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National status of *in vitro* conservational strategies for *Leptadenia reticulata* (Retz) Wight and Arn

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Abstract
Leptadenia reticulata (Jivanti) is a versatile medicinal plant, used from ancient periods as a natural remedy for many diseases due to its healing property. Lots of Indian researcher performed work for phytochemical screening, pharmacological effects and for its propagational strategies. Through *In vitro* propagation scientists are trying to proceed on new path which help to conserve *Leptadenia reticulata* and also help to discover hidden properties of this important medicinal plant. This work highlights some important work done by Indian researchers in field of isolation and characterization of phytochemical constituent and plant tissue culture of *Leptadenia reticulata* (jivanti).

Keywords: *In vitro*, Jivanti, *Leptadenia reticulata* Phytochemical, Pharmacological, Plant Tissue Culture.

Introduction
Leptadenia reticulata (Retz) Wight & Arn belongs to family Asclepiadaceae is an important medicinal plant. This plant is commonly known as Jivanti or Dori or Swarn, and is distributed in tropical and sub-tropical parts of Asia and Africa. In India, it is found in Gujarat, sub - Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India, ascending up to a altitude of 900 metres. (Rajeswari J *et al.* 2014) [12]. Jivanti is jeevana tonic that boosts energy level of the body, according to ayurveda. It is beneficial for the patient who suffer from weak debility or a lack of energy. It also increases longevity, memory enhancement, immunomodulation and adoption. Lactogenic, anabolic and galactogogue effect was also observed in it. Plant is also of great value in 'tridoshas' (Vatta, Pitta and Kapha) (Kaushik *et al.* 2013) [6].

Phytochemistry and Pharmacology
Phytochemical analysis of *Leptadenia reticulate* was performed by many researchers, and important constituents like in roots carbohydrates, steroids, coumarins, flavonoids, tannins, phenolics and alkaloids have been reported. (Chetankumar *et al.* 2012) [3]. the major phytochemical compound is stigmasterol. It also contains ß–sitosterol α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, stigmasterol, hentriacontanol, a triterpene alcoholsimiarenol and apigenin (Kaushik *et al.* 2013) [6]. Gc-Ms Analysis of Whole Plant of Leptadenia Reticulata was done (Rajeshwari J *et al.* 2014) [12]. They characterized 31 different components, some of them are presented as given below:-
Biological Activities of Phytochemical Compounds Identified in Ethanol Extract of Whole Plant *Leptadenia Reticulata*

Phytochemical Compounds	Biological Activities
2-Propanone, 1-(ethylthio)-	No Activity reported
Glycerin	Flavor
1-Penten-4-one, 2-acetyl-1-dimethylamino- ((Z)- or (E)-)	No Activity reported
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Antimicrobial, Anti-inflammatory, Antiproliferative Antioxidant, Automatic nerve activity
Ethyl hydrogen succinate	No Activity reported
á-d-Allopyranoside, methyl 6-deoxy-2-O-methyl-	No Activity reported
Benzenecarboxylic acid	Antifungal agents, Food preservatives
2-Furancarboxaldehyde, 5- (hydroxymethyl)-	Antimicrobial, Preservatives
2-Methoxy-4-vinylphenol	Antioxidant, Antimicrobial, Anti inflammatory

Due to presence of aforesaid phytochemical constituents *Leptadenia reticulata* shows certain pharmacological activities like Anticarcinogenicity against Dalton's Ascitic Lymphoma (Sathiyanarayanan, 2008), immunomodulatory and antioxidant activity. CDRI and NDRI, Lucknow reported that *Leptadenia reticulata* promotes eyesight, good in chest congestion, galactogogue, antibacterial, hypotensive, anti-cancerous and anti-oxidant. Hepatoprotective activity of *Leptadenia reticulata* stems was reported against carbon tetrachloride-induced hepatotoxicity in rats (Nema *et al.* 2011) [10]. *Leptadenia reticulata (Retz)* is one of component of at least 43 marketed poly herbal formulations which are used against wide range of health ailment and physiological disorders. So *Leptadenia reticulata (Retz)* has been used to recovered various physiological, bacterial or even as a galactogogue in ruminant, cancer and asthma. (Sonara *et al.* 2013) [18].

Plant Tissue culture

Researchers show their interest to conserve and propagate this important medicinal plant as Parabia *et al.* (2007) [11], achieved multiple shoot production and organogenesis by using different combination of plant growth regulators IBA and Kn.

Sathyanarayana (2007) successfully cultured somatic embryo by the combination of MS, Sucrose, NAA and BAP, all stages of embryo formation heart shape, cotyledonary etc observed in this work.

