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PROTECTIVE EFFECT OF *MALUS PUMILA* ON *E. COLI* INDUCED PERITONITIS IN *ALBINO WISTAR* RATS AND ITS ANTIOXIDANT ACTIVITIES

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ABSTRACT

Infectious or non-infectious peritonitis leads to systemic inflammation due to violation of the peritoneum which is often fatal. Evidences suggest that apple peel has a wide range of polyphenols having antioxidant activity and its consumption has been linked with improved health benefits. The aim of the present investigation was evaluate the in vitro antimicrobial, *in vitro, in vivo* antioxidant and protective potential of the methanolic and aqueous extract of *M. pumila* in *E. coli* induced model of peritonitis in albino wistar rats. Rats were pre-treated with 200 mg/kg and 400 mg/kg/bwt dose for 3 days and fourth day with *E. coli* (1×108 CFU/ml) strain and consecutively 3 days treatment. Mortality was monitored for 14 days. After the death of rats or completion of the experiment rats were sacrifice and kidney were used for our protocol. Colonies were count and statically analysis was done. Results showed dose dependent anti-microbial activity. Thus the aqueous extract of *M. pumila* exhibited significant protection against *E. coli* induced peritonitis in normal rats. It significantly reduced the viable cells of *E. coli* when inoculated in rats. Activity is attributed to flavonoids and polyphenols. The present study thus suggests that aqueous extract of *M. pumila* significantly reverses peritoneal infection by *E. coli* in rats. It can be suggest that this medicinal formulation will be used as herbal medicine with no side effects. The high content of phenolic compounds, antioxidant activity, and antimicrobial activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants. Thus, consumption of apple fruits along with peel might be recommended to gaining better nutritive benefits.

KEYWORDS: *M. pumila*, Peritonitis, Antioxidant, Antimicrobial, *E. coli*

INTRODUCTION

A fruit and vegetable diet can be a remedy for several diseases. Various investigations have shown that fruits are a natural source of dietary fibre, trace elements and antioxidant compounds [1, 2]. Apple (*Malus pumila* Mill) is the fruit of malus plant. It belongs to the family Rosaceae and has been widely cultivated in various parts of the world for centuries. At present, the annual global apple production is about 70 million tons, thus making apple the third largest consumed fruit next to bananas and oranges [3]. Apples contain a relatively high concentration of polyphenolics and their consumption has been linked with improved health due to their effectiveness in several chronic diseases [4, 5]. Many review articles are available on apple products to claim beneficial effects on

cardiovascular disease, cancer, pulmonary function and age-related cognitive decline [6, 7]. Apples are composed of different tissue types (peel, cortex, core and seed) and each tissue type contains a different composition of phytochemicals [8]. Unpeeled fruits possess higher contents of bioactive compounds as compared to peel ones [9]. Moreover, the polyphenolic content of apple peel extract is six times higher than that of the fresh extract. The apple fruit pulp contains mainly catechin, phloretin glycoside, procyanidins and caffeic acid whereas the peel possesses all these compounds and has flavonoids such as anthocyanins, quercetin glycosides and cyanidin glycoside in addition, which are absent in the pulp [10]. There are reports of beneficial effects of apple peel phytochemicals against a variety of experimentally induced pathological conditions [11]. It is reported to have an inhibitory effect on low density lipoprotein (LDL) oxidation [12], anti-proliferative property [13], depletion of reactive oxygen species (ROS) generation during stress conditions [14], antihypertensive activity [15], a-glucosidase inhibitory property [16] and protective property against damaged mitochondria and DNA [17]. Apple peel has also been reported to regulate metastasis [18]. The oral administration of apple extracts has been shown to inhibit AP-1 transactivation which involves signal transduction of MAP kinase, thus inhibiting cancer formation [19]. In addition, the intake of apple polyphenols is inversely proportional to coronary atherosclerosis by inhibition of lipid peroxidation [20]. Peritonitis is a serious disease, due to the inflammatory response in the serous membrane lining the abdominal cavity and viscera. The immediate answers to peritonitis are hyperthermia, bowel distension, hyperaemia, accumulation of gases and liquids, hypovolemia and pain. At the same time, there are cardiac, respiratory, renal and metabolic responses. It is also high contribution of fibroblasts that produce fibrin, responsible for the formation of intra-abdominal adhesions [21-24]. Although often the treatment of peritonitis include mechanical removal of contaminants through peritoneal washings with saline, antibiotics and abdominal integrity restoration associated with modern intensive and surgical care units, currently peritonitis still accounts for approximately 50% of deaths consequent to sepsis [25-28]. Given popular use of *M. Pumila* in treating diseases and previous research demonstrating antimicrobial and antioxidant activity. The objective of this study was to estimate the total phenolic content, flavonoid content, antioxidant activity, antimicrobial and protective potential of the methanolic and aqueous extract of apple peel in *E. coli* induced model of peritonitis in albino wistar rats.

