

Propagation, phytochemistry and pharmacology of *Plumbago indica* - a review

ABSTRACT

Aims: *Plumbago indica* is a medicinal plant widely grown in the tropics and temperate region to use in traditional systems of medicine. Different parts of this plant are used to treat rheumatoid arthritis, dysmenorrhea, cancer, leprosy, syphilis, rheumatism, paralysis, headache, leukoderma, enlarged glands, scabies, ophthalmia, dyspepsia, haemorrhage, piles, flatulence, loss of appetite etc. Various kinds of researches were carried out in different countries on propagation and pharmacology of this medicinal plant. Scientifically proven data were concentrated on this paper to facilitate reliable convenience data source and encourage further studies.

Methodology: Scientific data published on full papers, abstracts about morphology, growth, propagation, traditional medicinal uses and pharmacology of *P. indica* over 70 years (1949-2020) by researchers in different countries were collected and categorized under suitable topics.

Results: According to literature *P. indica* leaves, stems, roots contain phytochemical compound that are responsible for its medicinal properties. Tissue culture of this plant can be used as effective propagation method to fulfill the increasing demand of raw materials (dried plant parts) for medicinal preparations as well as preserve the plant in their natural habitat.

Value: *P. indica* is used to treat vast range of diseases in traditional medicinal systems in different countries and currently pharmacological experiments are conducting to prove it scientifically. Plumbagin present in *P. indica* roots was already identified as a potential anti-cancer agent and subjected to more research interest. Therefore this review article helps new researchers to get wide knowledge about the plant and its pharmacology.

Keywords: Plumbaginaceae, *Plumbago indica*, Plumbagin, anti-cancer, pharmacology

1. INTRODUCTION

Genus *Plumbago* belongs to family Plumbaginaceae that has about 24 genera and about 400 species. Genus *Plumbago* comprises of 18 species that grow as shrubs or perennial herbs. *Plumbago amplexicaulis* Oliv., *Plumbago aphylla* Bojer ex Boiss., *Plumbago auriculata* Lam., *Plumbago ciliate* Engl., *Plumbago caerulea* Kunth, *Plumbago dawei* Rolfe, *Plumbago europaea* L., *Plumbago glandulicaulis* Wilmot-Dear, *Plumbago indica* L., *Plumbago madagascariensis* M. Peltier, *Plumbago montis-elgonis* Bullock, *Plumbago pearsonii* (L.) Bolus, *Plumbago pulchella* Boiss., *Plumbago stenophylla* Wilmot-Dear,

Plumbago scandens L., *Plumbago tristis* Aiton, *Plumbago wissii* Friedr. and *Plumbago zeylanica* L. are the species that belong to genus *Plumbago*. Hairy calyx is a characteristic feature of this genus [1,2]. These species are native to warm temperature to tropical regions of the world [3] and have wide geographical distribution that some of species grow under saline or limestone conditions [4]. *P. indica* is considered as one of the most important medicinal plants belongs to Family Plumbaginaceae. *P. indica* is an erect or spreading half woody plant widely used in Ayurveda, Siddha, Unani and Homeopathy. This species is originated in Sikkim and Khasi hills of India and migrated to other neighboring countries including Sri Lanka [5]. *P. indica* is cultivated as a medicinal plant as well as an ornamental plant throughout the tropic and temperate region [6]. *P. indica* is widely cultivated in South India, Philippines, Kenya, Tanzania, Zimbabwe, Mozambique, Madagascar [5], Africa, Europe, Indonesia, China, Malaysia and Arabian Peninsula for harvesting its tuberous roots for medicinal purpose [7].

P. indica has wide range of pharmacological activities against many diseases. Currently plumbagin is mainly extracted from *P. indica* roots [8]. As plant does not produce seeds, shoot cuttings are mainly used for the vegetative propagation [9]. This plant has a slow growth and take considerably long period of time for roots to be ready for medicinal purposes [8]. *P. indica* rapidly decline from their natural habitat due to over exploitation for commercial uses [10]. Normally distractive harvesting is done as the root system of this plant used in preparation of traditional medicines [9]. Therefore *in vitro* plant propagation methods are used as an alternate strategy for sustainable conservation and propagation of *P. indica* [10].



Plate 01: *P. indica* (a) Plants (b) Flowers (c) Tuberous roots

P. indica plays a vital role in traditional medicine because it contains different types of active organic compounds which have therapeutic effect on several diseases [11]. Roots are used after purification to reduce toxicity in Ayurvedic preparations. India, China and other Asian countries traditionally use different parts of *P. indica* to treat rheumatoid arthritis, dysmenorrhea and cancer. Root extract is traditionally used to treat leprosy and syphilis in Myanmar [5]. Juice of leaves and roots is mixed with vegetable oil to prepare ointment for rheumatism, paralysis, leprosy [12], headache [5], leukoderma, enlarged glands and scorpion-sting [13]. Milky juice of this plant is used for treat scabies, leukoderma [12], ophthalmia and as an antiseptic agent [13].

