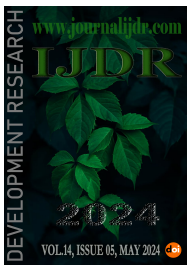
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 01, Issue, 05, pp. 65665-65668, May, 2024
https://doi.org/10.37118/ijdr.28329.05.2024



RESEARCH ARTICLE **OPEN ACCESS**

EVALUATION OF DERMAL TOXICITY AND ANTIOXIDANT PROPERTY OF *ZANTHOXYLUM OXYPHYLLUM* EXTRACTS

Kushal Rabha; Pritam Mohan; *Sumitra Debnath; Archana Hazarika; Himangshu Baruah and Fulmoni Kalita

Department of Veterinary Pharmacology & Toxicology, M.V.Sc, College of Veterinary Science, AAU, Khanapara, Guwhati, India

ARTICLE INFO

Article History:
Received 17th February, 2024
Received in revised form
26th March, 2024
Accepted 18th April, 2024
Published online 30th May, 2024

Key Words:

Zanthoxylum oxyphyllum; Antioxidant Properties; Acute dermal toxicity.

Corresponding author: Sumitra Debnath

ABSTRACT

Zanthoxylum oxyphyllum is a tropical plant, which is traditionally used in the treatment and management of various conditions including analgesic, anti-inflammatory, skin infection and wounds. The aim of this study was to investigate antioxidant property and acute dermal toxicity of *Zanthoxylum oxyphyllum*. For evaluation of antioxidant properties DPPH radical scavenging activity and Nitric oxide scavenging activity was performed. Oxidation is the integral part of aerobic process of life. It involves the transfer of electrons or hydrogen via a chemical reaction from a substance to an oxidizing agent leading to the production of free radicals. These free radicals which are highly reactive in turn initiate a chain of reactions that lead to cellular damage. This present study showed Both the hydroethanolic and chloroform extracts of the selected plant was positive for antioxidant properties. Acute dermal toxicity of *Zanthoxylum oxyphyllum* was determined in rabbit according to OECD guideline 402. Both the extracts were non-sensitizing to rabbit skin and caused very little or no irritation in intact and damaged skin rabbits treated with single and multiple doses. Irritation scores did not differ significantly between the control and treatment groups, according to the acute dermal toxicity study.

Citation*: *Kushal Rabha, Pritam Mohan; Sumitra Debnath, Archana Hazarika, Himangshu Baruah and Fulmoni Kalita, 2024. *"Evaluation of dermal toxicity and antioxidant property of zanthoxylum oxyphyllum extracts". International Journal of Development Research*, 14, (05), 65665-65668.

INTRODUCTION

Herbal medicines, which have been used as the foundation of health care across the world since the dawn of time, are still extensively utilized and play a significant role in international trade with transactions worth billions of dollars. The therapeutic, pharmacological and economic significance is still recognized, but to varying degrees in the world. Plant componentsare essential for pharmacological research and drug development, not only as direct therapeutic agents, but also as starting materials for drug synthesis or else models for pharmacologically active molecules. According to the United Nations convention on biological diversity, the conservation and sustainable use of biological diversity is critical for meeting the food,health and other needs of the world's growing population, for which access to and sharing of genetic resources and technologies is required.Legislative regulations on medicinal plants have not evolved in accordance with a structured controlled paradigm. Governments identify medicinal plants, herbs and products derived from them in various ways and countries have taken diverse approaches to licensing, dispensing, manufacturing and trade to assure their safety, quality and efficacy. Despite the long history of herbal medicine use, only a limited number of plants species have been investigated for potential medicinal uses. A far lesser number plants, extracts, active substances and products have safety and effectiveness evidence.

