REVIEW OF LITERATURE
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The plants used by the tribals of South Gujarat as medicines number over two hundred. Among these, *Argyreia boseana* Sant. & Pat., *Curculigo orchioides* Gaertn. and *Hemidesmus indicus* (L.) R. Br., were selected for this doctoral research work. The plants were chosen on the basis of their availability and their usage by indigenous groups for various medicinal purposes. All three plants are found growing in the South Gujarat forest regions and are used by the indigenous tribal groups living there to cure various ailments, which includes some unreported and unscrutinized uses, which has furthered our interest on working on these plants.

2.1. *Argyreia boseana* Sant. & Pat.

2.1.1. Botanical description:
*Argyreia boseana* Sant. & Patel (Synonyms: *Argyreia festiva* Wall., *Argyreia malabarica* Woodrow, *Argyreia hookeri* Cooke), commonly known as Kumbharao, belongs to the family Convolvulaceae. It is an extensive climber with the stem grooved, glaborous or slightly pubescent. The leaves of the plant are ovate-cordate to narrow-lanceolate, usually pubescent or silky beneath. Flowers are showy and white, which is rare in the genus. The cyme is generally many flowered, often capitate with bracts often conspicuous. The flowers generally have 5 sepals and they are of leathery texture. The fruits are a little enlarged and often red coloured from within. The corolla of the plant is unfundibuliform or somewhat tubular with plicate limb and short lobed. It bears 5 stamens with oblong or straight stamens and annular disk. The ovary is 4 celled, has 4 ovules, with style filiform, and stigma globose. The fruit is of a berry type. The seeds are fewer and remain embedded in the fruit pulp (Kirtikar & Basu, 1975).

2.1.2. Geographical distribution:
The plant flowers during the months of August-September. It is reported to be found in Mahabaleshwar, Rohal, Ghosalel, Chaukull and Xudal-ghotge in Maharashtra (Anonymous, 1946; Almeida, 1996).

Though the plant has been reported in the ‘Flora of Maharashtra’ as endemic to India, it is not mentioned in important documents like ‘Flora of Gujarat’ (Shah, 1978), in the report prepared by the Gujarat Ecological Commission titled ‘Threatened Biodiversity: Baseline Information...
(2001)' and the official list of flora of the Shoolpaneshwar Sanctuary titled ‘The Eco-environmental studies of Sardar Sarovar Environ’ (1992). However, the plant has been traced in the South Gujarat Forests (D’Cruz, 2002).

The plant is rare in distribution and is currently endangered due to over-exploitation. Earlier, the plant abounded in the locality neighbouring the forest nursery in Dedipada region, but it has presently been almost wiped out from the same region and is restricted to the inner recesses of the Dedipada Forests, South Gujarat (D’Cruz, 2002).

2.1.3. Reported uses:
Scientific reports on this plant are not available. On the other hand, the traditional reports for the plant suggest that the leaves are antiphlogistic and the roots are cathartic, bitter, aphrodisiac, diuretic and used in gonorrhoea, rheumatism and diseases of the nervous system (Anonymous, 1962).

However, in a related species belonging to the same genus, *Argyreia nervosa*, the seed extracts were reported to possess hypotensive activity (Rastogi & Mehrotra, 1990).

2.1.4. Traditional medicinal and other uses:
A serious disease that merits medical scrutiny is described by the Vasavas, a tribal community inhabiting the South Gujarat Forest region, as “Nagot”. Symptoms include headache, body swelling, intense stomachache, oedema below chest region, fever and a hazy mind. The symptoms are very similar to liver cirrhosis. In stomachache, body swelling and severe headache root extract is given (D’Cruz, 2002).

2.1.5. Phytochemical studies:
There are no phytochemicals reported from the plant as no scientific work has been carried out. However, in a related species belonging to the same genus, *Argyreia speciosa* several important chemicals like 1- Triacontanol, β- sitosterol, Epifriedelinol and its acetate isolated from the plant have been reported (Masur et al., 1971). Ergometrine, Caffeic Acid and Ethyl Caffeate were isolated from seeds of the plant (Agarwal & Rastogi, 1974). Several species belonging to *Argyreia* genus also contain lysergic acids. The oil contains Oleic acid as the major component (Batra & Mehta, 1985).
2.1.6. Pharmacology of *Argyreia*:
The roots of *Argyreia speciosa* are alterative and tonic. Powdered roots are given in milk to cure synovitis and syphilis. The leaves of the plant are antiphlogistic and is also used in curing skin diseases. The powdered roots are soaked seven times during seven days in the juice of the tubers of *Asparagus racemosus* and dried. The resulting powder is given in doses of a quarter to half a tola with clarified butter for about a month. This improves the intellect, strengthens body and prevents effects of age. Roots of this plant forms an ingredient of a compound powder *Ajmodadi Churna* which is useful in rheumatic affections and hemiplegia (Nadkami, 1996; Sheth, 2004).

The oil extracted from the seeds of *Argyreia speciosa* show moderate antiseptic activity against several Gram +ve and Gram -ve bacteria and phytopathogenic fungi (Rastogi & Mehrotra, 1990).

2.1.7. Patents:
As the plant still remains unexplored and underresearched, there are no patents found on the plant. However, in related species, *Argyreia nervosa* and *Argyreia speciosa* patents of various medicinal and other uses have been reported, which are listed in the table below with their patent numbers and titles.

**Table. 2.1. List of patents granted on *Argyreia nervosa* L.**

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Source: www.freepatentsonline.com

2.2. *Curculigo orchioides* Gaertn.

2.2.1. Botanical description:
*Curculigo orchioides* Gaertn. is a plant belonging to family hypoxidaceae/ Amaryllidaceae. It is a seasonal perennial and while the underground tubers mature and grow through out the year,
the aerial parts are seen sprouting only during the monsoon season. The rootstock of the plant is stout, short or elongate, sometimes up to 30 cms., with copious fleshy root fibres. The leaves are sessile or petiolate, 15-45 by 1.3-2.5 cms, linear or linear-lanceolate, membranous, plicate, glabrous or sparsely softly hairy. The tips of the leaves sometimes on reaching the ground, root and base sheathe. The scape of the plant is very short, clavate, flattened, with pedicles, bracts and ovary hidden among the leaf sheaths.

The flowers of the plant are bright yellow, distichous, the lowest in the raceme 2 sexual, the upper male; bracts lanceolate, membranous.

Perianth segments 13-17 mm long, elliptic-oblong, acute, hairy on the back. The stipes are very slender, 1/3-2.5 cm long. The stamens are small, filamentous, very short, anthers linear.

The ovary of *Argyreia* flowers is lanceolate, the cells 6-8 ovulate; stigma with 3 cleft. The fruits are capsule type and are 13 mm long, hypogaeous, 1-4 seeded, with a slender beak, having spongy septa (Kirtikar & Basu, 1975). The seeds are oblong; testa deeply grooved in wavy lines, black and shining.

2.2.2. Common Names:

2.2.3. Geographical distribution:
The plant is found in all parts of India beginning from subtropical regions of Himalayas, Kumaon to Assam and in the Western Ghats from Konkan to southwards from near sea level to around 2300 m in rock crevices and lateritic soil. The plant is also distributed across Japan, Malaysia, Australia and China (Nasir, 1970; Pal & Jain, 1998). In Temperate regions of Asia in China in regions like Fujian, Guangdong, Guangxi, Guizhou, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang and also in Eastern Asian region Japan in Honshu (Wu, 1994), Kyushu, Ryukyu Islands, Shikoku; Taiwan the plant has been reported.
In tropical Asia along with Indian Subcontinents mentioned earlier, the plant has been seen growing in Nepal, Pakistan, Sri Lanka, Cambodia, Myanmar, Thailand, Vietnam, Indonesia, Malaysia and Philippines.

2.2.4. Environmental status of the plant:
The plant has been reported to be endangered in several official lists of endangered species including the Red list of threatened plant species. There are many possible reasons for that:

1. The plant is seasonal and grows only during monsoon seasons. Thus, the plant is available only for 3-4 months every year.
2. The flowers are few and the plant has not been seen bearing seeds. If at all there are seeds, they are not viable.
3. The plant reproduces through bulbils, which fall on the ground and germinate into a new plant. In most of the regions of Gujarat, because of the irregularities in rains, the bulbil formation doesn’t necessarily take place in a season and thus the plants have limited scopes to multiply themselves.
4. The species is highly medicinal, but not considered for systematic cultivation. Thus, to meet the demand of raw materials, the plants are unscrupulously uprooted from wild, which are then never replenished (CAMP Reports, 1995).

