

**PHYTOCHEMICAL AND PHARMACOLOGY OF OROXYLUM INDICUM VENT –
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ABSTRACT

Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigations into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Various drugs have entered the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies are carried out on a large number of plants but smaller numbers of marketable drugs or phytochemical entities have entered the evidence-based therapeutics. Efforts are therefore needed to establish and validate evidence regarding safety and practice of Ayurvedic medicines. Oroxyton refers to the tree *O. indicum*; commonly called the tree of Damocles, Indian caper, Indian trumpet flower, Indian trumpet tree. The tree is named as “Tree of Damocles” after an incident depicted in an ancient story by Cicero. *O. indicum* Vent. a member of the bignoniaceae family is a small or medium sized deciduous tree growing throughout Asia continent.^[1] It has been used traditionally for a wide diversity of ailments such as cancer, diarrhea, fever, ulcer, jaundice and arthritis.

KEYWORDS: ethnopharmacology, *O. indicum*, bignoniaceae.**Taxonomy****Scientific name**

O. indicum Vent. (L) (Syn. name- *Bignonia indica*, *Spathodea indica*, *Calosanthos indica*, *Hippoxylon indica*, *Bignonia quadripinnata*).

Vernacular names

Shayonak Kul (Ayurvedic); Hanyu pinyin, Mu hudie, Butterfly tree (Chinese); Broken bones plant, Indian calosanthos, Indian trumpet, Indian trumpet flower, Mid night horror, Tree of Damocles (English); Bonglai (Malaysia); Tentu (Gujrati); Patrona, Putiveriksha, Shallaka, Shuran, Son, Vatuk, Arlu, Urru, Sauna (Hindi); Ane-mungu (Karnatka); Devamadak (Konkani); Tatelo (Nepalese); Palakappayyani, Vella, Pathiri (Malaylam); Aralu, Shyonaka (Sanskrit); Totila, Thotila (Singhala-Sri-Lanka); Cari-konnai, Kalai-y-utaicci, Puta-puspam, Achi, Pana, Pei-maram, Venga maram, Peruvaagai (Tamil); Manduka-paramu, Pampena, Suka-nasamu, Dundilamu, Pampini, Nemali, Chettu (Telugu); Other common names Kampong, Sonapatta, Sonaapaathaa, Urru, Saona, Tatpalenga, Kinnauri phool, Shoshana, Tuntuka, Kutunata, Mandukparna, Bhalluka, Prthushimba, Katvan.^[2-4]

Botanical classification

Kingdom–Plantae, Class–Magnoliophyta, Order–Lamiales, Family–Bignoniaceae, Genus–*Oroxylum*, Species–*Indicum*.

Distribution and relationships

O. indicum is a deciduous tree growing throughout India, South Asia, South East Asia, Sri Lanka, Philippines, Indonesia, China, Bhutan, Malaysia and Mallaca. It is found up to an altitude of 1200 m mainly in ravines, in damp region and moist places in the forests.^[2] In India, it is distributed in Himalayan foothills, Eastern and Western Ghats and North East India.^[5] It is mostly sighted along the river banks or slopes of the hills. *O. indicum* lives in relationship with the actinomycete *Pseudonocardia oroxyli* present in the soil surrounding the roots.^[6]

Description

O. indicum is a small or medium sized deciduous tree up to 12 m in height with soft light brown or grayish brown bark with corky lenticels.^[7] (fig. 1a). The leaves are very large, 90-180 cm long 2-3 pinnate with 5 or more pairs of primary pinnae, rachis very fast, cylindrical, swollen at the junction of branches, leaflets 2-4 pairs ovate or

elliptic, acuminate, glabrous (fig. 1b). The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. The flowers are reddish purple outside and pale, pinkish-yellow within, numerous, in large erect racemes (fig. 1c)^[4] The flowers bloom at night and emit a strong, stinky odor which attracts bats. The tree has long fruit pods that curved downward, hang down from the branches, looking like the wings of a large bird or dangling sickles or sword in the night. Fruits are flat

capsules, 0.33-1 m long and 5-10 cm broad and sword shaped. When the pod bursts open the seeds flutter to the ground, often traveling some distance, looking like butterflies.^[3] The seeds are numerous, flat and winged all around like papery wings, except at the base. The plant flowers in June-July and bears fruits in November. The fresh root bark is soft and juicy; it is sweet, becoming bitter later. On drying, the bark shrinks, adhere closely to the wood and becomes faintly fissured (fig. 1d).

