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Phytochemical and Pharmacological Activities of *Zantoxylum armatum.* DC.: An Overview.

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ABSTRACT

Zanthoxylum armatum DC. (Family: Rutaceae) is a branched, scandent, or erect shrub or a small tree, 6 m tall or more, with dense foliage and found in the hot valleys of the Himalayas from Jammu to Bhutan and in Eastern Ghats in Orissa and Andhra Pradesh in India, Nepal and Pakistan. The plant has been commonly used in traditional system of medicine for the treatment of fever, skin sensitivity, anti-inflammation, chest infection, Dental problems, and digestive problems and in scabies. In view of the immense medicinal importance of *Z. armatum*, this review was aimed at compiling all currently available botanical, phytochemical, pharmacological and ethno-medicinal information on *Z. armatum*. Information in the biomedical literature has indicated the presence of a variety of medicinally-important chemical constituents in *Z. armatum*. Pharmacological studies by various groups of investigators have shown that *Z. armatum* possesses significant biological and pharmacological activities, such as antibacterial, antifungal, antiviral, anti-inflammatory and antioxidant properties.

Keywords: *Zanthoxylum armatum*, ethno-medicinal uses, phytochemistry, Pharmacological activities.

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INTRODUCTION

Plants containing inborn potentially active ingredients used to cure disease or relieve pain are called medicinal plants [1]. Plants play a therapeutic and restorative role in protecting human beings from the adverse effects of diseases and other complications, thus considered to have a beneficial role in healthcare system. That is the reason that large proportion of population of the developing countries still rely on herbal medicines. Despite their importance, medicinal plants are seldom handled within an organized manner and most are of them are exploited with little or no respect for the future [2]. Significant increase in medicinal plants usage has been recorded continuously both for traditional users and pharmaceutical industry. Medicinal plants provide opportunities for biological screening, methods useful for the industry and trends in the pharmacological investigations of natural products. Plants are the natural and most easy accessible source of therapeutically active biological principles, thus there is a dire need to screen out plant for development of new drugs. For this purpose plants have been assayed widely but still large number of them has not arrived to the conventional health care system [3, 4]. Therefore, search for new drugs from microorganisms, fungi, plants and animals must be persistent and these can be the sources of innovative and prevailing restorative agents for newer, safer and accessible drugs [5]. Now a day, due to advancement of modern and new sophisticated methods, plant scientists are taking more interest in exploring new drugs from natural and biologically active compounds of the plants, which could be serve as inexhaustible resources for pharmaceutical industries [6]. *Zanthoxylum armatum* is one of the most important medicinal plants in Indian medicinal Literature. Almost all parts of this plant are used in Indian traditional system for the treatment of various ailments and the significant medicinal properties was further reported through scientific investigation. However, detailed information on this plant is not available. Keeping this in view the present review was focused on the botany, phytochemistry, pharmacology and ethno-medicinal uses of Z*. armatum*.

Botany

Timur (*Zanthoxylum armatum*)

A B C D

Figure 1: Z*anthoxylum armatum* (A & B) twig with unripe fruits (C) twig with ripened fruits (d) Dried fruits.

Taxonomic position of *Zanthoxylum armatum* DC

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Rutales

Family: Rutaceae

Genus: *Zanthoxylum*

Species: *armatum*- DC.

English names: Bamboo-Leaved Prickly Ash, Prickly Ash, Toothache Tree, Winged Prickly Ash, Wing leaf Prickly Ash

Other common name

Hindi – Darmar, nepali dhaniya, tejphal, tumuru, **Bengali** – Gaira, tambul, **Oriya** – Tundopoda, **Sanskrit** – Tumburu, dhiva, gandhalu, tejovati, **Manipuri**- Mukthrubi, **Nepali-**Timur, Nepali peeper, **Urdu**- Dambrary, Tamu.

