PHYTOCHEMICAL SCREENING, NUTRITIVE VALUES AND SOME BIOLOGICAL ACTIVITIES OF SEED (KERNEL) OF *ZIZIPHUS MAURITIANA* LAM. (ZEE)

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Abstract

The main aim of the present work is to study phytochemical screening, nutritive values and some biological activities of seed (kernel) of *Z. mauritiana* Lam. (Zee). For preliminary phytochemical test, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, tannins, steroids and terpenoids were determined but cyanogenic glycosides and reducing sugars were absent in the Zee seed. The nutritive values such as crude proteins, ash, fibers, moisture, carbohydrates and fats were found to be 39.91 %, 4.27 %, 15.29 %, 7.23 %, 12.97 % and 27.56 % respectively. Moreover, methanol, ethyl acetate, ethanol extracts were revealed antimicrobial activity (inhibition zone diameters = 11~18 mm) against all tested microorganisms while pet-ether and watery extracts did not show activity. Ethanol extract was possessed potent antimicrobial activity than methanol and ethyl acetate extracts. Antiproliferative activity of ethanol and watery extracts of *Z. mauritiana* Lam. against Hela (Cervix) and A 549 (Lung) cancer cell lines were screened by MTT assay. The IC_{50} value of watery extract was found to be 170.36 μg/mL against cervix cancer. Both ethanol and watery extracts did not show anti-inflammatory activity and cytotoxicity effect.

Keywords: antimicrobial, antiproliferative, Hela, A549, MTT assay, anti-inflammatory, cytotoxicity

Introduction

Plants are valuable gift of nature. Especially, medicinal plants have been used as traditional remedies since ancient time. Medicinal plants have a great importance in the field of research because they are safe to use for the communities. There are so many herb plants which produce a variety of bioactive constituents of known therapeutic values (Ghasham *et al*., 2017)

A medicinal plant, *Ziziphus mauritiana* Lam belongs to the family Rhamnaceae and genus is *Ziziphus*. It is a tropical and subtropical fruit tree widely distributed in many Asian countries such Afghanistan, Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka, Vietnam, Thailand, Africa and Australia (Mahajan *et al*., 2009). It is wild plant as well as ber seeds are spread by birds, native animals and humans who eat the fruit and expel the seeds. Till now, around 40 species of *Ziziphus* are in record and out of which *Z. mauritiana* Lam. is very common, especially in dry places (Ghasham *et al*., 2017).

Z. mauritiana Lam. is a spiny, every green shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter, spreading crown, stipular spines and many dropping branches (Sukirti *et al*., 2012). The seeds of *Z. mauritiana*, a species close to *Z. Jujuba*, used to treat insomnia and reduce the body temperature and sweat. *Z. mauritiana* seeds have been also used as sedative and hypnotic drugs in many Asian countries. According to Literature, the seeds contain large amounts of fatty oil and proteins, sterols, and triterpenoid compounds (betulin and betulinic acid) and also contain a large amount of vitamin C (Sena *et al*., 1998)

In literature, many studies reported that *Z. mauritiana* have some medical benefits such as antioxidant, anti-microbial, anti-diarrheal, anti-diabetic, hepto protective and anti-cancer

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(Abdallah *et al*., 2016). The current study aimed to evaluate nutritional value, anti-microbial, in-vitro anti-proliferative and anti-imflammatory activities of seed extracts.

Materials and Methods

Collection and Preparation of Plant material

Seeds of *Z. mauritiana* Lam. were collected from Kyauk Pantaung Township, Mandalay Region in March, 2018. The sample was identified by Department of Botany, University of Yangon. The seed were dried in shade for up to one week and the dried seeds were crushed to fine powder using electronic mill. And then this powdered sample was kept in air tight container protected from moisture until used.

Preliminary Phytochemical Test

The dried powder samples were used to determine the major phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compound, reducing sugar, saponins, starch, tannins, steroids and terpenoids by using standard procedures (M-Tin Wa, 1972, Marini-Bettolo *et al*., 1981).

