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Evaluation of the antimicrobial activity of gooseberry and wild apple fruit juices of the northeast region of India

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ABSTRACT

A large number of wild fruits belonging to the north eastern region of India are considered to be nutritionally abundant and traditionally used for the treatment of various ailments. In the present study, juices of gooseberry and wild apple have been evaluated for their antimicrobial activity in its consumable form as a potential source of natural anti-infective agent. The fruit juices were screened for their antibacterial and antifungal activities qualitatively, using the agar well diffusion method followed by quantitative assessment by determining the minimum inhibitory concentration and minimum bactericidal concentration. The study revealed that both the fruit juices exhibited broad spectrum antibacterial activity. Considerable activity against drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and extended spectrum β-lactamases producing Gram-negative bacteria was observed. Thus, this study highlights the antibacterial efficacy of wild apple and gooseberry fruit juices and augments the beneficiary properties of highly nutritional fruit juices that are specific to Indian region.

KEY WORDS: *Docynia indica*, drug resistance, *Emblica officinalis*, polyphenols, tannins

INTRODUCTION

There has been a remarkable progress in the development of antibiotics. Yet, infectious diseases remain a major threat to public health worldwide. Moreover, there has been an alarming increase in antimicrobial resistance among the microbial pathogens causing various infections, thereby rendering numerous classes of antibiotics ineffective. There is, therefore, an increasing need to search for new antimicrobial agents (Bag *et al.*, 2012). With growing interest in eco-living lifestyle, increased attention is being paid to the natural antimicrobial agents.

The foothill of eastern Himalayas offers a potential source of underutilized edible fruits. The region is explicitly rich in the mineral content which is apprehended in its native flora and fauna (Rymbai *et al.*, 2016). A number of fruit juices have long been used and recommended for their use as an anti-infective agent in traditional medicine by the local tribes. These fruit juices have the properties of bioavailability and retention of certain minerals by bioactive polyphenolic compounds (Bansode and Chavan, 2013). Not only are these fruit juices cost-effective but also with disassociated side effects, which make them better alternatives for the currently available antibiotics (Bag *et al.*, 2012; Bansode and Chavan, 2013).

Indian gooseberry (*Emblica officinalis*) commonly referred as "Amla" fruit has been traditionally associated with numerous health benefits. It is described as light green, round to transversely spherical fruit with astringent taste (Rudrappa, 2009). Indian gooseberry has been reputed to contain the highest proportion of vitamin C and antioxidants (Shishoo *et al.*, 1997). Other health benefits reportedly include diuretic activity, anti-diabetic, detoxifying effects, strengthening of body organs, and strengthening of immune cells among others (Facts, 2016).

Another fruit used in traditional medicine is the wild apple located in parts of eastern Asia. Wild apples (*Docynia indica*) vary from their domesticated counterparts in being one-third their size and are bitter to sour in taste

(Rymbai *et al*., 2016). It is known as crab apple in English and locally as "Sohphoh Khasi." It is popular and regarded as the "health fruit" among the tribes of Meghalaya and Nagaland. The medicinal benefits accredited include stimulation of appetite, improved digestion, reduction in bloating, treatment of heartburn, controlling high blood pressure, and anti-diabetic activities (NagalandPost, 2012; Rymbai *et al.,* 2016).

These fruit juices with their nutritional benefits are known to exhibit antimicrobial activity. This feature roots for an important strategy in the establishment of alternative therapies to treat infection or avoid critical side effects associated with antibiotic therapies as these juices are completely natural in the source. Therefore, the present study was undertaken to evaluate the antimicrobial activity of the fruit juices of gooseberry and wild apple grown in northeast India.

MATERIALS AND METHODS

Fruit Juices

The gooseberry and wild apple fruit juices were obtained from Exotic Juices Pvt. Ltd., Manipur, India.