Distribution of *Zanthoxylum armatum*

Zanthoxylum armatum DC is commonly known as timur, found in the hot valleys of the Himalayas from Jammu to Bhutan at altitudes of 1,000-2100 m and in Eastern Ghats in Orissa and Andhra Pradesh at 1,200 m., in India, Nepal and Pakistan.

Botanical description of *Zanthoxylum armatum*

It is a branched, scandent, or erect shrub or a small tree, 6 m tall or more, with dense foliage. Leaves are compound, imparipinnate with 3-7 foliolate and pellucidpunctate. Petiole and rachis are winged. Leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. The branches are armed with thorns of up to 2 cm. Flowers born axiliary, minute and polygamous. Calyx 6-8-acute, lobed, petals absent. Male flowers with 6-8 stamens with rudimentary ovary and female flowers with 1-3 carpals. Ovary 1-3 locular. Fruit small drupes with red colour, splitting into two when ripe. Seed are rounded and shining black. Its fruits and seeds are edible and used as potherb species. According to farmers, timur grows best on sites with deep, moist soils that are also exposed to the sun. This is reflected in the occurrence of many timur shrubs or small trees around cultivated farmland. Farmers mention that the trees are mainly disseminated by birds, who like the fruits. During digestion, seeds are scarified, which stimulates germination. Timur can also be propagated vegetatively from branch cuttings or seeds. Timur flowers regularly around April to May and produces constant fruit yields over the years. However, hailstorms in spring can destroy the flowers. Due to their carminative, stomachic and anthelmintic properties [7], the fruits, seeds, and bark of timur are extensively used in indigenous medicines.

Propagation of *Zanthoxylum armatum*

It is generally propagated through seeds, but occasionally through soft wood cuttings and air layering.

Ethno-medicinal uses

Z. armatum is used locally as medicinal plants and fuel wood species. Fruits and seeds are edible and used as potherb species. The plant is used for Pneumonia and tick infestation [8]. Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for toothache, dyspepsia, as a carminative and stomach ache. Seeds are used as condiment and flavouring agent. Wood is used to make walking sticks [9, 10].

Powdered fruit is mixed with *Mentha* species and table salt, eaten with boiled egg for chest infection and digestive problems [11]. The fruits and seeds are used as an aromatic tonic for fever and dyspepsia. Fruit is used for toothache, dyspepsia, as a carminative and stomach-ache. Seeds are used for condiment and flavouring agent. An extract of the fruits is reported to be effective in expelling roundworms [7]. Because of their deodorant, disinfectant, and antiseptic properties, the fruits are also used to treat dental problems, and their lotion is used against scabies. Steam distillation of dried fruits yields an essential oil that has deodorant and antiseptic properties; it is used in soaps and dental preparations. Powdered fruit is mixed with *Mentha* sp. and table salt, eaten with boiled egg for chest infection and digestive problems [11]. The oil obtained by steam distillation of the fresh plant shows antifungal activity. The bark is pungent and used to clean teeth. Due to its appealing aroma and valuable perfume, timur is used in the manufacture of several health-care products. Most of this manufacturing takes place in India, which therefore has a well-established commercial outlet for dried timur fruits [12,13].

Phytochemistry of *Zanthoxylum armatum*

Several alkaloids have been isolated from the stem-bark and root-bark. The fruits of this species yielded essential oils. A number of alkaloids has been isolated and reported from the various parts of the *Zanthoxylum armatum* DC. berberine (bark), dictamnine (stem-bark), magnofluorine(0.02% as picrate), xanthoplanine (0.01% as picrate) (wood and bark), magnofluorine (0.17% as picrate), xanthoplanine, skimmianine, dictamnine and -fagarine *(Wealth of India 2005).* A new amide designated as armatamide along with two lignans, asarinin and fargesin, α- and β-amyrins, lupeol, and β-sitosterol-β-D-glucoside – has been isolated from the bark of timur. The structure of the new compound was deduced by spectral and chemical analysis as N-(4'-methoxyphenyl ethyl)-3, 4-methylenedioxy cinnamoyl amide [14].

