



## Phytochemical Screening and Anthelmintic Activity of *Zizyphus rugosa* Lamk

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### ABSTRACT

*Zizyphus rugosa* Lamk. an edible medicinal plant is grown in Central Western Ghats. The fruit is commonly known as famine fruit. The objective of the study was to phytochemical screening and anthelmintic activity of *Zizyphus rugosa*. About 500 gm of air dried powdered material of unripe fruit of *Zizyphus rugosa* was subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 hours in the order of increasing polarity of solvents. The condensed crude extracts were used for preliminary screening of phytochemicals. The extract showed the +ve response for alkaloids, phenols and flavonoids and –ve response for tannin, lignins, steroids and saponins. The phytochemical screening of secondary metabolites reveals that alkaloids, phenols and flavonoids showed the +ve response whereas tannin, lignins, steroids and saponins showed –ve response. The quantitative analysis of alkaloids, phenols and flavonoids reveals that the alkaloid was dominant in both two-yearly average values. Further, alkaloid is followed by flavonoids and phenols in their concentration. The extracts of petroleum ether, chloroform and ethanol of unripe fruit of *Zizyphus rugosa* were screened for its anthelmintic activity against *Pheretima posthuma*. The time of paralysis and death of *Pheretima posthuma* was determined against all the extracts of petroleum ether, chloroform and ethanol in the concentration of 25, 50 and 100 mg/ml. The extracts of petroleum ether, chloroform and ethanol showed paralysis as well as death of worms in a less time as compared to albendazole (25 mg/ml). Of the three extract the petroleum ether extract showed more effective than compare other two extract. Of the three extracts petroleum ether and ethanol extract in the concentration of 25 mg/ml showed equal time of paralyzing time. Subsequent increase in different concentration of extracts did not show the much differed in time of paralysis as well as death of worm.

**Keywords:** Anthelmintic activity, *Pheretima posthuma*, phytochemicals, *Zizyphus rugosa* Lamk.

### INTRODUCTION

Most of the drugs from plants which have become important in modern medicine had a folklore origin and are traditional in system of medicine. Species of fruits trees in the *Zizyphus spp* are examples of multipurpose plants with great potential for ethno medicinal use all over world.<sup>1</sup> Different species of the genus *Zizyphus* like *vulgaris*, *sativa*, *jujuba* etc belong to the family Rhamnaceae showed hypoglycemic activity.<sup>2</sup> *Zizyphus rugosa* Lamk is one of the famine edible medicinal plants of Central Western Ghats. The pulps of the fruits are the source of macro and micro elements and proximates with negligible amount of toxic elements. The pulp of the fruit is the resources to prepare juice and dosa a popular south Indian dish. The pulp of the fruit can be used to establish cottage based industries to produce juice and to provide employment and to generate income by the local people.<sup>3</sup> The dish which is prepared can be used as an additional food Further, the bark of root and stem leaves and flowers are used in the preparation of herbal formulations.<sup>4</sup> The fruit is described as demulcent and enter into the treatment of throat and broncho-pulmonic irritations and the dried powdered leaves and fruits are applied topically in the treatment of boils.<sup>5</sup> The phytochemical studies reported that Cyclopeptide alkaloids and six flavones glycosides<sup>6</sup> and one saponine<sup>7</sup> were isolated from root bark of *Zizyphus rugosa*. The root

bark extract of *Zizyphus rugosa* in water, chloroform, ethyl acetate and methanol were subjected to determine anti-inflammatory and analgesic activities.<sup>1</sup> Pandey *et al*<sup>7</sup> isolated rugosanine–A: N-formylcyclopeptide alkaloid and uncharacterized isoquinoline from the stem bark of *Zizyphus rugosa*. The antibacterial, anti-fungal and  $\beta$ -glucuronidase inhibitory activity of the extract of leaves and bark of *Zizyphus rugosa* and *Zizyphus oenoplia* were studied.<sup>2, 9</sup> The extensive work has been done on uses, medicinal potential and herbal formulation. The present study is aimed to phytochemical screening and anthelmintic activity of *Zizyphus rugosa* Lamk.

