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## Research Paper

# Phytochemical and antibacterial assay of *Zanthoxylum rhetsa* (Roxb). DC

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## Abstract

*Zanthoxylum rhetsa* is a tall deciduous tree, commonly known as *Indian prickly ash* in English and *Kothumurikku* or *Mullilavu* in Malayalam. The present investigation aims to analyze qualitatively the different phytochemicals present in the fruits of *Z. rhetsa* and evaluate its antioxidant properties as well as antibacterial activity. The fruit extracts from the collected sample, during November, were tested for the presence of bioactive compounds. Various phytochemicals like carbohydrates, alkaloids, flavanoids, coumarins, cardiac glycosides, phenols, terpenoids, tannins, saponins, phlobatannins, steroids, anthraquinones etc were analysed. The modified ferric ion reducing method was used to assay antioxidant property<sup>1</sup>. Phytochemical analysis of methanol and ethanol extract of fruit indicated the presence of alkaloids, flavonoids, terpenoids, proteins, carbohydrates, triterpenes, saponins, coumarins, steroids and chalcones. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* exhibited sensitivity towards methanol, ethanol and ethyl acetate extracts of fruit. *Serratia* was not sensitive to the extracts of fruit. The antioxidant property was promising. The present study revealed that *Z. rhetsa* contains principles with antioxidant as well as antibacterial property.

**Keywords:** *Zanthoxylum rhetsa*, Phytochemicals, antioxidant properties, antibacterial activity.

## Introduction

*Zanthoxylum rhetsa* is a tall deciduous tree of Rutaceae family, which is commonly known as *Indian Prickly Ash* in English and *Kothumurikku* or *Mullilavu* in Malayalam. It is seen widespread in the Western Ghats, where it grows up to a height of 35 metres. The bark is normally 15-20 mm thick and mottled with conical prickles. It is a native of warm, temperate and subtropical area worldwide. It is distributed in Konkan, Deccan, Mysore, Malabar, Anamalai and Travancore at low elevation Orissa, Chittagong, Pegu. Also found in Assam and Meghalaya and in eastern Western Ghats of peninsular India.

Characteristic secondary metabolites of *Zanthoxylum* species includes lignoids, xantholectin, sesamin, essential oils, alkaloids, amides, flavonoids, terpenes, steroids and coumarins. Most flavonoids apart from catechins are present in plants as beta glycosides<sup>2</sup>. Therapeutically terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, antihelminth and counter irritant<sup>3</sup>. Many tannin containing drugs are used in medicine as astringent. They are also used as healing agents in inflammation, burns, piles etc.<sup>4,5</sup>. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats<sup>6</sup>. Certain compounds ameliorate pathophysiological conditions in Streptozotocin induced diabetes<sup>7</sup>. Spooning a group of natural products like, cardiac glycosides occur in stem spines of *Z. rhetsa* have great pharmaceutical importance due to their

relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin etc.<sup>8</sup>. The fruits of *Z. rhetsa* are aromatic, astringent, stimulant and stomachic. They are used in treatment of asthma, bronchitis heart troubles, toothache & rheumatism. The skin of fruit is credited with astringent and digestive properties. Oil obtained by steam distillation is used as traditional remedy for cholera. It is further applied as antiseptic and disinfectant. The peels of fruit, seed, as well as oil extracted from fruit are used for medicinal purposes. A decoction of bark is taken initially as a cure for pain in the chest. The bark is pounded and mixed with oil then used extremely as a remedy for stomach pains.

