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Phytochemistry, Micropropagation and Pharmacology of near threatened plant *Pseudarthria viscida* (L.) Wight & Arn

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Abstract
Pseudarthria viscida (L.) Wight & Arn., is a beneficial medicinal plant of India used as traditional medicine to treat several diseases. Due to its potential utilization as a therapeutic agent, it was incorporated in the high trade sourced medicinal plants. Phytochemical analysis has shown the presence of important compounds contributing to the therapeutic value of the plant. This plant has several pharmacological properties supported by *in vitro* and *in vivo* studies. Major activities include antibacterial, antioxidant, cytotoxic, antidiarrhoeal, antipyretic, hepatoprotective, anti-inflammatory, antidiabetic, analgesic and wound healing activities. Most of the therapeutic activities of the roots of this plant have been ascribed to its phytocompounds such as flavonoids. Indiscriminate utilization of this plant makes it vulnerable medicinal plant. Limited research has been conducted on micropropagation and conservation of the plant. Therefore, the present review compiles the micropropagation, phytochemical and pharmacological data to enable the researchers for future research.

Keywords: *Pseudarthria*, Dasamoola, anticancer and hepatoprotective

Introduction
Pseudarthria viscida is one of the valuable Indian ethnomedicinal plants with commercial and medicinal values. It is one among the "Dasamoola" and also an important constituent of several Ayurvedic medicine preparations such as Dasamoolarishta, Agastyaharitakirasayana, Anuthaila, Brahma Rasayana, Sudarshanachurna, Narayanathaila and Dhanuantharaghrita described in ancient scripts [1, 2]. This plant has many pharmacological properties mainly the roots have more therapeutic activities to treat fever, rheumatism, bronchial asthma, hemorrhoids [3, 2], diabetes mellitus [4, 5, 6], heart diseases and blood disorders [7]. Other properties like antihypertensive [8], antioxidant [9, 10], antiulcer [11], antifungal [12], antidiarrheal [7, 13] and antitumor [14, 6].

Traditionally it has been used in the treatment of rheumatism, bronchial asthma, haemorrhoids, diabetes mellitus, blood disorders, fever, antihypertensive, antioxidant, antiulcer, antifungal, antidiarrheal and antitumor [15, 14, 6, 3, 2, 4, 5, 7, 8, 9, 10, 11, 12, 13]. The roots are astringent, sweet, bitter, emollient, digestive, constipating, antihelmintic, cardiotonic, febrifuge and tonic. The roots of PV are one of the ten components used in the formulation of Dasamoola, which is a combination of ten medicinal plants and majorly the roots are used for the preparation in Ayurveda. The roots are used to treat intermittent fever, urinary diseases, edema, tumours, anorexia, flatulence, diarrhoea, vomiting and piles [3, 12, 9, 5].

The genus Pseudarthria has 6 scientific plant names of species rank, validated taxonomically and accepted as species names [16] *viz*., *Pseudarthria viscida* (L.) Wight & Arn., *Pseudarthria confertiflora* (A. Rich.) Baker., *Pseudarthria crenata* Hiern., *Pseudarthria fagifolia* Baker., *Pseudarthria hookeri* Wight & Arn., *Pseudarthria macrophylla* Baker. Recently, a new species *Pseudarthria panii* was identified in Asia [17]. Due to limited availability and excessive utilization this plant is being red listed and needs conservative measures for the proper utilization of its pharmaceutical potential in the treatment of diseases [18]. Therefore this review will provide a comprehensive overview of its micropropagation, phytochemical and pharmacological properties.