Pharmacological activities of *Zanthoxylum armatum*

Pharmacognostic studies and physicochemical properties of the *Zanthoxylum armatum* leaf-

Essential oils from *Zanthoxylum armatum* leaf (ZVO) were hydro distillated and GC-MS analysis was carried out for identification of various components by Naveed et al, [15].

Anti-inflammatory

Bergapten, a coumarin extracted from the plant exhibited significant inhibition of the production of pro-inflammatory cytokines namely tumour necrotic factor -α (TNF- α) and interleukin-6 (IL-6) by PBMCs stimulated with lipopolysaccharide in concentration-dependent manner [16]. Also linalool and linalyl acetate are known to acquire inflammatory activity [17].

Antispasmodic activity

Rabbit's jejunum preparations: Experiments on rabbit's jejunum preparations were carried out as following [18]. Slaughtered animals were dissected to open abdomen, and jejunum portion(s) were extracted and kept in freshly prepared Tyrode's solution, aerated with carbogen gas (5% Carbon dioxide and Oxygen mixture) to keep them alive and ready for use. Quiescent sub-maximal doses of acetylcholine (0.3 µM) to the tissues were used when needed for keeping the tissue viable and alive [19]. About 1.5 cm length tissue was mounted in 10 ml tissue bath containing Tyrode's solution and stabilized for 25-30 minutes. All the processes were carried out at 37+ 1oC with constant aeration and kept under 1 gram pressure. On attaining reproducible response, test samples at the doses of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, and 10.0 mg/ml were applied to the bath solution [18, 19]. The processes were repeated thrice (n=3) and fall in spontaneous activity was observed to be change of the sample tested. For the determination of possible mode of action, the tissue was pre-treated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction [20]. The extract was then applied in cumulative manner to obtain a dose dependent curve and relaxation. Intestinal responses data were recorded using Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) attached with Power lab (Model No: 4/25 T) AD Instruments, Australia. Data was recorded at range of 20 mv, low pass 5 Hz X 10 gain using input 1, rate 40/S. Results were expressed as% of KCl induced contraction. Student "t" test was used at 95% confidence interval (CI). 'P' values less or equal to 0.05 was considered as statistically significant [19].

Anti-bacterial activities

Antibacterial activities of the plant were carried out by agar well diffusion method as used in our previous study [21]. Bacterial strains were first cultured on nutrient broth and incubated for 24 hours prior to experiments. Nutrient agar was melted, cooled to 40oC and poured into sterilized petridishes. Wells were then bored in media using 6mm diameter with the help of sterile metal cork borer and keeping a distance of 24 mm between two adjacent wells. 4-8 hour old bacterial culture was spread on the surface of nutrient agar with the help of sterilized cotton swab. These processes were repeated thrice turning the plate 60o between each streaking. About l00 µl of 3 mg/ ml of respective extract, dissolved in DMSO was then added to the wells. Other wells were supplemented with DMSO and 10 µg Imipenem served as positive and negative controls. The plates were then incubated for 24 hours at 37oC. The plates were then observed for zones of inhibition. All the experiments were conducted in triplicate.

Anti-fungal activities

Seven days old fungal cultures (PCSIR labs Lahore Pakistan), test samples, sabouraud dextrose agar, Dimethyl sulphoxide (DMSO), screw cap test tubes, micropipettes, autoclave, incubator, standard antibiotic (Miconazole). Twenty four mg of crude extract was dissolved in 1 ml sterile Dimethyl sulphoxide (DMSO) serving as a stock solution. 4 ml Sabouraud dextrose agar (SDA) growth media was transferred to each screw capped tube, under sterile conditions and autoclaved at 121oC for 15 minutes. These tubes were then allowed to cool to 50oC and 400 µg/ml test sample was added to non-solidify SDA tubes, which were then allowed to solidify at room temperature. Next each glass tube was inoculated with 4 mm diameter piece of inoculum removed from 7 days old fungal culture, whereas, agar streak was employed in case of non-mycelial growth. Other media supplemented with DMSO and miconazole antibiotic were used as a negative and positive control respectively. The tubes were incubated at 28} 1oC for 7 days. Cultures were observed twice weekly during incubation. Growth in the media was estimated by measuring linear growth (mm) in the in media loaded with sample, DMSO and miconazole respectively and then percentage inhibition of fungal growth was calculated as follows

$$\% \text{ Mycelia inhibition} = Gn - Gt / Gn \times 100$$

Where, Gn= Mycelial growth in normal, Gt= Mycelial growth in test [22]

