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A comprehensive review: *Holarrhena pubescens*

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Abstract

Holarrhena pubescens, also known as Kurchi, is an herb with bioactive compounds such as alkaloids, tannins, flavonoids, and glycosides. It has been traditionally used for treating various diseases. Studies have demonstrated its antidiarrheal, antimalarial, hepatoprotective, antioxidant, anti-inflammatory, anticancer, anti-wound-healing, and anti-diabetic activities. It restricts intestinal motility and lessens water and electrolyte flow, minimizing the frequency and severity of diarrhoea. Its bark extract shows potent anti-plasmodial activity against *Plasmodium falciparum*. It enhances antioxidant defence, reduces oxidative stress, and limits the release of pro-inflammatory cytokines, exhibiting hepatoprotective and anti-inflammatory properties. Its alkaloids boost insulin secretion and lower blood sugar levels, and its tannins and flavonoids promote angiogenesis and collagen formation, speeding up wound healing. It also inhibits cancer cell growth, potentially making it useful in cancer treatment. Its chemical analysis shows the presence of numerous bioactive substances. The present study aims to gather well-structured information related to ethnopharmacology, phytochemical makeup, and pharmacological potential of *H. pubescens*. All information on *Holarrhena pubescens* related to its various attributes carried out in this review with use of different electronic database viz. SHUATS e-journals, Science direct, CABI, PubMed, Scopus, Google scholar, J-Gate, and secondary meta data collected from Academia.edu, LinkedIn, Research Gate etc. The present review summarises botanical description, vernacular names, Pharmacognostical profile, medicinal and folk uses, reported phytochemicals, novel and cosmetic herbal formulations, toxicological reports etc.

Keywords: Phytoconstituents, ethnopharmacology, angiogenesis, Kurchi, extraction

Introduction

Although the existence of medicinal plants has been recognised for ages, human civilisations were not aware of their significance as a source of life-saving medications. Ancient medical literature such as the Rig Veda and Sushruta Samhita in Ayurveda, Dioscorides' De Materia Medica, the Ebers Papyrus from ancient Egypt, and the Pen Tsao from China all developed after this. The main concept of Ayurveda, which is the predominant traditional medical system in India, is the existence of three "doshas," or bodily systems, called kapha, pitta, and vata. In some parts of India, the Unani and Siddha medical systems are also significant. According to these systems, the presence of specific components in a balanced condition promotes good health, while their imbalance results in a variety of ailments ^[1]. The transition from natural to synthetic pharmaceuticals happened towards the end of the 19th century, and since then, the usage of phytomedicine has steadily decreased ^[2]. Although though synthetic medications are widely used, some traditional medical practises, such as Ayurveda, Unani Tibetan, and traditional Chinese medicine, are still practised today. They frequently utilise medicinal plants, which serve as a source for identifying chemical compounds and applying them to cure a variety of illnesses ^[3].

Holarrhena pubescens Synonym *Holarrhena antidysenterica* is a medicinally important plant of Africa as well as tropical and subtropical regions of Asia. It is an Indian medicinal tree that may grow up to 4 feet in circumference and 30–40 feet in height. It is a tree Native to the tropical Himalaya and Assam, it is widespread in Indian and Pakistani forests ^[4]. It is frequently used in Indian medicine to treat ailments like bleeding piles, diarrhoea, amoebic dysentery, liver disorders, and irritable bowel syndrome. The plant has an astringent, bitter flavour. Many disorders are traditionally treated with it (Table 1), and pharmacological and clinical investigations support its use for a variety of cutaneous, intestinal, and diabetic condition. Due to its many therapeutic benefits and lack of hazardous side effects, *H. pubescens* is regarded as the most beneficial medicine ^[5]. Kurchi is the name given to the stem bark of this plant, which contains astringent, antidiarrheal, antidysenteric, anti-anthelmintic, stomachic, febrifugal, digestive, and tonic characteristics. Medicinal properties of *H. pubescens* summarised in table 1 ^[6].

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Fig 1: *Hollarrhena Pubescens* (a) Leaves ^[49] (b) flowers ^[53] (c) Seeds ^[55] (d) fruits ^[44]

Table 1: Medicinal properties of *H. pubescens*.

Sr. No.	Disease	Medicinal Property
1.	Animal bites	A remedy for dog, bug, scorpion, and snake bites ^[7] .
2.	Indigestion	Appetizer, stomachic ^[8]
3.	Blood-related ailments	Anaemia, blood infection, blood purifier, haemorrhage, nose bleeding ^[9]
4.	Body pain	Pain reliever for rheumatoid arthritis, knee pain, headaches, and body aches ^[10]
5.	Brain-related disorders	Improves depression and other nervous disorders, acts as memory enhancer ^[11]
6.	Cold and throat-related ailments	Cold, cough, and throat infection expectorant ^[7, 11]
7.	Dental or oral ailments	Analgesic for toothache ^[12]
8.	Dermatological problems	Action against warts, dermatitis, leukoderma, pimples, ringworm, scabies, skin allergies, abscesses, boils, bruises, and other skin conditions ^[13]
9.	Diabetes	Regulates blood sugar ^[7, 14]
10.	Fever	Antipyretic, febrifuge for intermittent fever, pyrexia ^[15]
11.	Gastrointestinal disorders	Active against colic problems, intestinal ulcers, stomach aches, dyspepsia, flatulence, cholera, diarrhoea, and dysentery as well as food poisoning, gastroenteritis, and indigestion ^[8]
12.	General health	Muscle strength, obesity, tonic ^[15, 16]
13.	Gynaecological disorders	Easy delivery, leukorrhea, toning up vaginal tissues after delivery ^[17, 18]
14.	Joint- and muscle-related ailments	Active against arthritis, rheumatism ^[19, 20]
15.	Liver complaints	Useful for bilious disorders, bile infection, jaundice ^[21, 22]
16.	Piles	Active against piles, fissures, fistula, haemorrhoids ^[23, 24]
17.	Respiratory disorders	Active against asthma, bronchitis ^[25, 26]
18.	Urogenital disorders	Controls urination, cystitis, diuretic, dysuria, urinary problem, urinary tract infection, urine tract burning sensation ^[26]
19.	Intestinal Parasites	Anthelmintic for internal worms such as threadworms, tapeworms, and Guinea worms ^[27]

Geographical Distribution

It is indigenous to South Central China, Cambodia, Myanmar, Thailand, Vietnam, Laos, India, Nepal, Bhutan, Pakistan, Bangladesh, Mozambique, Kenya, Northern Tanzania, Zaire, Zambia, and Zimbabwe. It was first introduced in South-east China, Hainan, Taiwan, and Mauritius; however, it is uncertain whether it is present in Malaysia ^[4].

