THE PLANT *O. indicum*:

*O. indicum* (Syonakh) is an important medicinal plant used in folk medicine, to cure numerous diseases. It is used in Ayurvedic medicine as astringent, antipyretic, aphrodisiac, carminative, diuretic, stomachic and for respiratory disorders (John, 2001). The plant is also reported to possess diuretic, antifungal, antibacterial, anti-inflammatory, and anti-arthritic activity (Warrier et al., 1995). The leaves and stem bark of the plant are reported to contain flavonoids namely, oroxylin-A, scutellarin, baicalein and chrysin (Sankara and Nair, 1972). Flavonoids such as baicalein is reported to possess anti-inflammatory (Hong et al., 2002), anti-ulcer (Kennouf et al., 2003), antioxidant (Ng et al., 2000), hepatoprotective (Neidworok et al., 1999) and immunomodulatory activities (Lien et al., 2003), while baicalein and chrysin both are reported to have antibacterial, antifungal and antiviral activities (Kujumgier et al., 1999; Tahara et al., 1987). Additionally, biochanin-A possesses tumor necrosis factor-α and anti-fungal activity (Knight and Eden., 1996). Ellagic acid is a prime polyphenolic compound (Jadhav and Laddha, 2004).

The stem bark, root and root bark of *O. indicum* were used as a traditional medicine in India, Thailand and other Asian countries. The seed of the plant has been widely used in the treatment of acute or chronic bronchitis, pharyngitis, pertussis, cough and other respiratory disorders. It was reported that this plant possess anti-inflammatory, antioxidant, anticancer, antimicrobial, gastroprotective and immunostimulant activities. In Peninsular Malaysia, the bark of the plant has been used in dysentery, while the leaves are used to treat stomach ache, rheumatism and to help healing of wounds (Burkill, 1966). In Myanmar, Laos and Philippines, the roots and trunks are used as an astringent and as a tonic in dysentery (Perry, 1980).

The stem bark is reported to possess various pharmacological activities and strong anticancer properties, which may be due to its antioxidant potential (Lambertini et al., 2004; Costa-Lotufo et al., 2005). Its decoction act as a powerful anticancer medicine, particularly against nasopharyngeal cancer in Maram Naga village of Senapati district, Manipur (Mao, 2002). The plant is also used for the treatment of abdominal tumors in Asian folk medicine (Soe and Myo Ngwe, 2004).
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Literature Review

In India, roots of the plant are used in Ayurvedic preparation called “Dasamoola”, which is used as astringent, anti-inflammatory, anti-helminthic, anti-bronchitic, anti-leucodermaic, anti-rheumatic, anti-anorexic and for treatment of leprosy and tuberculosis (Gupta et al., 2008). It is also used in other Ayurvedic formulations such as brahma, rasayana, chyavanaprasa awalwha, amartarista, dantyadyarista, narayana taila, dhanawantara ghrita, etc. The root bark is a constituent for several other Ayurvedic medicines, which are used as an astringent, bitter tonic, stomachic and as anodyne. It is also included in tonic formulations, such as Chyawanprash (Gupta et al., 2008). The fruit is used to treat a broad variety of diseases, including haemorrhoids, bronchitis, cholera, smallpox and colic. A decoction or broth is taken to relieve pain and inflammation of rheumatism and osteoarthritis and it can also be applied topically.

The tribal people of northern Thailand used bark to treat cuts and burns. The decoction of bark is used by pregnant women during labour pain to facilitate child birth, but its use by pregnant women may leads to abortion. The root bark is used as an aphrodisiac and appetizer. The fruits of the plant are used as anthelmintic, in heart disease, bronchitis, and in piles (Anonymous, 2007).

Pharmacological activities:
Several pharmacological and nonpharmacological investigations have been carried out on the plant and its phytoconstituents which can be categorised as below:

Anti-inflammatory activity
Upaganlawar et al. (2009) carried out in vivo studies in carageenan induced rat paw edema model and reported that the aqueous extract of plant leaves exhibit significant anti-inflammatory activity at dose level of 150 mg/kg b.w. and 300 mg/kg b.w. at 5 h in carageenan induced rat paw edema inhibiting release of prostaglandin like substances. Laupattarakasem et al. (2003) reported that significant pharmacological activity can also be seen in rat hind paw edema test. They also inferred that flavonoids may be responsible for this activity and the extract also reduced the release of myeloperoxide.
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Anti-hepatotoxic activity

Leaves of *O. indicum* are widely used as a prophylaxis for liver disorders in Indian systems of medicine. Tenpe *et al.* (2009) reported anti-hepatotoxic activity of petroleum ether, chloroform, ethanol or aqueous extracts of leaves at a dose of 300 mg/kg b.w. when administered to diseased rats, against CCl₄ induced hepatotoxicity and among them ethanol extract was found more effective. CCl₄ and *O. indicum* treated rats showed significant reduction in liver and kidney profile and significant increase in total protein.