The availability of this plant is constrained to only 4 months each year. The plant starts growing with the onset of the monsoon season and disappears with the cessation of rains. Because of its significant medicinal properties the plant has been exploited by various means such as cattle grazing in the forests, collection of leaf litter by local people and the growth of a weed named Chromalena odorata (Augustine & D’Souza, 1997).

Various Reports of Botanical Survey of India and NBRI (National Botanical Research Institute) report the plant as an endangered species. According to the data published by the Government of India, Curculigo orchioides Gaertn. is required by the Pharma/Ayurveda industries to the tunes of 2.25 tonnes/annum and is reported to be in short supply to meet this demand (Planning Commission of India, 2005).

2.2.5. Cultivation Details:
A group of scientists in Aromatic Plants Research Station, Kerala carried out a cultivation study on two different biotypes of Curculigo orchioides Gaertn. and concluded that that Panamkuzhi
variety had an active growth phase of 7 months and after that on harvest, it gave the highest rhizome yield (Joy et al., 2004).

The same group later reported that the dry matter production and yields of *Curculigo orchioides* were the highest at 25 per cent shade and 10 x 10 cm spacing due to improved plant height, number of leaves and canopy spread and also a higher chlorophyll-a and a+b contents and harvest index. The content of primary metabolites and curculigoside in rhizome was higher at closer spacing. The uptake of nutrients was higher under shaded condition and closer spacing due to higher plant densities and dry matter production (Joy et al., 2004).

2.2.6. Reported uses:

*Curculigo orchioides* Gaertn. is a potent medicinal plant as it possesses several disease curing properties ranging from aphrodisiac, hypoglycaemic, anti carcinogenic, anti asthmatic, tonic, wound healing, spasmylytic, vasoactive, immunosuppressant, hepatoprotective etc. The tubers are also used as pesticides (Ignacimuthu, 1998).

*Curculigo orchioides* is reported to be a rejuvenator and aphrodisiac. The rhizome extract is reported to be hypoglycaemic and anti carcinogenic (Dhar et al., 1962). It is also effective against hemorrhoids, diarrhoea, jaundice and asthma. The rhizomes are directly applied to the cuts for wound healing (Asolkar et al., 1992). It is also reported to be an anticarcinogenic, spasmylytic, hypoglycemic and antiasthmatic (Bisht & Nayar, 1960; Chandel, 1996).

2.2.6.1. Aphrodisiac property: *Curculigo orchioides* Gaertn. is mainly famous for its aphrodisiac property as it is believed to help in over coming sexual debilities in males and works as a sexual tonic for female. In a study, ethanolic extract of rhizomes was evaluated for its effect on orientation behavior and spermatogenesis in albino rats. A change in orientation behavior was assessed by orientation towards female, towards environment, towards self and type of mobility. Administration of 100 mg/Kg of ethanolic extract had pronounced effect on orientation of male towards the female rats. The increased spermatogenesis in treated group was confirmed by change in histo-architecture as evidenced by increase in number of spermatocytes and spermatids. These findings support the folk use of this plant as an aphrodisiac (Chauhan and Dixit, 2008).

2.2.6.2. Inhibitors in Alzheimer's disease: New polyphenol classes have been tested against amyloid-α peptide aggregation. Scientists have identified four novel polyphenols which could
be efficient fibril inhibitors in Alzheimer's disease: malvidin and its glucoside and curculigosides B and D (Riviere et al., 2008).

**2.2.6.3. Vasoactive properties**: Vasoactive properties of two new glucosides, which were found to be present in tissue cultured shake flask cultures was analysed in rat (Valls et al., 2006).

**2.2.6.4. Immuno stimulant effects**: Two phenolic glycosides were studied for their effect on macrophage migration index (MMI), haemagglutination (HA) titre, plaque forming cell (PFC), PHA-induced blast transformation of lymphocytes (BTL) and delayed type hypersensitivity (DTH). Significant immunostimulant activity was found in purified glycoside-rich fraction isolated from the ethyl acetate extract (Lakshmi, 2003). In another experiment, cell mediated immunity was checked in immunosuppressed mice and similar results were obtained (Bafia & Mishra, 2005).

**2.2.6.5. Hepatoprotective activity**: Methanolic extract of the tubers was investigated for its hepatoprotective activity using carbon tetrachloride (CCL₄) intoxicated rat liver as the experimental model. Various Biochemical parameters were checked and the findings proved that the hepatotoxic rats showed signs of returning towards normalcy in MEC co-administered animals, thus corroborating the antioxidant efficacy of MEC (Venukumar & Latha, 2002).

**2.2.6.6. Anti microbial activity**: The roots of Kalimusli (Curculigo orchioides) can be fractionated with different solvents and screened for their antimicrobial and antitumor activity. Antifungal activity is then screened using agar plate method, and antibacterial activity of the extracts are determined by disk diffusion method.

In another study, Curculigo orchioides rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (Staphylococcus aureus and Staphylococcus epidermidis) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa and Salmonella typhimurium) bacteria. Because the steam distilled preparation was found to be the most active amongst the different extracts, its antibacterial activity against the aforementioned strains was compared to the standard antibiotics gentamycin, ampicillin, doxycycline and erythromycin. Only the clinical isolate of S. aureus showed more sensitivity towards water extracts than the standard
strain. Also, the steam distilled fraction was more effective against Gram-positive strains than Gram-negative strains (Nagesh, 2008).

2.2.6.7. Anti tumour activity: Antitumor activity was screened against a human breast cancer cell line (MCF-7). The antitumor activity of different fractioned extracts of Kalimusli was reported to be due to the presence of saponins, which are glycosides (Varshney and Sharma, 1996; Singh & Gupta, 2008).

2.2.6.8. Anti diabetic activity: The ethanolic extract of the tubers was investigated to study the effect on blood glucose level. The antihyperglycemic efficacy of ethanolic extract of the rhizome was evaluated in normal, glucose loaded and alloxan induced diabetic rats. Both alcohol and aqueous extracts were tested with alloxan-induced diabetic rats. Blood glucose levels were evaluated on the 7th, 14th, and 21st days. Doses of 500 and 1000 mg/kg body weight of both extracts produced significant (p < 0.001) hypoglycemic activity in alloxanized rats when compared with diabetic control (Madhavan, 2007).

2.2.6.9. Anti asthmatic activity: The ethanol extract of Curculigo orchiodes Gaertn. was evaluated for antiasthamatic activity by using various in vitro and in vivo animal models and the results of these studies indicated anti asthmatic potentials of the ethanolic extract of Curculigo orchioides (Pandit et al., 2008).

2.2.6.10. Anti oxidant activity: The methanolic extracts of dried tubers of Curculigo orchioides Gaertn. showed anti oxidant activity to a significant level (T'ang et al., 2004).

2.2.6.11. Effects of bone strength: It was found that administration of the rhizome extract prevented bone loss in the trabecular bone of the tibia in ovariectomized rats without affecting the weight of the body and the uterus, and increased serum phosphorus, calcium, and OPG levels, decreased serum DPD/Cr, TRAP, ACTH, and corticosterone levels, but did not alter serum TNF-α, IL-6, and ALP levels in ovariectomized rats (Cao, 2008).

2.2.7. Ayurvedic uses: 
According to Ayurveda, Black Musli is bitter-sweet to taste with a peculiarly sweet after-taste that remains even after digestion. Further, the roots are heating, aphrodisiac, alterative, appetizer,
fattening, useful in piles, biliousness, fatigue, blood diseases etc. According to Unani system of medicine, roots are carminative, tonic, aphrodisiac, antipyretic and useful in bronchitis, ophthalmia, indigestion, vomiting, diarrhoea, lumbago, gonorrhoea, gleet, hydrophobia, joint pains etc. The roots are of use in Seeth Veeryam, polyuria, white leprosy, aphrodisiac, prameham (Leucorrhoea) with constant discharge.