Morphological Description

H. pubescens is a deciduous tree with oblong and elliptic leaves, Corymbose cymes inflorescence with white, fragrant flowers. Corolla lobed and oblong. Fruits have white markings and have long, terete follicles. The seeds are linear-oblong and glabrous. It bears fruit from August to October and flowers from April to July. The roots of the tree are thick

and fibrous, and it produce a bitter, yellowish-white coloured latex when cut. Morphology of *Hollarrhena pubescens* summarised in table 2 and depicted its various parts of plant in figure 1 ^[28].

Botanical Classification

Hollarrhena pubescens is a plant species belonging to the family Apocynaceae, which is a large family of flowering plants that includes about 415 genera and over 5,500 species. The genus *Hollarrhena*, to which *Hollarrhena pubescens* belongs, comprises about 15 species of small trees and shrubs native to tropical regions of Asia and Africa.

The botanical classification of *Hollarrhena pubescens* is summarised in table 3 ^[29].

Table 2: Morphology of *Hollarrhena pubescens* ^[28]

Serial No.	Feature	Description
1	Leaf shape	Oblong or ovate, with a pointed tip and smooth margins
2	Leaf size	10-20 cm long and 5-10 cm wide
3	Leaf surface texture	Velvet-like pubescence
4	Flower colour	Creamy-white to yellowish-brown
5	Flower shape	Tubular, with five petals and a five-lobed corolla
6	Fruit type	Capsule, 10-15 cm long, with numerous seeds
7	Bark colour and texture	Greyish brown with vertical fissures and corky texture when mature
8	Root	thick, fibrous roots and release a bitter, yellowish-white latex

Table 3: Botanical Classification ^[29]

Sr. No.	Taxonomical Level	Classification
1	Kingdom	Plantae
2	Phylum	Angiosperms
3	Class	Eudicots
4	Order	Gentianales
5	Family	Apocynaceae
6	Genus	Holarrhena
7	Species	<i>Holarrhena pubescens</i>

Vernacular Names: Common vernacular names are summarised in table 4.

Table 4: Vernacular Names ^[30, 31]

Sr. No	Language	Names
1	Hindi	Indrajab, Karwa inderjau, Kauriya, Kuda, Kura, Kurchi, Tita-indrajao (seeds)
2	Urdu	Inderjau talkh
3	Sanskrit	Bhadrayava, Girimallika, Indrayava (seeds), Kutaj, Kutaja, Kalinga (tree), Sakra sakhin, Sakravija (seeds), Vatsakavija, Vatsika
4	Bengali	Kurchi, Kureya, Kutaja, Tita-indrajau
5	Malyalam	Kadalapala, Kadavelapparintholi, Kotakappala
6	Marathi	Kadu-indrajau, Kuda, Pandhara-kuda
7	Tamil	Kashappu-vetpalarishi, Kirimllikai, Kudasappalai, Kuluppalai-virai (seeds), Kutaca-p-palai, Mlaimllikai, Veppalai
8	Telugu	Amkuda, Amkuda-vittulu, Girimallika, Indravrakshamu, Kakakodise, Kodisepala, Kondamalle, Kutajamu
9	Arabi	Lisan-ul-asafir-almurr
10	English	Bitter oleander, Conessi, Easter tree, Jasmine tree, Ivory tree, Kurchi, Tellicherry bark
11	French	Ecoree-decodaga
12	Persian	Indarjavi-talkh, Zaban-e-kunjashk-e-talkh

Phytoconstituents/Phytochemicals

There are several phytochemicals known to exist in *H. pubescens*. The major constituents are steroidal alkaloids, flavonoids, triterpenoids, phenolic acids, tannin, resin, coumarins, saponins and ergosterol.

Phytochemicals reported in stem bark and seeds

Phytochemicals found in stem bark and seeds are summarised in table 5.

Table 5: Phytochemicals isolated from stem bark and seeds of *H. pubescens* ^[32]

Sr. No.	Compound Name	Chemical Formula
1	Conessimine/Isoconessimine	C ₂₃ H ₃₈ N ₂
2	Conessine	C ₂₄ H ₄₀ N ₂
3	Isoconessine	C ₂₄ H ₄₀ N ₂
4	Conarrhimine	C ₂₁ H ₃₄ N ₂

Phytochemicals reported in stem bark only are summarised in table 6.

Table 6. Phytochemicals Isolated from Stem Bark of *H. pubescens* ^[33, 34]

Serial Number	Compound Name	Chemical Formula
1	Holarrhidine	C ₂₁ H ₃₆ N ₂ O
2	Cuspareine	C ₃₂ H ₄₂ N ₂ O ₄
3	Kurcholessine	unknown
4	Conessidine	C ₂₁ H ₃₂ N ₂
5	(3),-N-Methylholarrhimine	C ₂₂ H ₃₈ N ₂ O
6	-N-Methylholarrhimine	C ₂₂ H ₃₈ N ₂ O
7	NNN'-N'-Tetramethylholarrhimine	C ₂₅ H ₄₄ N ₂ O
8	Trimethylkonkurchine	C ₂₄ H ₃₈ N ₂
9	Holarrifine	C ₂₄ H ₃₈ N ₂ O ₂
10	Kurchenine	C ₂₁ H ₃₂ N ₂ O ₂
11	Holarrhessimine	C ₂₂ H ₃₆ N ₂ O
12	Holarrhine	C ₂₀ H ₃₈ N ₂ O ₃
13	Conkurchinine	C ₂₅ H ₃₆ N ₂
14	Kurchamine	C ₂₂ H ₃₆ N ₂
15	7 α -Hydroxyconessine	C ₂₄ H ₄₀ N ₂ O
16	Kurchilidine	C ₂₂ H ₃₁ NO
17	Neoconessine (isomer of conessine)	C ₂₄ H ₄₀ N ₂
18	Holadysenterine	C ₂₃ H ₃₈ N ₂ O ₃
19	Kurchessine	C ₂₅ H ₄₄ N ₂
20	Lettocine	C ₁₇ H ₂₅ NO ₂
21	Kurchimine	C ₂₂ H ₃₆ N ₂
22	Holarrhenine	C ₂₄ H ₄₀ N ₂ O
23	Holarrhimine/Kurchicine	C ₂₁ H ₃₆ N ₂ O
24	Holacine	C ₂₆ H ₄₄ N ₂ O ₂

