

EVALUATION OF PHYSIOCHEMICAL PARAMETERS ON THE FRUIT OF *Zanthoxylum limonella* Alston (FAMILY- RUTACEAE)

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ABSTRACT

The Plant *Zanthoxylum Limonella*, belongs to the family Rutaceae is a climbing shrub, capable of growing up to 50 meter height and used as a traditional herbal drug in Ayurveda. It is popularly used for the treatment of cardiac, respiratory diseases, tooth infection as well as against stomach infection. The standardization of dried, matured pieces of the fruit and also in powdered form was carried out in the present study. The study includes macroscopical analysis along with physicochemical parameters such as ash and extractive values, fluorescent analysis, different chemical behavior and preliminary phytochemical screening. The total ash, acid insoluble ash and water soluble ash values were NMT 0.89%, 0.59% and 0.06% respectively. The alcohol soluble extractive and water soluble extractive values were NLT 8.17% and 4.05% respectively. The LOD at 105°C was NMT 6.9%. The results of the studies would be of immense value in evaluating the quality and purity of the drug sample.

Keywords: *Zanthoxylum Limonella*, Standardization, Physicochemical standards.

INTRODUCTION

Zanthoxylum Limonella is the tribe Zanthoxyleae, subfamily Rutoideae, family Rutaceae, is the large genus of aromatic, prickly dioecious (or rarely monoecious) tree or shrubs, which is distributed mainly in the pantropics but also in the subtropics. The genus dealt with in this article has substantial synonymy with the genus *Fagara*.¹ The bark of *Z. Limonella* is noted for its febrifugal, sudorific and diuretic properties.² *Zanthoxylum limonella* is called "Veerasingam pattai" in vernacular language. It is popularly used for the treatment of cardiac, respiratory diseases, tooth infection as well as against stomach infection.³

Figure 1. *Z. Limonella*



The various parts of *Z. limonella* are used as traditional medicine in India. The bark is bitter and is used as a tonic and to cure rheumatism.⁴ Essential oil extracted from the fruit is commonly known as 'Mullilam oil'; it is

frequently used as anti-inflammatory, antiseptic, anticholera, diarrhea and hypocholesterlamic; fruits are used as pickle.⁵ Several alkaloids have been isolated from the stem bark.^{6,7} Vitamin E has been detected from the seed oil⁸ and aromatic components have been isolated from the essential oil.⁹ The main component isolated from the bark was lupeol.¹⁰ The plant also used in making of traditional rosaries in Manipur.¹¹

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Taxonomic Studies¹²

Domain: Eukaryota
Kingdom: Plantae
Subkingdom: Viridiplantae
Phylum: Tracheophyta
Subphylum: Euphyllophytina
Infraphylum: Radiatopses
Class: Magnoliopsida
Subclass: Rosidae
Superorder: Rutanae
Order: Rurales
Suborder: Rutineae
Family: Rutaceae
Genus: *Zanthoxylum*
Specific epithet: *limonella* - (Dennst.) Alston
Botanical name: *Zanthoxylum limonella* (Dennst.) Alston

Zanthoxylum limonella L. (Dennst.) Alston belongs to the family Rutaceae. It is a climbing shrub, capable of growing up to 50 meter height and the cylindrical stem has small spikes on the surface. *Zanthoxylum limonella* commonly inhabits temperate regions of Assam, Bhutan, Himalaya, Tamil Nadu (Kolli hills) and Kerala.

MATERIALS AND METHODS

Collection and Authentication

Fruits of *Zanthoxylum limonella* were collected from three distinct regions like Kerala, Mumbai and Delhi, in the month of February 2009 in a quantity sufficient for all the experiments in a single batch. The plant material was authenticated by NISCAR, Delhi. The *Zanthoxylum Limonella* fruits were washed under running tap water and shade dried (30°C, 50 ± 5% relative humidity) for 15 days. The shade dried plant material was powdered using a dry grinder to get the coarse powder (sieve no. 10/44). The powder was stored in air tight container for

further use.