There are number of reports that roots of this plant are used to treat early stages of leukoderma, baldness of head. Roots are used in Java as veterinary medicine for expelling worms from horses. Root extract is used as microfilaricide because it is worked effectively against filarial parasite in cattle [5]. Tincture of the root is used to treat secondary syphilis, leprosy, dyspepsia, hemorrhage, piles, flatulence, loss of appetite and other digestive complaints. The roots or root extract of this plant is used as abortifacient, antifertility medicine and oral contraceptive [13, 14, 15]. The roots of *P. indica* ingested directly to cervix or applied as a paste using abortion stick. It is also rarely used as homicidal poison [7]. *P. indica* root extract is used to prepare adaptogenic drug for cancer patients in traditional medicinal treatments in Thailand [16]. Due to this vast range of medicinal uses of *P. indica*, these plants are over exploited from their natural environment and subjected to severe threat of disappearance from its natural habitats. Therefore, cultivation of *P. indica* using either conventional propagation or tissue culture should be increased to fulfill the commercial demand. Scientifically proven data regarding different propagation techniques were concentrated in this paper to encourage the production of plant materials through cultivation. According to literature, *P. indica* has been widely using in different traditional medicinal systems throughout the world. Currently pharmacological experiments are conducting to prove its different biological activities scientifically. Important findings of pharmacological researches were summarized in this review article to help new researchers to get wide knowledge about its pharmacology.

2. MORPHOLOGY

P. indica is perennial herb or small shrub grows well under warm tropical climate. These small shrubs are grown up to 1.0 – 1.5 m height and stem is erect, trailing or climbing. The stem is simple or branched from the base [5]. Leaves are about 10 cm in length, ovate-elliptic in shape, simple, having alternate arrangement with entire margine and exstipulated. Base of the leaf is tapering in to a short somewhat clasping petiole [12].

Bisexual, regular and pentamery flowers are red in color and inflorescence on glabrous elongated spike or raceme which has 10-30 cm length. Flowers have ovate shaped 2-3 mm bracts and 8-9 mm long, tubular shape, glandular and red calyx. Five sepals are fused to form the calyx [5]. Five petals are fused in to 2.5 cm long, spreading, apiculate, tubular silverform corolla. Filaments of stamens are as long as the corolla tube. Anthers are exerted just beyond the throat. Ovary is superior, five carpellary, unilocular with basal placenta and one anatropous ovule. There are five stigmas on about 2.2 cm long style. Pollinated flowers form membranous circumscissile capsule which enclosed by persistent calyx [12]. *Plumbago* species show carnivorous behavior during flowering. Glands on *Plumbago* sepals produce resinous secretion that helps to capture small insects. *P. indica* is capable of producing Proteases in response to the stimuli as insects or decomposing insects [17]. Fruit and seeds are not known in this species. Therefore vegetative propagation is the main propagation method, thus in nature this species shows a low genetic variability [6].

P. indica has cylindrical irregularly bending stout root system that has short transverse shallow fissures at the regions of the bents. Roots have light yellowish brown color smooth surface which exude juice from fresh cut surface. Healthy *P. indica* plant can produce 18-20 stout roots per plant [18]. Roots turn in to dark brown to blackish brown when dry and release a characteristic strong pungent odor [19].

3. PROPAGATION

3.1 Conventional propagation of *P. indica*

There are number of reported conventional propagation methods. In some instances *P. indica* is used as intercropping in coconut and rubber plantations [20]. *P. indica* is one of medicinal plant that has high adaptability to grow under low light intensity [21]. Stem cutting (15 cm), leaf bud cutting, cutting with two internodes, shoot tip cutting, stump cutting were tested and observed the number of days for root initiations, shoot emergence and to attain 30 cm height. Shoot tip cutting method was taken minimum days for root initiations (12.33 days), shoot emergence (7.38 days), attain 30 cm height (129.00 days) and produced highest number of roots (27.17 per plant) after six months when compare with other conventional propagation methods [22].

Two node semi hard wood cuttings were initially planted in polybags containing a potting mixture. After three months, rooted cuttings were planted at a spacing of 70 cm x 15 cm x 50 cm (length, width and height) ridges as intercrop of 20 year old coconut plantation. Maximum fresh (88.63 g/plant) and dry root weights (22.73 g/plant) were observed 18 months after planting. Cow dung (8 tons per hectare) was used as basal dressing at the time of planting. Top dressing was done by using 25: 25:25 kg per hectare two months after planting. Second dose of cow dung (8 tons per hectare) was applied after ten months of planting [20]. This species can be used as intercrop with mature strands of *Hevea* species without affecting the latex yield of the plants. *P. indica* has given higher yield when it grows as intercrop in coconut plantation than growing in open area [21]. Semi-hardwood two node cuttings have shown higher survival (84%) one month after planting when it dipped in 500 ppm IBA solution for 30 seconds and highest number of roots (4.37) was observed after three months in stem cuttings that dipped in 500 ppm Indol-3-butyric acid (IBA) solution for 60 seconds. Stem cuttings that planted after dipping in distilled water for 60 seconds has only shown 60% survival after one month and 2.29 roots per stem cutting after three months [23].

P. indica grows well in rich, moist and well drain soil containing areas which have 25 °C – 35 °C with the pH of 5.5-6.0. Plant shows stunted growth when it grows below pH 5.0 or above pH 7.0. High yield of root can be obtained when plant uprooted between 12 to 18 months after planting in the field [6].

3.2 Tissue culture of *P. indica*

Plant tissue culture is a technique which uses to grow plant from cells, tissues or organs that obtain from mother plants on sterile growth media containing all necessary nutrients and plant growth regulators under controlled environment conditions [24]. Murashige and Skoog (MS) medium, Linsmaier and Skoog (LS) medium, Gamborg (B5) medium and Nitsch and Nitsch (NN) medium are reported to be media used frequently for tissue culture. Not only that plant growth regulators used by different researchers also varies depending on the explant used [25].