### MATERIALS AND METHODS

#### Study area

The Kuvempu University Campus is 230 hectares, it is on the latitude of 13° 42' 20" N and longitude of 75° 13' 22" E. The campus is surrounded from the north by Gonibeedu, south by B.R. Project the east from Nellisara and from the west by Umblebylu. The area receives the rainfall between 741.1 mm and 1500.48 mm/year. The year is divided into three district seasons summer, winter and rainy. The vegetation type is moist deciduous type. The *Zizyphus rugosa* which has been grown widely in the Kuvempu University Campus has been identified and selected for study.



### Collection of plant material and preparation of sample for analysis

The mature unripe fruit samples were collected from the Kuvempu University campus during February and March 2009 and 2010. The fruit samples were washed and shade dried. Having dried fruits samples were powdered and used for further analysis.

### Preparation of extract

The shade dried fruit material is powdered using mixer grinder and subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 hours in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals.<sup>8</sup>

### Preliminary screening of secondary metabolites

#### Preparation of extract

The shade dried fruit material is powdered using mixer grinder and subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 hours in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals.<sup>8</sup>

#### Preliminary screening of secondary metabolites

The successive extract was subjected to preliminary phytochemical testing for the detection of major chemical groups. The preliminary phytochemical screening was carried out according to the recommended standard procedures.<sup>8,19</sup>

#### Test for alkaloids

**Mayer's test/Wagner's test/Dragendorff's test:** Two ml of extract was treated with 1 ml of 1% HCL and boiled for few minutes. One ml of the above mixture was treated with 6 drops of Mayer's reagents/Wagner's reagent /Dragendorff's reagent. The formation of Creamish precipitate/Brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids.

#### Test for phenols

**Phenol test:** The crude extract was mixed with 2 ml of 2% solution of FeCl<sub>3</sub>, a blue green or black colouration indicated the presence of phenols.

**Ellagic acid test:** The crude extract was mixed with a few drops of 5% mixture containing glacial acetic acid and 5% sodium nitrate solution. A muddy yellow, olive brown, niger brown or deep chocolate colour indicated the presence of phenols.

#### Test for flavonoids

**Shinoda test:** 3 ml of each extract was treated with 5 ml of 95% ethanol and few drops of concentrated HCL and 0.5gm magnesium turnings, formation of pink, reddish or brown colour indicated the presence of flavonoids.

**Alkaline reagent test:** The crude extract was treated with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

**Zinc-hydrochloric acid reduction test:** 4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added. The appearance of red colour indicated the presence of flavonoids and orange colour indicates the presence of flavones.

**Flavonoid test:** 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. The appearance of yellow colour indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to portion of each extract. A yellow colouration indicating the presence of flavonoids.

#### Test for tannins

Crude extract was mixed with 2 ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black coloration indicated the presence of tannins.

#### Test for lignins

When the extract was treated with 2% furfuraldehyde. The formation of red colour indicated the presence of lignins.

#### Test for steroids

**Salkowski's test:** The crude extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added carefully and shaken gently. The reddish brown colour indicated the presence of steroids.

**Liebermann-Barchardt's test:** The extract was treated with a 2 ml acetic anhydride and mixed with 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of blue-green ring indicated the presence of steroids.

#### Test for glycosides

Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was poured into another test tube containing 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. The formation of the brown ring at the interphase indicated the presence of cardiac glycosides.

#### Test for saponins

**Foam Test:** The crude extract was treated with 5 ml of distilled water in test tube. It was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

**Haemolysis test:** 2 ml each of 18% sodium chloride solution was taken in two test tubes. 2 ml of distilled water was added to one test tube and the 2 ml of filtrate was added to another test tube. Few drops of blood was added



to the both the test tubes. Mixed and observed for haemolysis under microscope.

