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NATIVE AMERICAN ETHNOBOTANY AND TOXICOLOGICAL ANALYSIS OF THE *ZANTHOXYLUM* GENUS

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ABSTRACT

Zanthoxylum americanum, otherwise known as northern prickly ash, is an aromatic shrub or small tree native to North America. It is used in several traditional medicines to cure various diseases. This shrub has been known to act as a dermatological aid, antifungal, toothache remedy, and a cold and cough remedy[1].

A wide range of chemical compounds found within the plant have shown promising pharmacological characteristics. These include coumarins, alkaloids, and furanocoumarins which come from the stem, root, bark, berries, and many other parts of the plant. Some of the most important furanocoumarins found in *Z. americanum* extracts are psoralen, 8-MOP, and imperatorin, and it is these compounds that give the fruit, husk, and leaf extracts their broad spectrum of antifungal activity[2]. Furanocoumarins are phototoxic by covalently binding to DNA forming adducts which allow *Z. americanum* to be used in phototherapeutic applications against fungal infections[2,3]. Furanocoumarins also correlate to the antimicrobial activity of *Z. americanum*[2]. The chemical compounds within *Zanthoxylum americanum* make it a common therapeutic for many infections and other skin conditions.

Documenting and analyzing how other species within the *Zanthoxylum* genus can be used in traditional medicine is important to further understand the properties that cause toxicity or have therapeutic effects. This review documents the ethnobotany, chemistry, and toxicity/bioactivity of other species within the *Zanthoxylum* genus including *Z. armatum* DC. and *Z. acanthopodium* DC.

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INTRODUCTION

The *Zanthoxylum* genus belongs to the Rutaceae family which consists of over 200 species including trees and shrubs in warm subtropic areas around the world[4]. Some are more commonly used in Northeastern United States, while others are found in places like India, Nepal, and Indonesia. *Zanthoxylum* comes from the word *Xanthoxylum* which in Greek derives to "zanthon xylon" or yellow wood[5]. Many articles and authors use *Zanthoxylum* and *Xanthoxylum* interchangeably[5]. Species in the *Zanthoxylum* genus have distinct features. They have the presence of recurved spines on the trunk and branches[5]. The leaf shapes can be varied having up to 15 pairs of leaflets[5]. The seeds of the fruits are either red or black with a slight shine[5,6]. The fruits can contain one to five aromatic carpels[5,7]. Species in the *Zanthoxylum* genus have been used in traditional medicine to treat fungal infections, stomach ailments, inflammation, and rheumatic diseases[4]. They are importance sources of edible fruits, oils, and medicinal plants[5]. The major components of *Zanthoxylum* species are furanocoumarins, monoterpenes, and alkaloids. They have shown potential biological activities such as antioxidant, antibacterial, antimicrobial, and antifungal.

ZANTHOXYLUM ARMATUM DC.

ETHNOBOTANY

Zanthoxylum armatum DC. is an erect shrub or small tree found in the hot valleys of the Himalayas and India[8]. The seeds, bark, and fruit are used to treat various diseases. The fruits, branches, and thorns are used to help elevate toothaches whereas the seeds and bark are used in fever, indigestion, and cholera treatment[9,10]. There are also some studies showing that *Z. armatum* DC has the potential to help manage diabetes.

Figure 1: Zanthoxylum armatum. (a) A mature flowering plant (b) Young fruits (c) Ripe fruits. Reconfigured from Phuyal et al. (2019)[11].

Table 1: Ethnomedicinal uses of Zanthoxylum armatum. Reconfigured from Phuyal et al. (2019)[11]

Ailments/Use	Parts used
Abdominal pain	Fruit decoction
Anthelmintic	Fruits and seeds
Cold and cough	Fruits
Dyspepsia	Fruits
Flavoring agents	Seeds
Gastrointestinal disorders	Fruits
Insecticides/pesticides	Branches
Rheumatism	Fruits
Stomachache	Fruits
Toothache	Fruit/seeds
Tuberculosis	Seeds

CHEMISTRY

Essential oil extractions from *Zanthoxylum armatum* DC branches and leaves contain β-Terpinene (45.56%), piperitone (33.47%), and 3-carene (8.88%)[12], all of which are monoterpenes. However, different places of origin and harvest time seem to cause varying chemical composition for the same plant essential oil[12]. This is seen with *Z. armatum* DC as varied harvest times resulted in a change of main components to 2-undecanone (19.75%), followed by 2-tridecanone (11.76%) and β-caryophyllene (9.88%)[12,13]. Essential oils extracted from Kashmir and Jummu contained linalool, linalyl acetate and limonene[14,15].The main constituents of the essential oils of *Zanthoxylum armatum* DC are consistently monoterpenes which have demonstrated insecticidal as well as antibacterial activities[16]. It has even been used to treat some cancers [16]. Crude methanol extracts of *Zanthoxylum armatum* DC are seen to also contain phenolic and flavonoid compounds like tambulin that show antimycotic potential[11].