Anthelmintic activity

Downing *et al.* (2000) evaluated anthelmintic activity of *O. indicum* against equine strongyle eggs and compared it with most effective deworming agents, i.e. ivermectin. 0% hatching, 0% viability of eggs and larvae and delay of hatching of eggs were reported at a dose of 2×10⁻¹ g/mL, 2×10⁻⁴ g/mL and 2×10⁻⁵ g/mL respectively. The results suggested that *O. indicum* may be an appropriate anthelmintic.

Anticancer activity

Various models have been used to perform numerous studies to prove the anticancer potential of *O. indicum*. Narisa *et al.* (2006) reported that 95% ethanol extract of *O. indicum* showed cytotoxic activity at a concentration of 0.05% against the Hep-2 cell lines. Roy *et al.* (2007) reported baiacalein caused inhibition of 50% of HL-60 cells at 25-30 μM concentrations. The exposure of HL-60 cells to 10 or 20 μM baiacalein for a period of 36-48 h causes inhibition of its proliferation and was associated with S or G2M phases cell accumulation. Although, induction by apoptosis and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) may also be associated with proliferation inhibition at a higher dose. The results showed that baiacalein has anti-tumor effects and for which *O. indicum* extract is used in cancer therapy.

Nakahara *et al.* (2001) reported with an Ames test inhibition of mutagenicity of Trp-P-1 by methanolic extract of *O. indicum*. Baiacalein with IC₅₀ value of 2.78±0.15 μM works as major antimutagenic constituent. High content of baiacalein (3.95±0.43%, dry weight)
was identified as the potent antimitagenic constituent of *O. indicum* which acts as desmutagen and leads to inhibition of the N-hydroxylation of Trp-P-2.

Tepsuwan *et al.* (1992) observed that after oral administration of the nitrosated *O. indicum* fraction in vivo shows genotoxic and cell proliferative activity in stomach mucosa of male F344 rats. Administration of the nitrosated *O. indicum* fraction induced in the stomach pyloric mucosa a dose-dependent DNA single-strand scission after 2 h at doses of 1 g/kg b.w. and 2 g/kg b.w. Induction of DNA elution rate constant increase about 18-fold is observed at a dose of 2 g/kg b.w., and doses of 0.7-2.8 g/kg b.w. induced upto 11-fold dose-dependent increases in the stomach pyloric mucosa replicative DNA synthesis after 16 h at doses of 0.25-2.0 g/kg b.w., increases in the stomach pyloric mucosa after maximum 4 h is observed upto 100-fold in ornithine decarboxylase activity. The results suggested genotoxic and cell proliferative activity is present in *O. indicum*.

Costo-Lotufo *et al.* (2005) showed that *O. indicum extract* causes toxicity to tumour cell lines tested with an IC₅₀ value 14.2 µg/mL for HL-60, 17.2 µg/mL for B-16 ,19.6 µg/mL for CEM, and 32.5 µg/mL for HCT-8. It also Inhibit the cell cycle progression from the first cleavage (IC₅₀ = 13.5 µg/mL) on the sea urchin eggs. All these findings concluded that anticancer compounds could be present in *O. indicum* extracts.

**Immunostimulating activity**

Zaveri *et al.* (2006) reported immunomodulatory activity measures of delayed-type hypersensitivity and immune responses to sheep red blood cells (SRBC haemagglutinating antibody [HA] titre) in rats using n-butanol fraction of *O. indicum* root bark (100 mg/kg b.w., once daily for twenty-two consecutive days). The histopathologic analysis in treatment group showed an increase in cellularity of lymphoid tissues, e.g., T-lymphocytes and sinusoids. The extent of edema is lower in control rats as compared to that in drug-treated rats of triple antigen-mediated immunological edema model enhancing DTH reactions. Besides this, the antioxidant potential of the drug raises the levels of catalase, reduced glutathione and superoxide dismutase and significantly reduced the whole blood malondialdehyde content. On the basis of all these findings, the reported immunomodulatory activity of *O. indicum* confirmed its ability to increase specific immune
responses (both humoral and cell-mediated) as well as its antioxidant potential. This study also provides reasonable explanation on the use of plant in various immunomodulatory formulations of Ayurveda, like Chyavanprash, etc.