### Quantitative analysis

Quantitative estimation of alkaloids, phenols and flavonoids were carried out according to the recommended standard procedures.<sup>19-21</sup>

### Estimation of alkaloids

A 500 mg of fruit sample was extracted with methanol and the methanol extract was condensed and 20 ml dilute acetic acid (1: 5) was added. The mixture was shaken well in a separating funnel. The acetic acid layer was collected and added with 25 ml N- hexane and chloroform. This was shaken again in a separating funnel for 3 times and pH was adjusted to 8 using sodium hydroxide solution and shaken for 30 min. In a separating funnel, the chloroform layer was collected, washed with water and pH was adjusted to 11 to 12 by adding ammonium hydroxide and the chloroform layer collected and filtered using dry filter paper. The filtrate was transferred to a pre-weighed beaker and dried under reduced pressure at 60°C for 6 h. The amount of alkaloids was calculated using the formula.

$$\text{Total percentage of alkaloid} = \frac{\text{The Weight of alkaloid residue (x)}}{\text{Wight of sample (W)}} \times 100$$

x = Weight of the residue

y = Weight of the empty evaporator dish

z = Weight of the empty dish + alkaloid residue

Total (X) = Z – Y

### Estimation of phenol by Folin ciocalteu's reagent method

Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce blue coloured complex (molybdenum blue)

500 mg of fruit sample was weighed and it was grinded it with a pestle and mortar in 10 times volume of 80 % ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was saved. The residue was re-extracted with five times the volume of 80 % ethanol centrifuge and pooled the supernatants. The supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water (5 ml). The aliquots were pipette out (0.2 to 2 ml) in to test tubes. The final volume was adjusted to 3 ml with distilled water 0.5 ml of folin- Ciocalteu's reagent was added. 2 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution was added after 3 minutes. Mixed thoroughly, the test tube was kept in boiling water for exactly one minute. Cooled and the absorbance at 650 nm against a reagent blank. Simultaneously, a standard graph was prepared by with different concentration of catechol. The phenol concentration was found out with standard graph and the phenol concentration was expressed as mg/100 gm fruit material. The phenols are expressed in percentage.

### Estimation of flavonoids

500 mg of fruit sample was added with 10 ml methanol, homogenized and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for the estimation of flavonoids. 1 ml supernatant was transferred to a 25 ml conical flask and diluted to 2 ml with distilled water, 4 ml of vanillin reagents was added rapidly from a burette (within 10-15 seconds) to flask A. 4 ml of sulphuric acid was added to flask B into flask C and 4 ml of vanillin reagent and 2 ml of water was added and this was considered as blank. The content of flasks A and B were shaken in a water bath below 30°C and they were transferred to room temperature for exactly 15 minutes. The absorbance was measured at 500 nm against 47 % sulphuric acid (flask D). The absorbance of the contents of flask B and C were subtracted from that of A. Alternatively; the absorbance of the content of A+D against B+C could be used. A standard curves was prepared using phloroglucinol and amount of flavonoid (mg/g) was calculated. The flavonoids values are expressed in percentage.

### Anthelmintic activity

The method of Anuj Kumar *et al*<sup>21</sup> was followed with modification for the screening of anthelmintic activity which was evaluated on adult Indian earthworm, *Pheretima postuma*.

**Worms:** Indian earthworm *Pheretima posthuman* (Annelida) was collected from horticultural department at N.R.Pura of Chikamagalur, Karnataka. The average size of *Pheretima posthuma* was 6-8 cm. They were washed with water to remove dirt.

**Chemicals:** Albendazole, double distilled water and saline (6% dextrose)

### Procedure:

The fruit extract of *Zizyphus rugosa* were evaluated for anthelmintic activity in *Pheretima posthuma* (earth worm) of nearly equal size (6-8 cm). *Pheretima posthuma* is used due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability of earthworms, they have been used widely for the initial evaluation of the anthelmintic compounds. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into five groups of six earth worms in each and placed in eight petri dishes containing the extract solutions or the reference drugs as mentioned below.

Group-1: Received double distill water as the control

Group-2: Received Albendazole suspension at a dose of 25 mg/ml which served as the standard

Group-3: Received petroleum ether/chloroform/ethanol extract at a dose of 100 mg/ml

Group-4: Received petroleum ether/chloroform/ethanol extract at a dose of 50 mg/ml



Group-5: Received petroleum ether/chloroform/ethanol extract at a dose of 25 mg/ml

All petri dishes were kept under room temperature. The living or viable worms were kept under close observation. Observation was made for time taken to complete paralysis (PT) and death (DT) for individual worms. Each worm was frequently applied with external stimuli which stimulates and induce movement in earthworms, if alive. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading of the body colour. The motionless worms were then transferred at 40° C to confirm that they were dead.

