IJPSR (2022), Volume 13, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 October 2021; received in revised form, 15 February 2022; accepted, 05 May 2022; published 01 July 2022

BIOLOGICAL ACTIVITY OF THE INDIGENOUS FRUITS AND VEGETABLES OF THE INDIAN HIMALAYAN REGION

Murtaza Gani [*1] and Tanveer Alam [2]

Department of Chemistry [1], KLDAV PG College Roorkee - 247667, Uttarakhand, India.
Affiliated to Department of Chemistry [2], HNB Garhwal University (A Central University) Srinagar - 246174, Uttrakhand, India.

Keywords:

Antibacterial, Antifungal, Minor, extracts, Indigenous

Correspondence to Author:
Murtaza Gani

Department of Chemistry,
KLDAV PG College Roorkee - 247667, Uttarakhand, India.

E-mail: kmurtazakmg@gmail.com

ABSTRACT: This work aims to evaluate the antimicrobial potential of ethanol, methanol, and water extracts of cherry (*Prunus avium)* and acetone extracts of quince *(Cydonia oblonga)*, hexane & ethyl acetate extracts of Handh (*Taraxacum officinale)*, acetone & methanol extracts of sustchal (*Malva neglecta*) of the selected minor fruits and vegetables of Indian Himalayan Region. Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations (MIC) of different plant extracts against Gram-positive *(Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two strains of fungus (*A. niger, A. flavus)*. The extracts exhibited both antibacterial and antifungal activities against tested microorganisms. The concentration increase of the extracts resulted in the increase of inhibition zone values resulting in the increase in the antimicrobial activities of the extracts. Both pulp and peel of Quince (*Cydonia oblonga)* exhibited significant antimicrobial activity. Acetone Quince (*Cydonia oblonga)* and Handh (*Taraxacum officinale*) extract showed significant antibacterial activity ($P < 0.05$) against all tested bacterial strains.

INTRODUCTION: Food spoilage is one of the big problems faced by the food industry. The growth of microorganisms predominantly causes food spoilage or deterioration [1]. Most of the bacterial strains got resistant to certain antibiotics due to mutations in genes, changes in their structures, and indiscriminate use of antibiotics to treat infectious diseases, which led to most antibiotics' ineffectiveness.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(7).2697-02

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(7).2697-02

This generated a renewed interest in herbal medicines [2]. For the genus Taraxacum, only a few studies concerning its antimicrobial properties have been reported, which is due to the presence of terpenoids, triterpenoids, steroids, coumarins, phenols, saponins, flavonoids, flavones, flavonols, chalcones, phlorotannins and cardiac glycosides in antimicrobial extracts. Still, neither compound isolation nor further identification was performed [3].

In the case of *Malva neglecta*, the entire plant exhibits therapeutic properties, but in general, the pharmacological effects of Malva are assigned to the leaves and flowers, especially due to the presence of some flavonoids and mucilages in these parts [4]. For *Cydonia oblonga*, studies on antimicrobial activity of different aerial parts of the

quince plant demonstrated that its leaf and fruit are effective against gram-positive and gram-negative bacteria [5]. The crude extract of quince fruit polyphenols showed antibacterial activity against the Gram-negative bacterium *E. coli*. For Prunus avium, it has been shown that its extract can exhibit antimicrobial activity against pathogenic oral bacteria [6]. In addition, it has been shown that a blend of cherry has effects of natural antimicrobials on the inhibition of *Listeria monocytogenes* [7]. The aim of the present work was to evaluate the microbial activity of the selected minor fruits and vegetables of the Indian Himalayan Region.

MATERIAL AND METHODS:

Antimicrobial Activity: To assess the antimicrobial activity of the extracts, the following microorganisms were used, *Staphylococcus aureus*for Gram-(+) bacteria, *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella sp.* strain for Gram-(-) bacteria, the yeast *Candida albicans* and the mould *Aspergillus niger*, while fungal strains *Aspergillus flavus* and *Aspergillus niger* were used for antifungal activity. Susceptibility of the test organism to the extract was determined by employing the standard disk or well diffusion technique [8, 9]. Briefly, the bacterial suspension in potato count broth (PCB), adjusted to 0.5 McFarland turbidity and evaluated using a serial 10-fold dilution method, was spread plated on count agar medium (PCA) in order to give a population of 10^8 colony-forming units (cfu)/ plate.