MATERIALS AND METHODS

Plant material

Apples (*M. pumila*) of the red delicious variety were purchased from local fruit shop New Market Bhopal (M.P). The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *M. pumila is* 119/Bot/Saf/67.

Chemicals and reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Bacterial strain

The test organisms *Escherichia coli* (*MTCC* 2075) were obtained from the stocks of the Pinnacle Biomedical Research Institute, Bharat scout guide bhawan, Shyamla hills, Bhopal, (M.P.).

Extraction

Apple peel was washed and rinsed properly. They were dried in shade and powdered mechanically. About 500 gm of the powder was extracted with different organic solvents viz; methanol, aqueous and allow to standing for 2-3 days. At the end of the third day extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [29].

Qualitative analysis of phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids, polyphenols and terpenoids in various extracts of apple. The above phytoconstituents were tested as per the standard method [30].

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in extracts. For this TPC and TFC are determined. Methanol and aqueous extracts of *M. pumila* are subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenolic content estimation (TPC)

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (20-100 μg/ml) of gallic acid was prepared in methanol. Concentration of 100 μg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and it was then incubated at room temperature for 30 min with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g) [31].

Total flavonoid content estimation (TFC)

Different concentration of rutin (20 to 100 μg/ml) was prepared in methanol. Test sample of near about same polarity (100 μg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution were added and allowed to stand for 5min and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 min. Absorbance was determined at 510 nm against water as blank. Total flavonoid content was calculated by the standard regression curve of rutin/ quercetin [32].

***In vitro* Antioxidant Activity**

DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.*, 2006 [33] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different

concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *M. pumila* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50ºC for 20 min separately, and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve [34].

Reducing Power (%) = (As / Ac) × 100

Here, Ac is the absorbance of control and (As) as is the absorbance of samples (extracts) or standards.

Anti bacterial sensitivity assay

Well diffusion method

The agar well diffusion method technique [35] was used to determine the antibacterial activity of the plant extracts. Inoculation was done on sterile nutrient agar media plate using 18 hours old culture. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labeled accordingly; 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48 hours in upright condition. The Experiment was repeated triplets and the mean values were calculated.

Minimum inhibitory concentration (MIC) Assay

The MIC is the lowest concentration of a substance that inhibits the growth of fungi within a defined period of time. The MIC is expressed in mg/ml. Broth dilution is a susceptibility testing technique in which serial dilutions (usually two-fold) of an antifungal agent are made in a liquid medium that is inoculated with a standardised number of organisms and incubated for a prescribed period [36,37].

***In vivo* study**

Animals

All ethical and handling guidelines were followed as set by Indian Legislation and approved by Institutional Animal Ethics Committee. All animals were procured and housed in animal house maintained under standard hygienic conditions. All animals were given standard diet (Golden Feed,

New Delhi) and water regularly. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/ERe/S/15/CPCSEA). Protocol approval reference no. PBRI/IAEC/PN-45.

Acute oral toxicity

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight [38].

Treatment

Animals were housed in a group of five in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden feed, New Delhi) and water, *ad libitum.* The environment was also regulated at 25 ± 1 0C with 12/12 h (light/dark) cycle. Animals were further divided in four groups with six animals in each group.

Group I: **Vehicle treated with *Escherichia coli* treated group:** *Escherichia coli* (1×10^8 CFU/ml) were dissolved in normal saline and were administrated by oral route at a dose of 10ml/kg body weight.

