Chapter 3.

REVIEW OF LITERATURE

3.REVIEW OF LITERATURE

3.1 Mahamrutyunjaya Rasa :

It is a formulation containing a mixture of herbs and minerals used as a cardio active formulation prescribed in the *A.S.S. Rasa Rasayana Prakarana.* The dose of the formulation is 125 mg twice a day[73]. The formula of the formulation is given in Table 3.1.

Table 3.1 Formula of *Mahamrutyunjaya Rasa*[74].

Ingredients	***Quantity***
Visa	1Part
Brihati	1Part
Pippali Kana	1Part
Marica	1Part
Gandhaka	1Part
Tankana	1Part
Hingula	2Part
Jambira	Q.S

The formulation is manufactured by a number of industries. Some of them are listed in Table 3.2

Table 3.2 List of Manufacturers of *Mahamrutyunjaya rasa.*

Manufacturer	**Amount per pack**
Baidyanath	20 tabs
Dabur	40 tabs
Pune Rasashala	20 tabs

The ingredients present in the formulation have been studied to some extent and their medicinal nature is well known. The following is the description of all the ingredients present in the formulation.

3.2) Aconite Root:

Aconite roots have been used for centuries as a medicinal herb and was referred to in Indian Ayurvedic medicine some 3,000 years ago, in the ancient traditional Chinese Shennong Herbal some 2,000 years ago, as well as in the French Pharmacopoeia in 1884. The present scenario shows its widespread utility in the international market. The name Aconite is derived, according to Pliny, from the Black sea port, Acone. The genus Aconitum is a relatively large one, there being some sixty well-defined species, nearly half of which have been used in medicine. Indian Aconite is derived from *Aconitum ferox Wall.*, a plant growing in the Himalayas and in Nepal[76].

(a)

(b)

Fig 3.1. (a)Picture of *Aconitum ferox* plant.(b) Picture of dried Aconitum root.

3.2.1) Definition:

Aconitum ferox also known as *Aconitum virorum* is a species of monkshood, in the family Ranunculaceae. It is also known as the Indian Aconite. "The dried tuberous roots of *Aconitum ferox, Wall.* (Ranunculaceae), without the presence

or admixture of more than 5 percent of stems or other foreign matter, and yielding not less than 0.5 percent of the ether-soluble alkaloids of Aconite." [77]

3.2.2) Synonyms[75]*:*

Table 3.3 Synonyms of *Aconitum ferox*

Sr. No.	Language	Names
1	Sanskrit	Visha, Vatsnabha
2	English	Aconite root, Monkshood, Wolfs-bane, Wolfroot, Friar's cap, Cuckoo's cap, Blue rocket
3	Hindi	Mithazehar, Bish or Bikh, Bachnag
4	Bengali	Katbish, Bisha
5	Gujarati	Shingadiovachnag, Nagpuri bachnag
6	Tamil	Vaishnavi
7	Marathi	Bachnab

3.2.3) Habitat[75]*:*

The plant is available in the temperate sub-alpine Himalayas, from Sikkim to Garhwal. It is abundant at Sandakphu, which is the highest point of Darjeeling hills. The greater part of the drug is generally supposed to be derived from *A.ferox. A. luridum, H.f. and T.*, is found in Sikkim, *A. Lycoctonum, Linn.*, from Kashmir to Kumaon, *A.napellus, Linn.*, along the temperate alpine Himalaya in four varieties, viz;, proper, rigidum, multifidum and rotundifolium. *A. palmatum, Don.*, in the Eastern temperate Himalaya from Garhwal to Manipur. The last species is considered by the natives of Sikkim not to be poisonous.

3.2.4) History[75]*:*

Hindu writers mention that not less than eighteen kinds of *Bish* or poison, of which ten are said to be unfit for medicinal use on account of their extremely poisonous properties which they exaggerate to such an extent as to say that their touch is fatal; of the eight kinds which may be used, that known as *Teliya. Bachnag* is said to be the best; it is of a yellowish brown colour, and in shape like a deer's horn. *Bish* as a name for Aconite appears to have been known to the Hindus from the earliest ages, but the word appears to have been applied also to any poisonous root.

3.2.5) Ethnopharmacology[75]*:*

Aconite root is recommended in diseases arising fom cold humours and atrabilis, and also in leprosy, cough, asthma and ulceration of the throat. *Bish* is much used as an external application, the root being formed into a paste (*lep*) and spread upon the skin as a remedy for neuralgia and other painful affections, such as boils and internally it is prescribed in fever and rhematism, but is generally mixed with a number of other drugs, both mineral and vegetable; moreover, it undergoes a process of purification by being boiled in milk or cow's urine, which must considerably diminish its activity. In native prescriptions for cough, asthma, and fevers. Aconite is combined with borax and aromatics, sulphur and croton seeds are added if there is constipation. The famous Indian pill for snake-bite contains aconite root, white arsenic, yellow arsenic, red arsenic, herb of *Aristolochia bracteata,* fruit of *Randia dumetorum* in equal parts. These drugs are rubbed down on a stone with the juice of the betel pepper-leaf, and made into pills, the size of the seed of *Abrus precatorius* (about 2 g). The dose is one pill every five minutes rubbed down with betel leaf juice until three pills have been taken. European physicians in India were in the habit of using *Bish* as a substitute for ordinary Aconite root, and it has of late years been in Europe as a source of Aconitine. Modern physiological research shows that Aconite applied externally acts as a local irritant and narcotic, producing numbness and tingling. Introduced into the circulation in large quantity it causes sudden paralysis of the heart-muscle, which appears to be due to the action of the poison upon the vague roots; smaller, but poisonous doses, cause disturbance of the respiration, muscular weakness, vascular depression and death. Therapeutic doses cause reduction of the force and frequency of the circulation muscular inertia and slight tingling in the extremeties or lips. Atropine and digitalis have been used with success in cases of poisoning by aconite; they appear to restore the power of the heart by counteracting the effects of the poison upon the vagus roots.

3.2.6) Varieties of Aconites[76]

Table 3.4.Indian Aconites of Commerce according to new Classifications

Sr.No.	Names of Type	Species of Varieties include in Type
1	Napellus	*A. napellus, A. ferox var. laciniatum and A. ferox var. spicatum*
2	Atrox	*A. ferox Var. atrox, A. ferox, var. polyschiza.*
3	Anthora	*A. heterophyllum* and *A.paperatum*

Stapf (1995) divided the Indian aconites into three types according to their being annual, perennial and biennial:-

1) Gymnaconitum type (annual duration) *A. gymnandrum*
2) Lycoctonum type (perennial duration) *A. laeve, A. luridum, A. moschatum*
3) Napellus type (biennial and normally paired).

3.2.7) Description[76]*:*

Aconitum is a deciduous perennial plant that grows up to 1.0 meter height and 0.5 meters wide and prefers many types of soil. They are tall and erect with the stem being crowned by racemes of large and eye-catching blue, purple, white, yellow or pink zygomorphic flowers with numerous stamens. They are distinguished by having one of the five petaloid sepals (the posterior one), called the galea, in the form of a cylindrical helmet; hence the English name monks-hood has been given. There are 2 - 10 petals, in the form of nectaries. The two upper petals are large. They are placed under the hood of the calyx and are supported on long stalks. The tubers are tapering (1 to 2 cm thick), dark brown externally and white within with a horny and starchy fracture. Roots are paired, occasionally separated due to the breakage. They are ovoid, conical and small portions of stem sometimes is attached, tapering downwards to a point. It is 5 cm long and 0.4-1.8 cm thick,. It gradually decreases in thickness towards tapering end. It is wrinkled longitudinally as well as transeversely and rough due to root scars, dark brown to blackish brown in fracture. The stem is cartilaginous and hard with white cambium, odour indistinct, taste slightly bitter followed by a strong tingling sensation.

3.2.8) Cultivation[75]:

Aconite prefers a soil slightly retentive of moisture, such as a moist loam, and flourishes best in shade. It would probably grow luxuriantly in a moist, open wood, and would yield returns with little further trouble than weeding, digging up and drying. It can be raised from seed, sown 1/2 inch deep in a cold frame in March, or in a warm position outside in April. It takes two or three years to flower from seed. Propagation is usually by division of roots in the autumn. The underground portion of the plants are dug up after the stem has died down, and the 'daughter' roots that have developed at the side of the old roots are selected for replanting in December or January to form new stock, the young roots being planted about a foot apart each way. The young shoots appear above ground in February. Although the plants are perennial, each distinct root lasts only one year, the plant being continued by 'daughter' roots.