Test Cultures

Pure cultures of Gram-negative organisms: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Enterobacter aerogenes* ATCC 13048, *Proteus mirabilis* ATCC 21100, *Salmonella abony* ATCC 6017, *Shigella boydii* ATCC 8700, *Pseudomonas aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603(Extended Spectrum β-Lactamases, ESBL); Gram-positive organisms: *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 43300 (methicillin-resistant *S. aureus,* MRSA), and *Enterococcus faecalis* ATCC 51299 (vancomycin-resistant enterococci, VRE); fungal organisms: *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 were included in the study. The sources of these cultures were NCCS, Pune, India and HiMedia Labs Pvt. Ltd, Mumbai, India.

Screening for Antimicrobial Activity

The fruit juices were screened for its antibacterial and antifungal activities using agar well diffusion method (Hindi and Chabuck, 2013; Momand *et al.,* 2014). The antibacterial activity was assayed using Mueller-Hinton (MH) agar while Sabouraud dextrose agar was used for assessment of antifungal activity. Molten media containing 100 µL of test inoculum (1.5×10^8 cfu/mL) were poured into sterile Petri dishes and allowed to solidify. Wells of 6 mm diameter were bored and test samples were subsequently added to each well. The test samples included unfiltered fruit juice (UFJ) and particles of fruit juice. Furthermore, standard antimicrobials, amikacin (30 µg/mL) for antibacterial activity, and ketoconazole (60 µg/mL) for antifungal activity were included and sterile distilled water was used as the negative control for both. The plates were refrigerated for 1 h to diffuse the samples and incubated at 37°C for 24 h for antibacterial and at room temperature for 48 h for antifungal activities. The inhibition zone diameter of the test samples was measured and compared with the corresponding standard drugs to determine its efficacy. The % activity was calculated with the below formula:

Formula (Rojas *et al.,* 2006):

$$\%\ \text{Activity} = \frac{\text{Zone diameter of test sample - Zone diameter of negative control}}{\text{Zone diameter of standard}} \times 100$$

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the fruit juices showing antimicrobial activity were determined using broth dilution method (Pandey *et al.,* 2011; Hindi and Chabuck, 2013). The confirmation of both MIC and MBC of the fruit juice/s against all test bacteria was determined by growth on solid media (Jayana *et al.,* 2010; Pandey *et al.,* 2011).

Dilutions of the test samples using double strength MH broth in the case of UJF and single strength MH broth for particles were prepared in required concentration. The UFJ was analyzed at 100%, 75%, 50%, and 25%; while the particles were used at 800 mg/mL, 600 mg/mL, 400 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL concentrations. An inoculum of 100 µL of test organism (1.5×10^8 cfu/mL) was added to each concentration and incubated at 37°C for 24 h. Post incubation, all the samples were plated onto MH agar plates.

RESULTS AND DISCUSSION

In the wake of increasing drug resistance toward existing antimicrobials, evaluation of alternate natural antimicrobial agents is the need of the hour. With their highly documented uses in ancient medicine, gooseberry and wild apple were easy choices. In the present study, the commercially available gooseberry and wild apple fruit juices from Exotic juices Pvt. Ltd. were tested for their antimicrobial properties.

The acquired fruit juices were slightly acidic in nature. The clear supernatant (unfiltered) juice and pulp (particles) were individually assessed for their antimicrobial activity.

Screening Using Agar Well Diffusion Method

The fruit juices were initially screened using agar well diffusion method for their antibacterial and antifungal activity (Figures 1 and 2). Amikacin and ketoconazole were used as standards for antibacterial and antifungal activities, respectively. Amikacin, an aminoglycoside is a potent broad-spectrum bactericidal agent effective against a wide range of infections (Gonzalez and Spencer, 1998). Ketoconazole is a derivative of imidazole and has been documented to be active against *Candida* and *Aspergillus* (Robinson *et al.,* 2011; Spampinato and Leonardi, 2013).