Analysis of Nutritional Values

Nutritional values such as proteins, ash, fibers, moisture, carbohydrates and fats of *Ziziphus mauritiana* Lam. were examined by using procedures (AOAC, 1990) at Small Scale Industries Department, Yangon, Myanmar.

Determination of moisture content

Sample (2 g) was placed in the moisture dish, which had previously been dried and cooled in air-tight desiccators, and accurately weighed. The dish with the sample was placed in an oven and dried for 30 min at 100 °C. Then, they were removed from the oven and cooled in the air-tight desiccators at room, temperature and weighed. The procedure was repeated until the loss in weight had not been changed. The moisture content can be calculated by the following formula.

$$\text{Moisture } (\%) = \frac{\text{loss in weight}(\text{g})}{\text{weight of sample (g)}} \times 100$$

Determination of ash content

Sample (2 g) was introduced into a predried and cooled porcelain crucible and accurately weighed. Then, it was heated gently over a burner until the sample was thoroughly charred. The crucible and content were then transferred to the muffle furnace at 600 °C for two hours until the residue was free from carbon. Then the crucible containing residue was cooled in a desiccators and weighed. Heating, cooling and weighing were repeated until constant weight was attained. The ash content of the sample was calculated using the following equation.

$$\text{Ash } (\%) = \frac{\text{weight of residue}(\text{g})}{\text{weight of sample (g)}} \times 100$$

Determination of fats content

About (10 g) of sample was weighed, placed in a cloth bag and the bag was then placed in a soxhlet extractor. Petroleum ether (b.pt. 60-80 °C) was poured into the extractor until some of it overflowed into the flask. The flask was heated on a water bath. The extraction was assumed to be

completed when a small amount of extract placed on a watch glass did not leave any residue on evaporation of solvent. A duration of about 8 h was required for complete extraction. The pet-ether was removed by simple distilled until the volume of the pet-ether was remained to about 10 mL. The last tract of the solvent was then removed by placing the content in an oven at about 100 °C) until the constant weight was obtained. The fats content of sampled was calculated by the following equation.

$$\text{Fat (\%)} = \frac{\text{weight of fat}\left(\text{g}\right)}{\text{weight of sample (g)}} \times 100$$

Determination of fibers content

About (2 g) of samples were accurately weighed and introduced into 500 mL round bottomed flask. Then, 1.25 % sulphuric acid (200 mL) was poured into the flask. The flask was connected with reflux condenser and digested for about 30 min. The flask was connected was rotated with hand every few minutes in order to mix the contents and remove particles from the sides. The contents in the flask were filtered through a linen filter supported in a Buchner funnel with water suction pump and washed to free from acid with boiling distilled water. The residue was then washed down into the flask with 1.25 % sodium hydroxide (200 mL) and boiled for about 30 min, rotating the flask in 5 min intervals. After boiling, the flask was removed and filtered through the same linen filter used in acid hydrolysis. The residue was washed thoroughly with hot distilled water until free from alkali. Then, the residue was heated in an oven at 100 °C until the constant weight was obtained. The crucible and content were then ignited in a muffle furnace at a dull-red heat (approx., 600 °C) until all organic matter had been destroyed (approx., 20 minutes). The contents of the crucible were cooled and weighed. Heating, cooling and weighing were repeated until a constant of sample was calculated by the following equation.

$$\text{Fiber}\left(\%\right) = \frac{\text{weight of fiber}\left(\text{g}\right)}{\text{weight of sample (g)}} \times 100$$