Callus produced by *in vitro* propagation could either be vegetative or embryonic. Induction of callus is the first step in indirect organogenesis. Callus induction was achieved by culturing *P. indica* inter nodal segments in MS medium supplemented with 2.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.5 mg/L Kinetin (Kn) [26]. Light green nodular calli have been produced by nodal explants in MS media supplemented with 3.0 mg/L 6-Benzylaminopurine (BAP), 1.5 mg/L Kn and 1.0 mg/L 1-Naphthaleneacetic acid (NAA) or 2.0 mg/L BAP and 0.5 mg/L picloram or 3.0 mg/L BAP and 2.0 mg/L Kn. White friable calli (embryonic callus) were produced by MS medium supplemented with 1.5 mg/L BAP, 0.5 mg/L Indole-3-acetic acid (IAA) and 1.5 mg/L 2, 4-D or 2.0 mg/L BAP or 2.0 mg/L BAP and

2.0 mg/L IAA from nodal explants. Leaf explants have produced white friable callus in MS media supplemented with 1.5 mg/L BAP, 0.5 mg/L IAA and 1.5 mg/L 2, 4-D or 2.0 mg/L BAP or 2.5 mg/L BAP and 1.0 mg/L NAA [11]. Greenish yellow friable callus development from stem segments cultured on MS medium supplemented with 1.0 mg/L 2,4-D and 0.1 mg/L BAP has observed within 14 days [27]. Highest callus induction percentage from leaf disc explants (82.4 ± 1.1) was observed in MS medium supplemented with 2.0 mg/L NAA and 0.25 mg/L Kn. However, calli turned into brown after eight weeks of culturing and this condition was controlled by adding 0.25 mg/L ascorbic acid to the media [28]. Somatic embryogenesis is the formation of plants through embryo like structures from somatic callus. MS medium supplemented with 1.5 mg/L Kn, 0.5 mg/L GA3 and 0.1 mg/L NAA was optimum for embryo formation (86.8 ± 0.8) and highest number of somatic embryos per culture was reported as 66.4 ± 0.6 . MS medium supplemented with 0.10 mg/L Kn was gave higher germination percentage (62.0 ± 1.1). Initial induction of embryogenic phase was strongly depended on auxin, cytokinin and gibberellic acid concentrations. However, auxin and gibberellic acid were not required for subsequent growth and development of embryos into plantlets [28]. Acetylsalicylic acid was used to induce somatic embryos from cell suspension culture of *P. indica*. MS medium supplemented with 8.32 μ M ASA and 5.06 μ M IAA was gave higher number of somatic embryos per culture (216) [29].

Shoot induction from the callus was achieved by culturing callus on MS medium supplemented with high concentrations of cytokinins. Calli induced shoots in the presence of 2.0 mg/L BAP and 1.0 mg/L NAA [26]. Green color nodular calli were produced by MS media supplemented with 3.0 mg/L BAP, 1.5 mg/L Kn and 1.0 mg/L NAA or 1.5 mg/L BAP, 0.5 mg/L IAA and 1.5 mg/L 2, 4-D or 3.0 mg/L BAP, 2.0 mg/L Kn and 1.0 mg/L NAA or 2.5 mg/L BAP and 2.0 mg/L NAA or 2.5 mg/L BAP, 1.5 mg/L Kn and 1.5 mg/L NAA and those calli were kept in the same media for 45 days to undergo differentiation [11].

BAP was more effective for shoot proliferation than kinetin. MS medium supplemented with 2.0 mg/L BAP was given the highest number of shoots per nodal explant (5.2 ± 0.4) [27]. In the presence of 1.5 mg/L BAP and 0.3 mg/L NAA nodal explants gave 80% shooting response with highest number of shoots per explant (13.62 ± 0.8) [30]. Nodal explants cultured on MS medium supplemented with 1.5 mg/L BA and 1.0 mg/L IAA have given earliest bud initiation (7.94 ± 0.22 days), maximum shoot length (5.33 ± 0.19 cm) and Maximum shoot proliferation (5.28 ± 0.26 shoots) [31]. Shoot induction was also done by using leaf explants. Leaf explants which cultured on MS medium supplemented with 6.66 μ M BA and 2.69 μ M NAA gave highest shoot induction percentage (80.0 ± 6.0), highest number of shoots per explant (105.0 ± 0.3) and highest shoot length (3.1 ± 0.30) after 35 days of culture [32]. Nodes on the stem that were in one to five positions have negligible difference in shoot forming frequency and number of buds form. However, shoots emanated from youngest nodes were shorter (0.92 ± 0.19) cm than mature nodes (2.3 ± 0.50) [33]. *In vitro* conservation of shoot buds was achieved by using Mannitol. Shoot buds heights were reduced up to 83.1% in media with PGR and 93.3 % in media without PGR when Mannitol was added to the culture media. Mannitol (20 g/L) has the ability to conserve shoot buds in same media for eight months without any dead plantlet [34].

Micro shoots which cultured on MS medium supplemented with 1.11 μ M BA and 1.44 μ M GA3 gave the highest percentage of shoot elongation (65.0 ± 5.0) and highest shoot length (2.0 ± 0.0 cm) [32]. Shoot elongation was also achieved by auxin and cytokinin. Shoot buds that cultured on MS medium supplemented with 0.5 mg/L BAP and 1.0 mg/L IAA have given highest shoot length (3.23 ± 0.13 cm) [11].

