

# Evaluation of anti-fatigue and antioxidant activities of ethanolic leaf extracts of *Annona squamosa* and *Annona reticulata*

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## ABSTRACT

The present work aimed to estimate the possible antioxidant and anti-fatigue effect of ethanolic leaf extracts of *Annona squamosa* and *Annona reticulata*. *In vitro* antioxidant activities of leaf extracts were investigated through hydroxyl and phosphomolybdenum assay methods. The anti-fatigue activity of leaf extracts (75 and 150 mg/kg) was elucidated by a weight-loaded forced swimming test, and the potential mechanism was explored by determination of fatigue-related biochemical parameters like blood lactate and Serum urea nitrogen in mice. Results indicated that significant antioxidant activity was observed by ethanolic leaf extract of *A. squamosa* compared to ethanolic leaf extract of *A. reticulata* and the results are comparable with that of standard Ascorbic acid. *In vivo* experimental studies, leaf extracts could evidently extend exhaustive swimming time of mice, inhibit the increase of blood lactic acid (BLA) and decrease serum urea nitrogen (BUN) of mice after swimming. Considering all these, the leaf extracts possessed appreciable efficacy to alleviate fatigue, and the mechanism might be associated with favorably modulating the process of energy consumption, metabolism, and attenuating oxidative stress injury. The results provided an important basis for developing the leaf extracts as a novel antioxidants and for treating fatigue.

**Keywords:** Anti-fatigue activity, antioxidant activity, *Annona squamosa*, *Annona reticulata*.

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## INTRODUCTION

*Annona squamosa* L and *Annona reticulata* L. plants belongs to family Annonaceae. They have multipurpose with edible fruits and these two plants are one of the sources in medicinal and industrial products which are cultivated all over India for its edible fruit at a height of 3 to 8 meters with lateral branches and leaves are thin, simple with fine hairs underneath arranged alternately. *A. squamosa* is small shrub (or) well branched tree commonly known as Custard apple in English, Sitaphal in Hindi and Sitaphalam in Telugu, it has excellent source in vitamins and high energy. *A. reticulata* is a small deciduous (or) semi evergreen tree commonly known as bullocks-Heart in English, Ramphal in Hindi and Ramaphalamu in Telugu (Pandey and Barve, 2011; Saeed and Ahmad, 2017). These two plants are attributed with medicinal properties for the treatment of

epilepsy, dysentery, cardiac problems, fainting, malignant tumours, dysuria, ulcers, constipation, haemorrhage, diabetes, HIV, cancer, diarrhoea, anaemia, insecticidal and also used to treat various pathologies due to its anti-inflammatory, antimicrobial, antioxidant affects (Shirwaikar et al., 2004; Shivanna et al., 2019).

Fatigue is a weariness caused by exertion, exhaustion and it is a feeling of extreme physical/mental tiredness, arise from severe stress and hard physical (or) mental work. Fatigue acts as a blinker of various diseases. Antioxidants play an effective role in treatment and prevention of several diseases. An Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is the process of chemical reaction produces free radicals, leads to chain reactions which may damage cells. Antioxidants such as thiols (or) Ascorbic acid

(vitamin c) end these chain reactions. Antioxidants are mainly used as industrial chemicals and natural chemicals. Antioxidant used in industrial products prevents oxidation and natural chemicals found in foods and body tissue which have beneficial health effects (El-Chaghaby et al., 2014). Oxidative stress is an imbalance between the excessive formation of ROS and limited Antioxidant defence (Pizzino et al., 2017). Very severe physical exercise also causes oxidative stress in body due to excess generation of oxygen derived free radicals. While doing exercise large amount of oxygen is used of about 4-5% of total oxygen consumed during respiration which results in acceleration of free radical generation. Reactive oxygen species responsible for a greater number of degenerative diseases like cancer, wound healing, atherosclerosis, diabetes and also responsible for exercise induced protein oxidation and being muscle to fatigue (Taniyama and Griendling, 2003). Antioxidants can delay (or) provides protection for living organisms from damage caused by uncontrolled production of ROS and protein damage, lipid peroxidation, DNA strand breaking due to their redox properties, which help them to act as hydrogen donors, reducing agents and free radical scavengers, strong chelators of metal ions (Srividya et al., 2013).

So far much pharmacological work has not been reported on the Antioxidant and Antifatigue activities on the leaves of *A. squamosa* and *A. reticulata*. Therefore, the present study aims to investigate the Antioxidant and Anti-fatigue activities on the ethanolic leaf extracts of *A. squamosa* and *A. reticulata*.

