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EVALUATION OF PHYSICOCHEMICAL, PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF ZANTHOXYLUM RHETSA (ROXB). DC. BARK.

Veena Naik[1], Subrahmanya P[2]

[1]PhD scholar, Department of Dravya Guna Vijnana, Alva's Ayurveda Medical College, Moodabidri.
[2]Professor & Head, Department of P G Studies in Dravya Guna Vijnana, Alva's Ayurveda Medical College, Moodabidri.

Corresponding Author: drsp28@gmail.com

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ABSTRACT

The plant *Zanthoxylum rhetsa (*ROXB). DC. (Indian prickly ash) is commonly available in Dakshina Kannada District of Karnataka State, widely distributed in Western Ghats and subtropical areas, and is used in many traditional practices; it is known for its folklore use in the varicose vein, the condition of Varicose vein known as Siraja Granthi in Ayurveda. To explore the folklore utility of the plant *Z. rhetsa* therapeutically, the plant's bark is subjected to evaluate its Physicochemical, Phytochemical, and Antioxidant properties. These procedures are carried out using API guidelines (Ayurvedic Pharmacopeia of India). The drug was obtained from its natural habitat and authentified. Phytochemical studies revealed the presence of flavonoids, saponins, tannins, carbohydrates, and proteins. The DPPH Assay has shown dependent antioxidant activity.

Keywords: *Zanthoxylum rhetsa (*ROXB). DC, folklore medicine, *Siraja granthi*, DPPH Assay

INTRODUCTION

According to WHO, Traditional medicine is defined as the sum of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health and the prevention, diagnosis, improvement or treatment of physical and

mental illness[1], in which herbs play a vital role. Herbs are a boon to humanity because of their unique properties, which are explained for various ailments. The same plants or their source plants have been used for some other diseases by folklore practitioners for a long period [2]. these medicine has been used for humans since ancient times, and even today, 80% of the population depends on traditional medicine for initial treatments[3], these medicines are not only for diseases but also to maintain health. *Zanthoxylum rhetsa* (Roxb.) DC is one such source plant for the drug '*Tumburu'* mentioned in Ayurveda for its utility in diseases like *Kushta*, *Shoola*, and *Krimi* [4], which belong to the family Rutaceae. The plant is also traditionally used as an antidiabetic, antispasmodic, diuretic and anti-inflammatory agent and bears significant antinociceptive and antidiarrheal activities. This plant's fruits and stem bark are traditionally used as a stimulant, astringent, stomachic and digestive and are prescribed for urinary infection, dyspepsia, heart troubles, toothache, asthma and bronchitis[5]. This plant contains many chemical substances like terpenoids, xanthyletin and sesamin, alkaloids, flavonoids and sabinene[6]. The different parts of this species have been used in traditional medicine. The bark has diuretic actions and is used in managing dental caries[7]. The folklore practitioners of Dakshina Kannada District are successfully utilising the bark powder mixed with lemon juice as an external application in the case of varicose veins. So far, it has not been explored scientifically. Such folk plants need to be revalidated scientifically. Hence, a Physicochemical, Phytochemical and Antioxidant properties study of *Z rhetsa (*Roxb) DC. The drug is taken here.

Materials and methods

Plant material—The plant material was collected from Moodbidri Alvas Ayurveda Medical College, and the sample was authenticated by the Dravya Guna professor. The stem bark was dried in the shade for two months. Then, the shade-dried plant was subjected to coarse and fine powdering.

Materials required: Shade-dried stem powder of *Zanthoxylum rhetsa* (Roxb) DC, Ethanol Extract, and Aqueous Extract. The procedure is enlisted below, which is followed according to the guidelines of API[8] , Total ash, Acid-insoluble ash, Water-soluble ash, Determination of extractable matter, Moisture content, Phytochemical screening, TLC, HPTLC, and DPPH Assay.

Physicochemical test procedure

Total ash

Place about 2-4 g of *Z. rhetsa bark churna* material, accurately weighed, in a previously ignited and tared crucible. It was spreading the material in an even layer and igniting it by gradually increasing the heat to 500-600 °C until it was white, indicating the absence of carbon. Cooling in a desiccator and weighed without delay and calculated the content of total ash in mg per g of air-dried material.