**Antimicrobial activity**

Kawsar et al. (2003) conducted anti-microbial activity of various extracts of *O. indicum* using disk diffusion method against fourteen pathogenic bacteria, among which five were gram-positive and nine were gram-negative and seven pathogenic fungi. The result showed mild to moderate activity against all bacteria and fungi and little activity against bacteria but moderate activity against fungi by using crude ethyl acetate and methanol extract respectively. The minimum inhibitory concentration values of the flavonoid compounds isolated from *O. indicum* lies between 64-128 µg/mL which were determined against *E. coli, S. aureus, B. subtilis,* and *S. dysenteriae*. Thatoi et al. (2008) confirmed this study using different strains. Ali et al. (1998) reported a strong antifungal activity of *O. indicum* dichloromethane extract against dermatophytes and wood rot fungi.

**Gastro-protective activity**

Zaveri et al. (2007) reported gastroprotective activity of 50% alcoholic extract and different fractions of *O. indicum* against ethanol-induced gastric mucosal damage, among which alcoholic extract of different fractions (100-300 mg/kg) showed significant decrease in gastric ulceration. The n-butanol fraction pretreatment in WIRS-model showed significant antioxidant and antiulcer activity in gastric mucosal homogenates and reveals significant gastroprotective effect against both WIRS-induced and ethanol induced gastric ulcer rats. Gastro-protective activity was found responsible by presence of flavonoids in *O. indicum*.

**Antiarthritic**

Laupattarakasem et al. (2003) tested aqueous and ethanol extract of *O. indicum* in rat peritoneal leukocytes for in vitro release of myeloperoxidase (MPO). The results
showed a significant effect i.e. 60% inhibition of MPO released by the use of aqueous extract.

**Antiproliferative**

Lambertini *et al.* (2004) studied the antiproliferative activity of *O. indicum* on human breast tumour cell lines and results indicated that *O. indicum* has antiproliferative activity against MCF7 and MDA-MB-231 breast cancer cell lines.

**Photocytotoxic activity**

Ong *et al.* (2009) reported the photocytotoxic activity of *O. indicum* leaves methanol extract against promyelocytic leukaemia cell line HL-60. The cell line HL-60 was irradiated with broad spectrum light source of 9.6 J/cm² in four replicates after incubation with 21 μg/mL of crude extracts for 2 h. Viability of cells was measured by Colorimetric MTT protocol after 24 h. Pheophorbide-a was used as the positive control and control without irradiation.

**Unidentified chemical constituents:**

The chemical constituents of *O. indicum* are always the centre of interest for the researchers. A number of secondary metabolites have been reported from various parts of the plant, like flavonoids, glycosides, alkaloids, tannins, terpenoids, etc.

The leaves have been reported to contain flavones and their glycosides oxorindin, baicalein and scutellarein. Leaves also contain an anthraquinone, aloe-Emodin (Khare, 2007; Dey *et al.*, 1978). Bark of the root is reported to have chrysins, baicalein, oroxylin-A and dihydrobaicalein. Heartwood yield beta-sitosterol and an iso-flavone, prunetin. Its root and stem contains three flavones *viz.*, oroxylin A (5,7-dihydroxy-6-methoxyflavone), baicalein (5,6,7-trihydroxy flavone) and chrysin (5,7-dihydroxy flavone). It also contains pterocarpan and rhodioside with p-hydroxyphenyl ethanol and cyclohexanols (Vasanth *et al.*, 1991; Dey *et al.*, 1978; Theobald *et al.*, 1981). Four other flavonoids, chrysins, baicalein, baicalein-7-O-diglucoside (Oroxylin B) are also reported from the plant. In Indian system of medicine the various parts of plant like root, bark, stem and leaf are
prescribed for snake bite (Kirtikar et al., 2003). Leaves are used for treating an enlarged spleen and to alleviate headaches and ulcers and also reported for its analgesic and antimicrobial activity (Singh et al., 2002). Several tribes of India used bark and seeds of the plant to treat fever, pneumonia and respiratory troubles (Panghal et al., 2010; Patil et al., 2008). It is also used to cure various stomach disorders (Rout et al., 2009). Seeds are used as a digestive and to treat boils and wounds. The root is used as astringent, tonic and is anti-inflammatory, aphrodisiac, expectorant, and anthelmintic. The bark is diuretic and stomachic and bark, root bark and root decoction is useful in diarrhea and dysentery. Root bark and seeds are carminative, stomachic, diaphoretic and used as an astringent and tonic. Root bark is also used to treat bile problems and cough (Kunwar et al., 2009).