India is the birthplace of traditional medicinal plants such as Siddha, Ayurveda and Unani, in which various plants are utilized to cure human diseases. Traditional medicines are still used by about 65% of the world's population for primary healthcare. Herbal resistance is sweeping the globe. They have clearly been appreciated for ages for their medicinal, flavorful and aromatic characteristics, but for a long time they were overshadowed by synthetic products of modern civilization. Folk medicine is typically described as traditional medicine practiced by nonprofessional healers or reflected in local custom or knowledge and generally includes the use of natural, particularly herbal, treatments. Traditional medicine is defined by the World Health Organization (WHO) as "the health practices, approaches, knowledge and believes incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercise, applied singularly or in combination to treat, diagnose and prevent illness or maintain well- being." People turn to nature in search of protection and security after discovering the adverse effects and negative consequences of modern medicinal system. The vast store house of herbal medicine is proving to be a blessing to our civilization. Apart from their nutritional importance, plant derived chemicals may be useful therapeutic weapons in the battle against a variety of human and animal ailments, making them vital in traditional medicine for treating a variety of disease. Plant medications, often known as herbal medicines, have been widely utilized to treat variety of ailments.

Northeast India is endowed with a large potential reservoir of plant species bearing diverse chemical constituents with possible high medicinal value. According to a recent report, the Indo-Burma region is one of the 25 hotspots of the world with more than 13,500 plant species, of which 7,000 species are endemic. North East India is known for its rich biodiversity (Borah *et al.*, 2012). The state of Assam is home to some of the world's most diverse ecosystems, including tropical rainforests, deciduous forests, riverine grasslands, bamboo orchards and several wetland habitats, many of which are now protected as national parks and reserved forests.Ethnobotanical studies demonstrate how indigenous plants are used by people of a specific culture and religion, as well as how they are classified and identified. Some edible plants have a significant economic value and are closely tied to the state's indigenous tribes' socioeconomic growth. *Zanthoxylum oxyphyllum* is one of 12 species of Zanthoxylum found in this part of India (Borah *et al.*, 2012). *Zanthoxylum oxyphyllum* under the family Rutaceae, commonly known as Mezenga in Assam grow in tropical and temperate regions (Pirani *et al*., 1993). They are represented by thorny, dioecious shrubs or small trees with dense foliage and prickly trunk and leaves with a strong and pungent taste (Chayee *et al.,* 1996). The tender shoots of this plant are cooked and eaten as a vegetable in Assam which acts as blood purifier, help in reducing incidence of leucoderma and are useful against stomachtrouble (Buragohain et *al.*, 2011). Seeds of most Zanthoxylum fruits are rich in oil containing large amounts of alkaloids and unsaturated fatty acids possessing noteable antioxidant activity (Xia L *et al*., 2010). Fruits are used as spice and help in digestion. The stem bark yielded indoquinazoline alkaloid and is commonly applied in rheumatism, varicose ulcers, varicose veins, skin diseases and leg pains. Moreover, it is also used in relieving inflammation, fevers and hypotension (Arun and Paridhavi 2012). The bark and root extracts of *Z. oxyphyllum* has been shown to have antiproliferative activity against the growth of human keratinocytes (Kumar and Muller, 1999). Besides it has stimulant, stringent and digestive properties and is also used in dyspepsia and diarrhoea (Medhi *et al.* 2009). The present study was undertaken to evaluate the antioxidant properties and acute dermal toxicity of *Zanthoxylum oxyphyllum* hydroethanolic and chloroform extracts of the leaves.

MATERIALS AND METHODS

Plant collection and processing: The plant *Zanthoxylum oxyphyllum* was identified and characterised by Botanical Survey of India. The leaves of *Zanthoxylum oxyphyllum* were collected from the Goalpara district of Assam, near Meghalaya border. The collected leaves were thoroughly washed with fresh water to remove soil and dust particles, as well as other debris. The leaves are then shade dried at room temperature for about 15-20 days. The shade dried leaves of the plant was ground to fine powder in a Laboratory Willey Mill. They were regularly turned on and off to prevent fermentation, rot and other damage, which was then stored at 4˚C in airtight containers with proper labelling until the required extracts were prepared for the experiments.

Preparation of hydroethanolic and chloroform extracts: Hundred (100) grams of powdered *Z. oxyphyllum* leaves were suspended in 1 litre of 1:1 ethanol and distilled water solution and 1 litre of chloroform solution and kept for 9 days with intermittent stirring every 8 hours. Finally, on the tenth day, the suspension was passed through muslin cloth to remove the coarse material, followed by Whatman filter paper 1. The solvents used for extraction of the bioactive compound were evaporated with the help of a rotary evaporator and the semi-solid residue from the rotary evaporator was subjected to further solidification by heating for 24 hours in a hot water bath at very low temperature. The remaining compound on the petri dish is the hydro ethanolic and chloroform extract.

