of the plant. [92-95]

The flower of *P. rosea* contains pigments of 3-rhamnosides (pelargonidin, cyanidin, delphinidin and kaempferol) together with a mono and di-galloylglucose.[21,22]

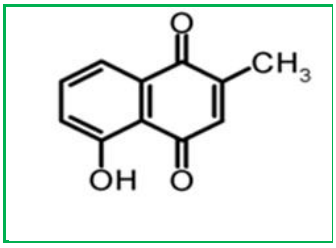

Figure 2: Structure of plumbagin

Origin and distribution of plumbagin

A new glimpse to the origin and distribution of plumbagin was obtained from the report on isolation and identification of plumbagin from *Diospyros olen* (Ebenaceae), a plant indigenous to New Caledonia. New Caledonia is considered as one of the world's most critically endangered and botanically most important hotspots as it was a part of the ancient megacontinent "Gondwanaland" which got split into parts of present day Antarctica, Chile, New Zealand, New Caledonia, Australia, New Guinea and other small isolated islands. As it remained isolated from the rest of the world's landmasses, it preserves a snapshot of Gondwanan forests. While the original plant source of plumbagin, *Diospyros*, is indigenous to the old world it is still retained in related plants after a possible period of 350 million years.[96]

Plumbago rosea accumulates maximum plumbagin in the roots, whereas in *P. auriculata* and *P. zeylanica*, high yields are from the leaf and stem parts respectively.[97] Though plumbagin is the characteristic feature of three families Plumbaginaceae, Droseraceae and Ebenaceae, some other plants of the families Ancestrocladaceae and Dioncophyllaceae also possess plumbagin (Table 4).

Plumbagin is also present along with a series of other structurally related naphthoquinones in the roots, leaves, barks and wood of *Juglans regia* (English walnut, Persian walnut, and California walnut), *Juglans Cinerea* (butternut and White walnut) and *Juglans nigra* (Black walnut).[108,109]

Isolation and characterization of plumbagin

Although plumbagin was first isolated in 1829[23], a detailed study on its extraction, isolation, crystallization, solubility and chemical reactions were reported by Thomson.[24] Isolation of plumbagin using petroleum ether was reported by Roy and Dutt.[110] Katti and Patwardhan[111] isolated plumbagin from the root barks. Budzianowski[112] described the isolation of plumbagin and some rare naphthoquinone glucosides from the shoots of *Drosera gigantean*. Different extraction techniques were reported in *P. scandens* and plumbagin was isolated through solvent extraction.[31] Centrifugal liquid–liquid partition chromatography (counter-current chromatography) was used to isolate plumbagin from *P. auriculata* using hexane: ethyl acetate: methanol: water (40:10:10:2, v/v) as solvent system.[31] The solubility of plumbagin in supercritical carbon dioxide has been determined spectroscopically.[113] Several published reports are available for the isolation of plumbagin.[80,114] A Thin Layer Chromatography method for determination of plumbagin in hairy root cultures of *P. rosea* was reported by Yogananth and Basu.[16]

An HPLC method for the isolation of plumbagin was reported in *Drosera* sp.[80] A rapid, accurate and simple HPLC method for estimation of plumbagin was developed by Gupta.[115] HPTLC method was also developed for isolating plumbagin from *Drosera burmanii.*[100] Quantification of plumbagin was determined through HPTLC method and reported 0.17% in *P. rosea*, 0.04 in *P. capensis* and 0.01% in *P. zeylanica.*[94] An HPTLC method for isolation of plumbagin was also described by Pawar.[116]

Plumbagin was successfully synthesized through chemical process by Fieser and Dunn.[25] According to Mallavadhani[95] there are two pathways (Shikimic acid and mevelonic acid) involved in the biosynthesis of plumbagin. In higher plants, synthesis of plumbagin and other naphthaquinone (Isoshinanolone, droserone) is through acetate-malonate pathway.[117] The amount of plumbagin synthesized in plants is influenced by various factors including growth, age and flowering of the plant as well as its locality, conditions of soil and season of the year. Plumbagin content is high in older plants grown in dry soil.[6] Stress mediated production of plumbagin is also reported in *Ancistrocladus heyneanus* in which plumbagin synthesis is very high when the plant is attacked by a holoparasite *Cuscuta reflexa.*[26]

Physicochemical properties of Plumbagin

Colour, molecular weight and solubility of Plumbagin ($C_{11}H_8O_3$) were reported.[27] Plumbagin shows a spectrum with absorption bands at 212, 266, 410 and 423 nm.[76] The λ max of plumbagin in ethanol is 220 or 226 nm in the UV range and 418 nm in the visible range. The visible maximum of plumbagin in alkali is 526 nm.[107]

Pharmacological studies on Plumbagin

Malaria larvicidal and macrofilaricidal activity have been reported with root extracts of *P. rosea*[30,32] Activity against herpes simplex virus type 1 was reported with ethanol extract of the leaves of *P. rosea.*[31,3] Anti ovulatory and estrogenic activity was also reported with extracts from leaf.[118] The 24 h LD 50 values of ethanolic extract of *P. rosea* in mice were 239.88 mg and 1148.15 mg/kg body weight for intra peritoneal and oral routes respectively.[119] Anti cancerous and antimicrobial activities are reported by several workers.[31,33,35,36,120,121] Its action against HIV-1 has been reported by the Developmental Therapeutics Programme of the National Cancer Institute.[122] Prolongevity[38] and tumour angiogenesis inhibition was also reported.[39] The pharmacological activities are summarized (Table 5).

