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Dual role of select plant based nutraceuticals – as antimicrobial agents to mitigate food borne pathogens and as food preservatives

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The present work displays the unique dual role of commercially important nutraceuticals from plants where they potentiate the therapeutic effect of commercial antibiotics to combat food pathogens. They also effectively enhance the food preservative value of nisin by doubling the shelf life of fruit juices.

Many food stuffs have the nature of getting spoiled over a time period and therefore require protection. Food spoilage is another purview of concern caused by bacteria and fungi. Apparently, the microbes that spoil food also affect humans and are termed food pathogens which later can become serious threats and cause wide outbreaks of critical diseases. The fact of demand that food stuffs should be preferably fresh, minimally processed, ready-to-eat and with better shelf life poses a major challenge for food industry[1]. Adding to these challenges is the safety of absolutely essential preservatives added to protect these foods which otherwise becomes impossible without their use. One such destiny of safe protecting molecules are nutraceuticals which are industrial products used in/as food for their nutritive/therapeutic value. They could be nutrients, plant products or dietary supplements. In 2013, the commercial growth of dietary supplements was 19.5 % per year and plant products at 11.6 % estimating a global nutraceuticals market of USD 117 billion[2]. Natural products as food preservatives have long been researched due to their relative safety and additive value such as nutrition, colour, taste and as antioxidants. They are thus preferred by consumers over artificial preservatives such as butylated hydroxytoulene (BHT) due to their carcinogenic property[3]. Therefore, natural products from plants are now being heavily trialled as food preservatives in various food stuffs[4].

On the other hand, these safe plant nutraceuticals can also be researched in managing food borne illnesses. The structural diversity and complexity of these molecules make the microbes puzzling to implement antimicrobial evasion strategies, commonly termed as 'drug resistance'. Thus, these molecules have dual utilities as drugs in pharmaceutical and as preservatives in food industry realms.

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† *Electronic Supplementary Information (ESI) available: Description of experimental procedure, Tables of Antimicrobial and synergistic profiles, Tables of 5 log reduction studies, Time kill curves, growth curves of microbes in fruit juices*
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Shikonins and their stereoisomers, alkannins are commercially important naphthoquinones which are projected to be used as nutraceutical food supplements[5]. They are also extensively used as a food colour (additives), fabric dye and in cosmetic formulations[6]. Another nutraceutical molecule of high demand is Ursolic acid (UA), a pentacyclic triterpene which is commonly used as dietary supplements for body builders[7]. It has been used in commercial body building supplements such as Labrada®, Premium powders® etc. On par with above molecules is Vasicine, a long discovered alkaloid molecule from leaves of *Adathoda vasica*. It has always been used as potential therapeutic agent as bronchodilator and other respiratory illnesses[8]. However, it is also been used as a dietary supplement (Morpheme remedies®, Vasaka and Himalaya® herbals, Vasaka) to treat allergic respiratory problems[9].

Even though the above described plant metabolites have been established commercially for their utility in food industry as nutraceuticals or food additives, their bioactive properties such as antimicrobial potential especially against food pathogens needs further in-depth exploration. This indeed might provide insights concerned with the utility of these molecules in realms of therapy and food preservation.

The major limitation for the use of plant products as food preservatives is because, their amounts added in food stuffs exceeds organoleptic acceptance by consumers. Examples of such substances are essential oils which even though approved as safe, bring out changes in odours and flavours in food stuffs[10]. Therefore, it becomes necessary to bring down the concentration of added natural preservatives to acceptance level. Such applications could be possible with use of combinations which could not only bring down the amount but also could keep the microbial resistance towards the added preservatives at check

Thus, the present study initially explores the synergistic antimicrobial profile of stated select nutraceuticals isolated from different plants, along with commercial antibiotics to combat common food pathogens, thus evaluating their utility in pharmaceutical domain. Further insights were interpreted based on studies of these safe antimicrobial nutraceuticals in preserving fruit juices (Apple, orange and red grapes) which are one of most common and easily spoiled food stuffs. Studies were conducted in both commercial pasteurised real fruit juices and freshly prepared domestic juices and the effect of these nutraceuticals in extending the shelf life of the products were determined, thus exploring their utility in food industry.