Determination of protein content

Accurately weighed sample (0.5 g) was introduced in the dry Kjeldahl's digestion flask. Potassium sulphate (0.2 g) and copper sulphate (0.05 g) were added to the flask. Concentrated sulphuric acid (10 mL) was then poured into the flask in such a way as to wash down any solid adhering to the neck and the contents were shaken until well mixed. The flask was placed in the neck of the flask. The contents were digested and heated over a small flame so that the liquid boiled gently. Digesting was contained until the mixture become clear and almost pale green color. Then, the flask completely to the steam distillation for about 30 min. 40 % NaOH (100 mL) was also added in it to make the mixture strongly alkaline. Before 40 % NaOH was poured into the apparatus, the tip of the condenser dipped beneath the surface of 0.005 M sulphuric acid (50 mL) of a conical flask (receiver-flask). The steam liberated from boiling water in flask was passed through the mixture in apparatus. When ammonia evolved was carried by steam and condensed was assumed to be completed 15 min after boiling of the solution. Then, the receiver flask was removed and the tip the condenser was washed with distilled water by means of washing bottle into the receiver.

The excess acid remained unreacted with ammonia was titrated with standard sodium hydroxide solution. The percentage of protein content can be calculated by using the following calculation.

$$\text{Protein (\%)} = \frac{0.014 \times 100 \times (v_1 - v_2) M_B \times 6.25}{W}$$

Where,

v_1	=	Volume (mL) of NaOH solution used in blank
v_2	=	Volume (mL) of NaOH solution used in test
M_B	=	Molarity of NaOH solution
W	=	Weight (g) of the sample
0.014	=	Milliequivalent weight of the nitrogen

Determination of carbohydrate content

The carbohydrate present in foods include, starch (glycogen in animal tissue), dextrin, mono and disaccharides. The total carbohydrate content of any food can be obtained as the difference between 100 and the sum of the percentage of moisture, protein, fat, ash and fiber. Although individual carbohydrates can, if necessary, be estimated separately by chemical methods, the described above is sufficiently accurately for practical nutrition work.

Determination of energy values

The energy value of the samples was calculated by the following equation.

Energy value (kcal/100g) = (4 × protein) + (4 × carbohydrate) + (9 × fat)

Antimicrobial Activity

The antimicrobial activity of different crude extracts such as watery, ethanol, ethyl acetate, petroleum ether and methanol extracts were determined against six strains of microorganisms, *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Escherichia coli*, by employing agar well diffusion method at Pharmaceutical Research Department (PRD), Ministry of Industry , Yangon, Myanmar. (Abalakal *et al*., 2010)

(a) Preparation of Nutrient Agar Medium

To a mixture of 1 g of meat extract, 1 g of peptone, 0.5 g of NaCl and 1.5 g of agar powdered were placed in a sterilized 250 mL conical flask, 100 mL of sterile distilled water were added to obtain nutrient agar medium. The resulting mixture was heat to dissolve the contents. Then the pH of the resulting solution was adjusted to 7.2 with 0.1 M NaOH solution. It was sterilized in an autoclave at 121 °C for 15 min.

(b) Screening by Agar Well Diffusion Method

About 20-25 mL of agar medium contained test organisms poured into the sterile petri-dishes under alseptic condition near the flame of the spirit burner and left the agar solid, the cork bower about 10 mm in diameter was sterilized and made a well in the agar plate previously described. Then the extract samples were introduced into the well (about 0.2 mL). They were then incubated at 36 °C for 24 h. The formation of inhibition zone around the well was observed. This observation indicates the presence of antimicrobial active compounds in the extract.

Screening of Antiproliferative activity by using MTT assays

In *in vitro* antiproliferative activity of ethanol and watery extracts of the fruits of *Z. mauritiana* Lam .was determined against two human cancer cell lines such as A 549 (lung cancer)

and Hela (human cervix cancer). These tests were done by the procedure described by Win *et al*., (2015) at Department of Natural Products Chemistry, Institute of Natural Medicine, and University of Toyama, Japan.