The rhizomes are pounded with ajwain and the decoction is given to unconscious children. It is also prescribed in piles, jaundice, asthma, diarrhoea and gonorrhoea. It is considered to be a demulcent and an aphrodisiac (Kirtikar & Basu, 1975).

Both Hindu and Mahomedan medical literatures mention white and black Mûsali. In the Râja Nirghanta, the plant is described as Hemapushpi, “having golden flowers,” and is considered to be alterative, tonic, restorative, and useful in piles, debility and impotence. It enters into the composition of several medicines intended to act as aphrodisiacs and restoratives. The plants of two years or more age have the best potentials to cure the ailment. These selected plants are washed and sliced with a wooden knife, threaded upon a string, and dried in the shade; when dry they may be powdered. The dose is 180 grains, to be beaten up with an equal quantity of sugar in a small glass of water or milk until it forms a thick mucilage. Treatment to be continued for forty days, abstinence from mental and physical exercise being enjoined. Mûsali is prescribed for asthma, piles, jaundice, diarrhoea, colic, and gonorrhoea; it is considered to be demulcent, diuretic, tonic, and aphrodisiac, and is often combined with aromatics and bitters (Dymock, 1890).

2.2.8. Traditional medicinal and other uses:
The bulbs of the plant are useful in scorpion bites. They are prescribed usually combined with bitters and aromatics in the form of electuary, the dose being a teaspoonful twice a day. In case of Gonorrhoea and dysuria, menorrhrea, leucorrhoea and menstrual derangements the drug is given with warm milk and sugar in doses of two drachms. In case of piles, asthma, jaundice, diarrhoea and colic pains, the tubers are first washed and then cut with wooden knife, they are then dried in the shade and then given with equal quantity of sugar in a glass of milk in the form of a thick mucilage (Nadkarni, 1996). It has also been used in our Indian Medicinal system mainly as an energizer and as aphrodisiac (Nadkarni, 1982).
The Lodha Community prescribes root paste about 7 g with decoction of 9 long peppers as a cure for stomach ulcers. They give root paste with curd (3:1) to women suffering from leucorrhoea. For the cattle suffering from fever, the Lodhas give paste of 21 roots about 1 cm long with Pulta (Croton roxburghii) leaf paste and santals apply fresh root paste to cattles to stop bleeding (Pal & Jain, 1998).

The Santals take the powder of dried root stocks as a cure for dyspepsia. The mundas give rhizome paste with goat milk and black pepper seed powder (4:3:1) as a tonic against impotency. In the Phillipine islands, the plant part is used for the treatment of various skin diseases.

The traditional healers of Chhattisgarh use both Kali (Curculigo orchioides) and Safed Musli (Chlorophytum sp.) together in common herbal formulations used as sex tonic and aphrodisiac. In indigenous systems of medicine, Kali (Black) Musli is used more frequently as compared to the Safed Musli. The traditional healers of Chhattisgarh Plains prepare the herbal formulation using Black Musli, Safed Musli, Satavar (Asparagus racemosus) and Mochras (Gum of Semal). All herbs are used in equal amount. This formulation is recommended with a glass of cow milk, daily at night. This herbal formulation is supposed to be consumed at least for a month during the winter season. The healers recommend it to the patients of different age groups for various purposes. The young patients having the problem of spermatorrhoea, night pollution etc. are advised to take it till complete cure. In old age, this formulation helps in maintaining sexual health. It is also believed that the formulation can be applied in form of aqueous paste externally on male genitals for getting more instant effect (Oudhia, 2003).

2.2.9. Phytochemical studies:
The rhizome contains the saponins, Curculigo sapcnins A, B, C, D, E, F, G, H, I, J, K, L and M; the sapogenins, Curculigenin A, B, C; the phenolic glycosides, Corchioside A, Curculigoside B, the Chlorophenyl glycosides, Curculigines B and C, a triterpene alcohol, Curculigol, a pentacyclic triterpene, an aliphatic compound, 31-methyl-3-xo-20-ursen-28-oic acid, 25-hydroxy-33-methylpentatricontan-6-one along with N-acetyl-N-hydroxy-2-carbamic acid methylester, 3-acetyl-5-carbomethoxy-2H-3,4,5,6-tetrahydro-1,2,3,5,6-oxatetazine, N,N,N’,N’ tetra Methylsuccinamide, henriciacontanol, α-sitosterol, Stigmasterol, Cycloartenol, and Sucrose. Curculigo saponin C and F promote proliferation of spleen lymphocytes very significantly. Curculigosaponin F and G increase the weight of the thymus in vivo in mice. A peptide curculin
C, containing 114 amino acids, has been isolated from the fruits, for use as a flavour modifier. Curculin elicits a sweet taste. Alcoholic extract of the plant shows adaptogenic, anti-inflammatory, anti convulsive, sedative, androgenic activities. It is believed in traditional Chinese medicine, that Curculignins Rhizoma consists of dried rhizome containing Curculigoside is used as a tonic. Curculigoside has been identified as the characteristic compound having immunological and protective property of the Rhizome (Gupta et al., 2005).

The principle constituents present are starch 43.48%, tannins 4.15%, enzymes 14.18% and ash 8.6%. Apart from this it also contains glycoside, orcinol-1-O-beta-D-apiofuranosyl-beta-D-glucopyranoside, curculigoside, syringic acid.

From the rhizomes of Curculigo orchioides Gaertn. two phenolic glucosides namely orchioisides A and B were isolated besides four known compounds and their structures were elucidated (Gupta et al., 2005).

A new orcinol glucoside, orcinol-1-O-α-D-apiofuranosyl-(1->6)-α-D-glucopyranoside, was isolated from the rhizomes of Curculigo orchioides Gaertn., together with seven known compounds: orcinol glucoside, orcinol-1-O-α-D-glucopyranosyl-(1->6)-α-D-glucopyranoside, curculigoside, curculigoside B, curculigoside C, 2,6-dimethoxy benzoic acid and syringic acid. The structures of these compounds were elucidated using spectroscopic methods. The antioxidant activities of these isolated compounds were evaluated by colorimetric methods based on their scavenging effects on hydroxyl radicals and superoxide anion radicals, respectively. All the compounds showed potent antioxidative activities (Wu et al, 2006).

The roots contain saponins, tannins, mucilage, starch, sapogenins, curculigenin, phenolic glycosides, orchioisides, curculigoside, curculigines B & C, a triterpene alcohol, curculigol, a petacyclic triterpene, cycloartenol and sucrose (Sheth, 2006). Phytochemical analyses have been done by several groups and some pharmacologically important chemicals have also been established, like flavanone glycoside-I, three steroids, six triterpenoids, three saponins and other metabolites [Garg et al., 1989; Rao & Mishra, 1996; Rao et al., 1978; Rekha & Reddy, 1998; Tandon & Shukla, 1995; Xu et al., 1992].
Two new aliphatic hydroxyl ketones—27-hydroxytriacontan-6-one and 23-hydroxytriacontan-2-one (Mistra et al., 1984), 21-hydroxytetracontan-20-one and 4-methylheptadecanoic acid from rhizomes, Curculigine A, N-Acetyl-N-Hydroxy-2-Carbamic Acid methyl ester, 3-acetyl-5-carbomethoxy-2 H-3,4,5,6-tetrahydro-1,2,3,5,6-oxotetrazine (Mehta & Dubey, 1983) and N, N, N', N'-tetramethyl succinamide, a new orcinol glycoside—Corchioside A (Gupta et al., 2005), Hentriacontanol, sitosterol, Stigmasterol, Cycloartenol and Sucrose were isolated (Garg et al., 1989). Thirteen various saponin compounds were isolated from Curculigo roots, which included Curculigenin A, Curculigosaponin A, Curculigosaponin D, E and F (Xu et al., 1991).

The other reported important compounds are Lycorine, Resin, Tannin (1.45%), Fat starch (48.48%) and ash 60% containing oxalate of calcium (Duke, 1991). 5,7- dimethoxy-dihydromyricetin-3-O-α-L-Xylopyranosyl, α- D-Glucopyranoside, Yuccagenin (Kirtikar and Basu, 1975).

Lakshami et al. have identified new phytoconstituents from the ethyl Acetate fraction of the tubers of Curculigo. The compounds are n-decan-3-yl pent-3'-en-1-oate; n-heneitriacont-13-en-5; 10-diol hex-2-en-1-oate; n-hexadec-9; 11-dienyl cinnamate; n-tridecanyl-hex-2; 4-dien-1-oate (Lakshami et al., 2004).