For the disk diffusion test, sterile paper discs (6 mm) were added of the test sample (20 µL) and placed onto the inoculated agar surface. After cultivation at 37 (bacteria)/ 27 °C (*C. albicans*) for 24 h or 22 °C for 4-12 days (*A. niger*), the resulting inhibition zone diameters were measured.

A 100 µL amount of the diluted working extract or pure phenolic solution and 100 µL of the bacterial suspensions (5×10^5cfu/mL) were added in the microwells. The plates were incubated aerobically at 37 °C for 24 h. Bacterial growth was revealed by the presence of turbidity and a pellet on the well bottom. MICs were determined as the first well in ascending order that did not produce a pellet. To confirm MIC and establish MBC, 25 µL of broth was removed from each well and inoculated on PCA plates.

After overnight incubation at 37°C, the number of surviving organisms was determined (MBC was determined when 99.9% of bacteria were dead).

RESULT AND DISCUSSION:

***Taraxacum officinale* (Handh):** The antibacterial effects of the extracts of *T. officinale* were evaluated against four uropathogenic bacteria **(Table 1)**. Evaluation of the Taraxacum extracts revealed that the Hexane extract inhibited the growth of *S. aureus* by 85% at 200 µg/mL and was a more effective inhibitor for the Gram-positive than for the Gram-negative *strains* **(Table) 1**.

K. pneumoniae was the lowest inhibited by the Hex extracts. EtOAc extract showed low activity against E. coli with 1600 µg/mL and growth inhibition with a 92% value. However, this extract did not exert an antibiotic effect on Gram-positive bacteria S. aureus in the concentrations tested **(Table 1)**. Similar results have been reported where the ethanol extract of leaves of *T. officinale* had low antimicrobial activity against *S. aureus, E. coli,* and *Salmonella abony* [10]. In addition, it was observed that as the extract concentration increased, so does the bacteria growth inhibition. This inhibitory effect may be due to phenolic compounds, terpenes, tannins, flavonoids, alkaloids, and/or proteins in the plant extracts. Such compounds had been reported to have an active effect on the bacterial cell membrane, which may destroy these microorganisms [11, 12].

In studying the antipathogenic properties of genus Taraxacum to combat infectious diseases, *T. officinale* is the most studied species, but it has shown various results depending on the extraction characteristics or on the bioassay performed. Omit lines 32-39.

Micrococcus luteus and *Vibrio cholera* with MIC values of 1.0 mg/mL and 12.5 mg/mL, respectively, but did not show any activity against *S. aureus, Enterobacter faecalis, Enterococcus bacteria, V. cholera, Bacillus subtilis, Pseudomonas aeruginosa, K. pneumonia*, or *E. coli* [13]. This work provides preliminary information for the development and use of natural medicine with extracts of *T. officinale* in the control of disease against uropathogenic bacteria as antimicrobial agents.

TABLE 1: ANTIBACTERIAL ACTIVITY OF N-HEXANE AND ETHYL ACETATE EXTRACT OF *TARAXACUM OFFICINALE*

Percentage of growth inhibition (%)/MIC (µg/mL) at 24 h*				
Extracts	***E. coli***	***S. aureus***	***K. pneumoniae***	***P. mirabilis***
Hex	69 ± 2.1	85± 3.3	49 ± 0.0	68 ± 0.8
EtOAc	92 ± 0.9	0 ± 0.0	-	-
Chloramphenicol	89 ± 0.0	-	91 ± 0.0	86± 0.0
Streptomycin	-	82 ± 1.1	-	-

*Mean of triplicates ± standard deviation of three replicates; (-) not tested.

***Malva neglecta* (Sustchal):** The antimicrobial activities of *M. neglecta* extracts against different microorganisms were assessed according to inhibition zone diameter. Results are presented in to change **Table 2**. The acetone and methanol extracts were active on all microorganisms tested with different zone diameters indicating weak (inhibition zone < 12) and moderate antimicrobial activity (inhibition zone < 20-12).

The acetone extracts of *M. neglecta* showed moderate activity against all microorganisms tested. These results are in lieu of Mansour *et al.* [13]. The most effective reason for the selected plants is the presence of certain phytochemicals like glucosinolates, alkaloids, flavonoids, rhamnose, galactose, galacturonic acid, glucuronic acid, phenolic acids, tannins and volatile oils.