25	Holafrine	C ₂₉ H ₄₆ N ₂ O ₂
26	Holadysone	C ₂₁ H ₂₈ O ₄
27	Holacetine	C ₂₁ H ₃₂ N ₂ O ₃
28	3 α -Aminoconan-5-ene	C ₂₂ H ₃₆ N ₂
29	Dihydroisoconessimine	C ₂₃ H ₄₀ N ₂
30	Conamine	C ₂₂ H ₃₆ N ₂
31	Conkurchine	C ₂₀ H ₃₂ N ₂
32	Pubadysone	C ₂₁ H ₂₆ O ₃
33	Puboestrene	C ₂₀ H ₂₄ O ₃
34	Pubamide	C ₂₁ H ₂₇ NO ₃
35	Pubescimine	C ₂₄ H ₄₀ N ₂ O
36	Kurchinidine	C ₂₁ H ₂₉ NO ₂
37	Kurchinine	C ₁₉ H ₂₄ O ₃
38	Pubescine	C ₂₂ H ₂₆ N ₂ O ₄
39	Norholadiene	C ₂₁ H ₂₉ NO
40	Holadiene	C ₂₂ H ₃₁ NO
41	Holonamine	Unknown
42	RegholarrhenineA	C ₂₂ H ₃₁ NO ₂
43	RegholarrhenineB	C ₂₁ H ₂₉ NO ₂
44	RegholarrhenineC	C ₂₂ H ₃₄ N ₂
45	RegholarrhenineD	C ₂₃ H ₃₈ N ₂ O
46	Regholarrhenine E	C ₂₅ H ₄₄ N ₂ O ₂
47	Regholarrhenine F	C ₂₅ H ₄₄ N ₂ O
48	Rutin	C ₂₇ H ₃₀ O ₁₆

Phytochemical from leaves is summarised in table 7.

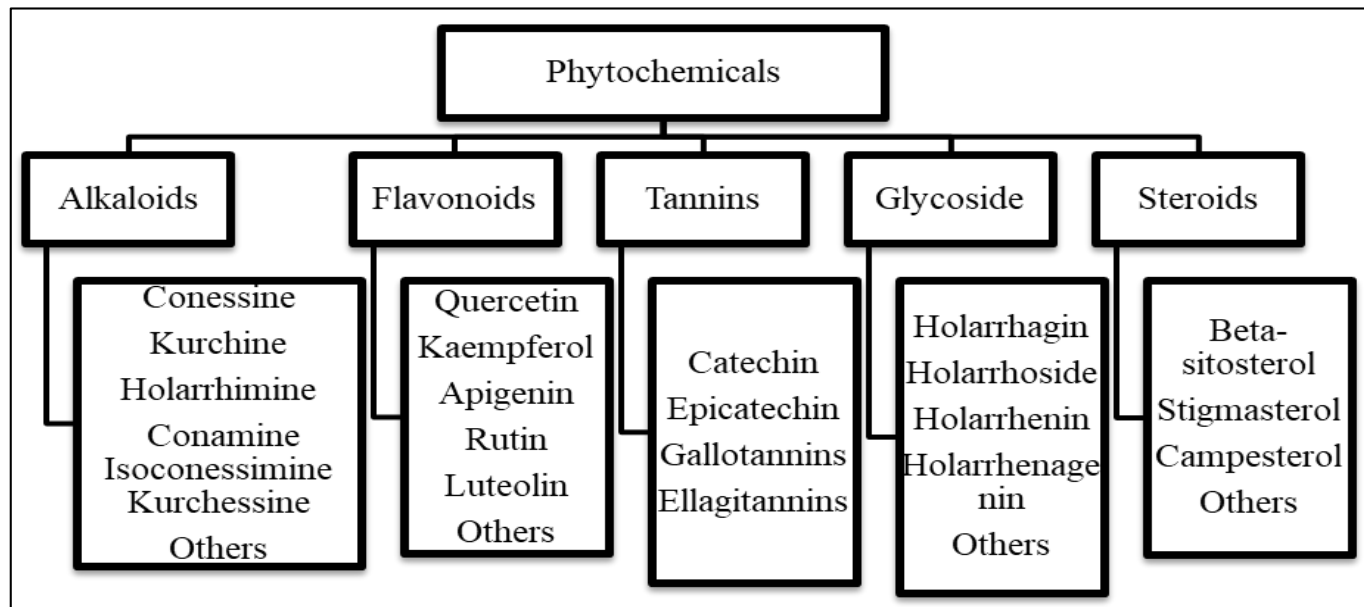
Table 7: Phytochemicals Isolated from Leaves of H. Pubescens [34, 35]

Sr. No.	Compound Name	Molecular Formula
1	Holantosine-B	C ₂₈ H ₄₅ NO ₅
2	Holantosine-E	C ₂₈ H ₄₇ NO ₆
3	Holantosine-C	C ₂₈ H ₄₇ NO ₆
4	Holantosine-D	C ₂₈ H ₄₅ NO ₅
5	Holantosine-A	C ₂₈ H ₄₇ NO ₆
6	Holantosine-F	C ₂₈ H ₄₅ NO ₅
7	Holarosine A	C ₃₀ H ₄₇ NO ₆
8	Holantosine-B	C ₂₈ H ₄₅ NO ₅
9	Holaricine	C ₂₁ H ₃₂ N ₂ O ₃
10	Kurchiphyllamine	C ₂₃ H ₄₇ NO ₂
11	Holarosine A	C ₃₀ H ₄₇ NO ₆
12	Holantosine-B	C ₂₈ H ₄₅ NO ₅

Phytochemicals Isolated From seeds summarised in table 8.

Table 8: Phytochemicals Isolated from Seeds of H. Pubescens [36, 37]

S. No.	Compound Name	Chemical Formula
1	Antidysenteric	C ₂₃ H ₃₆ N ₂ O
2	Conimine	C ₂₂ H ₃₆ N ₂
3	β -sitosterol	C ₂₉ H ₅₀ O
4	Conessine	C ₂₆ H ₄₀ N ₂ O ₄
5	Kurchine	C ₂₇ H ₄₁ NO ₄