It is reported that, MS medium supplemented with 0.5 mg/L IBA alone gave the highest root differentiation percentage (80.0 ± 5.0) and the highest dry root weight (4.3 ± 0.0 g). The highest fresh root weight (28.1 ± 0.01 g) was observed with 0.5 mg/L IAA [32]. Half MS medium supplemented with 0.4 mg/L IBA gave maximum number of roots (11.76 ± 0.3) when compare with the other concentrations of IBA and IAA [30]. Strength of the MS was reduced to one quarter and added different concentrations of IAA with different sucrose percentages to induce roots. Highest number of roots per shoot (23.50 ± 2.54) was observed in $\frac{1}{4}$ MS medium supplemented with 0.75% (w/v) sucrose and 0.5 mg/L IAA. But highest root length (4.50 ± 0.26 cm) was observed in $\frac{1}{2}$ MS medium supplemented 1.5% (w/v) sucrose and 0.5 mg/L IAA [11].

in vitro grown *P. indica* plantlets were directly introduced into red soil, vermiculite and farmyard manure (1:1:1) mixture, however survival rate was reduced to 10% after three weeks acclimatization. Therefore, direct introduction of tissue cultured plants into soil is not a suitable acclimatization method [32]. Rooted *P. indica* culture tubes were kept in room temperature for seven days before transferring into pots. Plants were reared under control environment conditions for another three weeks (temperature: 30 ± 2 °C, light: 1500 lux and 80% humidity). Then plants were exposed to outdoor condition and 85% survival was observed [35].

4. PHYTOCHEMISTRY

P. indica is rich in different types of alkaloids, flavonoids, saponins, glycosides and tannins [36].

Table 1: Phytochemicals present in different parts of *P. indica*

| Plant part | Phytochemicals |
|-----------------|---|
| Leaves and stem | Apigethenin, Cuteolin, 7-O-gencsides, Amyrin, Palmistic acid, Sitostrol [30], Plumbaginol [37], Campesterol, Plumbagin, Stigmasterol and 6-hydroxy plumbagin [38]. |
| Inflorescence | Pelargonidin, Cyanidin, Delphinidin [39], Kaempferol and Monogalloylglucose and Digalloylglucose [5]. |
| Roots | Plumbagin, 6-hydroxy plumbagin, Droserone, Elliptinone, Plumbagic acid, 3,3 –biplumbagin, Lactone, Ayanin, Azaleatin, Arachidyl alcohol [5],[40], Myricetin-3,3',5',7-tetra methyl ether, Ampelopsin 3',4',5',7-tetramethylether, Plumbagic acid, Roseanoic acid [41], α -naphthylamine, Myricyl palmitate, Palmitic acid and β -sitosterol [5]. |

Plumbagin (5-Hydroxy-2-methyl-1, 4-naphthoquinone) is one of the naturally occurring bioactive organic compound which is isolated from roots of *P. indica* [42]. It is a simple

hydroxy naphthoquinone which is commercially important for its broad range of pharmacological activities [43]. Plumbagin is yellow needle shaped crystals which is soluble in alcohol, acetone, chloroform, benzene, and acetic acid and slightly soluble in hot water. Plumbagin has melting point ranging 78-79 °C [5]. Plumbagin standard solution was stable in both refrigerated condition and ambient condition [44]. Plumbagin content of tissue cultured field grown plant roots (1.80 ± 0.25 g per 100g of dry row material) was higher than conventionally field grown (1.33 ± 0.15 g per 100g of dry row material) and hydroponically grown (1.08 ± 0.01 g per 100g of dry row material) plant roots. Dry callus (0.26 ± 0.02 g per 100g of dry row material) contain considerably low amount of plumbagin when compare with root extracts [45]. *P. indica* roots contain high amount of plumbagin than *P. capensis* and *P. zeylanica* roots. *P. indica*, *P. capensis* and *P. zeylanica* roots contained 0.569 mg%, 0.429 mg%, 0.247 mg% of plumbagin respectively [46, 47]. *P. indica* roots contained high plumbagin yield (19.13×10^{-2} %) while *P. zeylanica* (1.1×10^{-2} %) and *P. auriculata* (2.1×10^{-2} %) contained higher amount of plumbagin in stem and leaf [48]. Ethanol was the most suitable solvent for *P. indica* root extraction than ethyl acetate, isopropanol, dichloromethane and diethyl ether. Ethanol has given high yield of the crude extract (11.5% w/w) when compare with other solvents [40].