However, variations in the biochemical composition of Curculigo orchioides Gaertn. due to genetic, geographical and climatic factors were observed, which can ultimately affect the herbal composition (Savithri et al., 2005).

2.2.10. Pharmacology of Curculigo orchioides:

The roots are reported to be bitter, sweet, carminative, tonic, aphrodisiac, antipyretic, useful in bronchitis, ophthalmia, indigestion, vomiting, diarrhoea, lumbago, dyspnoea, gonorrhea, gleet, hydrophobia, pains in the joints according to Unani literature.

The rhizome is prescribed for asthma, piles, jaundice, diarrhoea, colic, and gonorrhea. It is considered to be demulcent, diuretic, tonic and aphrodisiac and is often combined with aromatics and bitters. (Kirtikar & Basu, 1975).

The experiments showed that ethanol extract of Curculigo orchioides had adaptive effects, such as enhancing tolerance towards high temperature and hypoxia. It also had sedative,
anticonvulsant and androgen-like effect. Besides, it increased the immunological activity of mice (Chen et al., 1989).

Along with many other plants, Curculigo orchioides can warm the kidneys and strengthen the yang (Chun-Chun, 1998).

2.2.11. Toxicity studies
No toxic side effects have been reported for the plants at usual concentrations. Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines 420 in Swiss mice at a dose level of extracts up to 10 g/kg (Pandit, 2008).

2.2.12. Patents granted on Curculigo orchioides Gaertn.
Total 24 patents have been obtained on the various properties of the plant, which have been listed below.

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Source: www.freepatentsonline.com

2.3. *Hemidesmus indicus* (L.) R. Br.

*Hemidesmus indicus* (L.) R. Br., a twining shrub, is commonly known as 'Indian Sarsaparilla' and in some regions also as 'Anant Mul' and 'Sariva'. The plant belongs to family Asclepiadaeae. This is a common medicinal plant: widely used in Indian systems of Medicines and also an official drug in Indian Pharmacopoeia (Anonymous, 1996) and British Pharmacopoeia (Anonymous, 2003).
2.3.1. Botanical description:
It is a perennial, slender, laticiferous, twining or prostrate, wiry shrub with woody root stock and numerous slender, terete stems having thickened nodes. The leaves of *Hemidesmus indicus* are simple, opposite, very variable from elliptic to oblong to linear-lanceolate, apiculate, acute or obtuse, entire, leathery, shiny, dark green above and paler beneath, variegated with white above, silvery white and pubescent beneath. The flowers of this plant are greenish purple in colour and are crowded in sub sessile cymes in the opposite leaf axils. The corolla is five partite, lobes thick, ovate-oblong, rugose within; corona single, coralline with five stamens having incurved filaments and distinct pollinial bages which are spherical, closely pressed to the brownish caudicle. The fruits are slender, follicles cylindrical, around 10 cm in length, tapering to a point at the apex. The seeds are flattened, black, ovate-oblong, silvery white (Sivraj & Balchandran, 1994).

*Hemidesmus indicus* (L.) R. Br. generally flowers during August to January.

2.3.2. Common Names:
The plant is known as Sugandhi, Anantmul, Gopimulam, Sariva or Saribha in Sanskrit. In English it is called Indian Sarsaparilla or Country Sarsaparilla. Magrabu, Salsa, Kalisar, Hindi salsa are the Hindi common names of the plant species. In Punjabi and Bengali it is known as Anantmool. In Marathi it is known as Upersari and Dudhasali. In Persian its known as Ushba-Hindi, Yasmine Barri, Aushabhe-hindi. In French the plant is known as *Periploca des Indes*, Recine de Salsepareille. In Telugu, Sagandhipala, Pala Sugandhi. In Tamil and Malayali the plant is known as Nannri, Kizhanna, Naru-ninti and in Kannada it is known as Namada-beru; Sughanda-palada-gida.

2.3.3. Geographical distribution:
This shrub is found growing in almost all the moist regions of India and shows best vegetative growth during the monsoon season (Anonymous, 1959). The plant is found most commonly in Bengal, Bombay region and is extended upto Travancore and Ceylon (Nadkarni, 1990).

2.3.4. Medicinal Importance:
Roots of this plant has shown many medicinal properties and has been used since ancient times for medication of inflammation, respiratory disorder, skin diseases, fever, asthma, eye diseases, kidney and urinary disorder, rheumatism, diarrhoea and bronchitis. It has also been used in combination with other drugs for snake bites (Cragg et al., 1997).
2.3.5. Reported uses:
The plant possesses several disease curing activities, which include anti asthmatic, anti cancer, anti microbial, hepatoprotective, immuno stimulant and many others.

The stem, leaf and root extracts are extensively used as blood purifiers, diuretic, antirheumatic and antidiarrhoeal agents (Chopra et al., 1980; Das et al., 2003), antimicrobial activity (Hiremath et al., 1997; Jain & Basal, 2003), antioxidant and antithrombotic activity (Ravishankara et al., 2002; Mary et al., 2003), and has also been found to counteract atherogenesis caused in Type II Diabetic models (Murshed et al., 2001). The milky latex of this plant is also used for relieving inflammation of the eye (Kirtikar & Basu, 1935). The compound, (2-hydroxy-4-methoxy benzoic acid), purified from the roots of *Hemidesmus indicus* (L.) R.Br. showed venom neutralizing effect (Alam & Gomes, 1998). The extract obtained from roots is used for preparation of soft drinks (Hill, 1952; Sarasan et al., 1994).

2.3.5.1. Anti carcinogenic activity: Roots of *Hemidesmus indicus* (L.) R. Br. contain protective activities against hepatocarcinogenesis and severa other cancers (Iddamaldeniya et al., 2003; Wemeke et al., 2004; Anup & Jagadeesan, 2003).

2.3.5.2. Anti venom activity: Inflammation induced by Viper venom and Propiono bacterium acne are reported to be treated by root extract possibly by reducing reactive oxygen species and inflammatory cytokines IL8 and TNF(Y(Jain & Bansal, 2003).

2.3.5.3. Rheumatism curing activity: A paste of root powder applied topically is used to treat swellings, inflammation and chronic rheumatism.

2.3.5.4. Anti nociceptive activity: The ethanolic extracts of roots has also been reported to show significant anti-nociceptive effects in mice and the effects were studied by inducing pain through acetic acid (writhing test), formalin (Paw licking test) and hot plate test in mice. The dose was administered orally in dose range of 25, 50 and 100mg/kg. The extract showed dose-dependent anti-nociceptive effect in all models for anti-nociception and it blocked the neurogenic and inflammatory pain (Verna et al., 2005). However, at molecular level, the most compelling evidence so far is its ability to inhibit the binding of transcription factor the nuclear factor kappa B (NF-kB) to DNA at low concentrations (Lampronti et al, 2005).
2.3.5.5. **Antimicrobial activity:** Essential oil of *Hemidesmus indicus* (L.) R. Br. exhibited marked antibacterial activity against both gram positive and gram negative bacteria at lower concentrations, but failed to show appreciable anti-fungal activity (Prasad *et al.*, 1983). Chloroform and ethanol (95%) extracts of *Hemidesmus indicus* showed antifungal activity against *A. niger* (Hiremath *et al.*, 1997). The glycosides of *Hemidesmus indicus* roots inhibited *S. typhimurium* induced pathogenesis non-specifically, by reducing bacterial surface hydrophobicity and perhaps also by mimicking host cell receptors, thereby blocking its attachment to host cell and further pathological effects (Das and Devaraj, 2006). The ethanolic extract of *Hemidesmus indicus* (L.) R. Br. stem showed broad spectrum antibacterial activity against all Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) and the MSSA strains with inhibition zone size of 11-44 mm. The antibacterial activity was maximum with the acetone fraction. Similarly, the synergistic interaction was also evaluated with certain antibiotics like Z-lactam antibiotics. *Hemidesmus indicus* along with extracts of *A. calamus* and *P. zeylenica* showed synergism with cefuroxime (Aqil *et al.*, 2006).

