Chapter II

REVIEW OF LITERATURE

II.1 Herbs with reported Anti-inflammatory action

Alcoholic extracts from the tubers of the roots of *Cyperus rotundus* (Motha)\textsuperscript{1} and the whole plant of *Celastrus paniculata* (Malkangni)\textsuperscript{2} were found to exhibit anti-inflammatory activity.

*Tinospora cordifolia* Miers., commonly known as Giloa, Gulacha and Gulvel in Hindi, is used in the form of powder and water extract in arthritis.\textsuperscript{3} The naturally occurring *Tinospora crispa* at Chiang Mai in Thailand has been found to inhibit carrageenan-induced foot pad edema. The usefulness of a modified dosage of this plant extract for the clinical treatment of various types of inflammation has been suggested.\textsuperscript{4}

Singh et al.\textsuperscript{5} showed that the alcoholic extracts from the leaves of *Hibiscus rosa-sinensis* (Jasum), and the pure glycoside (Plumieride) obtained from the roots of *Nerium indicum* (Kaner) possessed anti-inflammatory activity. Since the extracts have low toxicity and high safety margin, they may
be assessed for their usefulness in the Ayurvedic system of medicine in cases of fever, pain and rheumatism.

Singh et al.\(^5\) revealed the potent anti-inflammatory activity of an alcoholic extract from the defatted seeds of ashwagandha (*Withania somnifera*), a reputed Indian medicinal plant used in the Ayurvedic system of medicine for ailments including rheumatism. Singh et al.\(^6\) showed that an alcoholic extract of ashwagandha in 500 mg./kg. dose in rats caused 30-35% inhibition of carrageenan-induced inflammation as compared to 30% inhibition recorded with 5 mg./kg. indomethacin and 60% inhibition with 100 mg./kg. phenylbutazone. Against mycobacterial adjuvant-induced arthritis in rats, ashwagandha in 500 mg./kg. dose was found to exhibit 40-60% inhibition as compared to average 70% inhibition caused by phenylbutazone (100 mg./kg.) and average 90% inhibition caused by 5 mg./kg. indomethacin. Anbalagan and Sadique\(^7\) have investigated the powdered root of ashwagandha for its anti-inflammatory properties. It exhibits less anti-inflammatory activity in the suppression of edema when compared to phenylbutazone but it has got more influence on acute phase reactants suggesting its possible beneficial action on inflammation. As the studies on its antistress adaptogenic activity have revealed that the plant caused increase of adrenocortical activity, its anti-inflammatory activity was probably mediated through this mechanism. Sahni and Srivastava\(^8\) showed that oral administration of ashwagandha at a dose of 1g./kg. produced significant anti-inflammatory activity on chronic inflammatory action induced by cotton pellet granuloma in rats. Cimetidine, cyproheptadine and diclofenac significantly potentiated the anti-inflammatory activity of *Withania somnifera* by 31.9, 27.6 and 69.0 per cent. respectively on cotton pellet-induced granuloma.
m,p-dimethoxy and m-hydroxy p-methoxy substituted arylidene analogues of methyl ester of 3-keto beta-boswellin acid synthesized by the condensation of 3-keto beta-boswellic acid, isolated from oleogum resin of *Boswellia serrata*, with various substituted aromatic aldehydes were shown to have moderate anti-inflammatory activity in rats by Gupta et al.\(^9\) The therapeutic properties of *Commiphora mukul* have been reviewed by B. Bhat.\(^10\) Vyas and Shukla\(^11\) tested purified guggulu (*Commiphora mukul*) for its anti-rheumatic activity and inferred that guggulu acts as digestive and analgesic agent without any toxic reaction or adverse side-effect. Later it was shown by Dwirejua et al.\(^12\) that the aqueous extracts of the resins of *Boswellia dalzielli*, *Commiphora incisa* and *Commiphora mukul* showed anti-inflammatory action.

A glycosidal fraction isolated from the leaves of *Maesa chisia* reveals its anti-inflammatory properties in experimental animals.\(^13\)

A medicinal plant *Azadirachta indica* (neem) grows wild in the dry forests of Burma and India, but is naturalized in Nigeria and other West African countries. The effect of extracts of the leaf and bark of the plant as a more potent prostaglandin synthetase inhibitor than acetylsalicylic acid has been shown by Okpako.\(^14\) Due to the role played by prostaglandins in diverse inflammatory processes, it became necessary to investigate the anti-inflammatory effect of *Azadirachta indica* extract, using acetylsalicylic acid and indomethacin as reference substances. Okpanyi and Ezeukwu\(^15\) showed the neem leaf extract to have a pronounced anti-inflammatory (rat paw edema) effect.
The alcoholic extract of neem leaf was proved by Koley et al.\textsuperscript{16,17} to possess a significant and dose-dependent anti-inflammatory activity both in carrageenan-induced acute inflammation and formaldehyde-induced subacute inflammation in rats. The anti-inflammatory action of neem leaf extract was also proved by Chattopadhyay.\textsuperscript{18}

It was also shown by Koley et al.\textsuperscript{16,17} that the doses of alcoholic extract of neem leaf needed to produce anti-inflammatory actions had no ulcerogenic effect on the gastric mucosa of rats. The oral LD\textsubscript{50} of neem leaf alcoholic extract in mice was found to be 22g./kg.

Chattopadhyay et al.\textsuperscript{19} proposed a possible mechanism of the anti-inflammatory activity of \textit{Azadirachta indica} leaf extract. The anti-inflammatory effect may be due to antagonism of the deleterious effect of 5-HT and PGE\textsubscript{1} on blood vessels.

Nimbudin, a bitter principle isolated from neem oil, has been screened by Pillai et al.\textsuperscript{20} for its potential usefulness as an analgesic and anti-pyretic agent in rheumatic conditions. They found that nimbudin, in 100 mg./kg. dose level, showed significant analgesic effect.

Anti-inflammatory activity was confirmed for the plants \textit{Antidesma menas} (PX) (Euphorbiaceae) and \textit{Casimiroa edulis} (PX) (Rutaceae).\textsuperscript{21} The plants \textit{Viburnum odoratissium} (PX) (Caprifoliaceae), \textit{Halenia elliptica} (PL) (Gentianaceae), \textit{Dictamnus albus} (PL) (Rutaceae), and \textit{Leptorhabdos parviflora} (PL) (Scrophulariaceae) were also found to possess anti-inflammatory activity.\textsuperscript{22}
The plants *A. neelgerryana* (PL) (Asteraceae), *Garcinia anomalala* (PX) (Clusiaceae), *Argyreia sericea* (PX) (Convolvulaceae), *Taxillus recurvus* (PX) (Loranthaceae) and *Syzygium calophyllifolium* (PX) (Myrtaceae) were found to possess anti-inflammatory activity but low.\(^{22}\)

Kolaviron, a biflavonoid extract of *Garcinia kola*, was found to inhibit turpentine-induced joint edema, carrageenan-induced paw edema and pleurisy and brewers' yeast-induced pyrexia in rats. The anti-inflammatory activity of kolaviron was found to be comparable to that of phenylbutazone and acetylsalicylic acid.\(^{23}\)

The acute and subchronic anti-inflammatory activity of *Scabiosa atropurpurea* L. has been studied by Saenz Rodriguez et al.\(^{24}\) who found that it is active against acute inflammations. The ethanolic extract of the roots of *Aegle marmelos* yielded marmin, which showed anti-inflammatory effect in carrageenan-induced paw edema in rats.\(^{25}\) Calves fed *Pteris aquilina* revealed very mild inflammatory reaction and infiltration of a few polymorphonuclear cells.\(^{26}\) *Zizyphus xylopyra* bark extract administered intraperitoneally in albino rats exhibited anti-inflammatory activity.\(^{27}\)

The influence of crude aqueous extracts of *Epilobium hirsutum*, *Epilobium dodonaei*, *Epilobium roseum*, *Epilobium montanum* and *Epilobium adenocaulon* on the carrageenan-rat paw edema and the prostaglandin biosynthesis in the perfused rabbit ear was studied by Hiermann.\(^{28}\) From all *Epilobium* species investigated so far, only *Epilobium angustifolium* reported earlier and *Epilobium montanum* were found to depress the formation of the rat paw edema. The active principle of *Epilobium angustifolium* is located in the leaves.
Hall et al.\textsuperscript{29} showed that the mono-and di-functional esters of helenalin (isolated from *Baldwina augustifolia*) exhibited potent anti-inflammatory action in rodents. It was also demonstrated that the mono-functional esters were less active than the di-functional esters and the parent agent.

Anti-inflammatory activity was shown by *Achyrocline satureioides* (Lam.) DC. Compositae extract.\textsuperscript{30} The anti-inflammatory conditions could be related to the presence of the flavonoids.

Middleton et al.\textsuperscript{31} reported that certain citrus flavonoids possessed in vivo anti-inflammatory activity. Later studies were undertaken to define the anti-inflammatory activity of hesperidin, an important flavanone of *Citrus* sp. The results showed that hesperidin possesses significant anti-inflammatory effect.\textsuperscript{32,33}

Varde et al.\textsuperscript{34} showed that the oral administration of 2 ml. of the boiled coconut oil extract of the rhizome of *Acorus calamus* and leaves of * Ocimum sanctum* and *Ocimum basilicum* produced 45\% inhibition of the carrageenan-induced paw edema, 13.6\% inhibition of cotton pellet granuloma formation and 61\% inhibition of croton oil granuloma pouch inflammatory response in rats.

90\% ethanol extract of leaves\textsuperscript{35} and 90\% ethanol, aqueous and petroleum ether extracts of the stem\textsuperscript{36} of *Strobilanthes heyneanus* were found to possess anti-inflammatory effect. Distinguishing pharmacognostic characters of *Nilgirianthus heyneanus* (Nees) Bremek. leaves having anti-inflammatory activity have been reported by Shantha et al.\textsuperscript{37}
Current knowledge on the sources of flavonoids and their reported anti-inflammatory activity have been summarized by Alcaraz and Jimenez. Lee Song Jin et al. demonstrated that significant anti-inflammatory activities were found especially in naturally occurring flavone and flavonol glycosides (15-29% inhibition) although the flavonoid derivatives tested showed less anti-inflammatory activity than hydrocortisone or indomethacin. Chalcone and flavanone derivatives were not significantly active. In general, flavonol glycosides of kaempferol-type were found to have a higher oral anti-inflammatory activity than that of flavonol glycosides of quercetin-type in mice.

Mannan from *Candida albicans* inhibited both inflammation and destructive arthritic changes. But mannan from *Saccharomyces cerevisiae* showed less effect. However, acetolysate of *Saccharomyces cerevisiae* mannan as well as simple methyl-α-D-mannopyranoside inhibited both inflammation and destructive arthritic changes to a similar degree as mannan isolated from *Candida albicans*. The effect, which is not dose-dependent indicates its possible immunoregulatory mechanism. This is the first time a therapeutic effect of simple carbohydrates on rat adjuvant arthritis has been described.

Woo Won Sick and Jae Soo Choi showed that the hexane fraction of the methanol extract of the roots of *Bupleurum longiradiatum* (*Umbelliferae*) exhibited anti-inflammatory activity. Fractionation of the hexane extract resulted in the isolation of arborinone as an active principle. The anti-inflammatory activity of the whole essential oil of *Bupleurum fruticescens* and its major components was investigated by Martin et al. in the rat hind paw edema model induced by carrageenan or by PGE$_1$. The anti-inflammatory
activity shown by the essential oil can be attributed to the two major components: α-pinene and β-caryophyllene. α-pinene needs the integrity of the adrenal glands to exert its anti-inflammatory activity, as opposed to β-caryophyllene which was active in adrenalectomized animals also.

Arvind Manohar Mujumdar et al.\textsuperscript{43} showed that piperine (1-peperonyl piperidine), isolated from \textit{Piper nigrum} Linn., exhibited significant anti-inflammatory activity both in acute and chronic models of inflammation in animals. The anti-inflammatory action of extracts of \textit{Momordica charantia} was studied by Jawahar Lal et al.\textsuperscript{44}

50% ethanolic,\textsuperscript{45} methanolic,\textsuperscript{46} aqueous\textsuperscript{46} and coconut oil\textsuperscript{47} extracts of fresh \textit{Ocimum sanctum} leaves, the volatile oil\textsuperscript{45} obtained by the extraction of the fresh leaves and the fixed oil\textsuperscript{45,48,49} extracted from the seeds were found to possess anti-inflammatory activity. The dual inhibition of cyclooxygenase and lipoxygenase by the fixed oil could account for its anti-inflammatory activity.\textsuperscript{50} It was concluded that the anti-inflammatory activity of the fixed oil appeared to be due to its unsaturated fatty acids in general and linolenic acid in particular.\textsuperscript{51}

Banerjee et al.\textsuperscript{52} reported the anti-inflammatory activity of petroleum ether extract of \textit{Ricinus communis} Linn. Garcia Dolores et al.\textsuperscript{53} proved \textit{Scrophularia frutescens} L. to be a potential anti-inflammatory agent.

Emodin, an anthraquinone derivative, isolated from the whole plant of \textit{Rhamnus triquerta} Wall., in 15 mg./kg. dose (ip), exhibited anti-inflammatory activity against carrageenan-induced pedal inflammation in rats.\textsuperscript{54} Chang Cheng-Hsiung et al.\textsuperscript{55} isolated emodin, the major component of the
chloroform fraction from *Ventilago leiocarpa* Bunge. (Rhamnaceae) and reported its anti-inflammatory effect on carrageenan-induced edema in rats.

Onion (*Allium cepa* Linn.) and garlic (*Allium sativum* Linn.) are two important *Allium* species with several medicinal properties. Garlic was proved to have a retardative effect on the development of atherosclerosis.\(^{56,57}\) It was also found that garlic powder manifests direct anti-atherogenic action in vitro and also in vivo.\(^{58}\) Biju and Augusti\(^{59}\) showed that not only the obnoxious oil of garlic but a palatable protein of this vegetable is also of great value as an anti-atherogenic agent. Thiosulphinates (allicins) and cecaenes (onion principles) have been shown to possess anti-inflammatory properties.\(^{60}\) This action, in part, is related to inhibition of inflammatory cell influx by thiosulphinates and cecaenes.\(^{61}\)

Anti-inflammatory effect of a *Pluchea indica* root extract was evaluated by Sen and Chaudhuri.\(^{62}\) Further studies by Sen et al.\(^{63}\) revealed the significant anti-inflammatory activity of the methanolic fraction of the extract of roots of *Pluchea indica* Less. on paw edema induced by arachidonic acid, platelet activation factor and compound 48/80. There was significant inhibition of spontaneous as well as compound 48/80-induced histamine release from mast cells.

Neolupenol, a pentacyclic triterpene isolated from *Pluchea lanceolata* flowers, was studied by Kaith,\(^{64}\) to determine its anti-inflammatory activity against carrageenan-induced rat paw edema. The degree of edema inhibition was found to increase with dose as well as time interval and was found to be maximum at 300 minutes. Neolupenol, when administered at 100 mg./kg. per body weight, was found to exhibit 70% edema inhibition which was
greater than that of the reference compound, ibuprofen (50 mg./kg. per body weight, 65% inhibition, 300 minutes).

Seeds of *Aphanamixis polystachya* (Wall.) Parker, Meliaceae (Rohituk), are used for treating rheumatism. A sensitive and relevant model was found for evaluating anti-inflammatory activity: Papaya latex-induced rat paw inflammation by Gupta et al.

Individual aqueous/alcoholic extracts of *Populus tremula*, *Solidago virgaurea* and *Fraxinus excelsior* exhibited significant anti-inflammatory activity. The anti-inflammatory properties of *Populus tremula* were mainly deduced from its components, salicylates. The coumarin components of *Fraxinus excelsior* were proved to have a variety of pharmacological properties, i.e., inhibition of T-cell activation and of the arachidonic acid cascade.

Anti-inflammatory effect of thick extractions of the schrot of fruit of *Hippophae rhamnoides* L. was studied by Sabynich et al. *Pongamia pinnata* (L.) Pierre grows on moist environment along rivers and streams all over India. Seeds of the plant have been described as a useful ayurvedic remedy for fever, abdominal colic, inflammation (externally) and gout. The anti-inflammatory property of *Pongamia pinnata* seeds was well documented by Agharkar. A sesquiterpene, furoic acid which is an anti-inflammatory metabolite of the soft coral *Sinularia* spp. has been synthesized by Williams David and John Faulkner.

Ginger (*Zingiber officinale*) is described in ayurvedic system of medicine to be useful in inflammation and rheumatism. The anti-inflammatory activity was proved and it was also suggested that at least one of
the mechanisms by which ginger shows its ameliorative effects could be related to inhibition of prostaglandin and leukotriene biosynthesis, i.e., it works as a dual inhibitor of eicosanoid biosynthesis.\textsuperscript{74,75}

Melek et al.\textsuperscript{76} demonstrated the significant dose-dependent anti-inflammatory activity of aqueous alcoholic extract of \textit{Atractylis carduus}.