Acid-insoluble ash

To the crucible containing the total ash, added 25 ml of hydrochloric acid, covered with a watch glass and boiled gently for 5 minutes. Rinsed the watch glass with 5 ml of hot water and added this liquid to the crucible. Collect the insoluble matter on an ashless filter paper and wash it with hot water until the filtrate was neutral. Transferred the filter paper containing the insoluble matter to the original crucible, dried it on a hotplate and ignited it to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, then weighed without delay and calculated the content of acid-insoluble ash in mg per g of air-dried material.

Water-soluble ash

Add 25 ml of water to the crucible containing the total ash and boil for 5 minutes, collected the insoluble matter in a sintered glass crucible or on ashless filter paper, washed it with hot water and ignited it in a crucible for 15 minutes at a temperature not exceeding 450 °C, subtracted the weight of this residue in mg from the weight of the total ash, calculated the content of water-soluble ash in mg per gm of air-dried material.

Determination of extractable matter

This method determines the number of active constituents extracted with solvents from a given amount of herbal material. Method 1. Hot extraction-Place about 4.0 g of *Z. rhetsa bark churna* material, accurately weighed, in a glass-stoppered conical flask. 100 ml of

ethanol was added and weighed to obtain the total weight, including the flask. Shaked well and allowed to stand for 1 hour. By attaching a reflux condenser to the flask and boiling gently for 1 hour; cool and weighed. Readjusting

to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shaked well and filtered rapidly through a dry filter. Transferring 25 ml of the filtrate to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105 °C for 6 hours, cooled in a desiccator for 30 minutes, then weighed without delay. Calculated the content of extractable matter in mg per gm of churna material. Method 2. Cold maceration - Place about 4.0 g of *Z. rhetsa bark churna* material, accurately weighed, in a glass-stoppered conical flask. Macerated with 100 ml of water for the plant material for 6 hours, shaking frequently, then allowed to stand for 18 hours. Filtered rapidly, taking care not to lose any solvent, transferred 25 ml of the filtrate to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105 °C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. Calculated the content of extractable matter in mg per g of *churna* material.

Moisture content

Loss on drying (gravimetric determination)

Placed about 2-5 g of *Z. rhetsa* bark *churna* material, accurately weighed by heating it in a muffle furnace up to 110degree Celsius, recorded the sample weight after one hour and again heated the sample, cooled it and recorded the weight, and repeated this process until constant weight is obtained.

Phytochemical screening -The Phytochemical screening results were done as per the reference from the standard reference[9] for alkaloids, tannins, flavonoids, glycosides, phenols, steroids, triterpenoids and carbohydrates, saponins

Phytochemical Qualitative Analysis plant extracts and methanolic and ethanolic aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods: by two samples1**)**50mg of the extracts were dissolved in dilute HCL and filtered, and 2) with kwatha preparation of *Z. rhetsa* bark *churna.*

Chromatographic procedure-70 grams of *Z. rhetsa* bark *churna* were mixed with 750 ml ethyl alcohol and ran through the Soxlett apparatus for six cycles. The solution obtained was taken, and the alcohol was separated. The obtained extract was taken in china dish and kept on water bath for complete dry. The dried extract was obtained, and two extracts were prepared: 1) 2gm of the drug was scraped, 20 ml of ethyl alcohol was added and made solution 2) kwatha prepared of *Z. rhetsa* with 1part drug with 16 parts of water, then analysed by TLC using silica gel TLC plates for the chromatographic profile.

HPTLC -1gm of powdered *Z. rhetsa* bark *churna* bark powder was suspended in 10 ml of ethanol and kept for cold percolation for 14 hr and filtered 3,6and 9µl of the above samples were applied on a precoated silica gel F_{254} on aluminium plates to a band width of 7mm using linomat 5 TLC applicator. The plate was developed in Ethyl acetate: Methanol: Water: Diethyl ether (3.0: 5.0: 2.0: 0.5). The developed plates were visualized in Short UV, Long UV and then devised with vanillin sulphuric acid reagent and scanned under 365nm Rf colour of the spots and densitometric scan were recorded.

DPPH Assay

The antioxidant activity of the aqueous extract *Z. rhetsa* was measured using DDPH scavenging activity. Vitamin C was used as a standard to compare the antioxidant activity of the extract. The extract has shown dependent antioxidant activity as that of Vit C. The EC50 of ascorbic acid was found to be 3.42± 0.34g/ml, while the EC50 of the extract was found to be 68± 0.46µgm/ ml.