Group II: **Standard drug treated group:** Ofloxicin was dissolved in normal saline and was administrated by oral route at a dose of 5m g/kg body weight

Group III: **200 mg/kg extract treated group:** Extract was dissolved in normal saline and was administrated by oral route at a dose of 200m g/kg body weight.

Group IV: **400 mg/kg extract treated group:** Extract were dissolved in normal saline and was administrated by oral route at a dose of 400mg/kg body weight.

Preparation for bacterial inoculums

In brief, *E. coli* strain (MTCC 2075) were grown on nutrient broth medium (3 g of beef extract, 5 g of peptone and 5 g of NaCl, pH 7, sterilized by autoclaving at 120°C for 30 min.) from a single colony and incubated at 37°C for 16-18 h at 37^oC to obtain stationary growth phase cultures. The bacteria were then centrifuged (200 rpm) for 10 min at 4°C and the pellets were resuspended in PBS to an OD of 0.1 at 660 nm, with a spectrophotometer, corresponding to 10^8 CFU/ml [39].

Systemic infection by E. coli

To produce infection, the rats were induced by the intra-peritoneal with suitable inoculums in a volume of 0.2 to 0.25 ml. After infection, the rats were observed twice daily and animals exhibiting profound inanition or an inability to reach food and water were sacrificed. The experimental design involved administration of each of the three test agents by daily oral dosing for a period of 14 days. dosing regimens were started on days -7,-6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6, 7 and relative to the day of challenge (day 0) with 1×10^8 CFU of *E. coli*/ ml. Before and after the challenged day animals were treated with 200 mg/kg body weight and 400 mg/kg body weight *M. Pumila peel* extract and 5 mg/kg body weight Ofloxicin respectively. Survival was monitored for all experimental groups till

14 day. These conditions were in accordance with those of previously described method [40], with slight modification. The pathological status of the rat was determined by visual examination of internal organs after their death or sacrifice at the completion of the experiment. All surviving rats were killed by cervical dislocation on 15 day determination of the numbers of CFU of *E. coli* per gram from the kidney [41, 42]. This determination was made by aseptically removing and weighing both kidneys, homogenizing kidneys in w/v ml of saline with a high speed Homogenizer (Remi RQ-124A) and Kidney burden was determined by culturing of homogenates in physiological saline followed by plating 0.1 ml aliquots onto Nutrient agar plates. The plates were incubated at 37°C, and the number of colonies was enumerated after 48 h of growth [43]. All animal care procedures were supervised and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal.

Bacterial clearance

E. coli was determined in the different groups by sampling retro blood at various intervals after 16 hrs and up to 14 days (at which time maximum mortality will be recorded). The sample was serially diluted and plated on Nutrient agar media. Incubate it at 24 hours at 37°C temperature. After incubation colony count was determine by colony counter method.

***In vivo* Antioxidant activity**

Lipid peroxidation (LPO) assay

LPO is an autocatalytic process, which is a common consequence cell death. This process causes peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the LPO process. MDA is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of LPO. This method described by *Okhawa et al.* (1979) [44], is as follows: The 10 % w/v tissues are homogenized in 0.15 M tris HCl buffer (pH 7.4) with a Teflon glass homogenizer. LPO in this homogenate is determined by measuring the amounts of MDA produced primarily. 0.2 ml tissue homogenate with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA are added. The volume of the mixture is made up to 4 ml with distilled water and then heated at 95°C on a water bath for 60 minutes using glass balls as condenser. After incubation the tubes are cooled to room temperature and final volume was made to 5 ml in each tube. 5 ml of butanol:pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 minutes. After centrifugation at 3000 rpm for 10 minutes, the upper organic layer is taken and its OD is taken at 532 nm against an appropriate blank without the sample (This absorbance will be of total MDA formed). The levels of lipid peroxides are expressed as n moles of TBARS/mg protein using an extinction coefficient of 1.56×105 ml/cm.