Experimental Animals: The acute dermal toxicity studies were carried out on New Zealand albino rabbit. Four male rabbits weighing between 2.7 and 3.2 kg were used in the experiment. The animals were kept in stainless steel cages at the animal house, at the Department of Veterinary Pharmacology and Toxicology, Khanapara, under ambient temperature, light and relative humidity. Food and water were provided ad libitum. Prior to the start of the test, the animals were acclimated to laboratory conditions for 7 days.

Ethical Approval: The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of experimental animals (CPCSEA) guideline and Institutional Animal Ethical Committee with the ethical clearance number 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/20-21/862 dated 31/07/2021 at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Khanapara.

Phytochemical analysis of extracts: The hydroethanolic and chloroform extracts of *Zanthoxylum oxyphyllum* were phytochemically analysed for the presence of various active principles such as steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes, and saponins.

Acute dermal toxicity: The experiment was performed in accordance with the OECD guidelines 402. The rabbits were shaved about 10% of their body surface area on day 0 of the test period to allow for the application of the test substance. A test site of about 6 cm^2 was used, while the right site was used as a control site. The rabbits were individually caged and left alone for 24 hours. On the first day of the test period, the oil was applied to intact and abraded skin at a dose of 0.5ml/site and was covered with gauze patches secured with adhesive tape. Patches are applied to areas of skin that have been abraded by scoring through the stratum corneum, but not sufficiently to cause bleeding, in addition to intact patches in patching skin (Lansdown, 1972). After that, the rabbits were returned to their cages. The patches were removed after 24 hours, and the skin response was assessed.

Evalution of antioxidant properties of *Zanthoxylum oxyphyllum* leaf extract

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- absorbance of test}}{\text{Absorbance of control}} \times 100$$

DPPH radical scavenging activity: The DPPH radical scavenging activity was measured according to the method of Cotelle *et al.* (1996) with some modifications. DPPH radical reacts with antioxidants compounds that can donate hydrogen and gets reduced. DPPH when acted upon by an antioxidant is converted into diphylpicryl hydrazine. 3 ml reaction mixture containing 200 µL of DPPH (100 µM) in methanol and 2.8 ml of sample (at various concentration 3- 110 µg/ml) in methanol were incubated at 37 °C for 30 mins and absorbance of text mixture was read at 517nm using UV vis spectrophotometer. The concentration viz. 50 µg/ml, 80 µg/ml and 110 µg/ml of samples were taken here. The percentage inhibition of DPPH radical was calculated by comparing the result of the test with dose of the control using the following formula:

Nitric oxide scavenging activity: Nitric oxide radical is generated from the nitroprusside solution at physiological pH (Marconi *et al.,* 1994 and Sreejayan and Rao, 1997). 1ml of sodium nitroprusside was mixed with 1 ml of sample of different concentration (3- 110 µg/ml) in phosphate buffer pH 7.4. three different concentrations viz., 50 µg/ml and 110 µg/ml of samples were taken for each sample. Butylated hydroxyl toluene (BHT) was considered as standard for it. The mixture was then incubated at 25 °C for 150 minutes. To 1 ml of incubated solution, 1 ml of griss reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546nm by using UV Vis spectrophotometer. Percentage of inhibition was calculated.

RESULTS

The dried and powdered leaves of *Zanthoxylum oxphyllum*were extracted using a standard technique. The hydro ethanolic extract

yield was found to be 14% and that of chloroform extract yield was found to be 6 %.

Phytochemical analysis: The hydroethanolic and chloroform extracts of *Zanthoxylum oxphyllum* were qualitatively analyzed for the presence of different active phytoconstittuents. The extracts were found to be positive for alkaloids, steroids, flavonoids, phenols, carbohydrates, Diterpenes, Triterpenes.

