PLANT PROFILE

Aganosma dichotoma K. Schum

Aganosma dichotoma K.Schum (Figure 1) belonging to family Apocynaceae, is a large climber with very stout stem, commonly known as Malati.

Figure 1: Plant and root of *Aganosma dichotoma* K. Schum

Geographical distribution

Blume was first to introduce *Aganosma* under the class of *Echites* which attained generic status by G. Don with other species based on specimens in Wallich's herbarium (Middleton, 1996). Eight species of *A. dichotoma* are known and distributed in India, China, Philippines, and Indonesia. In India, it mainly extends throughout Assam, Bihar, West Bengal, Orissa, Andhra Pradesh and Tamil Nadu.

Taxonomical description

Kingdom	:	Plantae
Subkingdom	:	Tracheophyta
Division	:	Magnoliopsida
Class	:	Dicotyledon
Order	:	Gentianales

Family : Apocynaceae

Genus : Aganosma

Species : *Aganosma dichotoma* K. Schum

Vernacular Names

Hindi : Malati

Sanskrit : Jati

Telugu : Pallamale

Tamil : Adavimalati

Kannada : Malatilata

Bengali : Haparmali

Botanical description

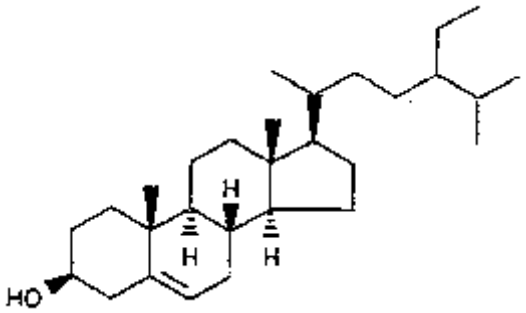
The plant is a large climber with glabrous, sparsely strigose or tomentose stem. Leaves: petiole 5 - 28 mm long; blade ovate to elliptic, apex acuminate, base rounded, rarely obtuse or weakly cordate, 2.8 - 14.2 x 1.3 - 4.8 cm, 1.3 - 2.8 x as long as wide, 2 - 4(- 6) pairs of lateral nerves, very strongly ascending, glabrous, pubescent only on abaxial midrib or pubescent all over abaxially. The inflorescence is lax, terminal, sparsely to densely pubescent, 3 - 20.5 cm long; pedicels 2.8 - 23 mm long. Sepals narrow lanceolate, 12 - 25 x 1 - 3.7 mm, 4 - 15 x as long as wide, longer than corolla tube, tomentose, with colleters in the corners inside. Corolla white; tube 8 - 13 mm long; lobes obovate, falcate, 7.3- 20 x 4.6- 14 mm, 1 - 2.4 x as long as wide, 0.8- 1.7 x as long as tube; very sparsely puberulent to puberulent outside, glabrous in the throat, pubescent in 5 rows behind anthers. Stamens inserted at 3.5 - 6.2 mm from base of corolla tube, 0.4 - 0.5 of tube length; anthers 4.4 - 5.8 x 0.9 - 1

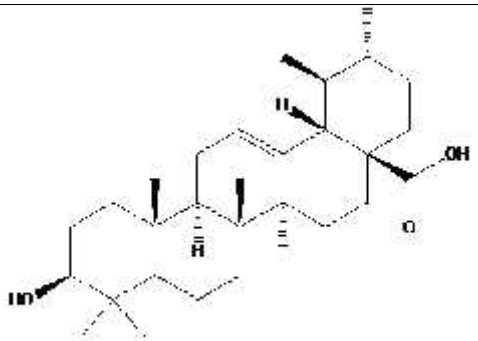
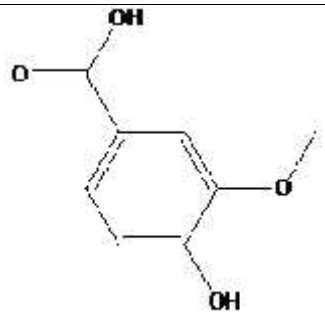
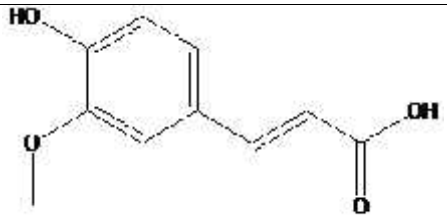
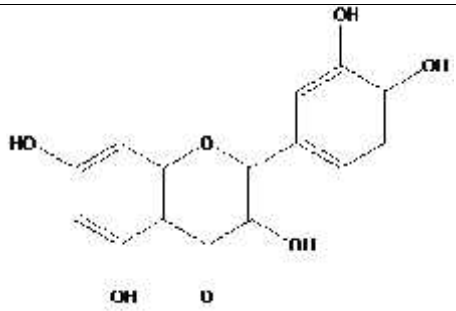
mm, 4.7 - 6.1 x as long as wide. Disk narrower at the top, 5-dentate or with some, but not all, lobes free, 0.9 - 1.9 mm long, 1.3 - 2.4 x as long as ovary. Ovary 0.7 - 0.8 mm long, pubescent; style 2.8 - 6.4 mm long; pistil head 1.9 - 2.7 mm long. Fruit fusiform, divergent to parallel, tomentose to glabrous, 6.3 - 21 x 0.7 - 1 cm. Seeds 10.5 - 18 x 4.3 - 6.6 mm; coma 1.4 - 3 cm long. (Middleton, 1996)

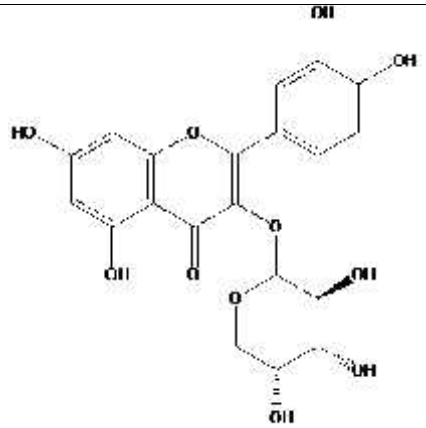
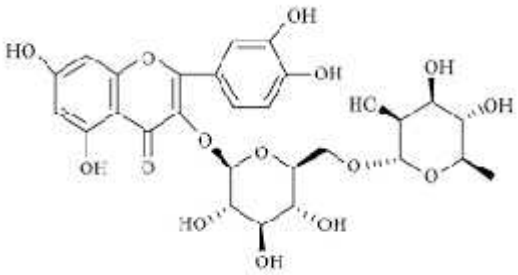
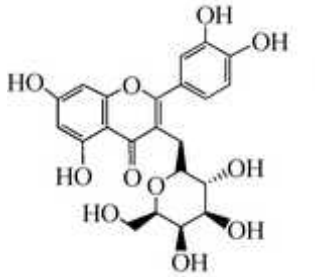
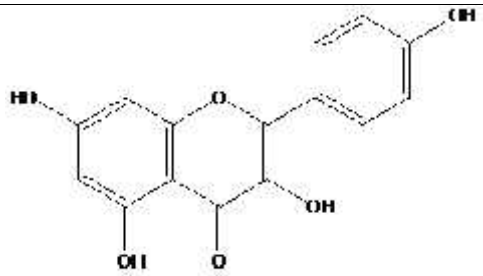
Chemical constituents

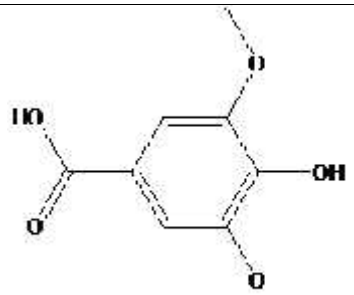
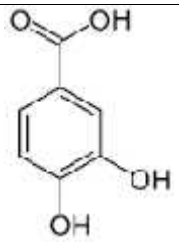
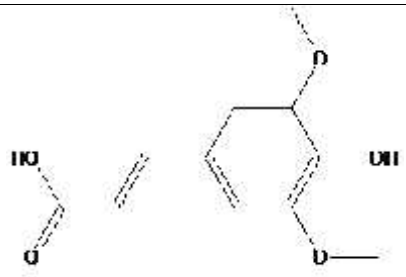
Phytoconstituents present in flowers of *A. dichotoma* includes β-sitosterol, ursolic, vanillic and ferulic acids, quercetin and its glycosides, rutin, hyperin, isoquercetin and quercetin- 3 arabinosides. Leaves contain β-sitosterol, quercetin, kaempferol, kaempferol-3 arabinoside, kaempferol-3 galactoside, vanillic, syringic, protocatechuic, ferulic and sinapic acid. The structure of the compounds is as follows (Table 1):

Table 1: Structures of compounds isolated from *Aganosma dichotoma*

S.No.	Name	Structure	Reference
1.	β-sitosterol		Sekhar *et al*., 1985; Sri Ramana *et al*., 1985

2.	Ursolic acid		Sekhar *et al*., 1985
3.	Vanillic acid		Sekhar *et al*., 1985; Sri Ramana et al., 1985
4.	Ferulic acid		Sekhar *et al*., 1985; Sri Ramana *et al*., 1985
5.	Quercetin		Sekhar *et al*., 1985; Sri Ramana *et al*., 1985

6.	quercetin- 3 arabinosides		Sekhar *et al*., 1985
7.	Rutin		Sekhar *et al*., 1985
8.	Hyperin		Sekhar *et al*., 1985
9.	kaempferol		Sri Ramana *et al*., 1985

10.	Syringic acid		Sri Ramana *et al*., 1985
11.	Protocatechuic acid		Sri Ramana *et al*., 1985
12.	sinapic acid		Sri Ramana *et al*., 1985

Traditional Uses

- *A. dichotoma* plant root has been used traditionally in various ailments including emesis, anthelmintic, bronchitis, leprosy, skin disease, ulcers, inflammation, purulent discharges from the ear and disease of mouth while flowers are used in disease of eye and leaves are used in biliousness (Kritikar and Basu, 1975; Vaidya and Kashyap, 2000).
- According to Vedavathy (2004), root powder is used in Stones removal in the urinary tract and bladder in Chittoor district of Andhra Pradesh. Root powder is given with milk early in the morning for a period of two weeks.

- In Bangladesh plant is traditionally used in rheumatic pain (Akber *et al*., 2011)
- *A. dichotoma* is also used as antiseptic, anodyne and also used as an ingredient in massage oils for paraplegia, neuralgia, sciatica. (Khare, 2007)
- Stem bark of *A. dichotoma* along with *Grewia tiliaefolia* mixed with old tamarind stored for 3 years is ground with crab and the skin of monitor lizard and then made into tablets and administered orally twice daily, to treat Malaria, fits, chicken pox, witchcraft and as antidote to poison in Visakhapatnam district of Andhra Pradesh (Rao *et al*., 2011).