Pal Siddhara and Chaudhuri\textsuperscript{77} showed that the methanolic fraction of the fresh leaf methanolic extract of \textit{Bryophyllum pinnatum} exhibited significant anti-inflammatory activity on different experimental models. The leaf extract significantly decreased the activity of serum aminotransferases: serum AST [serum aspartate aminotransferase] and serum ALT [serum alanine aminotransferase] as well as hydroxyproline concentration and increased ATP-ase concentration. The inhibitory effect on arachidonic acid and glucose oxidase-induced inflammation suggest that almost definitely the \textit{Bryophyllum pinnatum} leaf extract is not a cyclooxygenase inhibitor.

Cajucarinolide and isocajucarinolide, two new clerodane diterpenes isolated from the cortices of \textit{Croton cajucara} (Euphorbiaceae), were demonstrated to possess anti-inflammatory activity.\textsuperscript{78}

Caceres Armando et al.\textsuperscript{79} revealed that hot water infusion of seeds of \textit{Moringa oleifera} exhibited a significant inhibition of carrageenan-induced edema at 1000 mg. dried plant material per kg. Later Ezeamuzie et al.\textsuperscript{80} showed that root of \textit{Moringa oleifera} contains anti-inflammatory principle(s) that may be useful in the treatment of both the acute and chronic inflammatory conditions.
Essential oil from *Originum tyytanum*, laurel oil, tetramethylpyrazine, one of the alkaloids contained in *Ligusticum wallichii* and ferulic acid, a phenolic compound contained in *Ligusticum wallichii* and *Angelica sinesis* were suggested to have anti-inflammatory effect. Significant anti-inflammatory activity has been observed in the total benzene extract of *Swertia chirata* against acute, subacute and chronic models of inflammation.

*Tripterygium wilfordii* Hook f, a medicinal herb in traditional Chinese medicine, was found to be a potent immunosuppressive inhibitor of type II collagen-induced arthritis. Anti-inflammatory oleanane triterpenes were produced from *Tripterygium wilfordii* cell suspension cultures by fungal elicitation. The elicited production has been developed for commercial application in the light of the successful treatment of rheumatoid arthritis with *Tripterygium* extracts. The active principle responsible for the anti-inflammatory actions in *Tripterygium* was found to be diterpenoid containing triepoxides, but information on its chemistry was limited to the woody part of the root and the root bark. Later leaves of *Tripterygium* were studied and two novel diterpenoids were isolated. Anti-inflammatory activity of triptolide (isolated from *Tripterygium wilfordii* Hook f) was studied and it was found that a high dose of triptolide can stimulate the pituitary-adrenal axis.

The anti-inflammatory effects of *Aneococilus formosanus*, *Ganoderma lucidum* and *Gynostemma pentaphyllum* were studied, by Lin Jei-Min et al., against carrageenan-induced paw edema. The water extracts of *Gynostemma pentaphyllum* and *Ganoderma lucidum* were shown to be active against carrageenan-induced edema. *Gynostemma pentaphyllum* was found to have an activity even greater than indomethacin. But *Aneococilus formosanus* showed a delayed onset of anti-inflammatory activity: four hours
post-carrageenan administration. However, *Anoectochilus formosanus* significantly decreased the acute increase in serum aminotransferases [serum AST (serum aspartate aminotransferase) and serum ALT (serum alanine aminotransferase)] level caused by carbon tetrachloride. Histological changes such as necrosis, fatty change, ballooning degeneration, inflammatory infiltration of lymphocytes and Kupfer cells around the central vein were simultaneously improved by the treatment of *Anoectochilus formosanus*.

The dried aqueous extract of *Harpagophytum procumbens* (Pedaliaceae) exerted significant dose-dependent anti-inflammatory effect from the dose 100 mg. of dried secondary roots/kg. The main iridoid glycoside, harpagoside, of the plant does not appear to be involved in its anti-inflammatory properties.\(^9^0\)

Ternatin, a flavonoid isolated from *Egletes viscosa* Less., was also found to have anti-inflammatory effect.\(^9^1\) Bearberry leaf (*Arctostaphylos uva ursi* L. Spreng.) was shown to increase the inhibitory effect of dexamethasone on the allergic and inflammatory models.\(^9^2\) The extracts of *Vanda roxburghii* roots in petroleum ether, chloroform and methanol showed 54.2%, 42.1% and 21.9% anti-edema activity, respectively.\(^9^3\) Magnolol, isolated from *Magnolia officinalis*, inhibited mouse hind-paw edema induced by carrageenan, compound 48/80, polymyxin B and reversed passive Arthus reaction.\(^9^4\) *Quillaia saponaria* Molina. (Panama), Rosaceae, has produced statistically significant reduction of edematogenic response.\(^9^5\)

A dry extract from the above-ground part of *Desmodium canadense* was found to significantly affect the stages of inflammation. Apparently, the pharmacotherapeutic activity of the extract is a result of the presence of
flavonoids. The anti-inflammatory activity of the leaves of *Vinca rosea* Linn. was studied in experimental animals. Jain et al. demonstrated that the ethanolic extract of the stem bark of *Murraya koenigii* showed anti-inflammatory effect in carrageenan-induced inflammation in rats. A dose of 1 g./kg. inhibited ca 65.21% of inflammation. The methylene chloride extract of *Tanacetum microphyllum* aerial parts exhibited anti-inflammatory activity and yielded two anti-inflammatory flavonoids.

Germano et al. found that the dried crude extract of *Petiveria alliacea* showed an inhibitory effect on the cotton pellet granuloma and croton oil dermatitis. The activity was more pronounced by topical administration than by the oral route.

Gupta Sarita et al. evaluated the anti-inflammatory activity of isoplumbagin and lawsaritol, isolated from the stem bark and roots of *Lawsonia inermis*, against carrageenan-induced paw edema in rats. Phenyl butazone, isoplumbagin and lawsaritol at the oral dose of 100 mg./kg. exhibited 61, 60 and 40 per cent inhibition in comparison with controls. Isoplumbagin showed a significant anti-inflammatory activity similar to that of phenyl butazone.

The ethanolic extract of *Caralluma tuberculata* produced significant inhibition of carrageenan-induced paw edema and cotton pellet granuloma in rats. Anti-inflammatory activity of baicalein, a flavonoid from *Scutellaria baicalensis* Georgy roots, was greater in the chronic inflammation model than observed in the rat carrageenan-induced paw edema. It was also suggested that inhibition of the 5-lipoxygenase pathway of arachidonic acid metabolism may be one of the mechanisms of baicalein’s anti-inflammatory activity.
A protein fractionated and purified from the larva of *Parasa sinica* Moore. CCP (ip), has a significant anti-inflammatory effect on ear edema induced by croton oil in mice.\(^{104}\) Its ID\(_{50}\) is 1.6 mg./kg., but a dose of 2.5 mg./kg. can also significantly inhibit the rat ankle edema induced by carrageenan and egg white.

Velutinol A, from *Mandevilla velutina*, was found to be an anti-inflammatory agent.\(^{105}\) Jin Jingji et al.\(^{106}\) showed that *Caragana microphylla* Lam. can antagonize the inflammation induced by carageenan, hot water and croton oil. It can also inhibit the proliferation of granuloma, blood capillary permeability, phagocytic function of mononuclear phagocyte system, and synthesis or release of PGE\(_2\) at the inflammed part.

Anti-inflammatory activity of the iridoids: kutkin, picroside-1 and kutkoside from *Picrorhiza kurrooa*, was reported by Bhattacharya and Maiti.\(^{107}\) Padmaja et al.\(^{108}\) reported the anti-inflammatory activity of the saponifiable fraction of the petroleum ether extract of the root of *Ixora coccinea* Linn. (Rubiaceae), in carrageenan-induced paw edema in albino rats. Anti-inflammatory activity was found in the water fraction of methanol extract of the leaves of *Emblica officinalis* Gaertn.\(^{109}\)

Mongold *et al.*\(^{110}\) found that the aqueous alcoholic extract of the dried leaves of *Ribes nigrum* exhibited potent anti-inflammatory properties as shown by the inhibition of carrageenan-induced acute inflammation, cotton pellet granuloma and Freund adjuvant-induced arthritis in rats. It did not show any CNS sedative or narcotic effects. Thus the drug may be a useful alternative to anti-inflammatory drugs presently available.
It was reported by Lin Yong Cheng et al.\textsuperscript{111} that the valdivones, diterpene esters from the South African soft coral \textit{Alcyonium valdivae}, inhibit chemically-induced inflammation in the mouse ear assay.

Zarzuelo et al.\textsuperscript{112} have made studies on the anti-inflammatory actions of four species of the genus \textit{Sideritis}. \textit{S. incana} var. \textit{virgata}, \textit{S. funkiana} ssp. \textit{funkiana}, \textit{S. funkiana} ssp. \textit{talaverana} and \textit{S. hirsuta} were shown to be effective anti-inflammatory agents by intraperitoneal route; \textit{S. hirsuta} being the most active when administered orally. De Las Heras et al.\textsuperscript{113} examined extracts of \textit{Sideritis javalambrensis} Pau. in hexane, dichloromethane, ethyl acetate and methanol for their anti-inflammatory activity against adjuvant-carrageenan-induced inflammation and compared their activities with phenylbutazone. In the chronic stage of inflammation, the hexane- and methanol- extracts showed the greatest inhibitory activity. None of the extracts inhibited inflammation in the acute phase. Two purified plant products were obtained from the anti-inflammatory extracts of \textit{Sideritis javalambrensis} and their anti-inflammatory activities were evaluated and compared with aspirin, sodium salicylate and indomethacin.\textsuperscript{114}

Shimizu Mineo and Takashi Tomoo\textsuperscript{115} found that the diethyl ether-soluble fraction of the methanolic extract of the leaves of \textit{Aucuba japonica} Thunb. (Aoki) showed an anti-inflammatory effect when topically applied to rats. E-phytol, phytone, bis (2-ethylhexyl) phthalate and friedelin were isolated as active anti-inflammatory components inhibiting carrageenan-induced paw edema. The most effective, E-phytol, inhibited histamine-induced paw edema more potently than the compound 48/80-induced edema, suggesting that the mechanism of action is due to inhibition of the H\textsubscript{1} receptor. Aoki (\textit{Aucuba japonica} : leaves). Sekisyou (\textit{Acorus gramineus}),
Yoobaihi (*Myricae cortex*) and Sidareyanagi (*Salix babylonica* : leaves and branches) were also shown to have an anti-inflammatory action when used as bathing agent, though the effect varied depending on the extraction temperature.\textsuperscript{116} Extract of Sekisyou and Aoki in boiling water had little effect.

Park Eun-Hee and Mee Ja Shin\textsuperscript{117} showed the aqueous extract of *Gleditsiae spina* to have an anti-inflammatory action. Taguchi Kyoji et al.\textsuperscript{118} reported quercitrin (from *Houttuyniae herba*) to have an inhibitory effect on acute inflammation.

The ethanolic extract of *Cissus trifoliata* root showed marked anti-inflammatory activity in carrageenan-induced edema in rats and mice, and in chronic models of formaldehyde and adjuvant-induced arthritis in rats.\textsuperscript{119} It reduced the cotton pellet granuloma, and heat-induced hemolysis of rat erythrocytes in vitro. Singh and Dixit\textsuperscript{120} found that the ethanolic extract of aerial parts of *Trianthema portulacastrum* Linn. exhibited anti-inflammatory activity. Fractionation of the extract showed that the acetone-soluble fraction of the extract is responsible for the activity.

Isoflavonoids such as daidzein and puerarin from *Pueraria radix* exhibited significant anti-inflammatory activity at a dose of 2 mg./mouse.\textsuperscript{121} Santonin, a sesquiterpene lactone, commonly found in the plants of Compositae family was found to have significant anti-inflammatory activity on acute inflammatory processes.\textsuperscript{122} It also showed a significant inhibitory effect on granuloma formation. Martin-Aragon et al.\textsuperscript{123} showed that oral administration of a lyophilized infusion of *Tuberaria lignosa* leaves significantly inhibited adjuvant-carrageenan-induced edema.
Kubo Michinori et al.\textsuperscript{124} reported the importance of Corydalis tuber as an anti-inflammatory agent. A methanolic extract from Corydalis tuber (\textit{Corydalis turtschaninovii}) inhibited an increase in vascular permeability in mice induced by acetic acid, and reduced acute paw edema in rats induced by compound 48/80 or carrageenan. The extract suppressed the development of adjuvant-induced edema in arthritic rats. Alkaloidal components from the methanolic extract from Corydalis tuber: dehydrocorydaline, d-glaucine and 1-tetrahydrocoptisine were also found to be effective in both the acute and chronic phases of inflammation. Thus the crude drug Corydalis tuber can be considered to exert anti-inflammatory activity.

It was confirmed by Duwiejua et al.\textsuperscript{125} that the aqueous alcoholic extracts of \textit{Polygonum bistorta}, \textit{Guaiacurn officinale} and \textit{Hamamelis virginiana} contained anti-inflammatory substances.

Akihisa Toshihiro et al.\textsuperscript{126} reported the anti-inflammatory activity of 7-oxo-10\(\alpha\)-cucurbitadienol, a triterpene isolated from the seeds of \textit{Trichosanthes kirilowii} Maxim. 3-epikarounidiol, 7-oxoisomultiflorenol and 3-epibryonolol, isolated from the seeds of \textit{Trichosanthes kirilowii}, also showed marked inhibitory activity against 12-0-tetradecanoyl phorbol-13-acetate (TPA)-induced ear inflammation in mice.\textsuperscript{127} Later Ozaki Yukihiro et al.\textsuperscript{128} suggested that the anti-inflammatory and analgesic effects induced by ethanol extract of the fruit of \textit{Trichosanthes kirilowii} may be due to the seeds contained in it.

The n-hexane extract of \textit{Bidens campylototheca} Schultz Bip. ssp. \textit{campylototheca} (Compositae) and the five polyacetylenes, isolated from the
plant, showed significant in vitro inhibition of cyclooxygenase and 5-lipoxygenase.\textsuperscript{129} \textit{Rubia cordifolia} Linn. (Rubiaceae), an important component of the ayurvedic system of medicine, is used as an anti-inflammatory drug.\textsuperscript{130}

Singh Surjeet et al.\textsuperscript{131} found that the water-soluble fraction of \textit{Paederia foetida} displayed anti-inflammatory activity in carrageenan-, dextran- and histamine-induced edema in rats and mice. Anti-inflammatory activity was also observed in chronic models of adjuvant and formaldehyde arthritis in rats. A single dose of the aqueous suspension of the dried latex of \textit{Calotropis procera} was effective to a significant level against the acute inflammatory response.\textsuperscript{132}

Lapidin, a bicyclic sesquiterpene from \textit{Ferula linkii} Webb., has a dose-dependent anti-inflammatory effect in the carrageenan-induced edema, which at the higher dose (20 mg./kg.), was comparable (65\% inhibition of paw swelling at 3 hours) to that obtained after indomethacin (10 mg./kg.).\textsuperscript{133}

Roots of \textit{Angelica pubescens} were found to have anti-inflammatory activity.\textsuperscript{134} The anti-inflammatory and analgesic constituents from the roots were related to peripheral inhibition of inflammatory substances and to the influence on the central nervous system.

Betulin, betulinic acid and ursolic acid, the triterpenes which were isolated from \textit{Diospyros leucomelas}, showed anti-inflammatory activity in the carrageenan and serotonin paw edema tests and TPA and EPP ear edema tests.\textsuperscript{135}
The evaluation of a commercially available *Solidago gigantea* Herb. extract (urol mono) by Leuschner revealed pronounced anti-inflammatory properties in the rat with respect to a reduction of the carrageenan-induced rat paw edema. A direct comparison with diclofenac sodium revealed that a high dose of the extract of *Solidago gigantea* Herb. possesses the same anti-inflammatory efficacy as diclofenac sodium.

The flavonoid-rich fraction of the leaf of *Baphia nitida* Lodd. (Leguminosae) was found to have the ability to dose-dependently inhibit the inflammatory condition on the rodents. This explains the traditional use of the leaves of this plant as an anti-inflammatory agent. Mengi and Deshpande proved the ocular anti-inflammatory activity of the roots and leaves of *Butea frondosa*. Lee Song Jin et al. found that the n-butanol fraction of *Lonicera japonica*, showed anti-inflammatory activity in mice and rats. Loniceroside A, lonicerin and loganin, isolated from the n-butanol fraction of *Lonicera japonica*, showed anti-inflammatory activity comparable to aspirin. Reanmongkol Wantana et al. demonstrated the inhibitory effect of an alkaloid extract of the stem bark of *Hunteria zeylanica* Gard. on acute edema formation.