Results - The results, screened for a physicochemical test such as total ash, acid insoluble and water-soluble ash in Table no 1, Extractive values such as Water-soluble extract and acid soluble extract in Table no 2, loss on drying in Table no 3, phytochemical analysis Table no 4 and 4A, TLC in Table no 5 and HPTLC in table no 6 and DPPH Assay in table no 7, has performed by using standard protocol

Physicochemical tests

Total ash value	9.099 %w/w
Acid insoluble ash	0.0855w/w
Water soluble ash	0.089%w/w

Table no 1

Extractive value

Method	In Alcohol (alcoholic) (b)	In Distill water (aqueous)(a)
Hot extraction method	13.2%w/w	19.73%w/w
Cold maceration	23.6%w/w	8.96%w/w

Table no 2(extractive value)

Loss of drying value

Loss on drying	8.65%

Table no 3

Phytochemical screening

Phytochemical screening

	Kwatha(water extract)	Alcohol extract
Alkaloids		
Mayers test	-	-
Wagner's test	-	-
Hager's test	-	-
Dragendroffs test	-	-
Flavonoids		
Alkaline reagent test	-	-
Lead acetate test	-	-
Shinoda test	+	+
Tannin test		
Gelatin test	-	-
Diterpenesand triterpenoids		
Copper acetate test	-	-
Saponin test		
Froath test	+	
Foam test	+	
Glycosides		
Legals test	-	-
Proteins and amino acid		
Xanthoproteic test	-	-
Ninhydrin test	-	-
Carbohydrates		
Molishs test	-	+
Benedicts test	-	

Barfoeds test	-	
Fehling's solution	-	+
Phenolics compounds		
Ferric chloride	-	+
Lead acetate test	-	+
Phytosterols		
Salkowski test	-	-
Liebermann test	-	-

Table no 4 (phytochemical screening)

Alkaloids	Absent
Flavonoids	Present
Tanin	Absent
Diterpenes, triterpinoids	Absent
Glycosides	Absent
Saponin test	Present
Phenolics	Present
Phytosterols	Absent
Proteins and amino acid	Absent
Carbohydrates	Present

Table no 4A(phytochemical screening)

Chromatograph results

	Mobile phase	Under long UV rays (alcoholic extract)	Spots (aqueous extract)	Rf value	As shown in images
Alkaloids	Benzene (22.5ml)+ ethanol (2.5ml)	One florescent spot	No separation (tailing band)	8.4	1
Glycosides	Ethyl acetate ((10ml) butanol (10ml) water (7.5)	Two florescent spots	No separation	1.09, 1.01	2
Flavonoids	Ethyl acetate (10ml) formic acid (1ml) glacial acetic acid (1ml) water (2.5ml)	One spot	One spot	1(alcoholic extract), 0.85(aqueous extract)	3
Terpenoids	Toluene (18ml) Ethyl acetate (2ml)	Two spots	No separation	0.52, 0.75	4