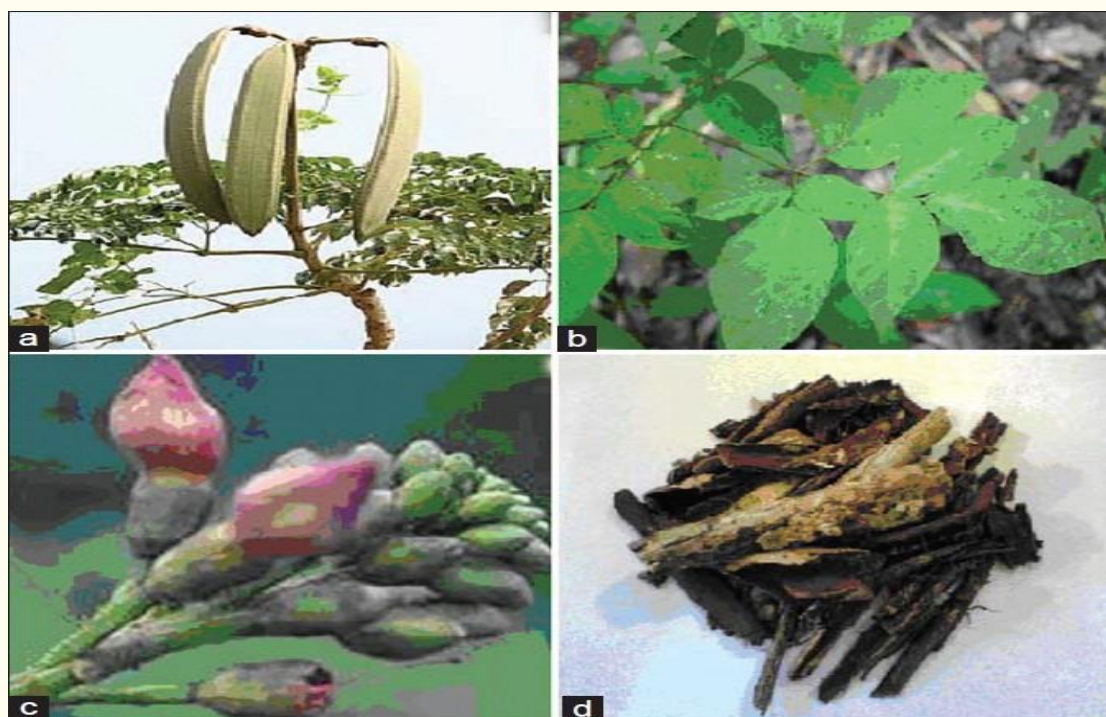


Fig. 1: *Oroxyllum indicum*: (a) tree (b) leaves (c) flowers (d) wood bark.

Ethnobotany

The tree is often grown as an ornamental tree for its strange appearance. The sword like fruit or a branch of the plant is used by the farmers to kill crabs in wet paddy fields. A paste made of the bark is applied to wounds of animals to kill maggots.^[3] In Thailand, the fruits and flowers of the plant are consumed as a vegetable.^[8]

Traditional uses

Roots are sweet, astringent, bitter, acrid, refrigerant,^[9] antiinflammatory, anodyne, aphrodisiac, expectorant, appetizer, carminative, digestive, anthelmintic, constipating, diaphoretic, diuretic, antiarthritic, antidiabetic and febrifuges. Tonic is useful in dropsy, cough, sprains neuralgia, hiccup, asthma, bronchitis, anorexia, dyspepsia, flatulence, colic, diarrhea, dysentery, strangury, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever. Root bark is used in stomatitis, nasopharyngeal cancer and tuberculosis.^[4,10] Leaves are used as stomachic, carminative and flatulent. Leaf decoction is given in treating rheumatic pain, enlarged spleen.^[4] ulcer, cough, and bronchitis. Mature Fruits are acrid, sweet, anthelmintic, and stomachic. They are useful in

pharyngodynia, cardiac disorders, gastropathy, bronchitis, haemorrhoids, cough, piles, jaundice, dyspepsia, smallpox, leucoderma and cholera.^[3] Seeds are used as purgative. Dried seed powder is used by women to induce conception. Seeds yield non-drying oil used in perfume industry. The seeds are ground with fire soot and the paste is applied to the neck for quick relief of tonsil pain. The seeds are used in traditional Indian Ayurvedic medicine, included in famous tonic formulations such as *Chyawanprash*. Bark decoction is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases. The medicated oil of *O. indicum* in sesame oil base instilled into ears mitigates the pain in otitis.^[2,3]