**Active constituents of *O. indicum*:**

Prunetin (Fig. 2.1) is an O-methylated isoflavone, a type of flavonoid. It has been first isolated from the bark of *Prunus emarginata* (Douglas ex Hook.) Walp. (Oregon cherry) by Finnemore in 1910 (Shriner and Hull, 1945). Pea roots isolated prunetin can act as an attractant for *Aphanomyces euteiches* zoospores (Yokosawa et al., 1986). It also acts as an inhibitor of human liver aldehyde dehydrogenase (Sheikh and Weiner et al., 1997).

![Fig. 2.1 Chemical Structure of Prunetin.](image)

Aloe emodin (Fig. 2.2) is an anthraquinone present in aloe latex as exudates. It has a strong stimulant-laxative action. Aloe emodin may increase carcinogenicity of some kind of radiation, but do not show carcinogenic effects when applied directly to the skin. Aloe
emodin is found in the gel, sap or leaves of Aloe vera (L.) Burm.f., the bark of Frangula (Rhamnus frangula P. Mill synonym. Frangula alnus Mill.) and Cascara Sagrada (Rhamnus purshiana var. tomentella (Benth.) K.L. Brandegee), the leaves of Senna (Cassia angustifolia M. Vahl) and the rhizome of Rhubarb (Rheum rhabanticum L.). It has a marked anti-viral effect against both herpes simplex virus (HSV) type 1 and 2 in vitro (Badgwell et al., 2004).

![Chemical Structure of Aloe emodin](image)

**Fig. 2.2** Chemical Structure of Aloe emodin.

Oroxindin (Fig. 2.3) is an O-methylated flavone, a type of phenolic chemical compound (Hui et al., 2002). It is a wogonoside, more accurately a wogonin glucuronide isolated from O. indicum (L.) Kurz, Scutellaria baicalensis Georgi (Ramachandran Nair and Joshi, 1979), Bacopa monnieri (L.) Wettst., and Holmskioldia sanguinea Retz. (Chaudhuri et al., 2004). Preliminary in vitro studies have shown that it may have anti-tumor properties (Lin et al., 2011; Gao et al., 2011). It has also been found to possess anticonvulsant effects. It acts as a positive allosteric modulator of the benzodiazepine site of the GABA\(_\text{A}\) receptor (Park et al., 2007).
Baicalein (5,6,7-trihydroxyflavone) is the flavonoid component of Nepalese and Sino-Japanese crude drugs (Deschamps et al., 2006; Hsieh et al., 2007). Baicalein (Fig. 2.4) a major flavone of Scutellariae baicalensis Georgi roots and inhibits the 12-lipoxygenase (12-LOX) pathway of arachidonic acid metabolism, which inhibits cancer cell proliferation and induces apoptosis. It is the aglycone of baicalin.

The flavonoid acts as an anti-inflammatory agent and has been shown to inhibit certain types of lipoxygenases. It also has antiproliferative effects on ET-1-induced proliferation of pulmonary artery, smooth muscle cell proliferation via inhibition of TRPC1 channel expression (Lin et al., 2011). In animal research baicalein showed possible antidepressant effects (Xiong et al., 2011). Baicalein also act as an inhibitor of CYP2C9 enzyme, which is responsible for the metabolism of drugs in body (Si et al., 2009). A derivative of baicalin is a known prolyl endopeptidase inhibitor (Tarrago et al., 2008).

