Chapter 2

Review of Literature
2. REVIEW OF LITERATURE

2.1 THE GENUS ZANTHOXYLUM

The genus Zanthoxylum belongs to the family Rutaceae, subfamily Rutoideae, in the tribe Zanthoxyleae. Zanthoxylum originates from the word Xanthoxylum which derives from Greek word "xanthon xylon" that means "yellow wood", hence both terms Xanthoxylum or Zanthoxylum are used by the authors.

The tribe Zanthoxyleae has two taxa, Fagara L with over 240 species and Zanthoxylum L. having only 15 species (Fish and Waterman, 1973). Brizicky in 1962 suggested that Fagara be a sub-genus under Zanthoxylum on the basis that the simple parianth of Zanthoxylum was a secondary condition derived from Fagara by abortion of some or all of the sepals and Hartley in 1966 combined the two taxa under Zanthoxylum (Fish and Waterman, 1973). The genus is a rich source of various chemicals such as alkaloids, amides, flavanoids, lignans, sterols and terpenes etc. (Medhi et al., 2013). Zanthoxylum genus consists of about 549 species scattered all over the world mainly in tropical and temperate regions.

2.2 MEDICINAL USES OF ZANTHOXYLUM SPECIES

The genus Zanthoxylum has wide ethnobotanical importance; some species are used as sources of pharmaceutical and cosmetic raw materials (Bafi-Yeboa et al., 2005) and others are used as antiplasmodial, larvicidal, anti-inflammatory, analgesic, antinociceptive, antibiotic, hepatoprotective, cytotoxic, antiproliferative, anthelmentic, antiviral, antifungal, anticonvulsant and anti-oxidant activities (Negi et al., 2011). As per Kritikar and Basu (1983), the fruits are used as digestive appetizer, to cure asthma and bronchitis, eliminate pain, disease of mouth, teeth and throat, treat heart diseases, piles, disorder, dyspepsia and diarrhoea. Oliver (1982), states that the root bark and
leaves of many Zanthoxylum species are used in many medicinal preparations for curing stomachache, toothache, cough, leprous ulcerations, urinary and venereal diseases, rheumatism and lumbago (Negi et al., 2011).

Figure 8: Photograph of Zanthoxylum alatum Roxb.

Isolation and Characterization of Anticancer Principles from Zanthoxylum alatum Roxb.
2.3 GENERAL DESCRIPTION OF ZANTHOXYLUM ALATUM

*Zanthoxylum alatum* Roxb. (ZA) (Syn. *Zanthoxylum armatum* DC., Family, Rutaceae), is a shrub, having a strong aromatic odour generally known as ‘prickly ash’ or ‘Timur’ or ‘Kababe Tejal’. It is an armed scandent or erect, 6 m. tall or more, with dense foliage, found in the hot valleys of the Himalayas (Wealth of India, 1998). The bark, fruits and seeds are extensively used in indigenous system of medicine as carminative, anti-inflammatory, stomachic and anthelmintic. The bark is pungent and used to clean teeth and exhibit antiproliferative activity against the growth and multiplication of human keratinocytes (Kumar et al., 1999). The stem exhibited hypoglycaemic activity in preliminary trials in experimental rats (Rahman and Zaman, 1989). The seeds are used in fever and dyspepsia, anti-inflammatory (Paridhavi and Agrawal, 2006).

2.4 GEOGRAPHICAL SOURCE

ZA is a deciduous shrub or small tree which grows in well drained alluvial, black soil. In India, it is found in the warmer valleys of the Himalaya from Jammu and Kashmir to Assam and Khasi (1,000 to 2,100 m), in the Eastern Ghats in Orissa and Andhra Pradesh (1,200 m) and the lesser Himalayan regions in the northeastern part of India for example, Naga Hills, Meghalaya, Mizoram, and Manipur (Kala et al., 2005) (Nadkarni, 2002; Baqer, 1989; Shinwari et al., 2003).
2.5 COMMON NAMES

English - Toothache tree
Hindi - Tumru, Timbur, Tejphal
Marathi - Tejbal
Oriya - Tejbala
Bengali - Tejovati, Tum
Punjabi - Tejbal, Tezbal
Urdu - Tamur
Nepal - Timur
Manipuri - Mukthrubi

2.6 TAXONOMICAL CLASSIFICATION

Domain - Eukaryota
Kingdom - Plantae
Subkingdom - Viridaeplantae
Phylum - Tracheophyta
Subphylum - Euphyllophytina
Infra phylum - Radiatopses
Class - Magnoliopsida
Subclass - Rosidae
Superorder - Rutanae
Order - Rutales
Suborder - Rutineae
Family - Rutaceae
Subfamily - Rutaceae
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Tribe - Zanthoxyleae
Genus - Zanthoxylum
Species - alatum (Medhi et al., 2013)