A 50% ethanolic extract from a dried whole body of *Agkistrodon blomhoffii* blomhoffii Boie. was found to have a prophylactic effect on hyperlipidemia, preventing the development of atherosclerosis. The ethanol extract of the plant *Hippocratea excelsa* was proved to be effective against both exudative-proliferative and chronic phases of inflammation. Both the infusion and the methanolic extracts of *Psoralea glandulosa*, *Cuscuta chilensis* and *Cestrum parqui* showed anti-inflammatory properties. A
number of plants such as *Calluna vulgaris*, *Corylus avellana*, *Geum urbanum*, *Juniperus communis*, *Polygonum aviculare*, *Potentilla erecta* and *Salix caprea*, used in Swedish traditional medicine to treat inflammatory diseases were tested and proved to be pharmacologically active.\textsuperscript{144}

Carvalho Jose et al.\textsuperscript{145} noted that the carrageenan-induced rat hind paw edema was significantly inhibited by oral administration of 300 mg./kg. of the aqueous extract of the fruits of *Caesalpinia ferrea* Mart. (Leguminosae). The water-extracts of several species of the Chinese drug *scolopendra* from different habitats were observed to inhibit the increased permeability of abdominal blood capillaries and ear inflammation in mice.\textsuperscript{146}

Gene Rosa et al.\textsuperscript{147} reported that the butanolic fraction derived from the aqueous extract from aerial parts of *Baccharis trimera* shows strong anti-inflammatory and analgesic properties which seem to be due, at least partly, to the inhibition of prostaglandin biosynthesis. The active constituents were found to be mainly saponins in which echinocystic acid (or its enantiomer) is the major aglycone, and also rutin.

Yegnanarayan et al.\textsuperscript{148} compared the anti-inflammatory activity of various extracts of *Curcuma longa* Linn. It was found that in carrageenan-induced edema, the anti-inflammatory activity of 40 mg./kg. of water extract was similar to that of 5 mg./kg. indomethacin. In the granuloma pouch method, water extract was the most potent and its activity was similar to that of hydrocortisone. Petroleum ether extract was the most potent, in the cotton pellet test, and its activity was similar to that of indomethacin. Claeson et al.\textsuperscript{149} based on the findings of their experiments, proposed that the
non-phenolic linear 1,7-diarylheptanoids, isolated from a Thai medicinal plant, *Curcuma xanthorrhiza* (Zingiberaceae) represent a novel class of topical anti-inflammatory agents.

Loggia et al.\(^\text{150}\) confirmed that the *Ginkgo biloba* leaf extract and some of its components, (ginkgolides, bilobalide, a biflavonic fraction and some pure biflavones) exhibited an anti-inflammatory action with a potency comparable to that of indomethacin. Agshikar et al.\(^\text{151}\) showed that a methanol extract of *Acanthus ilicifolius* Linn. exhibited statistically significant anti-inflammatory activity in carrageenan-induced edema in rats. Gracza Lajos et al.\(^\text{152}\) isolated rosmarinic acid from *Symphytum officinale* and showed that it is the main constituent of *Symphytum officinale* with anti-inflammatory activity.

The anti-inflammatory action of tea-leaf saponin, a mixture of saponins separated from leaves of *Camellia sinensis* var. *sinensis* was investigated by Sagesaka Yuko et al.\(^\text{153}\). They found that tea-leaf saponin inhibited rat paw edema induced by carrageenan in a dose-dependent manner. Activation of hyaluronidase, one of the enzymes involved in inflammatory reactions, was inhibited by tea-leaf saponin. It was also found that tea-leaf saponin antagonized the action of leukotriene D\(_4\), one of the chemical mediators of inflammatory reactions. Any symptom of toxic reaction was not observed when tea-leaf saponin was administered orally to mice at a dose of 2000 mg./kg.

Lazarova et al.\(^\text{154}\) found that administration of verapamil exerts a preventive anti-atherosclerotic effect only in therapeutic doses (0.25 mg./kg.).
Kasi Viswanadham et al.\textsuperscript{155} have showed that verapamil in minimum therapeutic equivalent dose, failed to show anti-inflammatory activity as did sub-anti-inflammatory dose of aspirin (54 mg./kg.), however when combined with sub-anti-inflammatory dose of aspirin, significant inhibition of carrageenan and cotton pellet-induced inflammation was observed. The anti-inflammatory activity of this combination treatment was almost comparable to that of the anti-inflammatory dose of aspirin (200 mg./kg.).

Rhizoma \textit{Cynanchi stauntonii} was found to exhibit obvious anti-inflammatory effect on croton oil-caused mouse ear swelling on intraperitoneal injection.\textsuperscript{156} Del Carmen Recio et al.\textsuperscript{157} isolated two anti-inflammatory principles quercetin-3-0-xylosyl (1\textendash 2) rhamnoside and quercetin-3-0-rhamnoside from the methanol extract of the leaves of \textit{Erythrospermum monticolum} (Flacourtiaceae) and evaluated their effects on a chronic topical inflammation model. Aethiopinone (I), an o-naphthoquinone diterpene from \textit{Salvia aethiopis} L. roots, has been evaluated for anti-inflammatory activity by Hernandez-Perez Margarita et al.\textsuperscript{158} The compound showed a pharmacological profile similar to other non-steroidal anti-inflammatory substances in reducing the edema induced by carrageenan.

\textit{Ranunculus japonicus} extract was found to inhibit the paw edema of rats induced by carrageenan, ear swelling of mice caused by xylene, mice vascular permeability increase induced by acetic acid, and granuloma formation in rats.\textsuperscript{159} Ansari and Ali\textsuperscript{160} have made studies on the analgesic and anti-inflammatory activity of six tetracyclic triterpenoids, pistacigerrimones A, B, C, D, E and F, isolated from the galls of \textit{Pistacia integerrima} and showed that pistacigerrimones C and D gave highly significant results. Ekpendu et
al.\textsuperscript{161} proved the petroleum ether and methanol extracts of \textit{Mitracarpus scaber} leaves to be favourable in treating inflammatory disorders. The results of investigations of Voitenko et al.\textsuperscript{162} on the analysis of anti-inflammatory effect of spiritual nastoykas which are made of roots and flowers of \textit{Echinacea purpurea} revealed the anti-inflammatory activity of the preparation.

"Ham-Hong-Chho" is a folk medicine in Taiwan, derived from the entire plants of \textit{Bidens pilosa} L. var. \textit{minor} (Blume) Sherff (Compositae), \textit{Bidens pilosa} L. and \textit{Bidens chilensis} DC. The anti-inflammatory effect of aqueous extracts of the three plants against paw edema induced by carrageenan and chronic arthritis induced by complete Freund's adjuvant were determined in rats. The results indicated that paw edema induced by carrageenan was significantly decreased by treatment with aqueous extracts (150 or 300 mg./kg.) of all the three plants and that the effect of \textit{Bidens pilosa} var. \textit{minor} was the most potent. But, only extracts (500 mg./kg.) of \textit{Bidens pilosa} L. var. \textit{minor} and \textit{Bidens pilosa} L. significantly decreased the paw edema induced by complete Freund's adjuvant.\textsuperscript{163}

Kulkarni et al.\textsuperscript{164} demonstrated that an ayurvedic indigenous formulation containing \textit{Withania somnifera}, \textit{Boswellia serrata}, \textit{Curcuma longa} and zinc was very efficient in rheumatoid arthritis.

Pandey et al.\textsuperscript{165} found the oral administration of Vatahari gugul, a composite drug containing \textit{Sapindus trifoliatus}, \textit{Commiphora mukul}, \textit{Emblica officinalis}, \textit{Terminalia chebula} and \textit{Terminalia belerica} and fomentation with \textit{Ricinus communis} leaves to be effective in the treatment of rheumatic diseases. Oral administration of composite herbal preparation,
Chandramarutha Chenduram and local application of Vathakesari Taila was also efficient in patients with rheumatic diseases.\textsuperscript{166} A compound ayurvedic preparation Dhanwantara gutika was favourable in the prevention of inflammatory effects.\textsuperscript{167}

On long term usage, Rumalaya, (a herbal mixture marketed by Himalaya Drug Co.), was found to be effective and useful for decreasing pain and retarding the onset of disability in rheumatoid arthritic cases, with almost no side effects, unlike most other non-steroidal anti-inflammatory agents.\textsuperscript{168} Rasonadi Kvatha, a combination of Rasona, Sunthi and Nirgundi was found to be useful in the treatment of amavata-rheumatoid arthritis.\textsuperscript{169} Triphala, a combination of equal parts of three myrobalans, showed maximum significant acute anti-inflammatory activity of 44.34\% in maximum dose. Significant anti-arthritis activity was also observed.\textsuperscript{170} Tsumura-shuuji-bushi-matsu, a herbal medicine produced from \textit{Aconiti} tuber, was proved to have a good analgesic effect in inflammed conditions.\textsuperscript{171}

Aqueous/ alcoholic extracts of \textit{Populus tremula}, \textit{Solidago virgaurea} and \textit{Fraxinus excelsior}, tested in three different combinations, reduced the paw edema to varying degrees and also dose-dependently inhibited the arthritic paw volume. The anti-inflammatory activity of the combinations was respectively comparable to the tested doses of diclofenac.\textsuperscript{67} Phytodolor N, a combination of the plants, \textit{Populus tremula}, \textit{Solidago virgaurea} and \textit{Fraxinus excelsior}, was proved to have anti-inflammatory and anti-rheumatic properties, often comparable to non-steroidal anti-inflammatory drugs, but with little or no side effects.\textsuperscript{68} Testing the activity of the water-soluble compounds of corresponding dry extracts, the activity of \textit{Fraxinus excelsior}
with an apparent $I_{50}$-value of 0.008% (w/v) by far dominates the inhibitory overall effect of the combination ($I_{50} = 0.014\%$, w/v).\textsuperscript{172} It was also concluded that the beneficial activities of the complete drug may at least in part be due to the reported anti-oxidative functions of the individual components.\textsuperscript{173}

An open study was made on the comparative efficacy of 'R' compound, an indigenous formulation, and aspirin in the treatment of polyarticular rheumatoid arthritis on 104 patients.\textsuperscript{174} Brahmi Rasayan (an ayurvedic medicinal mixture, whose major constituent is Brahmi leaves) suppressed various experimentally induced inflammatory reactions and did not show any gastric irritation in anti-inflammatory doses.\textsuperscript{175} It is suggested that it may partially mediate its anti-inflammatory activity by interfering with the action and/or synthesis of prostaglandins and also perhaps by stabilization of the lysosomal membranes. Its anti-inflammatory activity is comparable to that of indomethacin.

Sandhika, a compound herbal drug, showed significant anti-inflammatory activity at the dose of 0.25 g./kg. body weight. It was also suggested that Sandhika, which is an indigenous drug for inflammation with no detectable adverse effect, might be acting through scavenging the free radicals.\textsuperscript{176} Topical application of Shimotsu-to, a traditional Chinese herbal prescription, (5% in water) was found to suppress an acute edema of rat hind paw induced by 1% carrageenan.\textsuperscript{177}
II.2 Medicinal Principles of the Selected Indigenous Drugs

*Allium cepa* Linn. (Onion) and *Allium sativum* Linn. (Garlic)
Family: Liliaceae

Onion and garlic are two important *Allium* species which have been studied for their therapeutic uses as antibiotic, anti-diabetic, anti-oxidant, anti-atherogenic, anti-cancer, hypocholesterolaemic and fibrinolytic effects. The botanical name *Allium* is derived from the celtic word *all* which means pungent and it betrays the presence of a host of remarkable flavorants and odorants all having in common one element, sulphur.

Of the two *Allium* species, garlic is more effective than onion in its biological action. Onion is more common in human diet than garlic and hence its importance cannot be underestimated. Biological action of *Allium* products are ascribed to organosulphur compounds having allyl (CH$_2$=CH–CH$_2$–) or its isomer propenyl (CH$_3$–CH=CH–) group. Presence of these organosulphur compounds is the characteristic of this genus. These organosulphur compounds react with other systems through their sulphur-sulphur or sulphur-oxygen linkages and alkenyl side chains (unsaturated propenyl or allyl groups), garlic has the more active allyl group (CH$_2$=CH–CH$_2$–), while onion has a less active propenyl (CH$_3$–CH=CH–) or saturated methyl (CH$_3$–) or propyl (C$_3$H$_7$–) group.

Augusti has extensively studied the chemical constituents of onion and garlic and reported as below. Various chemical contents present in onion and garlic are given in Table II.2a.
Table II.2a. Various components of onion (*Allium cepa* Linn.) and garlic (*Allium sativum* Linn.)

<table>
<thead>
<tr>
<th>Contents</th>
<th>Onion</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical contents (g./100 g. wet wt.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>87 - 93</td>
<td>61.3 - 86.3</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.9 - 1.5</td>
<td>2.2 - 6.2</td>
</tr>
<tr>
<td>Fat</td>
<td>0.2 - 0.4</td>
<td>0.2 - 0.3</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>5.2 - 10.5</td>
<td>9.5 - 27.4</td>
</tr>
<tr>
<td>ash</td>
<td>0.7</td>
<td>0.6 - 1.5</td>
</tr>
<tr>
<td>Energy (Kilo cal.) per 100 g. wet weight</td>
<td>23 - 38</td>
<td>39 - 140</td>
</tr>
<tr>
<td><strong>Bulk elements (mg./100 g. wet wt.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>190 - 540</td>
<td>50 - 90</td>
</tr>
<tr>
<td>P</td>
<td>200 - 430</td>
<td>390 - 460</td>
</tr>
<tr>
<td>K</td>
<td>80 - 110</td>
<td>100 - 120</td>
</tr>
<tr>
<td>Na</td>
<td>31 - 50</td>
<td>10 - 22</td>
</tr>
<tr>
<td>Mg</td>
<td>81 - 150</td>
<td>43 - 77</td>
</tr>
<tr>
<td>Al</td>
<td>0.5 - 1</td>
<td>0.5 - 1</td>
</tr>
<tr>
<td>Ba</td>
<td>0.1 - 1</td>
<td>0.2 - 1</td>
</tr>
<tr>
<td>Fe</td>
<td>1.8 - 2.6</td>
<td>2.8 - 3.9</td>
</tr>
<tr>
<td><strong>Sulphur, Chlorine and trace elements (mg./100 g. wet wt.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.8 - 7</td>
<td>0.1 - 0.7</td>
</tr>
<tr>
<td>B</td>
<td>0.6 - 1</td>
<td>0.3 - 0.6</td>
</tr>
<tr>
<td>Cu</td>
<td>0.05 - 0.64</td>
<td>0.02 - 0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>1.5 - 2.8</td>
<td>1.8 - 3.1</td>
</tr>
<tr>
<td>Mn</td>
<td>0.5 - 1</td>
<td>0.2 - 0.6</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;0.5</td>
<td>0.3 - 0.5</td>
</tr>
<tr>
<td>S</td>
<td>51</td>
<td>65</td>
</tr>
<tr>
<td>Cl</td>
<td>36</td>
<td>43</td>
</tr>
<tr>
<td><strong>Vitamins (mg./100 g. wet wt.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.3</td>
<td>0.25</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.1</td>
<td>Traces</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>Folic acid</td>
<td>16.0</td>
<td>-</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>Retinol</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>(μg./100g. wet wt.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(green onion)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In addition, onion also contains small amount of vanadium (680-750 μg./100 g.) and carotene (630-890 μg./100 g.). All types of free amino acids (8.2-10 mg./100 g.) are also present in onion. Arginine and glutamic acid are in abundance. Fatty acids, palmitic, oleic and linoleic acids, account for 3/4th of the fats in onion. Ratio of total unsaturated/saturated fatty acids is 1:9. Sulphur containing compounds are important in Allium spp., but the concentrations of L-cysteine, L-cystine and L-methionine are relatively low, indicative of their rapid metabolism. A range of other involatile sulphur compounds are also found in onion and garlic (Table II.2b).

Steam distillation of onion bulbs and garlic cloves gives essential oil known as onion oil (0.005%) and garlic oil (0.1-0.2%) respectively.

Onion oil: It has acid taste and unpleasant odour. Gas chromatography of the oil revealed the presence of monosulphides \( (R_1-S-R_2) \), disulphides \( (R_1-S-S-R_2) \), trisulphides \( (R_1-S-S-S-R_2) \), tetrasulphides \( (R_1-(S)_4-R_2) \) and thiols \( (RSH) \). Alkyl or alkenyl di- and trisulphides are primarily responsible for the flavour of cooked onion.

Garlic oil: It contains diallyl disulphide (60%), allyl propyl disulphide (6%) and various polysulphides and monosulphides. On vacuum distillation, allicin, i.e., diallyl disulphide oxide, \( (C_2H_5-S(S(O)C_3H_5) \) is obtained. Enzyme alliinase acts on S-allyl cysteine sulfoxide (SACS) and produces allicin, pyruvic acid and NH₃.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Onion</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-cystine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-methionine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-methyl L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-propyl L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(2-propenyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(carboxyethyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(carboxypropyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>trans S-(1-propenyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(carboxyisopropyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(carboxymethyl) L-cysteine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-methionine sulphoxide</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-(2-propenyl) cysteine sulphoxide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S-methyl cysteine sulphoxide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S-propyl cysteine sulphoxide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S-allyl cysteine sulphoxide</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+ ) - present; and (-) - not present
Allicin type compounds $R_1$-$SS(O)$-$R_2$ are the immediate reaction products formed in crushed onions and garlic and on storage rearrange into $R_1$-$SS$-$R_2$ and $R_1$-$SS(O_2)$-$R_2$. The former then change into different polysulphides ($R_1$-(S)n-$R_2$). On heating they may form cyclic disulphide compounds (dithiins) also.