Quality control parameters¹³

Coarse powder of the plant fruit was used to perform quality control parameters such as total ash, acid insoluble ash, water soluble ash, extractable matter, loss on drying, foaming index and swelling index etc. Three determinations were carried out for each parameter.

Preliminary phytochemical studies^{14,15}

Preliminary phytochemical screening which is performed to establish a chemical profile of a crude drug.

Fluorescence analysis of extract and drug powder¹⁶

The fruit powder as such, after treatment with various solvents was subjected to fluorescent analysis. Observations were made under visible light and under UV light of short wave length and long wave length separately.

Behaviour of the drug powder with different chemicals, reagents & solvents¹⁶

Colour of drug powder was observed by using different reagents (NaOH, conc HNO₃, conc HCl, conc H₂SO₄, 5% acetic acid, 5% FeCl₃, ammonia, picric acid) and solvents in natural light.

Thin layer chromatography¹⁷

Slurry of silica gel G was prepared in distilled water and poured over a glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at 100-120°C (30min) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (1cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase, and allowed to run 3/4th of the height of the prepared plate.

RESULTS AND DISCUSSION

Organoleptic evaluation

Fruits of *Zanthoxylum limonella*, when examined for

Table 1. Organoleptic evaluation of *Zanthoxylum limonella* fruit.

S no	Character	When fresh	After drying	Powder
1	Colour	Green	Dark brown	Dark brown
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Bitter	Bitter	Bitter
4	Shape	Globular	Globular	-
5	Texture	Soft	Hard	-

Table 2. Quality control parameters of *Zanthoxylum limonella* fruit

S no	Particulars	Kerala	Delhi	Mumbai	Limit
1	Total ash	0.89%	0.82%	0.74%	NMT 0.89%
2	Water soluble ash	0.06%	0.05%	0.04%	NMT 0.06%
3	Acid insoluble ash	0.52%	0.59%	0.55%	NMT 0.59%
4	Ethanol(90%)soluble matter	8.17%	8.25%	8.96%	NLT 8.17%
5	Aqueous soluble matter	4.05%	4.75%	4.92%	NLT 4.05%
6	Loss on drying	6.5%	6.3%	6.9%	NMT 6.9%

Table 3. Preliminary phytochemical analysis of various fruit extracts of *Zanthoxylum limonella*.

S no	Chemical Constituents	Pet.ether ext.	Chloroform ext.	Ethanol ext.	Aqueous ext.
1	Carbohydrates	-	-	-	+
2	Proteins	-	-	-	+
3	Alkaloids	-	-	++	-
4	Saponins	-	-	-	++
5	Tannins	-	-	++	-
6	Flavonoids	-	-	++	-
7	Steroids	+	++	+	-
8	Fixed oil	+	+	+	+
9	Volatile oil	+	+	+	+
10	Phenolic comp.	-	-	++	-
11	Glycosides	-	-	+	++
12	Starch	-	-	-	+

++ = Intensely present, + = Present, - = absent.

organoleptic properties were found green coloured with a characteristic odour. Taste of globular fruits was bitter. (Table 1, Figure 2)

Figure 2. *Zanthoxylum limonella* fruits.



Quality control parameters

Total ash, acid insoluble ash, water soluble ash, extractable matter, loss on drying, foaming index and swelling index etc were found as table 2.

Preliminary phytochemical studies

The phytochemical tests of the fruit extracts shown the presence of carbohydrates, alkaloids, tannins, steroids, triterpenoids, flavonoids etc. (Table 3)

Fluorescence analysis of extract and drug powder

Fruit powder was treated with different reagents and observed for fluorescence under visible light and under UV light (short and long wavelength). (Table 4)

Behaviour of the drug powder with different chemicals, reagents & solvents

Fruit powder was treated with different reagents (NaOH, conc HNO₃, conc HCl, conc H₂SO₄, 5% acetic acid, 5% FeCl₃, ammonia, picric acid) and colour change was observed in natural light. (Table 5)

Thin layer chromatography

TLC profile of different fruit extracts were found as per table 6 when developed TLC plates were observed under UV light.