2.3.5.6. **Anti diarrhoeal activity:** The methanolic extract of root was proved to possess anti-diarrhoeal activity during both *in vivo* and *in vitro* studies. Thus, the plant constituents can be used as an alternative medicine for antibiotics or as a supplement to antibiotics to treat diarrhoea and other food borne diseases caused by multidrug resistant strains. (Das & Devaraj, 2006).

2.3.5.7. **Anti-ulcerogenic activity:** The aqueous ethanolic extracts of the roots of *Hemidesmus indicus* var. indicus were screened for anti-ulcer activity on animal models in wistar strain albino rats. The roots showed anti-ulcer activities and significantly reduced the formation of gastric and duodenal lesions in rats induced by various ulcerogenic procedures and cyto-destructing agent. It has muco-protective activity by selectively increasing prostaglandin (Anoop & Jagadeesan, 2003). Therefore it provides another alternative for ulcer treatment. It aims at enhancing the defensive factors so that the normal balance between offensive and defensive factors is achieved (Dharmani & Palit, 2006).

2.3.5.8. **Anti hyperlipidemic activity:** The cell culture extract of *Hemidesmus indicus* (L.) R. Br. was studied in normal and hypercholesterolemic rats for various lipid profiles in serum, tissues and fecal matter. Along with the atherogenic diet cell culture *Hemidesmus indicus* lowered the levels of serum, tissue and fecal lipid levels (Bopanna *et al.*, 1997). Recent *in vivo* studies...
using a ethanol-induced experimental model of hyperlipidemia showed that 2- hydroxy 4-methoxy benzoic acid (HMBA) is the bioactive molecule. Oral treatment of HMBA at 200 g/kg was shown to significantly reduce the ethanol-induced elevated plasma and hepatic levels of total cholesterol, triglycerides, lipoproteins, phospholipids and free fatty acids in rats. This was accompanied by elevated lipoprotein lipase (Saravanan & Nalini, 2007).

2.3.5.9. Hepatotonic and hepatoprotective activity: Prabakaran et al. (2000) found out that the oral consumption of 70% exthanolic extract of the plant, if given for 15 days, significantly prevented isoniazid induced hepatotoxicity in rats. Similar results were reported by Srivastava and Shivanandappa in 2006.

2.3.5.10. Antileprotic activity: Aqueous extract of roots of the plant exhibited bacteriostatic activity in mice infected with Mycobacterium leprae. P-methoxy salicylic aldehyde present in the extract was considered to be responsible for the activity (Gupta, 1981). Ethanolic extract also delayed the cutaneous hypersensitivity stimulation effects (Atai et al., 1986).

2.3.5.11. Chemoprotective activity: It was found that the roots of Hemidesmus indicus (L.) R. Br. were effective as chemopreventive agents in skin and were capable of ameliorating cumene hydroperoxide induced cutaneous oxidative stress and tumour promotion in a dose dependant manner (Atal et al., 1986; Shetty et al., 2005).

2.3.5.12. Radioprotective activity: Radioprotective effect of Hemidesmus indicus (L.) R. Br. root extract on lipid peroxidation in rat liver microsomes and plasmid DNA when examined showed higher rates of protection to microsomal membranes as evident from reduction in lipid peroxidation values. The extract could also protect DNA from radiation induced strand breaks (Shetty et al., 2005).

2.3.6. Ayurvedic uses:
The plant is reported to be one of the top twenty ayurvedic plants used in ayurvedic formulations (Patwardhan et al., 2004). Hemidesmus indicus (L.) R. Br. is one of the important ayurvedic plants and is in use till date in many formulations like Saarivadi kwatha, Sarivadyasava, Sarivadi vati, Sarivadyavaleh, Syrup, Tinctures of Hemidesmus indicus, Sarivadi Him, Pushyanug Churna etc. It has also been used in Homeopathy as a blood purifier.
In *Ayurveda* and *Siddha*, the plant is reported to have many curing properties and is known as: *Mathuda-rasam*, *tktarasam*, *seethe-veeryam*, *Mathura-vipaka*, *snigdham*, *kapham*, *vathararktam*, *kushtam*, *jwaram*, *prameham*, *pittam daham*, *arochakam*. It is also used to cure syphilis (Nadkami, 1990).

According to *Ayurveda*, root is cooling, aphrodisiac, antipyretic, alexiteric, antidiarrhoeal, astringent to bowels and useful in treatment of fevers, foul body odour, asthma, bronchitis, blood disorders, leucorrhoea, dysentery, diarrhoea, thirst, burning sensation, piles, eye troubles, epileptic fits, poisoning, rat bites etc. According to *Unani* system of medicine, root and stem are laxative, diaphoretic, diuretic and useful in treatment of syphilis and leucoderma. Roots are useful in hemicrania, joint pains and syphilis whereas stem is good in treatment of brain, liver and kidney related diseases. It is also useful in treatment of urinary discharges, uterine complaints, paralysis, cough, asthma etc. In central India, a special “Herbal Mala” is made from the root pieces of Anantmool and Semal (*Bombax ceiba*) which is used in the treatment of Marasmus. They also prepare a special herbal tea from the bark given twice a day for treatment of impurities of blood. Sometimes ‘Kevatch’ (*Mucuna pruriens*) and ‘Gokhru’ (*Tribulus terrestris*) are also added in this mixture. The natives use the roots internally in treatment of premature graying of hairs, jaundice, eye related diseases. A decoction is prepared by adding roots of anantmool, *Vetiveria zizanioides*, dried ginger, *Cyperus rotundus* and *Holarrhena antidysenterica* for the treatment of chronic fever and appetite. To take away extra heat from body, root powder is fried in ghee and given to the patients for up to one month. The root is also used with cow milk for treatment of renal calculi.

The root is an alterative tonic, diuretic, demulcent, diaphoretic and carminative. It is said to be good for gout, rheumatism, colds, fevers and catarrhal problems as well as for relieving flatulence, skin problems, scrofula and ringworms. It is blood purifier and said to be promoting health and cures all kinds of diseases caused by vitiated blood. It is useful in venereal diseases, herpes, skin diseases, arthritis, rheumatism, gout, epilepsy, insanity, chronic nervous diseases, abdominal distention, intestinal gas, debility, impotence and turbid urine in Ayurvedic system. It also purifies the urino-genital tract, blood and helps cleanse the mind of negative emotions; therefore it is useful in many nervous disorders.
It promotes health and vigor. Decoction of stalks and leaves is used for skin eruptions, hearing disorders, fevers etc. Root decoction helps in skin diseases, syphilis, elephantiasis, loss of sensation, hemiplegia, loss of appetite, blood purification and for kidney and urinary disorders (Lampronti et al., 2005).

The root is sweet, bitter with a flavour and is cooling, aphrodisiac, antipyretic, alexiteric, antidarrheal, astringent to the bowels, cures leprosy, leucoderma, itching, skin diseases, fevers, foul odour from the body, loss of appetite, asthma, bronchitis, tridosha- a disease of blood, leucorrhea, Kapha, Vata, dysentery and diarrhoea, thirst, burning sensation, useful in piles, rat bite poisoning, eye troubles, epileptic fits in children and children’s wasting diseases (Kirtikar & Basu, 1975).

2.3.7. Traditional medicinal and other uses

In Unani, the plant has been reported to cure syphilis, leprosy, resolvent, liquefying, diaphoretic, diuretic, cure for diseases of brain, liver, stomach, kidney and uterus. Because of its cold and moisterous nature it is topically applied on ulcers (Nadkami, 1990).

The roots of Indian Sarsaparilla are alterative, tonic, demulcent, diaphoretic and diuretic and its decoction with hing and ghee is used to cure loss of appetite, diarrhoea, nausea and vomiting. The root paste is useful in curing skin diseases, ulcerations and swellings. It also cures chronic cough in children when the root bark infusion in given in milk. Hemidesmus roots re useful in Leucorrhea, Gonorrhoea and constitutional syphilis, urinary troubles, stones in kidney and bladder and its latex is useful in inflammation of eyes. The roots are also reported to be useful in chronic rheumatism, hemiplegia and in weakness and asthma (Meloo, 1995; Bhattacharjee, 2001).