Distinctive flavours of various *Allium* species reflect varying amounts of alkenyl cysteine sulfoxides ($R$-$S$-(O)-$R_2$-$CH$-$CH(NH_2)$-$COOH$). In onions, S-propenyl cysteine sulfoxide ($CH_3$-$CH$ $=$ $CH-S$-(O)-$CH_2$-$CH(NH_2)$-$COOH$) is the precursor of lachrimatory principle, $CH_3$-$CH$ $=$ $CH$-$SHO$, according to the Nobel laureate Arturi Virtanen.\(^{179}\) The enzyme for its production is alliinase. In garlic, S-allyl cysteine sulfoxide ($CH_2$-$=CH$-$CH_2$-$S$-$CH_2$-$CH(NH_2)$-$COOH$) is the precursor for the antibiotic principle, allicin. In onion allicins, $R$ = CH$_3$ or C$_3$H$_7$.

S-allyl cysteine sulfoxide, originally called alliin, was the first S-alkenyl cysteine sulfoxide to be isolated by Stoll and Seebeck\(^{180}\) during their work (1947-48) on antibiotic action of garlic. Remaining alkyl and alkenyl sulfoxides, S-methyl and S-propyl cysteine sulfoxide from onion and garlic and S-propenyl cysteine sulfoxide from onion, were isolated by
Virtanen and Matikkala\textsuperscript{181} in Finland. By the action of alliinase on S-propenyl cysteine sulphoxide no allicin type compound is formed, but only the lachrimatory factor, propene sulphenic acid, \( \text{CH}_3\text{-CH} = \text{CH-S(O)H} \), is formed. An alternative structure, \( \text{CH}_3\text{-CH}_2\text{-CH}=\text{S}=\text{O} \) is also suggested for the lachrimatory factor.\textsuperscript{179}

Allicin has been synthesised and purified by liquid chromatography and identified by IR and mass spectrometry by Jansen et al.\textsuperscript{182} Allicin, an active ingredient released from garlic is a systemic vasodilator that acts by an unknown mechanism. It has been shown that the pulmonary vasodilator responses to allicin are independent of the synthesis of endothelial-derived relaxing factor or the activation of soluble guanylate cyclase.\textsuperscript{183}

Allicin of garlic is known as Russian penicillin as it is active against many bacteria that are resistant to antibiotics.\textsuperscript{199} Lilianna\textsuperscript{184} reported that bacteria resistant to 8 major antibiotics showed a high response towards garlic extract which contains allicin as the main active principle. Mathew and Augusti\textsuperscript{185} in 1973 reported its anti-diabetic action. The anti-diabetic effects of ethereal oil of onion and garlic was studied by Brahmachari and Augusti\textsuperscript{186,187} and later by Jain.\textsuperscript{188} A compound called, ajoene (C\textsubscript{9}H\textsubscript{14}S\textsubscript{3}O), a potent anti-thrombotic agent was isolated from garlic by Apitz-Castro et al.\textsuperscript{189} Ajoene is apparently unstable and may rearrange to polysulphides, \( \text{R}_1\text{-}(\text{S})_n\text{-R}_2\), \([n=2 \text{ or more}].\)

\[
3\text{C}_3\text{H}_5\text{-S(O)-S-C}_3\text{H}_5 \longrightarrow 2\text{C}_3\text{H}_5\text{S(O)CH}_2\text{CH} = \text{CH}
\]

\[
\begin{array}{c}
\text{Allicin} \\
\text{CH}_2 = \text{CH-CH}_2\text{-S} \\
\text{Ajoene}
\end{array}
\]
In garlic, neutral lipids predominate over phospholipids and glycolipids. A wide range of γ-glutamyl derivatives of amino acids, predominantly those containing sulphur or their derivatives have also been reported in onion and garlic (Table II.2c). All essential amino acids in required amounts or even more than that are present in garlic proteins. Particularly the sulphur containing amino acids, viz. cysteine and methionine and certain other amino acids like arginine in high quantities may have a role in the anti-atherogenic effects of garlic proteins. Histidine, lysine and cysteine are present in higher proportions in garlic protein than in the proteins of common cereals. There is a link between garlic and the L-arginine – nitric oxide pathway. Amino acid analysis of garlic powder demonstrates that it is a rich source of arginine, the precursor for nitric oxide. However, neither arginine nor alliin-derived products were found to be responsible for the activation of nitric oxide synthase by garlic in cell-free homogenates. Cysteine and methionine were detected in the roots of garlic. The garlic bulbs were found to be rich in glutamine, tyrosine and cysteine. Concentrations of γ-glutamyl derivatives of L-methionine, S-methyl-L-Cysteine and trans-S(1-propenyl)L-cysteine sulphonide have been reported to be present in Allium to the tune of 5-13; 5-19 and 130-200 mg./100 g. fresh weight respectively.

Anthocyanins from red onion and garlic, pectins from onion skin and garlic, quercetin from yellow outer skin of dry onion and garlic bulbs, kaempferol glycosides from garlic and onion bulbs have been reported.
Table II.2c. List of γ-glutamyl derivatives of amino acids present in onion and garlic

<table>
<thead>
<tr>
<th>Onion</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-L-glutamyl-S-(propenyl)L-cysteine sulfoxide</td>
<td>γ-L-glutamyl-S-methyl-L-cysteine sulfoxide</td>
</tr>
<tr>
<td>γ-L-glutamyl-S-(2-carboxypropyl) L-cysteine</td>
<td>γ-glutamyl-S-allyl-L-cysteine</td>
</tr>
<tr>
<td>Glutathione: glutathione-cysteine disulphide</td>
<td>γ-glutamyl-S-propyl-L-cysteine</td>
</tr>
<tr>
<td>Glutathione-γ-glutamyl-cysteine-disulphide</td>
<td>γ-L-glutamyl-S-allyl-mercapto-L-cysteine</td>
</tr>
<tr>
<td>S-sulphoglutathione γ-glutamyl-L-methionine</td>
<td>γ-L-glutamyl-S-methyl-L-cysteine</td>
</tr>
<tr>
<td>Valine, isoleucine, leucine, phenyl alanine, tyrosine, S-methyl cysteine</td>
<td>γ-L-glutamyl-S-(2-carboxy propyl) cysteinyglycine</td>
</tr>
<tr>
<td>γ-L-glutamyl-S-methyl-L-cysteine</td>
<td></td>
</tr>
<tr>
<td>γ-L-glutamyl-S-(2-carboxy propyl) cysteinyglycine</td>
<td></td>
</tr>
<tr>
<td>γ-L-glutamyl - S-methyl - L-cysteine sulfoxide</td>
<td></td>
</tr>
</tbody>
</table>

Pectins, flavonoids, sulfoxides etc. are good hypolipidemic agents. Flavonoids and sulfoxides also act as anti-oxidants. The active principles involved in the anti-oxidant activity were considered to be S-alkenyl cysteine sulfoxides\(^{197}\)(alliins) and quercetin and its flavone aglycone analogues.\(^{198}\)

Two new steroid glycosides: alliospiroside A and alliofuroside A have been isolated from the generative organs of onion.\(^{200}\) Four flavonol glycosides obtained from the wet scales of the deep purple onion were identified using a
combination of TLC, PC, UV-visible and \textsuperscript{1}HNMR analyses as quercetin-3'-glucoside, 4'-glucoside, 3,4'-diglucoside and isorhamnetin-3-glucoside. The occurrence of the isorhamnetin derivative is important due to its contribution to the anti-microbial property of onion.\textsuperscript{201}

Small amount of protocatechuic acid, phloroglucinol and its carboxylic acid and their methyl esters and pyrocatechol in outer skin and free sterols and their esters and ferulic acid in fleshy scales of onion have been reported. Garlic contains cholesterol, campesterol, \(\beta\)-sitosterol and stigmasterol.\textsuperscript{61}

Tannins isolated from the skins of red onions also exhibit anti-oxidant properties. The presence of prostaglandin A, or a compound possessing similar blood pressure reducing and chemical properties was isolated by Attrep et al.\textsuperscript{202} Garlic contains a group of structurally related biologically active thioglycosides, scordinins, which have been used as tonic in the oriental countries.\textsuperscript{61}

Eric Block\textsuperscript{203} has studied the organosulphur chemistry of the genus, \textit{Allium} and reviewed their physiological functions also. Benjamin Lau\textsuperscript{204} and K. C. Goyal\textsuperscript{190} have made extensive studies on the constituents of garlic.

According to the traditional medical literature,\textsuperscript{205} onion is a good source of vitamins and is useful in cattarrh, chronic bronchitis, fever and dropsy. In colic and scurvy it is used by mixing with common salt. Mixed with mustard oil in equal proportion, it is applied locally to treat rheumatic pain and inflammation. Fresh onion extract is bactericidal. Onion is used in malarial fever. Onion oil contains a heart stimulant. It increases pulse volume and the frequency of systolic pressure and coronary flow and, stimulates the intestinal smooth musculature and uterus. It promotes bile production and reduces
blood sugar.\textsuperscript{206} Onion extracts when given orally to diabetic dogs or when injected into rabbits\textsuperscript{207} show an action similar to that of insulin. In addition to hypoglycemic principle, a hyperglycemic substance (possibly thiols) is also present.\textsuperscript{61}

In ancient Indian medical literature (Sushrut Samhita), garlic has been documented as a curative agent for numerous diseases, viz. hemorrhoids, rheumatism, dermatitis, abdominal pain, cough, leprosy, loss of appetite, loss of weight, parasitic infestations and pyrexia. Garlic oil is used as a powerful antiseptic and vermifuge.\textsuperscript{199} Garlic clove was known as a home remedy in the east due to its prophylactic and curative properties.\textsuperscript{205} Garlic has been used as an anti-diabetic in Norway and Middle Europe\textsuperscript{208} Garlic is considered as a medicine to treat ailments of heart in Ayurvedic therapy.\textsuperscript{209}

Hyperlipidemia is a high risk factor in cardiovascular diseases\textsuperscript{210,211} and it is found that the incidence of cardiovascular disease is low in countries where garlic is widely used, the hypolipidemic action of garlic is pertinent in this context. Garlic and onion have a hypolipidemic action.\textsuperscript{203,212,213,221,222} Navneet Kumar Gupta\textsuperscript{223} reported the reduction of lipid synthesis in the liver of mice exposed to \textsuperscript{45}Ca internal irradiation by administration of garlic oils. Cholesterol lowering action of the garlic extract in patients was reported by Augusti\textsuperscript{214} for the first time. S-allyl cysteine sulphoxide, isolated from garlic is active in controlling hypercholesterolemia.\textsuperscript{215} \textit{Allium sativum} was found to be effective in cardiovascular diseases.\textsuperscript{216,217} Garlic dialysate has a depressant effect on automaticity and tension development in the heart, suggesting a beta- adrenoceptor blocking action produced by the garlic dialysate.\textsuperscript{218} The anti-oxidant and hypolipidemic effects of garlic oil, onion oil and alliins were reported.\textsuperscript{215,219,220} Popov et al.\textsuperscript{224} found the aqueous extract obtained from
1 mg. of the garlic preparation to be anti-oxidatively as effective as 30 nmol of ascorbic acid and/or 3.6 nmol of α-tocopherol. It was also found that the protection against lipid oxidation provided by garlic extract was concentration dependent, as was the protection provided by many synthetic anti-oxidants.²²⁵,²²⁶ Biju et al.²²² showed that both garlic oil and garlic proteins exhibit hypolipidemic effect. Even though the oil was found to be more effective, the garlic protein is more palatable and free from obnoxious smell. *Allium* products can counteract harmful effects of alcohol.²²⁷ Free radicals present in cigarette smoke could be reduced by garlic.²²₈ The obnoxious oil of garlic and a palatable protein of this vegetable were found to be anti-atherogenic agents.⁵⁹ The high percentage of cysteine in garlic protein and the reactive disulphide group in the oil may be responsible for their beneficial effects. Effects of garlic extract on platelets were studied by Yongqtan et al.⁵⁶ in experimental atherosclerosis of rabbits. Enhanced platelet activities were found to be inhibited by garlic extract and they concluded that garlic extract had a favourable effect in the prevention of atherosclerosis. The retardative effect of garlic on the development of atherosclerosis was also reported by Mirhadi et al.⁵⁷ Later the direct anti-atherogenic-related action of garlic powder was studied by Orekhov Alexander et al.⁵⁸ using cell culture and they suggested that garlic powder manifests direct anti-atherogenic-related action not only in vitro but also in vivo.

Block²⁰³ demonstrated the anti-asthmatic effects of onion extracts. Allicins and ceapaenes, the most active components exert a series of pharmacological effects such as inhibition of cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, inhibition of histamine release and leukotriene biosynthesis, prevention of bronchial
asthma in guinea pigs after inhalation of allergens and platelet activating factor and inhibition of inflammatory cell influx.

Allicins and cepaenenes were shown to possess anti-inflammatory properties.60 This action, in part, is related to inhibition of inflammatory cell influx by allicins and cepaenenes.

S-allyl cysteine sulphoxide present in garlic and S-methyl cysteine sulphoxide present in onion are active hypocholesteremic agents.178,212 It was reported by Sheela and Augusti215 that S-allyl cysteine sulphoxide, isolated from garlic, is active in controlling hypercholesteremia. Garlic oil, onion oil and alliins have hypolipidemic actions.215,219,220 Mechanism of their action as suggested by Augusti61 includes inactivation of thiol enzymes that promote lipid/cholesterol synthesis by organosulphur compounds of Allium. Secondly these compounds can reduce the levels of NADPH in tissues so that adequate amounts of NADPH not be available for fat and cholesterol synthesis.229,233

Organosulphur compounds are weak insulinogenic substances and they inactivate the thiol group which may destroy insulin.185 Garlic and onion products were found to be weak hypoglycemic and insulinogenic agents but they can effectively retard bad effects of diabetes, viz. loss of weight, glycosuria, derangements in the metabolism of carbohydrates, lipids and proteins.186-188,215,219,220,227,233-241 D. Farva et al.220 showed that daily administration of garlic oil to the streptozotocin-diabetic rats for 2 months very significantly decreased the raised levels of glucose, cholesterol and triglycerides in serum and liver, and total lipids and proteins in liver.

Platelet-aggregation-inhibitory effect of garlic and onion extracts has been attributed to adenosine, allicin, alliins, ajoene, polysulphides and
In view of in vivo findings of Lawson et al.\(^{242}\) and observations of Cavallito and Bailey\(^{243}\) and Wills\(^{244}\) that allicin reacts immediately with SH group of free or enzyme bound cysteine, forming S-(allylthio) cysteine, it is unlikely that allicin itself is found in blood after consumption of garlic. Indeed S-(allylthio) cysteine may be responsible for much of the biological activities of garlic within the body.\(^{242}\) Components of freshly cut garlic and onions were found to inhibit platelet aggregation through inhibition of cyclooxygenase and related enzymes.\(^{245}\) Block\(^{203}\) reported that allicin inhibit human platelet aggregation in vitro without affecting cyclooxygenase or thromboxane synthase activity or cyclic AMP levels, possibly by influencing calcium movement. Ajoene was found to inhibit platelet aggregation.\(^{246}\) Ajoene was also found to alter the conformation of a hemoprotein implicated in platelet activation. Ajoene can also inhibit adhesive interactions of human neutrophils and consequently affect in vivo superoxide anion formation. This gives an illustration of anti-oxidant property of garlic and onion products.\(^{203}\) Makheja et al.\(^{247,248}\) showed that garlic and onion oil fractions containing allicin or polysulphides significantly inhibited platelet aggregation induced by ADP, arachidonic acid or collagen. Anti-aggregatory activity of these oily fractions is due to their blockage of thromboxane and prostaglandin biosynthesis through inhibition of fatty acid oxygenases. Methylallyl trisulphide was shown to have more potent activity than other sulphides.

Anti-microbial effect of allicin on both gram negative and gram positive bacteria is considerable even at a 1:100,000 dilution.\(^{61}\) The anti-microbial property of garlic was demonstrated by Louis Pasteur in 1858.\(^{199}\) The anti-bacterial effect of aqueous garlic extract was investigated against *Helicobacter pylori* by Cellini Luigina et al.\(^{230}\) The concentration of aqueous
garlic extract required to inhibit the bacterial growth was between 2-5 mg/ml. The anti-bacterial effect of onion was also reported.249

In view of the culinary importance of alliaceous plants as well as the unique history of their use in folk medicine, cancer preventive effect of Allium plants were discussed.203 Unsaturated polysulphides in Allium species inhibit tumour promotion250,251 perhaps by enhancing glutathione-dependent detoxification enzymes. It is strange that steam distilled onion oil can also function as a weak tumour promoter as well as antipromoter in 7, 12 dimethyl benz (a) anthracene-initiated mouse skin cancer.203 Injection of tumour cells treated with garlic extract induced tumour immunity in mice. Hirao et al.252 showed that the observed anti-tumour effects cannot be solely due to the presence of low molecular weight compounds but instead even protein-like high molecular weight compounds have anti-tumour properties.