After the cell growth, the 70-100 % cell in the medium was aspirated with aspirator. The cell was washed with PBS (5 mL) for 2 times. The cells are trypsinased with trypsin (4 mL) and incubated for 2–3 minutes. And then the medium (1 mL) was added to stop trypsinization. The cell suspension was transferred to 15 mL centrifuge tube. The tube (cell suspension) was centrifuged in the refrigerated centrifuge machine (3000 rpm) with the same centrifuge tube for 3 minutes. After the centrifugation, the supernatant was carefully removed without disturbing the cell pellet and the cell was found at the bottom of the centrifuge tube. The cell in the centrifuge tube was added with fresh medium (3 mL) gently to the side of the tube and slowly pipetted up and down 2 to 3 time to re-suspend the cell pellet. The number of cell was counted with Haemacytometer.

The cell solution (10 µL) was mixed in the Tryphan blue (40 µL). The chamber and the covered slip were cleaned with alcohol (70 % EtOH). The chamber was dried and the overslip was fixed in position. The cell was harvested and the 10 µL of the cell was added to the Haemacytometer (do not overfill). And then the chamber was placed in the inverted microscope under a 10X objective and phase contrast was used to distinguish the cell. The cell was counted in the large, central gridded square (1 mm^2). The gridded square was circled and multiplied by 10^4 to estimate the number of cell per millimeter. The number of cell was counted by the following equation,

No. of cell in stock = counted cell/4 × 10^4 × dilution factor × volume of stock cell solution

After the cell counting, the cell was added with 120 mL (120 × 10^3 µL) of medium for 12 plates. 10 mL (100 µL) medium of cell was filled in 96 well plates. The cell in 96 well plates was incubated in an incubator for 24 hours.

After the incubation, the medium was removed by absorption machine (very carefully) and washed with 100 µL PBS solution. And then 100 µL of the different concentrations of sample and control solution was added in the 96 well plates. The sample solutions in 96 well plates with cells were incubated in an incubator for 72 hours. The sample solution with cell and medium was added with 100 µL MTT reagent. And then the 96 well plates were incubated in an incubator for 3 hours. After the incubation, the absorbance of each solution was measured at 570 nm by using UV-visible spectrophotometer. The percent cell viability activity was calculated by the following equation.

$$\% \text{ Cell viability } = \frac{(\text{Abs }(\text{test sample}) - \text{Abs }(\text{blank}))}{(\text{Abs }(\text{control}) - \text{Abs }(\text{blank}))} \times 100$$

Where,

$\text{Abs}_{(\text{test sample})}$ = absorbance of test sample solution
$\text{Abs}_{(\text{control})}$ = absorbance of DMSO solution
$\text{Abs}_{(\text{blank})}$ = absorbance of MTT reagent

IC_{50} (50 % inhibitory concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x} - x_1)^2 + (\bar{x} - x_2)^2 + \ldots (\bar{x} - x_n)^2}{(n - 1)}}$$

Where,

X = average % inhibition

$x_1, x_2, \ldots, x_n$ = % cell inhibition of test sample solution

n = number of times

Anti-inflammatory and cell viability activity

Anti-inflammatory activity of the samples was evaluated by NO inhibition assay according to the method of Jin *et al*., (2012) with some modifications. The RAW264.7 cells was cultured in α-MEM supplemented with 10 % heat incubated fetal bovine serum, and 1 % penicillin (10,000 U/mL)-streptomycin (10 mg/L). When the cell proliferation reaches about 70 % confluency, the cells were harvested using cell scraper and diluted to a suspended in fresh medium. The 100 µL of cells (4 × 104/well) were seeded in the 96-well plates and incubated for 24 h at 37 °C in a humidified atmospheric containing 5 % CO_2. The cells were then treated with 50 µL each of LPS (100 mg/mL) and different doses of samples for 24 h. NO production was monitored by measuring the accumulation of nitrite in the culture supernatant using Griess reagent (Schmidt et al., 1996). In brief, 100 µL each of the supernatant from 96-wells was mixed with equal volume of Griess reagent (0.5 % sulfanilamide and 0.05 % naphthylenediamide dihydrochloride in 2.5 % H_3PO_4) in the new 96 well plates and allowed to stand for 10 min at room temperature. The absorbance at 540 nm was measured using microplate reader. L-NMMA monoacetate was used as a positive control. On the other hand, the effect of the samples on the cell proliferation was evaluated by MTT solution (5 mg/mL) in the medium was added. After 3 h incubation, the medium was discarded and 100 µL each of DMSO was added to dissolve the formazan crystals and the absorbance at 570 nm was recorded by microplate reader. The percentage of NO inhibition and that of cell viability was calculated as follows:

$$\text{NO inhibition } (\%) = \frac{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{sample})}}{\text{Abs}_{(\text{control})}} \times 100$$