The most successful criteria of pharmaceutical industry in searching plants for new therapeutic agent in the various field of biomedicine is its ethnomedicinal use. The anaesthetizing effect of alpha-hydroxy-sanshool was reported, which is an alkylamide compound produced by *Zanthoxylum*. This compound also had potential as commercial product to reduces skin irritation. It also induce numbing sensation, a unique sensation of mild electric shock or "pins and needles" in the mouth<sup>9</sup>. In some parts of the India, seed oil is used against baldness and bark powder is used for toothache<sup>10</sup>. At the industrial level *Z. armatum* has been reported to contain high amount of linalool<sup>11</sup>, a compound used commercially as a precursor to vitamin synthesis and also in the production of soaps, detergents and insecticides. Another species, *Z. rhetsa* have shown the presence of variety of compounds including monolignols, coumarine alkaloids and ligans namely 3,5-demethoxy-4-geraniyloxy cinnamyl alcohol, xantholetin, sesamin as well as zanthorhetsamide. Ahsan et al., (2014)<sup>12</sup> reported the presence of quinoleneterpene alkaloids viz., chelerybulgarine, 2-epi simulano quinoline, 2,11-dimethoxy vepridimerine B and rhetsidimerine, in roots and bark of the plant. More than 15 useful compound were reported from this species, of which 1, 2 benzene dicarboxylic acid and diisooctyl ester were the major components. Antimicrobial agents such as antibiotics are emerging from a variety of micro organism and multiple drug resistant causes serious threat to the treatment of infectious diseases. Hence plant derived antimicrobial agents received considerable attention in recent years<sup>13</sup>. To confirm the antimicrobial activity and to ascertain the parameters associated with it, the plants had to be tested against an appropriate microbial model. The effects of plant extracts on bacteria have been studied by a very large number of researchers all over the world. The plant extracts and phytochemicals with known antimicrobial properties had great significance in therapeutics. In the present work, the dried fruit of *Zanthoxylum rhetsa* was evaluated for its phytochemical potency as well as antimicrobial properties.

## Materials and Methods

For the present investigation, fruits were collected during the premonsoon season from Pattambi, Palakkad district of Kerala. For the qualitative phytochemical analysis, the plant extracts were tested for the presence of bioactive compounds. Carbohydrates, alkaloids, flavanoids, coumarins, cardiac glycosides, phenolics etc were qualitatively analysed using standard procedures. Terpenoids, tannins, saponins, phlobatannins, steroids, anthraquinones etc were also analysed. Modified Ferric ion reducing antioxidant assay<sup>1</sup> was adopted for assaying the antioxidant activity. The shade dried fruits were powdered and stored in closed vessel for the study. The Dried extracts were prepared with volatile solvents viz., Methanol, Ethyl acetate and Ethanol. The extracts were kept in sterilized micro centrifuge tube and stored in refrigerator for further use. Stock solution of 100 mg/1 ml dose was prepared by suspending 100 mg dried extract in 1 ml of dimethyl sulphoxide (DMSO).

Bacterial strains obtained from stock cultures maintained in the Department of Botany, Govt. Sanskrit College Pattambi, which were initially procured from P.S.G College, Coimbatore were used for the study. The gram negative bacterial strains viz., *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella sp.* and gram positive strain *Staphylococcus aureus* was used for antibacterial assay. The antibiotic Cephataxim was used as positive control.

## Results and Discussion

*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* exhibited sensitivity towards methanol, ethanol and ethyl acetate extracts of fruit. Methanol, ethanol and ethyl acetate extract of fruit exhibited moderate zone of inhibition against *Escherichia coli*, *Pseudomonas* and *Klebsiella* (Table 2). Fruit extracts were effective against *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas aeruginosa*, *Escherichia coli*, which was about 50% of the inhibition exhibited by the positive control (Table 2).

On analysis of antibacterial properties of *Z. rhetsa*, Methanol, Ethanol and Ethyl acetate extracts of fruit showed more bactericidal property against *Staphylococcus aureus* than other four types of bacteria. Among these three extracts, methanol extract showed considerable activity when compared to ethanol and ethyl acetate fractions (Table 2). Against *Pseudomonas aeruginosa*, ethyl acetate fraction showed better activity than other two extracts, and ethanol extract showed lowest activity (Table 2), and for the bacteria *Escherichia coli* ethanol extract showed better activity than other two extract. The antibiotic cephotaxim was kept as a positive control and the antibacterial activity of the positive control was also recorded. Qualitative phytochemical analysis of ethanol and methanol extract of the fruit indicated the presence of alkaloids, flavonoids, terpenoids, proteins, carbohydrates, triterpenes, saponins, coumarins, steroids and chalcones (Table 1). The ethyl acetate extract of the fruit contains the alkaloids and steroids. The importance of saponins and terpenoids has been reported earlier. Therapeutically terpenoids exercise a broad range in its mode of actions like antiseptic, stimulant, diuretic, antihelminth and counter irritant<sup>3</sup>.