Superoxide dismutase (SOD) activity was determined using NBT method

The assay involves the production of superoxide from O_2 (using reduced β-nicotinamide adenine dinucleotide (NADH) as a reductant, and phenazine methosulphate (PMS) as a catalyst in the presence of an indicator, nitro blue tetrazolium (NBT), which turns blue when reduced by superoxide. The color change during the reaction was monitored spectrophotometrically in the visible range at 560 nm. When SOD enzyme is added to the reaction, (superoxide scavengers i.e., antioxidants) compete with NBT to react with superoxide. The percent inhibition of NBT reduction was used to quantify superoxide-scavenging. Prepared 10 % w/v tissue homogenate in 0.15 M Tris HCl or 0.1 M phosphate buffer. Centrifuged at 15000 rpm for 15 min at 4 °C. Supernatant (0.1 ml) was taken consider it as sample and 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M) + 0.1 ml phenazine methosulphate (186 μM) + 0.3 ml of 300 μM Nitroblutetrazolium + 0.2 ml NADH

(750 µM) were added. Incubated at 30°C for 90 sec. 0.1 ml glacial acetic acid was added. Stirred with 4.0 ml n-butanol. Allowed to stand for 10 min, centrifuged and separated n-butanol layer. OD at 560 nm was taken (taken n-butanol as blank) and concentration of SOD was expressed as units/g of liver tissue. Absorbance values were compared with a standard curve generated from known SOD [45,46].

One unit of SOD is the amount of enzyme that inhibit the rate of reaction by 50 %

Interpretation will be based on % inhibition is to SOD conc. Curve

% Inhibition = (Δ Absorbance $_{control}$ - Δ Absorbance $_{sample}$)/ Δ Absorbance $_{control}$ x 100

Glutathione (GSH)

DTNB is reduced in presence of GSH to produce a yellow compound. The reduced chromogen is directly propoertional to GSH conc. And its absorbance can be measured at 412 nm. Prepared 10 % w/v tissue homogenate in 0.1 M phosphate buffer (pH 7.4). 0.2 ml of homogenateis taken and added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA (0.0430 gm in 100 ml D/W) to precipitate the tissue proteins. The mixture is allowed to stand for 15 min prior to centrifugation for 10 min at 2000 rpm. The supernatant (400 µl) is then transferred to a new set of test tubes and added with 1.8 ml of the Ellman's reagent (5,5-dithiobis-2- nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes are made up to the volume of 2 ml with distilled water. After completion of the total reaction, solutions are measured at 412 nm against blank (water). Absorbance values were compared with a standard curve generated from known GSH [47, 48].

Calculation

1. **In Blood**

   = A sample x 66.66 mg/dL

   = A sample x 2.22 mmol/dL

2. **In Tissue**

   = (A sample x 66.66)/ g tissue used (mg/ g tissue)

   = (A sample x 2.22)/ g tissue used (mmol/ g tissue)

Histology

During the collection of the tissue from body of rat for the study of structural changes, pieces of tissue were cut washed and transferred in 10% formalin solution. After that various staining were done. Then slide were examined under microscope.

Biostatical interpretation

All data are presented in Mean ±SD. Data were analyzed by One Way ANOVA followed by Bonfeffoni's test. $P<0.05$ was considered as level of significance (n=4).

RESULTS AND DISCUSSION

Phytochemical analysis of methanolic and aqueous extract of *M. Pumila* peel showed the presence of carbohydrate, alkaloids, flavonoids, phenolics, tannin, saponins, triterpenoids Table 1.

Table 1 Result of phytochemical screening of *M. Pumila*

Tests	Methanolic extract	Aqueous extract
Carbohydrates		
Molish	+	+
Fehlings	+	+
Benedit's	+	+
Protien & amino acids		
Biurets	-	+
Ninhydrin	-	+
Glycosides		
Borntrager	-	+
Killer killani	-	+
Alkaloids		
Mayers	+	-
Hagers	+	-
Wagners	+	-
Saponins		
Froth	+	+
Flavonoids		
Lead acetate	+	-
Alkaline reagent test	+	+
Treterpenoids & Steroids		
Salwoski	+	+
Libberman Burchard	+	+
Tannin & Phenolics		
Ferric chloride	+	+
Lead acetate	-	+
Gelatin	-	+

Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and the TPC in aqueous and methanolic extract was found to be 0.120 and 0.093 mg/g equivalent to gallic acid. Results shown in Table 2 & Fig 1.