3.2.9) Collection and Drying[75]:

The leaves, stem, flowering tops and root are dried. The roots should be collected in the autumn, after the stem dies down, but before the bud that is to produce the next year's stem has begun to develop. If allowed to remain in the soil, the buds that crown the daughter roots begin to grow, in the late winter, and this growth exhausts the strength of the root, and the proportion of both starch and alkaloid it contains is lessened. The roots are first well washed in cold water and all the rootlets are trimmed off, and then dried, either entire, or longitudinally sliced to hasten drying. Drying may at first be done in the open air, spread thinly, the roots not touching. It is not complete till the roots are dry to the core and brittle, snapping when bent. Aconite root as found in commerce is, however, often yellowish or brownish internally with the stellate markings not clearly shown, probably from having been collected too early. It should be lifted in the autumn of the second year.

3.2.10) Classical Ayurvedic formulations[74]:

- *Mahamrutyunjaya rasa*
- *Mrutyunjaya rasa*
- *Ativisadi curna*
- *Balasanjivani curna*

- *Balacaturbhadra*
- *Srngyadi curna*

3.2.11) Chemical Constituents[77-88]*:*

Diterpenoid nitrogenous bases occur in the species of Aconitum. The diterpenoid alkaloids may be divided into two broad categories: the norditerpenoid alkaloids that are based on a hexacyclic C_{19} skeleton and those based on the C_{20} skeletons. Biogenetically, all these alkaloids are probably derived from tetracyclic or pentacyclic diterpenes in which the nitrogen atom of methylamine, ethylamine, or β-aminoethanol is incorporated in the norditerpenoid skeleton and in the C_{20} diterpenoid skeleton to form a substituted piperidine ring.

The norterpenoid alkaloids commonly called aconitines, may be subdivided in four groups based on four different skeleta. These groups are defined as:

1. Aconitine-type: These alkaloids possess the skeleton of aconitine, in which position C-7 is not oxygenated or substituted by any other group except hydrogen.
2. Lycoconitine: These alkaloids possess the skeleton of lycoctonine, in which C-7 is always oxygenated. C-7 may be substituted with OH, OMe or a methylenedioxy group bridging the oxygen at C-8.
3. Pyrodelphinine type: These alkaloids posses a modified aconitine skeleton with a double bond between C-8 and C-15. The pyro-type derivatives have been known for many years as pyrolytic degradation products of aconitine-type alkaloids.
4. Heteratisine type: These alkaloids possess the skeleton of heteratisine, in which a δ-lactone moiety is present.

Aconitum ferox contains Aconitine type of Norditerpenoid alkaloids. Fig. 2.2 shows the main alkaloids in the Aconitine type of alkaloids.

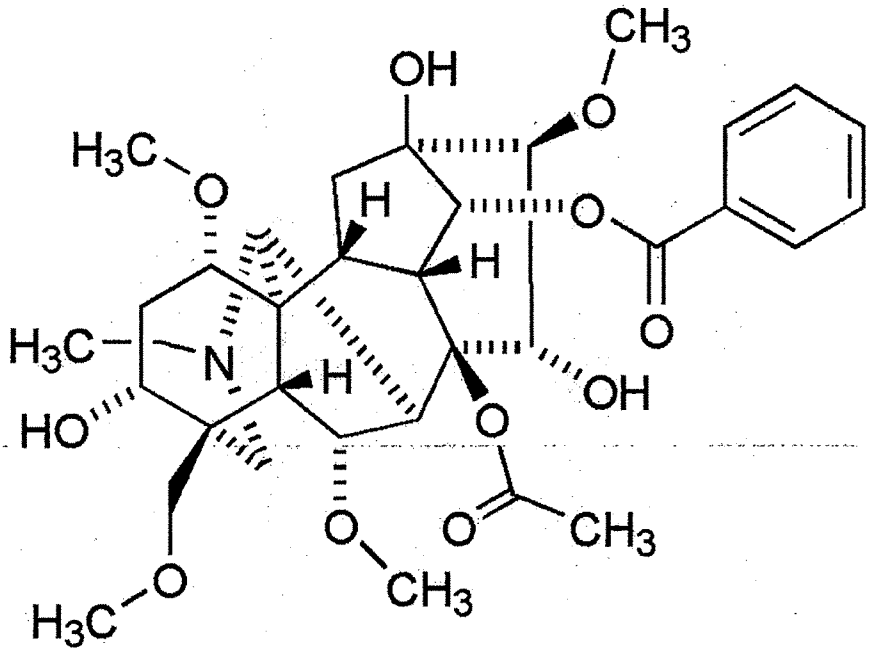

Fig. 3.2 Structure of Aconitine.

Aconite root contains from 0.3 to 1% alkaloidal matter, consisting of Aconitine - crystalline, acrid and highly toxic - with the alkaloids Benzaconine (Picraconitine) and Aconine. Aconitine, the only crystallizable alkaloid, is present to the extent of not more than 0.2%. Aconite acid, starch, etc., are also present. On incineration, the root yields about 3%. The Aconitines are a group of highly toxic alkaloids derived from various species of Aconite, and whilst possessing many properties in common are chemically distinguishable according to the source from which they are obtained. The Aconitines are divided into two groups: (1) the Aconitines proper, including Aconitine, Japaconitine and Indaconitine, and (2) the Pseudaconitines - Pseudaconitine and Bikhaconitine.

This disparity between Aconites is a very important matter for investigation, though perhaps not so serious from a pharmaceutical point of view as might at first appear, since in the roots of several different species the alkaloid is found to possess similar physiological action; but this action varies in degree and the amount of alkaloid may be found to vary considerably. Tinctures vary enormously as to strength, some proving seven times as powerful as others.

3.2.12) Poisoning[75]*:*

The symptoms of poisoning are tingling and numbness of tongue and mouth and a sensation of ants crawling over the body, nausea and vomiting with epigastric pain, laboured breathing, irregular and weak pulse, skin cold and clammy, features bloodless, giddiness, staggering, mind remains clear. All the species contain an active poison Aconitine, one of the most formidable poisons. It exists in all parts of the plant, but especially in the root. The LD_{50} value of aconitine for mice per injection about 0.15mg/kg b.w.

3.2.13) Pharmacological Activity:

Antitumor Activity[89]*:*

The antitumor properties were investigated against human tumor cell lines, A172, A549, HeLa and Raji, respectively, by a cell growth, a clonogenic assay, cell cycle distribution, cell cycle related molecules and gamma H2AX expression. The novel compounds derived from C_{20}-diterpenoid alkaloids showed a significantly suppressive effect in all cell lines. Suppressive effects of novel derivatives prepared from Aconitum alkaloids on tumour growth.

Anti inflammatory and Analgesic Activity[90]*:*

Aconiti tuber relieves neuropathic pain in the rat chronic constriction injury (CCl_4) model. Ten to 14 days after CCl_4 in the right hind paw, six groups of rats received oral placebo and doses at 0.5, 1, 2, 3, or 5 g/kg. It dose-dependently increased threshold of paw withdrawal and latency of paw withdrawal, which had been decreased due to CCl_4. The mechanism involves spinal kappa-opioid receptor mechanisms in a rat CCl_4 neuropathic pain model.

Antioxidant Activity[91]*:*

The DPPH radical scavenging activity of two flavonol glycosides obtained from ethanolic extracts of *Aconitum napellus sp. lusitanicum* was studied. The results showed a high DPPH antiradical activity of compound quercetin 3-O-(6-trans-caffeoyl)-beta-glucopyranosyl-(1-->2)-beta-glucopyranosyl-7-O-alpha rhamnopy-ranoside when compared with compound quercetin-3-sophoroside-7-rhamnopyranoside, rutin and ascorbic acid.

Anti-arthritic Activity[92]*:*

In acute inflammatory models, the paw edema of rats was induced by subcutaneous injection of carrageenan or pro-inflammatory mediators, including histamine, serotonin, bradykinin, and prostaglandin E(2) (PGE(2)) into the right hind paws of animals; while the ear edema of mice was induced by applying arachidonic acid or 12-O-tetradecanoylphorbol 13-acetate (TPA) on the ear surface. In nociceptive models, the tail-flick response induced by radiant heat stimulation was measured and the number of abdominal writhing episodes of mice induced by intraperitoneal injection of acetic acid were recorded.