TABLE 2: ZONES OF GROWTH INHIBITION (MM) SHOWING THE ANTIMICROBIAL ACTIVITY OF THE *MALVA NEGLECTA*

		Microorganisms				
		Inhibition Zone Diameter (mm)				
Extract	**Concentration**	***E. coli***	***S. pyogenes***	***S. aureus***	***P. aeruginosa***	***C. albicans***
Acetone	10 mg/ml	11±0.3	11±0.1	11±0.3	10±0.1	16±0.2
	20 mg/ml	13±0.2	12±0.3	12±0.2	11±0.2	16±0.3
	30 mg/ml	15±0.5	13 ±0.5	12±0.4	13±0.4	18±0.4
Methanol	10 mg/ml	11±0.2	11±0.1	10±0.2	12±0.1	12±0.3
	20 mg/ml	12±0.1	12±0.4	10±0.2	13±0.3	12±0.4
	30 mg/ml	14±0.2	13±0.3	13±0.3	14±0.2	14±0.1

Values are means ± SD of three separate experiments done in triplicate.

***Cydonia oblonga* (Quince):** The consistent and reproducible results were obtained using the standard disk and well diffusion techniques.

The antimicrobial activity was highest against the Gram-(+) *S. aureus* and the Gram-(-) *P. aeruginosa* bacteria, somewhat weaker against the *E. coli* and the yeast *C. albicans*, while with the *Salmonella sp*. and the mould *A. niger* no inhibition was obtained **(Table 3)**.

S. aureus was the most sensitive microorganism to the extracts examined in this study. Quince pulp extracts showed both bacteriostatic and bactericide activities. MICs and MBCs for peel extracts were found to be equal in **(Table 3).**

The agar diffusion assay showed that both quince pulp and peel extracts exhibited antimicrobial potentials with clear cut inhibition zones **Fig. 1.** The diameters of these inhibition zones increased with the concentration of polyphenolic compounds indicating that these molecules were responsible of the antimicrobial effects. The Omit sensitivity to the whole extracts or pure phenolic compounds varied widely. Rodriguez Vaquero *et al* reported that *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) were the most sensitive and the most resistant to flavonoid compounds than the other tested bacteria [14].

No inhibition of the mould *A. niger* growth was obtained at any concentration of the quince extracts in comparison to control culture. This finding may be expected since microbiological analyses of quince jams, which contain mainly the same phenolics as the fruit, revealed that some samples presented many yeasts and moulds [15].

The polyphenols, especially the chlorogenic acid, act in synergism with other components of the extracts to exhibit their total antimicrobial activities.

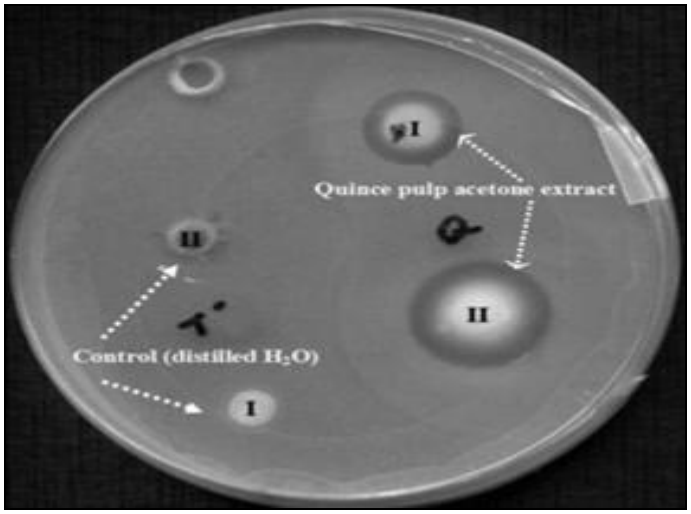

FIG. 1: *S. AUREUS* (ATCC6538) GROWTH INHIBITION: 20 AND 100 µL OF THE ACETONE QUINCE PULP EXTRACT WERE USED IN DISK (I) AND WELL (II) DIFFUSION ASSAYS, RESPECTIVELY

TABLE 3: ANTIBACTERIAL ACTIVITY OF QUINCE PULP AND PEEL EXTRACTS

Microorganism	***S. aureus* (ATCC6538)**		***P. aeruginosa* (ATCC 9027)**		***E. coli* (ATCC 8739)**		***C. albicans* (ATCC 14053)**	
Technique	Disk Diffusion	Well Diffusion	Disk Diffusion	Well Diffusion	Disk Diffusion	Well Diffusion	Disk Diffusion	Well Diffusion
Diameters of the Inhibition Zone (mm)								
Pulp Extract	16.5 ± 1.3	20.2 ± 1.4	11.4 ± 0.1	16.2 ± 0.4	W	08.2 ± 0.9	N	W
Peel Extract	22.3 ± 2.7	27.1 ± 2.6	15.5 ± 0.8	18.3 ± 0.9	08.6 ± 1.0	10.5 ± 1.1	W	09.6 ± 0.
MIC (µg/ml)								
Pulp Extract	10^2		5×10^2		10^3		5×10^3	
Peel Extract	10^2		10^2		5×10^2		5×10^3	
MBC (µg/ml)								
Pulp Extract	5×10^2		10^3		5×10^3		10^4	
Peel Extract	10^2		10^2		5×10^2		5×10^3	