To the best of our knowledge, there has been no previous report on use of these nutraceuticals explored for their synergistic antimicrobial profiling against food pathogens, and use of these plant

based nutraceuticals as food preservatives to extend the safety and shelf life of fruit juices.

Initially, we observed a commercial food supplement molecule β-Sitosterol-D-glucopyranoside from *D. bipinnata*, which worked synergistically with commercial antibiotics to combat common human pathogens[11]. Inspired by these observations, we isolated 4 more plant based nutraceuticals Acetyl shikonin (AS) and β,β dimethylacryl shikonin (BDMS) from *A. nobilis*, Vasicine from *A. vasica* and Ursolic acid (UA) from *C. buxifolia*†. These compounds were identified and confirmed based on spectral data and optical rotation studies (for shikonins). The spectral data of shikonins correlated with previous reports[12] and also for vasicine[8] and ursolic acid[13].

Antimicrobial studies were performed on both standard strains (Microbial Type Culture Collection, Chandigarh, India) and laboratory derived stable isolates obtained from spoilt food stuffs, juices and maintained in Nutrient agar slants at 4°C. All procedures for determination of Antimicrobial activity were done and inoculum size was standardized according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2015)[14]. Mueller Hinton Broth (MHB) was used to prepare inoculum and grown in incubator orbital shaker at 37°C for 4-8 h until the cultures attained turbidity of 0.5 McFarland Unit containing standardized inoculum size of 5×10^5 CFU ml^{-1} throughout the experiments. Minimum inhibitory concentrations of isolated compounds against food pathogens were carried out in triplicate using Resazurin Microtitre Assay (REMA)[15] with some modifications†. Both minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC) were determined for all the compounds against food pathogens.

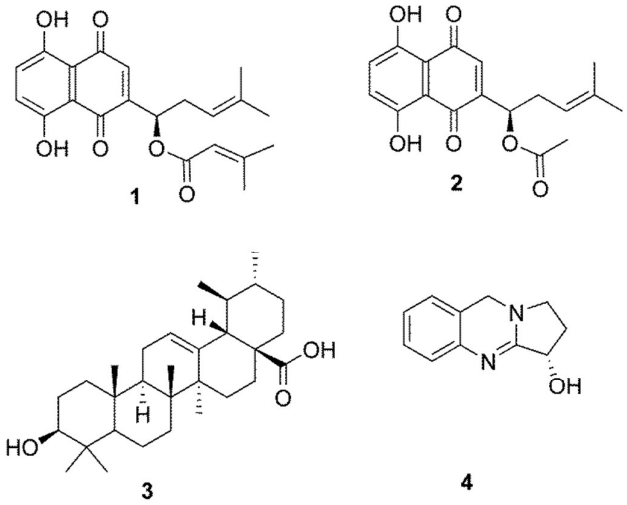

Fig. 1 β,β-dimethylacryl shikonin (**1**), Acetyl shikonin (**2**), Ursolic acid (**3**) and Vasicine (**4**).

To study the interaction of isolated bioactive compounds with other antimicrobial agents, combinations of the compound with commercial antibiotics were evaluated by the checkerboard test[16]. Pure compound combined with antibiotics at concentrations ranging from 1/32 × MIC to 4 × MIC were prepared in MHB with standard inoculum size of 5×10^5 CFU ml^{-1}. The fractional inhibitory concentration index (FICI) was found as the sum of the FICs of each of the drugs. FIC is defined as the MIC of each drug used in combination divided by the MIC of the drug when used alone. The interaction was defined as synergistic if the FIC index was less than or equal to 0.5; additive if the FIC index was greater than 0.5 and less than or equal 1.0; indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0. All experiments were done in triplicates and data represented in arithmetic average. Since, further studies were designed to apply these nutraceuticals as food preservatives, their synergistic profile were found with nisin which is an approved food preservative used commercially. Also, time kill curve analysis was done to evaluate the rate of bacterial inhibition by compound-antibiotic combinations alone since individual compounds had only moderate antimicrobial activity†.