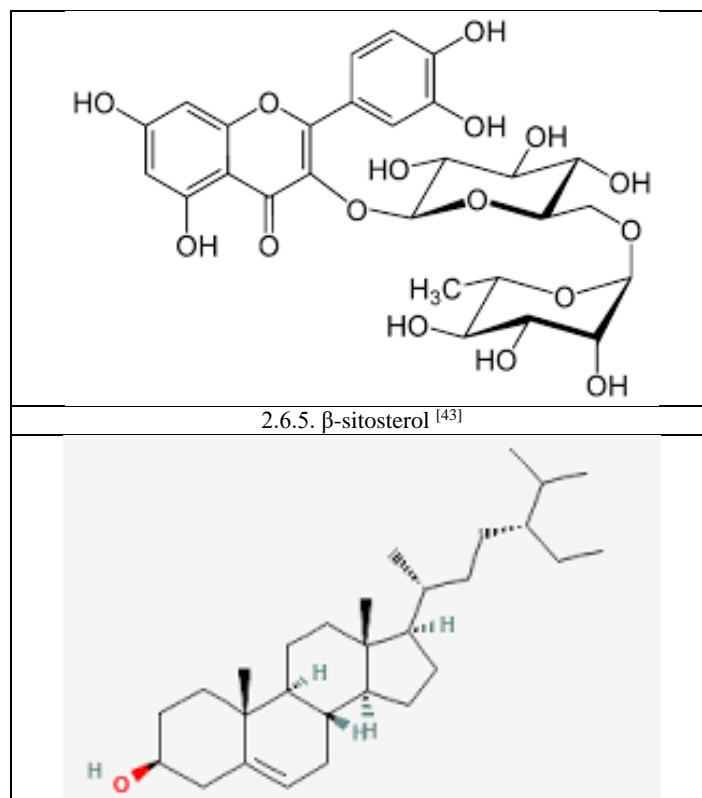


Flowchart 1. Phytochemicals Isolated from *Holarrhena pubescens* ^[38]

Structures of the major Phytochemicals

Table 9: Structures of the major Phytochemicals.

<p>2.6.1. Conessine ^[39]</p>
<p>2.6.2. Kurchine ^[40]</p>
<p>2.6.3. Cuspareine ^[41]</p>
<p>2.6.4. Rutin ^[42]</p>



Ethnopharmacology

In India and other nations, *Holarrhena pubescens* is a common medicinal plant used in traditional medicine to cure a variety of illnesses. Alkaloids, flavonoids, glycosides, and terpenoids are only a few of the plant's bioactive substances that contribute to its pharmacological effects.

Traditional uses of various plant parts of *Holarrhena pubescens*

Bark

- Its bark is widely used in Ayurveda medicine to treat piles, diarrhoea, leprosy, biliousness, and illnesses of the spleen ^[45, 46].
- Bark is used to treat headaches, piles, and heavy menstrual flow in unani medicine ^[47].
- According to the British Materia Medica, its bark can treat asthma, bronchopneumonia, stomach disorders, dyspepsia, diarrhoea, and dysentery in addition to acting as an antiprotozoal agent and treating malaria and chest infections ^[48].

Leaf

- *H. pubescens* leaves are not thought to have any therapeutic benefit in Ayurveda.
- In Unani medicine, leaves are used to treat conditions including chronic bronchitis, urine discharges, wounds, ulcers, as well as to relax the muscles and regulate menstruation.
- They are also used as aphrodisiacs, tonics, astringents, and galactagogues ^[50].

Roots

Apparently, the roots have aphrodisiac and abortifacient properties ^[51]. Moreover, they are used to treat severe abscesses, gonorrhoea, ascariasis, malaria, and venereal infections ^[52].

Flora

Flowers are purportedly utilised in Ayurveda to cure leukoderma, blood and spleen illnesses, as well as anthelmintic and antidiarrheal conditions ^[54].

Seeds

- The seeds are used in Ayurvedic medicine as an anthelmintic, astringent, and to treat hallucinations, dysentery, biliousness, leprosy, tiredness, skin problems, and bleeding piles ^[55, 56, 57].
- Seeds are employed as a carminative, aphrodisiac, astringent, and lithotriptic in Unani medicine ^[58].
- They are utilised as an alexipharmic, antidiarrheal, cholagogue, and analgesic in Tibetan medicine ^[59].
- They are utilised as an astringent, anthelmintic, febrifuge, stomachic, anti-dysenteric, and anti-diarrheal in the native medical system of Bangladesh ^[60].
- They are apparently used in other regions of the world to treat diuresis, chronic chest infections, asthma, malaria, vaginitis, diabetes, arthritis, hematuria, epilepsy, bronchitis, diarrhoea, dermatitis, and jaundice ^[61], according to reports (Figure 1).

Table 10: Traditional uses of *Holarrhena pubescens* ^[45-60]

S No.	Traditional Use	Part of Plant Used	Preparation Method
1	Antimalarial	Bark, root	Decoction, infusion
2	Anti-diarrheal	Bark, seed	Decoction, powder
3	Anti-inflammatory	Bark	Decoction, poultice
4	Anti-fungal	Bark, leaf	Decoction, infusion, poultice
5	Antiseptic	Bark, leaf	Decoction, infusion, poultice
6	Treatment of skin diseases	Bark, leaf	Decoction, poultice
7	Treatment of diabetes	Bark, seed	Decoction, powder
8	Treatment of respiratory infections	Bark, leaf	Decoction, inhalation, smoking, poultice

Pharmacological activities

Pharmacological Activity	Part of plant used	Mode of action	Reference
Anti-diabetic activity	Seeds	Inhibition of hepatic key enzymes involved in glucose metabolism	Yadav <i>et al.</i> , 2011 ^[105]
Anti-inflammatory activity	Bark	Inhibition of pro-inflammatory cytokines and enzymes	Saha <i>et al.</i> , 2013 ^[70]
Antimicrobial activity	Stem bark	Disruption of bacterial cell membrane integrity	Karmakar <i>et al.</i> , 2014 ^[106]
Antioxidant activity	Bark	Scavenging of free radicals and inhibition of lipid peroxidation	Patel <i>et al.</i> , 2013 ^[107]
Antitumor activity	Seeds	Induction of apoptosis and inhibition of cell proliferation	Alam <i>et al.</i> , 2017 ^[101]
Antihypertensive activity	Bark	Vasodilation and calcium channel blocking effect	Banerjee <i>et al.</i> , 2012 ^[108]
Hepatoprotective activity	Bark	Restoration of liver function and reduction of oxidative stress	Chowdhury <i>et al.</i> , 2014
Anticonvulsant activity	Stem bark	Inhibition of glutamate-mediated neuronal excitation	Srivastava <i>et al.</i> , 2020 ^[92]
Antidepressant activity	Stem bark	Enhancement of monoaminergic neurotransmission	Sultana <i>et al.</i> , 2004 ^[80]

Table 11: Pharmacological activities of *Holarrhena pubescens* ^[63-84]

No.	Activity	Part of plant used	Mode of action
1	Antimalarial activity	Bark, seed	Inhibits the growth of Plasmodium species
2	Antidiabetic activity	Bark, seed	Lowers blood glucose levels and improves insulin ser
3	Anti-inflammatory activity	Bark, leaf	Reduces inflammation and swelling
4	Antimicrobial activity	Bark, leaf	Inhibits the growth of various bacterial and fungal sp
5	Antioxidant activity	Bark, leaf	Scavenges free radicals and protects against oxidative
6	Antitumor activity	Bark, seed	Inhibits the growth of various cancer cell lines
7	Hepatoprotective activity	Bark, leaf	Protects the liver against damage
8	Hypotensive activity	Bark, seed	Lowers blood pressure
9	Neuroprotective activity	Bark, leaf	Protects the nervous system against damage