## RESULTS AND DISCUSSION

### Preliminary screening of secondary metabolites

The result of preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous fruit extract of *Zizyphus rugosa* are presented in Table 1.

**Table 1:** Preliminary screening of secondary metabolites

Test for secondary metabolites	Hot Extraction			
	PE	CL	ET	AQ
<b>ALKALOIDS</b>				
Wagner's test	+	-	+	+
Dragendorff's test	-	-	+	+
Mayer's test	+	+	+	+
<b>PHENOL</b>				
Phenol test	+	+	+	+
Ellagic acid test	+	-	+	+
<b>FLAVONOIDS</b>				
Shinoda's test	-	-	+	+
Zinc-hydrochloride acid test	-	-	+	+
Alkaline reagent test	+	+	-	-
Flavonoid test	+	+	-	-
<b>TANNINS</b>				
Ferric chloride test	-	-	-	-
<b>LIGNINS</b>				
Lignins test	-	-	-	-
<b>STEROIDS</b>				
Salkowski test	-	-	-	-
Liebermann- Burchardt's test	-	-	-	-
<b>GLYCOSIDES</b>				
Keller-Killiani test	-	-	+	+
<b>SAPONINS</b>				
Foam test	-	-	-	-
Haemolysis test	-	-	-	-

Petroleum ether: CL-Chloroform: ET-Ethanol: AQ-Aqueous

### Alkaloids

Wagner's test and Mayer's test showed +ve response for petroleum ether, ethanol and aqueous extract but -ve response for chloroform extract of fruit of *Zizyphus rugosa*. Drangendorff's test showed +ve response for ethanol and aqueous extract, but -ve response for petroleum ether and chloroform extracts of unripe fruit of *Zizyphus rugosa*. The number of investigators Jigna Parekh and Sumitra Chanda<sup>12</sup>, Edeoga *et al*<sup>17</sup>, Vimalavady and Kadavul<sup>22</sup> screened for alkaloids from the extracts of different solvents in different medicinal plants.

### Phenol

Phenol test and Ellagic acid test showed the + ve response for the entire extract but ellagic acid test showed -ve response for chloroform extract. Velmurugan *et al*<sup>23</sup> reported + ve test for phenols for the root bark extract of hexane, ethyl acetate, methanol of root bark of *Psidium guajava*, Doss<sup>15</sup> also reported +ve results for the phenols of various medicinal plants of methanol extract.

### Flavonoids

Zinc hydrochloride acid test, shinoa's test showed -ve response for petroleum ether and chloroform extract, but in +ve response showed for ethanol and aqueous extracts. In case of Alkaline reagent and flavonoids test showed -ve response for ethanol and aqueous extract but +ve response for petroleum ether and chloroform extract. Various investigators (Krishnaiah *et al*<sup>16</sup>, Bindu Hima *et al*<sup>24</sup> and Krishnamurthy and Asha<sup>25</sup>) screened flavonoids from various parts of the plants.

### Glycosides

In case of killer- Killiani test showed -ve response for petroleum ether and chloroform extract but +ve response for ethanol and aqueous extract. Krishnaiah *et al*<sup>16</sup> reported cardiac- glycosides from the six Malaysian medicinal plants.

### Quantitative estimation of secondary metabolites

At the same time quantitative estimation of secondary metabolites were done for alkaloids, phenol and flavonoids for two years 2009 and 2010.

**Alkaloids:** The percent values of alkaloids ranged between 7.00 and 8.00 with an average of 7.70 %. The minimum of 7.00 and maximum of 8.00 % were recorded in the sample of 2010 and 2009 respectively (Table 2 and Fig.1). The number of investigators estimated secondary metabolites in the different parts of the medicinal plants<sup>6-10</sup> and recorded the percentage of alkaloids between  $0.28 \pm 0.12$  and  $5.63 \pm 0.20$  in *B.tinctoria* and *P.edulis* respectively. Krishnaiah *et al*<sup>16</sup> reported the percentage of alkaloids between  $0.24 \pm 0.03$  and  $0.52 \pm 0.12$  in *E. officinalis* and *A.indica* respectively. Edeoga *et al*<sup>17</sup> reported the percentage alkaloids between  $0.34 \pm 0.10$  and  $1.04 \pm 0.20$  from the medicinal plants. Mallikarjuna *et al*<sup>8</sup> observed the variation of quantity of alkaloids in different parts of the