![Fig. 2.3 Chemical Structure of Oroxindin.](image)

![Fig. 2.4 Chemical Structure of Baicalein.](image)
Scutellarin (Fig. 2.5) scutellarein 7-O-β-D-glucuronide, are the major bioactive flavonoid glucuronides isolated from a Chinese herb *Erigeron breviscapus* (Vaniot) Hand.-Mazz. This compound is also found in *Teucridium parvifolium* Hook.f., *Tripora divaricata* (Maxim.) P.D.Cantino (Grayer *et al.*, 2002) and *Scutellaria lateriflora* L. (Gafner *et al.*, 2003). Scutellarin is used in the treatment of various diseases and disorders such as cardiovascular diseases, sleep disorders, depression, migraine, pain, and memory impairment (Gafner *et al.*, 2003; Goh *et al.*, 2005). In China, it is widely used as a remedy for various purposes like dilating blood vessels, decreasing the viscosity of blood, reducing the blood platelet count and inhibiting platelet aggregation activity and improving microcirculation (Hong and Liu, 2004; Liu *et al.*, 2005). Scutellarin has been shown to induce apoptosis of ovarian and breast tumour cells *in vitro*.

![Chemical Structure of Scutellarin](image)

**Fig. 2.5** Cemical Structure of Scutellarin.

Oroxylin A (Fig. 2.6) is an O-methylated flavone, a chemical compound that can be isolated from medicinal plants *Scutellaria baicalensis* Georgi and *O. indicum* and both are most important medicinal herbs in traditional Korean, Chinese and Japanese medicine. Oroxylin A has demonstrated a dopamine reuptake inhibitor activity and is also a negative allosteric modulator of the benzodiazepine site of the GABA<sub>A</sub> receptor (Yoon *et al.*, 2013; Liu *et al.*, 2013) and has been found to improve memory consolidation in mice by elevating brain-derived neurotrophic factor (BDNF) levels in the hippocampus (Kim *et al.*, 2014).
Chrysin belongs to a class of flavonoids. It occurs naturally in honey and bee propolis (glue), and in plants such as the passion flower, silver linden and some Geranium species. Chrysin (Fig. 2.7) is used for bodybuilding, for treating anxiety, gout, HIV/AIDS inflammation, erectile dysfunction (ED), baldness and also for prevention of cancer. (http://www.webmd.com/vitamins-supplements/).

Pterocarpans (Fig. 2.8) are derivatives of isoflavonoids found in the family Fabaceae. It is a group of compounds which can be described as benzo-pyranofurano-benzenes (i.e. 6H [1]benzofuro[3,2-c]chromene skeleton) and can be formed by coupling of the B ring to the 4-one position. 2'-hydroxyisoflavone reductase and glyceollin synthase are the enzyme responsible for the conversion in Cicer arietinum L. (Tiemann et al., 1987), and production of phytoalexins in soybean and glyceollins (Welle et al., 1988).
Fig. 2.8 Chemical Structure of Pterocarpons.

β-sitosterol (Fig. 2.9) is one of several phytosterols with chemical structures similar to that of cholesterol differs only by the presence of an extra ethyl group. It is white in colour and waxy in nature. Alone or in combination with similar phytosterols β-sitosterol reduces levels of blood cholesterol and is sometimes used in treating hypercholesterolemia (Prager et al., 2002). It is also used for treating prostatic carcinoma and breast cancer, although the benefits are still being in the process of evaluation in the United States (Awad et al., 2008). Beta-sitosterol is an antioxidant able to increase the level of typical antioxidant enzymes, reduce DNA damage and the level of free radical in cells.

Fig. 2.9 Chemical Structure of β-sitosterol.
THE PLANT *M. spinosa*:

The literature survey reported that most of the plants of family Rubiaceae are of great medicinal value. By the use of these plants several ailments like ulcers, dysentery, athlete’s foot, diabetes, whooping cough, bronchitis, asthma, migraine, etc. are successfully cured. Some plants of this family showed miraculous curing properties for regulation of menses, ensuring the birth of male child, treatment of snake bite and scorpion sting. The decoction of whole plant is used in vertigo and root bark decoction is very effective in tetanus infection. Root paste is applied to relief painful urination. Leaf powder is a prescription to kill intestinal worms and with black pepper to cure diphtheria. The narcotic effect can be observed by seeds powder (Singh and Ali, 2012).

*M. spinosa* is used in traditional medicine for treatment of various diseases in Bangladesh. The fruit and the leaves are eaten. Fruits are used in the treatment of inflammation, fever, biliary complaints and hepatic congestion (Pervin *et al.*, 2012).

Leaves are used in bone fracture and powdered leaves are said to be good in the treatment of diphtheria and infusion of leaves is given for gurgling in diphtheria and also as fodder, but are of poor quality (Ghani, 1988; Yusuf *et al.*, 2009). The plant is also reported to be used traditionally in the treatment of skin irritation, abortion and renal diseases (Pullaiah, 2003). The dry fruit is believed to be narcotic and is reported to be used to cure boils and dysentery. Decoctions prepared from the fruit pulp in water are given orally in jaundice.

