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Antibiotic potential of few wild Edible fruits of Family Rosaceae

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ABSTRACT
Rubus niveus, Pyracentha crenulata and Pyrus pashia are the representative of family Rosaceae comprises of 95 genera and 2800 species, Rosaceae family is distributed worldwide and abundantly available in temperate to sub-tropical zones of the northern hemisphere.This study highlights the phytochemical screening and antibacterial activity of ethanolic and aqueous extracts of three wild edible fruits. Antibacterial activity was performed by disc diffusion method against the strain Salmonella typhimurium (MTCC 3224), Klebsiella pneumonia (MTCC 3384), Bacillus subtilis (MTCC 441), Aeromonas hydrophila (MTCC 646), and Pseudomonas aeriginosa (MTCC 103). The preliminary screening of phytochemical constituents of different fruit extracts showed the presence of flavonoids, tannins, polyphenols, saponins, carbohydrates and steroids. The ethanolic extract of Rubus niveus emerged as the most promising fruit with highest antibacterial activity against the selected bacterial strains followed by Pyrus pashia and Pyracantha crenulata respectively.
Keywords: *Antibacterial activity, phytochemical screening, Rosaceae, Wild edible fruits*

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INTRODUCTION

One third of the world population, specifically in the rural areas of African and Indian sub- continent are mostly depend on a traditional system of medication like Ayurveda, Unani and Siddha etc[1]. In the recent past, excessive use of commercial antibiotics leads to resistance in the population against various drugs; therefore, alternative antibiotics are on demand especially from the plant sources[2,3].The bioactive components such as phenolics, flavonoids, tannins, alkaloids and some others are enormously present in fruits and play a vital role in human health management[4,5].The Rosaceae family fruits are cosmopolitan in distribution and are abundantly available in Indian sub-continent[6,7]

Among these wild fruits *Pyrus Pashia* (Mehul),*Pyracentha crenulata* (Ghingaroo) and *Rubus niveus* (Kala Hisalu) have huge medicinal properties, flourished all over the mountain ranges of Kumaun Himalaya. Traditionally, Pyrus pashia is used in cuts, wounds, fungal infection and treatment of mouth infections digestive disorder, heart disease, skin disease, astringent, diuretic and anti-dysenteric properties[8,9]. Whereas Pyracentha crenulata fruits is used in the treatment of heart disorders, hypertension, diabetes, blood pressure and circulation system especially in case of angina [10]. Rubus niveus fruits is used for the treatment of oesophageal, liver, tongue infection, anticancer and anti inflammatory [11,12].The present study deals with antibacterial properties and phytochemical screening of these neglected wild edible fruits of Kumaun region.

Fig.1.(a) *Pyrus Pashia* (Mehul) ,(b) *Pyracantha crenulata* (Ghingaroo), (c) *Rubus niveus* (Kala Hisalu).

MATERIAL AND METHODS

Plant Material

The wild edible fruits *Pyracantha crenulata* (Ghingaroo), *Pyrus pashia* (Mehul) and *Rubus niveus* (Kala Hisalu) of the family Rosaceae were collected from the Nainital District of Kumaun region, Uttarakhand, during the month of May-July 2018 and were authenticated by Botanical Survey of India, Dehradun, Uttarakhand India and the voucher specimen number allotted 137,139 and 138 The fruits were oven dried at 40°c and moderately powdered with the help of mechanical grinder.

Preparation of Fruit Extract

The moderately crushed powder was extracted in increasing polarity solvent (Hexane, Ethanol and water) by Soxhlet apparatus. All extracts were concentrated under vacuum using rotary evaporator (IKA, Germany) followed by concentrated on water bath. Each time powdered material was air dried before extracting with next solvent. The various extracts were stored in air tight containers in refrigerator at 4°c for further studies.