Anti-Diarrheal Property

H. pubescens, also known as Velvet bean or Cowitch, has antidiarrheal properties. The ethanol extract of *H. pubescens* seeds significantly reduces diarrhoea in rats. Alkaloids isolated from *H. pubescens* seeds also exhibit antidiarrheal activity against enteropathogenic *E. coli*. ^[63] Phytochemical analysis of *H. pubescens* seeds extract shows the presence of alkaloids and flavonoids, which may be responsible for its antidiarrheal activity. *H. pubescens* leaves' aqueous and methanol extracts are effective against diarrheal pathogens. *H. pubescens* root bark aqueous extract inhibits stable toxin production, reducing intestinal secretions and virulence of enterotoxigenic *E. coli* strains. The aqueous extract of *H. pubescens* seeds is effective against bacterial strains such as *Staphylococcus aureus*, *E. coli*, *Shigella*, and *Salmonella typhi* ^[13].

Anti-diabetic Activity

The passage describes the potential use of *H. pubescens*, a plant, for treating diabetes in various medicinal systems. The extracts of the plant's seeds and leaves have been found to possess antihyperglycemic and hypoglycaemic activity in rats, which suggests that the plant may have potential therapeutic value for treating diabetes in humans. The plant's seeds have been proven to have antihyperglycemic effect in rats when given in methanol, aqueous, and petroleum ether extracts. The methanol extract of the seeds was especially effective against diabetic rats. At a concentration of 300 mg/kg, the ethanolic extract of the seeds effectively lowered diabetes in rats. Moreover, glucosidase, an enzyme that releases glucose from di- and oligosaccharides and aryl glucosides in the food, was shown to be inhibited by the hydro-methanolic seed extract of *H. pubescens*, enhancing glucose absorption from the intestine ^[64].

It has also been discovered that the leaves of *H. pubescens* can treat diabetes. Diabetes was successfully treated in rats by administering an ethanolic extract of the plant's leaves at a BW for 21 consecutive days. ^[65] Although more research is required to establish *H. pubescens*' safety and effectiveness in treating diabetes in humans, the results generally point to this

plant's promise as a therapeutic agent. Mechanism of Action: The study by Ali demonstrated that an extract of *H. pubescens* can inhibit intestinal α -glucosidase activity, which is an enzyme that breaks down complex carbohydrates into simple sugars that can be absorbed by the body. By inhibiting this enzyme, the extract was able to limit the absorption of carbohydrates, which can lead to lower blood sugar levels after a meal. The study found that the extract had an IC50 value of 0.52 mg/mL, indicating a significant inhibitory effect on α -glucosidase. This study suggested that glucosidase inhibition is a crucial strategy for reducing postprandial hyperglycaemia in diabetics ^[11].

Anti-diarrheal

In another study *H. pubescens* alkaloids reduced enteropathogenic *Escherichia coli* growth *in vitro* and castor oil-induced diarrhoea *in vivo*, indicating potential as an anti-diarrheal agent ^[66].

It is believed that phytochemicals including saponins, steroids, alkaloids, tannins, and flavonoids are what give plants their antidiarrheal properties. Alkaloids and flavonoids have been found in *H. pubescens* seeds extract, and they may be the cause of this activity. Aqueous and methanol extract of *H. pubescens* leaves were found effective against the diarrheal pathogens *Salmonella typhimurium*, *S almonella typhi*, *Vibrio cholerae* and *Vibrio alginolyticus* ^[67].

Diuretic Property

The aqueous seed extract of *H. pubescens* was observed to significantly increase urine output in Wistar rats. The excretion of Na⁺ and K⁺ ions was also found to have significantly increased. It has also been noted that *H. pubescens* chloroform extract increases urine output in a dose-dependent manner. Moreover, it was found that the levels of Na⁺ and K⁺ in the urine were higher, indicating that the diuretic impact is likely caused by an increase in electrolyte excretion ^[68].

Anti-Urolithic Property

It has been noted that the methanol extract of *H. pubescens*

seeds inhibits the growth of calcium oxalate crystals. It significantly reduces polyurea, Ca⁺⁺ excretion, Ca⁺⁺ crystal formation, and water intake when tested on male Wistar rats. These results imply that the plant may be able to lessen kidney stones.^[69]

Anti-inflammatory and Analgesic Properties

Animal models have shown that *H. pubescens* extract has anti-inflammatory and analgesic properties. In rats with colitis-induced colitis, a methanol extract of the plant's bark resulted in a drop in malondialdehyde and nitric oxide levels but an increase in glutathione and superoxide dismutase^[70]. Research point to the effectiveness of *H. pubescens*. being dose-dependent, with a 400 mg/kg dose inhibiting rat paw oedema by 74%. At a dose of 200 mg/kg, the chloroform extract had the greatest analgesic and CNS-depressant effects^[71]. Paw oedema was greatly reduced by the methanolic extract of *H. pubescens*, and the extract improves stress tolerance in animal models. It is suggested that peritoneal mast cells and prostaglandin pathways mediate the analgesic action^[72, 73].

Antioxidant/Free Radical Scavenging Properties

With 90% DPPH free radical inhibition, the aqueous and methanol extract of *H. pubescens* exhibits very potent free radical scavenging activity. Moreover, the hydroxyl and superoxide ions are greatly reduced by the methanol extract. Moreover, it reduces the conversion of Fe³⁺ into Fe²⁺. According to a different study, its use lessens the harm that OH ions duo to deoxyribose. The ethyl acetate portion also prevented lipid peroxidation, nitrite inhibition, and H₂O₂ degradation^[74].

The antioxidant capacity of *H. pubescens* was studied by Zahin using the ferric thiocyanate, thiobarbituric acid, and DPPH radical scavenging methods. By using this method, showed a moderate antioxidant effect, but a very low DPPH radical scavenging activity.^[75] The antioxidant properties of *H. pubescens* bark were assessed in a different study by Bhusal, and it was shown that both methanol and ethanol extracts exhibited substantial DPPH inhibition activity, but the hexane extract appeared to have the least activity^[76].

Inhibition of Acetylcholinesterase and CNS-Stimulant Activity

Five alkaloids, namely conimine, isoconessimine, conessine, conarrhimine, and conessimine, were investigated for CNS-stimulant action in a study, on alkaloids isolated from *H. pubescens*. The most active compound was conessimine. According to this study, these alkaloids may be employed to treat neurological diseases^[77] Another investigation using Swiss albino mice revealed that a methanolic bark extract significantly reduced grip strength and reduced locomotive activity, demonstrating a CNS depressive impact^[78].