To explore the applicability of selected compounds as antimicrobial agents, their antimicrobial profiles should be assessed. The MIC and MBC of selected compounds against common food pathogens have been depicted in Table S1†. The observations indicate that vasicine has better antimicrobial activity than other tested compounds with better MIC range. Acetyl shikonin also worked better compared to BDMS and UA. The activities were observed in both Gram positive and Gram negative strains of bacteria. Both standard strains and laboratory derived food isolates were used to observe the consistency of the activities obtained. The activities were compared to nisin, a bacteriocin peptide and allicin, a natural plant based antimicrobial agent. Nisin has been projected commercially as a food preservative since few years, where it is commonly used in preserving fruit juices and has been approved by FDA, USA[17]. Allicin is a natural compound found in extracts of garlics and is also being implemented to be used as food preservative since it is safe and highly antimicrobial[18]. Since, nisin is ineffective against Gram negative strains, no effect was found, whereas other compounds had wide antimicrobial activity.

Many natural compounds with less or moderate activity could work better when combined with other drugs[19]. Therefore, compounds in present study were also combined with commercial antibiotics and observations were noted. Surprisingly, all compounds depicted striking increase in their activity profiles against food pathogens. Most of the synergistic combinations were observed with ciprofloxacin followed by methicillin. These results are depicted in Table S2†. For example, both the shikonins and vasicine were observed to be synergistic with ciprofloxacin against *Staphylococcus aureus*. It was noted that of all the synergistic combinations observed, some were 1:4 and others were 1:1. No other ratios of combinations were found to be synergistic. Also, no antagonistic effect was found between compounds and antibiotics against any of the pathogens. These results thus depict the possible effect of these nutraceuticals which could be prescribed as supplements in order to increase the efficacy of antibiotics in managing food borne infections and diseases, thus suggesting their use in pharmaceutical industry.

Further synergistic profiling was done in combination with nisin, to analyse the efficacy of compounds to be used as possible food preservatives. The observed MICs of combinations are depicted in Table 1. Most of the synergistic combinations were found to occur against Gram positive bacteria, since nisin is ineffective against Gram negative bacteria. However, one exception was found in case of *Salmonella enterica* where nisin at 64 µg/ml and AS at 64 µg/ml yielded a combined MIC of 16 µg/ml. The calculated FICI value is 0.5. The combination studies against Gram negative bacteria with no clear MIC of nisin were mainly conducted to see whether the combination of nisin with the compound reduces MIC value of compound. Surprisingly, all combinations of compounds with nisin resulted in additive observation (reduction in MIC) except one (synergistic) as described above. In all these studies involving Gram negative bacteria, MIC of nisin was taken as that of compound itself and the experiments were done in a similar manner. Since, individual compounds show moderate activity against pathogens; time kill curve analysis was done for synergistic combinations of nisin with respective compounds. Fig S1† shows the time kill curves for all the four compounds in combination with nisin that yielded synergistic

antimicrobial profiles. The pattern rather follows a similar path where most of the pathogens are killed within 10-15 h with complete inhibition within 24 h. However, *Salmonella enterica*, the only Gram negative synergistic observation had a gradual kill curve pattern where the inhibition was not immediate and followed a linear like pattern with complete inhibition by 24 h.