## MATERIALS AND METHODS

### Collection of plant material

The fresh leaves of *Annona squamosa* and *Annona reticulata* collected near Rajahmundry, Andhra Pradesh, India country. The plant was authenticated by Dr. T. Raghuram, Taxonomist, Maharani College, Peddapuram and voucher specimen number given is 22126.

### Preparation of ethanolic leaf extracts

The freshly collected leaves of *A. squamosa* and *A. reticulata* were washed with water to remove dirt and sand particles and dried under shade for 40 days and they were grounded into powder using a mechanical grinder. The powder was extracted with 95% ethanol for 3 days, followed by hot percolation for 3 hours. Then it was filtered and distilled at 80°C. Then it was transferred into the empty china dish and evaporated to get an ethanolic extract and kept in anhydrous calcium chloride containing desiccators (Thilza et al., 2010).

### Preliminary phytochemical screening

Preliminary phytochemical screening of Ethanolic leaf extracts of *A.*

*squamosa* and *A. reticulata* were done to test the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats (Rajendran et al., 2004).

### *In vitro* anti-oxidant study

#### Hydroxyl radical scavenging assay

The scavenging ability of the sample extracts and standard ascorbic acid on hydroxyl radicals were determined according to the method described by (Smirnov and Cumbes, 1989) with some modifications. Briefly, individual sample extracts (1 ml) at different concentrations (0.05, 0.1, 0.3 and 0.5 mg/ml) was added to the reagent containing 1 ml 1.5 mM FeSO<sub>4</sub>, 0.7 ml 6 mM H<sub>2</sub>O<sub>2</sub> and 0.3 ml 20 mM sodium salicylate. After incubation for 1 h at 37°C, absorbance of the reaction mixture was read at 562 nm. The formula to calculate the percentage inhibition was:

$$\text{Scavenging ability on hydroxyl radicals (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A<sub>0</sub> is the control reaction absorbance (containing all reagents except the sample extract), and A<sub>1</sub> sample extract absorbance. Ascorbic acid was used as standard.

#### Phosphomolybdenum assay

The antioxidant activity of sample extracts and standard ascorbic acid was evaluated by the phosphomolybdenum method according to the procedure (Prieto et al., 1999). The assay is based on Mo (VI)–Mo (V) reduction by the extract and at acid pH leads to formation of a green phosphate/Mo (V) complex. 0.3 ml of extracts (0.05, 0.1, 0.3 and 0.5 mg/ml) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction solution was incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm.

### Experimental animals

Swiss albino mice of about 18 to 22 g of either sex were used for the study. Animals were housed in colony cages at ambient temperature of 25 ± 2°C, 12 h light/dark cycle and 50 ± 5% relative humidity with free access to food and water *ad libitum*. The animals were acclimatized to the laboratory environment for at least a week before experimentation. Food but not water was deprived overnight and during the experiment period. All the experiments were carried out during the light period (9:00-16:00 h). Each group consisted of 5 animals. The animal experiments were performed based on the Institutional Ethics Committee (IEC) approval and guidelines Reg. No. 1269/a/10/CPCSEA.

### Acute toxicity studies

Ethanolic leaf extracts of *A. squamosa* and *A. reticulata* were administered orally in dose of 50, 100, 200, 400, 800 and 1600 mg/kg to groups of mice (n = 6); observed for signs of behavioural, neurological toxicity and percentage mortality was noted 24 h later. The doses were administered to the animals. As per the OECD guideline 420, so far, no pharmacological activities have been

carried out on this plant species. Therefore, based on the other species in the same genus, doses were considered for acute toxicity studies. The extracts were found to be devoid of mortality at 1600 mg/kg.<sup>2</sup>

### Animal grouping

25 mice were divided into 5 groups of 5 mice each:

Group I – Control group which receives the vehicle (0.9% Normal Saline)

Group II – Animals treated with Ethanolic leaf extract of *Annona squamosa* of 75 mg/kg p.o

Group III – Animals treated with Ethanolic leaf extract of *Annona squamosa* of 150 mg/kg p.o

Group IV – Animals treated with Ethanolic leaf extract of *Annona reticulata* of 75 mg/kg p.o

Group V - Animals treated with Ethanolic leaf extract of *Annona reticulata* of 150 mg/kg p.o