Anti-Microbial Activity

The ethanol extracts of *H. pubescens* seeds exhibited antibacterial activity against enteropathogenic *Escherichia coli*. The petroleum ether extract of bark of *H. pubescens* also exhibited inhibition of *E. coli*. It showed a moderate activity when compared to other plants species.^[79]

According to research, the enteropathogenic *Escherichia coli* strain's adhesion to, INT407 cells causes cytoplasmic membrane damage, apoptotic bodies caused by chromatin condensation, and enlargement and damage to the

mitochondria took place. In enteropathogenic *Escherichia coli* treated with *H. pubescens* extracts, these effects were reduced^[63].

H. pubescens extracts in methanol demonstrated antibiofilm action against *V. cholerae*. Findings of gene expression experiments showed that aph A or aph B, the main regulator genes controlling both virulence and biofilm formation, are downregulated by use of both leaf and bark extracts.^[80]

The alkaloidal fraction of *H. pubescens* demonstrated antifungal efficacy. *H. pubescens* bark's antifungal properties against *Candida albicans* were significantly enhanced by a methanol extract^[81].

Anti-malarial activity

When tested Anti-malarial activity against *Plasmodium falciparum* and given to Swiss mice infected with *Plasmodium falciparum* isolates, chloroform extracts of *H. pubescens* bark demonstrated considerable *in vitro* and *in vivo* anti-malarial activity^[82, 83].

According to Nondo,^[75] ethanol and methanol extracts had substantial antiplasmodial action against *Plasmodium falciparum*. The fractions recovered from *H. pubescens* roots were also very effective against artemisinin and chloroquine resistant *P. falciparum*. The steroidal alkaloid conessine, which was isolated from the bark of *H. pubescens*, demonstrated anti-plasmodial activity in a different investigation^[26].

In mice infected with *P. berghei*, Verma examined petroleum ether and chloroform extracts of *H. pubescens* and shown that the bark actively reduces parasitemia^[82]. A crude extract of *H. pubescens* bark has been shown by Simonsen to have strong *in vitro* anti-plasmodial action against chloroquine sensitive *P. falciparum* strain^[84]. Conessine, kurchine, conessidine, isoconessine, conkurchicine, and holarrhimine are among the steroidal alkaloid chemicals that have been found in significant amounts in the plant's stem, root, and seeds. Conessine, which was extracted from the plant's stem, is the molecule assumed to be responsible for the antimalarial activity^[85]. A methanol extract of *H. pubescens* has been shown by Verma *et. al.* to suppress *P. berghei* growth with a 43% suppression rate^[83].

Novel Formulations

There is limited research on novel formulations using *Holarrhena pubescens*. Some of them are as follows:

Topical gel formulation: The goal of the study was to create a topical gel formulation for the treatment of skin infections that contained an extract of *Holarrhena pubescens*. Numerous microorganisms, such as bacteria and fungus, can cause skin infections, which are a widespread issue. Ayurvedic practitioners have long employed the plant *Holarrhena pubescens* to treat a variety of illnesses, including skin infections. The extract was made from the *Holarrhena pubescens* plant's bark using a solvent extraction technique, and its concentration of bioactive substances such alkaloids, flavonoids, and tannins was standardised. The gel was created utilising triethanolamine as a pH adjuster and a carbopol polymer as a gelling agent.

The agar well diffusion method was used to test the gel for its antimicrobial activity, and the results revealed that it exhibited good antimicrobial activity against a variety of bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The bioactive substances included in the extract of

Holarrhena pubescens, including tannins, alkaloids such as conessine and kurchine, flavonoids like quercetin and kaempferol. The flavonoids like kaempferol provide the gel its antibacterial properties. The gel's physical characteristics, including pH, viscosity, and spreadability, were also assessed. The gel viscosity, and spreadability were acceptable, which suggests that it may be applied to the skin without difficulty and is stable. The study comes to the conclusion that a topical gel formulation comprising an extract of *Holarrhena pubescens* has good antimicrobial action against a variety of bacteria, including *Staphylococcus aureus* and *Escherichia coli*, which are typical causes of skin infections

In summary, the study supports the potential of *Holarrhena pubescens* as a non-pharmacological therapeutic option for skin infections. For patients with skin infections, the creation of a topical gel formulation containing the plant extract may offer a secure and efficient therapeutic option [86].

Nanoparticle formulation: The goal of the study was to create a nanoparticle formulation for the treatment of liver cancer that contained bark extract of *Holarrhena pubescens*. The extract was made from the *Holarrhena pubescens* plant's bark using a solvent extraction technique, and its concentration of bioactive substances such as alkaloids, flavonoids, and tannins were standardised. The nanoparticles' particle size, zeta potential, and drug loading effectiveness were assessed after they were made using the biodegradable polymer poly (lactic-co-glycolic acid) (PLGA). *In vitro* testing on liver cancer cells determined the nanoparticles' anticancer efficacy. The findings demonstrated that the *Holarrhena pubescens* extract-containing nanoparticles exhibited potent anticancer properties and had the capacity to cause liver cancer cells to undergo apoptosis, or "programmed cell death." The bioactive substances in *Holarrhena pubescens* extract, including tannins, alkaloids such as conessine and kurchine, flavonoids like quercetin and kaempferol, are responsible for the nanoparticles' anticancer action. According to the study, *Holarrhena pubescens* may be used as a natural alternative for the treatment of liver cancer, and the creation of a nanoparticle formulation may give patients a secure and efficient therapy choice. The formulation of nanoparticles using PLGA as a biodegradable polymer makes them excellent for medication delivery applications because of biocompatibility and quick excretion from the body. The study's findings demonstrate that a nanoparticle formulation containing an extract of *Holarrhena pubescens* has potent anticancer properties and could cause liver cancer cells to undergo apoptosis. Although more research is required, to ascertain the nanoparticles' effectiveness *in vivo* and to assess their potential as a liver cancer treatment [87, 88].

Herbal shampoo formulation: In order to cure dandruff caused by *Malassezia furfur*, a herbal shampoo formulation including an extract of *Holarrhena pubescens* was formulated. The extract's bioactive components were standardised after it was extracted from the plant's bark. The extract, along with surfactants and preservatives, were used to create the shampoo. *In vitro* testing of the shampoo's antifungal activity against *Malassezia furfur* revealed positive results, indicating its promise as a dandruff therapy. The study shows the potential advantages of employing natural components in shampoo formulations and offers evidence that *Holarrhena pubescens* may be a natural alternative for the treatment of dandruff. To assess the shampoo's effectiveness

in vivo, more research is required [89].