The fragrant root barks are prescribed in case of dyspepsia, loss of appetite, fever, skin diseases and ulcerations especially of syphilis origin and also in chronic rheumatism and leucorrhea. Hot infusion of the root bark with milk and sugar is a good alterative and tonic especially for children in chronic cough and diarrhoea. Roots, powdered and mixed with cow’s milk is given in case of coloured urine and in instances of gravel and stangury (Nadkarni, 1990).

Lodhas prescribe root paste about 10 gm for treatment of leucoderma and apply the paste with common salt as a cure for eruption on tongues of children. The root decoction with the root
paste of *Asparagus racemosus* and paste of long peppers is useful in the treatment of gonorrhea. The decoction is administered with paste of *Eleusine coracana* seeds and milk (Pal & Jain, 1998).

The Santals give root paste with paste of black peppers to women for treatment of constitutional disorder and take root decoction with sugar as a refrigerant (Pal & Jain, 1998).

The Oranos give dried roots with warm milk as a cure for nervousness. They prescribe root paste with *Schleichera oleosa* seed oil for the treatment of skin diseases (Pal & Jain, 1998).

The Mundas give root infusion to children for treatment of tonsils. Other ethnic communities apply root juice on new cuts for stopping bleeding and for healing wounds. The root decoction with honey is used as a cure for abdominal tumours (Pal & Jain, 1998).

It is especially useful in the second and third stages of syphilis and its numerous manifestations like eruptions, syphilitic rheumatism etc., kidney and urinary disorders of various kinds and constitutional debility (Nadkami, 1990).

Root tied up in plantain leaves, roasted in hot ashes and then beaten into a mass with cumin and sugar and mixed with cow’s ghree, given twice daily can cure genitor-urinary diseases. Milky juice is put in inflamed eyes to cure the same (Nadkami, 1990).

2.3.8. Phytochemical studies:

The roots of the plant are woody and have a sweet taste. Anantmul is reported to contain pregnane glycosides, coumarino-lignoid, terpenoids and sterols (Kirtikar *et al.*, 1984). It also contains tannins, fatty acids, saponins, resin acid. Anantmul is reported to have anti-inflammatory and antipyretic activities, antidiarrhoeal effect, antinociceptive activity, antioxidant and antithrombotic activity, antitumor activity and hepatoprotective activity (Nadkami, 1989).

Air dried roots yield essential oil containing P-Methoxy salicylic aldehyde as the major constituent. The aroma of the drug is attributed to this aldehyde. Other constituents present in the roots are-β-sitosterol, alpha and β-amyrins, amyrin acetates Lupeol and its octacosanoate (Aneja *et al.*, 2008; Cragg, 1997), Tetracyclic triterpene alcohols, small amounts of resin acids, fatty acids,
tannins, saponins a glycoside and a ketone (Sheth, 2006; Rastogi & Mehrotra, 1990) have also been reported. The flavanoid glycosides recognized in the flowers, were Hyperoside, Isoquercitin and Rutin whereas in the leaves, only Hyperoside and Rutin were identified (Subramaniam & Nair, 1968). Tannins 2.5% present in leaves; roots are reported to contain Sitosterol (Chatterjee & Bhattacharya, 1955). Coumarins, triterpenoid saponins, essential oil, starch, tannic acid, triterpenoid saponins present. A stearopten smilasperic acid is also obtained by distillation with water (Joseph et al., 1981).

The plant is reported to contain significant amount of Rutin in leaves (Rajan et al., 2002), steroids in cultured tissues and mature plant (M.R Heble and M.S. Chadha 1978). The plant is employed in traditional medicine for gastric ailments (Jain and Singh, 1994) and mainly consists of essential oils and phytosterols like Hemidesmol, Hemides:erol and saponins (Sarasan et al., 1994). Coumarins, triterpenoid saponins, essential oil, starch, Tannic acid, Triterpenoid saponins are present (Gupta et al., 1992). A stearopten smilasperic acid is also obtained by distillation with water (Joseph et al., 1918). A novel pregnane glycoside viz. Hemindicusin was isolated from CHCl3: EtOH (3:2) fraction of Hemidesmus indicus (L.) R. Br. by using modern spectroscopic techniques and chemical transformations. The structure of this compound was assigned as calogenin-3-o-3-o-methyl-Y-Lrhamnopyranoside (Sethi et al., 2006).

Leaves: 2.5% tannins is present in leaves (Lampronti et al, 2005).

Stem: Two novel pregnane oligoglycosides demicunine and heminine from CHCl3: EtOH (3:2) soluble extract of dried stems of Hemidesmus indicus (L.) R. Br. (Sigler et al., 2000) as well as Desinine (Oberoi et al., 1985), Indicine, Hemidine (Prakash et al., 1991), Indicusin (Prakash et al., 1991), Hemidescine, Ermidine (Chandra et al., 1994), Medidesmine, Hemisine and Demicine (Deepak et al., 1997) have been reported in Hemidesmus indicus (L.) R. Br.

Root: Essential oil and triterpenoids are present in roots (Pandey et al., 1973). The phytochemical studies on the roots of Hemidesmus indicus (L.) R. Br. resulted in the isolation of one each of acyclic triterpenic acid; acyclic diterpenic ester and monocyclic sesterterpene ester and their structures have been established as 2; 6, 10, 14, 18, 22 hexamethyl tetracos-1 oic acid; n-octyl-2, 6, 10, 14-tetramethyl hexadec-7-ol-10-en-13- on-1-octanoate and n-non-2c-en-1c-yl, -13 (15, 19, 19- trimethyl-cyclohex-14, 16-dienyl) -2, 6, 10-trimethyltetradec-6-ol-13-on-1-oate.
along with known Z-sitosteryl glucuronate and Z-sitosterol, on the basis of spectral data analyses and chemical means (Roy, et al., 2002). The aqueous-ethanolic root extract is reported to contain alkaloids, tannins, phenols and saponins (Anoop & Jagadeesan, 2003). β-methoxy salicylic acid is reported to be present in aqueous extract of roots of *Hemidesmus indicus* (L.) R. Br. The ethanolic extract of root is reported to contain triterpenes, flavonoids, tannins, coumarins and glycosides. Roots are reported to contain Sitosterol (Chatterjee & Bhattacharya, 1955). The quantitative analysis was done on roots of *Hemidesmus indicus* (L.) R. Br. for saponins and tannins and showed 0.6% and 3.0% respectively and the qualitative analysis showed presence of carbohydrates, saponins, phytosterols, phenols, flavonoids, terpenoids, tannins and phlobatannins (Khanna & Kannabiran, 2007). Amongst the plethora of chemical entities reported, a small molecular weight aromatic compound, 2- hydroxy 4-methoxy benzoic acid (HMBA), has caught the attention as the bio-active principle of *Hemidesmus indicus* (L.) R. Br. A reverse phase HPLC method was developed and validated for the simultaneous determination of 2-hydroxy-4- methoxybenzaldehyde and 2-hydroxy-4-methoxybenzoic acid in root extracts of *Hemidesmus indicus* (L.) R. Br. (Sircar et al., 2007).

The roots of the plant contain Coumarin, a volatile oil, A crystallizable principle- hemidesmine and a crystalline stearoptin called smilasperic acid (Nadkarni, 1990).

2.3.9. Pharmacology of *Hemidesmus indicus* (L.) R. Br.: The herb is mildly immuno-suppressant. The aqueous, alcoholic and steam distilled fractions of the crushed roots had no significant diuretic activity. The 50% ethanolic extract of the whole plant did not exhibit any effect on respiration, normal blood pressure and also on pressor response to adrenaline and depressor response to acetylalcholine and histamine in experimental animals. The extract also had no antispasmodic effect on guinea pig ileum. A saponin from the plant is found to have antiinflammatory activity against formalin induced edema (Aneja et al., 2005). The antioxidant activity of methanolic extract of *Hemidesmus indicus* (L.) R. Br. root bark is evaluated in several *in vitro* and *ex vivo* models. Preliminary phytochemical analysis and TLC fingerprint profile of the extract was established to characterize the extract which showed antioxidant properties (Ravishankara et al., 2002).

Modern studies have confirmed the antibacterial activity of the root extract and essential oil. Clinical trials have shown a benefit in ringworm infection and for malnutrition. The clinically used
doses are considered safe and beneficial, but overdose can be toxic. *Hemidesmus indicus* has been shown to have significant activity against immunotoxicity and other pharmacological and physiological disorders (Sultana et al., 2003).