Values are means ± SD of three separate experiments done in triplicate. N= no antimicrobial activity, Ø) 6 mm; W= weak antimicrobial activity, 6 mm < Ø < 8 mm.

***Prunus avium* (Cherry):** The results are shown in **Fig. 2**. The lowest value of extract that exhibited the MIC80 effect was 0.5 mg/ml (*A. haemolyticum*) and the highest value for the breakpoint effect was 21.13 mg/ml (*S. typhymurium*).

Coccia *et al.*, assumed that bactericidal effect was expressed only at concentration higher than double MICs [16] tested bacteria is understandable, considering it is a conventional antibiotic.

Antibiotics, as well as synthetic substances, exhibit a much higher antibacterial effect than natural products, but they also exhibit side effects [17].

We also found that some concentrations of extract exhibit a beneficial effect on bacterial growth. Our study, as well as theirs, showed no antifungal activity [16]. The results of the present study show that cherry extract caused MBC, MIC99, MIC90, and MIC80 effects in lower concentrations on *E. coli*. This coincides with the findings of Kirsch *et al*., but they used sour cherry methanol extract and water extract [17].

Kołodziejczyk *et al.*, investigated the activity of cherry pomace ethanol extracts against *Salmonella choleraesuis* and *E. coli* and showed a reduction of bacteria numbers at doses higher than 2,500 µg/ml.

They noticed that extracts showed bactericidal activity at concentrations higher than 10,000 µg/ml [18].

Omit lines 12-15 MIC99 concentrations for S. enteritidis and E. coli were 5.60 mg/ml and 5.08, while MBC values were 6.07 mg/ml and 8.19 mg/ml, respectively.

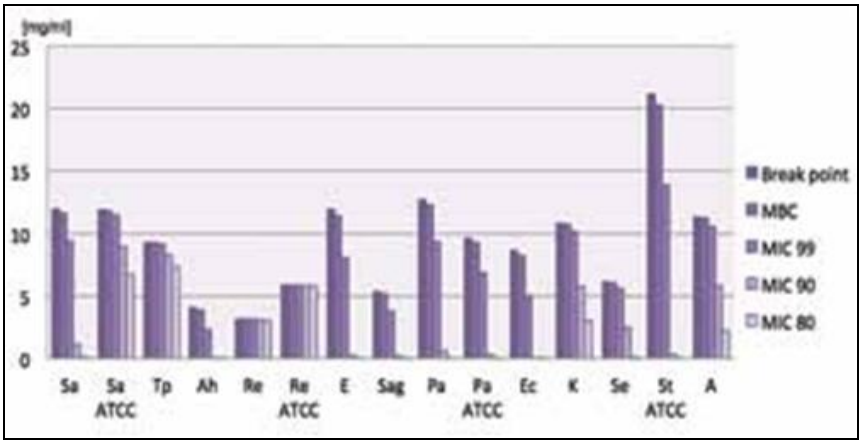

FIG. 2: ANTIMICROBIAL ACTIVITY OF CHERRY EXTRACT

CONCLUSION: The scope of the experiments included analysis of the antimicrobial activity of ethanolic, methanolic, ethyl acetate, hexane, acetone, and aqueous extracts against bacterial and fungal cultures and determination of the minimum inhibitory concentration of plant extracts tested microbial growth.

These results suggest that extracts have the potential as antibacterial uropathogenic disease agents. The findings suggest that these native plants have good antibacterial and antifungal properties and can be used for infection control and treatment of various diseases. It can also be a new source for antibiotics discovery and infection treatment.

Further studies are necessary to isolate and characterize the active components of the extracts\fractions and also to elucidate their mechanisms of action for this activity.

ACKNOWLEDGMENT: The authors are highly thankful to the Department of Chemistry, KL DAV PG College Roorkee Uttrakhand India for providing facilities of the current work.

Contribution of Authors: We declare that this work was done by the authors named in this article and the authors will bear all liabilities about claims relating to the content of this article.