Pharmacological uses

- According to Asolkar *et al*., (1992) Petroleum ether extract of the leaves of *A. dichotoma* possess anthelmintic activity against earthworms.
- The hypoglycemic effects of methanolic leaf extract of *A. dichotoma* were examined in Swiss-albino mice at doses of 200 and 400 mg/kg body weight and compared with standard Glibenclamide at a dose of 10 mg/kg body weight. Both doses of 200 and 400 mg/kg lowered the plasma glucose level in 3 hours administration with its standard drug Glibenclamide. The leaf extract of *A. dichotoma* showed significant hypoglycemic effect and increases the plasma insulin concentration (Khan *et al*., 2014).
- The methanolic leaf extract of *A. dichotoma* exhibited significant ($p < 0.05$) anti-diarrheal effect at a dose of 400 mg/kg body weight in castor oil induced anti-diarrheal assay. The peripheral and central analgesic actions were also

determined in methanolic leaf extract of *A. dichotoma* by using acetic acid-induced writhing and tail immersion methods. (Faruk *et al*., 2015)

- According to Day et al., (2014), free radical scavenging, brine shrimp lethality, antimicrobial and thrombolytic activities were evaluated in methanolic extract and fractions of leaves of *A. dichotoma*. Chloroform-soluble fraction demonstrated the highest free radical scavenging activity (IC_{50} value, 18.21µg/ml) and showed significant brine shrimp lethality having LC_{50} value of 3.98µg/ml. In thrombolytic assay, the carbon tetrachloride soluble fraction showed the highest clot lysis (30.48%). While petroleum ether, carbon tetrachloride, and chloroform-soluble fractions displayed weak to moderate antimicrobial activity with the zone of inhibition ranging from 7 to 14 mm.

GASTRIC ULCERS

An ulcer is a deep necrotic lesion commonly situated in the stomach generally in the proximal duodenum and seldom in the esophagus and jejunum. It is attributable to a shatter in the mucosal integrity close to the acid-secreting areas of the GIT (Chow *et al*., 1998). Gastric ulcers are primarily located along the lesser curvature of the stomach and morphologically gastric ulcers are usually round or oval and size is in between the 2-4 cm in diameter.

Pathophysiology of ulcer:

Peptic ulcers are formed by an imbalance between the aggressive factors (such as acid–pepsin, histamine secretion, bile acids, etc.) and defensive factors (such as mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins, epidermal growth factors) in the stomach (Lima *et al*., 2006) (Figure 2). Behavioral and environmental factors, for instance, high consumption of alcohol, poor diet, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), stress, smoking, genetic variation and infection with *H. pylori* are also responsible for gastric ulceration (Oliveira *et al*., 2011). The reactive oxygen species, especially hydroxyl radical also plays an imperative role in causing oxidative damage of mucosa in all types of ulcers (Das *et al*., 1997). Some of the important aggressive and defensive factors affecting the gastric ulceration of the stomach are listed below.

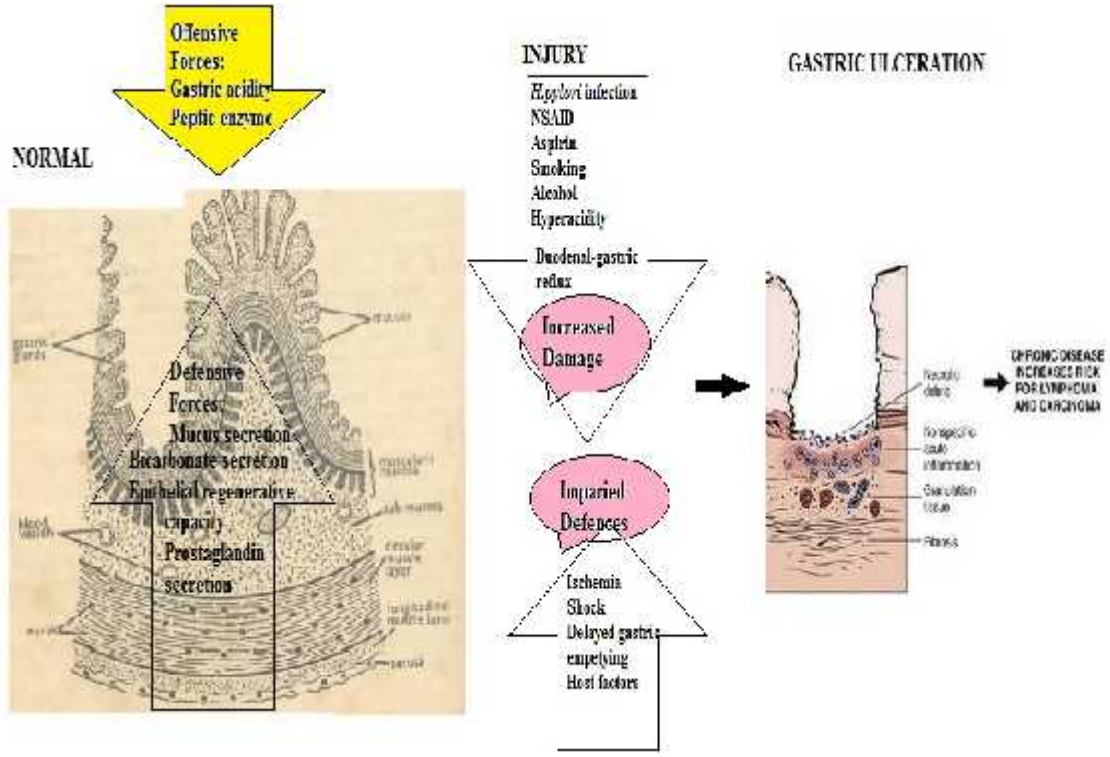


Figure 2: Pathophysiology and factors affecting peptic ulcer

Aggressive factors

a) ***Acid-Pepsin secretion***

Abnormalities in the secretion of gastric acid and pepsin are the main pathophysiology of gastric ulcer and about 50 % of gastric ulcer patients are associated with pepsin and acid hypersecretions (Szabo *et al*., 1998). Gastric acid secretion is mostly controlled by three principle secretagogues i.e. Histamine, acetylcholine, and gastrin. Parietal cells bear three receptors for these stimulators of acid secretion, H_2 receptors is responsible for release of histamine from specialized mast cells, muscarinic receptors is liable for the release of acetylcholine from the

vagus nerve and probably receptors approachable to endogenous circulating gastrin. Gastrin is responsible for the increased acid secretion either by direct stimulation of parietal cells or by the release of histamine from entero-chromaffin like (ECL) cells (Lindstrom *et al*., 2001).

b) *Histamine*

Histamine is present throughout the gastrointestinal tract, in ECL cells and constrained to the fundic mucosa of the stomach, mast cells, and nerves. Histamine shows their effects through H_1, H_2, H_3 and H_4 receptors, but only H_2 receptor is located on parietal cells in the fundic mucosa and they have also been present in the intestinal epithelium, immune cells and myenteric ganglia in humans and are potent stimulants of gastric acid secretion (Sander *et al*., 2006). Gastrin stimulates histamine secretion from the enetero-chromaffin-like cells via cholecysokinin-2 (CCK2) receptors on the ECL surface membrane (Bitziou and Patel, 2012). Histamine stimulates the H_2 receptor on the parietal cell and activates adenylate cyclase, leading to increasing of intracellular cyclic AMP concentrations and commencement of protein kinase A (PKA). Activation of PKA is responsible for phosphorylation of cytoskeletal proteins involved in transport of the H^+-K^+ -ATPase from cytoplasm to plasma membrane resulting in gastric acid secretion (Fykse *et al*., 2006).

(c) *Helicobacter pylori*

The *Helicobacter pylori* bacteria were discovered in the stomach of many patients in 1980 with gastritis and peptic ulceration and renowned to be a significant causative agent (Marshall, 1984). *H. pylori* are mainly involved in the majority of cases of both duodenal and gastric ulcer, both in the West and in developing countries and are

mainly believed to produce infection due to a unhygienic environment. The bacterial chromosome brings the outer inflammatory protein and a functional cytotoxin-associated gene island which increases virulence when it adheres to the gastric mucosa and probably causes ulceration. *H. pylori* infected patient have increased resting and meal-stimulated gastrin levels and decreased the production of gastric mucus and secretion of duodenal mucosal bicarbonate, all of which responsible for ulcer formation. *H. pylori* eradication reduces the incidence of ulcer recurrence—from 67 to 6 percent in patients suffering from duodenal ulcers and from 59 to 4 percent in patients with gastric ulcers (Hopkins *et al*., 1996).

(d) ***Oxidative stress and free radicals***

Oxidative stress has involved as one of the major pathogenic factors that directly impaired cells functions, promotes cellular organelles damage in the cells, particularly, mitochondria, lysosomes, and nucleus. The reactive oxygen species (ROS) such as superoxide anion (O^-_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH.) have been allied in the pathogenesis of many human ailments, including neurodegenerative disorder, stress, inflammation, diabetes and peptic ulcer (Bafna, 2005; Balaraman *et al*., 2004). ROS alter the physical, chemical and psychological factors and deteriorate the gastric mucosa resulting in the formation of gastric ulceration. Experimental and clinical data suggests that gastric mucosal damage by ethanol (Mizui *et al*., 1987; Pihan *et al*., 1987), non-steroidal anti-inflammatory drugs (Vaananenn *et al*., 1991; Yoshikawa *et al*., 1993) and by *H. pylori* (Davies *et al*., 1994) is also mediated through the ROS (Phull *et al*., 1995). Moreover, several kinds of stress are responsible for gastric ulceration by generating the ROS. The

fundamental primary product is lipid hydroperoxides, which are proficient for initiating lipid peroxidation chain reaction, and responsible for the formation of intracellular components such as lysosomal enzymes and other secondary oxidation products including aldehydes, hydrocarbons, acids, ketones and higher polymers which cause tissue damage. Among these, malondialdehyde (MDA) is mutagenic and carcinogenic, and its reaction with thiobarbituric acid is a marker of lipid peroxidation as thiobarbituric acid reactive substances (Dotan *et al*., 2004; Demir *et al*., 2003).

Defensive factors

a) Role of mucus or mucin secretions

The gastric mucosa is covered by a continuous layer of mucus gel, which has an erratic thickness of less than 500 μm (Allen *et al*., 1993). The composition of mucus is around 1% by weight salt and other dialysable components, 0.5-1% free protein, carbohydrate rich glycoprotein and 95% or more water. Mucin is a glycosylated glycoprotein component of mucus, accountable for the characteristic viscous gel forming property, alleged to be significant for the functional role of mucus. Their expression in the mucosa is regulated by several cytokines and endocrine hormones (Wittel *et al*., 2001). Mucus plays important role in the protection of mucosa from damage induced by acid, pepsin and other luminal toxins (Wallace, 2001). Profuse amount of gastric mucus is secreted during superficial mucosal injury and deliver a favorable micro-environment in repair by restitution. PGE_2 and PGI_2 of the gastric and duodenal mucosa are main pro–inflammatory cytokines responsible for mucus production and maintaining the cellular veracity of the gastric mucosa (Sairam *et al*.,

2002). In addition bicarbonate secretion is also alleged to be the first line of defense against various destructive ulcerogens and recent studies suggest that vagal cholinergic stimulation and luminal acid manage the gastric bicarbonate secretion. The bicarbonate transport into the mucus gel produced the protective zone is termed as 'mucus-bicarbonate barrier' and provide the gastroprotection against damaging factors (Fandriks and Jonson, 1990).