Simple protocol for high frequency *in vitro* regeneration of *Leptadenia reticulata* was described by Sudipta *et al.* (2011) [19]. Different concentration of Cytokinins was observed for multiple shoot induction using nodes, internodes, meristem as explants. The best response for multiplication was obtained in MS media supplemented with 0.25mg/l BA and 0.25mg/l Kn. Maximum numbers of roots were observed when the *in vitro* grown shoots were maintained on full strength MS media containing 2mg/l IBA followed by 200 mg/l activated charcoal.

Rathore *et al. (*2013) [13], used two different media combination MS medium + 5.0 mg/l of BAP + additives and MS + 1.0 mg/l of BAP + 0.1 mg/l of NAA and additives for shoot differentiation. The regenerated shoots were subcultured for further multiplication on MS + 1.0 mg/l BAP + 0.5 mg/l Kin + 2-iP (0.5 mg/l) and 0.1 mg/l of NAA + additives regularly after an interval of 3 weeks. They add ammonium sulphate in the medium resulted in increase in shoot number and promoted elongation. They successfully develop protocol for *in vitro* regeneration of shoots from petiole derived callus. Shoots regenerated *in vitro* by both processes were rooted *in vitro* on 1/4 strength of MS medium + 3.0 mg/l of IBA after 15–20 days. According to their results total dry biomass harvested per year was 2800 kg/acre.

Sudipta *et al.* (2013) [21], reported natural extract like tomato juice, carrot juice, coconut water etc on shoot induction and various carbon sources. Among carbon sources, maximum number of multiple shoots (5.20±0.21cm) and shoot length (5.1±0.16cm) was observed on Murashige and Skoog (MS) media supplemented with 2% sucrose. The maximum chlorophyll content (1.76±0.40mg/g tissue) was observed on MS media supplemented with 2% sucrose followed by 2% table sugar (1.42±0.5mg/ml). Highest protein content (16.3±0.24mg/ml) and total carbohydrate content (15.7±0.30mg/ml) was observed on media containing 3% sucrose. Among organic additives, 10% coconut water in MS media resulted highest number of multiple shoots (6.20±0.10), shoot length (4.72±0.06cm). Other parameters like chlorophyll content (1.7±0.02), total protein content (22.4±0.08) and total carbohydrate content (22.5±0.01) were also found to be superior on 10% coconut water supplemented media.

Biosynthesis of silver nanoparticles (Ag Nps) was carried out using methanol leaves extract of *Leptadenia reticulata* was performed by Kumara Swamy *et al.* (2015) [7] Characterization of Ag Nps were based on the observations of UV–visible spectroscopy, transmission electron microscopy, and X-ray diffraction (XRD) analysis. These Ag Nps were tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganisms (S. pneumonia (a) and K. Pneumonia) and antioxidant activity was performed using DPPH assay.

Significance of plant tissue culture of *Leptadenia reticulate*

Plant tissue culture emerge as a very versatile field of study through which one can modulate the protocol according to need of experiment as Sudipta *et al* (2011) [19], successfully developed media by using tap water and table sugar in place of sucrose by which the cost of media has been reduced. 1158 plants from a single explant within 133 days have been produced. They also reported suspension culture and secondary metabolite isolation. They obtained friable callus in MS media fortified Auxin secondary metabolite produced endogenously as well as exogenously. The result of their work is represent in table below

Qualitative analysis of the phytochemicals in the cells and spent medium of *Leptadenia reticulata*

Phytochemicals	Test Performed	Spent Medium	Dried cell
Alkaloid	Dragendorff's Test	+	+
Glycosides	Keller-Killiani	+	+
Saponin- anticancer, antifungal properties	Forth formations	+	+
Steroids- formulation of synthetic sex hormone29	Liebermann-Burchard reagent	-	+
Flavonoids- anticancer, antidiarrheal activity	Shinoda Test	+	+
Terpenoids - decrease the blood sugar	Salkowski Test	+	+
Anthraquinones	Ammonia Test	-	-
Reducing Sugar	Fehling Reagent Test	+	+
Tannin - wounds healing properties, antidiarrheal antibacterial activity	Neutral $FeCl_3$	+	+

PTC can also be mix with other biological techniques as Swamy *et al.* (2015) [7], synthesise nanoparticles (Ag Nps) of *Leptadenia reticulate.* Further, the *in vitro* cytotoxic effects of Ag Nps were screened against HCT15 cancer cell line and viability of tumor cells was also confirmed

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