Table 1: Classification (ILDIS World Database of Legumes (version 12, May 2014)

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Pseudarthria*
Species	*viscida*

Fig 1: *Pseudarthria viscida* in Nallamala forest (Telangana State)

Botanical Description and Geographical Distribution

Pseudarthria viscida (PV) is a perennial undershrub; branchlets densely villous, viscid. 2-3 ft. height, with slender stems, clothed with fine grey pubescence, undershurb. Leaves 3-foliate; terminal leaflet rhomboid, 4-16 X 4-5cm; petiloule 0.5cm; lateral leaflets 3.5-4.5 X 3-3.5cm, base cuneate-obtuse, margin entire, ciliate, apex acute, apiculate; petiloule 0.2cm; stipules 2. Flowers pink-red, 0.8cm, pedicel 4mm, in axillary and terminal racemes, 4cm. Calyx lobes 5, 2mm. Standard petal 2mm; wing petal 3mm; keel petal 3mm. Stamens diadelphous, 9+1, anthers 1mm, ovate; filament sheath 4mm. Ovary oblong, ciliate, unilocular, multicarpellary; style 1mm; stigma capitates. Pod flat, linear, 0.5cm; hooked, marginal hairs present. Seeds 4-6, reniform. Fl. & Fr.: Oct. - Jan. and it is belongs to the Fabaceae family [19, 20]. PV distributed throughout South India up to 900 meters in the hills and also in Gujarat and Tropical zone, Western peninsula, Ceylon, up to 3000 ft. and Timor. Recently, it was also reported to be distributed in the forest of Baratang Island in Middle Andaman group [21].

Table 2: Geographical Distribution of *Pseudarthria viscida* in India

State	Area of distribution in India	References
Andhra pradesh	Chittoor	[20].
Tamilnadu	Namakkal, Salem, Erode and Kuttralam	[22].
Kerala	Kottayam, Kanjicode and Trivendrum	[15, 5, 23].
Gujarath	Vadodara	[24].

Tissue culture studies

The pharmaceutical potential of PV was tapped for commercial cultivation due to increased mortality of seedlings in young stages, poor seed viability and germination rate as limitations [25]. PV being red listed plants needs to be conserved and micropropagation of such plants is necessary for the exploitation and utilization of their medicinal values. There are very few reports on tissue culture studies of PV [26, 15, 27].

Preliminary tissue culture studies using stem explants showed an average of 29.87 number of shoots on MS media supplemented 0.6 mg/L BAP, shoots were elongated on MS media with BAP+NAA (0.4 + 0.04 mg/l) and subsequently rooted on half strength MS medium+0.4 mg/L IBA [26]. A high-frequency regeneration protocol was standardized from cotyledonary node explants through callus induction. The frequency of callus induction was 96 % reported on MS medium supplemented with 1.5 mg/L 2, 4-D and 0.5 mg/L NAA followed by 97 % shoot regeneration from the generated callus with an average of 44.9 shoots per explants on MS medium with 3.0 mg/L BA and 1 mg/L NAA and finally, 98 % rooting was induced on MS medium IBA or NAA (0.5–4 mg/L) with an average of 3.3 roots per shoot after 45 days. The survival rate was 72.5 % [15].

Leaf, node and internodal segments explants were used and 98 % callus induction was reported from leaf explants on MS medium fortified with 1.5 mg/L 2,4-D + 0.5 mg/L Kinetin and 2.5 mg/L NAA+1 mg/L BAP. Shoot regeneration was shown from nodal explants on medium with 0.5 mg/L NAA+2.5 mg/L BAP after 28 days and rooting was observed from the medium with 2.5 mg/L IBA and 2 mg/L NAA after 29 days. Callus was cultured in suspension using abiotic (Salicylic acid) and biotic elicitors (Chitosan) enhanced the production of phenolic compound [27].