2.7 PLANT PART USED
Seeds, Fruits, Leaves & Stem-Bark

2.8 MACROSCOPY OF ZANTHOXYLUM ALATUM ROXB.
Figure 9, shows the images of different parts of the plant.

2.8.1 Stem Bark
Dried stem bark pieces are curved or channelled up to 8 cm in length and 0.2 to 0.3 cm in width, rough, longitudinally ridged and fissured with prominent large circular tubercular prickles or circular and shallow depressions left by them, numerous protruding lenticels, internally corrugated. Fracture is short, aromatic and pungent odour with astringent taste (Gupta et al., 2006).

2.8.2 Flowers
The flowers are small, yellow, usually unisexual, and borne in dense lateral panicles. The flowers are green or yellow, in dense terminal and occasionally axillary sparse panicles (Wealth of India, 1998).

2.8.3 Dried Fruits
Fruits are reddish-brown in colour, shape is sub-globose, mostly dehisced, follicles, consist of one seed in each follicle. Seeds are globose, glabrous, shiny black; up to 0.5 cm long, and about 0.3cm wide; pungent in taste with aromatic odour. The fruit is usually a
isolated carpel dehiscing ventrally, about 3 mm in diameter and tubercled (The Ayurvedic Pharmacopoeia of India, 1999).

2.8.4 Leaves
Leaves are imparipinnate or trifoliate, alternate, 5-23 cm long often with flattened prickles. Leaflets are up to 5 pairs, opposite, ovate to lanceolate, entire to glandular-crenate, acute to obtusely acuminate (Wealth of India, 1998).

Figure 9: Different Parts of Zanthoxylum alatum Roxb. (www.flowersofindia.net; www.nepl.com.np).

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2.9 MICROSCOPY OF ZANTHOXYLUM ALATUM ROXB.

2.9.1 Transverse Section of Stem Bark

Transverse section (TS) of stem bark shows well developed suberized cork followed by layers of collenchymatous cells, wide parenchymatous cortex and phloem transversed by somewhat wavy medullary rays becoming wider towards the pericyclic region, oil cells and phloem fibres.

Detailed TS of stem bark shows cork, the outermost tissue, consisting of 15 to 20 layers of brownish thick-walled suberized cells occasionally interrupted at places by lenticels, underneath the cork lies 5 to 8 layers of tangentially elongated cells of the collenchyma, remaining 10 to 20 rows of cortical cells being parenchymatous transversed with groups of fibres embedded with few sclereids. Phloem is transversed by wavy uniseriate, triseriate medullary rays becoming wider towards the periphery, isolated circular to oval oil cells and small groups of phloem fibres associated with idioblasts containing calcium oxalate crystals (prismatic) throughout the parenchymatous cells of the section (Gupta et al., 2006).

2.9.2 Powder Microscopy

Powder microscopy reported the presence of fragments of polygonal to rectangular thick-walled cork cells in surface view and section view, the later with underlying collenchymatous cells; abundant starch grains and prismatic crystals are scattered and embedded in the parenchymatous cells; tangentially and radially cut medullary rays associated with fibres and phloem parenchyma; fragments of crystal fibres; sclereids of various sizes and shapes with wide or narrow lumen and distinct transversally running pits; few oil globules are spread or embedded in the oil cells (Gupta et al., 2006).
2.10 CHEMICAL REVIEW OF *ZANTHOXYLUM ALATUM* ROXB.

2.10.1 Bark
Asarinin, fargesin, Dictamnine, 8-hydroxydictamnine, armatamide (amide), epieudesmine, eudesmine, β-sitosterol, (+) sesamin, (-) sesamin, Pluviatide, Lupeol, Vanillic acid, (Kalia et al., 1999; Xiaomeng et al., 1996; Deshpande et al., 1977; Deb et al., 1962), Zanthonitrile, berberine (Perry, 1980).

2.10.2 Fruits
Linalool, linalyl acetate, citral, geraniol, methyl cinnamate, limonene, sabinene.

2.10.3 Leaves
Methyl-n-nonyl ketone, linalool, uncharacterized sesquitepenes, tricosane (Wealth of India, 1998).

2.10.4 Carpels
Carples of the fruits yield an essential oil isomeric with turpentine having eucalyptus like odour & properties (Nadkarni, 2002).

2.10.5 Seeds
The essential oil of comprises of over 85% of hydrocarbon 1-α-phellandrene and linalool, an unidentified sesquiterpene in little quantities. Ramidi and Ali, 1998, isolated tambulin (flavonoid) from the seed.