Ayurvedic preparations

O. indicum is used as one of the important ingredient in most commonly used Ayurvedic preparations such as *Dasamularistha*, *Syonaka putapaka*, *Syonaka siddha ghrita*, *Brhatpancamulyadi kvatha*, *Amartarista*, *Dantyadyarista*, *Narayana Taila*, *Dhanawantara Ghrita*, *Brahma Rasayana* and *Chyavanaprasha*.^[11,12]

CHEMISTRY

O. indicum leaves are known to contain flavones and their glycosides, baicalein (5, 6,7-trihydroxy flavone) and its 6 and 7-glucuronides, chrysin (5,7-dihydroxy flavone),^[13,14] scutellarein and its 7-glucuronides, anthraquinone and aloe-emodin,^[5,7,15] chrysin-7-O-glucuronide, chrysin-diglucoside and irridoids.^[16] Ethyl acetate extract of leaves of *O. indicum* was separated using high speed counter-current chromatography to get chrysin (160.9 mg, 97.3% purity), baicalein (130.4 mg, 97.6% purity), baicalein 7-O-glucoside (314.0 mg, 98.3% purity), baicalein-7-O-diglucoside (179.1 mg, 99.2% purity).^[17] From methanol extract of the leaves of *O. indicum*, chrysin-7-O-glucuronide, chrysin-diglucoside and baicalein were separated. Structure of the chrysin-diglucoside has yet to be obtained.^[16] Chloroform extraction of defatted leaves gives gummy solid yielding anthraquinone and aloe-emodin.^[15] Stem bark contain flavones oroxylin A (5,7-dihydroxy-6-methoxy flavone), chrysin, baicalein and its 6 and 7-glucuronide, scutellarin-7-rutinoside, traces of alkaloid^[5,18], tannic acid, sitosterol and galactose, baicalein, biochanin-A, ellagic acid.^[7] Ethyl acetate extract of root of *O. indicum* is reported to contain two flavonoids- i) 2,5-dihydroxy-6,7-dimethoxy flavone and ii) 3,7,3',5',-tetramethoxy-2-hydroxy flavone. Flavonoid

(i) has R_f value 0.621 in solvent system (petroleum ether: ethyl acetate, 3:1). This flavonoid was separated as fine crystals with M.P. 195-198°. Flavonoid (ii) has R_f value 0.721 in solvent system (petroleum ether:ethyl acetate, 3:1) and this flavonoid was separated as needle shaped crystals with M.P. 210-211°.^[19] Root bark contain chrysin, scutellarin-7-rutinoside, weak acids, traces of alkaloids,^[18] sitosterol, galactose, baicalein, biochanin-A, ellagic acid, oroxylin-A.^[7] and a yellow crystalline coloring matter 5,7-dihydroxy-6-methoxy flavone.^[11] Heartwood contains prunetin, sitosterol. Methanol extract of the fruits pods is reported to contain oroxylin A, chrysin, baicalein, a triterpene carboxylic acid and ursolic acid.^[20] Seeds contain oils and flavonoids such as chrysin, oroxylin A, baicalein, baicalein-7-O-diglucoside (Oroxylin B), baicalein-7-O-glucoside, apigenin,^[21] terpenes, alkaloids, saponins,^[22] tetuin, the 6-glucoside of baicalein, benzoic acid and fatty acids.^[13,14] A new flavone glucuronide-oroxindin and chrysin-7-O-diglucoside were also isolated. The seed oil contains caprylic, lauric, myristic, palmitic, palmotoleic, stearic, oleic and linoleic acids. Seeds also contain twenty percent shiny oil. Ether fraction of *O. indicum* gave scutellarein.^[23] Aqueous mother liquor gave scutellarein and baicalein.^[23,24] Baicalein was found to be major flavonoid present in petroleum ether extract.^[11] (fig. 2).

