Research Article

In Vitro Antimicrobial Activity, Nutritional Value, Antinutritional Value and Phytochemical Screening of *Pyracantha crenulata* Fruit

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ABSTRACT

The present study focused to evaluating the in vitro antimicrobial activities, nutritional value, antinutritional value and phytochemical screening of wild edible fruit of *Pyracantha crenulata* were investigated by disc diffusion method against ten bacterial stains and three fungal stains. The ethanolic fruits extract of *Pyracantha crenulata* pulp showed significant activity (18mm, 17mm & 15mm) against *Shigella flexneri, Escherichia coli & Streptococcus pyogenes* against food poisoning bacteria. The fruits have been found to rich in nutrients such as crude protein, crude fiber and carbohydrates (5.13%, 7.40% & 24.88%) and also antinutritional as phenolic, saponins and flavonoids (1.83%, 1.56% & 3.12%) respectively and phytochemical screening of plant for the presence of carbohydrates, glycosides, alkaloids, tannins and resin.

Keywords: Antibacterial, Antifungal, Nutritional value, Antinutritional value, Phytochemical screening.

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. The Garhwal Himalaya is known for its rich bio-resources and ethanoculture diversity. The sources of drugs is mainly depends on these natural products from plant, animal, microorganism and minerals, which is treatment of human and animal disease. Medicinal plants represent a rich source of potent and powerful drugs. *Pyracantha crenulata* belongs to the family Rosaceae, is a popularly known as Ghingaroo in Uttarakhand state[1]. The fruit of this plant has been used in Garhwal folk and traditional medicine in the treatment of serious health conditions like heart disorders, hypertension, diabetes, blood pressure and circulation system especially in case of angina[2]. The leaves are found useful for antioxidant, immunomodulatory, anti-inflammatory activities and also use to make herbal tea. The pome fruit is orange-red and rich in sugar[3]. The food substances used as nutraceuticals contain antioxidants, minerals, vitamins, perbiotics, probiotics, polyunsaturated fatty acids certain phytochemical and dietary fibers[4]. This analysis revealed that, the fruits contained potent value of nutrients and antimicrobials as compared to the cultivated fruits with berry and 500gm fruits contain sufficient amount of nutrients required for per day a person.

MATERIALS AND METHODS

Plant Material: - The fresh parts of fruits of *Pyracantha crenulata* were collected from adjoining area of Dhunglwali village (Dist- Chamoli Uttarakhand) in the month of July – August 2012. The plant was authenticated by botanist Prof. R. D. Guar, Department of Botany and the voucher specimen number is GUH 7582. H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

Pyracantha crenulata leaves & fruits

Preparation of plant Extract

The plant material was separated into its selected parts (fruit and leaf) air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water)[5]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of fruit and leaf was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 500gm). The various concentrated extracts were stored in air tight container for further studies.

Media

Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

Bacterial Strains

Ten bacterial strains were used namely *Escherichia coli, Klebsiella pneumoniae, Enterobacter gergoviae, salmonella entericatyphim, shigella flexneri, Staphyloccus aureus, staphyloccus epidermidis, streptococcus pyogenes,* and *Bacillus cereus*, The bacterial strains were

supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, Customer no, 3921.

Fungal Strains

Three fungal strains were used namely *Candida albicans, Aspergillus flavus* and *Aspergillus parasiticus*, The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Antibacterial assay

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts [6 & 7]. Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

Antifungal assay

The antifungal activity was tested by disc diffusion method[8, 9]. The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

Nutritional & Antinutritional assay

The edible portion of fruit was analyzed for moisture, ash, fat[10]. Fiber as per method reported in AOAC. Total nitrogen was analyzed by microkjeldhal method [11] and for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat crude fiber and ash from 100% [12]. The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The antinutritional value analyzed by AOAC methods against salt standards. Ascorbic acid in fruits was estimated [13].

Table 1: Antibacterial activity of ten bacterial strains against *Pyracantha crenulata* plant fruits different extracts, disc size, 5 mm, Inhibitory zone size ±1 mm, mm means (millimetres) and – indicate (NIZ) no inhibitory zone.