The plant is used in traditional folk medicines (Bora and Kumar, 2003). Fruits and the bark of the plant are used to treat headache (Doley *et al.*, 2010), while the fruits and leaves are beneficial in diabetes, jaundice and other gastrointestinal disorders (Khan and Yadava, 2010; Sen and Chakravorty, 2011). Tender leaves, ripe fruits and seeds are useful to cure skin infections and pimples (Sen *et al.*, 2011; Das *et al.*, 2009; Buragohain, 2008); the leaf is also prescribed in indigestion and to treat dyspepsia (Nadkarni, 2005). Fruits are a good source of nutrient and are used to cure cough and as a refrigerant traditionally (Doley *et al.*, 2010; Nadkarni, 2005). The plant is also important for its abortifacient activity; seeds and fruits are used by several ethnic groups in India to induce abortion.
(Mitra and Mukherjee, 2009). It is also used against sun burn, as insect repellent and also as antiseptic (Ahmed and Borthakur, 2005).

Recently, two compounds were isolated from the fruits of *M. spinosa* which possess antimicrobial activity against *B. subtilis, K. pneuminiae, E. coli, S. aureus* and *C. albicans*. One was identified as oleanolic acid which possesses the highest antimicrobial activity (Buragohain, 2008). It has also been reported that methanol extract of *M. spinosa* possess antifungal activity (Goswami *et al.*, 2006). Seeds of *M. spinosa* chiefly contain 20% fatty oil, in which 80.4% are oleic acid. In addition, it contains scutellarein, baicalein, scutellarin, oroxindin, chrysin, oroxin A, B, baicalein, benzoic acid, tetuin, oroxylin A, hispidulin, apigenin, etc and leaves contain scutellarein, scutellarin and baicalin (Purkayastha, 2010).

**Pharmacological reports:**

**Antifungal activity**

Goswami *et al.* (2006) has reported the antifungal activity of the methanol extract of *M. spinosa*.

**Antibacterial activity**

Pervin *et al.* (2012) investigated the antibacterial activity of the ethanol extract of stem of *M. spinosa* against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Shigella dysenteriae* by disc diffusion and broth macro dilution assay. Minimum inhibitory concentration (MIC) of the extract was 500 µg/mL for *S. dysenteriae* whereas, 1000 µg/mL for *S. aureus, S. pyogenes* and *E. coli*.

The stem extract showed moderate antibacterial activity in both the assays. The extract showed antibacterial activity against *E. coli* in broth macro dilution assay, but in disc diffusion assay it is unable to inhibit the same organism. However, the MIC was obtained at a higher concentration (1000 µg/mL) in broth macro dilution assay than the extract content in the disc (500 µg/mL). Therefore, on the basis of this it is reported that the concentration may play a important role in latter experiments.

Rios *et al.* (1988) concluded that use of non polar compound(s) fail to diffuse in agar media and may show antibacterial activity in disc diffusion assay. Ghani (1988) and
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Yusuf et al. (2009) reported that the antibacterial activity of M. spinosa stem extract is relatively low than previously reported leaves extract by Chatterjee et al. (2009).

Cytotoxic activity

McLaughlin and Rogers (1998) had conducted the cytotoxicity test with brine shrimp lethality bioassay for identifying biologically active compound present in a crude extract.

The result of the works carried out by Borowitz et al. (1992), Anderson et al. (1991) and Meyer et al. (1982) indicate that the stem extract of M. spinosa might have biologically active compounds with enzyme inhibition, ion channel interference, antimicrobial, pesticidal and/or cytotoxic activities. The results of the above studies also showed that percent mortality of the shrimp napulii is directly proportional to the concentration of both the test extract and chloramphenicol. The obtained LD$_{50}$ of M. spinosa stem extract was 40 µg/mL, which is lower as compared to that of chloramphenicol and it was inferred that as a crude extract it is high which may be due to presence of more than one biological compound in the extract.