(b) ***Nitric oxide (NO)***

Nitric oxide is responsible for gastroprotection particularly by regulating the acidic and alkaline gastric secretion, mucus secretion and blood flow in the gastric mucosa, due to it is rapid reactivity with various oxygen species in the biologic system (Chandranath *et al*., 2002). According to Kato *et al*., (1998) NO formed by constitutive enzyme plays an important role in the intonation of gastric mucosal integrity by interacting with sensory neuropeptides and endogenous prostaglandins. Nitric oxide also inhibits the pentagastrin-induced acid secretion in rats (Esplugues *et al*., 1996), while *H. pylori* infection may encourage nitric oxide (NO) synthase expression and NO release, which in turn may inhibit acid secretion and reveal luminal alkalinization caused by bicarbonate leakage from the plasma into the gastric juice (Mannick *et al*., 1996).

(c) ***Role of cytokines and growth factors***

Cytokines are extremely important in strengthening the mucosal defense system by regulating the mucosal immune system. Several pro–inflammatory cytokines are involved in the pathogenesis of gastric ulcers, such as interleukins (IL–1β, IL–2, IL–6 and IL–8) and tumor necrosis factor (TNF–α). IL–1 has been reported to protect the

gastric mucosa mainly by stimulating the release of prostaglandin and NO and by inhibiting the gastric acid secretion, leukocyte adherence and ulcer–promoting mediators like platelet activating factor (PAF) and histamine from mast cells (Kaur *et al*., 2012). Additionally, pro-inflammatory cytokines cause an inhibition of somatostatin and gastrin releases from the D and the G cells, respectively. Growth factors like transforming growth factor (TGF− α) and vascular endothelial growth factor (VGEF) are local polypeptide hormones that alter the rate of cellular proliferation of their target cells carrying functional specific receptors and have an important role in protecting the gastric mucosa. VGEF play a major role in gastric ulcer healing by enhancing the stimulating process of angiogenesis, While TGF-α is released locally in the gastric mucosa, particularly when the mucosa is bare to topical irritants. According to Kobayashi *et al*., (1996), TGF-α responsible for the stimulation of the restitution and proliferation of mucosal cells, gastroprotection, vasodilatation, gastric adaptation to noxious substances, healing of acute and chronic lesions and inhibition of gastric acid secretion. TGF-α has been shown to share a common receptor (EGFR) and to accelerate ulcer healing due to stimulation of cell proliferation (Konturek *et al*., 1997).

Other factors affecting gastric ulcer

a) NSAIDS

NSAIDs are the most common grounds of peptic ulcer disease in patients without *H. pylori* infection. NSAIDs are accumulated in an ionized form intracellular and cause gastric injury by the damage of the gastric epithelium. After that, by changing the action of surface active phospholipids it reduces the hydrophobicity of the mucous

gel layer, followed by the suppression of the prostaglandin synthesis, which increases the neutrophils adherence to the endothelium of gastric microcirculation and causes injury (Siddique, 2014). In addition, NSAIDs inhibits the cyclooxygenase and reduces the formation of prostaglandins and effects produced by cyclooxygenase-2 i.e. enhancing gastric mucosal protection by stimulating mucus and bicarbonate secretion and epithelial cell proliferation and increasing mucosal blood flow (Collier and Pain, 1985).

b) *Ethanol*

Consumption of ethanol in an excessive quantity is considered to be one of the causative factors in the development of gastric ulcer (Franke *et al*., 2005). Alcohol causes the stomach cells to over secrete both acid and histamine, which make the stomach linings vulnerable to ulcer formation and ethanol also produces a marked contraction of the circular muscles of fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress and at the crests of mucosal folds leading to necrosis and ulceration. This reduces the secretion of bicarbonates and production of mucus and also increases the neutrophil infiltration into the gastric mucosa (Bardi *et al*., 2011). Szabo *et al* suggested that administration of ethanol causes a rapid and time dependent release of the endogenous cytokines (endothelin–1) into the systemic circulation, thereby leading to the development of the hemorrhagic mucosal erosions by vasoconstriction (Szabo *et al*., 1994).

c) *Smoking*

Continued smoking and chronic nicotine treatment with advancing age augments the secretion of HCl and pepsin and is also anticipated to alter the contents of gastric

juice and pepsin isoenzyme patterns. The increased gastric acid secretion is mediated through the prompt release of histamine after mast cell degranulation by H_2 receptors and due to the increased functional parietal cell volume or secretory capacity in smokers. Smoking and nicotine stimulate pepsinogen secretion by escalating chief cell number or with an augmentation of their secretory capacity. The adverse effects of cigarette smoke on ulcer disease in man are manifested as it slows the healing process of gastric ulceration and produces an increased ulcer re-occurrence via decreasing the gastric blood flow and angiogenesis (Kyoji *et al*., 1997).

d) *Diet*

Diet is also one of the lifestyle and behavioral factor distressing the ulceration of the stomach and duodenum. A fiber rich diet and vitamin A containing diet was reported to reduce the risk of duodenal ulcer (Harshman and Aldoori, 2004). Caffeinated beverages (eg. tea, coffee), cola type beverages, beer, and milk are potent stimulants of gastric acid secretion. Coffee produces acid output equal to 70 percent of peak acid output as compared to pentagastrin while 5% aqueous tea and coffee beverages act by decreasing PGI_2 synthesis. In addition, alcohol consumption and high dietary consumption of salt also cause ulceration of the stomach (Duggan, 2006).

e) *Stress*

There is substantial confirmation that supports the role of stressful life dealings in the etiology of PUD. An increase in free radical generation and acid-pepsin factors are responsible for stress-induced ulcers (Sairam *et al*., 2002). Stress may also increase the gastric motility, vagal overactivity, mast cell degeneration, reduces gastric mucosal blood flow and produce ulceration by the release of histamine with enhanced

acid secretion and reduced mucous production (Sen *et al*., 2009). Cold restrained stress induced ulcers are the result of autodigestion of gastric mucosal barrier, accumulation of HCl and generation of free radicals (Thamotharan *et al*., 2010).

f) *Blood group*

Patients with blood group 'O' are at increased risk of peptic ulcer as compared to other blood group patients (Nelson and Cox, 2005).

Clinical manifestations of Gastric Ulcer (Fauci *et al*., 2008)

- Epigastric pain (abdominal pain) characterized by burning or gnawing discomfort.
- Nausea, vomiting and weight loss.
- Dyspepsia.
- Insomnia.
- Bloating
- Bleeding, perforation and obstruction.

Management of Gastric Ulcer

Generally, gastric ulcer treatment is intended to relieve ulcer pain, healing the ulcer, preventing ulcer recurrence, and reducing ulcer-related complications as shown in figure 3. About 99% of gastric ulcers are caused by infection with the bacterium *H.pylori* or by use of NSAID. The goal of therapy in *H.pylori*-positive ulcer patients is to eradicate this bacterium. Successful eradication heals ulcers and reduces the risk of recurrence to less than 10% at 1 year. The goal of therapy in a patient with NSAID-induced ulcer is to heal the ulcer as rapidly as possible. Patients at high risk of developing NSAID ulcers should be switched to a COX-2 inhibitor or receive prophylactic drug co-therapy to reduce ulcer risk and ulcer related complications. A

number of synthetic drugs are available to treat ulcers but due to high side effects and less margin of safety of synthetic drugs, general people as well as researches have shifted their interest from synthetic drugs to herbal drugs. A list of plants which have been evaluated for its anti-ulcer activity has been described in Table 2. The table also includes the specific part which was used for the investigation.

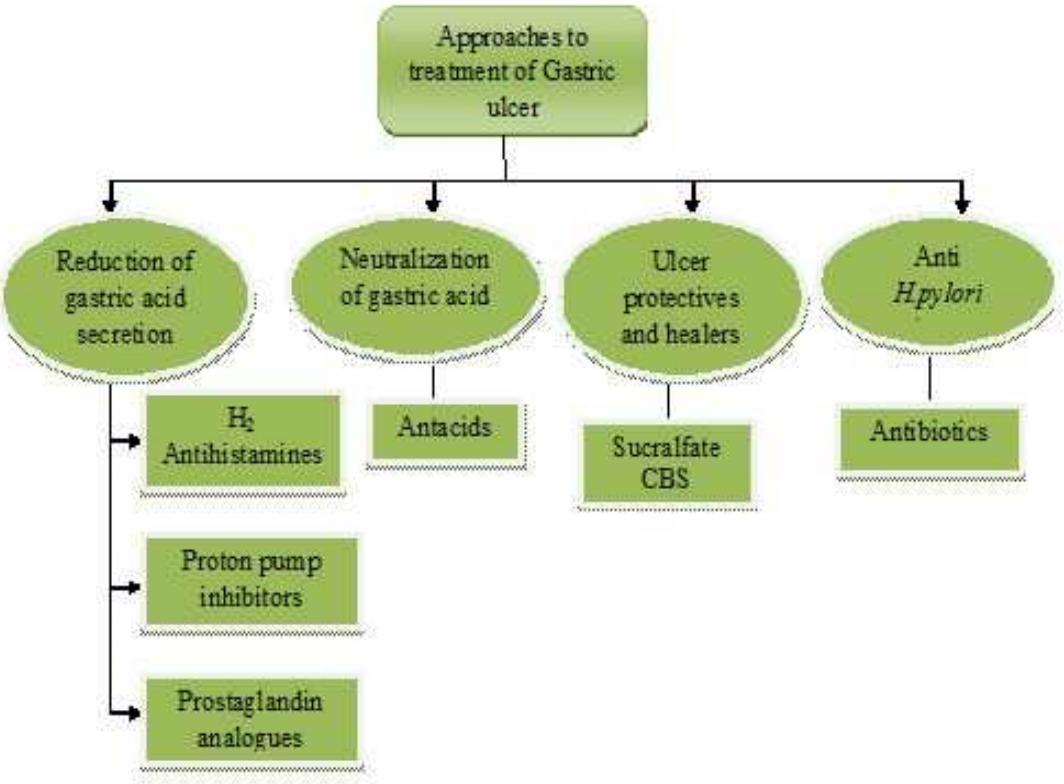


Figure 3: Treatment approach of gastric ulcer

Table 2: List of Medicinal plants with Anti-ulcer activity

Name of Plants	Family	Part used	References
Acacia catechu	Fabaceae	Root	Alambayan *et al*., 2015
Acacia nilotica	Fabaceae	seedless pod	Bansal & Goel, 2012
Acalypha indica	Euphorbiaceae	Whole plant	Kalimuthu *et al*., 2010
Aegle marmelos	Rutaceae	Unripe fruit	Dhuley, 2004
Albizia lebbeck	Mimosaceae	Bark	Balekar *et al*., 2013
Alstonia scholaris	Apocynaceae	Whole plant	Arulmozhi *et al*., 2012
Anamirta cocculus	Menispermaceae	Fruit	Satya & Paridhavi, 2012
Andrographis paniculata	Acanthaceae	Leaf	Wasman *et al*., 2011
Anogessius accuminata	Combretaceae	Leaf	Hemamalini *et al*., 2011
Anogeissus latifolia	Combretaceae	Bark	Govindarajan *et al*.,2006
Argemone mexicona	Papaveraceae	Aerial part	Das *et al*., 2011b
Argyreia speciosa	Convolvulaceae	Leaf	Jaiswal *et al*., 2011
Azadirachta indica	Meliaceae	Leaf	Chattopadhyay *et al*.,2004
Bambusa aruninacea	Graminae	Leaf	Muniappan & Sundararaj, 2003
Boswellia serrata	Burseraceae	Bark	Zeeyauddin *et al*., 2011
Brassica oleraceae	Brassicaceae	Leaf	Carvalho *et al*., 2011
Butea monosperma	Fabaceae	Root bark	Patil *et al*., 2009
Caesalpinia pulcherrima	Fabaceae	Aerial part	Sharma & Rajani, 2011
Camellia sinensis	Theaceae	Seed	Yoshikawa *et al* ., 2005