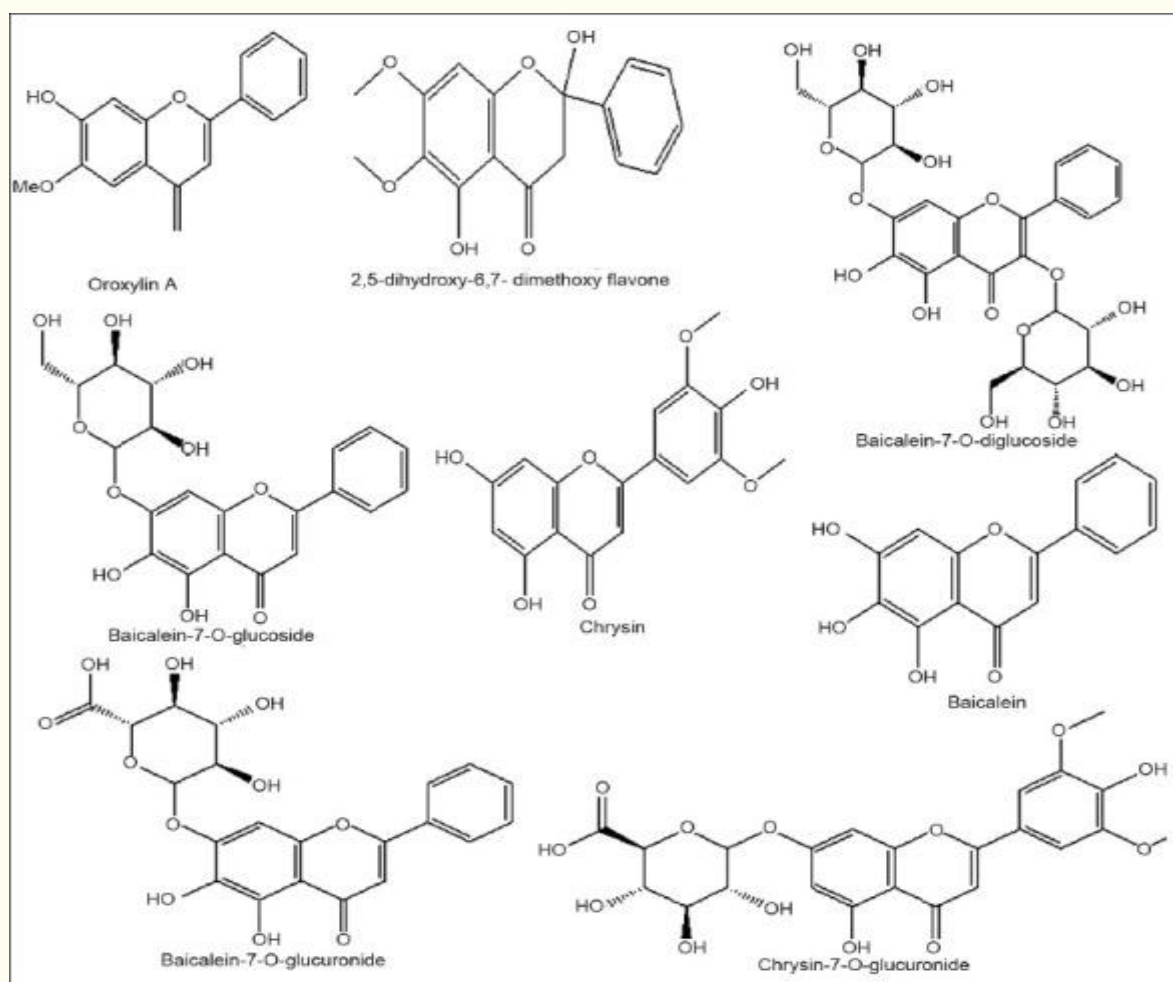


Fig. 2: Chemical structure of the biologically active compounds isolated from Oroxyllum indicum.