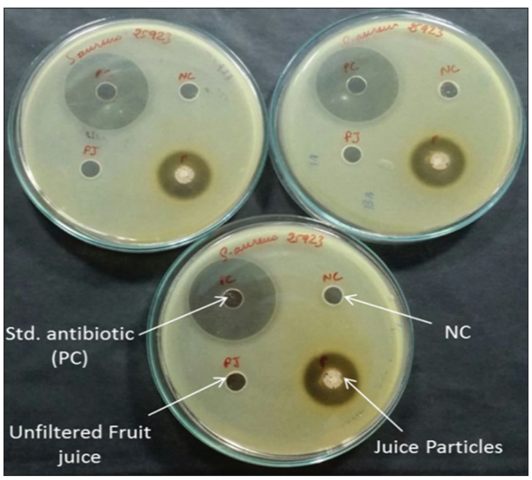

Figure 1: Screening of antimicrobial activity of gooseberry fruit juice (Legend: PC-positive control and NC-negative control)

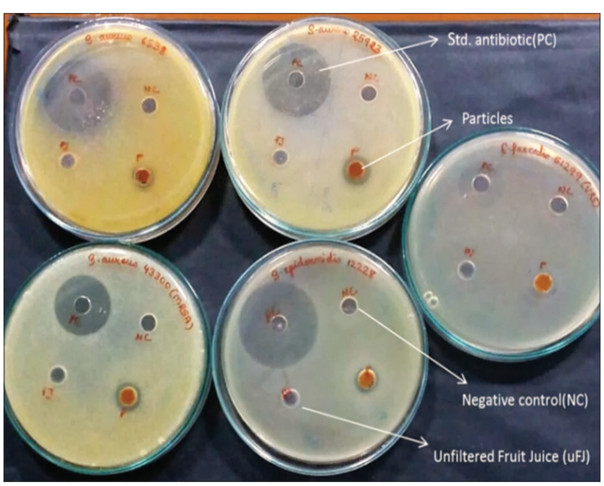

Figure 2: Screening of wild apple juice and particles against Gram-positive organisms

The zone diameter of Amikacin observed against the test bacteria were within the range specified by CLSI (CLSI, 2014). The activities of test samples (unfiltered fruit juice and particles) were determined in percentage by comparing its activity with amikacin for bacterial cultures and ketoconazole for fungal cultures.

A broad-spectrum antibacterial activity was exhibited by both the juices. The activity of UFJ was observed against selected organisms while particles exhibited activity against all test organisms (Figures 3 and 4). In the case of wild apple, no activity was exhibited by the unfiltered juice while the particles showed activity against all the test bacteria (Figures 5 and 6).

Anti-fungal activity was not observed against the two test organisms for yeast and mold namely *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404. However, there were other studies that report activity of gooseberry against *C. albicans* (Ahmad and Beg, 2001). Similarly, the activity of gooseberry against *Aspergillus* had been described by some studies and contradicted by others (Hasan *et al.,* 2016). The plant source, part of the plant used, a method of extraction and solvent of extraction plays an important part in case of activities (Zlotek *et al.,* 2015). The anti-candida activity

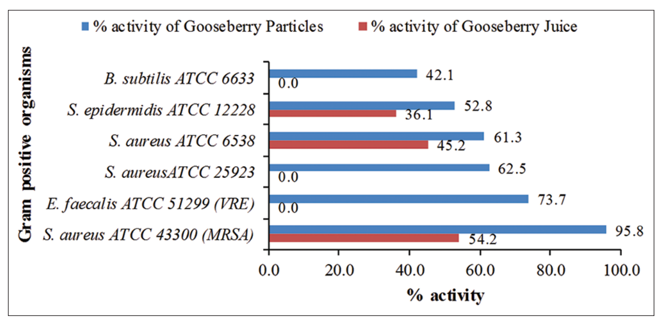

Figure 3: Activity of gooseberry fruit juice and particles against Gram-positive organisms