2.10.6 Phenolic Constituents
Two new phenolic constituents characterized as 3-methoxy-11-hydroxy-6,8-dimethylcarboxylate biphenyl and 3,5,6,7-tetrahydroxy-3′,4′-dimethoxyflavone-5-β-d-
xylopyranoside along with five known compounds, 1-methoxy-1,6,3-anthraquinone, 1-hydroxy-6,13-anthraquinone, 2-hydroxybenzoic acid, 2-hydroxy-4-methoxy benzoic acid and stigmasterol-5-en-3β-d-glucopyranoside has been found to be isolated from the seeds of ZA (Akhtar et al., 2009).

2.10.7 Essential Oil of *Zanthoxylum armatum* Extracted Through Hydrodistillation

Hydrocarbon fraction (17.35%) of ZA oil was much lower and oxygenated compounds comprised fairly high portion of essential oil (39.21%). Percentages of monoterpenes and sesquiterpenes were reported as 47.33% and 10.83% respectively. Oxygenated monoterpenes comprised major profile of chromatogram of essential oil i.e. 37.23% whereas monoterpenes hydrocarbons were 10.09%. Alcoholic percentage was much higher i.e. 26.76% and 15-hexadecanoic acid (6.58%) the only cyclic ester was found in relatively high %age (Amran et al., 2011).

2.10.8 A New Amide from *Zanthoxylum armatum*

A new amide designated as armatamide along with two lignans, asarinin and fargesin, alpha- and beta-amyrrins, lupeol and β-sitosterol-β-D-glucoside has been isolated from the bark of ZA. The structure of the new compound was characterized by spectral and chemical analysis as N-(4'-methoxyphenyl ethyl)-3, 4-methylenedioxy cinnamoyl amide (Kalita et al., 1999).
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2.11 ETHNOPHARMACOLOGICAL USES

The plant is used in traditional medical systems for abdominal colic, asthma, cancer, cholera, diabetes, cough, diarrhea, dysuria, fever, headache, hepatosis, microbial infections, toothache and worms, as well as considered useful in improving the blood circulation to affected parts and as a cardioprotective, analgesic, anti-inflammatory, pesticide, stomachic and tonic (Usmanghani et al., 1997; Duke et al., 2002).

2.11.1 Bark

Bark is used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The stem has exhibited hypoglycaemic activity in preliminary trials. The bark is pungent & used to clean teeth, employed to intoxicate fish, febrifugal, sudorific

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and diuretic properties (Wealth of India, 1998). The bark is used as an aromatic tonic in fever, dyspepsia, and cholera (Nadkarni, 2002).

2.11.2 Fruits
The fruits are used in dental troubles because of its antiseptic, disinfectant and deodorent properties. These are used in lotions for scabies (Wealth of India, 1998) and as spice. Fruit extract is reported to be effective in expelling round worms. The fruit is sweetish, bitter, hot; tasty and digestible; appetizer, anthelmintic, removes kapha and vata pain, tumours, abdominal troubles, useful in eye and ear diseases, diseases of lips, headache, heaviness, leucoderma, asthma, troubles of the spleen, difficult micturation (Nadkarni, 2002).

2.11.3 Seeds
The seeds are sharp with a good taste and smell, tonic, very astringent to the bowels and useful in diarrhea, carminative, bechic, pectoral, good in brain diseases and insanity, useful in stomatitis, strengthen the liver, purify the blood, remove foul smell from the mouth and are extensively used in the preparation of tooth powders & medicinal formulations (Wealth of India, 1998).