Ceiba pentandra	Bombacaceae	Root	Bhushan *et al*., 2011
Cordia myxa L.	Boraginaceae	Fruit	Abdallah *et al*., 2011
Curcuma longa	Zingiberaceae	Rhizome	Kim *et al*., 2005
Daucus carota L.	Apiaceae	Root	Nayeem *et al*., 2010
Desmodium gangeticum	Leguminosae	Leaf	Dharmani *et al*., 2005
Eugenia jambolana	Myrtaceae	Fruit	Chaturvedi *et al*., 2007
Ficus religiosa	Moraceae	Leaf	Gregory *et al*., 2013
Gardenia jasminoides	Rubiaceae	Fruit	Lee *et al*., 2009
Glycyrrhiza glabra	Papilionaceae	Root	Aly *et al*., 2005
Gymnosporia emerginata	Celastraceae	Leaf	Hemamalini *et al*., 2011
Ipomoea batatas	Convolvulaceae	Tuber	Panda & Sonkamble, 2012
Jasminum sambac	Oleaceae	Leaf	Alrashdi *et al*., 2012
Nigella sativa L	Rananculaceae	Seed	Sengupta *et al*., 2013
Phyllanthus amarus	Euphorbiaceae	Leaf	Shokunbi &Odetola, 2008
Piper cubeba	Piperaceae	Fruit	Parvez *et al*., 2010
Pongamia pinnata	Leguminoseae	Seed	Prabha *et al*., 2009
Potentilla fulgens	Rosaceae	Root	Laloo *et al*., 2013
Senecio candicans	Asteraceae	Leaf	Hariprasath *et al*., 2012
Syngonanthus bisulcatus	Eriocaulaceae	Scapes	Batista *et al*., 2013
Zanthoxylum zanthoxyloides	Rutaceae	Root bark	Boye *et al*., 2012

Pain

International Association for Study of Pain defined the pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1980). Pain can be classified as nociceptive and neuropathic in terms of therapeutic application. The peripheral nerve ending (nociceptor) pain transmits the impulse to the dorsal horn of the spinal cord, where it gets modified before onward transmission to the brain. Any pain allied with the stimulation of the nociceptor can be supposed to be a nociceptive pain. When, it is not caused by a stimulus applied to the nociceptor, but related with impulse generation within the pathway proximal to the nociceptor (this could be in the nerve, the spinal cord or the brain) is called neuropathic pain (Rajagopal *et al*., 2006).

Pathophysiology of pain (Patel *et al*., 2010)

Pain sensation could begin due to following reasons

1) Inflammation of the nerves. (Temporal neuritis)
2) Injury to the nerve and nerve ending with scar formation. (Surgical damage or disk prolapsed)
3) Nerve invasion by cancer. (Brachial plexopathy)
4) Injury to the structures in spinal cord, thalamus or cortical area that process pain information, which can lead to intractable pain, differentiation. (Spinal trauma)
5) Abnormal activity in the nerve circuits that is apparent as a pain.

Inflammation

Inflammation is a term derived from the Latin word "inflammare" meaning to burn, and it is a local reaction of living vascularized tissues to endogenous and exogenous stimuli. In 1794 Sir John Hunter accomplished: “inflammation is not to be considered as a disease, but as a valuable operation ensuring either to some aggression or to some diseases”. It is natural reaction of the body to protect itself from infection and strange substances. It involves a well-organized cascade of fluidic and cellular changes. It is recognizable grossly and histologically and has both beneficial and detrimental effects locally and systemically (Mesele *et al*., 2004).

Causes of inflammation

- Physical agents - mechanical injuries, alteration in temperatures and pressure, radiation injuries.
- Chemical agents- including the ever increasing lists of drugs and toxins.
- Biologic agents (infectious)- bacteria, viruses, fungi, parasites
- Immunologic disorders- hypersensitivity reactions, autoimmunity, immunodeficiency states etc.
- Genetic/Metabolic disorders- examples gout, diabetes mellitus etc. (Stankov, 2012)

Mediators of inflammation

The characteristic signs of inflammation are local redness, swelling, heat, pain, and loss of function. These manifestations are induced and controlled by a large number of chemical mediators as shown in figure 4.

Cytokines

Cytokines are small hormone-like polypeptides or glycoproteins having a molecular weight less than 30 kD. A variety of immune cells (monocytes, lymphocytes) and non-immune cells (endothelial cells) are responsible for the secretion of cytokines after stimulation by microbes or other cytokines. They bind to specific cellular receptors and acts at low concentration (Vilcek and Le, 1994). Binding with the receptor results signal transmission through intracellular messenger systems that activate transcription factors, and changes gene expression. Different intracellular signaling pathways are stimulated by different cytokine-receptor interactions. The receptors may also exist in soluble isoforms that have the ability to bind cytokines and modulate their activity. Most cytokines have multiple biologic effects, often overlapping the effects of others. The majority acts locally, but cytokines may also have systemic effects. Cytokines are important in host defense, but in high amounts, cytokines may be injurious to the host (Kilpatrick and Harris, 1998).

Chemokines

Chemokines are the largest family of cytokines in human immune physiology. They are small proteins that are distinct by four conserved cystine residues. Chemokines function by activating specific G protein–coupled receptors and induce cells to migrate through a concentration gradient. The role of inflammatory chemokines is to induce the migration of leukocytes to the injured or infected site. In addition, inflammatory chemokines activate the cells to mount an immune response and initiate wound healing (Rossi and Zlotnik, 2000). Nowadays, pharmaceutical industries conducting clinical development program by targeting the chemokine system because

their receptors are considered as a promising targets for the regulation of leukocyte infiltration in inflammatory and immune diseases (Kouji *et al*., 2011).

Kininis

The kinin is a group of polypeptides, found in blood, which ultimately produces bradykinin (BK). BK is a naturally occurring neuropeptide (plasma protein). It is a pharmacologically active kinin, which is considered as either cardioprotective or pro-inflammatory agent (Campbell *et al*., 2005). By asset of their ability to activate endothelial cells, leading to vasodilatation, increased vascular permeability, tissue-type plasminogen (t-PA) release, production of NO and mobilization of arachidonic acid, they participate in physiological (regulation of blood pressure, renal and cardiac functions) and pathological processes like inflammation (Moreau *et al*., 2005). BK is a part of Kallikrein-Kinin System (KKS), which is a complex of two substrates (kininogens) that activated by kallikreins enzyme and fabricate four inflammatory mediators (kinins) that bind to the B1 and B2 receptors of BK. The leucocyte conscription, initiation of inflammatory responses as well as the physiology of pain in inflammation is due to the B1-receptors. Currently, it has been determined that B1-receptors are mitogenic in fibrotic tissue (Medeiros *et al*., 2004).

Eicosanoids

Eicosanoids are short-lived, hormone-like substances present in tissues throughout the body. They function as mediators of a range of physiological responses such as inflammation, blood clotting, vascular dilation, and immunity. Eicosanoids can be divided into four classes: prostaglandins, leukotrienes, thromboxanes, and

prostacyclins. A large part of the inflammatory process is controlled exclusively by the prostaglandins and leukotrienes (Mark, 1997).

Prostaglandin

Prostaglandins (PG) are lipid autacoids derived from arachidonic acid and produced by the action of the enzyme cyclooxygenase (COX). PG play a key role in the generation of the inflammatory response in various disease, they are also a key factor in the physiological regulation of gastrointestinal and renal homeostasis. There are four principal bioactive prostaglandins generated in vivo: prostaglandin E2 (PGE2), prostacyclin (PGI2), prostaglandin D2 (PGD2) and prostaglandin F2α (PGF2α) (Ricciotti and Fitzgerald, 2011).

PGE2 is one of the most abundant PGs produced in the body and it is an important mediator of many biological functions, such as regulation of immune responses, blood pressure, gastrointestinal integrity, and fertility. PGE2 have a particular interest in inflammation because it is involved in all processes leading to the classic signs of inflammation: redness, swelling, and pain. Redness and edema arises due to amplified blood flow into the inflamed tissue through PGE2-mediated extension of arterial dilatation and increased microvascular permeability (Funk, 2001).

PGI2 is one of the most important prostanoids that regulates cardiovascular homeostasis. Vascular cells, including endothelial cells, vascular smooth muscle cells, and endothelial progenitor cells, are the major source of PGI2. In addition to its cardiovascular effects, PGI2 is an important mediator of the edema and pain that accompany acute inflammation (Kawabe *et al*., 2010).

PGD2 is a major eicosanoid that is synthesized in both the central nervous system (CNS) and peripheral tissues and appears to function in both an inflammatory and homeostatic capacity. In the brain, PGD2 is involved in the regulation of sleep and other CNS activities, which includes pain perception while in peripheral tissue PGD2 is produced mainly by mast cells, but also by other leukocytes (Urade and Hayaishi, 1999).

Leukotrienes

The name "Leukotriene" was introduced by the Swedish biochemist B. Samuelson, derived from the words leukocyte and three conjugated double bonds and it is eicosanoids lipid mediators which is responsible for the inflammatory response (Bailey and Martyn, 1985). Leukotrienes (LTs), including cysteinyl-LTs (LTC4, LTD4, and LTE4) and LTB4, are potent biological lipid mediators derived from arachidonic acid through the 5-lipoxygenase (5-LO) pathway (Paolo, 2010). LTB4 is an important mediator of inflammation and it is a potent chemotaxin for neutrophils and increases leukocyte adhesion to the blood vessel walls (Samuelson *et al*., 1987).

Platelet-activating factor (PAF)

Platelet-activating factor (PAF; 1-0-alkyl-2-acetyl-snglycero- 3-phosphocholine), a bioactive phospholipid mediator that has biological effects in normal cell function, as well as in cellular pathology and release of PAF can be a potent mediator of inflammatory responses (Teather *et al*., 2002). It is synthesized by a distinct membrane-bound acetyl transferase which catalyzes the transfer of an acetyl residue from Acetyl CoA to lyso-PAF, generated by the action of phospholipase A2 (PLA2) on phosphatidylcholine and lyso-PAF can then be acetylated by acetyl coenzyme A:

lyso-PAF acetyltransferase to form PAF (Mark *et al*., 1993). The primary role of PAF is to mediate intercellular interactions and it binds to its specific receptor that activates the cytoplasmic PLA2 and phospholipase C. PAF has a number of pro-inflammatory properties and *in-vivo* PAF causes increased vascular permeability, hypotension, decreased cardiac output, stimulation of uterine contraction, gastrointestinal disorders, acute bronchoconstriction, and leukocyte adhesion to endothelial cells (Honda *et al*., 2002). While *in-vitro* PAF can cause activation of platelets, polymorphonuclear leukocytes, monocytes, and macrophages and stimulation of glycogenolysis in perfused liver (Mark *et al*., 1993).