Bacterial Name		Erythromycin	Petroleum ether Extract		Chloroform Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Bacillus cereus	1272	12	-	-	-	9	10	13	10	11	8	10
Escherichia coli	729	14	-	-	-	8	-	-	11	14	9	9
Enterobacter gergoviae	621	13	-	-	-	-	8	10	12	12	8	9
Klebsiella pneumonia	432	11	-	-	-	9	8	9	10	11	9	10
Salmonella entericatypm	98	10	-	8	-	8	-	8	9	10	-	8
Shigella flexneri	1457	10	-	9	-	-	10	13	11	18	10	14
Staphyloccus aureus	902	11	-	-	-	9	11	14	13	11	9	12
Staphyloccus epidermidis	435	10	-	-	-	8	-	-	10	11	8	10
Streptococcs pyogenes	1925	12	-	-	-	-	-	10	11	15	9	13
Escherichia coli	443	13	-	-	-	-	-	9	12	17	9	11

Table 2: Fungal activities of three fungal strains against *Pyracantha crenulata* plant fruits different extracts, disc size, 5 mm, Inhibitory zone size ±1 mm, mm means (millimetres) and – indicate (NIZ) no inhibitory zone.

Fungal Name		Ketoconazole	Petroleum ether Extract		Chloroform Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Candida albicans	3017	10	-	9	-	-	-	8	-	11	-	9
Aspergillus flavus	2798	8	-	-	-	-	-	-	-	9	-	8
Aspergillus parasiticus	2796	9	-	-	-	-	-	8	-	10	-	9

Table 3: Nutritional value of *Pyracantha crenulata* plant fruits pulp.

Nutrients	Value
Moisture (%)	60.10 ± 0.15
Ash (%)	1.50 ± 0.08
Crude fat (%)	1.00 ± 0.25
Crude fibre (%)	7.40 ± 0.09
Total nitrogen (%)	0.82 ± 0.07
Total protein (%)	5.13 ± 0.04
Carbohydrate (%)	24.88± 0.16
Organic matter (%)	98.50± 0.22
Insoluble ash (%)	25.29 ± 0.05
Soluble ash (%)	74.71 ± 0.08

Table 4: Antinutritional value of *Pyracantha crenulata* plant fruits pulp.

Antinutrients	Value(mg100g-1)	Antinutrients	Value (g 100g-1)
Total saponins (%)	1.56±0.40%	Total phenolic (%)	1.83±0.05%
Total flavonoid (%)	3.12±0.50%	Total tannins (%)	0.66±0.25%
Total alkaloid (%)	0.15±0.10%		

Table 5: Phytochemical screening of *Pyracantha crenulata* plant fruits different extracts, (+) – Present, (-) – Absent.

Test	Pt. ether Extract	Benzene Extract	Chloroform Extract	Methanolic Extract	Ethanolic Extract	Water Extract
Carbohydrates/ glycosides						
(1) Molish test	(-)	(-)	(-)	(+)	(+)	(+)
(2) Fehling test	(-)	(-)	(-)	(+)	(+)	(+)
(3) Benedict test	(-)	(-)	(-)	(+)	(+)	(+)
Alkaloid						
(1) Mayer's test	(-)	(-)	(-)	(-)	(-)	(-)
(2) Dragondroff test	(-)	(-)	(-)	(-)	(-)	(-)
Flavonoids						
(1) Shinoda/pew	(-)	(-)	(-)	(+)	(+)	(+)
(2) Ammonia	(-)	(-)	(-)	(+)	(+)	(+)
Saponins	(-)	(-)	(-)	(+)	(+)	(+)
Tannins						
(1) Pyrogoll & catechol	(-)	(-)	(-)	(+)	(+)	(-)
(2) Gallic acid	(-)	(-)	(-)	(+)	(+)	(-)
Unsaturated sterol/triterpenes						
(1) Liebermann Burchard test	(-)	(-)	(-)	(+)	(+)	(-)
(2) Salkowiskis test	(-)	(-)	(-)	(+)	(+)	(-)
Resin	(-)	(-)	(-)	(+)	(+)	(+)
Phenolics compound						
(1) Ferric chloride	(-)	(-)	(-)	(+)	(+)	(+)
(2) Nitric acid	(-)	(-)	(-)	(+)	(+)	(+)
Protein and amino acid						
(1) Xanthoprotien	(-)	(-)	(-)	(+)	(+)	(+)

Preliminary Phytochemical Analysis

Preliminary phytochemical test of all different extracts of fruits powder of *Pyracantha crenulata* were performed for alkaloids, glycosides, carbohydrates, steroids, flavonoids, polyphenols, saponins, resin and tannins. However, alkaloids were absent or present in minor amount. The qualitative test of all extracts showed significant indication about the presence of metabolite which was detected by using standards methods[14].

RESULTS AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay. The results of

antibacterial, antifungal, nutritional & antinutritional value and phytochemical screening activity, table 1, 2, 3, 4, and 5, reveals that antibacterial, antifungal, nutritional & antinutritional and phytochemical screening activity of fruits of was evaluated against ten bacterial and three fungal human pathogenic strains.