### Invivo anti-fatigue activity

#### Forced swimming test

Mice were treated with ethanolic leaf extracts of *Annona squamosa* and *Annona reticulata* for a period of 4 weeks on the day of 28, 30 min after the extract administration forced Swimming test was performed individually with a constant loads corresponding to 10% of its body weight tagged to the tail in an acrylic plastic pool (50 cm x 50 cm x 40 cm) filled with water to a depth of 30cm and maintained at 25 ± 2°C. Exhaustion of the mice was determined by observing loss of coordinated measurements and failed to return to the surface within 10 s and then immediately swimming time was recorded. Then after mice were taken from the pool, dried with paper towel and then return to their cages after each session water in the pool was changed (Zhang et al., 2011).

#### Measurement of blood lactate contents of mice

Mice were treated with ethanolic leaf extracts of *A. squamosa* and *A. reticulata* for a period of 4 weeks blood lactate analyses was done. On the day of 28, blood samples were collected from the jugular vein of mice respectively. The blood lactate contents of the mice were tested according to the procedures provided in the commercial diagnostic kit of blood lactate (Zhang et al., 2011).

#### Measurement of serum urea nitrogen contents of mice

Mice were treated with ethanolic leaf extracts of *A. squamosa* and *A. reticulata* for a period of 4 weeks serum urea nitrogen (SUN) analyses was done. On the day of 28, blood samples were collected from the jugular vein of mice. The SUN contents of the mice were tested according to the procedures provided in the commercial diagnostic kit of SUN (Zhang et al., 2011).

### Statistical analysis

Data were analyzed by Graphpad INSTAT® version 3.0 software and presented as mean ± S.E.M. values. The statistical tests used

were one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. The levels of statistical significance ranged from  $p < 0.05$  to  $p < 0.001$ .

## RESULTS

The extraction yields of ethanolic leaf extracts were found to be 9.6% w/w and 8.2% w/w. The extract was dark green in colour.

### Preliminary phytochemical screening

The preliminary phytochemical screening of ethanolic leaf extracts of *A. squamosa* and *A. reticulata* revealed the presence of carbohydrates, flavonoids, glycosides, phenols, phytosterols, terpenoids, saponins, steroids, tannins, volatile oils (Florence et al., 2014; Zaman and Pathak, 2013).

### Acute toxicity studies

The extracts were found to be devoid of mortality at 1600 mg/kg. Hence, 1600 mg/kg was considered as LD<sub>50</sub> cutoff value.

### In vitro antioxidant activity

*In vitro* Antioxidant activity of ethanolic leaf extract of *A. squamosa* and *A. reticulata* was tested with different concentrations ranging from 50 to 500 µg/ml with different *in vitro* antioxidant methods. It was observed that free radicals were scavenged by the ethanolic leaf extracts of *A. squamosa* and *A. reticulata* compounds in a concentration dependent manner up to the given concentrations (Tables 1 and 2; Figures 1 and 2). The values obtained by ethanolic leaf extract of *A. squamosa* were comparable with that of standard ascorbic acid. The IC<sub>50</sub> values obtained indicates the *in vitro* antioxidant capacity of the extracts and ascorbic acid.

### Invivo anti-fatigue activity

The ethanolic leaf extract of *A. squamosa* and *A. reticulata* does not show any significant difference in the increased body weights of experimental groups compared to the control group (Table 3, Figure 3).

All the animals treated with ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows a significant increase in swimming time to exhaustion of mice compared to the control group.

**Table 1.** *In vitro* antioxidant activity of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* using hydroxyl radical scavenging assay.

Tested material	Concentration ( $\mu\text{g/ml}$ )	% inhibition $\pm$ SEM	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
Ethanolic leaf extract of <i>A. squamosa</i>	50	60.6 $\pm$ 0.58*	22.28
	100	72.05 $\pm$ 0.02*	
	300	85.54 $\pm$ 0.04*	
	500	93.55 $\pm$ 0.09*	
Ethanolic leaf extract of <i>A. reticulata</i>	50	55.33 $\pm$ 0.07	33.73
	100	65.60 $\pm$ 0.06	
	300	79.33 $\pm$ 0.07*	
	500	88.12 $\pm$ 0.04*	
Ascorbic acid	50	69.60 $\pm$ 0.32	24.54
	100	82.55 $\pm$ 0.26	
	300	83.46 $\pm$ 0.12	
	500	87.65 $\pm$ 0.16	

All the values are expressed as Mean  $\pm$  SEM, n = 3, \*  $p < 0.001$  when compared with standard values.