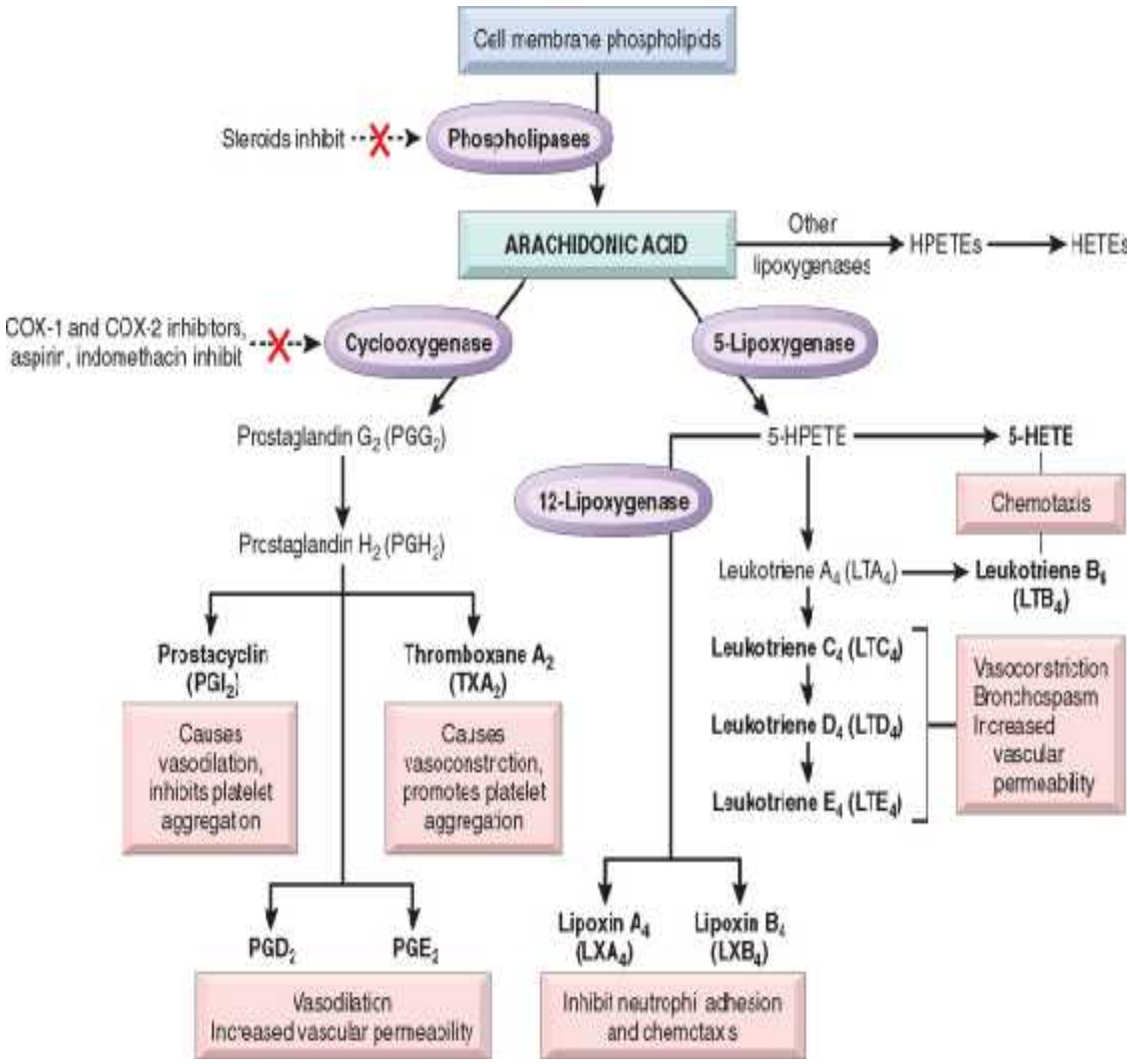


Figure 4: Catabolic pathway of Arachidonic acid (Kumar *et al*., 2015)

Herbal Ayurvedic remedies for inflammation

Several plants show anti-inflammatory activity that is used in the ayurvedic system of medicine, either singly or as mixtures. Various active compounds present in plant inhibits NF-κB, COX-2, 5-LOX, iNOS, TNF-α, IL-1β etc. which are responsible for their activity (Aggarwal *et al*., 2011). Table 3 enlists medicinal plants with anti inflammatory activity.

Table 3: List of medicinal plants shows anti-inflammatory activity

S.No	Plant Name	Family	Part used	Reference
1.	*Albizia lebbeck*	Leguminosae	Bark	Babu *et al*., 2009
2.	*Argyreia speciosa*	Convolvuceae	Root	Bachhav *et al*., 2009
3.	*Barleria prionitis*	Acanthaceae	Whole plant	Singh *et al*., 2013
4.	*Berberis aristata*	Berberidaceae	Root	Kumar *et al*., 2016
5.	*Boswellia serrata*	Burseraceae	Whole plant	Ismail *et al*., 2016
6.	*Calendula officinalis*	Compositae	Flower	Preethi *et al*., 2009
7.	*Cynodon dactylon*	Poaceae	Whole plant	Garg and Paliwal, 2011
8.	*Desmostachya bipinnata*	Poaceae	Root	Kumar *et al*., 2010
9.	*Garcinia mangostana*	Guttiferae	Fruit	Chen *et al*., 2008
10.	*Gymnema sylvestre*	Asclepiadaceae	Leaves	Malik *et al*., 2008
11.	*Indigofera tinctoria*	Fabaceae	Leaves	Sarkar *et al*., 2011
12.	*Kaempferia galanga*	Zingiberaceae	Whole plant	Vittalrao *et al*., 2011
13.	*Leucas cephalotes*	Labiatae	Whole plant	Baburao *et al*., 2010
14.	*Nigella sativa*	Ranunculaceae	Seed	Chehl *et al*., 2009
15.	*Ocimum sanctum*	Lamiaceae	Leaf	Mirje *et al*., 2014
16.	*Pandanus odoritissimus*	Pandanaceae	Leaves	Londokar *et al*., 2010
17.	*Phyllanthus amarus*	Phyllanthaceae	Leaves	Ofuegbe *et al*., 2014

18.	*Plumbago zeylanica*	Plumbaginaceae	Root	Dang *et al*., 2011
19.	*Rubia cordifolia*	Rubiaceae	Root	Kasture *et al*., 2001
20.	*Ruta graveolens*	Rutaceae	Aerial part	Ratheesh and Helen, 2007
21.	*Solanum nigrum*	Solanaceae	Berries	Ravi *et al*., 2009
22.	*Sphearanthus indicus*	Asteraceae	Flowers	Ali *et al*., 2011
23.	*Withania somnifera*	Solanaceae	Leaf	Shivamani *et al*., 2014
24.	*Xeromphis spinosa*	Rubiaceae	Bark	Das *et al*., 2009
25.	*Ximenia americana*	Oleaceae	Leaves	Shettar *et al*., 2015

Arthritis

Arthritis' means disease of or damage to the joints and comprises more than 100 different rheumatic diseases and conditions involves the breakdown of cartilage (normally protects a joint, allowing it to move smoothly), that also absorbs shock when pressure is placed on the joint, as during walking. Without the normal amount of cartilage, the bones rub together, causing pain, swelling (inflammation), stiffness and limited movement (Spector, 1990).

Types of Arthritis

Osteoarthritis: This is the most common type of arthritis. It is a degenerative disease primarily affects cartilage, which is the tissue that cushions the ends of bones within the joint. The cartilage begins to fray and may entirely wear away. The main characteristics of osteoarthritis (OA) are joint pain and stiffness. OA may also causes bone enlargement around the joints and occur in any joint, usually it affects the following areas like in the hand, shoulder, neck, lower back, hip, and knee (Gaby, 1999).

Rheumatoid Arthritis: Rheumatoid arthritis (RA) is a common autoimmune disease which is related with progressive disability, systemic complications, early death, and socioeconomic costs. It is a systemic inflammatory disease in which inflammation can occur in the lining of the joint that causes pain, stiffness, swelling, joint damage, and loss of function of the joints and supposed to be the result of a faulty immune system (Iain and Georg, 2011).

Juvenile Rheumatoid Arthritis: This is the most common form of arthritis in childhood, causing pain, stiffness, swelling, and loss of function of the joint. Rashes

or fevers are the main characteristics of it which may affect various parts of the body. It can continue for at least 6 weeks or often persists into adulthood that can result in major long-term morbidity, including physical disability (Beukelman *et al*., 2011).

Fibromyalgia: Fibromyalgia is defined as a common rheumatological syndrome characterized by widespread musculoskeletal pain particularly those of the neck, spine, shoulders, and hips accompanied by fatigue, sleep, memory and mood issues (Bertsias *et al*., 2010).

Systemic Lupus Erythematosus: Systemic lupus erythematosus (SLE) is the prototypic multisystem autoimmune disorder which can result in inflammation of and damage to the joints, skin, kidneys, heart, lungs, blood vessels, and brain. It is also known as lupus and etiology of SLE includes both genetic and environmental components with female sex strongly influencing pathogenesis. These factors lead to an irreversible break in immunological tolerance manifested by immune responses against endogenous nuclear antigens (Bertsias *et al*., 2010).

Spondyloarthropathies: spondyloarthritis is also known as spondyloarthropathy that related with inflammatory diseases including arthritis of the spine and peripheral joints as well as occur in the area where ligaments and tendons join to bones but it mainly affects the spine. The pain in the spine, legs, arms as joints, inflamed ligaments and tendons, skin rashes, eye, and intestinal problems are mainly associated with the spondyloarthritis (www.rheumatology.org).

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is common autoimmune disorder characterized by chronic inflammation, synovial membrane inflammation, and destruction of joints due to

progressive attrition of articular cartilage in the synovial joint through generation and infiltration of auto antibodies (Madhu and Harindran, 2014). It was first recognized in 1859 by Dr. A. Garrod when he described widespread joint pain and stiffness. RA was classified according to the 1987 American College of Rheumatology criteria (ACR) and 4 out of the 7 criteria to be met for the patient to be classified as having RA as mentioned in table 4 (Arnett *et al*., 1988).