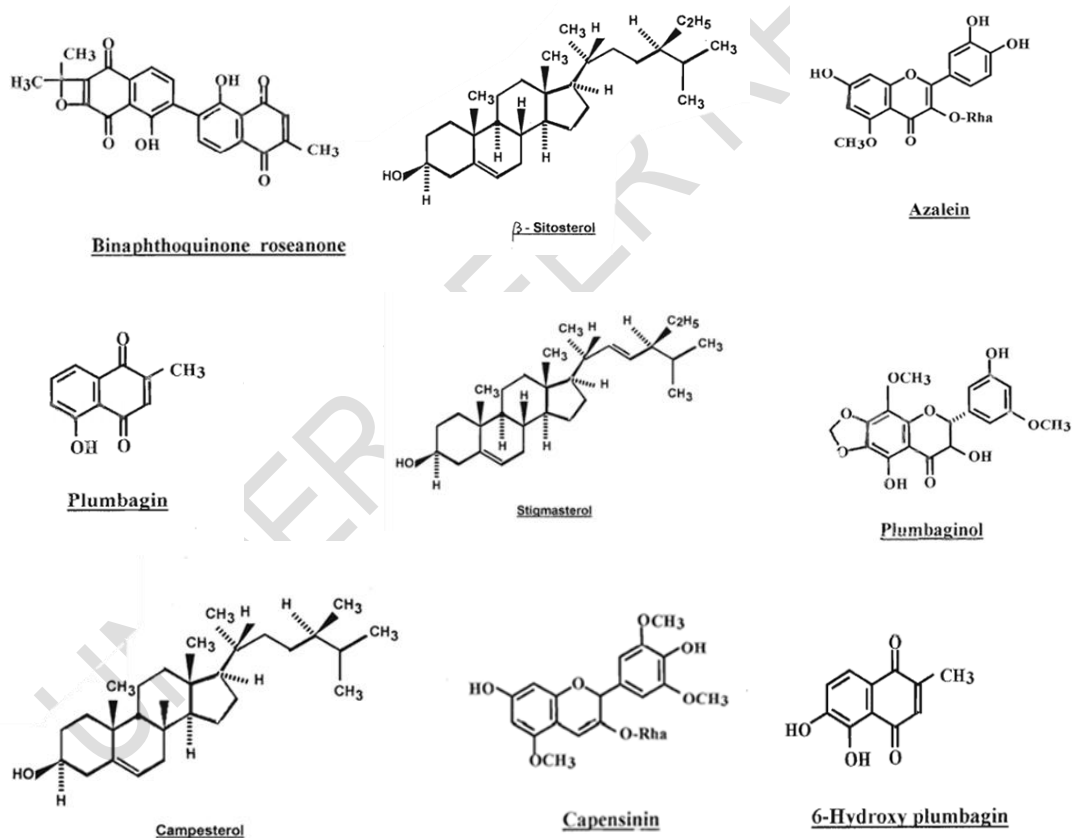


Plate 2: Structures of the chemical compounds present in *P. indica* (Arambewela and Silva, 1999)

The amount of phytochemicals present in the crude and the weight of the crude are depend on the type of solvent that used for *P. indica* plant extraction. Ethanol has given highest yield of the crude extract (11.5% w/w) and highest total content of the plumbagin derivatives (5.79 mg/g) when compare with ethyl acetate, isopropanol, dichloromethane and diethyl ether extractions [49]. Ethanol extraction of *P. indica* roots has given highest crude weight (5.50 gm) when compare with extractions of methanol (4.35 gm) and water (3.76 gm). Phenol content was also higher in ethanolic extract of *P. indica* roots (235 µg/ml) than methanol (147 µg/ml) and water (126 µg/ml) extractions [50]. *P. indica* root bark powder was extracted with absolute methanol by using ultrasound-assisted extraction and concentrated using rotary evaporator at 50 °C. The resulting yield was partitioned using 99 % ethyl acetate. According to the methanol extract analysis results, methyl 16-methylheptadecanoate, sec-Butyl isobutyl sulfide, 4-Amino-6-hydroxypyrimidine, Pyridine,4-(1,1-dimethylethyl), Succinic acid,di(3,5- dimethylcyclohexyl) ester, 8-Octadecenoic acid,methyl ester, 4-Hydroxypyridine 1-oxide, Cyclohexane,1,4-dimethyl-2-octadecyl, 2,Thiophenecarboxylic acid, 3,5-dimethylcyclohexyl ester and Hexadecanoic acid,methyl ester were detected but only methyl 16-methylheptadecanoate, 8-Octadecenoic acid,methyl ester and Hexadecanoic acid,methyl ester were shown more than 80% similarity. Ethyl acetate fraction was contained 1-Octyn-3-ol,3-O-(2-Amino-4-(carboxyiminomethyl)amino)-2,3,4,6-tetraeoxy-a-Darabinoheoxyranosyl)-D-chiro-inositol, Behenic alcohol, 5-Hydroxy-2-methyl-1,4-naphthalenedione, N-2,6-Dimethyl-4-[(trimethylsilyl)oxy]phenyl]-1,1,1-trimethylsilanamine, 1-Bromo-11-iodoundecane and 3,30 -Iminobispropylamine however only Behenic alcohol was shown more than 80% similarity [51].

5. EXPERIMENTAL PHARMACOLOGY

5.1 Anti-cancer activity

Herbal drugs contain a complex mixture of chemical components that have the ability to suppress cancer cell formation with minimal toxicity to normal cells [52]. Plumbagin is an important naphthoquinone, mainly found in the roots of plant species from Family Plumbaginaceae are used in treatment of cancer [42]. A study was conducted on SK MEL 28 human melanoma cell lines and lymphocytes which were treated with different concentrations of plumbagin and plant root extract of *P. indica*. Growth inhibition in a dose-dependent manner occurred by both when treated for 24 hours. SK MEL 28 cells showed a 50% cell proliferation inhibition at 84.74 µg/ml and 30.48 µg/ml when treated with plant root extracts and purified plumbagin respectively. Usually inhibition of proliferation occurs though mechanisms such as cell cycle arrest, DNA damage, apoptosis, and suppression of telomere and telomerase activity [53]. Nuclear morphology was analyzed quantitatively by staining and suggested that apoptotic cell death mechanism was more potentially involved in the anti-proliferative effect by plant root extracts compared to plumbagin. Furthermore, colony formation assay was also performed to compare the long-term effect of purified plumbagin and root extract on SK MEL 28 cells. Although root extract treated cells showed lesser colony formation over 10 days compared to purified plumbagin treated cells [52].