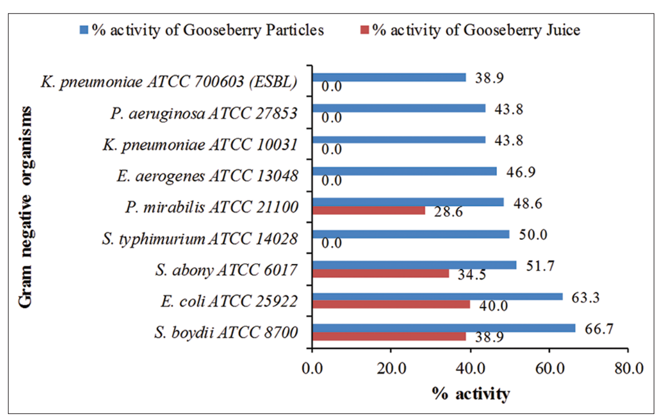

Figure 4: Activity of gooseberry juice and particles against Gram-negative organisms

may be therefore associated with a different source or extract.

MIC and MBC

The MIC and MBC were determined for both the UFJ and its particles. The juices in its neat concentration did not support the sustenance of any organisms. This may be attributed to the acidic nature of the juices. The MIC of the wild apple particles ranged from 50 to 400 mg for Gram-positive organisms and 50-800 mg for Gram-negative organisms. The MIC of the fruit juices has been tabulated in Tables 1-3. The MIC and MBC were confirmed by inhibition of growth on media plates (Figure 7).

In the present study, the fruit juice and particles of both gooseberry and wild apple showed strong activity against most of the tested bacteria. The results were compared with standard antibiotic drugs. Numerous mechanisms of antimicrobial activity of the fruits juices have been described. Although the exact target and mechanisms of the antimicrobial actions are not completely elucidated, the antimicrobial activity is mainly associated with the polyphenols and organic acids (Negi, 2012). Furthermore, in the case of natural products, identification of the specific site of action is difficult as various interacting reactions occur simultaneously (Davidson, 2001).

In the present study, gooseberry exhibited a higher antibacterial activity as compared to wild apple. Gooseberry fruits reportedly have a high fraction of hydrolysable tannins such as emblicanin A and B, punigluconin, and pedunculagin among others (Sawant *et al.,* 2012; Dasaroju and Gottumukkala, 2014). Studies have previously reported the presence of alkaloids such as phyllemblin; flavonoids such as kaempferol and phenolic compounds including ellagic acid and gallic acid (Dharmananda, 2003; Habib-ur-Rehman *et al.,* 2007). The phytochemicals of wild apple have also been studied earlier and it is reported to contain high amounts of phenolic compounds, mainly, gallic acid and rutin-like flavonoids (Shende *et al.,* 2016). The variation in the polyphenol and organic acid content has been attributed to seasonal and regional variations. The gooseberry and wild apple juices in this study are from a highly conserved region the north east region of India.

The antimicrobial activity of gooseberry and wild apple may be accredited to their phenolic compounds and flavonoids (Cowan, 1999). Phenols influence the protein to lipid ratio, membrane functioning, and ion channels while the catechins are known to disrupt the