**Table 2.** *In vitro* antioxidant activity of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* using phosphomolybdenum assay.

Tested material	Concentration ( $\mu\text{g/ml}$ )	Absorbance $\pm$ SEM (nm)
Ethanolic leaf extract of <i>A. squamosa</i>	50	0.18 $\pm$ 0.015*
	100	0.446 $\pm$ 0.026*
	300	0.753 $\pm$ 0.014*
	500	0.963 $\pm$ 0.017*
Ethanolic leaf extract of <i>A. reticulata</i>	50	0.026 $\pm$ 0.012
	100	0.046 $\pm$ 0.017
	300	0.12 $\pm$ 0.017
	500	0.176 $\pm$ 0.012
Ascorbic acid	50	0.181 $\pm$ 0.32
	100	0.381 $\pm$ 0.18
	300	0.621 $\pm$ 0.23
	500	0.973 $\pm$ 0.36

All the values are expressed as Mean  $\pm$  SEM, n=3, \*  $p < 0.001$  when compared with standard values.

The maximum forced swimming times was recorded (Table 4, Figure 4).

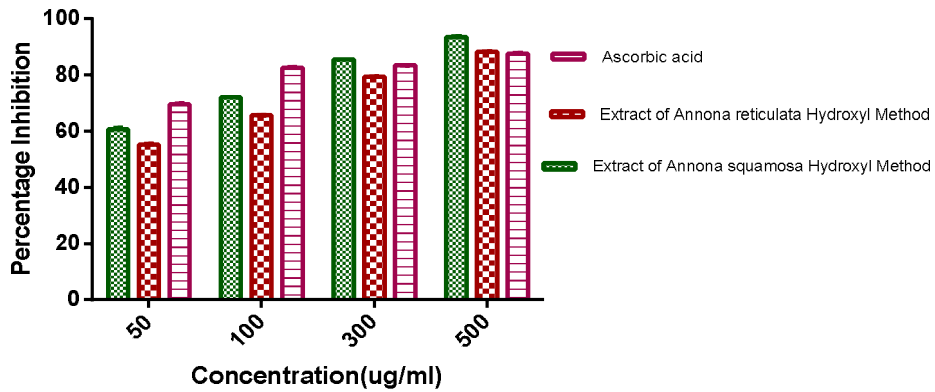
Therefore, ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows the anti-fatigue activity.

After swimming exercise, all the animals treated with ethanolic leaf extracts of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows significantly lower blood lactate contents compared to control group. The results indicated that the extracts of *A.*

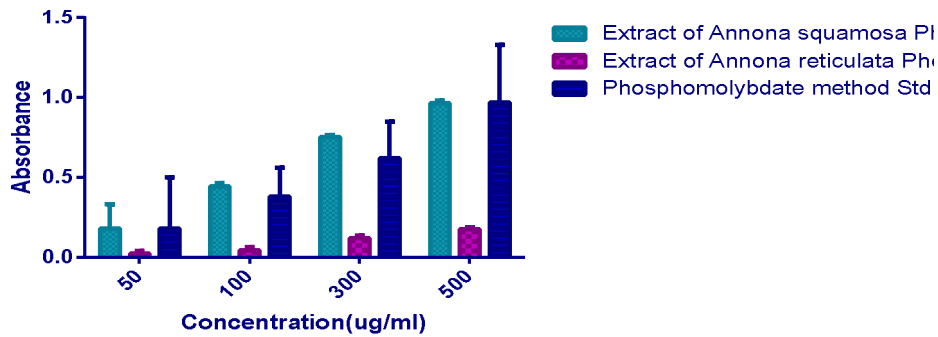
*squamosa* and *A. reticulata* could effectively delay the onset of fatigue by reducing the blood lactate contents in the blood (Figure 5, Table 5).

Therefore, ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows the anti-fatigue activity.

After swimming exercise, all the animals treated with ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows a significant decrease in serum urea nitrogen (SUN) contents



**Figure 1.** Effect of ethanolic leaf extracts of *Annona squamosa* and *Annona reticulata* and ascorbic acid against hydroxyl radicals.