Even though the mechanism is uncertain genotoxic effects of *P. indica* are also identified. In *in vitro* human lymphocytes genotoxic activities of the ethanolic extracts from *P. indica* roots was determined by sister chromatid exchange assay. No linear dose response was observed although at low concentrations such as 12.5, 25.0, 50.0 µg/ml but tended to increase as the concentration increased. In highest concentrations of 500 µg/ml very few mitotic cells were observed. The ability to cause very few mitotic cells signifies high genotoxicity and cytotoxicity which serve well in acting as a chemotherapeutic compound [16]. Several studies have been conducted in relation to prostate cancer and plumbagin [54,55,56]. In a study conducted on mice harboring PTEN-P2 tumors in the prostate and on

prostate tissue *in vivo* with plumbagin resulted in tumor regression in castrated mice, but not in intact mice. Using RNA-seq and bioinformatic methods to gain a better understanding on the cellular response of plumbagin was conducted and it was suggested that dihydrotestosterone production in the testes may prevent cell death due to plumbagin treatment, but the underlying mechanism is not understood [54]. Previous studies suggested a combination of plumbagin and androgen deprivation therapy is required for tumor regression. Plumbagin alone can partially arrest prostate tumor growth and androgen deprivation therapy could arrest the growth of androgen-dependent PTEN-P2 tumors *in vivo* [55]. In a dose dependent manner purified plumbagin treatment decreased the viability correlated with apoptosis induction in human prostate cancer cell lines; PC-3, LNCaP and C42 by reactive oxygen species (ROS) generation and depletion of intracellular glutathione (GSH) levels. This was irrespective for prostate cancer cell lines androgen responsiveness or p53 status. The major non-protein thiol in cells essential for maintaining cellular redox status and changes in expression of genes involved in ROS metabolism is GSH. Plumbagin-mediated ROS generation and apoptosis occurred at 3 - 7.5 μ M concentrations of plumbagin [56].

Various genes responsible for cell proliferation, survival and carcinogenesis which are linked to tumor development and metastasis are included in STAT3 signaling pathways. In human breast cancers MDA-MB-231SArfp cell lines and down-regulate mRNA expressions of IL-1 α , TGF- β , MMP-2 and MMP-9 plumbagin inhibit the activation of STAT3 signaling [57]. Another study conducted on breast cancer cell lines highlighted inactivation of Bcl-2 and the DNA binding activity of NF-kappaB by plumbagin. This suggested the inhibition of cell growth and induction of apoptosis by plumbagin is in part due to inactivation of NF-kappaB/Bcl-2 pathway [58]. Plumbagin has suppressed cell viability and proliferation in esophageal squamous cell carcinoma whereas normal esophageal epithelial cells are not affected. Plumbagin treatments increased the proportion of cells in the G0/G1 phase and decreased the proportion of cells in the S phase of the cell cycle and also inhibit the activation of STAT3 signaling pathway [59]. Serial maceration extractions of *P. indica* leaf, root and normal plant roots decreased cell viability of stomach cancer cell lines (AGS) and breast cancer cell lines (MDA-MB-231) [60].

There are reports on chemotherapeutic effect of *P. indica* where studies suggested ethanolic extract of *P. indica* roots could be used with radiation treatments to enhance tumor killing effect [61,62]. The combine effect of radiation and ethanolic extract of *P. indica* roots used for treatment in S-180 solid tumor and Ehrlich ascites carcinoma showed increased tumor killing effect. However, it was observed that extract alone showed weak tumor killing ability [61]. Previous studies, via many molecular mechanisms, such as targeting apoptosis, autophagy pathway, cell cycle arrest, anti-invasion, and antimetastatic pathway demonstrate that *P. indica* plays a significant role in anticancer activity, but not a mutagen [63].

5.2 Antimicrobial activity

Methanol extract of *P. indica* root bark and a tannin compound isolated from *P. indica* exhibited potential antimicrobial activity and activity against methicillin resistant *Staphylococcus aureus* (MRSA). Tannins, ability to inhibit pathogenic activities, primarily fungal infections such as *C. albicans*, even at higher concentrations was observed. A higher antibacterial activity towards gram-positive bacteria (methanol extract - *Staphylococcus aureus*: 24.0 mm; *Bacillus cereus*: 21.3 mm and ethyl acetate fraction - *S. aureus*: 16.7 mm; *B. cereus*: 9.0 mm) than that for the gram-negative bacteria (methanol extract - *Escherichia coli*: 18.7; *Pseudomonas aeruginosa*: 10.7 mm and ethyl acetate - *E coli*: 10.0; *P. aeruginosa*: 7.0 mm) was demonstrated in disk diffusion and broth dilution assays. While Topoisomerase II DNA gyrase from a *S. aureus* strain is a possible target, the inhibitory

activity of plumbagin identifies potential scaffold for improving antibacterial activity because its strong interaction with the arginine residues [64].