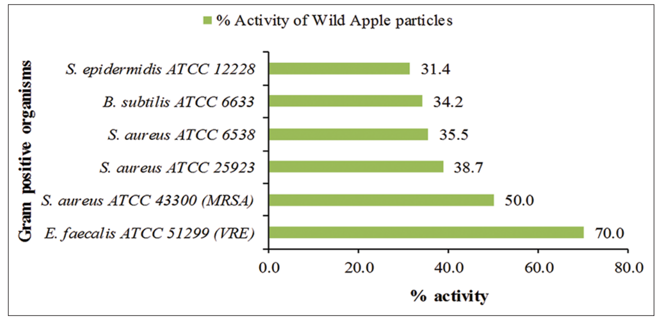

Figure 5: Activity of wild apple particles against Gram-positive organisms

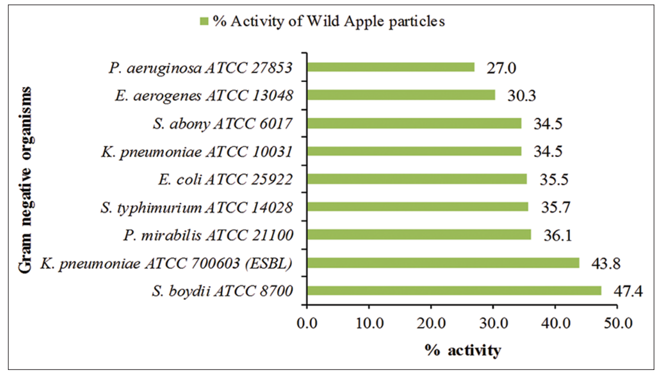

Figure 6: Activity of wild apple particles against Gram-negative organisms

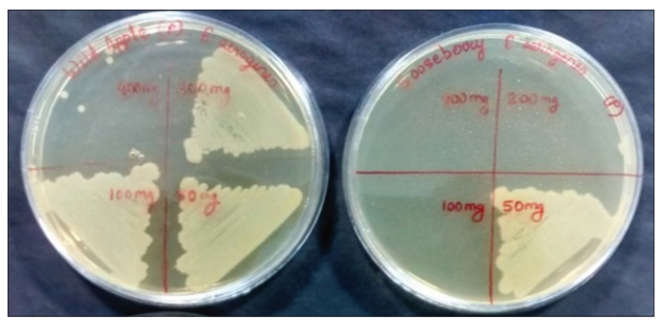

Figure 7: Minimum bactericidal concentration of particles

membrane integrity of the lipid bilayers. The flavonoid rutin present in wild apple has been reported to inhibit topoisomerase type II (Cushnie and Lamb, 2005). Kaempferolis known to have a high antibacterial activity against multidrug-resistant organisms such as MRSA and VRE (Xu and Lee, 2001). The antibacterial activity of kaempferol has been associated with the inhibition of protein kinase C (Shohaib *et al.,* 2011). Furthermore, alkaloids are thought to affect the genetic material in microorganisms thereby contributing to their antimicrobial activity (Negi, 2012).

The activity of both juices was observed against Gram-negative and Gram-positive organisms, with no particular

Table 1: MIC and MBC of gooseberry and wild apple fruit juices (unfiltered juices)

Test organism		Gooseberry fruit juice					Wild apple fruit juice				
		100%	75%	50%	25%	MIC/MBC (%)	100%	75%	50%	25%	MIC/MBC (%)
Gram-positive	*S. aureus* ATCC 25923	-	-	-	-	<25	-	-	-	+	>25-50
	S. aureus ATCC 6538	-	-	-	-	<25	-	-	-	-	<25
	S. aureus ATCC 43300	-	-	-	+	>25-50	-	-	+	+	>50-75
	S. epidermidis ATCC 12228	-	-	-	-	<25	-	-	-	+	>25-50
	E. faecalis ATCC 51299	-	-	-	+	>25-50	-	-	+	+	>50-75
	B. subtilis ATCC 6633	-	-	-	-	<25	-	-	-	-	<25
Gram-negative	*E. coli* ATCC 25922	-	-	-	-	<25	-	-	++	+++	>50-75
	K. pneumoniae ATCC 10031	-	-	-	-	<25	-	-	++	+++	>50-75
	K. pneumoniae ATCC 700603	-	-	-	-	<25	-	-	+	+++	>50-75
	E. aerogenes ATCC 13048	-	++	++	++	>75	-	-	-	+++	>25-50
	S. typhimurium ATCC 14028	-	-	-	++	>25-50	-	-	++	+++	>50-75
	S. abony ATCC 6017	-	-	-	++	>25-50	-	+	++	+++	>75
	P. mirabilis ATCC 21100	-	-	-	-	<25	-	-	-	-	<25
	S. boydii ATCC 8700	-	-	-	-	<25	-	-	-	++	>25-50
	P. aeruginosa ATCC 27853	-	-	-	++	>25-50	-	-	-	+++	>25-50