Table 4: The 1987 American College of Rheumatology criteria

S.No.	**Qualifying criteria**
1	Morning stiffness lasting > 1 hour before improvement
2	Arthritis involving 3 or more joints
3	Arthritis of the hand (particularly MCP, PIP and wrist joints)
4	Symmetrical distribution of joint involvement
5	Positive Rheumatoid factor
6	Rheumatoid nodules
7	Radiographic evidence of RA
MCP: metacarpalphalangeal joints, PIP: proximal interphalangeal	

Etiology of RA

At present, the prevailing hypothesis is that RA is likely to be multi-factorial with intense interaction between causative factors. The exact etiology of RA is not completely understood, but genetic susceptibility plays an important role and hormonal factors, infectious agents, and environmental factors are also responsible for the cause of RA (Klareskog *et al*., 2006).

Genetic factors

Genetic factor has been shown to play an important role in the development of RA and it was noted in 1970 that many RA patients had a variation in the human leukocyte antigen (HLA) region of chromosome 6 (6q21.3) compared to the general population. Twin studies associate genetic factors in RA, with concordance rates of 15 to 30% among monozygotic twins and 5% among dizygotic twins (Spector, 1990). The long-established association with the human leukocyte antigen (HLA)–DRB1 locus has been confirmed in patients who are positive for rheumatoid factor and these HLA-DRB1 alleles are suggested to be involved in the etiology of RA. Among other genes, the tyrosine-phosphatase gene PTPN22 on chromosome 1 are also susceptible to the development of RA (Arnett *et al*., 1988).

Environmental factor

Observational studies have acknowledged a number of factors may either increase or reduce the risk of developing RA. High birth weight increases the risk of RA while breastfeeding may reduce the risk. Diet and stress have also been considered to play a possible role in the disease progression. Studies have shown that higher consumption of olive oil, oil-rich fish, fruit, vegetables and beta-cryptoxanthin may have a protective effect on the development of RA, whereas lower consumption of foods rich in antioxidants, could be associated with an increased risk of RA, also high intake of red meat and low intake of vitamin C might play a role in the development of inflammatory polyarthritis (Pattison *et al*., 2004). During a lifetime, exposure to multiple factors may increases this risk, including poor socio-economic status, low level of education, smoking and geographical location.

Hormonal factor

The greater risk of rheumatoid arthritis among women is more common than men with a ratio of 3:1 and Changes in the female hormonal environment related to pregnancy, menopause, and breastfeeding have been suggested to play an important role in the development of RA. Studies have demonstrated that the female sex hormones estrogen and progesterone may alter immune function by inhibiting Th1 responses (Mattsson *et al*., 1991).

Infectious agent

Infectious agents (e.g., *Epstein–Barr* virus, *cytomegalovirus*, *proteus* species, and *Escherichia coli*) and their products (e.g., heat-shock proteins) have long been linked with RA. During infection immune complex is formed which may trigger the induction of rheumatoid factor, a high-affinity autoantibody against the Fc portion of an immunoglobulin, which has long served as a diagnostic marker of rheumatoid arthritis and is implicated in its pathogenesis (Iain and Georg, 2011). The majority of infections have a propensity to produce transient symptoms, but infections such as *Borrelia burgdoferi* can result in a form of arthritis virtually indistinguishable from RA, characterized by a chronic disease course and evidence of erosions (Mackenzie and Dawson, 2005).

Pathophysiology of RA

Rheumatoid arthritis is best characterized as an immune-mediated inflammatory disease (IMID). Various immune modulators (cytokines and effector cells) and signaling pathways are involved in the pathophysiology of RA and the presence and

activity of a number of pro-inflammatory chemokines and cytokines have established roles in disease pathogenesis.

The role of T cells

RA has been considered as predominantly a T cell driven disease for many years. The RA condition shows the disturbances in the normal balance of CD8+ cytotoxic T cells and CD4+ helper cells and the ratio of both the cells increases. T cells have also been found in large quantity in the synovial tissue, indicating a potential role in producing some of the clinical manifestations of RA (Kinne *et al*., 1997). The activation and infiltration of T cells and macrophages in the synovium result in the production of interleukin-1, -2, -6, -8, -10, -17; tumor necrosis factor-α (TNF-α); platelet-derived growth factor; insulin-like growth factor; and transforming growth factor β. These effector molecules are concerned in synovial tissue inflammation and proliferation, cartilage and bone destruction, and systemic effects (Kirkham *et al*., 2006).

The role of B cells

B cells contribute to RA pathogenesis not only through antigen presentation but also through the autoantibody production, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP). This occurs when activated T cells stimulate the transformation of B cells into plasma cells (Choy, 2012). B cells also infiltrate in the synovium and differentiate into plasma cells, producing polyclonal immunoglobulin and RF. As well, synovial fibroblasts are activated, releasing collagenases and activating metalloproteinase gene expression, which leads to the destruction of matrix tissues. The pannus formation with articular cartilage invasion,

periarticular erosions and osteoporosis, and joint swelling with destruction of periarticular structures are the net result of above activities (Stolt *et al*., 2003).

The role of cytokines

Cytokines are responsible for maintaining homeostasis between the actions of proinflammatory [e.g. tumor necrosis factor alpha (TNFa), interleukin-1 (IL-1)] and anti-inflammatory [e.g. tumor growth factor beta (TGF b) interleukin-10 (IL-10)] cytokines. Due to the up-regulation of pro-inflammatory cytokines balance between the two opposing subsets of cytokines are disturbed in RA patients (Agarwal and Malaviya, 2005; Brennan *et al*., 1998). The release of each cytokine triggers a specific cascade of events which are given in below table 5.

Table 5: Actions of cytokines that play major roles in RA pathobiology (Choy, 2012)

Cytokine	Role in the disease process
TNF-α	*Local effects* Increased monocyte activation, cytokine release, PG release. Increased poly-morphonuclear leukocyte priming, apoptosis and oxidative burst. T-cell apoptosis, clonal regulation, TCR dysfunction. Increased endothelial cell adhesion molecule expression, cytokine release. Decreased synovial fibroblast proliferation, collagen synthesis. Increased MMP and cytokine release. *Systemic effects*

	Acute-phase protein production, HPA axis dysregulation (fatigue and depression), CVD promotion.
IL-6	*Local effects* Osteoclast activation, Neutrophil recruitment, Pannus formation via promotion of VEGF production, B-cell proliferation and antibody production, T-cell proliferation, and differentiation. *Systemic effects* Acute-phase protein production, Anaemia, CVD promotion, Osteoporosis, HPA axis dysregulation (fatigue and depression).
IL-1	*Local effects* Increased synovial fibroblast cytokine, chemokine, MMP and PG release, Increased monocyte cytokine, reactive oxygen intermediate and PG release, Osteoclast activation, Endothelial cell adhesion molecule expression. *Systemic effects* Acute-phase protein production, CVD promotion, HPA axis dysregulation (fatigue and depression).
IL-17	Recruitment of monocytes and neutrophils by increasing local chemokine production, Facilitation of T-cell infiltration and activation, amplification of immune response (e.g. by induction of IL-6 production), increased synovial fibroblast cytokine and MMP release, Osteoclastogenesis and cartilage damage,

	Synergistic activity with IL-1β, TNF-α, and IFN-γ.
VEGF	Angiogenesis, contributing to pannus formation.

Diagnosis

There is no unique test for the identification of RA but the diagnosis is made by recognizing a pattern of signs and symptoms such as prolonged morning stiffness that may be improved by activity, polyarthralgia or polyarthritis (or both), joint gelling, and fatigue. A serum rheumatoid factor is present in up to 75 percent in RA patient but is frequently negative in early disease. A more specific marker, anti cyclic citrullinated peptide 2 (CCP) antibodies, has recently been described and may be a useful marker in patients with early disease (Greiner *et al*., 2005; Liao *et al*., 2008). Characteristic radiographic findings of RA include periarticular osteopenia, joint space loss, and marginal joint erosions but these changes are not often seen in the early disease. Magnetic resonance imaging (MRI) is useful in detecting RA before radiographic changes can be detected. MRI is more sensitive in detecting erosions, and it is able to identify bone marrow edema and synovial hypertrophy. Ultrasonography is used occasionally in establishing a diagnosis of RA, and it is more sensitive in detecting synovial and tendon inflammation (Hoving *et al*., 2004).

Treatment of RA

The goal to be achieved in the treatment of RA is to reduce inflammation in the joints, relieve pain, prevent or slow joint damage, reduce disability and provide support to help the patient live as active a life as possible (Sizova, 2008). Non-steroidal anti-inflammatory drugs (NSAIDS) and Corticosteroids have potent anti-

inflammatory effects may be considered to reduce pain, stiffness, and swelling. The 20th century led to introduce the several diseases modifying anti-rheumatic drugs (DMARDs) for the management of RA. Various conventional DMARDs are recommended including methotrexate, gold, leflunomide, hydroxychloroquine, and sulfasalazine but methotrexate is an influential immunosuppressive and anti-inflammatory agent, which used as a first-line treatment for RA patients (Lee and Weinblatt, 2001).

The anti-TNF-α biological agents are a newer form for treatment of RA which included TNF-alpha inhibitors (adalimumab, certolizumab, etanercept, golimumab, and infliximab) but also some more recently developed compounds with different mechanisms, such as abatacept, rituximab and tocilizumab. But they are costly, require parenteral administration, and have been associated with serious and opportunistic infections and lymphoma. Physiotherapy, Physical exercise, occupational therapy, and rehabilitation play important roles in the management of RA (Fishman and Bar-Yehuda, 2010). NSAIDs and DMARDs in combination are the more efficient approach towards treatment of RA. However, besides being expensive they also inflict severe well known adverse effects such as gastrointestinal ulcergenicity, teratogenesis, cardiovascular complication and renal morbidity (Zheng *et al*., 2014). Therefore, contemporary research focus is shifted towards the development of novel, efficient and herbal anti-arthritic agent with higher efficacy and minimum side effects. Anti-arthritic activity of some medicinal plants is enlisted in table 6.