Pathogens		BDMS	AS	UA	Vasicine
Staphylococcus aureus	C	1:1	1:1	1:1	1:1
(MTCC 96)	M	4	3	3	3
	R	A	S	S	S
Listeria monocytogenes	C	1:1	1:1	1:1	1:1
(MTCC 657)	M	4	3	3	3
	R	S	S	S	S
Bacillus subtilis	C	1:1	4:1	1:1	1:1
(MTCC 441)	M	4	12	8	4
	R	A	S	A	A
Bacillus cereus	C	4:1	4:1	1:1	1:1
(MTCC 1272)	M	12	12	6	6
	R	A	S	S	S
Enterococcus faecalis	C	4:1	4:1	4:1	1:1
(MTCC 439)	M	8	8	8	4
	R	S	S	S	S
Salmonalla enterica	C	4:1	4:1	4:1	4:1
(MTCC 9844)	M	32	16	64	8
	R	A	S	A	A
Escherichia coli	C	4:1	4:1	4:1	4:1
(MTCC 723)	M	16	8	16	8
	R	A	A	A	A
Vibrio cholera	C	1:1	1:1	-	4:1
(MTCC 3904)	M	64	16	32	32
	R	A	A	N	A
Klebsiella pneumonia	C	4:1	1:1	4:1	1:1
(MTCC 432)	M	64	16	32	16
	R	A	A	A	A
Shigella dysenteriae	C	4:1	4:1	-	1:1
(ATCC 23513)	M	64	8	512	8
	R	A	A	N	A
Pseudomonas aeruginosa	C	1:1	1:1	1:1	4:1
(MTCC 1688)	M	128	16	128	16
	R	A	A	A	A

Table 1: Synergistic antimicrobial profile of compounds in combination with nisin. C- Concentration ratio of nisin : Compound, M- MIC of combination (µg/ml), R- Result. S- Synergy, A- Additive, N- No interaction. BDMS- β,β dimethylacryl shikonin, AS- Acetyl shikonin, UA- Ursolic acid. For Gram negative bacteria, MIC of nisin was taken as equal to MIC of compound in combination against respective bacterium.

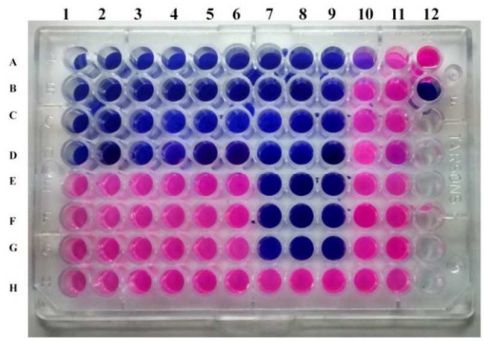

Fig. 2 Resazurin Microtitre Assay (REMA) for determining MIC of vasicine against one of the tested food pathogens, *Enterococcus faecalis*. Lanes 1-3: Vasicine, Lanes 4-6: nisin, Lanes 7-9: Allicin. Lane 10, 11: DMSO (10% v/v). A12: Negative control, B12: Un-inoculated blank, Blue colour: No microbial growth, Pink color: Microbial growth. MIC: 32 µg/ml (Vasicine, nisin), 4 µg/ml (Allicin).

Since, synergistic action of isolated molecules with nisin worked well in inhibiting food pathogens, the curiosity to push the isolated compounds to their maximum utility in food industry and to perceive their bioactivity with different dimension drove the experiments where the compounds were designed to act as food preservatives to extend the shelf life of common consumer preferred fruit juices- Apple, Orange and Red grapes. Both pasteurised commercial fruit juices (Tropicana® Pure premium and Dabur Real®) and fresh domestic juices were considered for the studies.

Initial experiments were done on fruit juices by plating these fruit juices onto Nutrient agar plates after regular serial dilution technique. The microbial plate counting was done to find the initial amount of microbes present in the juices. 5 log reduction studies† were done with two Gram positive strains, *S. aureus* and *L. monocytogenes*; and two Gram negative strains *S. enterica* and *E. coli* inoculated into fruit juices at 4-5 × 10^5 cells/ml. Concentrations of compounds at 4 × MBC – 1/32 × MBC were added to different sets of juices. Combinations with nisin were also experimented where combination ratios were taken from previous synergistic profile studies. The juices were kept at room temperature for 24 h. Then, aliquots were taken and plated onto nutrient agar and the concentrations which gave 5 log reductions over 24 h were tabulated. To juices with no initial microbial contamination, an initial inoculum of 4-5 ×10^5 CFU/ml of select pathogens, *L. monocytogenes,* and *S. enterica* was added in fruit juices. Various concentrations of compounds which gave 5 log reductions over 24 h were added to different sets of juices and growth curve analysis was done for 14 days. Further studies were done to check the extension of shelf life of juices. In this study, isolated compounds and nisin-compound ratios at various concentrations that gave 5 log reductions were added to 50 ml of juices. Then microbial counts were performed by taking aliquots from juices at regular time intervals (every 12 h). For Un-pasturized juices, studies were conducted for 7 days and for pasteurized juices it was 15 days. The juices were maintained at room temperature and a similar set of juices were maintained under refrigeration at 4°C. The actual food preservation profiles were tabulated through this study.