Diphenyl-1-picrylhydrazyl (DPPH) assay: The percentage of antioxidant activity (AA%) of each extract was assessed by DPPH free radical assay. The reactivity of the extracts prepared from *Zanthoxylum oxphyllum* were analysed with 2,2-Diphenyl-1-Picryl hydrazyl, a stable free radical which is got reduced by accepting hydrogen or electron from the donor molecule. The mean percent inhibition of DPPH radical by Chloroform and Hydroethanolic extracts at 50 µg/ml concentration were observed to be 28.03 ± 0.02 and 25.81 ± 0.60 respectively. Highest inhibition of radicals by both the extracts were found in the highest concentration group. Butylated hydroxytolune (BHT) was taken as the standard and it also showed dose dependant antioxidant property. The percentage of inhibition of DPPH radical by leaf extracts of *Zanthoxylum oxphyllum* are showed in Table 1.1.

Nitric oxide scavenging activity: *The* percentage of inhibition of Nitric oxide radicals by the leaf extracts of the plant are shown in Table 1.2. The mean percent inhibition of DPPH radical by hydroethanolic leaf extract at 50 µg/ml, 80 µg/ml and 110 µg/ml concentration were found to be 24.26 ± 0.23, 27.61 ± 0.21 and 31.50 ± 0.04 and 80.16 ± 0.08 respectively while that of chloroform extract showed inhibition percent as 20.16 ± 0.33, 23.19 ± 0.14 and 26.88 ± 0.42 in the prescribed concentration. Butylated hydroxytolune was taken as standard and showed inhibition percent as 41.13 ± 0.11, 59.40 ± 0.20 and 71.66 ± 0.09 in the prescribed concentration against the radicals.

Acute dermal toxicity: In acute dermal toxicity test, no erythema or oedema was seen in both the control animals and the test animals after 3 days of experimentation. In determining the dermal toxic effect of *Zanthoxylum oxyphyllum* in acute dermal toxicity test, no significant clinical change was observed in any of the treated groups except for the initial reaction within the first 30 mins of patch attachment when the rabbits tried to tear the patch off. The behavioral patterns and general appearance of the rabbits in the control and test groups were recorded after 1hr and 12 hrs post-application of test substances. No change meant that the manner in which the animals behaved after acclimatization did not alter when the skin was shaved and test substances were applied. No erythema or oedema was observed over the 14 days study period in both the control and *Zanthoxylum oxyphyllum* treated group.The irritation scores did not differ significantly between the control and treatment groups. The extract was non-sensitizing to rabbit skin and caused very little or no irritation in intact and damaged skin rabbits treated with single and multiple doses.

DISCUSSION

The present phytochemical analysis of hydroethanolic and chloroform extract of *Zanthoxylum oxyphyllum*revealed the presence of alkaloids, steroids, flavonoids, phenols, carbohydrates, Diterpenes, Triterpenes. In conformity with the present investigation, Ayangla *et al*. (2016) reported the presence of glycosides, coumarins, flavonoids, phenols, and tannins in crude ethanolic extracts of ZAL, ZOL, ZOS, ZRL, and ZR. He also reported that phenolic content was highest in the leaves of *Zanthoxylum oxyphyllum*. However, Borah *et al*. (2012) reported the presence of methyl heptyl ketone as being the main component of the leaves of *Zanthoxylum oxyphyllum*. Oxygen is an element obligatory for life where living systems have evolve to survive in the presence of molecular oxygen, which has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Shinde *et al*., 2012). Oxidative stress results when the balance between the production of reactive oxygen species (ROS) exceeds the antioxidant capability of the target cell (Ahmed *et al.,* 2009). The plant extracts and their confined constituents have always been an important part of different curative systems (Vanitha and Kathiravan, 2006). Plants are rich sources for natural antioxidants, the best known are flavonoids, vitamin C and other phenolic compounds (Laandrault *et al*., 2001). Polyphenols scavenge free radicals and inhibit the oxidative mechanisms that can lead to degenerative diseases. In the present study it was found that both the hydroethanolic and chloroform extracts of the selected plant was positive for antioxidant properties. Tiwari and Masood (1979) cited an alkaloid extracted from the defatted stem bark of the plant *Zanthoxylum oxyphyllum* as zanxyline and chemically named as 1-(4′methoxybenzyl)-6,7-dimethoxy N:N dimethyl 1,2,3,4 tetrahydroisoquinolinium hydroxide with the chemical formula $C_{12}H_{29}NO_4$.