Preparation of Culture Media

Nutrient broth was used for the culturing of bacterial strains. Loop-full of each bacterial culture was inoculated in the nutrient broth and incubated at 37°C for 72 hours in bacterial incubator. Nutrient broth, muller hinton agar, alcohol, distilled water of analytical grade were used in this study.

Phytochemical Screening

Phytochemical screening for the major chemical constituents of extracts of the selected fruit samples was carried out according to the standard procedures [13]. The fruit material was screened for the presence of alkaloids, carbohydrates, flavonoids, tannins, polyphenols, saponins, steroids and terpenoid.

Bacterial Strain and Antibacterial Assay:

Five bacterial strain were used namely Salmonella typhimurium (MTCC 3224), Bacillus subtilis (MTCC 441), Klebsiella pneumonia (MTCC 3384), Pseudomonas aeriginosa (MTCC), Aeromonas hydrophilla (MTCC 646).

The disk diffusion assay was carried out to perform antibacterial activity[14]. Firstly ethanolic extracts were dissolved in DMSO and water extract were dissolved in distilled water at two different concentrations 20mg/ml and 40 mg/ml. 20µl of bacterial inoculum was spread over the surface of a sterile muller hinton agar plates. Extracts were applied to filter paper disc (6 mm in diameter, Whatmann No.1,) and allowed to dry before placed on agar plate and plates were incubated at 37°C for 24hrs. After incubation, the diameter of inhibition zones was measured and antibacterial activity was calculated. Gentamycin (concentration 100µg/disc) was used as standard drug and ethanol, water and hexane were used as a negative control. Each test was performed in triplicate and results analyzed for statistical significance.

RESULT

The edible part of selected fruits was taken for their antibacterial and phytochemical evaluation and the findings are summarized in the table 1.The findings revealed that these fruits are nutritionally very rich and can be used as an economical alternative source of nutraceuticals specially curing various metabolic disorders and cardiac problem as evident from various investigations. Eight phytochemical tests were done to evaluate the presence and absence of bioactive compounds in selected wild edible fruits. The carbohydrates, protein, phenolics, flavonoids and saponins were present in ethanolic as well as in the aqueous extracts. Terpenoids were present in ethanolic extracts and oils and fats were absent in both the extracts. *Pyrus pashia* gave alkaloids in ethanolic extract and was absent in aqueous extract.

Table 1: Phytochemical Analysis of selected wild edible fruits *Pyracentha crenulata*, *Pyrus pashia* and *Rubus niveus*.

S.no.	Chemical constituents	Pyracentha crenulata		Pyrus pashia		Rubus niveus	
		Ethanol	Water	Ethanol	Water	Ethanol	Water
1.	Protein	+	+	+	+	+	+
2.	Carbohydrates						
(a)	Molish test	+	+	+	+	+	+
(b)	Fehling test	+	+	+	+	+	+
(c)	Benedict Test	+	+	+	+	+	+
3.	Alkaloids						
(a)	Mayer's test	-	-	+	-	-	-
(b)	Dragendroff test	-	-	+	-	-	-
4.	Saponins	+	+	+	+	+	+
5.	Phenolics and tannins	+	+	+	+	+	+
6.	Oils and Fats	-	-	-	-	-	-
7.	Flavonoids	+	+	+	+	+	+
8.	Terpenoids	+	-	+	-	+	-