Table no 5 TLC

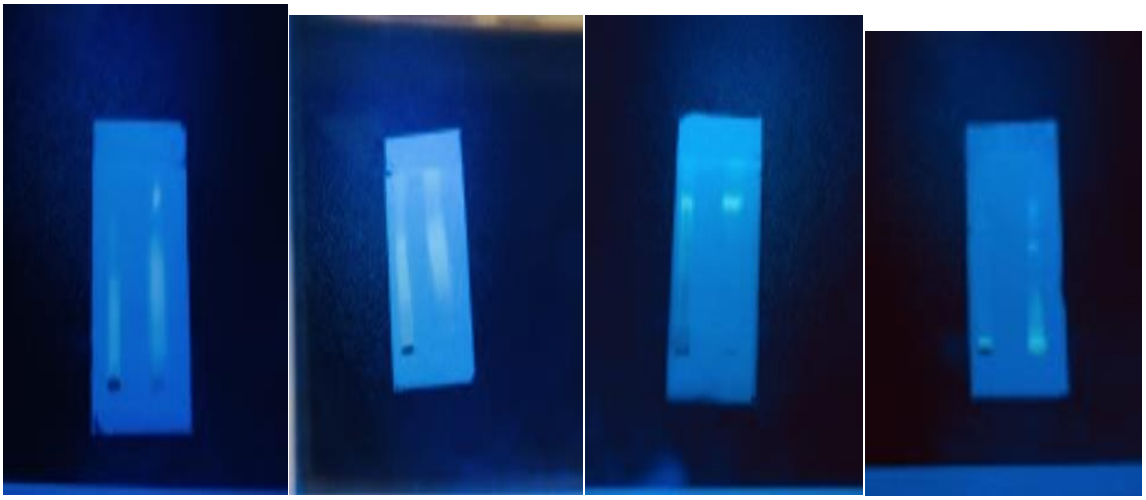
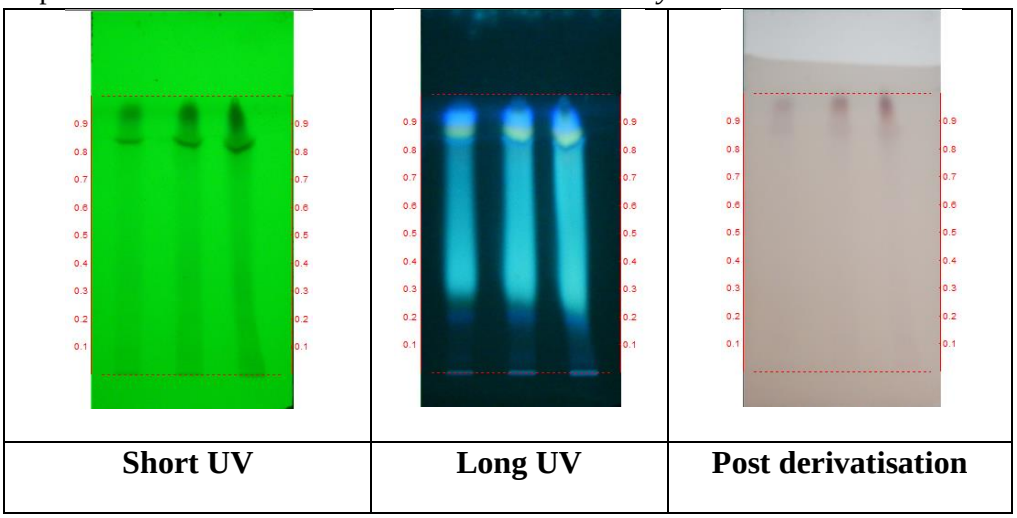


Image 1 **Image 2** **Image3** **Image 4**

Image 5 HPTLC photo documentation of methanol extract of *Zanthoxylum rhetsa* bark



Short UV	Long UV	Post derivatisation

TRACK 1 - ***Zanthoxylum rhetsa*** **bark** – 3µl

TRACK 2 - ***Zanthoxylum rhetsa*** **bark** – 6µl

Track 3 - ***Zanthoxylum rhetsa*** **bark** – 9µl

Solvent system –Ethyl acetate: Methanol: Water: Diethyl ether (3.0: 5.0: 2.0: 0.5)

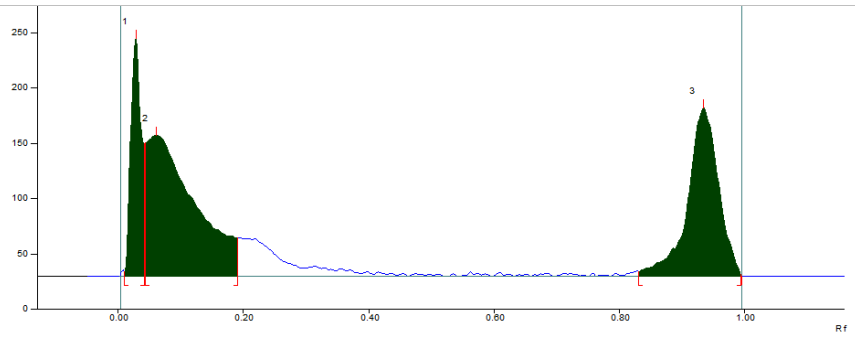
Table 6: Rf values of a sample of Zanthoxylum rhetsa bark