Phytochemical properties

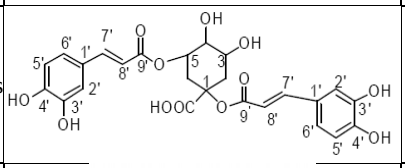
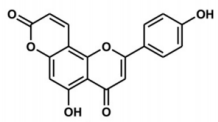
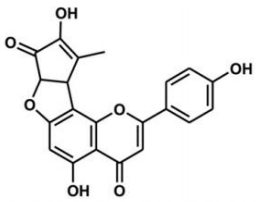
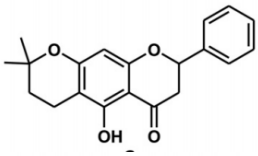
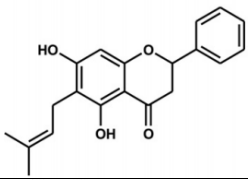
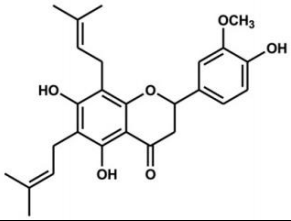
Phytochemical analysis of PV using GC-MS analysis identified 18 active compounds in methanol root extract [28, 29] and 20 volatile organic compounds in leaf extract [29]. The major compounds reported were 3-O-Methyl-d-glucose (61.33%), n-Hexadecanoic acid (12.66%), Oleic acid (7.93%) and 9, 12- Octadecanoic acid (4.88%), Neophytadiene [28, 29]. Of the different species of PV, phytochemical compounds isolated have been structurally elucidated majorly in *P. hookeri*.

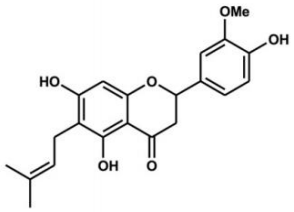
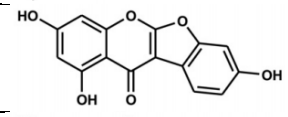
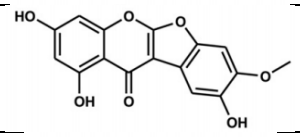
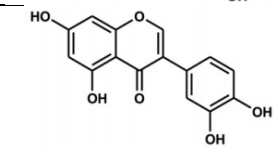
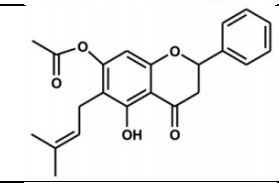
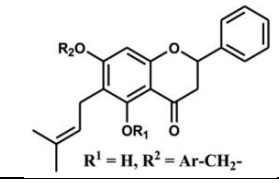
Table 3: The phytochemical compounds isolated from root and leaves of *Pseudarthria viscida*

Name of the Compound	Plant part	Reference
Butane, 1,1-diethoxy -3-methyl	Root	[28].
Hexanoic acid, ethyl ester		
Propane, 1,1,3-triethoxy		
1- Butanol, 3-Methyl- formate		
4H-Pyran-4-One,2,3-dihydro-3-5-dihydroxy-6-methyl		
Decanoic acid, ethyl ester		
Undecanoic acid		
3-O-Methyl-d-Glucose		
Tetra decanoic acid		
Oxirane, tetra decyl		

n-Hexadecanoic acid		
Hexadecanoicacid, ethyl ester		
5-Octadecene, (E)		
d-Mannitol, 1-decylsulfonyl		
9,12-Octadecanoic acid(Z,Z)		
Oleic acid		
Octadecanoic acid		
1-Monolinoleoglycerol trimethyl siliyl ether		
Hexadecen-1-ol, trans-9-	Leaf	[29].
2,4-Di-tert-butylphenol		
N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]		
1-Hexadecanol		
1,3-di-iso-propylnaphthalene		
E-15-Heptadecenal; Heptadecane,2,6,10,14-tetramethyl-		
Neophytadiene		
Phytol, acetate		
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate		
2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin		
n-Hexadecanoic acid		
Phthalic acid		
8-bromoctyl butyl ester		
10-Heneicosene (c,t)		
Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate		
1,2-15,16-Diepoxyhexadecane		
6,9,12,15-Docosatetraenoic acid, methyl ester		
1-Heptatriacotanol		
4,22-Stigmastadiene-3-one		

Table 4: Chemical structures of some phytochemical compounds of Pseudarthria Species

Name of compound	Class of compound	Structure	Biological activity	Pseudarthria Species	Ref.
1, 5 dicaffeoyl quinic acid	Polyphenolics		–	*P. viscida*	[30].
Pseudarflavone A	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	[31].
Pseudarflavone B	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
6,7-(2″,2″-dimethylchromano) flavanone	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
6-prenylpinocembrin	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
Hiravanone	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	