2.3.10. Toxicity studies:
No toxic effect has been observed if the plant is taken internally (Austin & Jagdeesan, 2002) at lower concentrations. However, in various studies it was observed that higher consumptions of the root powder at around 2500 mg/kg can cause mild to severe hepatotoxicity and hepatomegaly (Arseculeratne et al., 1985; Atal et al., 1985; Austin & Jegadeesan, 2002, 2003). However, it was mainly observed when aqueous extract was taken orally.

2.3.11. Patents granted on *Hemidesmus indicus* (L.) R. Br.:

There are around twenty patents granted on various properties of plants like anti-cigarette herbal formulation, anti-apoptotic botanicals, antioxidant, liver protective, a probiotic etc.

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Source: www.freepatentsonline.com
2.4. **Plant Tissue Culture:**

The status of the development of the plant tissue culture of these species is discussed below.

2.4.1. **Overview of Plant Tissue Culture:**

Plant Tissue Culture, also referred to as micropropagation or *in vitro* propagation/ multiplication is a technique in which a small part of the plant selected is inoculated into a synthetic medium that contains necessary micro as well as macro nutrients along with vitamins, amino acids, carbon source and phytohormones. It has both basic and applied as well as commercial applications (Thorpe, 1990).

Though the term was recommended to be used more restrictedly, these days it is generally used for the aseptic cultures of cells, tissues, organs, and their components under defined physical and chemical conditions *in vitro* (Street, 1977).

2.4.2. **Applications of PTC:**

After the 1960s, there was a dramatic increase in the applications of Plant tissue culture technique to various problems in basic biology, agriculture, horticulture and forestry through the 1970s and 80s. There applications were as discussed below:

2.4.2.1. **Cell behaviour:**

Nutrition was the earliest aspect of plant tissue culture that was investigated. Progress has been made since then in the culture of photoautotrophic cells (Yamada et al., 1978; Husemann, 1985). In vitro cultures, particularly cell suspensions have become very useful in the study of both primary and secondary metabolism (Neumann et al., 1985).

Morphogenesis or the origin of form is an area of research with which tissue culture has long been associated and one to which the technique has made significant contributions both in terms
of fundamental knowledge and application (Thorpe, 1990). Xylogenesis or tracheary element formation has been used to study cytodifferentiation (Dodds & Robers, 1985; Phillips, 1980; Fukuda & komamine, 1985).

2.4.2.2. Plant Modification and improvement:
Cell cultures have also played an important role in plant modification and improvement, as they offer advantages for isolation of variants (Flick, 1983). The somaclonal variation is dependent on the natural variation in a population of cells, either preexisting or culture induced, and is usually observed in regenerated plantlets (Larking & Scowcroft, 1981). Through plant tissue culture, it has been possible to generate a wide spectrum of mutant cells in culture (Jacobs et al., 1987). These include cells showing biochemical differences and resistance to various stresses or herbicides.

2.4.2.3. Pathogen free plants and germplasm:
Although these two uses of in vitro technology may appear unrelated, a major use of pathogen-free plants is for germplasm storage and the movement of living material across international borders (Thorpe, 1990). The ability to rid plants of viruses, bacteria and fungi by culturing meristem tips has been widely used since the 1960s. The approach is particularly needed for virus infected material, as bactericidal and fungicidal agents can be used successfully in ridding plants of bacteria and fungi (Bhojwani & Razdan, 1983).

2.4.2.4. Clonal propagation:
The use of tissue culture technology for the vegetative propagation of plants is the most widely used application of the technology. It has been used with all classes of plants from the very early times (Murashige, 1978). There are three ways by which micropropagation can be achieved. These are enhancing axillary bud breaking, production of adventitious buds directly or indirectly via callus and somatic embryogenesis directly or indirectly on explants.

Axillary bud breaking produces the smallest number of plantlets, but they are generally genetically true-to-type, while somatic embryogenesis has the potential to produce the greatest number of plantlets but is induced in the lowest number of plant species. Commercially, numerous ornamentals are produced, mainly via bud breaking (Murashige, 1990).
2.4.2.5. Product formation:
Since 1990 the continued expansion of the technique in the application of *in vitro* technologies to an increasing number of plant species has been observed. Tissue Culture techniques are being used with all types of plants, including cereals and grasses to ornamentals and medicinal plants. Using a variety of techniques like cell suspension techniques, several biofactories are being set up to generate secondary metabolites in bulk. Genetic modifications also have been given emphasis to generate newer plant species with desired traits.

2.5. Variations observed in plant tissue culture

2.5.1. Variations in progenies:
Several reports have been found indicating changes in genetic, biochemical and morphological changes in the progenies obtained through plant tissue culture. In certain cases, these are insignificant and does not alter the very purpose of micropropagation. However, in certain cases, such variations can prove to be detrimental to the whole process of micropropagation, especially if micropropagation is taken up for conservation of germ plasm.

The general belief is that when a plant is micropropagated through indirect organogenesis, the chances of encountering such variations are higher as the callus divides uncontrolled. However, the process of plant tissue culture involves three steps:

Differentiation $\rightarrow$ Dedifferentiation $\rightarrow$ Redifferentiation

In certain cases the dedifferentiation happens within the tissue by rearranging and redifferentiating cells that cannot be noticed in the form of callus. Thus many a times, plants, believed to be produced by direct organogenesis, are the product of a short term fast reorganization of tissues within the organ without any signs of callus. In such cases, ploidy level analysis along with other biochemical analysis becomes very important.

2.5.2. Variations in Ploidy levels:
Tissue culture has become an increasingly important propagation tool during the past 15 years. However, observations have been made in research studies and during commercial practice of micropropagated plantlets of features which differ from the original parent phenotype. Variation in propagules has a major impact on the commercial application of *in vitro* technologies. It is not clear in some systems whether multiple shoots arise via axillary buds or adventitious buds. When
micropropagating chimeral plants, this difference in bud origin can be ascertained by the appearance of adventitiously formed variant shoots. In addition, it is possible to study the number of cells or cell layers involved in the formation of adventitious shoots \textit{in vitro} based on the resultant plantlet phenotype.

Several strategies can be used to assess the genetic fidelity of \textit{in vitro} derived clones, but most have limitations. Karyological analysis, for example, cannot reveal alterations in specific genes or small chromosomal rearrangements (Isabel \textit{et al.}, 1993). Using the polymerase chain reaction (PCR) in conjunction with short primers of arbitrary sequence (Williams \textit{et al.}, 1990), randomly amplified polymorphic DNA (RAPD) markers have been shown to be sensitive for detecting variations among individuals between and within species (Carlson \textit{et al.}, 1991; Roy \textit{et al.}, 1992). RAPD markers have been used successfully to assess genetic stability among somatic embryos in spruce species (Isabel \textit{et al.}, 1993; 1996), among micropropagated plants of poplar (Rani \textit{et al.}, 1995) and ginger (Rout \textit{et al.}, 1997).

2.5.3. Variations in Biochemical parameters during Plant Tissue Culture:
Since the process of plant tissue culture for plantlet regeneration involves cyto differentiation, cyto dedifferentiation and cyto redifferentiation, at each stage of plant development, various parameters get altered. Some molecules are over expressed and some are repressed and thus metabolic processes are kept under check. Thus, biochemical parameters play an important role in designing developmental pathways for plants.

Visible manifestation of cell differentiation includes greening of callus, variation in the cell wall thickness and biogenesis of certain cytoplasmic organelles, such as plastids. Some tissues are specifically adapted for specialized functions, such as, secretion, storage, mechanical support and protection. Differentiation in such tissues involves differences in the basic metabolic pathways. The precise requirement for metabolites to bring about altered development can be fulfilled within the cell itself or through transport. Thus, explants require critical supply of metabolites: vitamins, phytohormones and nutrients when grown in aseptic condition. Similarly, callus cultures of certain plants require external supply of auxin and cytokinin to maintain cell division. These phenomena strongly support the tenet that cell differentiation involves the activation of certain genes and repression of others, which control different basic metabolic or anabolic pathways. Besides, hormones, several low molecular weight compounds, namely amino acids,
oligosaccharides and polyamines are also known to be involved in differentiation (Dey et al., 1998).