Anti-diabetic Activity[93]*:*

The anti-hyperglycemic action of Hei-Shug-Pian, the fire-processed product of the root of Aconitum (*Aconitum carmichaeli*), was investigated in streptozotocin-induced diabetic (STZ-diabetic) rats. Under treatment conditions wherein plasma glucose was lowered, the uptake of glucose into soleus muscle was increased and the incorporation of glucose into glycogen of hepatocytes was enhanced. The plasma glucose-lowering effect of Hei-Shug-Pian was eliminated by blockade of opioid mu-receptors. Thus, the plasma glucose concentrations of STZ-diabetic rats is lowered through activation of opioid mu-receptors of peripheral tissues, resulting in enhanced glucose utilization.

Cardiovascular effects[94]*:*

The effects of aconite alkaloids in Aconiti tuber, on the contraction and free intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) level in isolated rat thoracic aorta was investigated. Mesaconitine at 30 mM inhibited 3 mM phenylephrine-induced contraction in the endothelium-intact, but not endothelium-denuded, aortic rings. The effect of mesaconitine was dependent on external Ca^{2+} concentrations. The relaxation induced by mesaconitine was abolished by N(omega)-nitro-L-arginine methyl ester (0.1 mM, an inhibitor of nitric-oxide synthase), as well as the relaxation induced by acetylcholine. Acetylcholine induced relaxation in two phases in our conditions; the initial phase was transient and external Ca^{2+}-independent, and the second phase was sustained and external Ca^{2+}-dependent. Treatment with 100 nM thapsigargin, which depleted intracellular Ca^{2+} stores, inhibited acetylcholine-induced, but not

mesaconitine-induced, relaxation. Mesaconitine increased the $[Ca^{2+}]i$ level in endothelial cells by influx of Ca^{2+} from extracellular spaces.

In another study, the results suggested that the alkaloids can be grouped in Na^+ channel activating and blocking compounds, but none of the alkaloids seem to be suitable as analgesics because of the low LD50/ED50 values.

3.2.14) Analytical methods reported:

Different analytical methods have been reported for the determination of Aconitum alkaloids in aconite roots, which involved methods using, HPLC with UV detection (1-7), HPLC with tandem MS (8), LC-MS-MS (9), Capillary Electrophoresis (10), GC-MS (11) etc. Some groups have described LC-MS (12) methods for the analysis of Aconitum alkaloids in body fluids and tissues[95-106].

References:

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7) Ohta H, Seto Y, Tsunoda N, An optimized high-performance liquid chromatography (HPLC) method for benzoylmesaconine determination in *Radix Aconiti Lateralis Preparata* (*Fuzi*, aconite roots) and its products ***J. Chromatogr. B.*** (1997), 691:351.

8) Kasahara Y, Itou T. Simultaneous determination of aconitine analogues in Aconitum plants and foods that caused food poisoning by liquid chromatography with tandem mass spectrometry. ***Shokuhin Eiseigaku Zasshi.*** (2008), 49(2), 76-81.

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12) Ito K, Satoshi T, Funayama M, Mizugaki M, Quantitative analysis of *Aconitum* alkaloids in the urine and serum of a male attempting suicide by oral intake of aconite extract. (2000) ***J. Anal. Toxicol.*** 24: 348.

3.3) Solanum Roots;

This plant is of importance in Hindu medicines as the source of one of the drugs required for a number of formulations. In the *Nighantas* it bears the Sanskrit names of Bhantaki, Vrihati, Mahati, "large egg plant". In the Ayurvedic formulary of India, *Solanum indicum* (*brhati*) and *Solanum xanthocarpum* (*kantakari*), together, are termed as *brhari dvayam*[75].

(a)

(b)

(c)

Fig3.3 Pictures of (a) Plant parts, (b) Plant (c) Roots of *Solanum indicum*.

3.3.1) Defintion:

" The dried roots of ***Solanum indicum***, Linn. (Solanaceae), without the presence or admixture of more than 5 percent of stems or other foreign matter."

3.3.2) Synonyms [75]:

Table 3.5: Synonyms of *Solanum indicum*.

Sr. No.	Language	Names
1	Sanskrit	Brahati, Vrihati bhantaki
2	English	Indian night shade.
3	Hindi	Barhanta, Birhatta
4	Bengali	Byakura
5	Gujarati	Ubhi ringani
6	Tamil	Kari-mulli
7	Marathi	Dolimoola, Moti ringani

3.3.3) Habitat[75]:

It occurs throughout India to southern China and Malaya.

3.3.4) History[75]:

Brhati root forms one of the *Laghupancamula* meaning literally, minor five roots, of *Dasamula kvatha* (decoction of ten roots), one of the widely prescribed formulation of Ayurveda. *Maharsi Caraka* has categorized *brhati* as *kanthya* – benefical for the throat, *sothahara* – relieves oedema , *angamarda prasamana* – relieves bodyache and *hikka nigrahana* – anti hiccup.

3.3.5) Ethnopharmacology[75]:

Brhati is pungent and bitter in taste , pungent in the post digestive effect and is potent. It alleviates *kapha* and *vata dosas*. It possesses light, dry and sharp attributes.

The roots and fruits are used for medicinal purpose. The herb is useful both internally as well as externally. Externally, the fresh juice of *brhati* is applied in alopecia areata with honey. The mixture of powders of *brhati* fruit, *haridra* and *daruharidra* rhizomes is beneficial, topically, in pruritus vulvae to alleviate intense itching. In halitosis (bad breath), the gargle with the decoction of *brhati* is an effective deodorant. The inhalation of its seeds powder like snuff is a good stimulant in *samjnanasa*. The paste of *brhati* alleviates pain and itching. Internally, *brhati* is used in vast range of diseases. The fruit juice mixed with

honey and ghee prepared from cow's milk effectively curbs vomiting. The plant is beneficial in various digestive ailments like loss of appetite, abdominal pain, distaste, worms and colitis. In respiratory problems like colds, cough, asthma, sinusitis, pleurisy the decoction of its roots works well with the fruit powder of pippali (*Piper longum*). The cough due to kapha and vata are controlled with the decoction of its roots given along with the honey and ghee, respectively. Being hot and sharp in properties, brhati liquefies the phlegm and relieves the blocked mucous and clears off the respiratory channels. It is the best blood purifier, hence, benevolent in blood disorders. *Brhati* stimulates and strengthens the heart and ameliorates the oedema. It also works well in dysuria and urinary calculi as it is diuretic in action. In fever, it is of special benefit, as it digests for which, its decoction is recommended with *sunthi* and *dhanyaka.* The seeds boost uterine contraction, so are used in dysmenorrheal, amenorrhea and in difficult labour. The seeds also bestow an aphrodisiac action. In urinary disorders like dysuria, urinary stones and cystitis, the medicated ghee of its roots *brhati mula siddha ghrta,* is commonly used.

3.3.6) *Classical Ayurvedic Preparations*[74]:

The widely used preparations containing *Solanum indicum* are as follows:

- *Dasamularista*
- *Mahamrutyunjaya rasa*
- *Brhatyadi kvatha*
- *Brhati mula siddha ghrta*

3.3.7) Description[76]:

The trunk is trifling, but the branches are numerous, ligneous, and perennial, forming a large, very ramous shrub of several feet in height. The plant is armed with numerous, very acute and somewhat recurved spines. The young parts are downy; leaves solitary, or in pairs, petioled, ovate-lobate, downy and armed with straight spines on both the sides, from 2 to 4 inches long. The racemes are opposite to the leaves, supporting several long-pedicilled, middle-sized, pale blue flowers. The calyx is deeply 5-cleft and armed. The berries are erect, round, smooth and the size is similar to that of a marrowfat pea. When the berries are immature, they are deeply variegated and lighter green in colour and

when they ripen the colour changes to deep orange yellow. The roots well developed, long, ribbed, woody, cylindrical and pale yellowish-brown 1-2.5 cm in diameter. A number of secondary roots and their branches are also present. The surface is rough due to presence of longitudinal striations and root scars. The fracture is short and splinetary. There is no distinct odour and taste of the roots. It is a plant common all over India. Fruit and root contain wax, fatty acids and alkaloids (Solanine and Solanidine).

3.3.8) Toxicological Properties[76]:

Their toxicity is based on their anti-cholinesterase activity on the central nervous system. It leads to the disruption of cell membranes by complexation with membrane 3β-OH sterols, and changes the active transport of ions through membranes, resulting in disorders in the general body metabolism. Toxic symptoms in humans include neurological and gastrointestinal disorders, such as vomiting, stomach pain, increased heart rate and hallucinations.