6-prenyl-3′-methoxyeriodictyol	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
Boeravinone L	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
Desmoxyphyllin A	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
Orobol	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
6-prenylpinocembrin acetate	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	
7-benzyloxy-6-prenylpinocembrin	Flavonoids		Cytotoxic and antibacterial activity	*P. hookeri*	

Pharmacological properties

Antibacterial activity

Ethanol leaf extract PV at 100µg/ml showed prominent antibacterial against *Bacillus megaterium*, *E. coli*, *B. Subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and antifungal activity against *Candida albicans*, *Aspergillus niger* [32]. Leaf, root, stem and the callus extracts of PV displayed significant antifungal property against fungal pathogens responsible for crop plant and stored food grains diseases [12].

Antioxidant activity

In vitro antioxidant effect of root aqueous extract of PV by using DPPH, superoxide and nitric oxide assays exhibited free radical scavenging activity. EC_{50} values of the antioxidant activity are 19.50±1.25, 28.30±2.75 and 47.40±1.50 µg/ml. Polyphenol: 1,5 dicaffeoyl quinic acid was isolated from PV methanol extract [30]. Stem and root crude methanol extracts also showed potent antioxidant activity evaluated by DPPH assay [9].

Cytotoxic activity

Whole plant ethanolic extract of PV was evaluated for its cytotoxic effect on human colorectal cancer cell line - HT29, mouse muscle cell line - C2C12 and mouse, embryo fibroblast - 3T3 L1 cell lines. The extract at 1000 µg/ml concentration exhibited strong cytotoxicity against HT29 cell line [38]. Alkaloid fraction of the PV displayed cytotoxic effect on HeLa (Human cervical cancer line) and NIH 3T3 (mouse embryonic fibroblasts) cell lines. The corresponding EC_{50} values are 183.10±1.05 and 178.30±1.85 µg/ml respectively [30]. Leaf powder of PV subjected to microwave assisted extraction with ethanol showed *in vitro* anti-tumour activity against different cell lines L929, HCT 15, MCF7, SIHA and HeLa with IC_{50} values 17.17, 160, 18.72, 55, 60 50 µg/ml respectively by MTT assay [33].

Antidiarrhoeal activity

Ethanol root extract of PV at a dose of 200 and 400 mg/kg displayed dose-dependent inhibition of frequency of defecation in castor oil induced diarrhoea in Wistar albino rats [13].

Antipyretic activity

Ethanol leaf extracts of PV exhibit antipyretic activity on brewer's yeast induced pyrexia in Albino mice and Wistar rats. The extracts 200 and 400 mg/kg displayed a significant reduction in rectal temperature in a dose-dependent manner. The antipyretic property can be ascribed to the flavonoid composition of the extract. Increase in body temperature increased lipid peroxidation process coupled with increased oxidative stress. The antioxidant property of PV could decline the lipid peroxidation processes, thus decrease the body temperature [34].

Hepatoprotective activity

Ethanolic extract of PV displayed hepatoprotective effect on 7,12dimethyl benz[α]anthracene (DMBA) induced liver damage in Wistar albino rats. Oral administration of extract decreased levels of serum enzymes, neutralized lipid peroxidation and improved the enzymatic and non-enzymatic antioxidant defence system. This prevention might be due to the flavonoids present in the extract [35]. Ethanol extract of PV displayed hepatoprotective effect against N-Nitrosodiethylamine (NDEA) induced liver cancer in Wistar albino rats. The extract restored the antioxidant enzyme level

in the liver and showed a dose-dependent protective activity against the induced liver toxicity [35].

Anti-inflammatory and analgesic activities

Methanol root extracts of PV displayed significant anti-inflammatory activity against acute carrageenan-induced rat paw oedema in albino rats. The percent inhibition of oedema by the extract was 43.83% and 59.58% at 200 and 400mg/kg respectively. The toxicity studies showed the extracts to be safe and tolerable by the rats [36]. Likewise, ethanol root extracts of PV at 200 and 400 mg/kg showed 42.69% and 55.76% inhibition of edema respectively. Ethanol leaf and root extracts of PV decreased acetic acid-induced writhing response evident from analgesic studies [34, 24]. Whole plant ethanol extract of PV displayed anti-inflammatory activity in carrageenan-induced rat paw edema model in Wistar rats at a dose of 200 and 400 mg/kg body weight [22, 39]. Anti-inflammatory and analgesic activities can be ascribed to the root flavonoids targeting prostaglandins [24].