Table 6: Medicinal plants with Anti-arthritic activity

S.No	Plant Name	Family	Part used	Reference
1.	*Aconitum vilmorinianum*	Ranunculaceae	Root	Li *et al*., 2013
2.	*Alstonia scholaris*	Apocynaceae	Leaf	Arulmozhi *et al*., 2011
3.	*Aristolochia bracteata*	Aristolochiaceae	Whole plant	Chitme & Patel, 2009
4.	*Asystasia dalzelliana*	Acanthaceae	Leaf	Babushetty& Sultanpur, 2012
6.	*Capparis spinosa*	Capparidaceae	Fruit	Feng *et al*., 2011
7.	*Cassia auriculata*	Caesalpiniaceae	Leaf	Bandawane *et al*., 2014
8.	*Cassia uniflora*	Caesalpiniaceae	Leaf	Chaudhari *et al*., 2012
9.	*Cissampelos pareira*	Menispermaceae	Root	Amresh *et al*., 2007
10.	*Chenopodium album L*	Chenopodiaceae	Aerial part	Arora *et al*., 2014
11.	*Colchicum luteum* Baker	Liliaceae	Corm	Nair *et al*., 2011
12.	*Costus afer* Ker Gawl.	Zingiberaceae	Leaf	Anyasor *et al*., 2014
14.	*Cyathocline purpurea*	Asteraceae	Whole plant	Bihani *et al*., 2014
15.	*Elaeocarpus sphaericus*	Elaeocarpaceae	Fruit	Ramasamy *et al*., 2012
16.	*Ficus bengalensis*	Moraceae	Stem bark	Manocha *et al*., 2011
17.	*Glycosmis pentaphylla*	Rutaceae	Stem bark	Ramesh & Vijaya, 2012
18.	*Hybanthus enneaspermus*	Violaceae	Whole plant	Tripathy *et al*., 2009
19.	*Harpagophytum procumbens*	Pedaliaceae	Root	Andersen *et al*., 2004
20.	*Justicia gendarussa*	Acanthaceae	Leaf	Paval *et al*., 2009
21.	*Litsea cubeba*	Lauraceae	Root	Lin *et al*., 2013
22.	*Merremia emarginata*	Convolvulaceae	Whole plant	Purushoth *et al*., 2012
23.	*Machilus macrantha*	Lauraceae	Bark	Tatiya & Saluja, 2011

24.	*Mesua ferrea* Linn.	Cluciaseae	Seed	Jalalpure *et al*., 2011
25.	*Ocimum gratissimum*	Lamiaceae	Leaf	Madhu &Harindran,2014
26.	*Pongamia pinnata*	Fabaceae	Leaf	Arote & Yeole, 2011
28.	*Pistia stratiotes*	Araceae	Leaf	Kyei *et al*., 2012
29.	*Premna serratifolia* Linn	Verbenaceae	Wood	Rajendran & Krishna, 2010
32.	*Schefflera octophylla*	Araliaceae	Root bark	Chen *et al*., 2015
33.	*Syzygium cumini*	Myrtaceae	Seed	Kumar, 2008
34.	*Strychnos potatorum* L.	Loganiaceae	Seed	Ekambaram *et al*., 2010
35.	*Sida rhombifolia*	Malvaceae	Aerial part	Gupta *et al*., 2009
36.	*Vernonia anthelmintica*	Asteraceae	Seed	Otari *et al*., 2010
37.	*Vitex negundo* Linn	Verbenaceae	Leaf	Zheng *et al*., 2014
38.	*Wedelia calendulacea* L	Asteraceae	Leaf	Panchal *et al*., 2011
39.	*Xanthium srtumarium* L.	Compositae	Leaf	Patil *et al*., 2012
40.	*Xylopiaa ethiopica*	Annonaceae	Fruit	Obiri *et al*., 2014

Urolithiasis

The term urolithiasis is originated from three Greek words, ‘ouron’ (urine), ‘oros’ (flow), and ‘lithos’ (stone) which is the stone formation process in kidney, bladder, and urethra. Urinary tract and kidney stones are a worldwide problems associated with deposition of calcium, phosphates and oxalates (Singh *et al*., 2013). The population of all age groups from less than 1 year old to more than 70 years may be affected with this disease and their recurrence rate is approximately 10% within one year, 35% within five years, and 50% within 10 years. The stone forming belt in Asia has been extending across Sudan, Saudi Arabia, the United Arab Emirates, the Islamic Republic of Iran, Pakistan, India, Myanmar, Thailand, Indonesia and Philippines while in India, some parts of Maharashtra, Gujarat, Punjab, Haryana, Delhi, and Rajasthan are main stone belt region (Khaling *et al*., 2014).

Pathophysiology of urolithiasis

Mainly three events are responsible for the formation of calculi in urinary tract in the following sequence: urinary saturation, supersaturation, nucleation, crystal growth, aggregation of crystals, crystal retention, and, calculus formation (Figure 5). It may cause the intrusion in kidney drainage system and result in severe pain, bleeding, infection or kidney failure. Three different hypotheses are involved in the formation of stone and their growth.

1) The first hypothesis proposed that stones are formed due to the increased supersaturation of calcium oxalate in urine. After that, they can get fixed to the urothelium (frequently in the terminal section of the collecting ducts), and then grow slowly.

2) Second hypothesis suggests that formation of stone begins at the site of randall's plaque which reduces the urolith covering of renal papilla on which calcium oxalate and phosphate crystals are starts to deposited and then they can be retained during passing of urine by the kidney and serve as a nucleus for formation of future stone.

3) Third hypothesis included the formation of crystal nuclei in the lumen of a nephron and then stick to the apical surface of tubular epithelium which leads to crystal cell attachment. After that, crystal nuclei exposed to supersaturated ultra filtrate which enhance the further growth of this crystal, this would result in the plugging of nephron and lead to intratubular calcification (Evan, 2010).

Risk factors of urolithiasis

Gender and Race

In industrialized nations, the frequency of urolithiasis is higher in men than in women, with 50% recurrence rates in a lifetime (Wood *et al*., 2014). A study by NHANES (National Health and Nutrition Examination Survey) states that, men are more susceptible than women in the United state with a ratio of 3:1, prevalence was 7.1% in women and 10.6% in men while in other western countries it is 4.3% in women and 6.9% in men. It seems that women have lower concentrations of urinary calcium, oxalate and uric acid and higher concentrations of citrate than men. The stone disease occurs more often in white followed by Hispanics, blacks and Asians, irrespective of the geographic area concerned (Lopez *et al*., 2010).

Age

NHANES demonstrates that disease rate is increased in men up to the age of 65 years while 70 years in women (Krambeck *et al*., 2013). The age of onset of a lithic

disease depends on the nature of calculi. For example, in the first and second decades of life cystine stone begins to start, followed by calcium stones between the third and fifth decades, while uric acid stones usually found in later years, over 50 years old (Perdomo *et al*., 2015).

Geographic area

The incidence of urolithiasis depends on the geographical area, racial composition and socioeconomic status of the community. The possibility of urinary stone formation varies noticeably in different parts of the world. It affects populations of Asia (1-5%), Europe (5-10%), Brazil (5%), and North America (13%) (Perdomo *et al*., 2015). Western hemisphere (9.5% in Europe, 12% in Canada, 13-15% in the USA), are more prone to development of urinary calculi in adults than in the eastern hemisphere (5.1%). Although the highest risk has been reported in some Asian countries such as Saudi Arabia (20.1%) and South Western Asia represents a high-risk environment for urolithiasis (Lopez *et al*., 2010).

Diet

Dietary factor plays an important role in the pathogenesis of kidney stone formation, and risk of stone recurrence can be reduced by dietary modification. High intakes of dietary calcium, potassium, and total fluid decrease the risk of kidney stone formation, while supplemental calcium, sodium, animal protein, and sucrose may increase the risk for onset of disease. Around 10-20% of urinary oxalates arrive from dietary sources so the dietary reduction is advised for inhibition of calcium oxalate stone formation (Trinchieri, 2008).

Vitamin C acts as a precursor for formation of endogenous oxalates, so high doses

of vitamin C should be avoided in lithiasis patients. The excess animal protein (purine) is responsible for the uric acid stone formation. It is a by-product of purine metabolism and excreted in large quantities in the urine. Excess intake of protein produces urine with high uric acid concentration, supersaturation of urine uric acid, and a low pH, obligatory for formation of uric acid stones. There is no treatment is available for inhibition of uric acid crystal formation, so only dietary measures focus on reducing uric acid stone and enhancing urine volume (Singh *et al*., 2013).

Genetic Factor

Researchers have focused on identifying genes related to ureter calculi and made an effort to clarify the cause of urolithiasis and advancement in diagnosis and treatment of urolithiasis (Danpure, 2000). Recent genetic advances in urolithiasis indicate that polymorphism in manganese superoxide dismutase gene (Mn-SOD) is probably associated with urolithiasis through oxidative stress. Mn-SOD is one of the primary enzymes that scavenge potential harmful oxidizing species. According to Tugcu *et al*., (2007) substitution of A valine (Val) to alanine (Ala) at amino acid 16 has occurred in the mitochondrial targeting sequence of the Mn-SOD gene which is associated with an increase in urolithiasis risk.

Stress

Kidney stone formation may induce with stress which involves the hypophyseal-hypothalamus axis stimulation, leading to secretion of vasopressin which acts on the nephron at membrane of the collecting tubule and making it more permeable to water. Thus, water reabsorbed by it increases and causing the formation of hypertonic urine. Secondly, secretion of adrenocorticotropin acts through a

secondary hyperparathyroidism mechanism which increases serum calcium levels (Walters, 1986). Variation of cortisol, aldosterone, and catecholamines level in blood are occurred due to stress which activates the pituitary-adrenocortical axis and sympathetic-adrenal axis. Excretion of calcium in urine can be increased by cortisol, either by competing with aldosterone at the renal intracellular level or by inhibition of intestinal calcium absorption that affects bone metabolism (Arzoz *et al*., 2013).

Climate

The incidence of urolithiasis is higher in the tropics, especially in the summer. Where the risk of stone formation is annoyed by low urine volume due to high temperatures which increase sweating which lead to dehydration and causing reduced urine volume. The diuretic concentrations are increased which facilitates the crystallization of excreted substances in urine, thus triggering stone formation (Trincheiri *et al*., 2008). Hypotheses supported that hot climate induces the formation of urinary calculi due to dehydration. Additionally, sunlight exposure is also responsible for the stone formation which activates vitamin D and therefore increases the concentration of serum 25-hydroxyvitamin D that increases urinary calcium levels in summer (Wang *et al*., 2014).

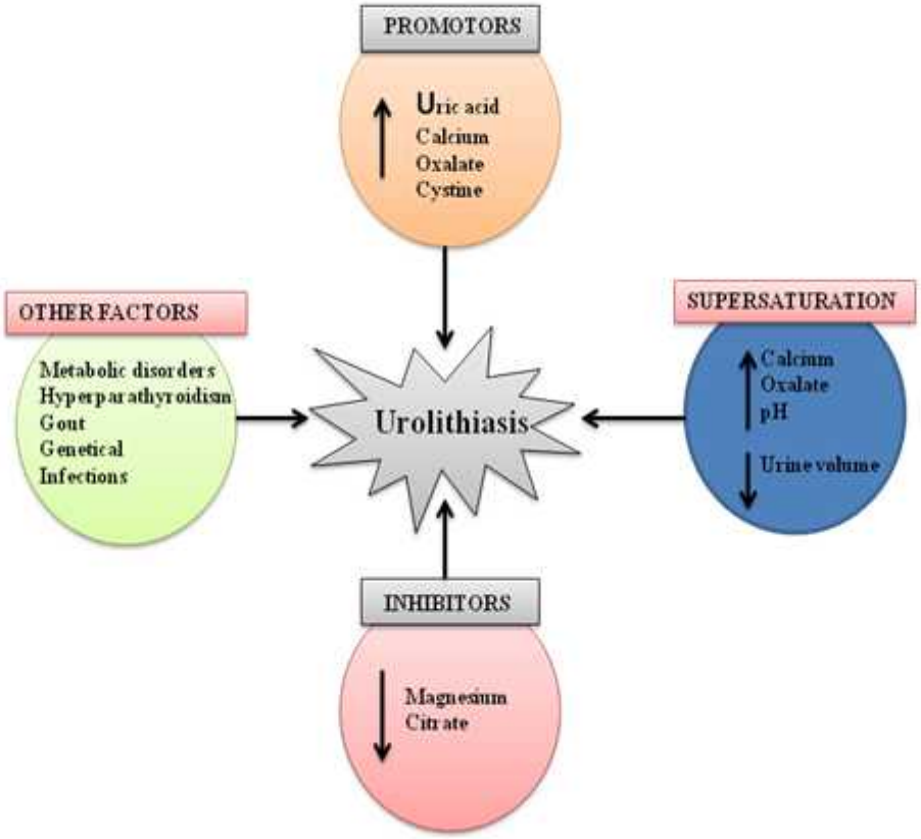


Figure 5: Pathophysiology of urolithiasis

Types of stone

Urinary stones can be classified according to its mineral composition. Calcium oxalate, struvite, uric acid and cystine stones are very common because these chemicals are present in the normal diet of a human being and build up bones and muscles. Uric acid is mostly present in all renal stones and arise in various sites in the urinary tract in the following order i.e. vesical < renal < ureteral < urethral but calcium oxalate is the chief component in the renal calculi (Khaling *et al*., 2014). Calcium containing stones are three types.

a) Calcium oxalate monohydrate (COM) or whewellite

b) Calcium oxalate dehydrate (COD) or weddellite,

c) Basic calcium phosphate or apatite

All above stones have occurred to an extent of 75-90% followed by magnesium ammonium phosphate (struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1% (Harsoliya *et al*., 2011). This struvite stone is also known as infection stone developed by infection in the urinary tract while uric acid stones are a bit less common and cystine stones are very rare. Cystine stones are greenish- yellow, flecked with shiny crystallites, rounded and moderately radio-opaque. Another type of stone is silicate or drug induced stones are very rarely seen, and can be occurred due to taking certain medications or herbal products and the subsequent build-up of chemicals from those products in the urine (Osborne *et al*., 2009).