**Figure 2.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* and ascorbic acid using phosphomolybdenum assay.

**Table 3.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on body weight of mice.

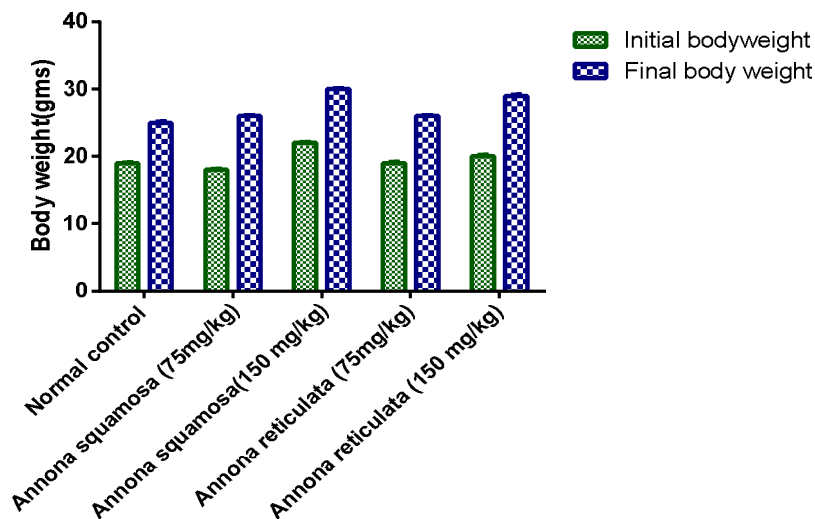
Groups	Body weights (g)	
	Initial	Final
Normal Control	19 ± 0.10	25 ± 0.18
Ethanolic leaf extract of <i>A. squamosa</i> (75 mg/kg)	18 ± 0.18	26 ± 0.14
Ethanolic leaf extract of <i>A. squamosa</i> (150 mg/kg)	22 ± 0.16	30 ± 0.14*
Ethanolic leaf extract of <i>A. reticulata</i> (75 mg/kg)	19±0.19	26 ± 0.16
Ethanolic leaf extract of <i>A. reticulata</i> (150 mg/kg)	20±0.21	29±0.18*

All the values are expressed as Mean ± SEM, n = 5, \*  $p < 0.001$  when compared with Control group.

compared to control group. The results indicated that the extracts of *A. squamosa* and *A. reticulata* could effectively delay the onset of fatigue by lowering the formation of BUN after exercise (Table 6, Figure 6). Therefore, ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) shows the anti-fatigue activity.

## DISCUSSION

The present study was evaluated to investigate the *invitro* antioxidant and *invivo* anti-fatigue activities of ethanolic leaf extracts of *A. squamosa* and *A. reticulata* in mice. Acute oral toxicity studies revealed that plant extracts of *A. squamosa* and *A. reticulata* is a non-toxic and non-

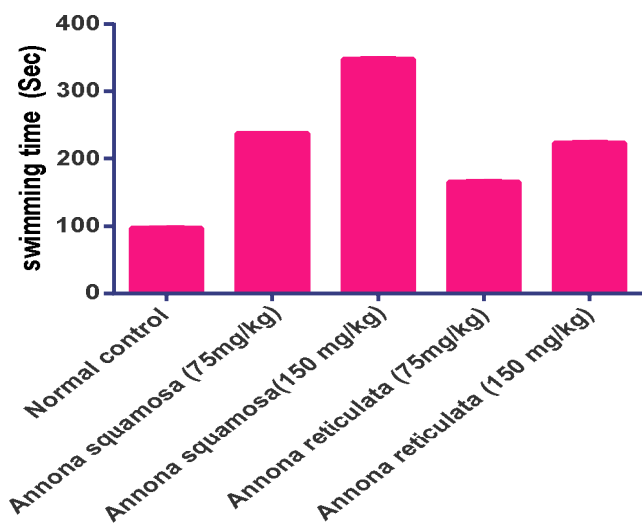


**Figure 3.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on body weight of mice.

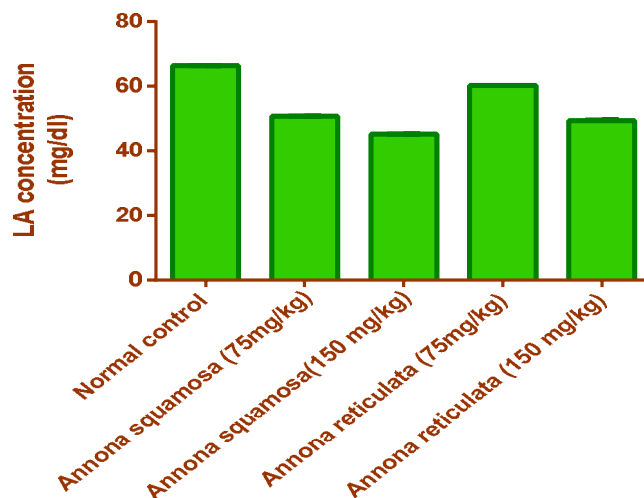
**Table 4.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on swimming time in mice.

