REVIEW OF LITERATURE

a) Pharmacological Review of Literature

Exhaustive literature review has shown that different extracts of various parts and the whole plant of *Aerva lanata* have been screened for various pharmacological activities, such as antidiabetic, antiurolithiatic, antioxidant, diuretic, anti-inflammatory, immunomodulatory, hepatoprotective, antimicrobial, cytotoxic properties.

- Yadav et al (2011), in their review on herbal plants used in the treatment of urolithiasis mentioned that the study gives evidences regarding mechanism of action of medicinal plants against experimentally induced nephrotoxicity. Hence, the review of the study has concluded that the herbal drug possesses nephroprotective activity and it has been proven by different animal models which gives many links to develop the future trials and also mentioned about the antiurolithiatic potentials of *Aerva lanata*.

- Ramachandran S et al (2011), evaluated poly-herbal formulation containing aqueous and ethanolic extracts of *Aerva lanata* for their antilithiatic activity on ethylene glycol induced lithiasis in Rats.

- Surendra KP et al (2011), in their review article reported that a wide range of plants and plant-derived products are used in folk medicine for the treatment of Urolithiasis as a prophylactic agent or as curative agent. Most of them found to be effective, but still the complete mechanism of action of these herbal drugs remains to be unclear. In present review authors discussed regarding the various mechanism of action through which phytotherapeutical agents exert their antiurolithiatic effect. Unlike allopathic...
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medicines which targets only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology and *Aerva lanata* is used as antulithotiatic and diuretic drug in India.\(^{24}\)

- Singh Sunder et al (2011), reported that they have screened the extract for antidiarrhoeal activity in rats and they found that doses of extract showed significant protection against PGE2 induced enteropooling, which might be due to the inhibition of synthesis of prostaglandins that is responsible for diarrhoea.\(^{42}\)

- Anantha D et al (2010) have reported that the aqueous and alcoholic extracts of seed and leaves of *Aerva lanata* have shown in vitro antihelmentic activity\(^{38}\).

- Rajesh R et al (2010), in their review on Phytochemical and pharmacological activities of *Aerva lanata* and have reported that aqueous and methanolic extracts of *Aerva lanata* shown various activities like lithiatic, diuretic, antimicrobial antitumour etc\(^{48}\).

- Veronika B and Saeed RK (2009), in their review on herbal medicines in the management of urolithiasis, made an exhaustive review of the plants for antiurolithiatic activity and mentioned that data obtained from in vitro, in vivo and clinical trials reveal that phytotherapeutic agents could be useful either as an alternative or as an adjunctive therapy in the management of urolithiasis. They mentioned that present review therefore critically evaluates the potential usefulness of herbal medicines in the management of Urolithiasis. They also reported that *Aerva lanata* is a reliable plant used for various indications in India \(^{34}\).
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➤ Sethi A and Sharma RA (2011), reported that there was a direct correlation between total phenolics present and antioxidant activities which could introduce phenols as the main antioxidant of *Aerva tomentosa* extracts and offering effective protection from free radicals. Hence the phenolic constituents present in *Aerva tomentosa* Forska is responsible for antioxidant activity of the plant\(^{51}\).

➤ Soundarajan P et al (2005), reported that aqueous suspension of *Aerva lanata* has reduced the crystal synthesizing enzymes and diminished the parameters of crystal deposition in kidneys. Also reported that *Aerva lanata* has produced reduction in the size of calcium oxalate crystals in ethylene glycol induced rats\(^{36}\).

➤ Liliana H V (2011), reported regarding the isolation of amyrin from several plants and have shown anti-microbial, anti-inflammatory and other interesting biological activities\(^{52}\).

➤ Chakraborthy (2011), reported that petroleum ether extract showed the presence of fatty acids, chloroform extracts showed the presence of triterpenes and sterols. Flavonoids, carbohydrates, amino acids and saponins were present in methanol extract and saponins, phenolic substances and tannins were present in the water extract of *Mirabilis jalap*\(^{58}\).