Herbal ointment: In order to cure skin infections brought on by germs like *Staphylococcus aureus* and *Candida albicans*, the study set out to create a herbal ointment formulation comprising an extract of *Holarrhena pubescens*. The extract's concentration of bioactive substances such as alkaloids and flavonoids was standardised after it was extracted from the bark of the *Holarrhena pubescens* plant. The extract from *Holarrhena pubescens* was combined with a variety of natural oils to create the herbal ointment.

Using the disc diffusion method, the herbal ointment's antibacterial activity was assessed *in vitro* against a variety of pathogens, including *Staphylococcus aureus* and *Candida albicans*. The outcomes demonstrated the ointment's good antibacterial efficacy against various pathogens, indicating its potential as a therapy for skin infection.

The study implies that the creation of an herbal ointment formulation could offer patients a secure and efficient treatment choice while also highlighting the potential of *Holarrhena pubescens* as a natural alternative for the treatment of skin infections. In ointment formulations, natural components like *Holarrhena pubescens* could assist lessen the usage of synthetic chemicals, which could have negative effects on the skin.

A herbal ointment formulation using an extract of *Holarrhena pubescens* has strong antibacterial action against a variety of pathogens, including *Staphylococcus aureus* and *Candida albicans*, according to the study's findings, which support this claim. To determine the ointment's effectiveness *in vivo* and to assess its potential as a treatment for skin infections, more research is required [90].

Antidiabetic tablet: The study was conducted in rats with induced diabetes. Rats with artificially induced diabetes were used in the investigation. The investigators employed the alloxan-induced diabetes model, which specifically kills the insulin-producing cells in the pancreas. This model is frequently employed in research on diabetes to assess the antidiabetic efficacy of novel medications or plant extracts.

The rats were split up into several groups, including a control group, a diabetic control group, and groups that received various dosages of the bark and seed extract tablet from *Holarrhena pubescens*. For 28 days, the tablet was administered orally once every day. Throughout the course of the investigation, the rats' blood sugar levels were monitored using a glucometer.

According to the study, the tablet considerably and dose-dependently lowered the blood glucose levels in the test animals. The tablet's highest dosage (500 mg/kg) was discovered to be the most successful at lowering blood glucose levels. Additionally, the tablet improved the rats' lipid profiles by lowering their levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol, all of which are cardiovascular disease risk factors.

The acute toxicity of *Holarrhena pubescens* bark and seed extract tablets in rats was also evaluated in the study. At doses up to 2000 mg/kg, the tablet was determined to be safe and well-tolerated, and no serious side effects were noticed. The study's overall conclusion is that the *Holarrhena pubescens* extract tablet has good efficacy and safety in experimental animals, and it has potential as an antidiabetic drug. However, additional studies are required to verify these results and explore the pharmacological capabilities of the active

ingredients in the extract^[91]

Eye drops: A formulation for eye drops that treats conjunctivitis with an extract of *Holarrhena pubescens* was produced and reported in a study in 2020. The conjunctiva, a thin layer of tissue that covers the white of the eye and lines the inside of the eyelid, becomes inflamed because of conjunctivitis, often known as pink eye, a common eye infection. Ayurvedic practitioners have long employed the plant *Holarrhena pubescens* to treat a variety of conditions, including infections of the eyes. The goal of the study was to make a formulation for eye drops containing an extract of *Holarrhena pubescens* that would exhibit strong antibiotic action against a variety of pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are frequently responsible for bacterial conjunctivitis. The extract was produced using a solvent extraction technique, and its concentration of bioactive substances such as alkaloids, flavonoids, and tannins were standardised. The agar well diffusion method was used to test the eye drops for their antimicrobial effectiveness, and the results revealed that the eye drops containing the extract of *Holarrhena pubescens* exhibited good antibacterial activity against all the pathogens examined. Rabbits were used as the study's animal model, and it was discovered that the eye drops were secure and well-tolerated in them. The bioactive substances included in the extract of *Holarrhena pubescens* are responsible for the eye drops' antibacterial properties. Conessine and kurchine alkaloid having antibacterial action against various microorganisms. There is evidence that flavonoids like quercetin and kaempferol have antibacterial properties as well. Tannins are polyphenolic substances with demonstrated antibacterial action through disruption of microorganism cell membranes. According to the study, a formulation for eye drops made using an extract of *Holarrhena pubescens* shows strong antimicrobial action against a variety of pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are frequently responsible for bacterial conjunctivitis^[92].

Marketed Formulations

There are several marketed formulations of *Holarrhena pubescens* for example Kutajadi Vati, Kutajghan Vati, Vatsakadi Vati, Vatsakadi Kwath or Kashayam, Kutajavaleha, Vijaya Churna, Kutajarishta, Kutajaveleh that are used in traditional medicine and in conventional medicine in certain parts of the world. Some are as follows:

Kutajghan Vati: It is a tablet made from the dried bark of *Holarrhena pubescens* that is used in Ayurvedic medicine for the treatment of diarrhoea, dysentery, and other gastrointestinal disorders. The tablet is usually taken with water after meals^[94].

Triphal Inderjau Kadwa: It is a beneficial remedy for digestive disorders. It improves digestive functions and helps to restore appetite. It can also aid flu-associated conditions, such as cold runny nose, frequent sneezing, and fever^[95].

Kurchicin: It is another formulation of *Holarrhena pubescens* that is used as an antimalarial drug in Nepal. It is an extract of the plant that contains conessine and other alkaloids. Kurchicin has been shown to have potent antimalarial activity and is considered an effective alternative

to conventional antimalarial drugs^[95].

Floristic Studies

Floristic studies of *Holarrhena pubescens* have been conducted to understand its taxonomy, distribution, morphology, anatomy, ecology, and pharmacological properties.

Taxonomy: A study by Muniappan analysed the molecular phylogeny of *Holarrhena pubescens* and its relatives within the family *Apocynaceae*. They used both chloroplast and nuclear markers to reconstruct the evolutionary history of these taxa and found that *H. pubescens* is a monophyletic group nested within a clade of related genera^[96].

Distribution: A study by Gangopadhyay investigated the distribution of *Holarrhena pubescens* in the Indian state of West Bengal. They found that the species is distributed in various forest types, including dry deciduous, moist deciduous, and semi-evergreen forests, and occurs at altitudes ranging from 50 to 1000 meters above sea level^[97].

Morphology: A study by Sasi examined the morphology of *Holarrhena pubescens* leaves and flowers. They found that the leaves are elliptic and have a pubescent surface, while the flowers are white, fragrant, and arranged in cymes^[98].