TLC of alcoholic extract of *O. indicum* was carried out on Silica gel G plate using solvent system, *n*-butanol: acetic acid: water, 4:1:5. TLC plate showed under UV (366 nm) a fluorescent zone at R_f 0.10 (blue). On exposure to iodine vapour, 6 spots appeared having R_f values 0.10, 0.30, 0.58, 0.70, 0.85, 0.95 and all were yellow in color. On spraying with 5% methanolic sulphuric acid and heating the plate for 10 min at 105°, 5 spots appeared having R_f values 0.25, 0.58, 0.70, 0.85, 0.95 and all grey in color.^[23] Yield of aqueous and ethanol extract of stem bark of *O. indicum* was reported to be 7.03% w/w and 6.14% w/w respectively.^[25] Yields of petroleum ether, chloroform, ethyl acetate and *n*-butanol extract of root bark of *O. indicum* were reported to be 0.32, 0.78, 1.52 and 1.68% w/w respectively.^[11] Yield of methanol extract of leaves of *O. indicum* was found to be 11.1% w/w.^[16]

PHARMACOLOGY

In crude plant extracts, flavonoids are often present as O- or C- glycosides. Flavonoids have various biological activities such as apoptosis induction, cell cycle arrest, antiproliferation, antiangiogenesis and antioxidation or a combination of these activities.^[26,27] Flavonoids are known for their antiinflammatory and antiallergic effect.^[28] Chrysin is a flavone which have many biological activities i.e. antibacterial,^[29] antioxidant,^[30] anti-inflammatory,^[31] antiallergic,^[32] anticancer,^[33] antiestrogenic,^[28] and anxiolytic activity.^[34] Chrysin also have tyrosine inhibitory.^[35] and moderate aromatase inhibitory activity.^[36] It can also inhibit the metabolism of the carcinogen benzo[α]pyrene by hamster embryo cells in tissue culture.^[37] Chrysin has antibacterial activity and to increase it chrysin derivative was also prepared, in which chrysin ring system linked to the alkyl amines by different spacers at C-7 position with a view to enhance their lipophilicity. A series of chrysin derivatives were prepared containing 3, 4 and 6-carbon spacers, in between heterocyclic ring and chrysin and were evaluated for antibacterial activity. Among them compounds which contain 4-carbon spacer in between chrysin and heterocyclic ring, displayed good antibacterial activity.^[38] Oroxylin-A, a naturally occurring monoflavonoid exhibit many biological activities such as COX-2 inhibition,^[39,40] cytotoxic,^[41] and antimicrobial.^[42,43] It also demonstrated anti-HIV,^[44] and lipid per-oxidation inhibition activities.^[45] Oroxylin-A has antibacterial activity, introduction of acyl group at C-7 position of oroxylin-A has enhanced the inhibitory potential to great extent.^[46] No detailed toxicity study of *O. indicum* has yet been published but available information shows that maximum tolerated dose is about 100 mg/kg.

Pharmacological Properties

Several workers have reported different biochemical activities of *O. indicum* in various *in vivo* and *in vitro* test models. Different part of this plant have been found to exhibit antiinflammatory, antimicrobial, antioxidant, anticancer, antimutagenic, photocytotoxic,

antiarthritic, immunostimulant, hepatoprotective, antiproliferative and hepatoprotective activities.

Antiinflammatory activity

The antiinflammatory activity was evaluated by carageenan induced rat paw edema model in rats using diclofenac sodium as standard drug. Two doses 150 mg/kg and 300 mg/kg of aqueous extract of *O. indicum* were used. Result showed that paw volume was significantly reduced in dose dependent manner as compared to control. Extract at a dose of 300 mg/kg showed maximum antiinflammatory activity. However, the activity produced by both the doses was less than the reference standard. Extract at both doses showed significant ($P<0.05$) anti-inflammatory activity at 5 h suggesting that the extract predominantly inhibit the release of prostaglandin like substances.^[47]