Groups	Swimming time (s)
Normal Control	96.36 ± 0.07
Ethanolic leaf extract of <i>A. squamosa</i> (75 mg/kg)	237.20 ± 0.09
Ethanolic leaf extract of <i>A. squamosa</i> (150 mg/kg)	348.3 ± 0.35*
Ethanolic leaf extract of <i>A. reticulata</i> (75 mg/kg)	164.73 ± 1.10
Ethanolic leaf extract of <i>A. reticulata</i> (150 mg/kg)	223.50 ± 0.71*

All the values are expressed as Mean ± SEM, n = 5, \*  $p < 0.001$  when compared with Control group.



**Figure 4.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on swimming time in mice.



**Figure 5.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on the content of serum LA concentrations in mice.

**Table 5.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on the content of serum LA concentrations in mice.

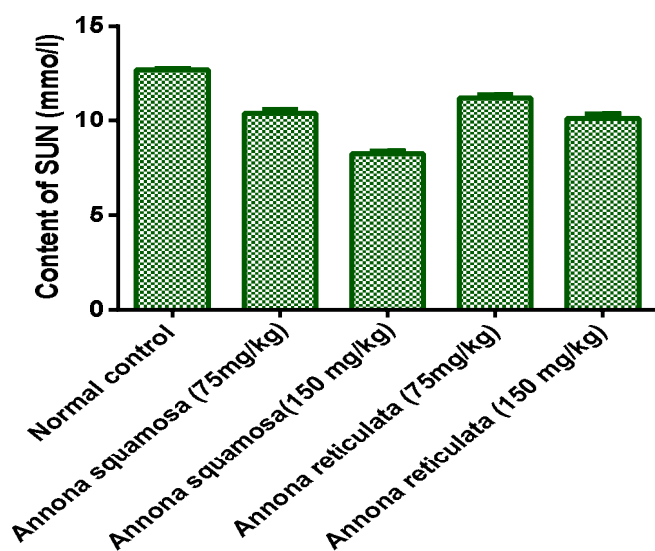
Groups	Serum LA concentration (mg/dl)
Normal Control	66.29 ± 0.09
Ethanolic leaf extract of <i>A. squamosa</i> (75 mg/kg)	50.62 ± 0.03
Ethanolic leaf extract of <i>A. squamosa</i> (150 mg/kg)	45.12 ± 0.04*
Ethanolic leaf extract of <i>A. reticulata</i> (75 mg/kg)	60.23 ± 0.03
Ethanolic leaf extract of <i>A. reticulata</i> (150 mg/kg)	49.39 ± 0.06*

All the values are expressed as Mean ± SEM, n = 5, \*  $p < 0.001$  when compared with Control group.

**Table 6.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on the content of SUN concentrations in mice.

Groups	Content of SUN (mmol/L)
Normal Control	12.71 ± 0.06
Ethanolic leaf extract of <i>A. squamosa</i> (75 mg/kg)	10.4 ± 0.22
Ethanolic leaf extract of <i>A. squamosa</i> (150 mg/kg)	8.26 ± 0.17*
Ethanolic leaf extract of <i>A. reticulata</i> (75 mg/kg)	11.21 ± 0.16
Ethanolic leaf extract of <i>A. reticulata</i> (150 mg/kg)	10.12 ± 0.27*

All the values are expressed as Mean ± SEM, n = 5, \*  $p < 0.001$  when compared with Control group.

**Figure 6.** Effect of ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* on the content of SUN concentrations in mice.

allergic. Since the extract did not produce any significant toxic effects in mice.

Annonaceae family plants are rich source of bioactive substances. Phytochemical tests helps in identifying new compounds which have therapeutic value. Preliminary phytochemical tests were performed by revealed the

presence of carbohydrates, flavonoids, glycosides, phenols, phytosterols, terpenoids, tannins, saponins, proteins, steroids (Florence et al., 2014) in the ethanolic leaf extract of *A. squamosa* and *A. reticulata*.