Anatomy: A study by Kumari investigated the anatomical features of *Holarrhena pubescens* stem and roots. They found that the stem has a circular shape and is characterized by a well-developed cortex, vascular bundles, and a pith, while the root has a typical dicot root structure^[99].

Pharmacognostical Studies

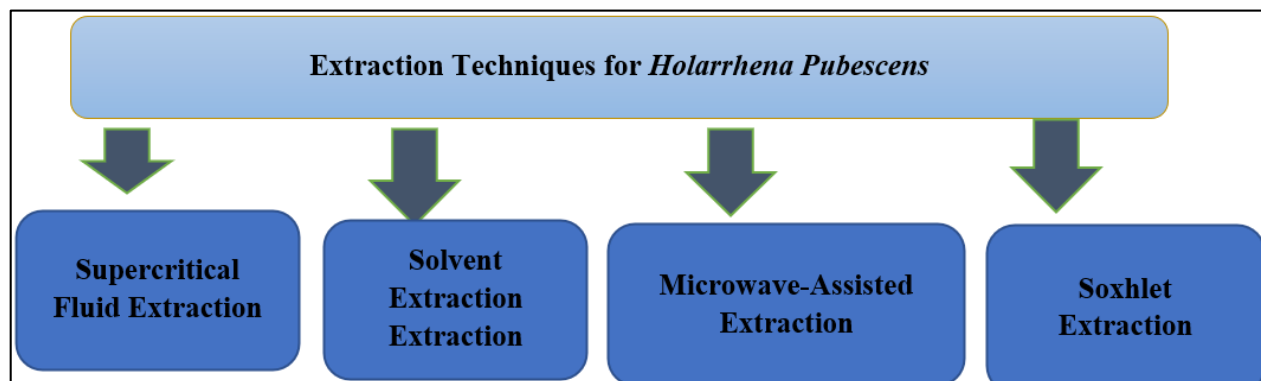
Organoleptic Characters: *Holarrhena pubescens* has a bitter taste and a faintly pungent smell, according to its organoleptic properties. The plant's bark, which is especially bitter, is frequently used in traditional medicine to cure a variety of diseases. The plant's leaves, which have a somewhat astringent flavour, are frequently used as a home treatment for diarrhoea and other gastrointestinal conditions. The plant's fruits have a flavour that is both somewhat bitter and woody. *Holarrhena pubescens*, which has a potent and distinctive flavour profile overall, is frequently used in conventional medicine to treat a variety of illnesses.

Powder Characteristics: The powder often ranges in colour from light to dark brown. The powder has a distinctive, slightly bitter smell. A bitter flavour can be detected in the powder. The powder has a fine, silky texture to it. When the powder is examined under a microscope, different features, including tracheids, sclereids, fibres, parenchyma cells, calcium oxalate crystals, and starch grains, may be visible. The powder contains a number of bioactive substances, such as alkaloids like conessine, conessimine, and kurchine, which are in charge of its therapeutic effects. Additionally, it has tannins, saponins, glycosides, and flavonoids. Insoluble in water, the powder is soluble in alcohol and diluted acids. Standardization: The study by Sharma established the pharmacognostic standards for *Holarrhena pubescens* bark. The macroscopic characteristics of the bark were described as brownish-black, rough, and fissured, with a bitter taste and characteristic odor. The microscopic features included the presence of cork cells, stone cells, and lignified fibers. The

physicochemical properties such as moisture content, total ash content, acid-insoluble ash content, and extractive values were also determined. The study concluded that these standards can be used to authenticate and standardize the plant material, ensuring its quality and efficacy for medicinal purposes. Anatomical characters Stomata are not present in the cork cells in the transverse section of *Holarrhena pubescens*. Small/rare clusters of calcium oxalate crystals are

seen in the mesocarp, which is covered with fibrous sclereids. The crystals are prismatic in shape. The trichomes are sessile and uniseriate. Sessile trichomes are affixed directly to the surface of the plant without a stalk or peduncle, in contrast to uniseriate trichomes, which have one row of cells^[100].

Extraction Methods



Flow Chart 2: Extraction Methods of *H. Pubescens* ^[101,102,103].

Adverse Drug Reactions

Holarrhena pubescens is generally considered safe for use as a medicinal plant. However, like any other medication or herbal supplement, it can cause adverse effects in some individuals. Here are some of the reported adverse effects associated with the use of *Holarrhena pubescens*:

- **Gastrointestinal disturbances:** *Holarrhena pubescens* may cause gastrointestinal disturbances such as nausea, vomiting, and diarrhoea. These effects are usually mild and self-limiting but may require medical attention in some cases^[104].
- **Allergic reactions:** Some individuals may develop an allergic reaction to *Holarrhena pubescens*, which can manifest as skin rash, itching, and swelling. In severe cases, it can lead to anaphylaxis, a life-threatening allergic reaction^[104].
- **Hypoglycaemia:** *Holarrhena pubescens* may lower blood sugar levels, which can lead to hypoglycaemia (low blood sugar). Individuals with diabetes who are taking medications to lower their blood sugar levels should use *Holarrhena pubescens* with caution and under medical supervision^[104].
- **Liver toxicity:** There have been reports of *Holarrhena pubescens* causing liver toxicity in some individuals. This effect is rare but can be severe and life-threatening^[104].

It is important to note that these adverse effects are not very common, and most individuals can use *Holarrhena pubescens* without any adverse effects. However, it is always recommended to use any herbal supplement or medication under the guidance of a qualified healthcare professional to minimize the risk of adverse effects.

Conclusion

Based on the findings discussed above, *Holarrhena pubescens* is a plant with a rich history of use in traditional medicine and has been found to have a variety of pharmacological properties. The plant includes a variety of phytochemicals, such as alkaloids, glycosides, and flavonoids, which oversee its therapeutic benefits. The plant useful in animal bites,

Indigestion, Blood-related ailments, Body pain, Brain-related disorders, Cold and throat-related ailments, Dental or oral ailments, Dermatological problems, Diabetes, Fever, Gastrointestinal disorders, General health, Gynaecological disorders, Joint- and muscle-related ailments, Liver complaints, Piles, Respiratory disorders, Urogenital disorders, Intestinal Parasites, anti-diabetic, anti-inflammatory, anti-microbial, and anti-tumor activity, among other notable pharmacological effects. *Holarrhena pubescens* possesses several morphological traits that are typical of its family in addition to its chemical characteristics. Cork cells, stone cells, and prismatic calcium oxalate crystals are some examples of these. The plant produces a fruit with a quadrangular shape and fibrous sclereids inside.

Overall, *Holarrhena pubescens* is a promising candidate for the development of new drugs for the treatment of a range of diseases. However, more research is needed to fully understand its mechanisms of action and to explore its potential therapeutic applications.

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