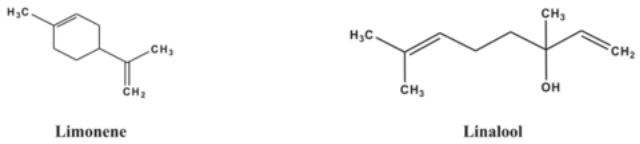


Figure 2: Terpene Structures in Z. armatum DC[14]

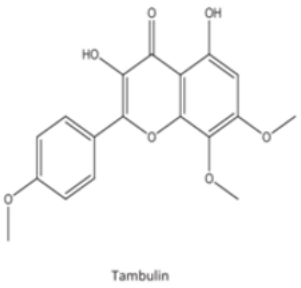


Figure 3: Flavonoid Structure in Z. armatum DC [11]

TOXICITY/BIOLOGICAL ACTIVITY

Zanthoxylum armatum DC methanolic and ethanolic fruit extracts evidenced antioxidant properties through free radical activity and radical scavenging activity[16,17,18]. Methanol extracts of leaves of *Zanthoxylum armatum* DC increased activity of antioxidant enzymes Superoxide dismutase, Catalase, and Glutathione in vivo[11]. This shows the potential of *Zanthoxylum armatum* DC as a natural antioxidant.

An ethanolic extract of *Zanthoxylum armatum* DC bark showed moderate activity against *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Streptococcus viridans*[11]. The hexane extract of the bark was found active against *M. luteus* and *P. multocida*[11]. Leaf essential oil of *Zanthoxylum armatum* DC exhibited strong activity against *M. luteus*, *S. aureus*, *E. coli* and *B. subtilis* but the methanol extract was found to be ineffective against all tested bacterial strains[11]. Different extracts seem to have different antibacterial activities which might be due to varying concentrations of chemical molecules shown to have antibacterial properties. This is important in targeting different bacterial strains to treat different bacterial diseases.

Zanthoxylum armatum DC also inhibits activity against microorganisms with the largest zone of inhibition being against *Bacillus subtilis*[16,19]. The ethanol extract shows inhibition of growth of both *Bacillus cereus* and *Bacillus thuringiensis*[19]. *Zanthoxylum armatum* DC can be an effective antimicrobial agent against *Bacillus* genus microorganisms.

Crude leaf extracts of *Zanthoxylum armatum* DC have shown to induce cytotoxicity through apoptosis and enhance caspase activation which leads to synergistic interactions among

chemotherapeutic drugs and *Zanthoxylum armatum* DC.[20]. *Zanthoxylum armatum* DC in tandem with chemotherapeutic drugs could be a novel anti-cancer treatment.

Zanthoxylum armatum DC essential oils from the leaves have been seen to have in vitro antifungal properties against fungal strains like *Trichophyton longifilis*, *Candida albicans*, *Fusarium solani*, *Microsporum canis*, *Aspergillus flavus* and *Candida glabrata*[11]. The oils also have antifungal activities against *Alternaria alternata*, *Alternaria brassicae*, *Curvularia lunata* which are crop pathogens[11]. These results show that *Zanthoxylum armatum* DC has potential to be used in agriculture against crop pathogens.

Table 2: Bioactivity of Zanthoxylum armatum and extracts. Reconfigured from Brijwal et al. (2013)[16].

Activity	Active Parts	Preparation
Antibacterial	Seeds/leaves	Essential oil
Antifungal	Leaves	Essential oil
Anti-inflammatory	Stem, bark Fruits Roots	Ethanolic extract Methanolic extract Ether extract
Antioxidative	Fruits	Methanolic extract Ethanolic extract
Antitumor	Leaves/fruits	Ethanolic extract

ZANTHOXYLUM ACANTHOPODIUM DC.