Methanol extract of *P. indica* was tested using disc diffusion method for determining antibacterial properties against four-gram positive bacteria *Bacillus subtilis*, *B. megaterium*, *B. cereus*, *S. aureus* and seven-gram negative bacteria, *P. aeruginosa*, *E. coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi*, *Vibrio cholera*, *Salmonella paratyphi*. Methanol extract had been shown varying degree of zone of inhibition ranging from 7.0 to 14.0 mm at 250.0 µg/disc. Zone of inhibition was increased up to 17.0 to 25.0 mm when concentration of methanol extract in disc increase up to 500 µg/disc [65,66,67]. Methanol extract of *P. indica* 500 µg /disc showed a significantly high zone of inhibition (17 – 25 mm) for gram positive bacteria like *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *Staphylococcus aureus* and zone of inhibition (18 - 23 mm) for gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *S. typhi*, *Shigella dysenteriae*, *S. sonnei*, *Vibrio cholera*. Anti-fungal activity of methanol extract of *P. indica* 500 µg /disc was shown zone of inhibition (20 - 27 mm) for fungi like *Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Cryptococcus neoformans*, *Microsporium spp.*, *Pityrosporum ovale*, *Trichophyton spp.* [68]. *P. indica* extract has shown anti-viral activity by showing inhibitory effect on influenza A (H1N1) pdm09 virus by inhibiting viral nucleoprotein synthesis and polymerase activity [69].

5.3 Anti-acne activity

Among skin disorders, commonly presenting one is Acne which affects almost everybody during adolescence. This occurs due to increased production of sebum followed by infections caused by micro-organisms like *Propionibacterium acnes*, *Staphylococcus epidermidis* bacteria and fungi like *Malassezia furfur* [70]. The potential antimicrobial activity of the *P. indicia* in agar disc diffusion method was determined for the anti-acne activity with different concentrations of acetone extracts. Plant extract at concentration of 10 mg/ml showed largest zone of inhibition in all three micro-organisms. Zones of inhibition at 10 mg/ml were 18.13 ± 0.06 mm in *S. epidermidis*, 12.06 ± 0.03 mm in *P. acnes* and 14.03 ± 0.03 in *M. furfur* [71]. Studies only focused on *in vivo* studies, while assessment of the efficacy and toxicity for these potent *P. indica* extracts as anti-acne medicine are yet to be found.

5.4 Antioxidant activity

Methanolic extract of roots of *P. indica* showed the presence of phenolic and non-phenolic compounds which are mainly responsible for anti-oxidant activity. *P. indica* roots extract has active compounds which have the ability to donate hydrogen to free radicals. The IC₅₀ value for methanolic extract of *P. indica* roots was 79.1 µg/ml while that for reference ascorbic acid was 4.2 µg/ml. For anti-oxidant activity can also be measured by the reducing power potential. Reducing power of methanolic extract of *P. indica* roots have been evaluated by the transformation of Fe³⁺ to Fe²⁺ through electron transfer ability. The reductive activity of the extract increased with the increasing concentration of the extract and when compared with the standard ascorbic acid, it has substantial reducing power in *P. indica* root extract was confirmed [36].

5.5 Anti-diarrheal activity

Anti-diarrheal activity of *P. indica* was observed using castor oil-induced diarrhea in mice Loperamide 3 mg/kg was used as positive control mice. Total number of 8.6 ± 1.20 feces for

total latent period (1.24 ± 5.25 hour) was observed in mice treated with 500 mg/kg and Loperamide 3 mg/kg was used as positive control. Loperamide showed total number of feces (6.4 ± 1.151) for total latent period (1.35 ± 6.02 hour) [68]. Plumbagin prevents secretory diarrhea by inhibiting calcium-activated chloride channels (CaCC) and cystic fibrosis transmembrane conductance regulators (CFTR) [72]. It significantly delayed intestinal motility and inhibited intestinal smooth muscle contractility without an evident impact on contractive frequency, indicating anti-diarrheal activity of *P. indica*.

5.6 Macro-filaricidal activity

Methanol extract of *P. indica* roots was tested against cattle filarial worm *Setaria digitata* at the worms' death was confirmed by doing post exposure in normal incubation media for 6-12 hours. Complete inhibition of mortality in cattle filarial worms were observed at crude extract concentration of 0.02 mg/ml within 100 minutes at 37 °C. Concentration of 0.01 mg ml⁻¹ crude extracts showed only 83.3% of the cattle filarial worms immobilized at an incubation period of 6 hours [73]. Although only few studies have been carried out so far on filaricidal activity, this indicates that there are filaricidal compounds present in *P. indica*.

5.7 Anti-fertility activity

There are studies on anti-fertility activity of *P. indica*. Anti-fertility activity of ethanolic extracts of *P. indica* roots in combination with aerial parts of *Aerva lanata* was evaluated using anti-implantation, abortifacient, and motility of Albino rat spermatozoa (*in-vitro*) models. Ethanolic extract of *P. indica* roots was administered orally for pregnant albino rats during 10th -18th days of their pregnancy. The percentage of abortions at 400 mg/kg b/w was 70%. Anti-implantation activity was also measured by administering ethanolic extract orally to pregnant albino rats within 1st to 7th days of pregnancy. *P. indica* showed 50% anti-implantation activity at 400 mg/kg b/w, while at lower concentrations no motility of rat spermatozoa within 60 seconds and no motility by 90 seconds at a concentration of 10% and 5%, respectively was recorded [74,75].