Funding Statement: The research work did not receive any funding from any source. This work was a part of research work for awarding a Ph.D. degree.

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES:

1. Pirbalouti AG, Jahanbazi P, Enteshari S, Malekpoor F and Hamedi B: Antimicrobial activity of some Iranian medicinal plants. Arch Biol Sci Belgra 2010; 62: 633-642.
2. Ahmad I, Mehmood J and Mohammad F: Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacology 2008; 62: 183-193.
3. Ragasa C, Apuada M and Rideout J: Terpenoids from *Taraxacum officinale*. National Research Council of the Philippines Research Journal 2009; 10 (1): 17–26.
4. Fabri RL, Nogueira MS, Dutra LB, Bouzada MLM and Scio E: Antioxidant and antimicrobial potential of *Asteraceae species*. Revista Brasileira de Plantas Medicinais 2011; 13 (2): 183–189.
5. Sajid SM, Zubair M, Waqas M, Nawaz M and Ahmad Z: A Review on Quince (*Cydonia oblonga*): A Useful Medicinal Plant. Global Veterinaria 2015; 14 (4): 517-524.
6. Seneviratne CJ, Wong RW and Hagg U: *Prunus mume* extract exhibits antimicrobial activity against pathogenic oral bacteria. Int. J Paediatr Dent 2011; 21: 299-305.
7. Williams RJ, Spencer JP and Rice-Evans C: Flavonoids: antioxidants or signalling molecules. Free Radic Biol Med 2004; 36: 838-849.
8. Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, Dessi S, Coroneo V and Cabras P: Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food Chem 2004; 52: 3530-3535.
9. Jayasuria H, Clark AM and Chesney JD: New antimicrobial filicinic acid derivatives from *Hypericum drummondii*. J Nat Prod 1991; 54: 1314-1320.
10. Ionescu D, Predan G and Rizea GD: Antimicrobial activity of some hydroalcoholic extracts of artichoke (*Cynara scolymus*), burdock (*Arctium lappa*) and dandelion (*Taraxacum officinale*). Bulletin of the Transilvania University of Brasov, Series II: Forestry, Wood Industry, Agricultural Food Engineering 2013; 6(2): 113–120.
11. Jassim AMN: Study of Some *Eucalyptus rostrata* Leaves Components and Effect of Its Extract on Different Microorganisms. Al-Mustansiriyah Journal of Science 2005; 16(2): 62– 71.
12. Kitts DD and Hu C: Dandelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation *in-vitro*. Phytomedicine 2005; 12 (8): 588–597.
13. Khan AM, Qureshi RA, Gillani SA and Ullah F: Antimicrobial activity of selected medicinal plants of

Margalla hills, Islamabad, Pakistan. Journal of Medicinal Plant Research 2011; 5(18): 4665–4670.
14. Mansouret SS, Oochak H, Darabpour E and Motamedi H: A survey on *Hibiscus rosa*-sinensis, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents. Asian Pac J Trop Med 2010; 3 (5): 351-355.
15. Rodriguez Vaquero MJ, Alberto MR and Manca de Nadra MC: Antibacterial effect of phenoliccompounds from different wines. Food Control 2006; 18: 93-101.
16. Ferreira IM, Pestana N, Alves MR, Mota FJM, Reu C, Cunha S and Oliveira M: Quince jam quality: microbiological, physicochemical and sensory evaluation. Food Control 2004: 15 (4): 291-295.
17. Tamara K, Suvajdzic LJ and Leovac V: Antimicrobial activity of sour cherry. Agro Food Industry Hitech 2016; 27 (1): 56-58.
18. Coccia A and Carraturo A: Effects of methanolic extract of sour cherry (*Prunus cerasus* L.) on microbial growth. Int J Food Sci & Technology 2012; 47 (8): 1620–1629.
19. Krisch J and Galgoszly L: Antimicrobial activity of sour cherry. Ann Fac Eng Hunedoara 2009; 7 (2): 131-134.
20. Kolodziejczyk K and Sojka M: Polyphenol composition, antioxidant capacity and antimicrobial activity of the extracts obtained from industrial sour cherry pomace. Industrial Crops and Products 2013; 51(1): 279–288.

How to cite this article:
Gani M and Alam T: Biological activity of the indigenous fruits and vegetables of Indian Himalayan region. Int J Pharm Sci & Res 2022; 13(7): 2697-02. doi: 10.13040/IJPSR.0975-8232.13(7).2697-02.