ETHNOBOTANY

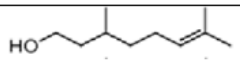
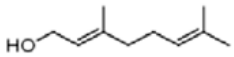
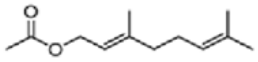
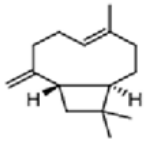
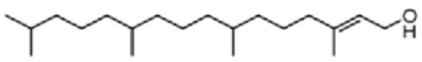
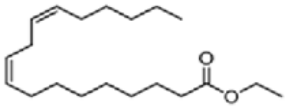
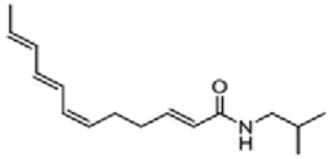
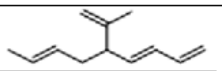
Zanthoxylum acanthopodium DC. is an aromatic shrub or a small tree that has distinct male and female flowers making it a dioecious plant[21]. It is often referred to as andaliman or Indonesian lemon pepper in Indonesia, Nepal, and India. It has closely compact leaves and a prickly stem[21]. The fruits, seeds and leaves have been traditionally used to treat stomachache, toothache, cough, and bronchitis[21,22]. The leaves have also been used as a spice, vegetable, and to kill or repel insects and pests[21,23]. In Indonesia, spices are not only valuable due to their flavoring in cooking but also in traditional medicine and keeping the body warm[24].

Figure 4: Zanthoxylum acanthopodium DC. fruits. Reconfigured from ("Zanthoxylum acanthopodium DC.", 2020)[25].

CHEMISTRY

Extracts of *Zanthoxylum acanthopodium* DC. commonly contain alkaloids, phenol compounds, flavonoids, tannins, triterpenoids, and many others[26]. Alkaloids, flavonoids, and tannins give andaliman its antimicrobial activity and phenolic compounds add to the antibacterial activity[28,29,30]. Flavonoids add to the antifungal activity of *Zanthoxylum acanthopodium* DC.[26,27]. β-myrcene, limonene, and citronellal are all seen in *Zanthoxylum acanthopodium* DC. essential oils and impact the unique flavoring of andaliman[31,32]. Geranyl acetate also plays a role in the fresh citrus and peppery smell of Z. acanthopodium DC.[30,33]. It also has shown to have antimycobacterial activity[34]. Different extracts of *Zanthoxylum acanthopodium* DC. have different phytochemical compounds as well as different amounts of them. It was found that there are more phytochemical compounds in *Zanthoxylum acanthopodium* DC. methanol fruit extracts compared to hexane extracts[30]. Another study found that there are roughly 20 compounds in the methanol extract and sixteen of them are terpenes and terpenoid derivatives[31]. The methanol extract of *Z. acanthopodium* fruit has geranyl acetate as the main compound with 26.72%[31]. This is similar to the n-hexane fraction that has geranyl acetate as 26.34%[35]. Another common compound found amongst *Z. acanthopodium* fruit extracts is geraniol, a monoterpene that has antimicrobial and antioxidant activity[35]. The ethyl acetate extract of *Z. acanthopodium* leaves revealed that the main compound was paulownin (93.17%), followed by lariciresinol (4.08 %) and bis (2-ethylhexyl) phthalate (1.58 %)[21]. Paulowin has been known to have antifungal properties[21,36].

Table 3: Identified chemical content of Zanthoxylum acanthopodium fruit. Reconfigured from Sibero et al. (2020)[31].

Compounds	Retention time	Peak area (%)	Structure
Citronellol	6.46	1.30	
Geraniol	6.82	7.31	
Geranyl acetate	8.52	26.72	
trans-Caryophyllene	9.27	1.51	
2-hexadecen-1-ol	16.99	1.72	
Ethyl linoleate	17.38	1.03	
Neoherculin	17.48	20.99	
5-(propenyl-2)-1,3,7-nonatriene	17.72	2.39	

TOXICITY/BIOLOGICAL ACTIVITY

Zanthoxylum acanthopodium DC. fruits' alkaloid fractions scavenge the free radical DPPH, with the chloroform fraction (pH 9) being the strongest antioxidant and the water fraction possessing the lowest antioxidant activity[37]. While the n-hexane crude extract fraction did not show antioxidant properties[37], the ethyl acetate extract did evidence antioxidant activity[38]. The alkaloids of *Zanthoxylum acanthopodium* DC. fruits act as lipid soluble antioxidants that prevent free radical striking[37,39]. This shows the potential of different *Zanthoxylum acanthopodium* DC. fractions to act as natural antioxidants.