3.3.9) Chemical Constituents:

Solanum glycoalkaloids are secondary metabolites formed from the same precursors as steroids. Cholesterol, cholesteranol, and cycloartenol are alternative precursors for aglycone biosynthesis. The aglycones have the C_{27} steroid skeleton of cholestane. Nitrogen is adapted to cholesterol derivatives from amino acids like glycine, arginine[115-116], or L-arginine[117]. The aglycones are divided into five different categories depending on their structure:

1)Solanidanes have fused indolizidine rings,

2)Spirosolanes have an oxa-azaspirodecane alkaloid portion,

3)22, 26-epiminocholestanes,

4)a-epiminocyclohemiketals, and

5)3-aminospirostanes.

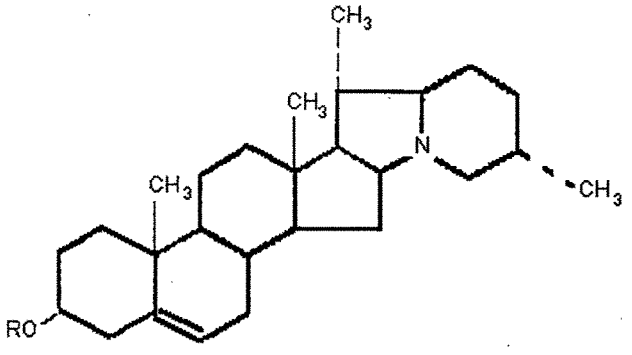
Most of the glycoalkaloids found in Solanum species belong to solanidanes and spirosolanes. The most common solanidanes are solanidine and its dihydrogenated form, desmissidine.

It has been reported that "solanine", which had been discovered in potato in 1820, was a mixture of two different glycoalkaloids, α-solanine and α-chaconine, both with solanidine as an aglycone but bound to different sugar

moieties[118-119]. The glycoalkaloids α-solanine and α-chaconine are generally present in plants, especially in *S. tuberosum*. The 22R, 25R epimer of solanidine has been detected in tubers of *S. vernei*, but this finding has not been reported elsewhere[120]. It has been suggested that solanidanes and spirosolanes are formed through the same biosynthetic route until the last steps, where etioline is transformed to either type of aglycone[117,121]. It is reported that diastereoisomeric spirosolanes are formed through different pathways. Immediately after formation, the aglycones are glycosylated by glucosyltransferase enzymes[122].

The fruit and root of *brhati* also contain wax, fatty acids and alkaloids solanine and solanidine. Disogenin, lanosterol, sitosterol, solasonnine, solamargine and solasidine have been isolated from the plant. A glycoalkaloid, solasonine on hydrolysis afforded solasodine, sugars, glucose, galactose and rhamnose. The oil from the seeds consists of the glycerides of lauric, palmitic, stearic, arachidic, oleic, and linoleic acids together with the phytosterols, sitoaterol and carpesterol.

Fig. 1. Structures of α-solanine and α-chaconine



solanidine R=H

α– solanine R= glu, gal, rham

α– chaconine R= rham, glu, rham

Fig 3.4 Structures of glycoalkaloids of *Solanum indicum*.

3.3.10) Pharmacological Activity

Glycoalkaloids are potentially toxic compounds that have a role in the plant's protection system. The ampiphilic nature of the glycoalkaloids contributes greatly to their anti-cholinesterase activity, aglycones as such being far less active than glycoalkaloids. Studies of the inhibition of acetylcholinesterase in *in vitro* studies have revealed that α-solanine and α-chaconine have about equal effects[123]. Subsequent research has shown that although the presence of sugar moiety is obligatory for such activity, the aglycone structure determines the activity level[124]. However, differences in sugar moieties are thought to contribute to cell membrane disruption via sterol

binding[125-126]. Both α-solanine and α-chaconine appear to induce changes in sodium active transport and in membrane potential on frog skin[127-128]. However, α-chaconine has been shown to be the most teratogenic compared to α-solanine[129]. Spirosolanes have shown lower activity than solanidanes, solanidine being the most teratogenic compared to solasodine[130].

Individual glycoalkaloids have been tested for their activity in biological systems. However, glycoalkaloids act synergistically, which means that the toxicities of individual glycoalkaloids do not predict the toxicities of mixtures of glycoalkaloids[131-132]. In this context, the need for reliable techniques, such as LC-MS, for glycoalkaloid analysis cannot be underestimated.

Some of the activities which have been studied in detail and reported are as follows:

Anti-Hypertensive activity[133]*:*

An ethanol extract of the fruit, standardized to contain > 0.15% chlorogenic acids, was tested orally in both normotensive rats and in those rendered hypertensive by twice daily intraperitoneal injection of N(W)-nitro-L-arginine methylester (L-NAME) for 1 week. The extract was either given at the same time as L-NAME or after the establishment of hypertension. The systolic blood pressure (SBP) was measured non-invasively using a tail cuff computer-aided monitoring device. Treatment of normotensive rats with the extract (30-300 mg/kg) for 4 weeks showed no hypotensive effect. Giving the extract (100 and 300 mg/kg) orally once daily during the 1 week hypertension induction period with L-NAME prevented the development of hypertension. Administration of the extract orally for 1 week after the establishment of hypertension tended to normalize the blood pressure. The extract showed good prophylactic as well as curative effect against L-NAME-induced hypertension, whereby its content of chlorogenic acids may play a minor role. Other constituents may be responsible for the antihypertensive action. The findings support further development of the extract as a potential therapeutically useful antihypertensive agent.

Immunostimulant Activity[134]*:*

Solanum indicum showed no toxicity to the brine shrimp (BST) nauplii, wheat rootlet growth (WRG) inhibition bioassay and lettuce seed germination (LSG) bioassay. It exhibited 75% inhibition to the growth of PPR virus.

Cytotoxic Activity[135]*:*

Both CHCl3 soluble (SI-IV) and insoluble (SI-V) fractions of the ethanolic extract (SI-I) showed cytotoxicity on seven cancer cell lines: Colo-205 (colon), KB (nasopharynx), HeLa (uterine cervix), HA22T (hepatoma), Hep-2 (laryngeal epidermoid), GBM8401/TSGH (glioma) and H1477 (melanoma). The purified constituents, SI-2 and SI-4 showed more potent effects by DEA and MTT assay. SI-2,3,4 and 5 also demonstrated cytotoxicity on cultured C6 glioma cells by PRE assay, ans SI-3,4 and 5 showed a tumor inhibitory effect *in vivo* in C6 glioma cells. In addition, SI-2 had an inhibitory effect on the DNA synthesis of C6 glioma cells at 10 mg/ml.

3.3.11) Analytical Methods Reported[136-142]**:**

Different analytical methods have been reported for the determination of the glycoalkaloids in potato tubers, which involved methods like colorimetry (1), HPLC with UV detection (2-3), GC (4), HPTLC (5), HPLC-EI-MS (6), Time resolved Flourescence (7), etc.

1) L. C. Baker, L. H. Lampitt, O. B. Meredith; Solanine, glycoside of the potato. An improved method of extraction and determination. ***Journal of the Science of Food and Agriculture***, 6(4), 197 – 202.

2) Hellenäs K.; Branzell C.; Liquid chromatographic determination of the glycoalkaloids α-solanine and α-chaconine in potato tubers. ***Journal of AOAC International.*** 1060-3271.

3) Edwards. J.; Cobba. H. ; Improved high-performance liquid chromatographic method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. ***Journal of Agricultural and Food Chemistry.***(1996), 44(9), 2705-2709.

4) Roosen-Runge C, Schneider E., On the determination of the solanum alkaloids solanine and chaconine, ***Z Lebensm Unters Forsch.*** (1977),164(2),96-97.

5) Patricia B., Charles K., Alfred N., Philippe H., Luc A. Determination of α-Solanine and α-Chaconine in Potatoes by High-Performance Thin-Layer Chromatography/Densitometry. ***Journal of AOAC International.*** (2000), 83(6), 1468-1473.

6) Britta Z., Gareth C., John D., Oliver F., Comparison of rapid liquid chromatography-electrospray ionization-tandem mass spectrometry methods for determination of glycoalkaloids in transgenic Wild-grown potatoes. ***Analytical Biochemistry*** 336 (2005) 178–186.

7) Maria A. Bacigalupo, Renato Longhi and Giacomo Meroni Alpha-solanine and alpha-chaconine glycoalkaloid assay in *Solanum tuberosum* extracts by liposomes and time-resolved fluorescence. ***Journal of Food Composition and Analysis***.17(5), (2004), 665-673.