The use of garlic in treating hypertension was reported.253 The mechanism of action of the garlic principles was reported to be due to their prostaglandin like effects by Rashid and Khan.254

Fibrinolytic property of onions was first reported by Gupta et al.255 and confirmed later by Menon et al.256 in people with fat-induced clotting of blood. Augusti et al.257 ascribed the fibrinolytic action of onion to its sulphur rich oil and an organosulphur compound cycloalliin. Reduced blood fibrinolytic activity is a defect that promotes thrombus formation in atherosclerotic vessels. Garlic was found to be able to increase clotting time and fibrinolytic activity.258 Augusti et al.257 suggested that these principles may weaken blood clots by opening S-S- cross-links between fibrin molecules through a disulphide exchange reaction and therefore render them more susceptible to natural fibrinolysis. Another suggested mechanism is that they may stimulate the
release of plasminogen activator from its site of production in the walls of veins elsewhere.

Protection from radiation, free radical production, and stress conditions are some other reported effects of garlic products. All these reports highlight the therapeutic values of onions and garlic. An anti-allergic substance was isolated from garlic by HPLC and was identified as (Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide [(Z)-ajoene]. by IR, FAB-MS and NMR. It was also found that the anti-allergic activity of (Z)-ajoene might be due to its inhibition of chemical mediator release. Garlic juice enhanced blood calcium level. The calcium content of bone ash was also increased whereas the phosphorus content was decreased. Garlic is used as a diuretic as well as a remedy for skin rashes, parasitic infestation, birth marks and snake and dog bites. The intravenous administration of chromatographically purified fractions of garlic (2, 4 and 6 μg./kg. dry weight) to anaesthetized rabbits elicits dose-dependent diuretic-natriuretic responses which reach a maximum 60 minutes after injection, and return to basal levels after 90 minutes. Garlic polysulphides are toxic to the larvae of mosquito and other insects. Garlic is also used in arthritis, heavy metal poisoning, constipation and athlete's foot. Zakhary Nadia reported the protective effect of garlic against schistosomal infestation. Lim-Sylianco et al. reported the anti-mutagenic effects of onion and garlic.

Excessive intake of Allium species may interfere with hemoglobin production and may lead to lysis of red blood cells. Prolonged feeding of high levels of raw garlic to rats has resulted in anaemia, weight loss and failure to grow. Uncontrolled use of onion may also lead to such effects. Abdominal hemorrhage and death of laboratory animals fed on excessive quantity of
garlic has been reported. Some people have an allergic response (e.g. dermatitis or asthma) to fresh garlic or onions. The negative as well as positive effects of *Allium* may be due to the action of their principles on thiol group systems. Excessive consumption of these principles may retard many enzymes and thereby retard protein synthesis. Therefore, only customary amounts of these spices may be used as part of diet, salads and pickles.

**Ocimum sanctum** Linn. (Malayalam: Tulasi) Family: Labiatae

*Ocimum sanctum* Linn. has been claimed to be valuable against a wide variety of diseases. The plant is used in the treatment of a number of ailments like dyspepsia, bronchitis, hepatic afflications, chronic fever and hemorrhage. Anti-hepatotoxic, hypoglycemic, radio protective, anti-ulcerogenic, anti-stress, anti-histaminic and anti-inflammatory activities of *Ocimum sanctum* Linn. have been reported. All parts of the plant have been reported to be pharmacologically active.

Skaltsa et al. have made a phytochemical study of the leaves of *Ocimum sanctum* Linn. Ursolic acid was isolated from the ether extract of the plant leaves. Campesterol, cholesterol, stigmasterol, beta-sitosterol, beta-carotene and methyl esters of myristic, palmitic, stearic, oleic, linoleic and linolenic acids were identified in the petrol extract.

Tulasi leaves on steam distillation give 0.7% essential oil. GLC analysis of the oil revealed the presence of 13 compounds of which eugenol (71.3%), carvaerol (3.2%), methyl eugenol (20.4%) and caryophyllene (1.7%) are the predominating constituents. The oil possesses anti-bacterial and insecticidal properties. It inhibits in vitro growth of *Mycobacterium*


tuberculosis and *Micrococcus pyogenes* var. aureus: in anti-tubercular activity. It has one-tenth of the potency of streptomycin and one-fourth that of isoniazid. The volatile oil extracted from the fresh leaves has been reported to have anti-asthmatic and anti-inflammatory activities.

Surender Singh et al. extracted the seeds of *Ocimum sanctum* Linn. with petroleum ether to yield a pale yellow coloured oil (fixed oil) and identified the unsaponifiable matter as β-sitosterol. They determined the physicochemical characteristics of the oil and the results are presented in the Table II.2d. The results of the GLC analysis of the fixed oil by Surender Singh et al. are presented in the Table II.2e. The data revealed the presence of mixture of five fatty acids. The fixed oil also has been reported to have anti-asthmatic and anti-inflammatory activities.

Table II.2d. Characteristics of fixed oil of *Ocimum sanctum* Linn.

<table>
<thead>
<tr>
<th>Yield</th>
<th>17.50% v/w with reference to dried seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>pale yellow</td>
</tr>
<tr>
<td>Consistency</td>
<td>viscous liquid</td>
</tr>
<tr>
<td>Density (at 25°C)</td>
<td>0.8750 g./cc.</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.472</td>
</tr>
<tr>
<td>Acid value</td>
<td>2.067</td>
</tr>
<tr>
<td>Iodine value</td>
<td>179.38</td>
</tr>
<tr>
<td>Saponification value</td>
<td>178.50</td>
</tr>
<tr>
<td>Ester value</td>
<td>176.43</td>
</tr>
<tr>
<td>Unsaponifiable matter</td>
<td>2.818%</td>
</tr>
</tbody>
</table>
Table 11.2e. Relative percentage composition of fatty acids in fixed oil of *Ocimum sanctum* Linn.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>11.69</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>3.19</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>13.82</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>52.23</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>16.63</td>
</tr>
</tbody>
</table>

Effect of *Ocimum sanctum* Linn. leaf extract was studied by Chattopadhyay et al.\textsuperscript{261} on paracetamol-induced hepatic damage in rats. The extract was found to protect the rats from hepatotoxic action of paracetamol as evidenced by significant reduction in the elevated serum enzyme levels.

Hypoglycemic effect of *Ocimum sanctum* Linn. leaf extract in normal and streptozotocin diabetic rats was studied by Chattopadhyay.\textsuperscript{262} Oral administration of alcoholic extract of leaves of *Ocimum sanctum* Linn. led to a marked lowering of blood sugar level in normal, glucose fed hyperglycemic and streptozotocin-induced diabetic rats. Further the extract potentiated the action of exogenous insulin in normal rats. The activity of the extract was 91.55% and 70.43% of that of tolbutamide in normal and diabetic rats respectively.

Dhanesh Kumar Jain\textsuperscript{260} reported that the essential oil (volatile oil extracted from the leaves of *Ocimum sanctum* Linn.) possesses anti-bacterial and insecticidal properties. Thaker and Anjara\textsuperscript{273} showed that the chloroform extract of *Ocimum sanctum* Linn. showed significant inhibition of pathogenic microorganisms and effective healing of infected wounds.
Administration of the plant extract showed variable but effective anti-stress activity by improving levels of SDH (succinate dehydrogenase). Effects of restraint stress (RS) and its modulation by *Ocimum sanctum* Linn. were evaluated by Sen *et al.* using some biochemical and biophysical parameters in rats. RS-induced elevations in blood glucose and urea levels were unaffected by pretreatment with *Ocimum sanctum* Linn. However, *Ocimum sanctum* Linn. lowered RS-induced cholesterol levels and the drug effectively lowered the RS-induced elevations in lactate dehydrogenase (LDH) and alkaline phosphatase. RS also induced (a) increased membrane protein clusterization, (b) increased membrane fluidity and (c) reduced membrane thickness in RBC membrane, whereas, the effects on the synaptosomal membrane were less marked. The RS-induced changes in RBC membrane dynamics were attenuated/reversed by *Ocimum sanctum* Linn. These biochemical and membrane changes during RS and their modulation by the adaptogens are discussed in light of the possible mechanisms of action of these agents, during such aversive stimuli.

The effect of *Ocimum sanctum* Linn. (Tulasi) on the humoral immune responses in experimental animals was studied by Mediratta *et al.* *Ocimum sanctum* Linn. enhanced anti-SRBC haemagglutination titre and IgE antibody titre, as measured by passive cutaneous anaphylaxis in experimental albino rats. Antigen-induced histamine release from peritoneal mast cells of sensitised rats in vitro was significantly inhibited by *Ocimum sanctum* Linn., which also antagonized responses to various spasmogens on isolated guinea pig ileum. *Ocimum sanctum* Linn. has been reported to modulate the humoral immune responses by acting at various levels in the immune mechanism such as antibody production, release of mediators of hyper sensitivity reactions and tissue responses to these mediators in the target organs.
Uma Devi and Ganasoundan have demonstrated the radioprotective properties of the extracts of leaves of *Ocimum sanctum* Linn. The effect is more pronounced for the water extract than the aqueous alcohol extract. There is an optimum dose for protection with the water extract, which is 50 mg./kg., above which the radioprotective effect decreases.

*Ocimum sanctum* Linn. extract has anti-ulcerogenic activity against experimental ulcers, and it is due to its ability to reduce acid secretion and increase mucous secretion.

50% ethanolic extract of fresh leaves, volatile oil (extracted from the fresh leaves of *Ocimum sanctum* Linn.) and fixed oil (extracted from the seeds of *Ocimum sanctum* Linn.) significantly protected guinea pigs against histamine and acetylcholine induced pre-convulsive dyspnoea.

50% ethanolic extract of fresh leaves, volatile oil and fixed oil inhibited the hind paw edema in rats against inflammation induced by carrageenan, serotonin, histamine and PGE-2.

Anti-inflammatory and analgesic action of the fixed oil were reported by Surender Singh et al. also. They demonstrated that the analgesic and anti-inflammatory activities are due to the triglyceride fraction of the fatty acids of the oil. *Ocimum sanctum* Linn. fixed oil contains 16.63% linolenic acid and hence the same could contribute to dual inhibition of arachidonate metabolism resulting in anti-inflammatory activity. The oil has also been found to inhibit ulceration induced by aspirin, indomethacin and alcohol, which could be due to leukotriene antagonistic activity. Similarly anti-ulcer activity against ulceration induced by histamine, reserpine and stress and anti-secretory activity of the oil could be attributed to its histamine antagonistic
and anti-cholinergic effects. Thus dual inhibition of cyclooxygenase and lipoxygenase by *Ocimum sanctum* Linn. fixed oil could account for the anti-inflammatory activity, and analgesic activity appears to be due to cyclooxygenase inhibitory, histamine antagonistic and anti-cholinergic effects, as the same has been reported to inhibit writhing response. It was thus suggested by Surender Singh et al. that anti-inflammatory and analgesic activity of *Ocimum sanctum* Linn. fixed oil are due to its unsaturated fatty acids in general and linolenic acid in particular.

The effect of feeding Tulasi leaves along with the normal diet, on the reproductive behaviour of adult male Wistar rats, was studied by Kantak and Gogate. There was a significant decrease in sexual behaviour score, when the dose of leaf extract was increased to 200 mg./kg. and 400 mg./kg.

*Ocimum sanctum* plant extract has been investigated by Prakash et al. to assess its anti-fertility activity on female albino rats. 50% ethanolic extract of leaves of *Ocimum sanctum* Linn. has depicted 37.5% anti-implantation activity when administered at a dose of 200 mg./kg. body wt.

*Phyllanthus niruri* Linn. (Malayalam: Kizhikanelli)
Family: Euphorbiaceae

*Phyllanthus niruri* Linn. is a herb which has been known for its varied therapeutic uses. *Phyllanthus niruri* Linn., commonly known for its usefulness in the treatment of jaundice, is also credited to be diuretic and useful to diabetics in the traditional literature. Also, compounds isolated from *Phyllanthus* species have been reported to have angiotensin converting enzyme (ACE) inhibitory activity and hypoglycemic activity in experimental animals.
Adventitious root, hairy root, shoot and crown gall \textit{(Agrobacterium)} cultures of \textit{Phyllanthus niruri} Linn. were established and the production of several phenolic compounds was analysed by Ishimaru Kanji et al.\textsuperscript{280} From hairy roots cultured in 1/2 Murashige - Skoog liquid medium, six phenolic compounds; gallic acid, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin 3-o-gallate and (-)-epigallocatechin 3-o-gallate were isolated. The production of these six phenols and the related compound (+)-catechin in tissue cultures were determined by HPLC. Roots of the mother plant cultivated under hydroponic conditions contained almost the same phenolic compounds as those in root cultures. On the other hand, leaves and stems of the cultivated plant contained hydrolysable tannins such as geraniin, corilagin and galloylglucose which were similarly observed in the plant collected in Peru.

The leaves of \textit{Phyllanthus niruri} Linn. were found to contain a bitter substance Phyllanthin.\textsuperscript{370} Dry leaves yield bitter principles hypophyllanthin (0.05\%) and phyllanthin (0.35\%); toxic to fish and frog.\textsuperscript{428}

In addition to the known lignans, nirtetralin, niranthin, hypophyllanthin, phyltetralin, phyllanthin, lintetralin, a new compound designated as phylester together with \(\beta\)-sitosterol, dotriacontanoic acid and a rare sterol, 24-isopropylcholesterol have been isolated\textsuperscript{281} from the hexane extract of the aerial parts of \textit{Phyllanthus niruri} Linn. The structure of the phylester has been elucidated as phthalic acid bis-2,5-dimethylhexyl ester on the basis of spectral and chemical evidences. Chemical studies on this plant have so far resulted in the isolation of lignans, flavonoids, triterpenoids and alkaloids.
Fractionation of an extract of *Phyllanthus niruri* Linn., based on its ability to inhibit [\(^{125}\text{I}\)]-ET-1 binding to A 10 cells (rat thoracic aortic smooth muscle cells), led to the isolation of three non-peptidic endothelin-1 (ET-1) antagonists, which have been identified as the lignans phyllanthin [1], hypophyllanthin [2] and nirtetralin [3]. These isolates were also found to inhibit [\(^{125}\text{I}\)]-ET-1 binding to the recombinant human ET\(_A\) receptor expressed in Chinese hamster ovary cells (CHO-ET\(_A\)), but were inactive against the recombinant ET\(_B\) receptor. The most potent compound was [2] with an IC\(_{50}\) value of 40\(\mu\)M. By means of a microphysiometer, [2] was found to attenuate ET-1-induced acceleration in the rate of acid extrusion from CHO-ET\(_A\) consistent with ET-1 antagonistic activity. Screening of synthetic 1-phenyl-tetrahydronaphthalene-derived analogues revealed that 5,8-dimethoxy-6-methyl-4-phenyl-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (BL-4170, 4) also inhibited [\(^{125}\text{I}\)]-ET-1 binding.

The analgesic effects of the hydroalcoholic extracts of *Phyllanthus urinaria*, *Phyllanthus tenellus*, *Phyllanthus niruri* and *Phyllanthus sellowianus* have been investigated in several models of nociception in mice by Santos et al. They confirmed that the hydroalcoholic extracts of the above plants exhibit potent and long-lasting anti-nociceptive activity in several models of pain, including the neurogenic analgesic component of the formalin test. The mechanism underlying their analgesic profile is presently unknown.

Agarwal et al. made studies on the use of two species of *Phyllanthus*. *Phyllanthus emblica* (fruit aqueous extract) and *Phyllanthus niruri* (leaf aqueous extract), in antagonizing the clastogenic effects induced by nickel chloride in mouse bone marrow cells. Both the crude aqueous extracts could effectively decrease the percentage of damaged cells as well as the frequency
of breaks per cell induced by three doses of the metal salt (10, 20 and 40 mg./kg. body weight). The protection afforded by the plant extracts is possibly due to the combined effect of the natural constituents of the plant rather than to any single component. The data emphasizes the importance of plant dietary supplements in alleviating metal cytotoxicity.

The plants belonging to Euphorbiaceae family were found to be commonly used in hepatoprotective formulations. A number of species of the genus *Phyllanthus* (Euphorbiaceae) have been tested for their efficacy as antivirals. Although the herbaceous species of subgenus *Phyllanthus* have been extensively used to treat jaundice, and have generally inhibited hepadnavirus DNAp, effects on chronic infection with hepatitis B virus (HBV) or related viruses have generally been negative. Reddy et al. showed that the ethanolic extract of *Phyllanthus niruri* Linn., exhibited statistically significant anti-hepatotoxic activity in terms of reduction in serum AST, serum ALT and serum bilirubin contents of CCl4-damaged group of albino rats. Agrawal et al. made a screening of *Phyllanthus niruri* Linn. on alcohol-induced liver cell damage in non-hepatectomized and partially hepatectomized rats. They found that alcoholic extract of roots and leaves of *Phyllanthus niruri* Linn. showed hepatoprotective effect in experimental rats. The root extract was more effective than the leaf extract. Jayaram et al. evaluated the safety of *Phyllanthus niruri* Linn. as an anti-hepatitis-B virus substance in in vitro and in vivo studies using mice as the model and vero cell-line as the tissue culture system and the study significantly proved the safety of the herb.