Table 2 Total phenolic content of extracts

S. No.	Conc. (µg/ml)	Aqueous Extract	Methanolic Extract
1	20	0.304	0.249
2	40	0.305	0.249
3	60	0.304	0.249
4	80	0.305	0.249
5	100	0.305	0.250
TPC		0.120 mg/gm equivalent to gallic acid	0.093 mg/gm equivalent to gallic acid

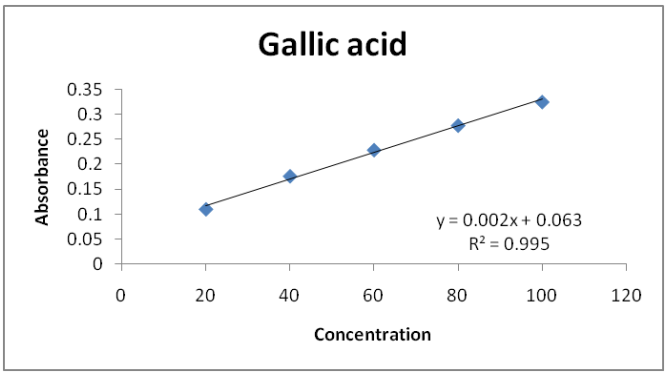

Fig. 1 Graph of estimation of total phenolic content

TFC was then calculated with respect to rutin taken as standard. The TFC in aqueous extract was higher than that of the methanolic extract with concentration being 0.083 mg/g equivalent to rutin Table 3 & Fig 2.

Table 3 Total flavonoid content of extracts

S. No.	Conc. (µg/ml)	Aqueous Extract	Methanolic Extract
1	20	0.176	0.141
2	40	0.176	0.141
3	60	0.176	0.141
4	80	0.175	0.141
5	100	0.175	0.141
TFC		0.083 mg/gm equivalent to Rutin	0.049 mg/gm equivalent to Rutin

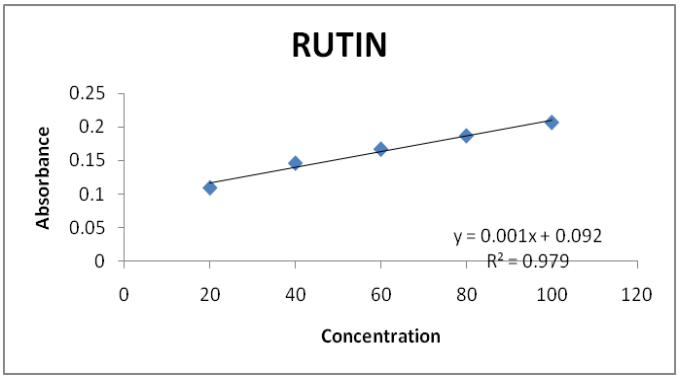

Fig. 2 Graph of estimation of total flavonoid content

Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard in both the tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed. % inhibition was higher in the aqueous extract where %

inhibition ranged from 44.63 % to 52.28 % while the values were lesser in methanolic extract ranging from 7.92% to 36.38 % Table 4 and Fig. 3.

Table 4 DPPH assay of ascorbic acid, methanolic, aqueous extract

S.No.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Methanolic Extract (% Inhibition)	Aqueous Extract (% Inhibition)
1.	**20**	48.22	7.92	44.63
2.	**40**	54.98	13.62	46.44
3.	**60**	61.39	19.13	47.11
4.	**80**	69.40	24.83	50.60
5.	**100**	74.38	36.38	52.28

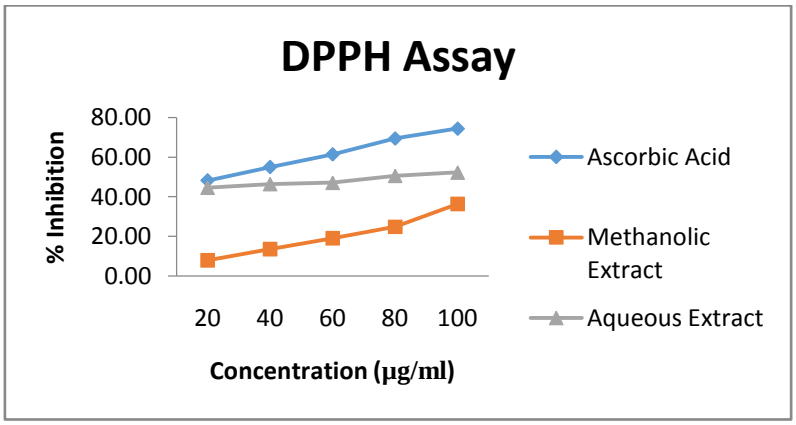

Fig. 3 DPPH Assay

The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. Reducing power assay was calculated in extracts and the values indicated a better activity Table 5& Fig 4.