3.4) Piper Species:

3.4.1) History:

Members of the botanical family Piperaceae were among the first cultivated plants[143]. Black pepper (*Piper nigrum*) and long pepper (*Piper longum*) are the best known species in this family and are probably among the most recognized spices in the world. Black pepper alone accounts for about 35% of the world's total spice trade. In addition, black pepper and long pepper have been used medicinally for centuries. In recent years, extensive research data on the phytochemistry and unique pharmacological actions of these plants have also become available. The Materia Medica of Ayurveda, which dates back to 6,000 B.C., has many references advocating the use of pepper in a variety of ailments, particularly those pertaining to the gastro-intestinal tract[144-151].

The earliest travelers from Europe who visited India described pepper cultivation on the Malabar coast[152]. Theophrastus mentions two kinds of pepper in the fourth century B.C., (most likely these were black pepper and long pepper). Discorides in the first century A.D. mentions black pepper and long pepper as well as white pepper, which is simply black pepper seed with its peel or pericarp removed. Black pepper and long pepper were among the spices from India on which the Romans levied import duty at Alexandria, around A.D. 176[153].

One reason spices in general, and pepper in particular, became so important in international trade was their popular culinary role. In those times, tough, heavily salted long-stored meat was standard fare, and spice additives made these meats more palatable, while simultaneously masking off-flavors.

3.4.2) *Chemical Constituents of Pepper*

Piperine is the active principle of black pepper (*Piper nigrum L.*) and long pepper (*Piper longum L.*). This is also the principal alkaloid of these plants. The piperine content is 3-9% and 3-5% (on dry weight basis) in *P. nigrum* and *P. longum* respectively.

3.4.2.1) Structure of piperine[155] :

Chemical names: 1-piperoyl piperidine (E,E) 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]piperidine.

Molecular weight: 285.33

Percentage composition:

C= 71.55% H=6.71% N=4.91% O=16.82%

Molecular structure:

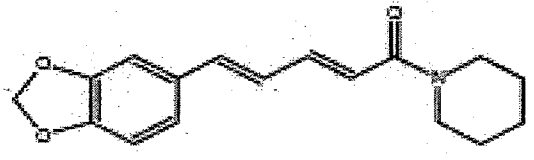


Fig. 3.5 Chemical Structure of Piperine.

3.4.3) Ethnopharmacology:

Piper species have been used in traditional medicine for intermittent fevers and to promote the secretion of bile. They are also recommended for neurological, broncho-pulmonary and gastrointestinal disorders, (including dyspepsia, flatulence, constipation and hemorrhoids)[144-152]. In Ayurveda, black pepper, long pepper and ginger are often used together in equal proportions in a preparation known as "trikatu", a Sanskrit word meaning "three acrids". Out of 370 compound formulations listed in the Handbook of Domestic Medicines and Common Ayurvedic Remedies, 210 contain either trikatu or its individual ingredients.[143] According to Ayurveda, the three acrids collectively act as "kapha-vatta-pitta-haratwam" which means "correctors of the three humors (doshas) of the human organism". Infusion is stimulant, carminative and alterative tonic more powerful than black pepper; also aphrodasiac, diuretic, vermifuge and emmenagogue. Externally used as rubefacient. Root is stimulant. Katu rasam, mathura vipakam, ushna veeryam, vatha kapha haram, lagu snigdam, rasayanam, vrishyam, clears ulcers, stimulates agni, in swasm, kasam, gulmam, soolam, etc., Old long pepper is more efficaciuos in medicine than fresh ones. Powdered long pepper administered with honey will relieve cough cold, asthma, hoarseness and hiccup. For catarrh and hoarseness a

mixture of long pepper , long pepper root, black pepper and ginger in equal parts is a useful combination.

3.4.4) Pharmacological Activity:

Antimalarial and Antipyretic:

The advantage of utilizing black pepper in the treatment of refractory intermittent fevers, which are symptomatic of malarial infections, is reported. Long pepper was discussed as a possible treatment for chronic malaria. [154] It was reported that long pepper was used for patients with chronic malaria with splenomegaly. Long pepper fruits were given in an increasing dose from 3 to 30, starting with 3 and increasing daily by 3 fruits. Subsequently the dose was decreased from 30 to 3 fruits, by reducing 3 fruits daily. Long pepper was boiled in milk and water and drank once a day in the early morning. Drinking this decoction reportedly caused cessation of malarial parasite multiplication and regression of splenomegaly.

Antiepileptic Activity:

In traditional Chinese medicine, black pepper has been used for the treatment of epilepsy.[157] Based on this traditional application, a new antiepileptic drug called Antiepilepserine has recently been synthesized by Chinese researchers. Antiepilepserine is a chemical relative of piperine, the main alkaloid phytochemical found in plants of the family Piperaceae. In traditional Middle Eastern medicine, black pepper has been used as a nerve tonic. Recently, the analeptic (nervous system stimulant) properties of piperine have been studied. Based on this research, piperine has been used successfully to counteract morphine-induced respiratory depression in experimental animals. [158]

Antiasthmatic drug:

Long pepper, and to a lesser extent trikatu, have been used in the treatment of asthma and chronic bronchitis in Ayurveda and Unani medicine. [153] In a study involving 240 children of different age groups suffering from frequent asthma attacks, long-term administration of long pepper fruits significantly reduced the frequency and severity of the attacks. Twenty-five patients in the study group showed no recurrence of asthma attacks, 161 showed clinical improvement, 47 did not benefit from the treatment, and 7 patients deteriorated. In another

study, 20 pediatric patients with asthma received long pepper in doses ranging from 9.35 to 15.75 gm daily for several weeks. As a result of this treatment all patients showed clinical improvement. [159]

Bioavailability enhancement :

The use of black pepper, long pepper, or trikatu is traditionally well-known in the treatment of a variety of gastrointestinal disorders, and all three act to improve digestion. In the 1920's Bose, an acknowledged author of "Pharmacographia Indica", reported an enhanced antiasthmatic effect of an Ayurvedic formula containing vasaka (*Adhatoda vasica*) when administered with long pepper[160]. In his "Pharmacopoeia Indica", Bose describes examples of his preparation which consists of juice from the vasaka leaves boiled with sugar, long pepper and butter; then this mixture was added to honey and given as a treatment for asthma. Through sustained experimentation and observation, ancient practitioners discovered herbal agents, such as pepper, which could increase the digestibility and efficacy of both nutrients and herbal drugs. The main purpose of trikatu's incorporation into numerous Ayurvedic formulations was most probably to enhance the efficacy of pharmacologically active ingredients. Several groups of investigators now attribute this bioavailability enhancing property of pepper to its main alkaloid, piperine. Piperine on hydrolysis with alkali gives piperic acid and piperidine. [161] The piperine content of pepper is directly proportional to its pungency. The biological properties of piperine have been extensively studied only in recent years[162-163]. The proposed mechanism for the increased bioavailability of drugs co-administered with piperine is attributed to the interaction of piperine with enzymes that participate in drug metabolism, such as mixed function oxidases found in the liver and intestinal cells. Interaction with the synthesis of drug chelating molecules in the body such as glucuronic acid has also been proposed.

Piperine may also interact with the process of oxidative phosphorylation, or the process of activation/deactivation of certain metabolic pathways, slowing down the metabolism and biodegradation of drugs. This action of piperine results in higher plasma levels of drugs, rendering them more available for pharmacological action. One of the first scientific experiments to confirm that

pepper could enhance the bioavailability of drugs was performed in the late 1970's by Atal and coworkers at the Regional Research Laboratory, Jammu-Tawi in India. These experiments revealed that *Piper longum* co-administered to rats orally with the drugs vasicine and sparteine increased the blood levels of vasicine by 232% and sparteine by more than 100% as compared to control animals who did not receive *P. longum*[161].

Thermogenic Activity:

Black and long peppers stimulate the skin as well as the tongue, thus they are also useful for topical application. They have broad antimicrobial, anti-parasitic and insecticidal properties. Peppers have been traditionally used as local anesthetics, but the mechanism of this analgesic (pain-relieving) action has only been recently described[163]. Piperine is thought to be the main phytochemical responsible for the analgesic action of pepper.

3.4.5) Analytical Methods Reported[164-175]:

A number of analytical methods have been developed for the estimation of Piperine in the plant materials of Piper species by Colorimetry (1), HPLC with UV detection (2-4), HPLC with DAD and electrochemical detection(5), LC-MS (6), HPTLC (7), Capillary electrochromatography(8). A number of methods have also been developed for the estimation of piperine in rat plasma using HPLC (9-10).

1) Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with draggendorff's reagent in plant materials. ***Journal of AOAC International*** (2003), 86(6), 1124-1127.

2) Verzele, M., Quereshi, S., *HPLC* Determination of *Piperine* in Pepper and in Pepper Extracts.***Chromatographia,*** (1980) 13(4), 241-243.