2.11.4 Leaves
The leaves on hydrodistillation yield oil which have antifungal activity (Wealth of India, 1998), insect-repellent activity and anthelmintic activity (Guleria and Kumar, 2006).
Table 4: The traditional uses of *Zanthoxylum alatum* DC. in different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Traditional uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>The seeds and the bark are used in the treatment of dyspepsia, fevers and cholera. Root, fruit, bark and leaf are used for catching the fish.</td>
<td>Chopra et al., 1986; Mathur et al., 1961</td>
</tr>
<tr>
<td>Pakistan</td>
<td>The decoction of dried fruit is used as a spice. Powder of dried fruits of <em>Zanthoxylum alatum</em>, <em>Trachyspermum ammi</em> seeds, <em>Mentha longifolia</em> dried leaves and black salt is taken with water during indigestion and cholera. Twigs are used as toothbrush during toothache and gum problems.</td>
<td>Barkatullah and Hussain, 2009; Abbasi et al., 2010</td>
</tr>
<tr>
<td>Japan</td>
<td>Seed is used for flatulence, digestion and depression.</td>
<td>Zaidi et al., 2009</td>
</tr>
<tr>
<td>Nepal</td>
<td>The decoction of the fruit is used for abdominal pain. Berries are antispasmodic, carminative and used for rheumatism and skin diseases. Bark is used for diabetes, cholera and asthma. Pickles from the fruits are useful for cold and cough, tonsillitis, headache, fever, high altitude sickness, vertigo/dizziness, limbs numbness, diarrhea and dysentery. Powdered dried fruits are taken with hot water to cure dysentery, diarrhea and stomachache.</td>
<td>Rajbhandari, 2001; Baral and Kurmi, 2006; Gevali and Awale, 2008</td>
</tr>
<tr>
<td>China</td>
<td>An infusion in vinegar is used to expel bugs or worms infecting ear. Plant lotion is used in the treatment of scabies.</td>
<td>Watson, 1930; Roi and Jacques, 1955</td>
</tr>
</tbody>
</table>
2.12 PHARMACOLOGICAL ACTIVITIES OF ZANTHOXYLUM ALATUM ROXB.

2.12.1 Anti-inflmmatory and Anti-nociceptive Activity

The ethyl acetate fraction of the alcoholic extract of the stems and roots of ZA possess anti-inflammatory and anti-nociceptive activity. For the anti-inflammatory and anti nociceptive study the Xylene-induced ear swelling in mice, Formalin test, acetic acid-induced writhing test has been used. By HPLC analysis of ethylacetate fraction eight lignans eudesmin, horsfieldin, fargesin, kobusin, sesamin, asarinin, planispine A and pinoresinol-di-3,3-dimethylallyl have been reported as the major components of this fraction (Tao et al., 2011).

2.12.2 Mosquito Repellent

The mosquito repellent property of oil of ZA has been studied against mosquitoes in mustard and coconut oil base and compared with standard repellent dimethyl phthalate (DMP). It showed protection in the both bases at all the concentrations. Repellents in mustard oil gave longer protection time than those in coconut oil. The oil gave significantly higher protection both in mustard (445 min) as well as coconut oil (404 min) than DMP at 0.57 mg/cm\(^2\) concentration (Das et al., 1999).

2.12.3 Piscicidal Activity

The piscicidal activity of the ethyl alcohol extract of the fruits of ZA has been evaluated on Mg\(^{2+}\) and Na\(^{+}\), K\(^{+}\)-ATPase activity in different tissues of Heteropneustes fossilis (air-breathing catfish). There is significant inhibition of enzyme activity in brain, liver and muscle tissues on exposure of fish to different concentrations of the extract. The inhibition was both dose and time dependent. Kinetic studies on Mg\(^{2+}\)-ATPase activity
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suggested that as piscicide, it is a non-competitive inhibitor (Ramanujam and Ratha, 2008).

2.12.4 Leech Repellent
Essential oil of the plant posseses leech repellent activity. Persistence of repellent properties of N,N-diethyl-meta-toluamide (DEET), dimethyl phthalate (DMP), N,N-diethyl phenyl acetamide (DEPA), 3 acetyl 2(2-6-dimethyl-5-heptenyl) oxazolidine (citronyl) and N-benzoyl piperidine (NBP) on cloth were tested against land leeches in evergreen rain and deciduous forests of Assam. ZA oil was at par with citronyl and exhibited better results than DMP and NBP though DEPA and DEET were found to be the best (Nath et al., 1993).

2.12.5 Inhibits Skin Sensitivity
A lipophilic fruit extract of ZA has been shown to reduce mouth irritation due to food. Dilution of extract with oleyl alcohol gives an ingredient of cosmetic which is easy to formulate and is endowed with a remarkable soothing effect based on inhibition of sensory irritation from sun bathing, depilation, shaving, insect bites, chemical treatments, etc. (Guglielmini and Cristoni, 2002).

2.12.6 Antitumor Activity
Crude extract of leaves and fruits of ZA has been reported to have cytotoxic potential. They were tested using brine shrimp assay. Significant inhibition was observed at all the test doses and was found to be dose dependent (Barkatullah and Muhammad, 2011). In the second report, a total of 11 methanol extracts obtained from four different Nepalese Zanthoxylum species were screened for their antiproliferative activity against

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the growth of human keratinocytes (HaCaT cells). The extract obtained from ZA bark was highly active with an IC$_{50}$ value of 11 µg/ml (Kumar and Muller, 1999).