➤ Yang Xie et al (2012), isolated and purified terpenoids from *Celastrus aculeatus* Merr by high-speed counter-current chromatography. The structures were identified by using spectroscopic methods including ultraviolet (UV), electron ionization mass spectrometry (EI-MS), hydrogen nuclear magnetic resonance \(^1\)HNMR and \(^{13}\)CNMR. They have reported that Nimbidiol and pristimerin were isolated from *C. aculeatus* Merr for the first time\(^{94}\).
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➢ Narendra Vyas et al (2012), reported that evaluate the antiurolithiatic activity of ethanolic extract of roots (ELC 200mg/kg) and oleanolic acid (O.A. 60 mg/kg, O.A. 80 mg/kg, O.A. 100 mg/kg) isolated from roots of Lantana camara in albino Wistar male rats using ethylene glycol induced Urolithiasis model.\(^9\)

b) Phytochemical Review of Literature

Exhaustive review of literature for Aerva lanata and other plants used as antiurolithiatic drugs (Pashanabheda) was made for the chemical constituents which are responsible for the activity. This has revealed the presence of some important phyto constituents in all these plants are responsible for antiurolithiatic activity.

➢ Fatemeh Fathiazad et al, (2006) reported that three flavonoids, apigenin, quercetin and rutin, have been isolated from waste tobacco leaves, and their identities have been confirmed by UV-visible, 1H-NMR and 13C-NMR spectroscopy. By using analytical HPLC, the amount of rutin present in the tobacco leaves, before and after fermentation, and also in waste tobacco leaves, has been determined as 1.5, 0.5 and 0.6%, respectively.\(^9\)

![Rutin](image)

Rutin
Perez RM et al (2008), reported regarding the antiurolithiatic activity shown by two important constituents 7-Hydroxy-2′,4′,5′-Trimethoxyisoflavone and 7-Hydroxy-4′-Methoxyisoflavone from *Eysenhardtia polystachya*.

Gallo and Sarachine MJ (2009) in their review on biological activities of Lupeol, a triteprenoid and its derivatives from different plants mentioned that it is responsible for various activities like anti-inflammatory, antiurolithiatic, antitumour, hepatoprotective, antimicrobial and antiprotozoal activities.

Nardev Singh (2009), reported that *Berginia ligulata* used very commonly as antiurolithiatic drug in formulations in India and it contains mainly sitosterol, stigmesterol, tannic acid and gallic acid.

Consolacion Y et al (2009) reported that the dichloromethane extract of the air-dried leaves of the endemic and endangered Philippine trees, *Ficus pseudopalma* and *Ficus*
ulmifolia afforded squalene, polyphenol, β-amyrin fatty acid ester, α-amyrin acetate and β-amyrin acetate. F. pseudopalma also yielded lupeol fatty acid ester, lupenone, oleanone, and ursenone, while F. ulmifolia also afforded lutein, lupeol acetate, β-carotene, phytol, α-amyrin fatty acid ester, sitosterol, and stigmasterol. Their structures were identified by NMR spectroscopy

Javed I and Mohammad A (2009) reported that the ethyl acetate extract of the roots of Citrus sinensis yielded a flavonoid. The compound was characterized as 5, 8-dihydroxy-6, 7, 4′-trimethoxyflavone on the basis of UV, I.R, mass and N.M.R (1H, 13C) spectral studies

Geetha K (2010) et al reported that triterpene β-amyrin was isolated from alcoholic extract (70 % v/v ethanol) of leaves of Salvadora persica and the effect of oral administration of beta-amyrin on calcium oxalate Urolithiasis has been studied in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium, phosphate and oxalate.
Yamunadevi et al (2010), reported that the Chromatographic fingerprint analysis of the methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 30 different types of steroids with 30 different Rf values from 0.04 to 0.97. Maximum number (11) of steroids has been observed in leaves followed by root.