3) Wood, A.B., Barrow, M.L., James, D.J., Piperine determination in pepper (*Piper nigrum* L.) and its oleoresins - a reversed-phase high-performance liquid chromatographic method ***Flav. Fragr. J.*** (2006), 3(2), 55 – 64.

4) Srinivas V. Pullela, Ashok K. Tiwari, UmaMaheswara S. Vanka, Anuradha Vummenthula, Hari B. Tatipaka, Krishna R. Dasari, Ikhlas A. Khan, Madhusudana R. Janaswamy HPLC assisted chemobiological standardization of α-glucosidase-I enzyme inhibitory constituents from *Piper*

longum Linn-An Indian medicinal plant. ***Journal of Ethnopharmacology***, (2006) 108(3),445-449.

5) Ternes W, Krause EL. Characterization and determination of piperine and piperine isomers in eggs. ***Anal Bioanal Chem.*** (2002), 374 (1), 155-160.
6) Friedman M, Levin CE, Lee SU, Lee JS, Ohnisi-Kameyama M, Kozukue N. Analysis by HPLC and LC/MS of pungent piperamides in commercial black, white, green and red whole and ground peppercorns. ***Journal of Agriculture and Food Chemistry***, (2008), 56(9): 3028-36.
7) Gopu CL, Aher S, Mehta H, Paradkar AR, Mahadik KR. Simultaneous determination of cinnamaldehyde, eugenol and piperine by HPTLC densitometric method. ***Phytochemical Analysis***, (2008), 19(2), 116-121.
8) Musenga A, Mandrioli R, Ferranti A, D'Orazio G, Fanali S, Raggi MA. Analysis of romatic and terpenic constituents of pepper extracts by capillary electrochromatography. ***J Sep Sci.*** (2007) 30(4), 612-619.
9) Bajad S, Singla AK, Bedi KL. Liquid chromatographic method for determination of piperine in rat pasma: application to pharmacokinetics. ***Journal of Chromatography B.*** (2002), 776(2), 245-249.
10) Bajad S, Johri RK, Singh K, Singh J, Bedi KL. Simple high-performance liquid hromatography method for the simultaneous determination of ketoconazole and piperine in rat plasma and hepatocyte culture. ***Journal of Chromatography A***, (2002), 949 (1-2), 43-47.

3.4.6) Piper Longum:

(a) (b)

Fig.3.6 Pictures of (a) *Piper longum* climber and (b) *Piper longum* Fruit.

3.4.6.1) Synonyms[75]:

Table 3. 6 Synonyms of *Piper longum*.

Sr. No.	Language	Names
1	Sanskrit	Pippali, trikana, tikshnatandula.
2	English	Long-pepper, Dried Catkins.
3	Hindi	Pimpli, Pipal, Pipli, Pipli-mool.
4	Bengali	Pipli, Pepul.
5	Gujarati	Pipara, Pipal.
6	Tamil	Pipili.
7	Marathi	Mothi

3.4.6.2) Habitat[75]:

This plant is indigenous to North-Eastern and Southern India and Ceylon, and cultivated in Eastern Bengal.

3.4.6.2)Parts Used[75]:

Immature berries (i.e., dried in the sun, and stems (roots).

3.4.6.3)Constituents[75]:

It contains an alkaloid piperine, a pungent resin, volatile oil, piperidine, starch, gum, fatty oil and inorganic matter. The volatile oil is yellowish in colour contains mainly α-phellandrene and caryophyllene.

3.4.6.4)Classical Ayurvedic Preparations[76]:

- *Pippalyasava*
- *Vardhamana pippali*
- *Causasti pippali*
- *Pippali khanda*
- *Sitopaladi curna*
- *Guda pippali etc.*

3.4.6.5) Description[75]:

The herb is monoecious, climber and many parts are finely powdery pubescent when young. Stem is often flexuous. Petiole is 1-3 cm long. The Leaves are ovate or elliptic. Leaf blade is membranous and dark green about 3-5 cm wide, 7-10.5 cm long. The apex is acuminate; base is cordate or oblique and opposite or alternate in arrangement. Male spikes are straight upto 5-8 cm long, 0.3-0.7 cm in diameter; peduncle is 0.5 cm long. The bract is orbicular, stalked; with 2 stamens. Female spikes are erect, 0.6-2 cm long, 0.2 cm in diameter; peduncle is 0.5 cm long. The bract is circular, peltate with 3 stigmas. Fruiting spike is straight up to 2.5 cm long. The drupe is globose, sessile, arranged densely on rachis. The flowering occurs from May to September.

3.4.7) Piper nigrum

(a) *(b)*

Fig. 3.7 Pictures of *Piper nigrum* (a) plant and (b) dried fruits

3.4.7.1) Synonyms[75]:

Table: 3.7 Synonyms of *Piper nigrum*.

Sr.No.	Language	Names
1	Sanskrit	Maricham, Maricha, Hapusha, Krishnam
2	English	Black pepper, Common pepper
3	Hindi	Gulmirch, Kalimirch
4	Bengali	Kalimirich
5	Gujarati	Kalomirich
6	Tamil	Milagu
7	Marathi	Kalamiri

3.4.7.1) Habitat[75]: This perennial climbing shrub is indigenous to Malabar and Travancore coasts, i.e., western coast of India.

3.4.7.2) Parts Used[75]: Dried Unripe fruit.

3.4.7.3) Constituents[75]: A volatile alkaloid Piperine or Pipirine, Piperidine, a balsamic volatile essential oil, fat, mesocarp contains chavicin and ash containing organic matter. Chavicin is a soluble pungent concrete resin.

3.4.7.4)Classical Ayurvedic Preparations[76]:

- *Maricyadi taila*

- *Trikatu*
- *Maricyadi gutika*
- *Marica ghrta*
- *Caturusana*
- *Pancakola*
- *Sadusana*
- *Svasakuthara*
- *Sodasa vati etc.*

3.4.7.5) Description[75]:

The plant is monoecious, and a stout climber rooting at the nodes. The Petiole is grooved about 0.8-1.5 cm long. The leaf blade is fleshy coriaceous, ovate to elliptic, 4-6 cm wide, 9-11 cm long. The apex is acuminate rounded to oblique base. The spikes with male and female flowers are present together which are 5-13 cm long and 0.3-0.5 cm in diameter. The peduncle is 1-1.5 cm long with 2 stamens and 3 stigmas. The fruiting spike is 7-10 cm long. The drupe is globose, sessile, arranged loosely on rachis. The flowering and fruiting occurs one year round.

3.5) Cinnabar

The crucial ingredient in many ayurvedic formulations, and alchemical preparations is mercury or quicksilver, which is used in five forms (*panchasthuta*): pure mercury (*rasa*), red sulphide of mercury (*Hingula*), mercuric perchloride (*viram*), mercurous chloride (*puram*), and red oxide of mercury (*rasacheduram*)[176].

Mercury occurs naturally as the metallic form and/or its sulfide ores such as cinnabar (HgS). A small concentration of mercury is found throughout the lithosphere, the atmosphere, the hydrosphere and the biosphere. The earth's crust contains 0.5 mg/kg, ambient air may contain 0.002-0.02 pg/ml, and sea water contains about 0.03 mg/ml. Mercury is also found in trace amounts in most animal and plant tissues[177].

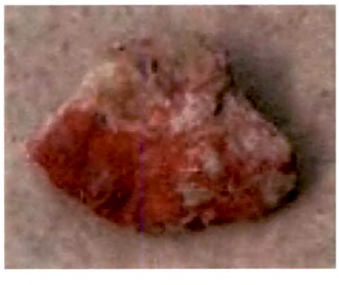


Fig3.8. Picture of Cinnabar

3.5.1) Physical Properties[178]:

Cinnabar is a colorful mineral that adds a unique color to the mineral color palette. Its cinnamon to scarlet red color can be very attractive. Well shaped crystals are uncommon and the twinned crystals are considered classics among collectors. The twinning in cinnabar is distinctive and forms a penetration twin that is ridged with six ridges surrounding the point of a pryamid. It could be thought of as two scalahedral crystals grown together with one crystal going the opposite way of the other crystal.

Luster is adamantine to submetallic in darker specimens. Transparency crystals are translucent to transparent.

Crystal System is trigonal.

Crystal Habits: Individual, well formed, large crystals are scarce; crusts and crystal complexes are more common; may be massive, or in capilary needles. Crystals that are found, tend to be the six sided trigonal scalahedrons that

appear to have opposing three sided pyramids. It also forms modified rhombohedrons, prismatic and twinned crystals as described above.
Cleavage is perfect in three directions, forming prisms. Fracture is uneven to splintery. Hardness is 2 - 2.5. Specific gravity is approximately 8.1+ (very heavy for a non-metallic mineral). It is almost insoluble in water, but can be transformed under strong acidic conditions into a soluble form.