Cytotoxicity

The cytotoxic activity of essential oil from the leaves of *Zanthoxylum armatum* was tested using brine shrimp assay following recommended method [23, 24]. About 20 mg of each extract was dissolved in 2 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to vials (3 vials /concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml, respectively. The solvent were allowed to evaporate overnight. 5 ml with seawater solution (38 g/L) were added to each vial. After 36 h of hatching and maturation of larvae as nauplii, 10 larvae were transferred to each vial using a Pasteur pipette. The vials were then placed at room temperature (25-27oC) under illumination. Other vials were supplemented with brine solution served as positive controls.

Phytotoxicity

The phytotoxic activity of essential oil of the leaves of *Zanthoxylum armatum* were evaluated using *Lamna minor* as test species following recommended procedure [23,24]. 15 mg of respective extract was dissolved in 15 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to the flask (3 flasks for each concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight under sterilized condition in laminar flow. 20 ml of E. medium was added to each flask. Other flasks (3 for each) were supplemented with E. medium and standard drug (Atrazine) served as negative and positive control. To each flask ten plants with 2-3 fronds were transferred and kept all the flasks under about 12 h day light conditions. Plants were observed daily and on each seventh day the numbers of fronds were counted.

Hepatoprotective activity

In the ethanolic extract of *Z. armatum*, a progressive increase has been recorded in the incidence of hepatic damage mainly due to the viral infection, hepatotoxic chemicals (alcohol), toxin in food (especially aflotoxins), peroxides (particularly peroxidised edible oil), pharmaceuticals (antibiotics, chemotherapeutics, and CNS active agents), environment pollutants and xenobiotics [25].

Biological activity of the isolated compounds from *Zanthoxylum armatum*

Various biological activities have been reported in *Zanthoxylum armatum* and some major ones are discussed below.

Mosquito repellent- The mosquito repellent property of its oil against mosquitoes in mustard and coconut oil base and compared with synthetic repellent dimethyl phthalate (DMP) as standard. It is afforded better protection in both the base at all the concentrations. Repellents in mustard oil gave longer protection time than in those in coconut oil. At 0.57 mg/cm^2 concentration the oil gave significant higher protection both in mustard (445min) as well as coconut oil (404 min) than DMP [26].

Piscicidal

The Piscicidal activity of the ethyl alcohol extract of the fruits was evaluated on Mg^+ and Na^+, K^+-ATPase activity in different tissues of carnivorous air-breathing catfish, *Hetropneustes fossilis* [27].

Leech Repellent

Essential oil of Z. armatum possesses leech repellent activity. Experiments on persistence of repellents properties of N,N diethyl phenyl acetamide (DEPA) N,N diethyl phenyl m-toluamide (DEET), 3-acety 2 (2,6-dimethyl-5-heptenyl) oxazolidine (citronyl), Dimethyl phthalate (DMP) and N-benzoyl piperidine (NBP) on cloth were tested against land leeches in evergreen rain and deciduous forests of Assam [28]. Results obtained were compared with volatile oil of it to evaluate its efficacy as leech repellent.

Inhibits skin sensitivity

A lipophilic extract of fruits was credited reducing mouth irritation due to food [29]. Dilution of this extract with oleyl alcohol gives ingredients of cosmetic which is easy to formulate and is endowed with a remarkable soothing effect based on inhibition of sensory irritation from sun bathing, shaving, depilation, insect bites, chemical treatments and other causes.

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