Short UV	Long UV	Post derivatization
-	0.21 (F. blue)	-
0.83 (Green)	0.83 (F. blue)	-
-	0.86 (F. blue)	0.87 (Purple)

***F – Fluorescent; L –Light; D – Dark**

0.28-Nitidine, 0.49-Chelerythrine, 0.83-Sanguinarine

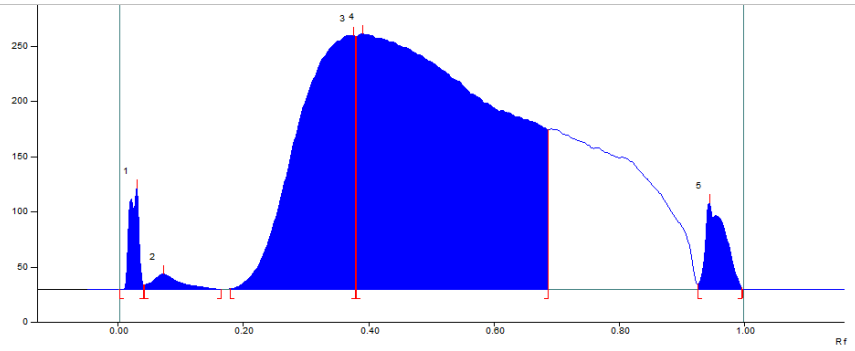
image 6. Densitometric scan of *Zanthoxylum rhetsa* bark



Track 3, ID: Zanthoxylum rhetsa bark

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.4 AU	0.03 Rf	215.0 AU	43.50 %	0.04 Rf	20.1 AU	2617.4 AU	17.30 %
2	0.04 Rf	120.4 AU	0.06 Rf	127.2 AU	25.74 %	0.19 Rf	34.7 AU	7094.1 AU	46.88 %
3	0.83 Rf	4.3 AU	0.94 Rf	152.0 AU	30.75 %	1.00 Rf	1.8 AU	5420.3 AU	35.82 %

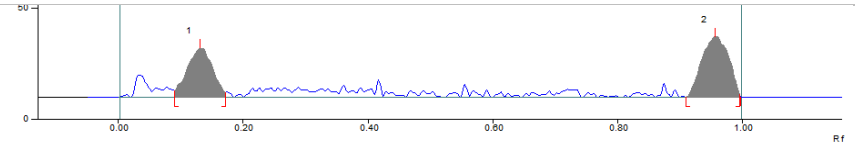
image 6a. At 254nm



Track 3, ID: Zanthoxylum rhetsa bark

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	91.4 AU	14.17 %	0.04 Rf	4.1 AU	963.4 AU	1.76 %
2	0.04 Rf	4.4 AU	0.07 Rf	14.0 AU	2.16 %	0.16 Rf	0.2 AU	439.8 AU	0.81 %
3	0.18 Rf	0.5 AU	0.38 Rf	230.0 AU	35.65 %	0.38 Rf	29.2 AU	15009.2 AU	27.48 %
4	0.38 Rf	228.8 AU	0.39 Rf	231.3 AU	35.86 %	0.69 Rf	44.5 AU	36435.5 AU	66.70 %
5	0.93 Rf	5.1 AU	0.95 Rf	78.4 AU	12.16 %	1.00 Rf	1.4 AU	1774.2 AU	3.25 %

Image 6b. At 366nm



Track 3, ID: Zanthoxylum rhetsa bark

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.09 Rf	2.9 AU	0.13 Rf	22.2 AU	44.81 %	0.17 Rf	2.3 AU	648.8 AU	43.32 %
2	0.91 Rf	0.1 AU	0.96 Rf	27.3 AU	55.19 %	1.00 Rf	1.2 AU	848.8 AU	56.68 %

Image 6c. At 620nm

Remarks

HPTLC was performed on the given sample of Zanthoxylum rhetsa bark. The respective tables and figures provide photo documentation, Rf values, a densitometric scan, and a 3-D Chromatogram.