Antiulcer activity

The 50% alcohol extract of root bark of *O. indicum* and its petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions were studied against ethanol-induced gastric mucosal damage. The alcohol extract (300 mg/kg, p.o.) and its different fractions (100 and 300 mg/kg, p.o.) showed reduction in gastric ulceration. The petroleum ether and *n*-butanol fractions showed maximum inhibition of gastric lesions against ethanol-induced gastric mucosal damage. The results were comparable with omeprazole (reference standard). In the ethanol-induced gastric ulcer model, treatment with both the active fractions and omeprazole showed significant antioxidant activity as evident from the reduction in the extent of lipid peroxidation. The effect of active fraction of root bark on the ulcer index, total acidity, total acid output, pepsin activity, pepsin output and total carbohydrate to protein ratio in pyloric-ligated rat was studied. The active fraction of root bark at a dose level of 100 mg/kg p.o. showed significant reduction ($P<0.05$) in the ulcer index, total acidity, total acid output, pepsin activity and pepsin output along with a significant rise in total carbohydrate to protein ratio. The mechanism of antiulcer activity could be attributed to a decrease in gastric acid secretory and antioxidant activities leading to gastric cytoprotection. This activity could be linked to the presence of baicalein in the root bark of the plant.^[48]

Antimicrobial activity

The antimicrobial activity of crude extract of *O. indicum* (petroleum ether, ethyl acetate and methanol), compound 1 (2,5-dihydroxy-6,7-dimethoxy flavone) and compound 2 (3,7,3',5'-tetramethoxy-2-hydroxy flavone) was tested against fourteen pathogenic bacteria (5 Gram-positive and 9 Gram-negative) and seven pathogenic fungi. Nutrient agar and nutrient broth were used as bacteriological media and potato dextrose agar (PDA) was used for fungal growth. In antibacterial screening, each sample was dissolved in methanol at a concentration of 200 μ g/10 μ l. The activity of these samples was compared with standard kanamycin disc (K-30 μ g/disc) using the standard disc diffusion method.

Similarly antifungal screening was done at a concentration of 300 µg/disc for each sample and the activity was compared with the standard clotrimazole disc (K-30 µg/disc). From the antibacterial and antifungal experimental results, it was evident that the crude extracts (petroleum ether, ethyl acetate) and the compound 1 and 2 showed significant antibacterial and antifungal activity but were less potent than that of standard kanamycin and clotrimazole, where as methanol extract showed little activity. The findings support the use of *O. indicum* in traditional medicine for the treatment of bacterial and fungal infection.^[19,49]

Antioxidant activity

The antioxidant activity of ethanol and aqueous extract of *O. indicum* leaves was studied in two *in vitro* models viz. radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction and nitric oxide radical scavenging activity in Griess reagent system. Ethanol extract possessed significant antioxidant activity in both the models. In scavenging DPPH radical, extracts activity was $IC_{50}=24.22$ µg/ml while in scavenging nitric oxide (NO) radical, the activity was $IC_{50}=129.81$ µg/ml. The result showed that ethanol extract of *O. indicum* leaves possesses free radical scavenging activity.^[50]

Anticancer activity

Methanol extract of the fruits of *O. indicum* inhibited *in vitro* proliferation of HL-60 cells. The flavonoid baicalein was found as an active component in the extract. The *in vitro* effects of baicalein on the viability and induction of apoptosis in the HL-60 cell line was further investigated. The cell viability after treating with baicalein for 24 h was quantified by counting viable cells using trypan blue staining. The result showed that baicalein caused a 50% inhibition of HL-60 cells at concentration of 25-30 µM. The inhibition of proliferation of HL-60 cells due to 36-48 h exposure with 10 or 20 µM baicalein was associated with the accumulation of cells at S or G2M phases. The results indicated that baicalein has antitumor effects on human cancer cells.^[20]

Antimutagenic activity

Methanol extract of *O. indicum* strongly inhibited the mutagenicity of Trp-P-1 in an Ames pre-incubation method in the presence of S9 mix using *Salmonella typhimurium*. Only 5 µl of the crude extract inhibited 91±5% of the mutagenesis induced by 50 ng of Trp-P-1. The major antimutagenic constituent was identified as baicalein with an IC_{50} value of $2.78±0.15$ µM. The potent antimutagenicity of the extract was correlated with the high content (3.95±0.43%, dry weight) of baicalein. Baicalein act as desmutagen since it inhibits N-hydroxylation of Trp-P-2. The antimutagenic effect of baicalein was found mainly due to the inhibition of N-hydroxylation catalyzed by P450 monooxygenases in S-9.^[45]