As seen in *Zanthoxylum armatum* DC, different extracts have different antibacterial activity. For *Zanthoxylum acanthopodium* DC., the ethyl acetate extract had higher antibacterial activity against *S. aureus* and *S. typhimurium* compared to the methanol extract which had higher antibacterial activity against *E. coli*[30]. Hexane extract of *Zanthoxylum acanthopodium* DC. fruits have shown to be active against *Mycobacterium smegmatis* and could be toxic to this bacterium through cell membrane damage[34]. It shows that andaliman or Indonesian lemon pepper can be effective against mycobacterial infection and *M. tuberculosis* due to the oleic acid, and geranylgeranyl acetate found in the fruits[34, 40,41]. The data shows the importance of *Zanthoxylum acanthopodium* DC in preventing bacterial infections which can lead to stomachaches and other gastric problems. Further research needs to be done on its potential to be a tuberculosis therapeutic.

Andaliman has shown to have antimicrobial activity as andaliman fruit extracts can inhibit the growth of microbes that are pathogenic and destroy foodstuffs[42,43]. Some microbes that *Zanthoxylum acanthopodium* DC. fruit extracts can inhibit are *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Aspergillus flavus*[42,43]. Essential oils from the leaves inhibit the growth of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*[42,44]. Methanol extract has the highest yield as well as the highest growth inhibition towards *Staphylococcus aureus* compared to the ethyl acetate, water, and hexane extractions[45]. The ethyl acetate extract inhibited growth towards *Staphylococcus typhimurium* and it was found that it gave the highest antimicrobial activities and the lowest minimum inhibitory concentration[45]. Many of the alkaloids in *Zanthoxylum acanthopodium* DC. are hypothesized to be responsible for the antimicrobial activity against *Staphylococcus aureus*[30,45]. An interesting finding is that *Zanthoxylum acanthopodium* D.C. fruit extract can be used to inhibit the growth of microbial spoilage in catfish fillet which means it could be quite promising in the food industry[30]. The data suggest *that Zanthoxylum acanthopodium* DC. can be used as an antimicrobial agent.

There have been very few studies done to show the antifungal activity of *Z. acanthopodium* D.C.. However, the flavonoids within the fruits have had some reported antifungal activity by inhibiting the growth of *A. alternata* and *C. lunata*[27,45]. Parts of the crude extracts of *Z. acanthopodium* D.C. showed moderate antifungal activity against two fungi named *Candida albicans* and *C. Krusei*[46]. Compared to the ethyl acetate and chloroform extracts, the petroleum ether extract had the greatest antifungal activity and the lowest minimum inhibitory concentration[46]. This shows that crude extracts especially the petroleum ether extract can be used

for antifungal activity, however further investigation into other extracts of *Z. acanthopodium* D.C. is necessary to fully understand its antifungal properties.

CONCLUSION

Zanthoxylum armatum D.C., *Zanthoxylum acanthopodium* D.C., and *Zanthoxylum americanum* are important medicinal plants as they have shown to have antioxidant, antibacterial, antimicrobial, and antifungal properties. Both *Z. acanthopodium* D.C. and *Z. armatum* have shown to be used as insecticides. *Z. acanthopodium* D.C. has also been used in the food industry for flavoring. Many of the biological activities these plants possess are due to the chemical compounds within the fruits, leaves, barks, and seeds. These compounds include furanocoumarins, alkaloids, flavonoids, terpenoids, and many others. *Zanthoxylum armatum* D.C., *Zanthoxylum acanthopodium* D.C., and *Zanthoxylum americanum*'s effectiveness in helping reduce fever, toothache, headaches, and other clinical symptoms make them an important part of traditional medicine. As evidenced in this review, the activities of these plants have been validated by several pharmacological studies. This suggests the potential biological applications of *Zanthoxylum armatum* D.C., *Zanthoxylum acanthopodium* D.C., and *Zanthoxylum americanum*. Further emphasis should be placed on how isolating and characterizing individual compounds and components can help establish a mechanism of action that can be targeted for medicinal purposes. Additional studies need to address acute and chronic toxicity of these plants before administering them for clinical use as a novel drug or therapeutic. However, the phytochemical and pharmacological studies establish a strong possibility that the traditional practices used by Native Americans could be transformed into modern scientific practices and medicinal uses to further develop the capabilities of medicinal plants and their therapeutic effects.

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