Hypoglycaemic and hypolipidemic effect

Sen et al. (2013) reported that from methanol, ethyl acetate and petroleum ether fraction of M. spinosa leaf, methanol extract was evaluated after twenty-one days of treatment in type-2 diabetic rats induced by high fat diet-alloxan. After twenty-one days methanol and ethyl acetate fraction displayed reduction in serum glucose level. Fractions demonstrated increase in body weight, high density lipid level in diabetic rats and significant decrease in triglycerides, total cholesterol, low density lipid, very low density lipid, and α-amylase level. Fractions showed significant hypoglycemic effect in glucose administered rats but no affect in normal animals.
**Chapter-2**

**Literature Review**

**Unidentified Chemical constituents:**

Recently, two compounds were isolated from the fruits of *M. spinosa* which possess antimicrobial activity against *B. subtilis, K. pneumoniae, E. coli, S. aureus* and *C. albicans*. One was identified as oleanolic acid which possesses the highest antimicrobial activity (Buragohain, 2008). Two new 19α-hydroxyursane-type triterpenes, 2α,3α,19α,24,28-pentahydroxyurs-12-ene and meyanthic acid, 3β-acetoxy-2β,19α,23-trihydroxyurs-12-en-28-oic acid along with one new aliphatic ester, myricyl pentadecanoate and five known compounds, 19α-hydroxyasiatic acid, oleanolic acid, myricyl alcohol, β-sitosterol and its glycoside were isolated from the methanol leaf extract of *M. spinosa* (Rudrapaul *et al.*, 2014).

**Active constituents of M. spinosa:**

1-Triacontanol (**Fig. 2.10**) is a fatty alcohol also known by name melissyl alcohol or myricyl alcohol. Triacontanol is a growth stimulant for many plants, especially roses, in which it rapidly increases the number of basal breaks. It is found in plant cuticle waxes and in bees wax. (http://en.wikipedia.org/wiki/Triacontanol).

![Fig. 2.10 Chemical Structure of myricyl alcohol.](image)

Oleanolic acid or oleanic acid (**Fig. 2.11**) is a naturally occurring triterpenoid and widely dispersed in food and medicinal plants. It can be found in Olive oil, *Phytolacca americana* L. (American pokeweed) and *Syzygium* P. Browne ex Gaertn., garlic, etc. It is comparatively non-toxic, hepatoprotective and posses antiviral and antitumor properties (Liu, 1995). Oleanolic acid was found to exhibit weak anti-HCV activities and weak anti-
HIV (Mengoni et al., 2002) but more potent synthetic analogs are being investigated as potential drugs (Fei et al., 2013).

Dinkova-Kostova et al. (2005) found an extremely potent synthetic triterpenoid analog of oleanolic acid which act as an inhibitor of cellular inflammatory processes while working in mouse macrophages with the induction of inducible nitric oxide synthase (iNOS) and of cyclo-oxygenase-2. Both are highly potent inducers of the phase-2 response (e.g., elevation of NADH-quinone oxidoreductase and heme oxygenase 1), and work against oxidative and electrophile stress as a protector of cells (Dinkova-Kostova et al., 2005). A study in mice found that oleanolic acid reduced sperm quality and motility and leads to infertility in mice (Mdhluli et al., 2002).

![Chemical Structure of Oleanolic acid.](image)

**Fig. 2.11** Chemical Structure of Oleanolic acid.

**EFFECT OF VARIOUS PLANTS ON MYCOTOXIN INDUCED TOXICITY:**

The term mycotoxin is derived from the Greek word ‘mycos’ meaning mould and the Latin word ‘toxicum’, which means poison. Mycotoxins are low-molecular weight secondary metabolites of fungi, occurs as a product of primary metabolic processes. It is usually associated with developmental processes because it occurs after balanced growth phase. Thus, sometimes mycotoxins are released by growing colonies at the sporulation
approximate time (Calvo et al., 2002), but the functions of mycotoxins are still a mystery. They are believed to protect the mould and act as a defence mechanism by excluding or poisoning animals, plants or other competing fungal species in the same environment. The particular secondary metabolites production such as mycotoxins, phytotoxins or antibiotics, is usually restricted to a small number of species and may be species or even strain specific (Smith and Moss, 1985).

They have always been a hazard to human being and domestic animals, but until the decade following 1970, their effects have not been largely studied.

Patial et al. (2013) reported protective action of sea buckthorn (Hippophae rhamnoides L.) against Ochratoxin A (OTA) induced nephropathy in Japanese quail with the inclusion of 2% sea buckthorn leaf powder in their feed and sea buckthorn leaf extract in their water.