Where, $\text{Abs}_{(\text{control})}$ and $\text{Abs}_{(\text{blank})}$ are the absorbance of the control group treated by LPS alone and the absorbance of the samples

$$\text{Cell viability } (\%) = \frac{\text{Abs}_{(\text{test sample})} - \text{Abs}_{(\text{blank})}}{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}}$$

Results and Discussion

Phytochemical constituents of *Z. mauritiana* Lam. (Zee)

Preliminary phytochemical investigation was carried out to know the secondary metabolites present in the seed (kernel) of *Z. mauritiana* Lam. The results are summarized in Table 1.

According to the results, it was observed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, tannins and steroids were present while cyanogenic glycosides and reducing sugars were not detected in seed sample. Therefore, It can be seen that the seed of Zee sample might contain potent secondary metabolites.

Table 1 Results of Phytochemical Investigation on *Z. mauritiana* Lam. (Zee)

No	Tests	Extracts	Test reagents	Observations	Results
1.	Alkaloids	1 % HCl	Wagner's reagent	Reddish brown ppt	+
			Mayer's reagent	White ppt	+
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrates	H_2O	10 % α- naphthol, Conc : H_2SO_4	Red ring	+
4.	Cyanogenic Glycosides	H_2O	Sodium picrate paper, Conc: H_2SO_4	No change in color	-
5.	Flavonoids	EtOH	Conc: HCl, Mg ribbon	Pink colour	+
6.	Glycosides	H_2O	10 % lead acetate	White ppt	+
7.	Phenolic Compounds	H_2O	10 % $FeCl_3$	Deep blue	+
8.	Reducing sugars	H_2O	Benedict's solution	No brick-red color	-
9.	Saponins	H_2O	Distilled water	Frothing	+
10.	Starch	H_2O	I_2 solution	Blue	+
11.	Tannins	EtOH	1 % $FeCl_3$	Greenish yellow	+
12.	Steroids	PE	Acetic anhydride, Conc: H_2SO_4	Greenish yellow	+
13.	Terpenoids	$CHCl_3$	Acetic anhydride, Conc: H_2SO_4	Pink color	+

(+) = Present, (-) = Absence, ppt = precipitate

Some Nutritional Values of *Z. mauritiana* Lam. (Zee)

The nutritional values of Zee were investigated by AOAC methods. Moisture content was determined by oven drying method and found to be 7.23 %.Larger the moisture content, the shorter the shelf-live. Proteins content was measured by micro-Kjeldahl method and it was observed 39.91%. The ash content was 4.27 % and measured by ashing in the muffle furnance. Fibers was determined by acid-alkali treatment and found to be 15.29%. Fats content was measured by Soxhlet extraction method using PE (b.pt. 60-80 °C) that was determined 27.56%. The total carbohydrate sand energy value can be calculated and found to be 12.97 % and 459.56 (kcal/ 100 g) respectively.

The result are summarized in Table 2 and from these data, it can be clearly seen that the protein contents is very high. Protein is important building block of bones, muscles, cartilage skin and blood. Therefore, the seed could be used as protein supplement for low protein legumes such as cereals. Next is fats content that fats from vegetable sources can help lower risk of heart attack, stroke and other major health problem. So, it was determined high nutritive values in the Zee seed sample.

Table 2 Results of Nutritional values on *Z. mauritiana* Lam.