+++: Luxuriant growth, ++: Growth, +: Few colonies, -: No growth, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Table 2: MIC and MBC of wild apple and gooseberry fruit juice particles against Gram-positive organisms

Test organism	Gooseberry particles (mg/mL)				Wild apple particles (mg/mL)			
	400	200	100	50	400	200	100	50
S. aureus ATCC 25923	-	-	+	++	-	+	+	++
S. aureus ATCC 6538	-	-	-	-	-	+	+	++
S. aureus ATCC 43300	-	-	+	++	-	++	++	++
S. epidermidis ATCC 12228	-	-	-	++	-	-	++	+++
E. faecalis ATCC 51299	-	+	++	++	-	+	++	++
B. subtilis ATCC 6633	-	-	-	-	-	-	-	-

+++: Luxuriant growth, ++: Growth, +: Few colonies, -: No growth, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Table 3: MIC and MBC of wild apple and gooseberry fruit juice particles against Gram-negative organisms

Test organism	Gooseberry particles (mg/mL)				Wild apple particles (mg/mL)				
	400	200	100	50	800	600	400	200	100
E. coli ATCC 25922	-	-	-	++	-	++	+++	+++	+++
K. pneumoniae ATCC 10031	-	-	-	++	-	-	+++	+++	+++
K. pneumoniae ATCC 700603	-	-	++	++	-	-	+++	+++	+++
E. aerogenes ATCC 13048	-	-	-	+++	+	++	+++	+++	+++
S. typhimurium ATCC 14028	-	-	+	+++	-	++	++	+++	+++
S. abony ATCC 6017	-	-	-	++	-	-	++	+++	+++
P. mirabilis ATCC 21100	-	-	+	++	-	-	-	-	++
S. boydii ATCC 8700	-	-	-	+	-	-		++	+++
P. aeruginosa ATCC 27853	-	-	-	+	-	-	++	+++	+++

+++: Luxuriant growth, ++: Growth, +:Few colonies, -: No growth, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

pattern. Previous studies have also reported a broad-spectrum antibacterial activity in gooseberry and wild apple fruit extracts (Ahmad and Beg 2001, Shende *et al.,* 2016). However, some remarkable results indicated that the juices and its particles had higher activity against the drug-resistant variants of bacteria such as MRSA, VRE, and ESBLs. This may be explained by the fact that the acquisition of drug resistance does not increase the innate virulence of the organism due to the cost of fitness incurred (Beceiro *et al.,* 2013).

The activities of the fruit juices observed in the present study were in its consumable form, unlike many previous studies wherein activity of crude extracts had been tested. Overall, this study highlights the antibacterial efficacy of wild apple and gooseberry fruit juices.

CONCLUSION

The activity of the gooseberry and wild apple fruit juices against enteric, gastric, respiratory, urinary, and skin bacteria makes them highly potential to be proposed as a good natural product to supplement the treatment of such infections. Furthermore, the efficacy of juices against these bacteria makes it broad-spectrum anti-infective of a natural source without any inherent side effects, unlike chemotherapeutic agents.

Plausible treatment of infections of the drug-resistant bacteria by natural products such as wild apple and gooseberry fruit juices can be achieved, but extensive studies need to be done before implementing such therapies. Furthermore, the synergistic activity of commonly used drugs may be evaluated. Nevertheless, regular consumption shall always be beneficial with its other benefits and check the control of such infections making it a good natural resource of supplemental therapy.

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