Kumar et al. reported that the alcoholic extract of leaves of *Phyllanthus niruri* Linn. at an oral dose of 250 mg./kg. produced a significant
decrease in the blood glucose level of alloxan-induced diabetic rabbits. The results were compared to that of an oral hypoglycemic agent, metformin (120 mg./kg.).

*Ricinus communis* Linn. (Malayalam: Avanakku)  
Family: Euphorbiaceae

*Ricinus communis* Linn. is a medicinal shrub, extensively used throughout the world.

D'Silva et al.\(^\text{293}\) isolated ribosome - inactivating protein or toxin as well as agglutinin from callus and cell suspension cultures that were established from seed explants of *Ricinus communis* Linn. The toxin, viz. ricin as well as the agglutinin viz. *Ricinus communis* agglutinin were synthesized in these cultures and were secreted into the medium. The lectins (toxin and agglutinin) were synthesized through several passages of cultured cells at levels which make them attractive as an alternative source of lectins. Biosynthesis of these carbohydrate-binding proteins was regulated by specific exogenous hormones and was positively correlated with the growth of the cultures indicating that toxin and agglutinin may have a role to play during cell division. The toxin and agglutinin were secreted into the medium providing a clue that they probably serve as defence molecules. Secretion of lectins into the medium facilitated easy isolation of the lectins. The synthesized lectins were biologically active and were found to be comparable with lectins from seeds, in terms of their electrophoretic actions.

O'Hare Mary et al.\(^\text{294}\) found that recombinant *Ricinus communis* agglutinin A chain produced in *Escherichia coli* had ribosomal RNA N-glycosidase activity and was approximately 10-fold less active than ricin A chain in a cell-free protein synthesis inhibition assay.
Wu June H et al. constructed and summarized the carbohydrate specificity of *Ricinus communis* agglutinin, in decreasing order by lectin determinants as II (Gal β 1→4 GlcNAc) > I (Gal β1→3GlcNAc) > E(Gal α 1→4 Gal) and B (Gal α1→3 Gal) > T (Gal β1→3 Gal NAc), while Tn (Gal NAc α1→Ser/Thr) is a poor inhibitor. (II, human blood group type II precursor sequence and T, Thomsen-Friedenreich sequence).

Solis Dolores et al. studied the binding of O-methyl and fluorodeoxy derivatives of methyl β-lactoside to the *Ricinus communis* toxin (RCA 60) and agglutinin (RCA 120) in order to determine the donor/acceptor relationships of the hydrogen bonds between the hydroxyl groups of methyl β-lactoside and the binding sites of the lectins. Analysis of the results indicates that both the C-3' and C-4' hydroxyl groups act as hydrogen bond donors to charged groups of both RCA 60 and RCA 120. The C-6' and probably also the C-2' hydroxyl groups participate both as donors and as acceptors of two hydrogen bonds with neutral groups of the lectins. And finally, the C-6 hydroxyl group possibly acts as a donor of a weak hydrogen bond to a neutral group in RCA 60, but not in RCA 120. The results provide a molecular basis to explain some features of the binding specificity of the lectins. Comparison of RCA 60 binding data with the recently refined X-ray crystal structure of the RCA 60-lactose complex shows similarities but also some discrepancies that can be attributed to the marked influence of the pH on the carbohydrate-lectin interaction.

Lord J. Michael et al. reported the structure, mode of action and some current applications of ricin. Ricin is an abundant protein component of *Ricinus communis* Linn. seeds (castor beans) that is exquisitely toxic to
mammalian cells. It consists of an enzymic polypeptide that catalyzes the N-glycosidic cleavage of a specific adenine residue from 28 S ribosomal RNA, joined by a single disulphide bond to a galactose (cell)-binding lectin. The enzymatic activity renders ribosomes containing depurinated 28 S RNA incapable of protein synthesis. The bipartite molecular structure of ricin allows it to bind to the mammalian cell surface, enter via endocytic uptake, and deliver the catalytically active polypeptide into the cell cytosol where it irreversibly inhibits protein synthesis causing cell death. Because of its cytotoxic potency, modified ricin is being used for the selective killing of unwanted cells and for the toxigenic ablation of cell lineages in transgenic organisms.

Jung-Yaw Lin and Su-Ying Liu. have made studies on the affinity gel electrophoresis of lectins purified from the seeds of *Ricinus communis* Linn. The mobilities of lectins on the gel showed various degree of retardation with their affinity toward their macromolecular ligand-agarose. *Ricinus* agglutinin which possessed the strongest affinity was retarded strongly, ricin C, and ricin B moderately, and ricin A, weakly. The concentrations of free moving ligand-galactose to reduce the retardation were also correlated very well with the potencies of affinities of lectins toward the ligand. The Kd values calculated from the retardations were 4.32 μM, 18.32 μM, 28.13 μM and 52.88 μM for *Ricinus* agglutinin, ricin C, ricin B and ricin A respectively. Affinity gel electrophoresis was found to be a simple and quick method for studying the interaction of lectins with their ligands.

Dua S and Amma M. K. P. have separated 2 acid phosphatases from 96h germinated castor bean seedlings by differential ammonium sulphate precipitation and purified by gel filtration chromatography. Both phosphatases
are active at around pH 4.8 to 5.2. Their $K_m$ values vary with the kind of
substrate used. They are inhibited by fluoride, arsenate and phosphate ions
and activated by $Fe^{2+}$ and $Mn^{2+}$ ions. Sulphhydryl groups are not required
for the activity of acid phosphatase I where as these are essential for acid
phosphatase II activity. Enzyme I was associated with haemagglutinin activity
where as enzyme II showed transferase activity.

Fox Brian et al.\textsuperscript{300} have made studies on the stearoyl-acylcarrier protein
$\Delta^9$ desaturase from \textit{Ricinus communis} Linn. A gene encoding stearoyl-acyl
carrier protein $\Delta^1$ desaturase (EC 1.14. 99.6) from castor was expressed in
\textit{Escherichia coli}. The purified catalytically active enzyme contained four
atoms of iron per homodimer. The desaturase was studied in two oxidation
states with Moessbauer spectroscopy in applied fields upto 6.0T. These
studies show conclusively that the oxidised enzyme contains two (identical)
clusters consisting of a pair of antiferromagnetically coupled ($J$>60 cm$^{-1}$,
$H = JS_1S_2$) $Fe^{3+}$ sites. The diferric cluster exhibited absorption bands from
300 to 355 nm; addition of azide elicited a charge transfer band at 450 nm.
In the presence of dithionite, the clusters were reduced to the diferric state.
Addition of stearoyl-CoA and $O_2$ returned the clusters to the diferric state. The
properties are consistent with assigning the desaturase to the class of
$O_2$-activating proteins containing diiron-oxo clusters, most notably
ribonucleotide reductase and methane monooxygenase hydroxylase.
Comparison of the primary structures for these three catalytically diverse
proteins revealed a conserved pair of the amino acid sequence-(AspGlu)-Glu-
Xaa-Arg-His-separated by about 100 amino acids. Since each of these
proteins can catalyze $O_2$-dependent cleavage of inactivated C-H bonds, it is
concluded that these amino acid sequences represent a biological motif used
for the creation of reactive catalytic intermediates. Thus, eukaryotic fatty acid
desaturation may proceed via enzymatic generation of a high-valent iron-oxo species derived from the diiron cluster.

Kula Jozef and Halina Sadowska\textsuperscript{301} manufactured (E)-2-nonenal from commercial castor oil by ozonolysis in acetic acid, followed by exposure of the resulting intermediate product to p-toluene sulphonic acid.

Visen et al.\textsuperscript{302} evaluated \textit{Ricinus communis} Linn. leaf extract for hepatoprotective, choleretic and anti-cholestatic activity. In a preliminary test with albino rats, an ethanol extract showed significant protection against galactosamine-induced hepatic damage. It also showed dose-dependent choleretic and anti-cholestatic activity, and hepatoprotective activity, as judged by the effects on hepatocytes isolated from paracetamol-treated rats. On fractionation of the ethanol extract, maximum activity was localised in the butanol fraction. Subsequent chromatographic fractionation and testing in the galactosamine model led to the isolation of two active fractions which in turn yielded two pure compounds: ricinine and N-dimethyl-ricinine. N-dimethyl-ricinine was found to be more active and it reversed the biochemical changes produced by galactosamine at a dose of 6 mg./kg. x 7 days.

Shukla et al.\textsuperscript{303} have demonstrated the dose-dependent choleretic, anti-cholestatic and hepatoprotective activity in rat observed with N-dimethyl-ricinine isolated from the leaves of \textit{Ricinus communis} Linn. The anti-cholestatic and hepatoprotective activity was seen against paracetamol-induced hepatic damage. The choleretic and anti-cholestatic activities were evidenced by an increase in the volume of bile and its contents. The hepatoprotective effect was evaluated by an increase in the per cent viability of hepatocytes (ex vivo) and by the reversal of altered enzymatic levels [AST (aspartate aminotransferase), ALT (alanine aminotransferase)] and alkaline
phosphatase] towards normal. The compound showed more potent activity than silymarin, a known hepatoprotective agent.

An alcoholic extract of *Ricinus communis* Linn. was not found to exhibit any hepatoprotective activity against alcohol-induced liver cell damage.\(^{288}\)

Banerjee et al.\(^ {52}\) found that petroleum ether extract of *Ricinus communis* Linn. exhibited significant anti-inflammatory activity against formaldehyde and adjuvant-induced rat’s paw arthritis. They also reported that *Ricinus communis* Linn. (150 mg./kg.) exhibited no significant analgesic activity. *Ricinus communis* Linn. was safe up to a dose of 1 g./kg. body weight in rats.

**Alstonia scholaris** Linn. (Malayalam: Pala) Family: Apocynaceae

Atta-ur Rahman et al.\(^ {305}\) have isolated the alkaloid 19,20-Z-vallesamine along with 19,20-E-vallesamine from the leaves of *Alstonia scholaris* Linn., a medicinal plant, and the stereochemistry has been deduced from NOE measurements. Again Atta-ur Rahman and Alvi\(^ {306}\) have made the isolation and identification of an indole alkaloid, alstonamine (C\(_{20}\)H\(_{22}\)N\(_2\)O\(_3\), M.P. 126 degree) and a sitisirikine type indole alkaloid, rhazimanine from the leaves of the plant. It also contains echitamine, echitenine, ditamine and echitamidine.\(^ {369}\)

Krishnaswamy and Purushothaman\(^ {307}\) have made studies on the anti-cancer activity of echitamine chloride, isolated from *Alstonia scholaris* Linn. Echitamine chloride, when given subcutaneously at 7.5 mg. and 5 mg./kg. body weight showed 80 and 53 per cent regression of tumour in Wistar strain of rats. Its ED\(_{50}\) was found to be 2.2 mg./kg. body weight. It was
also active against P 388 lymphocytic leukaemia at a dose of 16 mg./kg. body weight.

**Vitex negundo** Linn. (Malayalam: Karinchi) **Family: Verbenaceae**

Reddy and Radhakrishnaiah have made studies on the chemical systematics of Vitex. The quantified data on the chemosystematics of five species of Vitex indicate that they are closely related, and constitute a single cluster. On the basis of distribution of iridoids and ellagic acid, *Vitex pinnata* is recognized as a relatively primitive taxon.

Fruits of *Vitex negundo* Linn. contain an acid resin, as astringent organic acid, malic acid, traces of an alkaloid and a colouring matter. The seeds of *Vitex negundo* Linn. afforded a new lignan characterized as 6-hydroxy-4(4-hydroxy-3-methoxyphenyl) 3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde by spectroscopic methods.

The leaves of *Vitex negundo* Linn. contain a colourless essential oil of the odour of the drug, and a resin. An alkaloid nishindine was also isolated from *Vitex negundo* Linn. The structure of nishindaside, an iridoid from the leaves of *Vitex negundo* Linn., has been revised by Iwagawa Tetsuo et al.

*Vitex negundo* Linn. leaves are very efficacious in dispelling inflammatory swellings of the joints from acute rheumatism. Root is used in dyspepsia, colic, rheumatism, worms, boils and leprosy.

Ravishankar et al. have made studies on the pharmacology of *Vitex negundo* Linn. root. Petroleum ether and n-butanol extracts of roots of *Vitex negundo* Linn. were found to produce moderate CNS depression in
experimental albino mice. Butanol and ethanol extracts produced marked anti-parkinsonian effect. Chloroform extract markedly suppressed carrageenan pedal edema in rats. Petroleum ether extract protected mice against pentylenetetrazol convulsions. None of the extracts showed anti-psychotic and anti-depressant effect.

The ethanolic extract of *Vitex negundo* Linn. was studied by Nair and Saraf\textsuperscript{458} for its effect on the sensitised and non-sensitised guinea pig trachea using antigen and compound 48/80 respectively to examine its influence on mediator release and tracheal smooth muscle contraction. Histamine has been shown to contribute only to the initial phase of tracheal contraction while the release of lipoygenase derived arachidonic acid metabolites have been reported to contribute to the later sustained contraction. The ethanolic extract of *Vitex negundo* Linn. was found to significantly inhibit both the initial phase of contraction and the later sustained contracture of the trachea. The studies reveal that the ethanolic extract of *Vitex negundo* Linn. inhibits the release of histamine and products of arachidonic acid metabolism.

*Strobilanthes heyneanus* Linn. (*Malayalam*: Karimkurinji)

*Family: Acanthaceae*

Nair et al.\textsuperscript{35} have made a biochemical study on the anti-inflammatory effect of leaves of *Strobilanthes heyneanus* Linn. In preliminary studies, 90% ethanol extract of leaves was found to possess marked anti-inflammatory effect. A study of its effect on biochemical parameters involved in the inflammatory process showed that 90% ethanol extract of leaves significantly decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of serum and liver in a dose-responsive manner. It also decreased acid phosphatase activity of serum and liver. There is a marked increase in the
adrenal ascorbic acid content. It affected the total WBC count and liver protein levels.

Nair et al.\textsuperscript{36} later evaluated the pharmacological activities of extracts of stem of \textit{Strobilanthes heymeanus} Linn. using the solvents-petroleum ether (60-80°C), chloroform, 90% ethanol and water. The extract in 90% ethanol and in water exhibited marked aspirin-type analgesic, anti-inflammatory and immuno suppressant activities. Petroleum ether (60-80°C) extract showed anti-inflammatory, weak anti-convulsant activities and antagonized spasm induced in rat uterus using prostaglandin F\textsubscript{2α} and 5-hydroxy tryptamine. Chloroform extract possesses weak anti-convulsant activity and antagonized oxytocin-induced spasm in rat uterus.

\textit{Sida rhombifolia} Linn. (Malayalam: Anakurumthotti) and \textit{Sida retusa} Linn. (Malayalam: Kurumthotti) Family: Malvaceae

Sida contains an alkaloid, a fatty oil, phytosterol, mucin, resin, resin acids and potassium nitrate. The leaves and stem and root yield 0.085 per cent and the seed about 0.3 per cent alkaloid. The main portion of the alkaloid is ephedrine.\textsuperscript{360}

Root is considered as valuable in rheumatism.\textsuperscript{361} Mukerji\textsuperscript{360} reported the medicinal uses of the plant as: the bark of the root with sesame oil and milk is very efficacious in curing cases of facial paralysis and sciatica when caused by the inflammation of the nerves concerned. The leaves mixed with rice are given to alleviate the bloody flux.

Nadkami\textsuperscript{362} noted the uses of \textit{Sida retusa} Linn. as follows: roots of \textit{Sida retusa} are held in great repute in the treatment of rheumatism. Stems abound in mucilage and are employed as demulcents and emollients both for
external and internal use. *Sida* is used in calculus troubles and as a febrifuge with pepper. The mucilage is also used by chemists in oxidising mercury and also in treating scorpion-sting.

**Cocos nucifera** Linn. (Coconut) **Family: Palmae**

*Cocos nucifera* (coconut) tree grows throughout the world, especially in the lateral part of tropical belt. Nadkami\(^3\) reported the constituents and actions of *Cocos nucifera* Linn. as follows: enzymes-invertin, oxidase and catalase. Fresh kernel contains nitrogenous substances, fat, lignin, ash, palm sugar (glucose and sucrose) and inorganic substances. The milk in the coconut contains sugar (mannitol), gum, albumin, tartaric acid and mineral water. Ashes of the leaves contain a good deal of potash. Coconut oil contains free caprylic acid in addition to glycerides of lauric, myristic, palmitic and stearic acids. The analysis of the dried flesh of the nut yielded: moisture-2.60 to 6.95% and oil-60.0 to 71.0%.