No.	Parameters	Contents (%)
1	Proteins	39.91
2	Ash	4.27
3	Fibers	15.29
4	Moisture	7.23
5	Carbohydrates	12.97
6	Fats	27.56
7	Energy value	459.56 (kcal/100 g)

Screening of Antimicrobial Activity

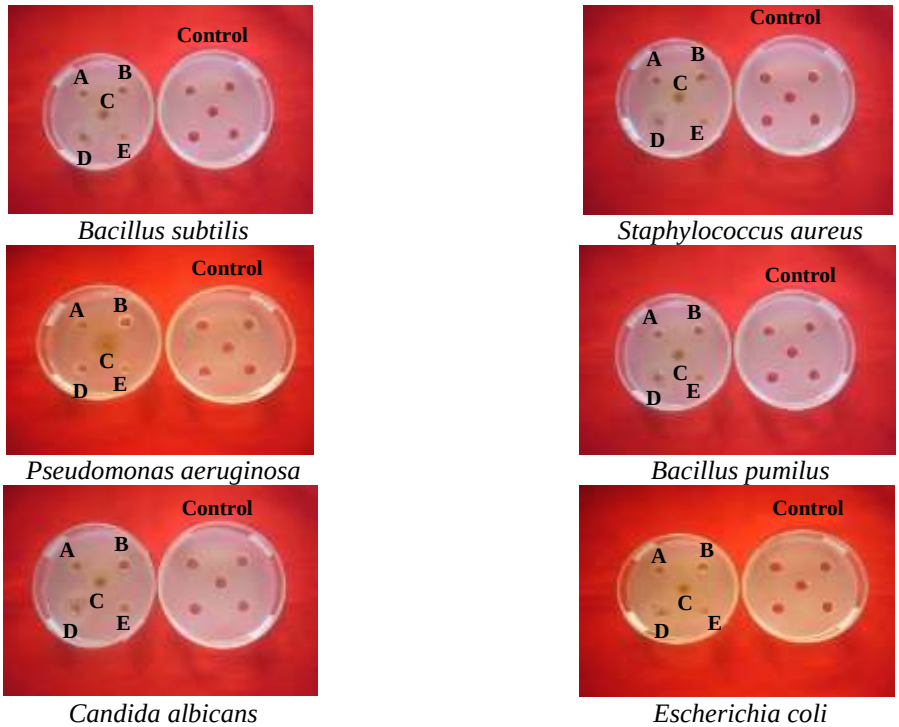
The antimicrobial activity of ethyl acetate, ethanol, methanol, pet-ether and watery extracts from *Z. mauritiana* Lam. were screened by agar well diffusion method. The resultant inhibition zone diameters are described in Table 3. A larger inhibition zone diameter usually means that the antimicrobial is more potent.

According to the result, ethanol, ethyl acetate and methanol extracts were revealed antimicrobial activity against all tested microorganisms (inhibition zone diameters = 11~18 mm) but pet-ether and watery extracts did not show the activity.

Table 3 Inhibition zone diameter of different crude extracts of *Z. mauritiana* Lam. against different strains of microorganism

Sample	Extracts	*B. subtilis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*B. pumilus* (mm)	*Candida albicans* (mm)	*E-coli* (mm)
Ziziphus mauritiaa Lam. (Zee)	Pet-ether	-	-	-	-	-	-
	MeOH	15 (++)	15 (++)	14 (++)	15 (++)	15 (++)	15 (++)
	EtOAc	11 (+)	12 (+)	12 (+)	12 (+)	11 (+)	12 (+)
	EtOH	15 (++)	16 (++)	14 (++)	16 (++)	18 (++)	17 (++)
	H_2O	-	-	-	-	-	-

Agar well–10 mm
10 mm ~14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)



Bacillus subtilis | *Staphylococcus aureus*
Pseudomonas aeruginosa | *Bacillus pumilus*
Candida albicans | *Escherichia coli*

A = PE Extract
B = MeOH Extract
C = H_2O Extract
D = EtOH Extract
E = EtOAc Extract