*S. potatorum* and they recorded the percentage of alkaloids between 1.30 and 2.20. The quantity of alkaloids may vary not only in the parts but also varied between fresh and preserved plant parts.<sup>8</sup> Alkaloids are nitrogen-containing compounds widely distributed in different plant group.<sup>22</sup> The alkaloids are the lead molecules of therapeutic importance and they are heterocyclic indole compounds which have proved to be having pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities.<sup>11</sup> The biological properties of alkaloids were studied.<sup>24-30</sup>

**Phenols:** The percent value of phenol ranged between 0.39 and 0.95 with an average of 0.6%. The minimum of 0.39% and maximum of 0.95% were recorded in the sample of 2009 and 2010 respectively (Table 2 and Fig.1). Doss<sup>12</sup> recorded percentage of phenol between  $0.16 \pm 0.10$  and  $12.85 \pm 0.28$  from different Indian medicinal plants. Krishnaiah *et al*<sup>16</sup> reported variation of percentage of phenol between  $0.024 \pm 0.13$  and  $0.719 \pm 0.23$  in the medicinal plant of Malaysia. Edeoga *et al*<sup>17</sup> observed variation of phenols in different medicinal plants of Nigeria. Mallikarjuna *et al*<sup>8</sup> reported variations of phenols among the different parts of the *S. potatorum*. Phenols constitute a large class of compounds in which a hydroxyl group (-OH group) is bound to an aromatic ring. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites and they possess biological properties such as atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Natural antioxidant is due to the presence of rich phenolic compounds such as flavonoids, phenolic acid and tocopherol etc.

**Flavonoids:** The percent values of flavonoids ranged between 0.22 and 1.23 with an average of 0.74%. The minimum of 0.22% and the maximum of 1.23% were recorded in the sample of 2009 and 2010 respectively (Table 2 and Fig.1). Flavonoids are group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and antioxidant enzymes and also act as anti-inflammatory agent. The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. They also inhibit microbes which are resistant to antibiotics. Flavonoids may help in providing protection against some diseases such as oxidative stress, cellular damage. Oxidative stress have been linked to cancer, ageing, antherosclerosis,

inflammation, ischemic injury and neuro degenerative diseases.<sup>22</sup>

The tannins, steroids and lignins were absent in the unripe fruit of *Zizyphus rugosa* Lamk.

#### Anthelmintic activity

The results of anthelmintic activity of petroleum ether, chloroform and ethanol extract are showed in Table 3.

From the above study it was observed that the extract of petroleum ether showed dose dependent anthelmintic activity as compared to a standard drug of albendazole. The petroleum ether extract showed paralyzing time of *Pheretima posthum*. The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be  $48.33 \pm 5.90$ ,  $38.33 \pm 0.02$  and  $29.55 \pm 4.60$  minutes respectively. In the mean time albendazole at a dose of 25 mg/ml caused paralysis in the above helminth in  $60.40 \pm 0.02$  minutes. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be  $60.30 \pm 0.11$ ,  $60.28 \pm 0.02$  and  $60.20 \pm 0.07$  and minutes respectively. In the meantime albendazole at a dose of 25 mg/ml caused death time of the above helminth in  $120.40 \pm 0.02$  minutes (Table 3). The petroleum ether extract showed paralysis as well as death of worms in a less time as compared to albendazole in case of *Pheretima posthuma* (Fig.2).

In case of chloroform extract showed dose dependent anthelmintic activity as compared to a standard drug albendazole. The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found  $54.33 \pm 0.06$ ,  $47.33 \pm 0.02$  and  $44 \pm 0.02$  minutes respectively. In the meantime albendazole at a dose of 25 mg/ml caused paralysis in the above helminth in  $60.40 \pm 0.02$  minutes (Table 3).