The inhibition percent of the extracts were found to be increased on concentration basis. More the concentration of the extract, more is its inhibition potential. The present finding is in good agreement with Patil *et al*. (2015). The antioxidant potential of the plant extracts may be gained by presence of functional groups on the leaf extract as they are good source of several antioxidant components such as β-carotene, gluthione, α-tocopherol, ascorbic acids and total phenols and flavonoids Patil *et al*. (2015). Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and anti-tumour activities. The metabolic conversion of arginine to citrulline leads to the formation of nitric oxide.

Table 1.1. Estimation of antioxidant properties of *zanthoxylumoxphyllum* extracts by dpph assay

EXTRACT	PARTICULARS	CONCENTRATION (*µg/ml*)	INHIBITION PERCENTAGE OF DPPH (%) Mean ± SE
Chloroform	*Zanthoxylum Oxphyllum*	50	28.03 ± 0.02
		80	35.11 ± 0.12
		110	39.52 ± 0.41
Hydroethanolic		50	25.81± 0.60
		80	29.13 ± 0.54
		110	35.07 ± 0.11
Standard	Butylated Hydroxyytolune	50	21.11 ± 0.18
		80	37.44 ± 0.25
		110	60.21 ± 0.57

Table 1.2. Estimation of antioxidant properties of *zanthoxylumoxphyllum* extracts by nitric oxide scavenging activity

Sample	Particulars	Inhibition Percentage of Nitric Oxide (%)		
		Concentration 50 (µg/ml)	Concentration 80 (µg/ml)	Concentration 110 (µg/ml)
		Mean ±SE	Mean ± SE	Mean ± SE
Hydroethaolic Extract	*Zanthoxylum oxyphyllum*	24.26 ± 0.23	27.61 ± 0.21	31.50 ± 0.04
Chloroform extract		20.16 ± 0.33	23.19 ± 0.14	26.88 ± 0.42
Standard	Butylated Hydroxyytolune	41.13 ± 0.11	59.40 ± 0.20	71.66 ± 0.09

These are carried out by specific Nitric oxide synthases in various cells such as fibroblast and endothelial cells. Nitrate and nitrite are known to be the by-products of decomposition of the compound sodium nitroprusside at a pH of 7.2. in her study observed that *M. arundinacea* extract decreased the amount of nitrite that was generated. This may be due to suppression of released nitric oxide may be partially attributed to direct nitric oxide scavenging as that of in our study. The scavenging of Nitric oxide by the extracts was increased in concentration dependent manner which is in good agreement with the findings of.In the present study, it was also found that a significant decrease in the nitric oxide radical due to the scavenging ability of extract and standard Butylated hydroxytoluene. Among the two extracts of the plant hydroethanolic showed an increased inhibition incomparison to chloroform extract. But the highest percent inhibition was noted in 110 µg/ml concentration in both of the extracts of plant. The scavenging activity of both hydroethanolic and chloroform extracts of *Zanthoxylum oxyphyllum* showed lesser in comparison to standard butylated hydroxyl toluene. The acute dermal toxicity studies were performed according to the OECD guidelines. This test is often a stepping stone for long-term toxicity tests as it sets the suitable dose for use in such studies and provides a gist of the dose-dependent relationship. In present acute dermal toxicity studies, the hydroethanolic extract of the plant produced no irritation. The irritation scores did not differ significantly between the control and treatment groups. The extract was non-sensitizing to rabbit skin and caused very little or no irritation in intact and damaged skin rabbits treated with single and multiple doses of chloroform extract as well. The skin irritation study on rabbit skin proved that the extracts in low and high concentrations produce no irritation to the skin.

Acknowledgement: We are thankful to Dept of Pharmacology & Toxicology, College of Veterinary Science, Assam Agricultural University, Khanapara for providing lab facility for carrying out research work.

Consent for publication: As a corresponding author, I Sumitra Debnath certify that the entire article is an original creation and none of the material in the manuscript has been published previously, or has not been accepted or considered for publication elsewhere. I also certify that I have not assigned, licensed, or otherwise transferred any right or interest in the manuscript to anyone.