High concentration of *P. indica* roots (800 mg/kg) extract on fetuses of mice suggested 100% fetotoxicity effect causing poisoning or degenerative effects in a developing fetus or embryo [76]. This could lead to abortions or congenital abnormalities. Acetone extract of stems and acetone and ethanol extract of leaves of *P. indica* also showed a temporary and reversible modification of the estrous cycle in rats when administered orally by prolonging the diestrus phase. Acetone extract of stems and acetone and ethanol extract of leaves of *P. indica* was exhibited estrogenic activity by increasing in diameter of the uterus, uterine weight, and thickness of the endometrial epithelium. According to literature, flavonoids and plumbagin compounds found in *P. indica* are known to exhibit antifertility activity [77,78]. When stems of *P. indica* were studied on estrous cycle at two dose levels, 200 and 400 mg/kg respectively using acetone extracts showed antifertility activity was shown in female Wistar rats [77]. A significant estrogenic activity was observed only when given together with ethinyl estradiol, the estrogenic activity produced by ethinyl estradiol causing an increase in the uterine wet weight.

5.8 Wound healing activity

Wound healing is a complex and dynamic process. A study was conducted among four groups; Group I, untreated control, Group II, topically treated with 0.5% w/w *P. indica* extracts, Group III, topically treated with 0.1% w/w plumbagin ointment and Group IV, topically treated with reference standard framycetin sulphate cream (1% w/w). Plumbagin ointment 0.1% w/w increased the rate of wound contraction, epithelization, collagenization

process in excision and incision wound models. Both *P. indica* and plumbagin showed significant increase of tensile strength by maturation of collagen through increasing the number of cross links and fiber strength when compared with the positive control framycetin sulphate cream [79].

5.9 Analgesic and anti-inflammatory activity

Male Wistar albino rats and mice were used to evaluate analgesic and anti-inflammatory activities in aqueous extracts of leaves of *P. capensis* and *P. indica*. A significant inhibition in the Carrageenan induced paw oedema at the dose of 300 mg/kg body weight was observed in comparison to the control group which was given standard drug Indomethacin. Inhibition of writhing scores for *P. capensis* and *P. indica* against 1% acetic acid were 67.32% and 70.29% respectively and Eddy's hot plate method exhibited an index of analgesia of about 68.29% and 45.2% respectively for *P. capensis* and *P. indica*. Additional studies are required by using more animals and to isolate the mechanism responsible for this pharmacological activity [80].

6 TOXICOLOGY

It is reported that, Plumbagin causes side effects including diarrhea, skin rashes, leukocytosis and increased serum phosphatase levels when high concentrations were administered to mice [81]. Therefore, toxic effect of the extract should be evaluated to identify the safe dose for treatments. Mice administered extracts through oral route showed high tolerance than intraperitoneal administration. The LD₅₀ of the extract by oral route was 1148.15 mg/kg and intraperitoneal administration was 239.88 mg/kg. Dark spots on liver, green color thyroid, increased weight of spleen and reduction of weight of liver, thymus, testes and kidneys were observed in male rats. Female rats showed a weight loss of thymus and increased weight in uterus on autopsy [82]. Cytotoxic activity of methanolic extract of *P. indica* was evaluated by using brine shrimp lethality assay and LD₅₀ was reported as 4.57 µg/ml [83]. GSH is non-enzymatic intracellular antioxidant which oxidized to GSSG during oxidative stress. Plumbagin and *P. indica* L. extract induce oxidant - anti oxidant imbalance in liver by depleting the hepatic GSH content while the hepatic GSSG content was increased. This increased hepatic oxidative stress causes chronic inflammation and cellular injury in liver [81].

Cytotoxicity of *P. indica* was measured by MTT assay on MDCK cell line and CC₅₀ value for cold macerated extract was 20.66 mg/ml while 15 hours Soxhlet extract shown 14.17 mg/ml CC₅₀ value [69]. IC₅₀ value of *P. indica* root extract on HGE-17 Cell lines was 178.29 µg/ml. cytotoxicity of *P. indica* root extract was lower than *P. zeylanica* (199.94 µg/ml) and *P. auriculata* (278.59 µg/ml) [84]. EC₅₀ values of aqueous and methanol extractions of *P. indica* root bark extracts on HeLa cell line were 781.9 ± 0.23 µg/ml and 42.5 ± 0.13 µg/ml respectively [51]. Niosomal plumbagin has shown less toxicity when compared with free plumbagin. There was a 70% mortality percentage when BALB/c mice were injected with 10 mg kg⁻¹ free plumbagin intravenously while intravenously injected niosomal plumbagin showed 30% mortality [85].

7. CONCLUSION

Genera *Plumbago* has a wide range of medicinal properties. Among them, *P. indica* contain vast range of phytochemical compounds that help to cure different kind of diseases in human and other animals. *P. indica* is widely used in Ayurveda, Siddha, Unani, and Homeopathy like traditional systems of medicine and currently subjected to different experimental research to find out its curing ability on cancer. Demand for dry *P. indica* plant

parts have increased dramatically over past few decades. Therefore, it is rapidly declining from their natural environment due to over exploitation for commercial uses. This plant is not produced seeds and has slow growth. Introduction of suitable propagation method will help to preserve it in their natural environment as well as it'll enough supply of raw materials for medicinal preparations. This article included scientifically proven data about its propagation, photochemistry and biological activities to encourage the future studies.

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9. CONSENT

It is not applicable.

10. ETHICAL APPROVAL

It is not applicable.

Significance of the study:

The study highlights the efficacy of "Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

11. DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY RESEARCH GRANT ASP/01/RE/SCI/2018/42 IN UNIVERSITY OF SRI JAYEWARDENEPURA.

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