Rout OP et al (2010), reported that the preliminary pharmacognostic and phytochemical investigation of leaf extract of *Coleus aromaticus* Benth. has shown the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, tannins, phenolic compounds and terpenoids. This plant has been used as antilithiatric drug traditionally.

Chakraborthy GS and Ghorpade P M (2010) in their article reported that *Calendula* contain Quercetin, confirmed by TLC and by qualitative test. Thus it was quantified using HPTLC a sensitive method for development of marker compounds. The method was carried out on precoated TLC aluminum plates with silica gel 60 GF as stationary phase using solvent system as Chloroform: Methanol (9.5: 0.5) with Rf value of 0.43. Quantitative analysis was carried out in the absorbance at 366 nm.

Yamunadevi M et al (2011), reported that the chemical profile Studies of *Aerva lanata* L. using HPTLC shown the presence of flavanoids. The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 21 different types of saponins with 21 different Rf values with range 0.01 to 0.98. Maximum number (9) of saponins has been observed in roots followed by stem (8). Among the three different saponins of reproductive parts (flowers and seeds), two saponins with Rf values 0.45 and 0.82 are unique to reproductive parts only. The saponins with the Rf value 0.42 is present commonly in all the vegetative parts of the plant. The
saponins with Rf values 0.46, 0.49, 0.63, 0.73, 0.80 and 0.92 showed their unique presence only in the stem. The saponins of root also showed their uniqueness by the expression 0.01, 0.11, 0.26, 0.54, 0.55 and 0.74 in the saponins profile.

Manoj Goyal et al (2011), reported in their review article on the phytochemistry and pharmacological aspects of *Aerva lanata* that phyto constituents present in the plant include alkaloids (ervine, methyleervine, ervoside, aervine, methylaervine, aervoside, ervolanine, and aervolanine), flavanoids (kaempferol, quercetin, isorhamnetin), lupeol, lupeol acetate benzoic acid, β-sitosteryl acetate and tannic acid. Pharmacological studies reported are diuretic, anti-inflammatory, hypoglycemic, anti-diabetic, antiparasitic, antimicrobial, hepoprotective, anti-uroolithiasis, antiasthmatic, antifertility and hypolipidemic properties.

Rajesh R et al (2011), have reported that *Aerva lanata* is an important source of medicinally important constituents such as O—acyl glycosides, beta Sitosterol, Daucosterol, Syringic acid, Vanillic acid, tyramine, Aervitrine which are important in the management of various diseases including diuresis and lithiasis.

Adepu A (2013) in their review reported that *Aerva lanata* is one of the most important medicinal plant used for many diseases and disorders. The presence of phytochemical constituents such as alkaloids, flavonoids, tannins etc and minerals such as sodium, potassium, calcium, chloride etc play a therapeutic role in pathologic conditions. The plant exhibited diuretic activity, anti-inflammatory, antihyperglycemic, urolithic, anti-hyperlipidemic and so on.
c) **Antimicrobial activity review of literature**

Exhaustive review of literature for *Aerva lanata* and other plants used as antimicrobial agents were carried out against microorganisms reportedly responsible in urinary tract infections.

- Zanetti G et al (2008) reported that urinary tract infections and urosepsis are complications which can precede or follow a kidney stone treatment. Often the stones themselves are the source of infection, whether they are infectious stones or not. Systemic infections are difficult to foresee, and neither a pre-operative negative urine culture nor an antibiotic prophylaxis avoid infectious complications for certain. The primary predictive risk factors of urosepsis are: patient conditions, urinary tract infection or a history of recurrent infections, characteristics of the stone, and anatomy of the urinary tract.

- Afshin SA et al (2011) reported the complications of urinary tract infection and urolithiasis in children of Iran. He has reported that urolithiasis remains a serious problem in children in their country. Family history of urolithiasis, urologic abnormalities, especially under the age of 5 years, metabolic disorders, and urinary tract infections tend to be associated with childhood urolithiasis.