Symptoms (Wells, 2000)

- Colic Pain
- Nausea and Vomiting
- Hematuria, Pyuria, Dysuria and Oliguria
- Renal tubular acidosis
- Hypercalciuria
- Hyperoxaluria
- Hypercitrateuria
- Hyperuricosuria

Current Management and Treatment

For treatment of urolithiasis initial step is to increase the daily fluid intake to at least 2.5 to 3L per day in conjunction with analgesic drugs and medications to examine salts that may increase or reduce formation of stones (Sean, 2005). The current approach for the treatment of urolithiasis is mainly associated with some allopathic agents like thiazide diuretics, alkali, allopurinol, penicillamine, analgesic, and probiotics. But most of them are not effective in all cases and have risks of long-term fertility (Samal *et al*., 2011). Despite these drugs some advanced techniques like extracorporeal shock wave lithotripsy and percutaneous nephrostolithotomy are also used but they have some side effects such as hemorrhage, tubular necrosis, and recurrence of renal stone formation and also these methods are costly and posses a great financial burden on society (Terlecki, 2007: Paik *et al*., 1998). From ancient periods, a number of medicinal plants playing an important role in treating the problem of renal calculi (Khan *et al*., 2010). Therefore, contemporary research focus is shifted towards the development of novel, efficient and herbal anti-urolithic agent with higher efficacy and minimum side effects (Table 7).

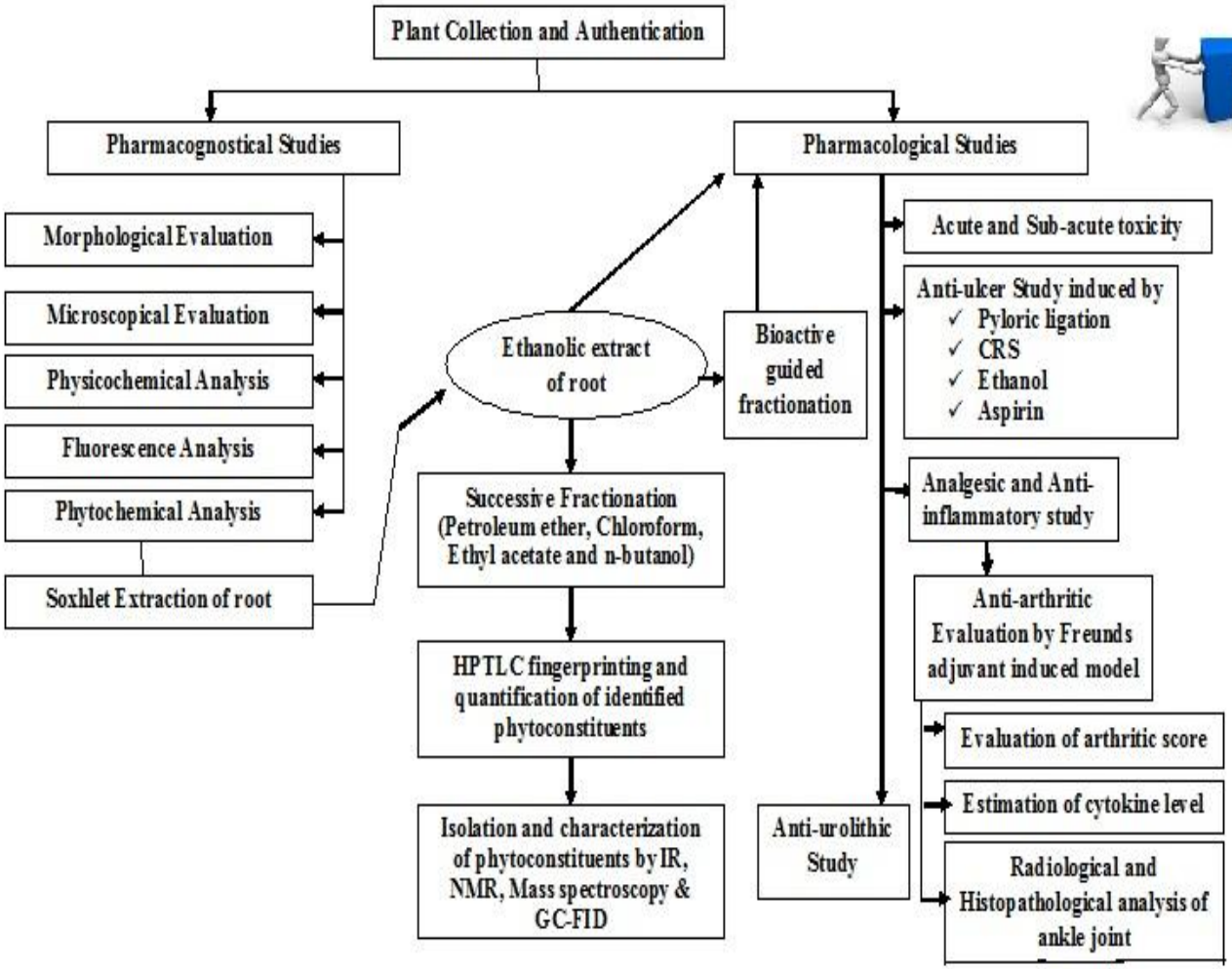
Table 7: List of medicinal plants with potent anti-urolithic activity

S.No	Plant Name	Family	Part used	Reference
1.	*Abutilon indicum L.*	Malvaceae	Leaf juice	Prachi *et al*., 2009
2.	*Acalypha indica Linn.*	Euphorbiaceae	Whole plant	Sathyaa *et al*., 2011
3.	*Achyranthes aspera L.*	Amaranthaceae	Root	Aggarwal *et al*., 2010
4.	*Aerva lanata*	Amaranthaceae	Whole plant	Soundararajan *et al*., 2006
5.	*Ageratum conzoides*	Asteraceae	Whole plant	Khan *et al*., 2011
6.	*Amaranthus spinosus*	Amaranthaceae	Root	Sharma *et al*., 2011
7.	*Amni visnaga*	Apiaceae	Whole plant	Yadav *et al*., 2011
8.	*Armoracia lopathifolia*	Crucifereae	Seed	Choubey *et al*., 2010
9.	*Benincasa Hispida*	Cucurbitaceae	Seed	Patel *et al*., 2011
10.	*Bergenia ligulata Wall.*	Saxifragaceae	Rhizome	Harsoliya *et al*., 2011
11.	*Bridelia crenulata Roxb.*	Euphorbiaceae	Stem bark	Singh *et al*., 2007
12.	*Caesalpinia huga L*	Caesalpiniaceae	Root	Chitme *et al*., 2010
13.	*Cansjeera rheedii*	Opiliaceae	Leaves	Vemurl *et al*., 2012
14.	*Cassia fistula*	Leguminosae	Wood bark	Ramesh *et al*., 2010
15.	*Cedrus deodara Roxb.*	Pinaceae	Heart wood	Ramesh *et al*., 2010
16.	*Corbichonia decumbens*	Molluginaceace	Leaf	Sharma *et al*., 2011
17.	*Cyclea peltata*	Menispermaceae	Root	Christina *et al*., 2002
18.	*Desmodium styracifolium*	Leguminosae	Whole plant	Hirayama *et al*., 2008
19.	*Dichrostachys cinerea*	Mimosaceae	Root	Jayakumari *et al*., 2011
20.	*Dolichous biflorus*	Fabaceae	Seed	Singh *et al*., 2010
21.	*Eleusine coracana*	Poaceae	Grains	Bahuguna *et al*., 2009
22.	*Ichnoccarpus frutescens*	Apocynaceae	Root	Anbu, 2011
23.	*Jasminum auriculatum*	Oleaceae	Flower	Bahuguna *et al*., 2009

24.	*Lantana camara*	Verbenaceae	Leaves	Mayee *et al*., 2011
25.	*Lawsonia inermis*	Lythraceae	Leaves	Kore *et al*., 2011
26.	*Moringa oleifera*	Moringaceae	Bark	Fahad *et al*., 2010
27.	*Nymphaea alba Linn.*	Nymphaeaceae	Leaves	Bhaskar *et al*., 2012
28.	*Origanum vulgare*	Lamiaceae	Aerial parts	Khan *et al*., 2011
29.	*Pergularia daemia*	Asclepiadaceae	Whole plant	Vyas *et al*., 2011
30.	*Pinus elderica*	Pinaceae	Fruit	Hosseinzadeh et al., 2010
31.	*Trachyspermum ammi*	Umbelliferae	Seed	Kaur *et al*., 2009

Plan of Work


PLAN


Plant Collection and Authentication
Pharmacognostical Studies
Pharmacological Studies
Morphological Evaluation
Microscopical Evaluation
Physicochemical Analysis
Fluorescence Analysis
Phytochemical Analysis
Soxhlet Extraction of root
Ethanolic extract of root
Bioactive guided fractionation
Successive Fractionation (Petroleum ether, Chloroform, Ethyl acetate and n-butanol)
HPTLC fingerprinting and quantification of identified phytoconstituents
Isolation and characterization of phytoconstituents by IR, NMR, Mass spectroscopy & GC-FID
Acute and Sub-acute toxicity
Anti-ulcer Study induced by
✓ Pyloric ligation
✓ CRS
✓ Ethanol
✓ Aspirin
Analgesic and Anti-inflammatory study
Anti-arthritic Evaluation by Freunds adjuvant induced model
Evaluation of arthritic score
Estimation of cytokine level
Radiological and Histopathological analysis of ankle joint
Anti-urolithic Study