Since somaclonal variation was first defined (Larkin & Scowcroft, 1981), it has been widely documented in tissue culture-raised plants at morphological, chromosomal, biochemical and molecular levels in many plant species and extensively reviewed (Dey et al., 1998). Plant off-types, i.e. non true-to-type and DNA rearrangements and alterations in copy number, genetically not identical to the mother plant are known. However, it is sometimes hard to differentiate these among the resulting plants. These plants can simply result from hardening errors and not arise from a epigenetic changes. These epigenetic changes might include the genetic make up of the plant. But in contrast to somaclonal changes, such of plant growth regulator, such as auxins, for process trait is not passed to their offspring through the sexual initiation. Nevertheless, these auxins are known to be cycled or might entirely disappear during plant maturation (Shawn, 2000).

Along with variations observed in biomolecules at various stages of development, significant changes in the levels of expressions has also been seen in case of plant enzymes. Various enzymes like Peroxidases, Polyphenoloxidases and others work as metabolic markers and their activities indicate normal metabolic processes in the regenerant tissues.

2.6. Current status of tissue culture work on the chosen plant species:

Leaf explants of Curculigo orchioides cultured on a Murashige and Skoog (MS) medium without cytokinins produced a limited number of plantlets that originated directly from the cut end of the midrib. Rhizomes responded by producing plantlets with 6-Benzyladenine (BA) (0.44–6.66 μM) A higher concentration of BA (2.22–4.44 μM) resulted in nodular callus that when transferred to cytokinin-free medium formed shoots. The shoots were rooted on media supplemented with either (0.54–5.37 μM) of 1-naphthaleneacetic acid (NAA) (0.57–5.71 μM) of Indole-3-Acetic Acid (IAA), or (0.49–4.90 μM) Indole-3-Butyric Acid (IBA). The plantlets could be hardened (Augustine & D’Souza, 1997). Similar results were also reported by Prajapati et al. and other groups (Wala & Jasrai, 2003).
An efficient protocol was developed for in vitro clonal propagation of *Curculigo orchioides* Gaertn. through apical meristem culture on Murashige and Skoog (MS) basal medium supplemented with 1.5 mg/l 6-benzyladenine (BA), 100 mg/l adenine sulfate (ADS) and 3% sucrose. Inclusion of indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA) in the culture medium improved the formation of multiple shoots. The highest frequency of multiplication was obtained on MS medium supplemented with 1.5 mg/l BA, 100 mg/l Ads, 0.25 mg/l IBA and 3% sucrose. The micro shoots were then induced into rooting by transferring them on MS medium containing 0.25 mg/l IBA and 2% sucrose and the plants were hardened (Francis et al., 2007).

Successful protocol for multiple shoot induction of *Curculigo orchioides* using shoot tip and rhizome disc was established in which a synergistic effect between 6-benzylaminopurine (BAP) and kinetin was observed on the regeneration of shoot buds from proximal rhizome disc rather than shoot tip explant. Optimum root induction was achieved on half-strength MS liquid medium supplemented with 1 mg/l of Indole-3-Butyric Acid (IBA). The plantlets were then hardened with higher rates of survival (Nagesh, 2008).

Many other groups of scientists have also worked on the plant tissue culture of *Curculigo orchioides* Gaertn. having either identical or closely similar results to the ones mentioned above (Suri et al., 2002; Dhenuka & Balakrishnan, 1999; Suri et al., 1999; Suri et al., 1998).

Three different elicitors viz., Methyl Jasmonic Acid, Salicylic Acid and Ethephon were tested on in vitro grown cultures of *Curculigo orchioides* Gaertn. It was found that all three of them influenced the production of curculigosides contents of leaves and the amount of Curculigosides produced were found to be higher (Nema et al., 2008).

2.6.2. Tissue culture reports: *Hemidesmus indicus* (L.) R. Br.:

Plant tissue culture of the plant species has been carried out by various groups of scientists. Patnaik and Debata (1997) reported *in vitro* multiplication of the plant through axillary bud culture.

A tissue culture protocol was developed for rapid multiplication of the species through multiple shoot cultures obtained in MS medium supplemented with BAP 1mg/l and 0.5 mg NAA. The
multiplication rate was found to be increasing on fortification of the medium with ADS. The rooting was done using IBA 2 mg/l and 1 mg/l of NAA (Misra et al., 2003).

Similar results were obtained using lower concentrations of BAP alone or in combination with a very little auxin for the production of multiple axillary shoots by various groups (Sarasan et al., 1994; Malathy & Pai, 1995; Sharma & Yelne, 1995; Patnaik and Debata, 1997).

In work carried out by Siddique et al. it was seen that MS media containing different concentrations and combinations of 2,4- D, Kinetin and NAA showed callus induction. The Highest growth of callus was obtained in 1 mg/l NAA and 2 mg/l Kinetin (Siddique et al., 2003).

NAA 1 mg/l with BAP 2 mg/l produced maximum number of shoots (7-8) per explant having minimum response time of 4 days. Maximum elongation of shoot buds was achieved within 30-35 days after inoculation (Saha et al., 2003).

2.7. Variations in Phytochemical compositions during Plant Tissue Culture:
In the search of alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Rao & Ravishankar, 2002). Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products.

Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformations of natural compounds has been demonstrated (Cheetham, 1995; Scragg,
1997; Krings & Berger, 1998; Ravishankar & Rao, 2000). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

Table below discusses some examples of such biochemicals which have been worked on to increase their yields in plant cell suspension cultures.

**Secondary Metabolites Produced in High Levels by Plant Cell Cultures**

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>PLANT SPECIES</th>
<th>YIELDS (% DRY WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shikonin</td>
<td><em>Lithospermum erythrorhizon</em></td>
<td>20 1.5</td>
</tr>
<tr>
<td>Ginsenoside</td>
<td><em>Panax ginseng</em></td>
<td>27 4.5</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td><em>Morinda citrifolia</em></td>
<td>18 0.3</td>
</tr>
<tr>
<td>Ajmalicine</td>
<td><em>Catharanthus roseus</em></td>
<td>1.0 0.3</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td><em>Coleus blumeii</em></td>
<td>15 3</td>
</tr>
<tr>
<td>Ubiquinone-10</td>
<td><em>Nicotiana tabacum</em></td>
<td>0.036 0.003</td>
</tr>
<tr>
<td>Diosgenin</td>
<td><em>Dioscorea deltoids</em></td>
<td>2 2</td>
</tr>
<tr>
<td>Benzylisoquinoline</td>
<td><em>Coptis japonica</em></td>
<td>11 5 – 10</td>
</tr>
<tr>
<td>Alkaloids</td>
<td><em>Thalictrum minor</em></td>
<td>10 0.01</td>
</tr>
<tr>
<td>Berberine</td>
<td><em>Coptis japonica</em></td>
<td>10 2 – 4</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td><em>Galium verum</em></td>
<td>5.4 1.2</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td><em>Galium aparine</em></td>
<td>3.8 0.2</td>
</tr>
<tr>
<td>Nicotine</td>
<td><em>Nicotiana tabacum</em></td>
<td>3.4 2.0</td>
</tr>
<tr>
<td>Bisocladrine</td>
<td><em>Stephania cepharcntha</em></td>
<td>2.3 0.8</td>
</tr>
<tr>
<td>Tripdiolide</td>
<td><em>Tripterygium wilfordii</em></td>
<td>0.05 0.001</td>
</tr>
</tbody>
</table>

Source: Misawa, 1994. FAO
2.8. Protective Roles of plants:

2.8.1. Anti microbial activity:

Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications (Dhar et al., 1968; Samy & Ignacimuthu, 1998, Dahanukar et al., 2000). The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents (Chopra et al., 1996; Baquero, 1997) that lead to repeated use of antibiotics and insufficient control of the disease (NCID, 2002). New prototype antimicrobial agents are needed to address this situation. This prompted us to evaluate plants as source of potential chemotherapeutic agents, antimicrobial activity based on their ethnomedical use.

2.8.2. Anti oxidant activity:

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial biomolecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell, 1994; Halliwell and Gutteridge, 1985). In recent years one of the areas which has attracted a great deal of attention is antioxidants in the control of degenerative diseases in which oxidative damage has been implicated. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Larson, 1988; Tripathi et al., 1996; Sreejayan & Rao, 1997; Vani et al., 1997).