3.5.2) Sources[76]:

Occurances include Almaden, Spain; Idria, Serbia; China and California.

3.5.3) History[176]:

Mercury is most widely used metal in *Rasa-Shastra* discipline of Ayurveda and to some extent most controversial also. According to Indian and Chinese alchemical traditions, in fact, it is mercury, not gold, that holds the highest position in the evolutionary rank of elements. It is the power and mystery of mercury that transforms base metals into gold of a quality far superior to that which is found in nature. When this purified gold is again "fixed" with mercury and absorbed into the body of an adept, it is said to have the power to prolong life. Thus the majority of the texts on alchemy, especially those concerned with mystical experiences and enlightenment, consider mercury rather than gold to be the center of this ancient science.
The essence of life in the human body is ojas – the intrinsic brilliance that enables the stream of life to flow continuously until it unites with cosmic existence, consciousness. Both ojas and virya are rasa, and rasayana, or alchemy, is the science of knowing the dynamics of ojas and virya. The counterpart of ojas and virya outside the body is mercury, which is also called "rasa" because mercury is the essence of all substances.
Taking mercury into the body increases rasa; by stabilizing mercury in the body, the practitioner of rasayana prolongs life.
Cinnabar, which contains mercury sulfide, has been used in Chinese traditional medicines for thousands of years as an ingredient in various remedies, and 40 cinnabar-containing traditional medicines are still used today. Cinnabar has been used in Chinese herbal medicine as a memory-enhancing drug for more than 2000 years. There is no doubt that its significant accumulation in tissues

and organs might produce neurological dysfunction e.g., a decrease in intellectual performance, including short-term memory, cognitive abilities and spatial learning.

Ayurvedic doctors maintain that only such purified mercury is fit for medicinal preparations. Ayurveda regards mercury as the master medicine for all diseases, and Ayurvedic preparations containing mercury are used to cure illnesses accompanied by the symptoms of dizziness, loss of memory, low energy, degeneration of bodily tissues, and damage to heart, kidney, liver, lungs, and brain. These are identical to the symptoms caused by mercury poisoning. This is in line with the homeopathic principle that similar cures similar, so it makes sense that mercury is the medicine for such problems, whether or not mercury poisoning is the case.

3.5.4) Pharmacological Activity[179]*:*

Although cinnabar is regarded as inorganic and insoluble, it can be absorbed in the gastrointestinal tract and then distributed to the brain, thus producing neurobehavioral toxicity. The critical brain mercury concentrations in association with specific neurobehavioral and concurrent neurobiochemical changes, including active avoidance responses, spontaneous locomotion and neuronal Na+/K+-ATPase activity, which are considered to be indicators of neurobehavioural deficits were compared in rats. Daily feeding with high doses (1.0 g/kg) of HgS and cinnabar, which were regarded as over-dosages or abused-dosages as in the reported cinnabar intoxication cases (Prakash et al., 1995) was carried out.

The results obtained indicated that oral HgS (1.0 g/Kg) did not produce marked central neurotoxicity but apparently improved acquisition (during 13 consecutive days of administration) and long-term memory (2 – 33 weeks after cessation of treatment) as compared with the vehicle controls. The possibility that the analgesic effects of mercurial compounds contributed to prolonged latency for escape in active avoidance responses was considered. By means of tail flick thermal hyperalgesic tests, it was found that cinnabar had no analgesic effect, but that MeHg had a reversible analgesic effect, which restored after discontinuous administration. This finding indicated that the irreversible

control neurotoxicity induced by MeHg was not totally due to the analgesic effect.

3.5.5) Toxicity[181]*:*

The toxicity of mercury is known to be highly dependent on its chemical form: organomercury is generally more toxic than inorganic mercury salts. Elementary mercury and insoluble HgS are the least toxic. Stable and insoluble forms such as HgS will have different consequences to other species of mercury. Heating cinnabar results in release of mercury vapor, which in turn can produce toxicity similar to inhalation of these vapors. The doses of cinnabar required to produce neurotoxicity are 1000 times higher than methyl mercury. Following long-term use of cinnabar, renal dysfunction may occur.

As reported in The Toxicological profile for Mercury, published by the U.S. Department of Health and Human Services, inhaling mercury causes nervous system disorders, which intensify and become irreversible with continued exposure. These include tremors, emotional instability, insomnia, headaches, memory loss, and loss of the ability to think clearly. Inhaling mercury also damages the respiratory system, inducing coughing, shortness of breath, and burning pains in the chest. In severe cases the lung tissue swells and fills with fluids. This can lead to pneumonia, emphysema, scarring and even collapse of the lung. Kidney damage, renal failure, rashes, fever, chills, and elevated white blood counts are also among the other consequences of inhaling mercury vapor. Death is caused by shock, cardiovascular collapse, acute renal failure, and severe gastrointestinal damage. In short, inhaling or swallowing mercury has a devastating effect on the respiratory, circulatory, nervous, gastrointestinal, muscular, and cardiovascular systems, and it damages the kidneys, liver, heart, brain, and reproductive organs.

The World Health Organization's guidelines maintain that the lowest level that could possibly be harmful to humans is 5 parts per million (ppm). This level is based on scientific results from the 1960s that placed the level at which risk begins at 50 ppm for most people; WHO then applied a safety factor of 10, deciding that a level of 5 or less is safe for even the most vulnerable populations.

Dimercaprol and succimer are effective chelation therapies for general mercury intoxication including cinnabar.

Cinnabar is chemically inert with a relatively low toxic potential when taken orally. In risk assessment, cinnabar is less toxic than many other forms of mercury, but the rationale for its inclusion in traditional Ayurvedic and Chinese medicines remains to be fully justified.

Although these adverse effects are well documented, their exact cause is open to question. Because the electron structure of mercury is loose, many other metals readily dissolve in it. According to the Chinese and Ayurvedic systems of medicine, it is these impurities in mercury, such as the presence of zinc, lead, and other minerals, that make mercury toxic.

3.5.6) Pharmacokinetics[180-183]:

A person can be exposed to mercury from breathing in contaminated air, from swallowing or eating contaminated water or food, or from having skin contact with mercury. Not all forms of mercury easily enter your body, even if they come in contact with it; so it is important to know which form of mercury you have been exposed to, and by which route (air, food, or skin).

The amount of mercury absorbed by Inhalation is 80%. Following ingestion, Absorption is

1.Less than 0.01% for metallic mercury,

2.Less than 10% for inorganic mercury (mercury used in Ayurvedic medicine),

3.More than 95% for organic mercury (methyl mercury).

Mercury can also be absorbed through the skin, but the amount is small compared to breathing or swallowing it. (The "true absorption" of a single oral dose of $HgCl_2$ was calculated to be about 20% at two different dose levels).

The analysis showed that cinnabar is insoluble and poorly absorbed from the gastrointestinal tract. Absorbed mercury from cinnabar is mainly accumulated in the kidneys, resembling the disposition pattern of inorganic mercury.

3.5.7) Purification of Ayurvedic Mercurial Preparations

All the purification processes lead to the elimination of impurities through mechanical/chemical treatment of the mercury, which is then followed by a prolonged heat treatment. The purification of cinnabar includes soaking in

lemon juice and replacing the juice about seven times. The final product is washed with hot water and dried.

Fig3.9. Pictures showing Purification of Cinnabar using lemon juice.

3.5.8) Sensitive And Selective Detection Methods For Cinnabar Determination[191].

A variety of detection methods have been used to detect cinnabar selectively and sensitively. These methods can be isted as follows: gravimetry, micrometry, radiometry, titrimetry, colorimetry and fluorometry, atomic absorption spectrometry (AAS) (cold vapour, electrothermal etc.), atomic fluorescence spectrometry (AFS), atomic emission spectrometry (AES) [spectrography, inductively coupled plasma-atomic emission spectrometry (ICP-AES), microwave-induced plasma-atomic emission spectrometry (MIP-AES), direct current plasma-atomic emission spectrometry (DCP-AES) etc.], neutron activation analysis (NAA), X-ray fluorescence (XRF), electron probe micro-analysis (EPMA), proton induced X-ray emission (PIXE) etc.), mass spectrometry (MS), electrometry (polarography, amperometry, voltammetry etc.), chromatography, and other miscellaneous methods.