Oxidative stress is the reason for the pathology of many diseases and conditions including diabetes, CVD, inflammatory conditions, cancer and aging by developing free radicals. Antioxidants help in providing resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms which prevents diseases. From the results, both the ethanolic leaf extract of *A. squamosa* and *A. reticulata* exhibits the antioxidant activity. Leaves of *A. squamosa* and *A. reticulata* are rich in flavonoids like rutin and hyperoside, this possess various biological properties related to antioxidant mechanisms. Significant antioxidant activity was observed by ethanolic leaf extract of *A. squamosa* with  $IC_{50}$  value 22.45 by hydroxyl radical scavenging assay and 0.97 nm absorbance by Phosphomolybdenum method at 500  $\mu$ g/ml concentration (Shirwaikar et al., 2004).

Body weights of the mice treated with *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) was measured at initial day (1<sup>st</sup> day) and final day (28<sup>th</sup> day) before the administration of ethanolic leaf extract of *A. squamosa* and *A. reticulata*. These extracts did not show exhibited any significant difference in the body weights of experimental groups compared to control groups.

Forced swimming test is one of the valid animal model

for screening anti-fatigue potential of novel compounds. Forced swimming test was performed in mice by standardizing the workload to reduce the swimming time by adding weights to animal chest or tail (10% to their body weight). The extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) enhanced the swimming time to exhaustion of mice compared to control group, this indicates that leaf extracts of *A. squamosa* and *A. reticulata* possess anti-fatigue activity. Finally the ethanolic leaf extract of *A. squamosa* had better anti-fatigue activity (could elevate the exercise tolerance by delaying the onset of physical fatigue) compared to the ethanolic leaf extract of *A. reticulata* (Zhang et al., 2011).

Blood lactate is the glycolysis product of carbohydrate under anaerobic conditions and glycolysis is the main energy source for intense exercise over a short time. Therefore, concentration of lactate acts as an identifier for judging the intensity of exercise with the blood lactate accumulation. Anti-fatigue agents act by increasing rapid removal of lactate (or) by reducing the glycolytic process which delays accumulation of lactate. The results revealed that extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) could effectively delay the onset of fatigue by reducing the Blood lactate contents in the blood, this indicates that the leaf extracts of *A. squamosa* and *A. reticulata* possess anti-fatigue activity (Zhang et al., 2011; Lamou et al., 2016).

Serum Urea Nitrogen (SUN) is one of the important biochemical parameter related to fatigue. There is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance. Protein metabolism process take place in the liver, during this metabolism process urea is formed as an end product. Protein is breakdown into amino acids which contain nitrogen. This nitrogen is used to generate energy or to develop other substances necessary to cell by removing the ammonium ion. The results revealed that the extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) could effectively delay the onset of fatigue by lowering the formation of Blood Urea Nitrogen (BUN) after exercise (Zhang et al., 2011; Lamou et al., 2016).

The above studies states that the ethanolic leaf extract of *A. squamosa* (75 and 150 mg/kg) and *A. reticulata* (75 and 150 mg/kg) could extend the swimming time to exhaustion of mice by decreasing the levels of blood lactate and SUN contents.

Previous pharmacological research articles states that increase in swimming time to exhaustion of mice by decreasing the levels of blood lactate and SUN contents possess anti-fatigue activity. Hence, the present study indicates that the ethanolic leaf extract of *A. squamosa* and *A. reticulata* has potent Antioxidant activity and Anti-fatigue activity that could elevate the exercise tolerance.

The current study provides evidence that the *A.*

*squamosa* ethanolic leaf extract has potent antioxidant activity and Anti-fatigue activity compared to ethanolic leaf extract of *A. reticulata*.

## CONCLUSION

In conclusion, the data suggested that ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* could extend the swimming time to exhaustion of the mice, as well as decrease the blood lactate and serum urea nitrogen contents. These results indicated that ethanolic leaf extract of *Annona squamosa* and *Annona reticulata* had anti-fatigue activity and could elevate exercise tolerance. However, further studies are necessary to clarify the detailed mechanism(s) involved in the anti-fatigue properties of ethanolic leaf extract of *A. squamosa* and *A. reticulata*. The antifatigue potential may be expressed through mechanisms that involve the antioxidant activity of the extract. Further studies are needed to determine the effect of the extract on chronic physical activity.

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