2.12.7 Hepatoprotective
ZA is described as a hepatoprotective in Ayurveda (the Indian system of medicine). The ethanolic extract of ZA has been reported to have hepatoprotective activity in CCl$_4$ induced hepatotoxicity in male wistar rats. Ethanolic extracts at doses 100, 200 and 400 mg/kg has been administered orally daily for 7 days. The hepatoprotective activity has been assessed using various biochemical parameters like alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase serum bilirubin, total protein and serum antioxidant enzymes along with histopathological studies of liver tissue. The substantially elevated serum enzymatic levels of serum transaminases, alkaline phosphatase and total bilirubin were significantly restored towards normalization by the extracts. Bark extracts significantly increased the levels of antioxidant enzymes such as superoxide dismutase, catalase and glutathione. Phytochemical analysis revealed presence of isoquinoline alkaloid, berberine, as well as flavonoids and phenolic compounds, which have been known for their hepatoprotective activities. ZA possesses significant protective effect against hepatotoxicity induced by CCl$_4$ which may be attributed to the individual or combined action of phytoconstituents present in it (Ranawat et al., 2009). The hepatoprotective activity of leaves of ZA in CCl$_4$ produced hepatotoxicity in rats has also been reported (Verma and Khosa, 2010).

2.12.8 Larvicidal Activity
The study has been done on larvicidal potential of essential oils of ZA against three mosquito species with varied activities. The larval sensitivity towards the oil was found in the same order (Cx. Quinquefasciatus >Ae. aegypti >An. stephensi) as for some other
essential oils tested against these mosquito species. The findings of the study, therefore, suggested the use of the whole essential oil from the seeds of ZA as a local resource in controlling mosquito larvae (Tiwary et al., 2007).

2.12.9 Antispasmodic, Antidiarrhoeal, Aronchodialator and in Cardiovascular Disorders

The crude extract of ZA has been tested for its possible spasmolytic effect in isolated rabbit jejunum, where it inhibits the spontaneous contractions, thus showing an antispasmodic effect.

In the in vivo study, ZA extract protected the mice against castor oil induced diarrhea. The induction of diarrhoea with castor oil results from the action of ricinoleic acid formed by the hydrolysis of oil, which produces changes in the transport of electrolytes and water, resulting in the generation of giant contractions of the transverse and distal colon (Croci et al., 1997). The observed Ca\(^{++}\) antagonist effect of the plant extract in this study may explain the use of this plant as antidiarrhoeal, as Ca\(^{++}\) antagonists have been known to be effective in gut motility disorders, such as abdominal cramps and diarrhoea.

The plant has been studied further for a possible bronchodilator effect. In trachea, the extract caused relaxation of both CCh and K+ induced contractions, like verapamil, suggestive of nonspecific relaxation, possibly mediated through Ca\(^{++}\) channel blocking (CCB)-like mechanism. Ca\(^{++}\) antagonists are known to be effective in congestive respiratory disorders and the presence of such activity, as observed in this study may explain the medicinal use of the plant in asthma. In view of the well established therapeutic potential of Ca\(^{++}\) channel blockers and the medicinal use of ZA in the cardiovascular disorders, the plant extract have been further studied for the possible vasorelaxant and cardio-depressant effects. When tested in isolated aorta preparations, it
caused inhibition of both PE and high K+-induced contractions, similar to that observed with verapamil (Gilani et al., 2010).

2.12.10 Antidysenteric
The dried fruits of the ZA has been reported to have anti dysenteric property. Five dry fruits are made into powder and the powder mixed in curry and taken once daily for three days (Kar and Borthakur, 2008).

2.12.11 Antioxidant
Antioxidant activity of defatted ethanolic extract of ZA leaves has been studied for its free radical scavenging property in different \textit{in vivo} models such as DDPH, nitric oxide, superoxide, hydroxyl radical and lipid peroxide radical scavenging models. The extract showed significant dose dependent free radical scavenging property in all the models except in hydroxyl radical inhibition assay. The extract showed the presence of high phenolic contents corresponding to 120.7 μg equivalent of pyrocatechol suggesting the plant to exhibit antioxidant activity (Verma and Khosa, 2008).

The radial scavenging activity of the ethanolic extracts of fruit has also been tested against two important radicals, DPPH and hydroxyl. The extract exhibits a stronger antioxidant activity against OH radical as it caused 47% scavenging of the radical at 2 mg/ml and also showed its ability to scavenge the radical even at a low concentration (0.25 mg/ml). The extract also showed its ability to quench the stable DPPH radical (IC$_{50}$ = 4.56 ± 1.3 mg/ml) which is less compared to standard ascorbic acid (IC$_{50}$ = 21.4 ± 1.6 μg/ml) (Batool et al., 2010).