Author's contributions: All the authors read and approved the final manuscript.

Conflict of Interest: The author declared no conflict of interest.

Data Avilability: Data will be available on request.

REFERENCES

Ahmad, IM.; Abdalla, MY.; Mustafa, NH.; Qnais, EY. and Abdulla, FA. 2009. Datura aqueous leaf extract enhances cytotoxicity via metabolic oxidative stress on different human cancer cells. *JJBS,* 2:9-1.

Arun K.K.V, Paridhavi M 2012. An ethno botanical phytochemical and pharmacological utilization of widely distributed species Zanthoxylum: A comprehensive overview. *InternationalJournal of Pharmaceutical Innovation;* 2(1).

Ayangla N.W, Neetu Singh, Ajay Kumar, (2016); Phytochemical analysis of plant species of genus Zanthoxylum*International Journal of Medicine and Pharmaceutical Science* 6 (1): 1-8.

Borah, R., Saikia, K., Talukdar, A. K., &and Kalita, M. C. (2012). Chemical composition and biological activity of the leaf essential oil of Zanthoxylum oxyphyllum. *Planta Medica*, *78*(05): 100.

Buragohain, J., Konwar, B.K. and Bordoloi, M.J. (2011). Isolation of an antimicrobial compounds from the tender shoots of Zanthoxylum. *Der Pharmacia Sinicia*, 2(6): 149.

Chayee, C.C.; Mau J.L.; Wu C.M.; (1996). Characteristics of the the steam distilled oil and carbondioxide extract of Zanthoxylum simulans fruits. *Journal of Agriculture and Food Chemistry;* 44(1):1096-1099.

Cotelle, N., Bernier, J. L., Catteau, J. P., Pommery, J., Wallet, J. C., and Gaydou, E. M. 1996. Antioxidant properties of hydroxy-flavones. *Free radical biology and Medicine*, 20(1): 35-43.

Kumar KC, S., & Müller, K. 1999. Lichen metabolites. 2. Antiproliferative and cytotoxic activity of gyrophoric, usnic, and diffractaic acid on human keratinocyte growth. *Journal of natural products*, 62(6): 821-823.

Marconi, G., Lutz, M. B., Granucci, F., Winzler, C., Paglia, P., Foti, M., and Ricciardi-Castagnoli, P. 1994. Retroviral immortalization of phagocytic and dendritic cell clones as a tool to investigate functional heterogeneity. *Journal of immunological methods*, 174(1-2): 269-279.

Medhi K, Purohit B.P, Gogoi A.J, Saikia S.P, Kanjilal P.B, Bhau B.S 2009. Molecular marker based genetic diversity analysis of two economically important plants (Zanthoxylum hamiltonianum and Nepenthes khasiana) of North-East India. Biodiversity in herbal medicine. IQAC Synod College and Akansha publishing house,New Delhi pages: 47-55.

Patil, S., Sivaraj, R., Rajiv, P., Venckatesh, R., & Seenivasan, R. (2015). Green synthesis of silver nanoparticle from leaf extract of Aegle marmelos and evaluation of its antibacterial activity. *Int J Pharm Pharm Sci*, *7*(6), 169-173.

Pirani J.R (1993). A new species and a new combination in Zanthoxylum (Rutaceae) from Brazil. *Brittonia*; 45(2):154-158.

Shinde, A.; Abd Elmouttale,AT.; Ashraf, T. and Mostafa, U.(2012). Effect of tomato and guava juices on oxidative stress in rats after strenuous exercise. *Jordan Journal of Biological Sciences*, 5:167-174.

Tiwari, K.P., and Masood, M. (1979) Zanoxyline-A new alkaloid from stem bark of Zanthoxylum oxyphyllum.Phytochem.

Vanitha, C. and Kathiravan, M.2006. Importance and scope of medicinal plants. *Agricultural Herbal Visi*, 15: 30-31.

Xia L, You J, Li G, Sun Z, Suo Y. (2010). Compositional and antioxidant analysis of Zanthoxylum bungeanum seed oil obtained by supercritical CO2 fluid extraction. *J Am Oil Chem Soc*, 88(7):1029-1036.

****