- Amutha K (2010) reported that methanolic extract of *Aerva lanata* showed considerable activity against *Proteus mirabilis* (43.33%), *Salmonella paratyphi* B (41.11%), *Bacillus subtilis* (37.78%) and *Candida tropicalis* (36.67%). *B. diffusa* showed good antimicrobial activity against *Bacillus subtilis* (32.22%), *Salmonella paratyphi* B (38.89%), *Candida tropicalis* (36.37) and *Proteus mirabilis* (34.44%). *C. tora* did not show any activity against *Staphylococcus aureus*, *P. aeruginosa*, *E. coli* and *Candida albicans*. Among all the four plants that were taken for study, *P. pinnata* showed very good activity against most of the pathogens.
Goyal M et al (2011) reported that ethyl acetate and methanol extracts of *Aerva lanata* whole plant showed interesting antimicrobial activities against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Shigella shiga, Shigella sonnei, Shigella flexneriae, Shigella boydii, Klebsiella, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Hensinela californica and Rhizopus oligosporum and petroleum ether, ethyl acetate and methanol extracts showed significant cytotoxic properties.

Payal Chawla (2012) reported that there are approximately 28 species of *Aerva* genus, but only a few species are medicinally useful of which *A. persica, A. lanata and A. familial* are of great value. A number of flavonol glycosides (e.g., aervanone, kaempferol-3-galactoside, isorhamnetin-3-O-beta-D-glucoside) have been reported from Acme, persica as major phytoconstituents and the minor constituents are betacyanins (glycine betaine and trigonelline), sterols and carbohydrates. Roots and flowers are reported to possess hypoglycemic, antioxidant, anthelmintic, analgesic, antimalarial, antiviral activities and medicinal properties against rheumatism and kidney troubles.

Muthukumaran P (2011) reported that the investigation carried out evaluated the antioxidative and antimicrobial activities of the methanol and aqueous extracts of *Aerva lanata* aerial parts. Three principal bioactive compounds such as saponins, flavonoids and tannins are positive for both the extracts, alkaloids are detected only in methanol extract and are absent in aqueous extract. Both methanol and aqueous extracts have shown promising antibacterial activity against gram positive bacteria viz. *B. subtilis and S. aureus*.

Gurumurthy H et al (2009) reported that the petroleum ether, chloroform and methanol extracts of *Aerva lanata* were screened against four gram-negative bacteria (*Escherichia coli ATCC 69314, Klebsiella pneumoniae NCIM 2719, Pseudomonas*...
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aeruginosa NCIM 2200 and Agrobacterium tumefaciens NCIM 2943) and two gram-positive bacteria (Staphylococcus aureus NCIM 2080 and Bacillus subtilis MTCC 441). They have performed in vitro antibacterial activity by agar well diffusion method. All the three extracts showed promising inhibitory activity against both gram-negative and gram positive bacterial strains tested. The extracts exhibited high degree of sensitivity against gram-negative bacteria. Among the three extracts, methanol extract was highly active and it had a particular good activity against Pseudomonas aeruginosa. Agrobacterium tumefaciens, Staphylococcus aureus and Bacillus subtilis and chloroform extract was highly active against Pseudomonas aeruginosa and Klebsiella pneumoniae. The most susceptible bacterium was E. coli.

Srujana M et al (2012) reported that The Antibacterial testing of stem extract of Aerva lanata was evaluated by Agar well diffusion method using gram positive bacteria like Staphylococcus aureus, Bacillus subtilius, gram negative bacteria like Escherichia coli, Klebsiella pneumoniae. Amongst the test extracts, the results suggested that, Ethyl acetate, Ethanol extracts of stem showed significant antibacterial activity compared with standard drug.

d) Molecular docking studies review

Exhaustive review of literature for Oxalate oxidase which has reportedly participated in Urolithiasis mechanism and its docking studies by different compounds.