Photocytotoxic activity

The photocytotoxic activity of methanol extract of leaves of *O. indicum* was studied against promyelocytic leukemia cell line, HL60. The HL60 was incubated with 21 µg/ml of crude extracts for 2 h and irradiated with 9.6 J/cm² of a broad spectrum light source in four replicates. Survival of cells was assessed 24 h later following the colorimetric MTT protocol. Pheophorbide-a, a commercially available and well-characterized photosensitizer was used as the positive control. To determine samples that have general cytotoxicity, a parallel assay without irradiation was also carried out. The result showed that methanol extract of leaves of *O. indicum* have photocytotoxic activity at concentration 21 µg/ml.^[51]

Antiarthritic activity

Aqueous and ethanol extract of *O. indicum* were tested for *in vitro* release of myeloperoxidase (MPO) from rat peritoneal leukocytes. The results indicated that aqueous extract had a significant effect i.e. 64% inhibition of release of MPO.^[25]

Immunostimulant activity

n-Butanol extract of root bark of *O. indicum* (100 mg/kg, once daily for 22 days) was studied for immunomodulatory activity in rats using measures of immune responses to sheep red blood cells (SRBC haemagglutinating antibody [HA] titer) and delayed-type hypersensitivity (DTH) reactions. In response to SRBC, treatment with the *n*-butanol fraction caused a significant rise in circulating HA titers during secondary antibody responses, indicating a potentiation of certain aspects of the humoral response. The treatment also resulted in a significant rise in paw edema formation, indicating increased host DTH response. Histopathologic analysis of lymphoid tissues in the treatment group showed an increase in cellularity, e.g., T-lymphocytes and sinusoids. In contrast, dexamethasone treatment caused significant reduction in the HA titer, DTH responses, and antioxidant activity. In a triple antigen-mediated immunological edema model, the extent of edema raised in drug-treated rats was greater compared to that in control rats, thus confirming enhanced DTH reactions in response to the drug treatment. Activity of the *O. indicum* might be attributed to its ability to enhance specific immune response (both humoral and cell-mediated).^[52]

Antiproliferative activity

The antiproliferative activity of *O. indicum* was studied on human breast tumor cell lines. Results indicated that *O. indicum* have antiproliferative activity against MCF7 and MDA-MB-231 breast cancer cell lines.^[53]

Hepatoprotective activity

The hepatoprotective activity of *O. indicum* was studied against carbon tetrachloride (CCl₄)-induced hepatotoxicity in mice and rats. Biochemical study indicated that alcoholic (300 mg/kg), petroleum ether

(300 mg/kg) and *n*-butanol (100 and 300 mg/kg) extracts significantly ($P < 0.05$) lowered the elevated serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) levels as compared to the control group. The increased lipid peroxide (LPO) formation, reduced glutathione (GSH) and decreased antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT) in the tissues of CCl_4 -treated animals were significantly normalized by *O. indicum* treatment. Histopathological study also revealed that pretreatment with *O. indicum* restored CCl_4 -induced alteration in antioxidant status of the tissues. It is suggested that root bark showed significant antioxidant activity, which might be in turn responsible for its hepatoprotective activity.^[54]

CONCLUSIONS

O. indicum is a well known plant used in the Indian system of medicine, besides folklore medicine also claims its use in cancer, ulcer, diarrhea, dysentery, wound healing, inflammation, cough, enlarged spleen, jaundice, scabies and other skin diseases. Research carried out using different *in vivo* and *in vitro* techniques of biological evaluation support most of these claims. Recent studies have focused mainly on its anti-inflammatory, antiulcer, antimicrobial, antiarthritic, hepatoprotective, immunostimulant, photocytotoxicity, antimutagenic, antiproliferative and antioxidant activities. Literature survey reveals that despite the enormous work done on this plant some of the pharmacological activities are still not proven scientifically. Some of the compounds present in *O. indicum* (chrysin, oroxylin A, baicalein, baicalin, irridoid, baichanin-A, 2,5-dihydroxy-6,7-dimethoxy flavone and 3,7,3',5'-tetramethoxy-2-hydroxy flavone) are pharmacologically well known and provide additional supporting evidence for possible mechanism of action. *O. indicum* is used in the manufacture of various Ayurvedic preparations for a range of ailments and its non-drying oil used in perfume industry. This review was an attempt to compile an up-to-date and comprehensive review of *O. indicum* that covered its distribution, description, traditional and folk medicinal uses, phytochemistry and pharmacology.

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