Eraslan et al. (2013) showed protective effect of pumpkin seed oil against aflatoxin induced oxidative stress in male Balb/c mice. They have observed that in those animals which received both aflatoxin and pumpkin seed oil showed closer value of the oxidative stress markers as that of control animals as compared to those animals which received only aflatoxin.

Solcan et al. (2013) investigated the hepatoprotective effect of sea buckthorn against toxicity induced by aflatoxin B1 (AFB1) and reported that when chickens were simultaneously dosed with AFB1 and an extract of sea buckthorn berries, subsequent histology of the liver showed a significant reduction of necrosis and fatty formation compared with chickens treated with AFB1 alone. Moreover, they have also showed that the levels of AFB1 residues in chicken livers were significantly reduced by sea buckthorn oil.

Yang et al. (2011) reported that 6-gingerol has a powerful defensive potential against the genotoxicity caused by Patulin (PAT) in HepG2 cells and proved that 6-gingerol notably decreased the micronuclei formation and DNA strand breaks caused by PAT. They have also shown that 6-gingerol effectively suppressed PAT-induced intracellular ROS
formation and 8-OHdG level. Moreover, they have found that pretreatment by 6-gingerol leads to attenuation of GSH reduction induced by PAT in HepG2 cells.

Rangsaz N et al. (2011) showed turmeric extract (Curcuma longa L.) can provide protection against the negative effects of aflatoxin on performance of broiler chickens where they displayed that there were no notable differences in body weight (BW), body weight gain (BWG), feed intake and feed conversion ratio (FCR) between groups fed turmeric and the control group as compared to the aflatoxin treated chicken.

Hassan et al. (2010) reported that ethanol extract of Aquilegia vulgaris L. induced its preventive effect against exposure to fumonisins which leads to toxicity in cells via the induction of oxidative stress in rats via increasing the antioxidant capacity, inhibition of lipid peroxidation and scavenging of free radicals.

Malekinejad et al. (2011) reported that Ochratoxin A (OTA) contamination in human foods and animal feeds could cause reproductive abnormalities, partly by interfering in oxidative stress system and exerts its toxic effects on testes whereas, melatonin and Glycyrrhiza glabra L. with antioxidant properties could satisfactorily protect rats against OTA toxic effects.

Yuan et al. (2010) suggested that Gynostemma pentaphyllum (Thunb.) Makino protects mouse male germ cells against apoptosis caused by Zearalenone through antioxidation and anti-apoptosis via the regulation of Bax and Bcl-2 expression.

Ben et al. (2009) investigated that Zearalenone (ZEN) induces toxicity via decreasing the sperm number, testosterone level and antioxidant enzyme in male Balb/c mice. They showed that this toxic effect can be reversed by the extract of Raphanus sativus L., which is rich in many antioxidant compounds and safe in counteracting the oxidative stress and protect against the toxicity resulting from ZEN.

Zorgui et al. (2009) reported that extract of Opuntia ficus-indica (L.) Mill possess anti genotoxicity property against Zearlenone Balb/c mice via protection of micronuclei, chromosome aberrations and DNA fragmentation.
Abdelwahed et al. (2008) have showed antimutagenic activity of extract of *Pituranthos tortuosus* (Coss.) Maire against aflatoxin B1 (AFB1). Moreover, they have also reported apoptotic and antiproliferative properties of these extracts taking two leukemia cell lines, L-1210 and K-562. They suggested that methanol, acetone, ethyl acetate and total oligomer flavonoid extracts showed the highest inhibition level of mutagenicity produced by the indirect mutagen AFB1.

Braicu et al. (2010) demonstrated the potent protective effect of flavan-3-ols against aflatoxin B1 (AFB1) cytotoxicity in A2780 epithelial cell line. They suggested that this protective effect may be due to the inhibitory effect of flavan-3-ols on AFB1-induced reactive oxygen species ROS formation and oxidative damage.

Abdel-Fattah et al. (2010) determined that aflatoxin could affect some hematological parameters and increase the enzyme levels of liver. Peroxidation reactions, arising in aflatoxin biotransformation may be the reason behind increase in these parameters and these reactions may inflict oxidative injury to cellular components. They reported that, when rats administered with aflatoxins contaminated diet were given white ginseng, it shows significant increase in biological parameters improvement as well as improvement of histopathological picture of the liver and kidney in different experimental groups. The results showed that the hepatic cellular injury produced by aflatoxins in rats was prevented by the vigorous protective action of white ginseng.