Figure 1 Inhibition zones of various crude extracts against microorganisms

Investigation of Antiproliferative Activity

In vitro antiproliferative activity of ethanol and watery extracts of *Z. mauritiana* Lam. (seed) was determined against two cancer cell lines, Hela (Human cervix) and A 549 (Lung).In this activity 5-Fluorouracil is used as a positive control. Antiproliferative agent is the ability of a compound to stop the growth of cells that means not allowing the cancer cells to multiply rapidly. The result was shown in Table 4. According to result, the watery extract of Zee was found to possess activity against Hela cell line (IC_{50} value = 170.36 μg/mL). However, ethanol extract did not show activity.

Table 4 Anti-proliferative Activity of Crude Extracts of *Z. mauritiana* Lam.

Sample		Hela			A549			
		20 μg/mL	**200 μg/mL**	**IC_{50}**	**20 μg/mL**	**200 μg/mL**	**IC_{50}**
EtOH	(seed)	57.60 ± 1.48	90.66 ± 4.88	>200	69.70 ± 8.41	70.00 ± 3.50	>200
H_2O	(seed)	86.24 ± 1.98	42.86 ± 1.77	170.36	77.88 ± 4.17	55.17 ± 0.35	>200
	2 μg/mL	10 μg/mL	20 μg/mL	IC_{50}	2 μg/mL	10 μg/mL	20 μg/mL	IC_{50}
5-FU	91.44 ± 4.95	85.22 ± 0.28	24.93 ± 6.22	15.84	136.23 ± 12.94	70.45 ± 5.59	47.89 ± 8.21	19.06

* 5-FU (5-Fluorouracil) = Positive control
A549 = Lung cancer cell line
Hela = Cervix cancer cell line

Screening of anti-inflammatory and Cell viability activity

Anti-inflammatory and cell viability activity of ethanol and watery extracts was investigated by MTT assay and L-NMMA is used as a positive control. Anti-inflammatory agents block some substances in the body that can cause cancer. The % NO inhibition result is inversely proportional to % cell viability. It can be clearly seen that in Table 4.The greater IC_{50} values of % cell viability than the % NO inhibition that the sample was possessed anti-inflammatory effect. Less IC_{50} value of % cell viability means that it was cytotoxicity effect. According to result, Zee seed sample did not possess anti-inflammatory activity and cytotoxicity effect.

Table 5 Anti- inflammatory Activity of *Ziziphus mauritiana* Lam. (Zee)

Sample		%NO inhibition			% Cell Viability		
		10 μg/mL	**100 μg/mL**	**IC_{50}**	**10 μg/mL**	**100 μg/mL**	**IC_{50}**
EtOH	(seed)	6.01±0.79	45.36±1.04	>100	84.09±1.29	85.63±3.81	>100
H_2O	(seed)	3.83±0.46	27.87±0.3	>100	72.35±2.33	50.54±2.55	>100
*L-NMMA		18.49±0.10	50.35 ±0.10	98.25	100.32±12.41	92.01± 1.02	>100

* L-NMMA (L-N monomethyl-L-argine) = Positive control

Can be concluded as extract that has anti-inflammatory effect

Conclusion

From the overall assessment, the following inferences could be deduced. There were rich phytochemical constituents in Zee seed sample. It was found that high nutritional values among them proteins content was observed in highest amount (39.91%).So, the plant source of protein could be explored for better supplement in food. For antimicrobial activity, ethanol extract showed potent antimicrobial activity with inhibition zone diameter ranges between 14-18 mm against all tested organism.

Nowadays, there are so many popular anti-cancer agent in medicinal plants. So, most of the people are interested in investigation of antiproliferative activity. Watery extract of Zee seed showed mild antiproliferative activity against Hela cell line (IC_{50} = 170.36 μg/mL. It was neither anti-inflammatory activity nor cytotoxicity effect in Zee sample. Therefore, the present study will contribute that seed of *Z. mauritiana* Lam. can be used as traditional medicine for some diseases.

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