+ = Present, - = Absent

Antibacterial screening of *Pyrus Pashia* (Mehul), *Pyracentha crenulata* (Ghingaroo) and *Rubus niveus* (Kala Hisalu) was performed using hexane, ethanolic and water fruit extracts against five bacterial strains i.e. *Salmonella typhimurium* (MTCC 3224), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumonia* (MTCC 3384), *Pseudomonas aeriginosa* (MTCC), *Aeromonas hydrophilla* (MTCC 646) using disc diffusion method. The findings of ethanolic and aqueous extract are summarized in Table 2 and 3, hexane extract didn't show any antibacterial activity. Ethanolic fruit extracts with 40mg/ml concentration showed higher antibacterial activity against all selected bacterial strain. Maximum zone of inhibition was observed in case of *Rubus niveus* against *Salmonella typhimurium* that is19.16±0.26mm, followed by *Pseudomonas aeriginosa* of 19.7±0.05mm and the minimum zone of inhibition was observed in case of *Pyrus Pashia*against *Pseudomonas aeriginosa*that is 7.8±0.15 mm. In aqueous extract, *Rubus niveus* showed the highest zone of inhibition against, *Aeromonas hydrophilla* that is9mm and minimum inhibitory zone was observed in *Pyrus pashia* against *Pseudomonas aeruginosa.* The average zone of inhibition at100µg/ml concentration of standard drug Gentamycin was observed 20mm, 18mm, 22mm, 16mm, 14mm against *B. subtilis, K. pneumoniae, P. aereginosa, S.typhimurium, A.hydrophila* respectively.

Table 2: Antibacterial activities of ethanolic extract of selected wild edible fruits against the bacterial strains.

Fruits	Bacterial species and zone of inhibition observed in mm					
	Fruit extract Concentration	*S.typhimurium*	*K.pneumoniae*	*B.subtilis*	*A.hydrophila*	*P.aeriginosa*
Pyracentha crenulata	20mg/ml	10±0.115	12.23±0.14	13.03±0.08	12.06±0.08	10.23±0.12
	40mg/ml	13.83±0.12	16±0.17	10.2±0.115	12.4±0.15	13.03±0.12
Pyrus pashia	20mg/ml	8.96±0.14	16.63±0.23	12.76±0.14	9.96±0.20	7.8±0.15
	40mg/ml	13.03±0.12	17.86±0.17	16.83±0.20	12.9±0.05	10.03±0.08
Rubus niveus	20mg/ml	14.56±0.29	13.8±0.11	15.86±0.12	13.86±0.08	12.8±0.17
	40mg/ml	19.16±0.26	17.86±0.03	16.83±0.14	17.83±0.08	19.7±0.05

Results are expressed as means ± SD for triplicates

Table 3: Antibacterial activities of aqueous extract of selected wild edible fruits against the bacterial strains.

Fruits	**Bacterial species and zone of inhibition observed in mm**					
	Fruit extract Concentration	*S.typhimurium*	*K.pneumoniae*	*B.subtilis*	*A.hydrophila*	*P.aeriginosa*
Pyracentha crenulata	20mg/ml	7.26±0.14	9.76±0.17	9.26±0.12	9.96±0.14	10.7±0.05
	40mg/ml	10.7±0.11	15.03±0.08	9.93±0.08	11.13±0.03	12.73±0.08
Pyrus pashia	20mg/ml	7.1±0.11	12.06±0.12	11.1±0.1	8.4±0.15	M.A
	40mg/ml	11.23±0.14	15.2±0.05	12.13±0.06	11.06±0.03	7.06±0.03
Rubus niveus	20mg/ml	11.33±0.08	9.3±0.11	8.06±0.03	13.23±0.14	13±0.11
	40mg/ml	13.13±0.03	14.53±0.14	8.1±0.05	16.4±0.06	15.5±0.20