**Table 1: Qualitative phytochemical profile of fruits of *Zanthoxylum rhetsa* using different solvents**

S. No.	Phytoconstituents	Ethyl Acetate	Ethanol	Methanol
1	Alkaloids	-	+	+
2	Flavonoids	+	+	+
3	Terpenoids	+	+	+
4	Phlobatannins	-	-	-
5	Anthraquinones	-	-	-
6	Tannins	-	-	-
7	Phenols	-	-	-
8	Cardiac Glycosides	-	-	-
9	Protein	+	+	+
10	Carbohydrate	+	+	+
10	Triterpenes	+	+	+
11	Steroids	-	+	+
12	Saponins	+	+	+
13	Coumarins	+	+	+
14	Chalcones	+	+	+

**Table 2: Antibacterial activity of fruits of *Zanthoxylum rhetsa* tested against micro organism**

S. No.	Solvent	Dosage (in mg)	Inhibition Zone (in mm)			
			<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
1.	Methanol	0.15	7	7	0	7
		0.20	11	8	7	8
		0.25	12	9	8	9
		0.30	13	11	11	10
2.	Ethanol	0.15	7	7	0	7
		0.20	8	7	7	8
		0.25	10	8	7	9
		0.30	10	8	10	11
3.	Ethyl acetate	0.15	8	7	7	7
		0.20	9	8	8	7
		0.25	11	9	8	8
		0.30	12	9	10	10

From plant saponin synthetic steroid is prepared to treat wide variety of diseases such as allergic and asthmatic conditions<sup>14</sup>. In the present investigation ethyl acetate, ethanol and methanol fractions contain saponins which are of therapeutic value. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats<sup>6</sup>. It also having anti-diabetic effects<sup>15</sup>. The dietary consumption of antioxidant rich fruits and vegetables reduce the occurrence of a class of diseases<sup>16</sup>.

The compounds with antioxidant property increase the activity of scavenging enzymes such as catalase and super oxide dismutase. In the present investigation, the antioxidant analysis, IC<sub>50</sub> value of in ethyl acetate, ethanol and methanol extract was 13, 48 and 33 respectively (Table 3). Similar reports indicated that the onion oil effectively combated the peroxidation damages induced by nicotine

**Table 3: The antioxidant activity of the solvent extracts of fruits of *Z. rhetsa***

S. No.	Solvent	Concentration (µg)	Absorbance	Inhibition	IC50 Value
1	Methanol	BLANK	.02		33
		1000	.03	33	
		1500	.05	60	
		2000	.11	81	
		2500	.14	85	
2	Ethanol	BLANK	.12		48
		1000	0.16	25	
		1500	0.25	52	
		2000	0.32	62	
		2500	0.36	66	
3	Ethyl acetate	BLANK	.02		13
		1000	.04	50	
		1500	.07	71	
		2000	.13	84	
		2500	.16	87	

**Table 4: Anti oxidant activity of Ascorbic acid**

Concentration (in µg)	Wavelength (in nm)	Absorbance	Inhibition %	IC <sub>50</sub> Value
Blank	700	.86		70.37
1000	700	1.14	25	
1500	700	1.50	44	
2000	700	1.68	49	
2500	700	1.73	50	

## Conclusion

Even though pharmacological industries have introducing new antibiotics day by day, resistance to these drugs by microorganisms has also increased tremendously during the last few decades. In general bacteria have the genetic ability to transform and acquire resistance to drugs, which are utilized as therapeutic agents. Different part of the medicinal plant have various medicinal properties. Generally the fruit are used as raw material. Natural products are considered as a clinically effective and safer alternative to the synthetic antibiotics. Krishnakumar *et al.*, reported that the constituents of the rice bran possessed microbicidal property.

The qualitative phytochemical analysis of the fruit extracts revealed the presence of pharmacologically important compounds, viz., alkaloids, flavonoids, terpenoids, protein, carbohydrate, triterpenes, steroids, saponins, coumarins and chalcones. The methanol extract of fruit of *Z. rhetsa* exhibited maximum zone of inhibition against *Staphylococcus aureus*. The ethyl acetate extract of fruit also showed better zone of inhibition against *Pseudomonas aeruginosa*. Ethanol and methanol extract of fruit show better zone of inhibition against *Escherichia coli*.

From the results it may concluded that the fruit extracts were useful against some human pathogenic microbes. The phytochemicals present in the plant constitute medicinal properties especially microbicidal against pathogenic bacteria. Further detailed investigations are required to identify the compound/s responsible for the antibacterial and antioxidant property.

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