Table 5 Result of reducing power assay

S. No.	Ascorbic acid	Methanolic Extract	Aqueous extract
1.	0.987	0.162	0.321
2.	1.032	0.174	0.334
3.	1.145	0.191	0.365
4.	1.159	0.214	0.386
5.	1.196	0.24	0.398

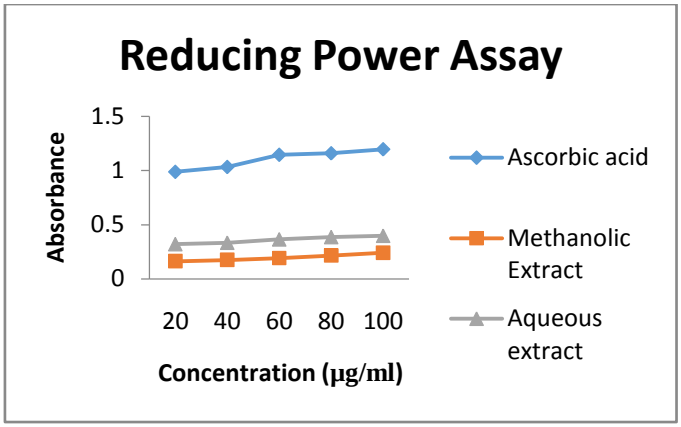

Fig. 4 Reducing power assay

In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 200 mg/kg and 400 mg/kg have been fixed as ED50 for present study Table 6.

Table 6 Acute oral toxicity of Extract

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

The methanolic extract was also tested for in-vitro antimicrobial test using well diffusion assay. The organisms tested were similar to the ones used in the aqueous extract. The values of zone of inhibition indicated that the maximum activity was against *klebsiella* while the minimum was against *B. cereus*. The aqueous extract shows better activity than the methanolic extract. The reason can be better solubility of the chemical constituents in the aqueous extract. .Evaluation of antimicrobial activity of extracts was recorded in Table 7 and illustrated in Fig. 5.

Table 7 Antimicrobial activity of methanolic and aqueous extract by well diffusion Assay

Methanolic Extract				
Organisms	**100 mg/ml**	**150 mg/ml**	**200 mg/ml**	**250 mg/ml**
B. cereus	5.50v0.577	6.75±0.500	7.75±0.500	9.50±0.577
Klebsiella	5.75±0.500	7.50±0.577	9.75±0.500	12.50±0.577
Salmonella	7.00±0.816	7.75±0.500	9.25±0.500	10.50±0.577
Actinomyces	5.50±0.577	7.50±0.577	9.75±0.500	11.75±0.500
Aqueous Extract				
B. cereus	12.75±0.500	13.50±0.577	15.25±0.500	16.50±0.577
Klebsiella	8.75±0.500	11.00±0.816	12.50±0.577	13.50±0.577
Salmonella	12.75±0.500	14.50±0.577	16.75±0.500	17.75±0.500
Actinomyces	8.50±0.577	11.00±0.816	13.75±0.957	14.75±0.500