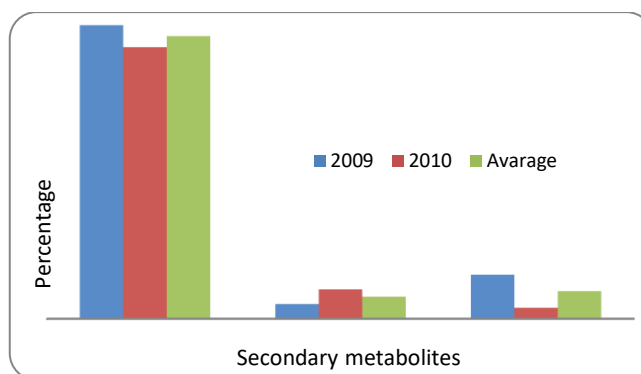
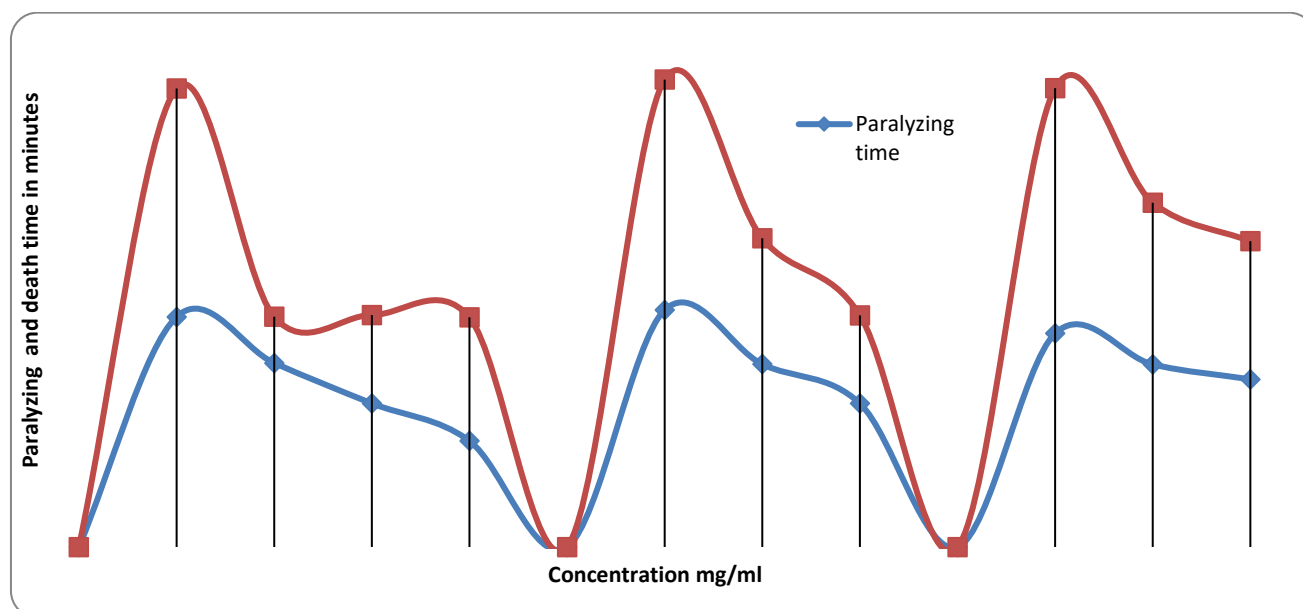


Figure 1: Quantitative variation of secondary metabolites

Table 2: Quantitative estimation of secondary metabolites

	Alkaloids				Phenol				Flavonoids			
	I	II	III	Mean $\pm$ SD	I	II	III	Mean $\pm$ SD	I	II	III	Mean $\pm$ SD
2009	8.00	8.00	8.00	$8.00 \pm 0.00$	0.39	0.44	0.44	$0.40 \pm 0.02$	1.23	1.23	1.23	$1.20 \pm 0.00$
2010	7.80	7.00	7.40	$7.40 \pm 0.40$	0.67	0.67	0.95	$0.8 \pm 0.16$	0.23	0.32	0.22	$0.30 \pm 0.06$
Average value	7.90	7.50	7.70	$7.70 \pm 0.40$	0.53	0.55	0.69	$0.60 \pm 0.09$	0.73	0.72	0.72	$0.75 \pm 0.06$





**Figure 2:** Variation of anthelmintic activity between standard drug and crude extracts of petroleum ether, chloroform and ethanol in mature unripe fruit of *Zizyphus rugosa* Lamk.