3.6) Sulphur:

Sulphur is the chemical element in the periodic table that has the symbol S and atomic number 16. It is an abundant, tasteless, odorless, multivalent non-metal. Sulfur, in its native form, is a yellow crystalline solid. In nature, it can be found as the pure element or as sulphide and sulphate minerals. It is an essential element for life and is found in two amino acids[187].

Fig3.10. Picture of Crude Sulphur.

3.6.1) Synonyms[75]:

Table 3.9 Synonyms of Sulphur.

Sr. No.	Language	Names
1	Sanskrit	Gandhaka
2	English	Brimstone
3	Hindi	Gandak, Gundhak
4	Bengali	Gandrak
5	Gujarati	Gandhak
6	Tamil	Gandhakam
7	Marathi	Gandhak

3.6.2) Source[75]:

A non-metallic element found free in beds of gypsum and in a state of sublimation in regions of extinct volcanoes; also in combination with several ores called pyrites as sulphates and sulphides of iron, copper, lead, zinc, mercury etc. In India, it occurs naturally in some parts, in Nepal, Kashmir, Afganistan and in Burma. It is a constituent of various vegetable and animal substances such as albumin etc. It is obtained by roasting, fusion or by sublimation.

3.6.3) Physical Characters[187]:

Sulfur forms the greatest number of well-characterised allotropes, some of which had been identified before sulfur was proven to be an element. The existence of at least 22 sulfur allotropes at ambient pressure has been demonstrated, with 18 of these structurally characterised. A further 4 allotropes can be generated at high pressure, and the structures of 3 of these have been determined. The densities of the solids produced at atmospheric pressure range between 1.9 and 2.2 g/ml, whilst that of the high pressure form reaches 6.6 g/ml.

Sulphur is available in the market in four forms:

1) Yellow variety or vitreous or precipitated sulphur or *Amlasar gandhaka,* occurs in semi-transparent crystals ressembling the translucent ripe fruits of the *Amalaki.* This is employed for internal use in combination with mercury.
2) The white variety known as roll sulphur is found in sticks about two inches in width and 3 to 5 inches long; the taste is bitter and astringent and the smell is nauseous. It is very brittle; it is somewhat sticky to touch. It being inferior to the yellow variety is preferred for external application.
3) The red variety is called *Rati Hirakasi* or *Lal gandhak*; it occurs in small, flat or irregular crystalline pieces of a shining orange, red, purple or brick dust colour. The taste is acrid and bitter. It burns with a faint blue flame and emits the smell of sulphur.
4) The black variety i.e., sublimed sulphur is a purified form of sulphur and is prepared by washing *Gandhaka* in milk. It is first dissolved in an iron ladle smeared with butter and then gradually poured into basin of milk. When cooled and solidified it is fit for use. It is a light yellow powder of a bitter astringent taste and of a peculiar smell.

3.6.4) Ethnopharmacology:

Patients allergic to modern sulpha drugs do not show allergic reaction when *Gandhaka Rasayana* is given (the difference is processing, Ayurvedic Sulphur compounds are purified and prepared as per Ayurvedic texts). The daily dosage during an Ayurvedic treatment is about 30-40 mg of mercuric sulphide. This usually is given in combination with processed aconite.

Sulphur is more often than not used together with mercury because it acts as a regulator of the latter's fluidity. It converts mercury to mercuric sulphide, a substance that is insoluble in mineral acids.

3.6.5) Pharmacological Action[75]:

Sulphur is described as of bitter astringent taste with a peculiar strong smell. It increases bile, acts as a laxative and alterative and its preparations also act as alterative, diuretic and insecticide. Sulphur, when taken internally and in small doses, becomes absorbed and may be detected in the sweat, milk and urine. It is a stimulant to the secreting organs such as skin and the bronchial mucous membranes. It has a specific action on the rectum and increases the haemorrhoidal secretions. In large doses it acts as a purgative. It readily combines with and fixes metallic mercury and is therefore extensively used in combination with that metal. In combination with jaggery or cream of milk, sulphur is given in diseases like haemorrhoids and in skin diseases. Internally it is given with milk or in the shape of a sulphurated butter.

Sulphur is useful in cough, asthma, consumption and general debility; also in enlargement of the liver and spleen, chronic fevers, etc.

It is used for treating leucoderma, hepatomegaly, ascites, peptic ulcer, eye diseases, cysts, chronic venera diseases and skin diseases.

It is a potent drug used for the preparation of Rasayanam, Pills, Bhasmas.

3.6.6) Toxicity[185]:

Sulphur is not a highly toxic substance. Improperly purified and irregularly prepared sulphur medicine if consumed over a long period causes toxic effects. The toxic features are yellowish discolouration of conjuctiva, pallor of the face, discoloration of the shin similar to the colour of ridged gourd flower, disfigured and blackish teeth, profuse hyperhydrosis with yellowish colour, urine discoloration, bad breath, dyspepsia and flatulence

3.6.7) Need of Purification[73]:

Detoxiction

Fig. 3.11. Picture depicting the purification of sulphur.

Detoxication implies removing the impurities inhibiting the potency of the medicine. Impurities of sulphur should be removed with the detoxifying agent. Four detoxifying agents are used generally. They are milk, henna, banana, stems and peels of tamarind.

3.7) Sodium Metaborate

Sodium metaborate is an alkaline salt with excellent buffering properties, consists of white crystalline granules. The high solubility of sodium metaborate can provide a much higher concentration of borate ions in solution than borax at the same temperature[192].

3.7.1) Synonyms[75]:

Table 3.10 Synonyms of Sodium metaborate.

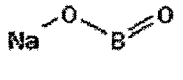
Sr. No.	Language	Names
1	Sanskrit	Tankana, Rasashodhan.
2	English	Sodium Biborate, Borax
3	Hindi	Tinkal, Sohaga
4	Bengali	Sohaga
5	Gujarati	Tankan-khar
6	Tamil	Venkaram
7	Marathi	Tankan-khar

3.7.2) Source[193]:

It occurs as a natural deposit. Crude borax is found in masses by evaporation of water, on shores of dried up lakes in India and Tibet; it is also obtained from the mud of lakes surrounded by hills in Nepal. In this crude state it is known as Sohafoor or tinkala. When purified by dissolving it in water, straining through cloth, evaporating to dryness and crystallizing, it is called borax.

3.7.3) Characteristics[194-195]:

It is composed of boric acid and soda. In the native state it exists as an impure saline incrustation of a dirty-white colour. It exists as crystalline tough mass or in the form of translucent irregular masses. Exposed to the air it becomes opaque. Formula: $NaBO_2 \cdot 2H_2O$ [or $Na_2B_2O_4 \cdot 4H_2O$]



Theoretical composition: Boric oxide- B_2O_3 34.19%, Sodium oxide: Na_2O 30.43%. Water of crystallization, H_2O 35.28%.

Anhydrous equivalent, $NaBO_2$ 64.62%

Molecular weight, $NaBO_2 \cdot 4H_2O$ 101.83

Specific gravity, 1.90

Sodium metaborate is stable at ordinary temperatures. If exposed to the atmosphere for extended periods, it will pick up carbon dioxide from the air and form sodium carbonate and borax. Sodium metaborate shows little tendency to cake except after prolonged storage or if it becomes severely wetted by rain or substantial water penetration. When stored under normal conditions of temperature and humidity, sodium metaborate is unlikely to cake or change chemically[196].

3.7.4) Purification[73]:

Borax is purified steeped for a night in whey and dried in the sun.

3.7.5) Uses[75]*:*

Borax is given internally in acidity of the stomach, amenorrhoea, dysmenorrhoea, menorrhagia, puerperal convulsions and to promote uterine pains during labour. As a solvent it is given in uric acid diathesis with good results. In the *Kaphaja* type of fevers a pill called *kapha-ketu rasa* made of aconite, borax and reduced conch-shell in equal parts, powdered, mixed well and soaked over three times in the juice of fresh ginger and made into pills of two parts each is given with honey and ginger-juice. This is used in all sorts of phlegmatic complaints from common catarrh to bronchitis and pneumonia even attented with discharges of the ears and the nose. In prolonged and tedious labours due to want of action or power in the uterus to expel the foetus, and in abortion in combination with cinnabar. In small doses it is given to children as a laxative. It is also used in the loss of appetite; painful dyspepsia, cough, asthma and diarrhoea in children. Diuretic, emmenagohue, astringent, antacid and local sedative and antiseptic. Externally borax is used in lotion for treatment of acne, freckles, chloasma etc., to stop itching in urticaria, psoriasis, pruritis pudendi, vulvi and sloughing ulcers.