Results are expressed as means ± SD for triplicates. M.A= Moderately Active

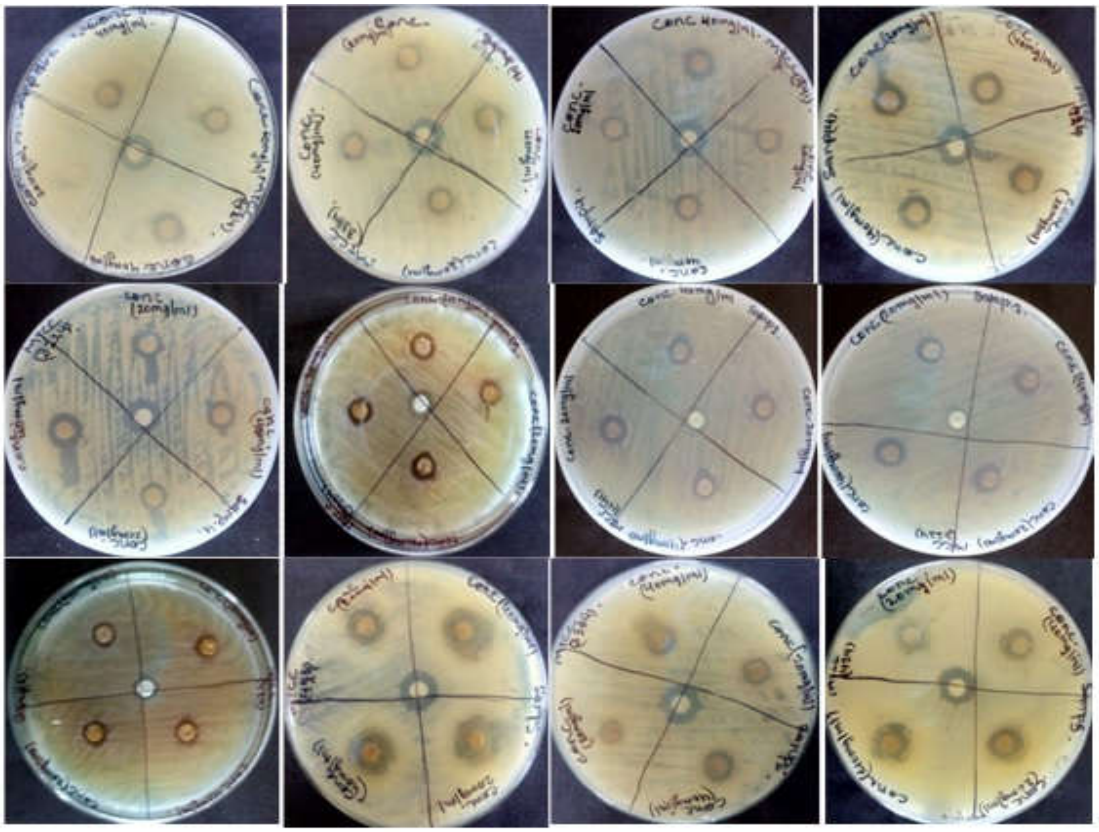

Fig2. Antibacterial activity of five bacterial strain against of *Pyrus pashia*(Mehul), *Pyracentha crenulata* (Ghingaroo) and *Rubus niveus* (Kala hisalu) fruit extract.

DISCUSSION

Rosacea family is considered as one of the richest representative having secondary metabolites of very high antibacterial activity, in recent past various studies have been made related to the nutraceutical values of fruits and it has been proved by many researcher that plant sources can provide healthy alternative into our everyday life. This study was aimed to evaluate the comparative antibacterial activity of *Pyracantha crenulata*, *Rubus neveus* and *Pyrus pashia* against five bacterial strains[14,15]. The result of phytochemical screening revealed that these wild edible plants are the rich repository of phytochemicals with nutritional and therapeutic values[16]. The evaluation of antibacterial activity revealed that most of the bacterial strains were sensitive to ethanol extract of *Rubus niveus* followed by *Pyrus pashia* then *Pyracantha crenulata.* According to the results it's proved that the *K. pneumonie* is highly sensitive to these three selected fruits extracts. The highest Zone of inhibition was found between 12 mm to 9mm in case of *K. pneumonie*.

CONCLUSION

The findings of the present study conclude that wild edible fruits need more attention to understand and evaluate their therapeutic and nutritional property. It is assumed that these plants and plant part can be cultivated with minimal economic inputs and could be of use for local farmers as sources of revenue simultaneously research investigation and result may yield new or highly potent phytochemicals.

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