Coconut milk is refrigerant, nutrient, aperient, diuretic and anthelmintic.\(^3\) Analysis of coconut water shows the presence of the vitamins of the B group.\(^3\) According to Nadkami,\(^3\) coconut water is cooling, refrigerant, demulcent and in large doses aperient. Water of unripe fruit is useful in thirst, fever and urinary disorders. Milk or water of the green fruit is a cooling refrigerant drink, useful in urinary disorders. It allays vomiting in bilious fevers. Root of coconut is used in uterine diseases. Ashes of the leaves are used in medicine. Aswal et al.\(^3\) reported the anti-bacterial activity of root of *Cocos nucifera* Linn.
**Hydrocotyle asiatica** Linn. (Malayalam: Kudakan) Family: Umbelliferae

Nadkami\(^\text{366}\) gave the constituents and actions of *Hydrocotyle asiatica* Linn. An oleaginous white crystalline substance vellarin is the active principle of the leaves, resin and some fatty aromatic body, gum, sugar, tannin: albuminous matter, salts—mostly alkaline sulphates. Vellarin has the odour and bitter persistent taste of the fresh plant; it is soluble in ethanol, ether, caustic ammonia and partially in hydrochloric acid. Leaves are dried in the shade so that no active principle is lost, powdered and kept in well stoppered bottles.

Indian physicians use this as an internal and external remedy in various skin diseases, ulcerations, eczema, psoriasis, leprosy, malaria and fevers, epilepsy, insanity, syphilis, amenorrhoea, enlargement of glands in abscess and in chronic rheumatism.

Chowdhury et al.\(^\text{367}\) reported that the alcohol extract, glycosidal fraction and individual glycosides of *Hydrocotyle asiatica* Linn. exhibited anti-spasmodic activity in rat ileum. It was also noticed that it did not show any anti-bacterial, hepatotoxic or nephrotoxic activities.

**Curcuma longa** Linn. (Malayalam: Manjal) Family: Zingiberaceae

The results of studies on *Curcuma* species showed that "yujin", recorded before the end of Ming dynasty was the tuber of *Curcuma longa*.\(^\text{313}\) Dhawan\(^\text{314}\) called turmeric as a gold mine.

Chromatographic separation and quantitative analysis of curcumin and related compounds were described by Tonnesen Hanne Hjorth and Jan Karlsen.\(^\text{315}\) The poor reproducibility of the quantitative analyses was due to the interaction between the 1,3-dicarbonyl group of the molecules and the
solid chromatographic support. Later a sensitive and rapid spectrofluorometric method for determination of microamounts of curcumin in curcumin spices and related flavours has been developed by Jasim Fadhil and Fatima Ali.\textsuperscript{316}

Verghese\textsuperscript{317} has isolated curcumin from \textit{Curcuma longa} Linn. rhizome. Curcumin, \textit{bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione}, is an yellow-orange dye. Zachariah and Nirmal Babu\textsuperscript{318} showed that curcumin and oleoresin levels were not affected by storage of fresh turmeric rhizomes. Dihydrocurcumin was isolated from the rhizomes of \textit{Curcuma longa} Linn. as a minor content of curcuminoids.\textsuperscript{319} A new curcuminoid, cyclocurcumin (IV), was isolated by Kiuchi Fumiyuki et al.\textsuperscript{320} from the nematocidally active fraction of turmeric, the rhizome of \textit{Curcuma longa} Linn., together with three known curcuminoids, curcumin (I), demethoxy curcumin (II) and bisdemethoxy curcumin (III). The structure of IV was elucidated on the basis of spectral data and confirmed by the partial synthesis from curcumin (I). Although the above curcuminoids were inactive when they were applied independently, the nematocidal activity increased remarkably when they were mixed, suggesting a synergistic action between them.

Curcumin is a modern drug.\textsuperscript{321} Curcumin, contained in the rhizome of the plant \textit{Curcuma longa} Linn., is a naturally occurring phytochemical that has been used widely in India and Indonesia for the treatment of inflammation. Soudamini et al.\textsuperscript{322} showed that the oral administration of curcumin significantly lowered the increased lipid peroxidation, in the rat tissues like liver, lung, kidney and brain, produced by carbon tetrachloride, paraquat and cyclophosphamide. Administration of curcumin was also found to lower significantly the serum and tissue cholesterol levels in rats, indicating
that the use of curcumin helps in conditions associated with peroxide induced injury such as liver damage and arterial diseases.

Nishigaki Ikuo et al.\textsuperscript{323} reported that administration of curcumin suppressed the increase in the peroxide level in the liver induced by carbon tetrachloride and in activities of serum aminotransferases (AST and ALT) in a manner dependent on the concentration of curcumin. Curcumin was also found to significantly inhibit lipid peroxidation induced by Cobalt-60 irradiation in rat liver and serum.\textsuperscript{323,325}

Sreejayan and Rao\textsuperscript{326} showed that curcumin inhibited lipid peroxidation induced by ferric ions, ferrous ions and ferric-ADP chelate (in the presence of ascorbic acid or NADPH) in rat brain homogenate and liver microsomes. This may represent one of the mechanisms through which curcumin exhibits anti-inflammatory and anti-cancer activities. Curcumin is known for its property of scavenging superoxide radical and hydroxyl radical. It has been shown to inhibit lipid peroxide formation in vitro.\textsuperscript{327}

Sreejayan and Rao\textsuperscript{328} compared three natural curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin), and acetylcurcumin for their ability to scavenge superoxide radicals and to interact with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radicals. The results showed that curcumin is the most potent scavenger of superoxide radicals followed by demethoxycurcumin and bisdemethoxycurcumin. Acetylcurcumin was inactive. Interaction with DPPH showed a similar activity profile. The study showed that the phenolic group is essential for the free radical scavenging activity and presence of methoxy group further increases the activity.
Srivastava et al.\textsuperscript{329} reported that curcumin inhibits platelet aggregation and alters eicosanoid metabolism in human blood platelets. Curcumin has an inhibitory effect on vascular smooth muscle cell proliferation.\textsuperscript{330} Cytotoxic and tumour reducing properties of curcumin were reported by Soudamini and Kuttan.\textsuperscript{331}

The pleiotropic cytokine tumour necrosis factor-\(\alpha\) (TNF) induces the production of interleukin-1\(\beta\) (IL-1), and, together, they play significant roles in many acute and chronic inflammatory diseases. They have been implicated in the pathogenesis of intracellular parasitic infections, atherosclerosis, AIDS and autoimmune disorders. Chan Marion Man-Ying\textsuperscript{332} showed that, in vitro, curcumin, at 5\(\mu\)M, inhibited lipopolysaccharide-induced production of tumour necrosis factor-\(\alpha\) and interleukin-1\(\beta\) by a human monocytic, macrophage cell line, Mono Mac 6. It was also demonstrated that curcumin, at the corresponding concentration, inhibited lipopolysaccharide-induced activation of nuclear factor kappa B and reduced the biological activity of tumour necrosis factor-\(\alpha\) in L 929 fibroblast lytic assay.

Curcumin has demonstrated phototoxicity to several species of bacteria,\textsuperscript{333,334} and also to mammalian cells,\textsuperscript{335} under aerobic conditions. The spectral and photochemical properties of curcumin vary with environment\textsuperscript{335,336} and solvent.\textsuperscript{336} The phototoxicity makes curcumin a potential photosensitizing drug which may find application in phototherapy.

Hussain and Chandrasekhar\textsuperscript{337} showed that curcumin reduced the incidence of cholesterol gall-stones, induced by feeding a lithogenic diet. Biliary cholesterol concentration was also significantly reduced by curcumin feeding.
Nagabhushan et al.\textsuperscript{338} reported the anti-mutagenicity of curcumin against environmental mutagens. With \textit{Salmonella typhimurium} strain TA 98, in the presence of S-9 mix of sodium azide, curcumin inhibited the mutagenicity of bidi and cigarette smoke condensates, tobacco and masher extracts, benzo [a] pyrene and dimethyl benzo [a] anthracene in a dose-dependent manner. Observations indicate that curcumin may alter the metabolic activation and detoxification of mutagens.

Sinha et al.\textsuperscript{339} demonstrated that curcumin might possess anti-5-HT action on gastric tissue. Curcumin in doses of 5, 10, 15 and 20 mg./kg. showed progressively increasing degree of protection against 5-HT-induced lesion.

Ukonan A and Ukonan C, two phagocytosis-activating polysaccharides, isolated from the rhizome of \textit{Curcuma longa} Linn., were analysed.\textsuperscript{340,341} The reticuloendothelial system-potentiating, anti-complementary and alkaline phosphatase-inducing activities of ukonan A and ukonan C and their degradation products were investigated\textsuperscript{340,341} and found as follows. The core structural features of ukonan A include a backbone chain mainly composed of \(\beta\)-1,3-linked D-galactose, \(\beta\)-1,4-linked D-xylose and \(\alpha\)-1,2-linked L-rhamnose residues. All of the galactose units in the backbone carry side chains composed of \(\alpha\)-L-arabin-\(\beta\)-D-galactosyl or \(\beta\)-D-galactosyl residues at position 6. The structural features of the arabinogalactan core of ukonan C include a backbone chain composed of \(\beta\)-1,3-linked D-galactose and \(\beta\)-1,4-linked D-xylose. All of the galactose units in the backbone carry side chains composed of \(\beta\)-1,6-linked D-galactosyl residues with or without terminal \(\alpha\)-L-arabinose units at position 3. Both ukonan A and ukonan C have remarkable effects on immunological activities. Periodate oxidation
caused the decrease in or disappearance of these activities. But the controlled Smith degradation product having the core structure of polysaccharide showed a pronounced effect on anti-complementary activity.

Gonda Ryoko et al.\textsuperscript{342} have made studies on the characterization of a neutral polysaccharide ukonan D. Ukonan D was isolated from the rhizome of *Curcuma longa* Linn. It produced a single band on electrophoresis and a single peak on gel chromatography, and its molecular mass was estimated to be 28000. It showed remarkable reticuloendothelia system-potentiating activity in a carbon clearance test. Ukonan D is composed of L-arabinose: D-galactose : D-glucose: D-mannose in the molar ratio of 1:1:12:0.2, in addition to small amounts of peptides moiety. Methylation analysis, carbon-13 nuclear magnetic resonance and enzymatic degradation studies indicated that its structural features include mainly both $\alpha$-1,5-linked L-arabino-$\beta$-3, 6-branched D-galactan type and $\alpha$-4,6-branched D-glucan type structural units. The influence of degradation with $\alpha$-amylase followed by the elimination of glucan side chains on its immunological activity was also discussed.

Nigam and Ahmed\textsuperscript{343} showed that the essential oil of *Curcuma longa* Linn. was obtained in a yield of 1.3%. The gas chromatographic examination of its essential oil revealed ar-turmerone to be its major constituent (59.687%), occurring along with numerous other trace elements. Nirmala Menon and Narayanan\textsuperscript{344} prepared nine fragrance chemicals from ar-turmerone.

Golding Bernard and Esteban Pombo-Villar\textsuperscript{345} have made studies on the structures of $\alpha$- and $\beta$-turmerone. Procedures were described for isolating turmerones from rhizomes of *Curcuma longa* Linn. and for chromatographically separating $\alpha$-from $\beta$-turmerone. Using a combination
of spectroscopic techniques these compounds were shown to be (R,6S)-2-methyl-6-(4-methylcyclohexa-2,4-dienyl) hept-2-en-4-one 2 (\(\alpha\)-turmerone) and (R, 6S)-2-methyl-6-(4-methylene cyclohex-2-enyl) hept-2-en-4-one 3 (\(\beta\)-turmerone). Syntheses of optically pure turmeronol A and ar-turmerone were achieved in a simple manner starting from ethyl (R)-3-hydroxybutanoate (4) of 100\% c.c.\(^{346}\)

The effect of turmeric oil and curcumin, isolated from *Curcuma longa* Linn., on fifteen isolates of dermatophytes, four isolates of pathogenic molds and six isolates of yeasts were studied by Apisariyakul Amphawan et al.\(^{347}\) The inhibitory activity of turmeric oil was tested in Trichophyton-induced dermatophytosis in guinea pigs. The results showed that all fifteen isolates of dermatophytes could be inhibited by turmeric oil at dilutions of 1:40-1:320. None of the isolates of dermatophytes were inhibited by curcumin. The other four isolates of pathogenic fungi were inhibited by turmeric oil at dilutions of 1:40-1:80 but none were inhibited by curcumin. All six isolates of yeasts tested proved to be insensitive to both turmeric oil and curcumin.

Dietary turmeric\(^{348}\) and its content curcumin\(^{322-326}\) lowers lipid peroxidation. Reddy et al.\(^{348}\) showed that dietary turmeric lowers lipid peroxidation by enhancing the activities of anti-oxidant enzymes. Srivastava et al.\(^{329}\) have reported that turmeric extracts inhibit platelet aggregation and modulate eicosanoid biosynthesis. Due to their eicosanoid-modulating property, it was suggested that the spices may serve to provide clues to drugs directed to arachidonic acid pathway enzymes as pharmacological targets. Curcumin, inhibited platelet aggregation induced by arachidonate, adrenaline and collagen. This compound inhibited thromboxane \(B_2\) production from exogenous \(^{14}C\) arachidonate in washed platelets with a concomitant increase
in the formation of 12-lipoxygenase products. Moreover, curcumin inhibited the incorporation of $[^{14}C]$ arachidonic acid into platelet phospholipids and inhibited the deacylation of arachidonic acid-labelled phospholipids (liberation of free arachidonic acid) on stimulation with calcium ionophore A 23187. The anti-inflammatory property of curcumin may, in part, be explained by its effects on eicosanoid biosynthesis.

According to Huang Huei-Chen et al.\textsuperscript{330} curcumin dose-dependently inhibited the proliferation of rabbit vascular smooth muscle cells stimulated by fetal calf serum. Curcumin had a greater inhibitory effect on platelet-derived growth factor-stimulated proliferation than on serum-stimulated proliferation. Cinnamic acid, coumaric acid and ferulic acid were much less effective than curcumin as inhibitors of serum-induced smooth muscle cell proliferation, suggesting that the cinnamic acid and ferulic acid moieties alone are not sufficient for activity and that the characteristics of the diferuloylmethane (curcumin) molecule itself are necessary for activity. Curcumin may be useful as a new template for the development of better remedies for the prevention of the pathological changes of atherosclerosis and restenosis.

The genotoxic effects of turmeric in mice was evaluated by Jain et al.\textsuperscript{349} Single acute dose treatment (500 mg./kg. body wt.) could not significantly induce micronucleated polychromatic erythrocytes but caused considerably higher chromosomal aberrations (6.22%).

Soudamini and Kuttan\textsuperscript{350} demonstrated that life-span of mice treated with a chronic lethal dose of cyclophosphamide was considerably increased by the simultaneous administration of turmeric extract or curcumin (40.8% and 91.7% respectively). Increased alanine aminotransferase (ALT), alkaline
phosphatase and thiobarbituric acid-reacting material in the liver were also reduced by turmeric and curcumin.

*Curcuma longa* Linn. is used by Ayurvedic practitioners for its anti-bacterial activity. Studies were made by Arun Prasad Basu\(^{351}\) to determine the anti-bacterial activity of *Curcuma longa* Linn. Anti-bacterial activity was compared with that of penicillin on Gram-positive and that of streptomycin on Gram-negative organisms. It was found that activity against Gram-positive and Gram-negative organisms was to a much lesser extent in comparison with penicillin and streptomycin. A pilot study of the use and efficacy of *Curcuma longa* Linn. in scabies was made by V. Charles and S. X. Charles.\(^{352}\) Efficacy of roots of *Curcuma longa* Linn. (turmeric) in the form of ointment was evaluated by Kumar Anil et al.\(^{353}\) in the treatment of full thickness open skin wounds created on either side of the vertebral column in the thoraco-lumbar region in twelve buffalo calves at different intervals till 30 days. Histological observations showed fibroblastic and angioblastic proliferation on the 7th day and formation of thin wavy collagenous fibres on the 15th day. The complete healing was observed on the 30th post-wounding day.

A study was carried out by Ratanabanang Koon Kavi et al.\(^{354}\) on the mode of action and some properties of a cobra neurotoxin inhibitor found in the extract of *Curcuma* Sp. (Zingiberaceae). When the principal post synaptic neurotoxin of the Thai cobra (*Najanaa* Siamensis) was mixed with an aqueous extract of *Curcuma* Sp. rhizome, the principal post synaptic neurotoxin was inactivated as tested in mice or in vitro using a rat hemidiaphragm preparation. The neurotoxin inhibitor was found only in the water insoluble fraction of the rhizome extract.
Chandra and Sadique\textsuperscript{355} showed that a preparation consisting of *Phyllanthus fraternus*, *Eclipta alba* and curcumin of *Curcuma longa* (25:15:10) was able to protect liver from injury caused by carbon tetrachloride injection. Patel et al.\textsuperscript{356} showed the hepatoprotective activity of *Curcuma longa* Linn.

Bhatnagar Upendra\textsuperscript{357} prepared the aqueous, petroleum ether and 95% ethanol extracts of *Curcuma longa* Linn. and tested in pregnant albino rats for its anti-fertility effect. The aqueous and ethanol extracts exhibited encouraging activity. The best anti-fertility effect was found at a dose of 100 mg./kg. body weight of aqueous extract which was 90%. Other extracts also exhibited encouraging effect with 60-70% resorptive loss.