**Table 3:** Anthelmintic activity of *Zizyphus rugosa* Lamk. in different extract

Groups	Concentration (mg/ml)	<i>Pheretima posthuma</i>	
		Paralyzing time in minutes	Death time in minutes
Distilled Water	-	-	-
Standard	25	60.40 ± 0.02	120.40 ± 0.02
Petroleum ether extract	25	48.33 ± 5.9	60.30 ± 0.11
	50	38.33 ± 0.02	60.28 ± 0.02
	100	29.55 ± 4.6	60.20 ± 0.07
Chloroform extract	25	54.33 ± 0.06	60.45 ± 0.01
	50	47.33 ± 0.02	60.43 ± 0.01
	100	44.00 ± 0.02	60.33 ± 0.01
Ethanol extract	25	48.5 ± 0.005	60.54 ± 0.07
	50	37.33 ± 0.03	60.19 ± 0.012
	100	35.66 ± 0.01	60.14 ± 0.01

The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 60.45 ± 0.01, 60.43 ± 0.01 and 60.33 ± 0.01 minutes respectively. In the meantime albendazole at a dose of 25 mg/ml caused death time of the above helminth in 120.40 ± 0.02 minutes. The chloroform extract showed paralysis as well as death of worms in a less time as compared to albendazole in case of *Pheretima posthuma* (Fig 2).

The ethanol extract showed paralyzing time of *Pheretima posthuma* with dose of 25, 50 and 100 mg/ml were found 48.50 ± 0.005, 37.33 ± 0.03 and 35.66 ± 0.01. In the meantime albendazole at a dose of 25 mg/ml caused paralysis in the above helminth in 60.40 ± 0.02 minutes. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 60.54 ± 0.07, 60.19 ± 0.012 and 60.14 ± 0.01 and minutes respectively. In the

meantime albendazole at a dose of 25 mg/ml caused death time of the above helminth in 120.40 minutes (Table 3). The ethanol extract showed paralysis as well as death of worms in a less time as compared to albendazole in case of *Pheretima posthuma* (Fig 2). Of the three extract the petroleum ether extract showed more effective than compare other two extract. Of the three extract petroleum ether and ethanol extract in the concentration of 25 mg/ml showed equal time of paralyzing time. Subsequent increase in concentration of extract did not show the much differ in time of paralysis as well as death of worm. The fruit extract of *Zizyphus rugosa* showed more effective less anthelmintic activity as compared to standard drug. The anthelmintic activity of *Zizyphus rugosa* may be due to the effect of active phyto-constituents i.e. alkaloids, phenols and flavonoids present in the extracts. Alkaloids may act on central nervous system and caused paralysis of the earthworm.<sup>29</sup> The effect would be due to presence of the steroidal alkaloid oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in local homeostasis which is essential for the development of helminthes. The extract exhibited significant anthelmintic activity at highest concentration of 100 mg/ml as compared with piperazine citrate as standard reference and distilled water as control.<sup>30</sup>

## CONCLUSION

The fruit of *Zizyphus rugosa* is a famine edible medicinal plants of Central Western Ghats. The extracts of petroleum ether, chloroform, ethanol and aqueous were screened for qualitative analysis. The qualitative analysis revealed that alkaloids, phenols and flavonoids are +ve response in all the extracts, whereas tannins, lignins, steroids and saponins showed -ve response. The quantitative estimation of

secondary metabolites revealed that alkaloids were dominant, which is followed by phenols and flavonoids. Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments however, there is need to further carry out advanced spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future. The anthelmintic property of petroleum ether, chloroform and ethanol showed paralysis as well as death of worms in a less time as compared to albendazole (25 mg/ml) in case of *Pheretima posthuma*. The wormicidal activity of alcoholic extracts suggests that it is effective against parasitic infections of humans. Further it is necessary to identify and isolate the possible active phytoconstituent or constituents which are exactly responsible for the anthelmintic action and study its other pharmacological properties for futuristic and cost effective pharmaceutical formulations.

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