The major striking applicability of the current study, is the application of these compounds in real fruit juice models, thus to find its direct effect in extending the shelf life of juices. Real fruit juices such as Tropicana® Pure premium and Dabur Real® are pasteurised food industrial products that contain no added preservatives[20]. If opened, the left over juice stays uncontaminated for 7-10 days only under refrigeration[21]. This is due to the fact that once opened, the sterile internal atmosphere of the package is tampered which leads to entry of food spoilage microbes. The reason that these juices do not contain preservatives is because of lower preference by consumers over the safety of the added preservatives. Therefore, the present study was designed to determine any possible extension in shelf life of pasteurised and non-pasteurised fruit juices under normal and refrigerated conditions.

Initial plating techniques revealed that pasteurised fruit juices contained no bacterial colonies. Standards defined by Food and Drug Administration, USA states that any food preservative should be able to produce 5 log reductions of microbes under standard conditions for any food stuff tested[22]. Thus, concentrations of compounds and their combinations with nisin that depicted 5 log reductions within 24 h were tested. The nisin + compound combination ratios were prepared based on results depicted in Table 1. The 5 log reduction observations are depicted in Table S3†. Since, number of cells of *S. aureus* did not exceed 10^2 CFU/ml throughout the experiment due to antimicrobial action of tested compounds; the risk that it might produce enterotoxins in juices was limited. This is because *S. aureus* produces toxins only at microbial load of >10^3 CFU/ml[23]. One striking observation was that the amount of compounds or compound-nisin combinations that gave 5 log reductions was less.

Juice	Fruit	Control		BDMS		AS		UA		VN		N		N+BDMS		N+AS		N+UA		N+VN	
Room temperature (21°C)																					
Fresh		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Apple	0	1	0	1	0	1	0	1	0	2	0	3	0	4	0	4	0	3	0	5
	Orange	0	1	0	1	0	1	0	1	0	2	0	3	0	4	0	4	0	4	0	6
	Grapes	0	1	0	1	0	1	0	1	0	1	0	3	0	4	0	4	0	3	0	5
Tropicana	Apple	2	3	2	3	2	3	2	3	3	4	3	4	4	5	4	5	4	5	4	5
	Orange	3	4	3	4	3	4	3	4	4	5	4	5	4	5	4	5	4	5	5	5
	Grapes	3	4	3	4	3	4	3	4	3	4	4	5	4	5	4	5	4	5	4	5
Dabur Real	Apple	2	3	2	3	2	3	2	3	3	4	3	4	4	5	4	5	4	5	4	5
	Orange	3	4	3	4	3	4	3	4	4	5	4	5	4	5	4	5	4	5	5	5
	Grapes	3	4	3	4	3	4	3	4	3	4	4	5	4	5	4	5	4	5	4	5
Under refrigeration (4°C)																					
Fresh	Apple	0	1	0	2	0	2	0	1	0	3	0	4	0	5	0	5	0	4	0	6
	Orange	0	2	0	2	0	2	0	2	0	3	0	4	0	5	0	5	0	5	0	7
	Grapes	0	1	0	2	0	2	0	1	0	3	0	4	0	5	0	5	0	4	0	6
Tropicana	Apple	3	4	4	6	4	6	4	5	5	8	5	10	6	13	6	13	6	11	7	14
	Orange	4	5	5	7	5	7	5	6	6	10	6	10	7	14	7	14	7	12	8	15
	Grapes	4	5	5	7	5	7	5	7	5	9	6	10	7	13	7	13	7	11	7	15
Dabur Real	Apple	3	4	4	6	4	6	4	5	5	8	6	10	6	13	6	13	6	11	7	14
	Orange	4	5	5	7	5	7	5	7	6	11	6	11	7	14	7	14	7	12	8	15
	Grapes	4	5	5	7	5	7	5	6	5	9	6	11	7	13	7	13	7	11	7	15