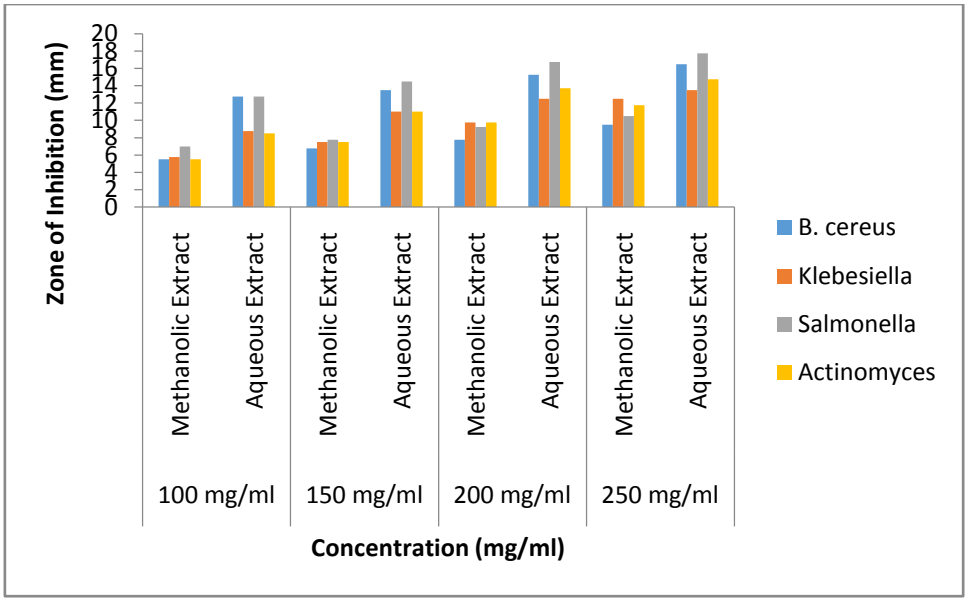

Fig. 5 Graph showing diameter of zones of inhibition of microbial growth for aqueous and methanol extracts of *M. Pumila* against various species of micro-organisms

In-vivo antimicrobial activity was tested by inducing peritonitis through *E. coli*. The activity of the extract was calculated by measuring the CFU/ml of the microorganisms. The experiment was done in groups of 4 containing control, standard (5mg/kg), extract (200 mg/kg) and extract (400 mg/kg). The results obtained indicated a dose dependent antimicrobial activity of the extracts; the extract given at a concentration of 400 mg/kg had better activity than the one administered at 200 mg/kg Table 8 & Fig. 6.

Table 8 *E. coli* induced Peritonitis

Groups	CFU/ml
Control	183.50±12.369
Standard (5 mg/kg bwt)	39.50±7.326
Extract (200 mg/kg bwt)	81.00±5.508
Extract (400 mg/kg bwt)	56.25±5.123

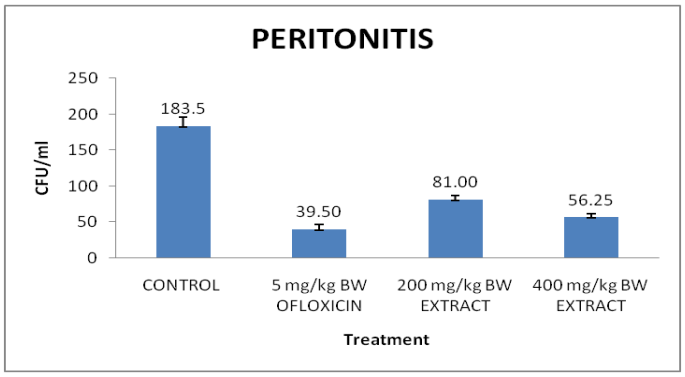

Fig. 6 *E. coli* Induced peritonitis

In-vivo antioxidant activity was measured through a set of enzymes including LPO, GSH and SOD. The levels were measured and it indicated that the extract had significant antioxidant activity however the results obtained were dose dependent the higher the dose (400 mg/kg) shown better the antioxidant activity Table 9.

Table 9 Effect of aqueous extract of *M. pumila* on antioxidant parameters of control and experimental animals

Groups	MDA Level	SOD Level	GSH Level
Control	88.55±6.37	195.36±46.42	0.210±0.023
Standard	31.9±2.193	276.98±18.89	0.434±0.117
Ex (200 mg/kg)	53.05±9.38	215.38±45.28	2.712±0.255
Ex (400 mg/kg)	44.6±3.92	256.32±78.4	3.460±0.242

CONCLUSION

Peritonitis continues to be an important problem in the health care system. *M. Pumila* has recently received some attention for its beneficial effects against several diseases. Our research study showed that the use of *M. Pumila* could effectively reduce the severity of acute peritonitis in our *E. coli* model. Further studies need to be done to verify the effect of phenolic compound, explore the mechanisms and promote the clinical use. An accurate biomarker for the early identification of peritonitis would be of great diagnostic value. An early finding of the correct diagnosis of peritonitis and the subsequent effective initiation of an appropriate treatment may help to lower the complication rate and to improve the prognosis.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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