DPPH Assay

		DPPH assay	
	Conc. (g/mL)	**Vitamin C**	***Zanthoxylum rhetsa* (Aqueous extract)**
	1	**40.50**	**21.34**
	2	**49.05**	**32.09**
	4	**54.55**	**42.95**
	8	**66.19**	**46.46**
	10	**67.08**	**48.89**
	20	**70.40**	**54.72**
	40	**74.82**	**57.35**
	80	**79.93**	**65.23**
	100	**81.17**	**70.17**
	200	**82.60**	**71.93**
	400	**83.07**	**74.18**
	800	**83.71**	**77.23**
	1000	**84.18**	**79.36**
		SE	
		4.76	6.73
		4.36	6.16
		0.92	1.30
		2.66	3.77
		4.10	5.79
		0.69	0.98
		2.44	3.45
		3.68	5.21
		2.92	4.13
		1.73	2.45
		0.87	1.23
		0.51	0.72
		0.23	0.32

Table 7(DPPH Assay)

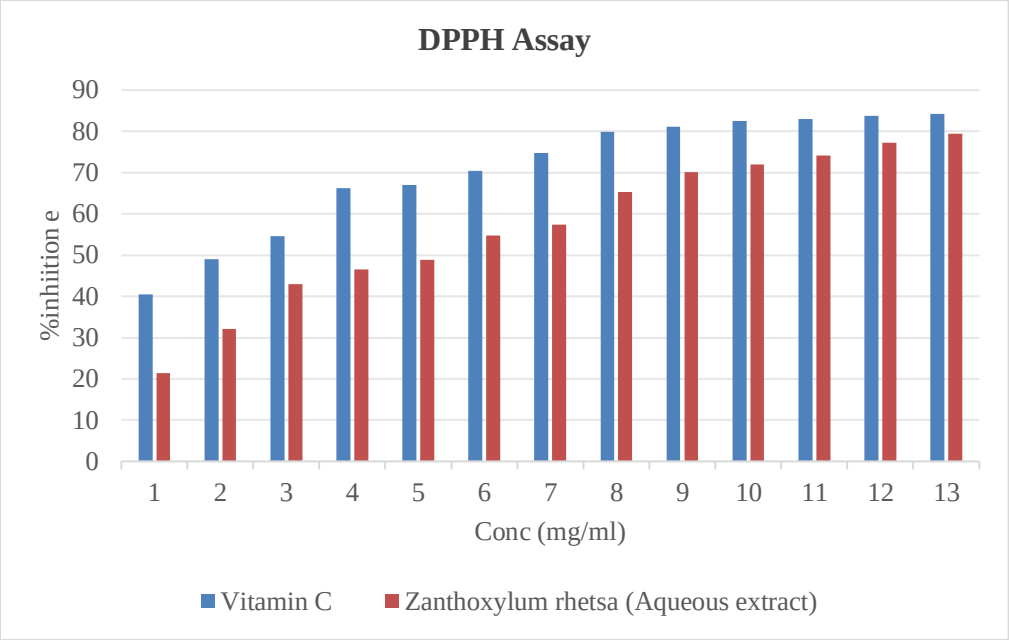


Image 7(DPPH Assay)

The extract has shown dependent antioxidant activity as that of Vit C. The EC50 of ascorbic acid was found to be 3.42± 0.34g/ml, whereas the EC50 of the extract was found to be 68.08 ± 0.46µgm/ ml, as depicted in Table 10 and Image 9.

DISCUSSION

The bark of *Zanthoxylum rhetsa*(ROXB).DC. has been used widely in traditional medicine for a long time; it is a drug used for different purposes and in other conditions related to health problems. The physicochemical test phytochemical screening, thin layer chromatography, high-performance thin layer chromatography, and DPPH assay are dealt with in this paper. The total cash values were determined and presented in percentage form; extractives values were also determined, the moisture content was moderate, TLC and HPTLC were determined for chemical constituents' presence, and the phytochemicals for chemical evaluation ,which were less toxic and biologically active. The bioactive compounds are responsible for the drug's action, and there are plenty in this drug; the compounds of this herb, such as carbohydrates, protein, saponins, flavonoids, etc, are present. DPPH assay has shown dependent antioxidant activity

CONCLUSION

This above-said tree is the primary source plant for *Zanthoxylum armatum*, *Tumburu*; it is used in several disorders. In this study, various chemical tests are performed in the two types of extracts (alcoholic and aqueous) of the stem part of *Zanthoxylum rhetsa* (Roxb.) DC, from the results of this study, concluded that the various extracts have multiple chemical constituents. All these procedures are performed for the genuinicity of the drug, and it may help for further studies.

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