Khobragade CN et al (2011) reported that in humans oxalate is end product of protein metabolism, with no enzyme remained to act on it. In conditions of its enhanced endogenous synthesis or increased absorption from the diet, oxalate accumulation leads to hyperoxaluria which can further lead to a number of pathological conditions including Urolithiasis. Present study, used Hordeum vulgare OxOx crystal structure.
(PDB ID 2ET1A) as a template for constructing 3D models of OxOx from *Triticum aestivum, Arabidopsis thaliana, Sclerotiana sclerotiarum*. Similarly Homology models for isoforms Ceriporiopsis subvermispora 336, C. subvermispora 422 were constructed by using template *Bacillus subtilis oxalate decarboxylase* (Oxdc) (PDB ID 2UY8A) by comparative modeling approach in SWISS MODEL, MODELLER, 3D JIGSAW and GENO 3D program server.\(^{114}\)

> Opaleye O et al (2006) mentioned in his article that Oxalate oxidase (EC 1.2.3.4) catalyzes the conversion of oxalate and dioxygen to hydrogen peroxide and carbon dioxide. In this study, glycolate was used as a structural analogue of oxalate to investigate substrate binding in the crystalline enzyme. The observed monodentate.\(^{115}\)

> Requena L (1999) reported that Oxalate oxidase (EC 1.2.3.4) catalyses the conversion of oxalate and dioxygen into CO\(_2\) and H\(_2\)O\(_2\). The barley (*Hordeum vulgare*) seedling root enzyme was purified to homogeneity and shown by metal analysis and EPR spectroscopy to contain Mn (II) at up to 0.80 atom per subunit. The involvement of Mn and neither flavin, Cu nor Fe in the direct conversion of dioxygen to H\(_2\)O\(_2\) makes oxalate oxidase unique. A model of the active site of the holoenzyme based on a homology model of the apoenzyme is proposed.\(^{111}\)
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- Dahiya T and Pundir CS (2013) studied and reported to reduce urinary oxalate excretion employed liposome encapsulated oxalate oxidase in animal model. EMA-oxalate oxidase encapsulated liposome caused oxalate degradation in experimental hyperoxaluria indicating that the enzyme could be used as a therapeutic agent in hyperoxaluria leading to urinary stones\textsuperscript{113}.

e) Analytical method development

Exhaustive review of literature for developing analytical method and its validation for Quercetin and other naturally isolated compounds was carried out.

- Oleszek WA (2002) reported in his article on chromatographic estimation of plant Saponins using TLC/HPTLC. He emphasized that the biological and spectrophotomeric methods still being used for saponin determination provide, to some extent, valuable results on saponin concentrations in plant material. Standardisation and identification of the peaks in HPLC chromatograms has been based on comparison of the retention times with those observed for authentic standards. But new hyphenated techniques, combining HPLC with mass spectrometry and nuclear magnetic resonance are developing rapidly and allow on-line identification of separated saponins. Capillary electrophoresis has been applied for saponin determination only in a limited number of cases and this method is still being developed\textsuperscript{117}.

- Vijaya Sri K (2009) reported that a simple reversed-phase liquid chromatography method was developed for the quantitative determination of Quercetin. The author reported that method was simple, sensitive and highly selective and involves single extraction of drug from plasma in (4:1) ratio of methanol: DMSO. The mobile phase was pumped at a flow rate of 1.0 mL/min and the effluent was monitored at 370 nm. The retention time of Quercetin was 2.72 min. The limit of detection of drug in plasma was found to be 0.2 g/mL\textsuperscript{118}.
Sajeeth CI et al (2010) reported that An HPTLC method was developed for the quantitative estimation of Gallic acid, Rutin, and Quercetin from aqueous and ethanolic extract Eruca sativa, precoated HPTLC silica gel 60 F254 as stationary phase and mobile phase for gallic acid Toluene: Ethyl Acetate: Formic Acid [7:5:1 v/v/v/v/v] and mobile phase for Quercetin and rutin, ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v/v/v/v]. Detection and quantification were performed densitometrically at λ 280 nm for Gallic acid, 280 nm Quercetin and 366 nm for Rutin. The standard Rf values of Gallic acid, Quercetin, and Rutin are 0.35±0.01, 0.98±0.01 and 0.34±0.02 respectively.\(^{116}\)