Table 4. Fluorescence (UV) analysis of fruit powder.

S no	Particulars of treatment	Under ordinary light	Under UV light	
			Short Wavelength (254nm)	Long Wavelength (366nm)
01	Fruit Powder as such	Greenish black	No florescence	No florescence
02	Powder + 50% H ₂ SO ₄	Light brown	Dark green	No florescence
03	Powder + 1N HCl	Sand stone	Reddish brown	No florescence
04	Powder + 50% HNO ₃	Golden brown	Water green	No florescence
05	Powder + 5% KOH	Reddish brown	Reddish green	No florescence
06	Powder + MeOH	Sand stone	Reddish green	No florescence
07	Powder + 1N NaOH	Reddish brown with yellow tinge	Dark reddish brown with yellow tinge	No florescence

*The sample from different sources were shown same behavior.

Table 5. Colour characters of fruit powder in different chemical reagents in natural light.

S no	Drug treatment	Fruit powder
1	Powder as such	Greenish black
2	Powder + NaOH	Yellow
3	Powder + conc HNO ₃	Brownish yellow
4	Powder + conc HCl	Green
5	Powder + conc H ₂ SO ₄	Brown
6	Powder + 5% acetic acid	Black
7	Powder + 5% FeCl ₃	Greenish black
8	Powder + Ammonia	Black
9	Powder + Picric acid	Greenish black

Table 6. TLC profile of different fruit extracts of *Zanthoxylum limonella* (under UV 256nm and iodine vapour).

Extracts	Solvent system	Detecting reagents	
		UV(256nm) (Rf values)	Iodine vapor (Rf values)
Alcoholic extract	Hexane: Chloroform: Ethanol(3:4:3)	8 (0.11, 0.15, 0.23, 0.35, 0.55, 0.58, 0.75, 0.81)	7 (0.11, 0.23, 0.35, 0.55, 0.58, 0.75, 0.81)
	Ethyl acetate: methanol (7:3)	5 (0.23, 0.28, 0.32, 0.39, 0.55)	5 (0.23, 0.28, 0.32, 0.39, 0.55)
	100% Chloroform	7 (0.24, 0.37, 0.53, 0.61, 0.67, 0.88, 0.98)	6 (0.24, 0.53, 0.61, 0.67, 0.88, 0.98)
Chloroform extract	100% chloroform	4 (0.13, 0.32, 0.41, 0.52)	4 (0.13, 0.32, 0.41, 0.52)
	Hexane: Chloroform: Ethyl acetate (2:5:3)	5 (0.19, 0.23, 0.34, 0.51, 0.62)	5 (0.19, 0.23, 0.34, 0.51, 0.62)
	Chloroform: benzene (2:8)	5 (0.18, 0.25, 0.28, 0.43, 0.74)	2 (0.25, 0.58)
Petroleum ether extract	Cyclohexane: Ethyl acetate	6 (0.12, 0.16, 0.27, 0.29, 0.38, 0.42)	5 (0.16, 0.27, 0.29, 0.38, 0.42)
	Petroleum ether: Diethyl ether(6:4)	4 (0.14, 0.47, 0.61, 0.71)	5 (0.14, 0.32, 0.37, 0.47, 0.61)
Aqueous extract	Butanol: Acetic acid: Water (5:3:2)	5 (0.21, 0.26, 0.32, 0.39, 0.52)	5 (0.21, 0.26, 0.32, 0.39, 0.52)
	Butanol: Acetic acid: Water(6:1:3)	5 (0.44, 0.71, 0.80, 0.89, 0.93)	5 (0.44, 0.71, 0.80, 0.89, 0.93)

CONCLUSION

The comparative and multidisciplinary approach of the study of *Zanthoxylum limonella* does help in understanding its identification, taxonomical determination and medicinal importance in depth. The adulterants in drug obtained from *Zanthoxylum limonella* can be identified by this investigation.

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