Antibacterial and antifungal activity

Pyracantha crenulata ethanolic fruits extract significant activity (18mm, 17mm & 15mm) against *Shigella flexneri, Escherichia coli & Streptococcus pyogenes* against food poisoning bacteria, the order of the species based on total antibacterial activity is as follows: *Shigella flexneri* > *Escherichia coli* > *Streptococcus pyogenes*.

Nutritional and antinutritional value

The level of nutrients such as crude protein, crude fat, crude fiber and carbohydrates (5.13%, 1.00%, 7.40% & 24.88%) and also antinutritional as phenolic, saponins, tannins and flavonoids (1.83%, 1.56%, 0.66% & 3.12%) present in rich amount as compared to cultivated edible fruit (e.g. Berry).

Phytochemical screening

The phytochemical analysis of *Pyracantha crenulata* fruits powder shows the presence of alkaloids, glycosides, carbohydrates, steroids, flavonoids, polyphenols, saponins, resin and tannins, however alkaloids were absent.

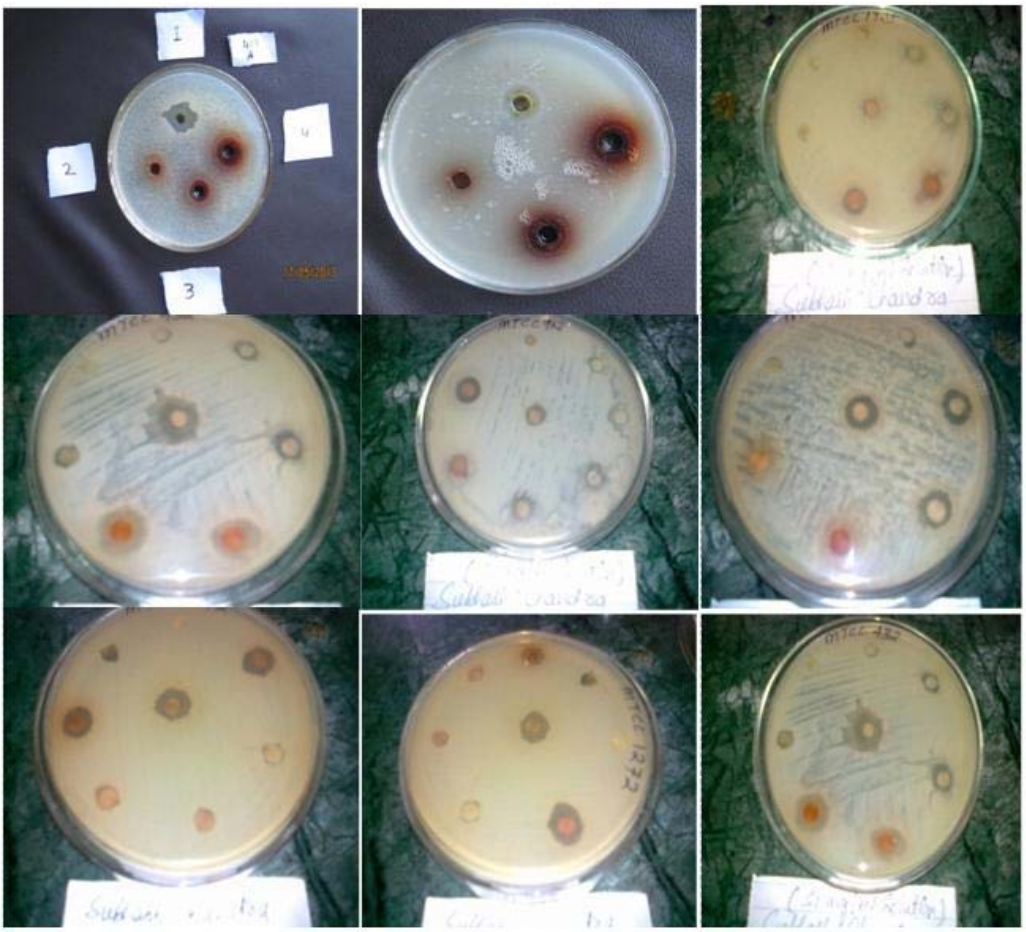

Figure 1: Antibacterial and antifungal activity of ten bacterial strains & three fungal strains against *Pyracantha crenulata* plant fruits different extracts.

CONCLUSION

The present study results focused on antimicrobial activity, nutritional activity, antinutritional activity and phytochemical screening of *Pyracantha crenulata* this investigation revealed that antimicrobial and antifungal activity against selected bacterial and fungal strains. Which encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with this plant. Even at low concentrations, these plant species contained potent nutritional value and also showed high antimicrobial and antifungal activity nearly equal to that of the commercial fungicide used as a positive control.

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