An incubation temperature coupled with 60% relative humidity was found to be conducive environment for maximum spoilage of *Curcuma longa* rhizome tissues infected with fungus. Higher and lower levels of temperatures and relative humidities had a retarding effect on the disease intensity.\textsuperscript{359}

\textbf{11.3. Biochemistry and Pharmacology of Natural Salts}

Common salt has been known since ancient times to be an essential ingredient of food for live-stock and man. It contains both sodium (Na) and chlorine (Cl), and is the main source of both the elements. The combination of sodium and chlorine as salt is widely distributed in nature where it occurs not only in the sea (2.68%) and other saline waters, but also in dry deposits as rock salt.

The value of common salt for man and other animals was probably recognized before recorded history.\textsuperscript{371} A strong craving for salt is exhibited by grazing animals. The value of supplementary salt for cattle was first
demonstrated by Boussing in 1847. Babcock (1905) observed that after two or three weeks without salt, lactating cows exhibited an abnormal appetite for it, but up to an year elapsed before any health effect was noted. Smith and Aines (1959) repeated and expanded this study and identified sodium rather than chlorine as the element primarily concerned. Osborne and Mendel (1918) showed that the rat required sodium, and Orent-Keils and co-workers in 1937 differentiated between sodium and chlorine deficiency in these species.

The animal body contains about 0.2% sodium. Some of this amount is located in the skeleton in an insoluble form but the larger proportion is found in the extracellular fluids where it undergoes a very active metabolism. Sodium is readily absorbed, mainly from the upper small intestine. The transport of sodium across intestinal epithelium appears to be dependent on a system of pumps and passive leaks located in cell membranes. Absorption of sodium also occurs from the rumen. In animals, approximately 80% of the sodium entering the gastrointestinal tract arises from internal secretions such as saliva, gastric fluids, bile and pancreatic juice.

Sodium in addition to potassium, maintains osmotic pressure and regulates acid-base metabolism. Sodium and chlorine are crucial to the maintenance of normal fluid volume and osmotic pressure relationships. Sodium has a major role in the transmission of nerve impulses and in maintaining proper muscle and heart contractions. The passage of nutrients into the cells and the excretion of waste products are controlled by the help of sodium and chlorine. Sodium ions must be present in the lumen of small intestine for absorption of sugars and amino acids. Insufficient sodium lowers the utilization of digested protein. Absorption of the several of the water
soluble vitamins (riboflavin, thiamine and ascorbic acid) may be sodium coupled. Water absorption in the intestine may also be closely linked to sodium ion transport.

The adult human body contains about 100 g. sodium. An average, about 4-5 g. sodium are ingested per day in the ordinary diet. If there is a deficiency of sodium in the diet, the urinary excretion falls to a very low level.

Sodium is excreted mainly in the urine as salt, with smaller amounts lost in feces and perspiration. Loss of salt through perspiration is a major excretion route for some species. Sodium may also be lost via secretion in milk. Excessive losses of sodium may occur from vomiting, diarrhoea or profuse sweating. Body concentrations of sodium is regulated by hormones acting to maintain a constant sodium:potassium ratio in the extracellular fluid. Aldosterone, secreted from the adrenal cortex, regulates the reabsorption of sodium from the kidney tubules. The anti-diuretic hormone of the posterior pituitary is responsive to changes in the osmotic pressure of the extracellular fluid; both hormones maintain a constant sodium:potassium ratio.

Potassium (K) has been known to be nutritionally essential element for man and animals. Sir Humphrey Davey first isolated potassium in 1807. Sidney Ringer in 1883 first recognized the importance of potassium in the animal tissue in perfusion experiment with frog hearts. He demonstrated that the perfused mammalian tissue requires a balance of sodium, potassium and calcium to function effectively. Since that time the physiological importance of potassium has been studied in far greater detail.

After calcium and phosphorus, potassium is the thirdmost abundant mineral in the animal body. It represents approximately 0.3% of the body's
dry matter of which two thirds is located in the skin and muscle. In contrast to sodium, potassium is present primarily inside the cells. The blood cells contain about 25 times as much potassium as is present in the plasma. Muscle and nerve cells also are very high in potassium, containing over 20 times as much as that present in the interstitial fluid.

Potassium is absorbed mainly by simple diffusion from the upper small intestine, but some absorption also occurs in the lower small intestine and large intestine. Large volumes of saliva (high in potassium) are continuously secreted by ruminants. So a significant amount of potassium in the rumen is derived from saliva. True digestibility of potassium is relatively high (95% or higher) for most feed stuffs.

Potassium carries out inside the cell many of the functions that sodium performs in the plasma and interstitial fluid, i.e. maintenance of acid-base relationships and proper osmotic balance. Sodium, potassium and chlorine are the three major electrolytes in the body which maintain cation-anion balance. Sodium is the major extracellular cation, providing greater than 90% of the total cations in the plasma and interstitial fluid; whereas potassium, the major intracellular cation, provides greater than 75% of the total cations within the cell.

Active transport mechanisms regulate the concentration of specific electrolytes in the extracellular and intracellular compartments. The intracellular-extracellular separation of sodium and potassium is handled by sodium pump. Maintenance of these concentration gradients is important for transport of substrates into and out of the cell as well as the regulation of the osmotic pressure. Potassium is important in the transport of oxygen and carbon dioxide through the blood and is responsible for at least half the
carbondioxide carrying capacity of the blood. Potassium has a major role in the transmission of nerve impulses and muscle contractions. An ionic balance exists between potassium, sodium, calcium and magnesium ions. These ions affect capillary and cell function and the excitability of nerve and muscle. For instance, potassium acts as a brake in regulating heart beat and suppresses heart flutter. It also helps to prevent tetany, convulsion and an unsteady gait. Potassium activates or functions as a cofactor in several enzyme systems. These include energy transfer and utilization, protein synthesis and carbohydrate metabolism. Some of the enzyme systems are influenced or activated by K⁺. Potassium has a role in the uptake of amino acids into cells. This may form the basis for the influence of potassium on growth.

Potassium must enter cells against a concentration gradient and thus active metabolic process is required. It is excreted into urine by both filtration and secretion. Fecal loss accounts for only about 13% of the total loss of potassium in cows, the remainder being excreted in the urine. In lactating cows, milk can account for 12% of potassium lost from the body.

In 1842 Choussat reported the first direct experiment in which calcium was shown to modify the composition of bone. Pigeons fed on a diet of wheat and water died with very fragile bones, particularly the sternum. When CaCO₃ was added to the diet, the bones were normal. The need for adequate supplies of calcium for the nursing mother was well established by 1900. Gestation and lactation weaken the mother's teeth. Variable amounts of calcium are present in almost all feed stuffs. Calcium content in natural feeds varies, depending on the species of plant or plant part analysed. Grains such as barley, corn, sorghum, oats and wheat are very low in calcium (0.02-0.1%).
Calcium (Ca) is a major mineral component of man. An adult human being weighing 50 to 70 kg. contains about 850 to 1400 g. of calcium. 99% of calcium is present in the bones. The blood contains about 10 mg./100 ml. of blood. Calcium and phosphorus are the two most abundant mineral elements in the animal body. Calcium and phosphorus make up over 70% of the total mineral elements in the body. The calcium:phosphorus ratio in bone is nearly constant and somewhat greater than 2:1. Bone contains considerable amounts of carbonate and nitrate and small amounts of magnesium, sodium, potassium, chlorine, fluorine and traces of other elements.

Normal adult bone has the following approximate composition: water 45%, ash 25%, protein 20% and fat 10%. The ash content is expressed most frequently for moisture-free, fat-free, bone. In mammals, the ash is made up of Ca-36%, P-17% and Mg-0.8%. Most of the calcium and phosphorus occur in bones in the form of calcium phosphate \[Ca_3(PO_4)_2\] and hydroxy-apatite \[Ca_{10}(PO_4)_6(OH)_2\]. The calcium phosphate of bone is deposited within a soft fibro-organic matrix composed of collagen fibres and to a much less extent, of mucopolysaccharidic gel. The protein matrix in bone is calcified when the proper levels of calcium, magnesium, phosphorus and other minerals are present. The animal continually needs calcium, phosphorus and other nutrients to maintain the bone in a strong condition.

Non-skeletal calcium plays important roles in a wide variety of essential functions in body metabolism. 1% of the body's calcium located outside of the bone is found in extracellular fluid, soft tissue and as a component of various membrane structures. Non-skeletal calcium occurs as the free ion bound to serum proteins and complexed to organic and inorganic acids. Calcium is essential for normal blood clotting: the calcium ion must be present for the
formation of thrombin from pro-thrombin, which reacts with fibrinogen to form the blood clot, fibrin.\textsuperscript{377} Calcium has a role as a cofactor in many enzymatic reactions, acting as an activator. [eg.- adenosine triphosphatase (ATPase)] or stabilizer of enzymes\textsuperscript{385} and it is necessary for secretion of a number of hormones and hormone releasing factors.\textsuperscript{386}

Lactose may promote absorption of Ca\textsuperscript{++} by interacting with the absorptive cells of the intestine to increase their permeability to calcium ions.\textsuperscript{387} However the percentage of absorption of calcium lowers with age and high calcium intakes or low vitamin D intakes.\textsuperscript{388} The level of dietary calcium influences calcium absorption, as high dietary levels depress the efficiency of absorption. Vitamin D stimulates active transport of calcium across intestinal epithelium.\textsuperscript{389} Vitamin D and parathyroid hormone (PTH) play an important role in the mobilization of calcium from bone to the extracellular fluid compartment.\textsuperscript{390} There is evidence that vitamin D functions in the distal renal tubules to improve calcium absorption. It has been shown that 1, 25-dihydroxy cholecalciferol functions in improving renal reabsorption of calcium.

Calcium is required for muscle contraction, membrane permeability, myocardial function and normal neuromuscular excitability. It is important in the regulation of heart beat, along with potassium and sodium. Synaptic transmission is likewise affected; acetylcholine may not be liberated in the total absence of calcium ions.\textsuperscript{391} Lowered levels of calcium result in increased excitability, whereas higher levels result in a pseudotranquilizing effect. Extremely low levels of serum calcium can result in tetany. Calcium is needed for efficient weight gain and feed utilization.
Lithium (Li) is used in the therapy of manic-depressive psychosis. However, results of studies carried out during the last few years indicate that lithium is essential in several species of animals. Lithium salts were first introduced into medicine by Garrod\textsuperscript{392} for the cure of gout. A later report by Cade\textsuperscript{393} that lithium salts given to ten manic patients resulted in the improvement in conditions of all has since resulted in an extensive use and testing of lithium in these disorders. Studies to date support the original conclusion that prophylactic lithium is of value in these patients.

The pharmacological actions of lithium salts are complex and knowledge about them is far from complete. Observations reveal that lithium, like tricyclic anti-depressants is a competitive reversible inhibitor of choline esterase.\textsuperscript{394} Lithium at 25 mM is an inhibitor of fluoride stimulated adenylate cyclase.\textsuperscript{395} It is an activator of epinephrine-stimulated adenylate cyclase.\textsuperscript{396} The latter result is of considerable interest because of the increased level of cyclic AMP found in the urine of manic patients. Lithium has also been shown to be an effective agent in the recovery of animals with Bovine Spastic Paresis (BSP), a disease of the central nervous system, including cerebral structures regulating specific muscle motoricity.\textsuperscript{397}

Anke et al.\textsuperscript{398} (1983) fed goats lithium deficient diets from 1976 to 1983 and reported that lithium deficient animals needed more insemination to become pregnant and had decreased milk production. Picket\textsuperscript{399} (1983) reported that rats fed on diets containing 3-5 ppb lithium (low lithium diets) had no difference in growth rate and behaviour when compared to those of the control group, which were fed on diets containing 500 ppb of lithium; however they reported significantly reduced reproductive performance.
The level of lithium used in therapy of manic-depressive psychosis varies with the type of mania. The dose recommended is 0.4 to 0.8 mmol/L for prophylaxis of bipolar and unipolar illness and 0.8 to 1.2 mmol/L for treatment of acute mania. Sudden ceasing of lithium causes the occurrence of new episodes of illness. Gradual discontinuation over four weeks is the withdrawal method of choice. But when lithium is reinstated, it is no longer effective in many cases.

Little information is available on the toxicity of lithium. The doses of lithium required to raise serum lithium level to therapeutic levels of 0.5 to 1.5 mEq/Litre for manic depressive disorders is approximately 500 mg./day. Signs of toxicity have been reported at only over twice that concentration. In human patients particularly women, the lithium treatment depressed the thyroid function, probably by blocking thyroxine release. In rats lithium has been shown to lower the uptake and turnover rate of $^{125}$I by the thyroid.

For humans the RDA (Recommended Dietary Allowances, 1989) allowances for calcium is the same for all ages except young infant. The sodium, potassium and calcium requirements of humans are shown in the Table II.3.
Table II.3. Sodium, potassium and calcium requirements for humans

<table>
<thead>
<tr>
<th>Humans</th>
<th>Sodium (Na) mg./day</th>
<th>Potassium (K) mg./day</th>
<th>Calcium (Ca) mg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>500</td>
<td>1600-2000</td>
<td>1200</td>
</tr>
<tr>
<td>Female</td>
<td>500</td>
<td>1600-2000</td>
<td>1200</td>
</tr>
<tr>
<td>Pregnant</td>
<td>569</td>
<td>1600-2000</td>
<td>1200</td>
</tr>
<tr>
<td>Lactating</td>
<td>635</td>
<td>1600-2000</td>
<td>1200</td>
</tr>
</tbody>
</table>

Common salt contains both sodium and chlorine and is the main source of both elements. It is added as an essential ingredient in bread and other food materials. Consumption of sodium chloride in excess has been associated with hypertension in certain sensitive individuals and low sodium intake has been recommended for such individuals. Several salt substitutes such as Sendha (rock salt), containing low amounts of sodium are now commercially available in India. A sample of rock salt was reported to contain 315 mg. sodium and 187 mg. potassium per gram.

Rock salt (Halite) is a mineral with chemical composition of NaCl. Sylvite with the composition of KCl is similar in structure and occurrence. Naturally occurring halite and sylvite are usually coloured brown to red. Both minerals occur primarily as stratified beds in sedimentary rocks of marine origin. Their origin is by the evaporation of sea water. Crystallisation of halite requires an 11 fold concentration of sea water by evaporation and this occurs mainly in subtropical maritime regions, as seen today along the southern coast of the Persian Gulf. Halite is colourless or white when pure; often yellow or red and sometimes blue. It has got a saline taste and it is soluble in water. Halite colours the flame deep yellow due to the sodium present.
Chapter III

SCREENING OF INDIGENOUS HERBAL EXTRACTS FOR ANTI-INFLAMMATORY ACTION: IN VITRO STUDIES USING RBC-MEMBRANE

III.1 Introduction

A large number of anti-inflammatory drugs are in therapeutic use. Several others are undergoing clinical trial. The drugs include corticosteroids and their synthetic derivatives, non-steroidal anti-inflammatory drugs, gold salts, anti-rheumatic methotrexate, cyclosporine, etc. Most of these drugs are known to produce from mild to serious side effects. There is a growing demand for inexpensive and safe treatment methods.

Inexpensive indigenous anti-inflammatory drugs are commonly used in indigenous systems of medicine. Active constituents such as phenolic compounds, flavonoids, coumarins, xanthones, lignans, triterpenoids, steroids, polysaccharides, alkaloids, etc. are reported to be responsible for the anti-inflammatory activity of the indigenous drugs.²⁷⁴
Decrease in the stability of the erythrocyte membrane was noted in polyarthritis and in rheumatoid arthritis, due to changes in protein or lipoproteins of cellular membrane of erythrocytes. A wide variety of drugs belonging to various pharmacological and chemical categories are known to protect erythrocytes against hypotonic hemolysis. Brown and Mackey and later Bhaskar Rao et al. showed that non-steroidal anti-inflammatory drugs protected erythrocyte membranes from hypotonic hemolysis. The study of the erythrocyte membrane stabilization is simple and rapid, though non-specific. It is useful as a preliminary screening test for the potential anti-inflammatory compounds.

In the present chapter, several synthetic non-steroidal anti-inflammatory drugs [beta-methasone sodium phosphate (Betnesol), diclofenac sodium (Nac-50), oxyphenbutazone (Suganril), indomethacin (Microcid), piroxicam (Dolone) and ibuprofen (Brufen-400)] and indigenous herbal extracts [roots of Sida retusa Linn. (Malayalam: Kurumthotti) and Sida rhombifolia Linn. (Malayalam: Anakurumthotti), water of Cocos nucifera Linn. (Coconut water), roots and leaves of Vitex negundo Linn. (Malayalam: Karinochi), Strobilanthes heymeanus Linn. (Malayalam: Karimkurinji) and Ricinus communis Linn. (Malayalam: Avanakku), the whole plant of Phyllanthus niruri Linn. (Malayalam: Kizhikanelli) and Hydrocotyle asiatica Linn. (Malayalam: Kudakan), bulb of Allium cepa Linn. (Onion) and Allium sativum Linn. (Garlic), rhizome of Curcuma longa Linn. (Malayalam: Manjal) and leaves of Alstonia scholaris Linn. (Malayalam: Pala) and Ocimum sanctum Linn. (Malayalam: Tulasi)] are screened for their anti-inflammatory action based on the observation that non-steroidal anti-inflammatory drugs protected erythrocyte membranes against hypotonic hemolysis.