Table 5: Reported pharmacological activities of Plumbagin

Activity	References
Abortifacient	Sattar[123]
Antiarthritic	Poosarla[124]
Anticancerous	Parimala and Sachdanandam[125]; Sugie[126]; Aziz[127]; Nazeem[47]
Anticoagulant	Santhakumari[128]
Antifeedent	Tokunaga[129]
Antifungal	Shin[104]
Anti-*helicobacter pylori*	Wang and Haung[130]
Anti-inflammatory	Dorni[131]
Antimalarial	Likhitwitayawuid[103]
Antimicrobial	Premakumari and Santhakumari[132]; Nadkarni[35]; Dindry[133], Dinda[36]; De-Paiva[31]
Antioxidant	Tilak[134], Tan[135]
Antiviral	Min[136]
Cardiotonic	Itoigawa[105]
Insecticidal	Kubo[137]
Leishmanicidal	Iwu[138]
Microfilaricidal	Mathew[32]
Prolongevity	Hunt[38]
Radiosensitizing	Nair[139]
Tumor Angiogenesis inhibition	Lai[39]
Neuroprotective	Luo[140]

Plumbagin is also toxic as it generates superoxide anion reactive oxygen species that can damage various biomolecules.[40] Plumbagin is a powerful irritant in small doses; it is sudorific and stimulates the central nervous system. On comparison with insecticides like azadiractins, pyrethrum, nicotine, rotenone and essential oils, plumbagin were found to be very much promising to control insect pests and vectors.[30,141,142] Members belonging to Plumbaginaceae have also been widely investigated and established their anti-malarial, anti-protozoan activities.[143,14] However, *Escherichia coli* is resistant to plumbagin.[37] The mechanism of antifeedent activities in carnivorus plants were studied and reported that plumbagin is the candidate responsible for rescuing the plants from predators.[129] In *Nepenthes khasiana* plumbagin acts as a molecular trigger to prey capture and digestion.[86]

Emerging evidence suggests that the anti-cancer properties of plumbagin are mediated by the inactivation of Akt/NF-kB signaling pathway as well as inactivation of MMP-9 and VEGF that are considered to be important for the processes of invasion, metastasis and angiogenesis.[144,45]

Plumbagin induces oxidative damage as a consequence of their ability to undergo redox-cycling[145] with the generation of reactive oxygen species (ROS), which mediate apoptosis in cancerous cells.[146-155] Earlier studies also showed that plumbagin induces apoptosis in human lung cancer A549 cells through JNK/P[53] pathway[44] and caused autophagic cell death in breast cancer by P13K/AKT inhibition.[143] A copper redox- cycling reactive oxygen dependent DNA damage was also reported for its anticancer properties.[153] It has also been reported to inhibit the activity of Topoisomerase II.[154] Plumbagin is shown to inhibit platelet aggregation *in vitro* and *in vivo*, *via.* Suppressing the binding of activated platelets to neutrophils and increased inhibition of intact neutrophils on platelet reactivity.[155]

Clinical studies for drug design and development

Mitosis inhibition, radiomimetic, nucleotoxic and cytotoxic effects of plumbagin was established through clinical studies.[41,156] Cell cycle arrest and induction of apoptosis through reactive oxygen species in human melanoma cells was also reported.[42] Successful labeling of plumbagin with[125], significant uptake of tumors in Swiss albino mice bearing fibrosarcoma and the potential use of iodinated plumbagin in tumor therapy was reported by Bapat.[157] Clinical studies revealed that plumbagin is useful in patients with common wart.[10] Tumour inhibitory effects against *Ehrlich ascites* carcinoma in mice was studied and low doses of plumbagin have significant tumour inhibitory effects.[31,3] Leads for development of drugs using plumbagin, especially for cancers, were obtained based on clinical studies. The anti-cancer effects of plumbagin have been reported in diverse cancer models such as prostate,[150] lung[44,158], cervical[159,139], ovarian[160], melanoma[42] and breast cancer including triple negative breast cancer.[143,161] Selectivity of plumbagin towards cancer cells was studied by several workers.[44-47] Pharmacokinetics and oral bio-availability of plumbagin in rats was also reported.[48]

CONCLUSION

The published literature on *P. rosea* showed that, the plant has many potential therapeutic uses including the treatment of cancer. *P. rosea* as a good experimental system like *Nicotiana tobaccum, Dacus carot*a and *Arabdosis* etc for various tissue culture related studies. Considering the immense medicinal and commercial potential of the species, there is an urgent need to conserve this medicinal plant for production and supply of quality planting material and mass production of the root drug. From this review it can be concluded that, the plant can be a good source of an anticancer drug. Future research with respect to clinical studies needs to be carried out to explore its uses.

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