Wound healing activity

Ethanol extract of PV exhibited significant wound healing activity by reducing the surface area of the wound as assessed by excision wound model for 12 days in Wistar rats. Ethanol extract in the form of ointment (10% w/w) applied topically on Wistar rats increased wound contraction, reduced the period for epithelialisation and high skin breaking strength was noticed [22].

Neuroprotective activity

Ethanol root extracts of PV demonstrated neuroprotective activity against β-amyloid (25-35)-induced amnesia in Swiss albino mice as evident by behavioral pattern using water maze and biochemical parameters. Treatment with extract improved the cognitive function, prevented the neurodegeneration and restored the neuronal function. Thus the extract can be used for Alzheimer's and dementia therapy [23].

Antidiabetic activity

The ethanol root extract displayed significant antidiabetic activity compared to glibenclamide in alloxan-induced diabetes in albino rats at a dose of 200 mg/kg bw. Histopathological studies showed the protection of pancreatic β-cells by ethanol extracts treatment, probably because of the flavonoids and tannins constituents of the extract [5].

Aqueous and ethanol root extract of PV exhibited antihyperglycemic property in STZ-induced non-insulin-dependent diabetes mellitus (NIDDM) rats at 250 and 500 mg/kg dose. Diabetic rats treated with aqueous extract retained the body weight compared to the diabetic control group. This could be due to the protective property of PV extracts in controlling muscle wasting. Aqueous extract of PV displayed prominently decreased glycated haemoglobin, serum creatinine, urea, LDH levels in PV treated diabetic groups as compared to the diabetic control. It also decreased GOT and GPT levels in the kidney and liver in diabetic rats [37]. Antihyperglycemic activity of PV root extracts could be contributed by the flavonoids, tannins, leucopelargonidin derivatives and phenolic substances present in ethanolic root extract and tannin, leucopelargonidin derivatives of the aqueous extract [5, 37, 6]. Whole plant ethanolic extract of PV showed anti-hyperglycemic and anti-hyperlipidemic activity in Streptozotocin-Nicotinamide induced type-II diabetic rats. Treatment with the extract significantly decreased blood glucose levels, SGOT and SGPT levels, and lipid profile [16].

Table 5: Antidiabetic activity of roots *Pseudarthria viscida*

Dose range tested (mg/kg body weight)	Solvent	Diabetes induction Chemical	Model used	Type of Diabetes	References
100 and 200	Ethanol	Alloxan monohydrate	Wistar Albino rats	Hyperglycemia	[5]
250 and 500	Water	Streptozotocin	Wistar Albino rats	Non-insulin-dependent diabetes mellitus	[37]
250 and 500	Ethanol	Streptozotocin (STZ) nicotinamide	Wistar Albino rats	Non-insulin-dependent diabetes mellitus	[6]

Conclusion

Historically, plants have always been at the rescue of human health for the prevention and treatment of diseases. Traditional herbal medicine has been practised in certain parts of the world. PV has been used since ancient times for the treatment of diseases. Phytocompounds extracted from PV have to be structurally elucidated and its biological activities have to be screened. Moreover, refinement of the extracts has to follow bioassay-guided fractionation for better activities. PV has displayed antibacterial, antioxidant, cytotoxic, antidiarrhoeal, antipyretic, hepatoprotective, anti-inflammatory, antidiabetic, analgesic and wound healing activities. The mechanisms of these therapeutic activities have to be explored. Furthermore, research is necessary to exploit the potential of flavonoid compounds and their mechanisms involved in treating the diseases. This plant has limitations in the micropropagation and standardization of regeneration protocols for better regeneration is necessary. The effects of biologically active compounds from this plant in combination with other drugs have to be determined. The large gap of information from *in vitro*, *in vivo* experiments and clinical studies using this plant compounds has to be minimized for their better utilization as therapeutic agents.

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