Je-Chiaun Ye et al (2010), in their research article reported that analytical method development is very essential to determine the amount of β-sitosterol in medicinal plants. Hence they have developed analytical method for β-sitosterol using HPLC.\(^{120}\)

Verma N (2013) reported that Extract yield at optimum condition was then analyzed by high performance liquid chromatography (HPLC) for quantifying bioactive flavonoid compounds. The wavelength for maximum absorption of Quercetin is 365 nm and the flow rate was maintained at 0.5 ml/min.\(^{119}\)

**Botanical Review of Literature of Aerva lanata** L. (Ex. Juss. Schult) \(^{16-19; 35-40;}\)

**Plant description:**

**Name:** *Aerva lanata* (Linn.) Juss. Ex Schultes)

Family: Amaranthaceae

**Taxonomy**

Kingdom: Plantae (Plants)
Sub-kingdom: Tracheobionta (Vascular plants)

Division: Magnoliophyta (Angiospermes, flowering plants)

Class: Magnoliopsida (Dicotylédones)

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Amaranthaceae

Genus: Aerva

Species: Aerva lanata (L.) A. L. Juss. Ex Schultes

Common names

Ayurveda: Paashaanabheda, Gorakshaganjaa, Aadaanpaaki, Shatkabhedi

Bengali: Chaya

Rajasthani: Bhui

Sindhi: Bhui, Jari

Punjabi: Bui-kaltan

Hindi: Gorkhabundi, Kapurijadi

Marathi: Kapurmadhura, Kapurimadhuri, Kapurphuti, Kumra
Morphology

Herb, erect or prostrate with a long tap-root, branched from near the base; branches many, pubescent or wolly-tomentose, striate.

Leaves alternate, 2-2 × 1-1.6 cm on the main stem, 6-10 × 5-6 mm on the branches, elliptic or obovate, or subotbicular, obtuse or acute, entire, pubescent above, more or less white with cottony hairs beneath; petioles 3-6 mm long, often obscure.

Flowers greenish white, very small, sessile, often bisexual, in small dense subsessile axillary heads or spikes 6-13 mm long, often closely crowded and forming globose clusters; bracteoles 1.25 mm, long, membranous, broadly ovate, concave, apiculate. Perianth 1.5-1.25 mm long; sepals oblong, obtuse, sometimes apiculate, silky-hairy on the back. Utricle broadly ovoid, acute; stigmas two, seed 0.85 mm in diameter, smooth and polished, black.

Active constituents—Aerva lanata contains

a) Alkaloids
b) Glycosides, Terpenoids, saponins
c) Polyphenolic compounds
d) Volatile oils, Fatty acids

Traditional Uses:

It is commonly used in Ayurvedic medicines and recommended in texts for various diseases. The plant is used as astringent, bitter, cooling, emollient, vermiuge, supparative, diuretic and lithontriptic. It is useful to treat boils, cephalalgia, cough, strangury and lithiasis. The plant has useful medicinal value; the extract is proved for
nephroprotective activity, diuretic effect, cytotoxicity and antioxidant, immunomodulatory effect, diuretic effect, anti-inflammatory effect, antimicrobial activity, hepatoprotective activity, antihyperglycemic effect.

Major uses are:

- Astringent,
- Bitter tonic,
- Emollient,
- Vermiuge,
- Supparative,
- Lithiatic
- Diuretic