Table 2: Preservation profile of compounds and nisin-compound combinations on fruit juices. A-Day at which atleast one CFU/ml was found, B- Day at which CFU/ml doubled the initial value. BDMS- β,β dimethylacryl shikonin, AS- Acetyl shikonin, UA- Ursolic acid, VN- Vasicine, N- nisin.

The permissible amount of nisin added to food stuffs is 250 ppm[17]. Throughout the study, this limit was not exceeded with respect to nisin. Even though compound or their combinations with nisin proved to yield 5 log reductions at certain concentrations within 24 h, it is likely that the juices that contain these agents could be still contaminated over days. This phenomenon is called 'Phoenix effect' where some of the microbial cells survive the long lag phase and regrow after some days. This phenomenon is seen in many food products[24]. Thus, shelf life studies were conducted for 14 days where model microbes *Listeria monocytogenes* (Gram positive) and *Salmonella enterica* (Gram negative) were inoculated in a model fruit juice (red grapes) of Tropicana®. Growth curve analysis was performed in all the fruit juice sets with respective nutraceutical compounds and their combinations with nisin added at 5 log reduction concentrations. Nisin alone served as positive control and juices without any antimicrobial agent was considered as negative control. The observations are depicted in Fig S2†. It was found that only vasicine was able to significantly inhibit microbial colonies of fruit juices at room temperature compared to nisin. However, nisin + compound combinations proved to be beneficial in inhibiting the microbial counts. Initial inoculum of 4 -5 × 10^5 cells were inhibited effectively for first 2 days in many combinations. However, over time, survived microbial cells started to multiply and the recovered amount of bacterial colonies were observed and plotted till 15 days. Remarkable observations such as vasicine + nisin combination worked to reduce and contain the microbial counts significantly. Ursolic acid which depicted moderate antimicrobial activity individually worked well in combination with nisin. Conclusively, nisin with BDMS, AS, UA and Vasicine inhibited microbial colonies in opened fruit juice packages until 7, 7, 8 and 9 days in case of Gram positive bacteria. Whereas, only nisin + vasicine combination inhibited Gram negative microbe until 7 days.

Inspired by the above observations, studies were designed to explore the ability these compounds and their combinations with nisin in actual food preservation. In these studies, the model fruit juices brand considered were two (Tropicana and Dabur) and the fruits considered were 3 (Apple, orange and red grapes). Along with Commercial Juices, domestic unpasteurised freshly prepared juices were also considered to successfully understand the utility of antimicrobial compounds. Table 2 elaborately depicts the effect of isolated nutraceutical compounds in preserving fruit juices. Since, commercial fruit juices were pasteurised, no microbial colonies were present in them as found through microbial plating and subsequent colony counting. However, this was not the case in fresh fruit juices. The amount and type of microbes present varied in different fruit juices. Juices over the study period were maintained under (i) room temperature (ii) Under refrigeration at 4°C. Controls were maintained under similar conditions except that they did not contain any antimicrobial agent. Since, the amount of microbes that could be permitted for safe consumption of fruit juices varies with respect to microbial strain and type of fruit juice; two set points were created to analyse the efficacy of food preservatives. (i) The day at which at-least 1 colony was found to occur in fruit juice. For unpasteurised fresh juices, this day was zero since they already contain microbial colonies due to handling and preparation. (ii) The day at which the initial microbial colony(s) doubled. For example, fresh apple juice contained 225 microbial colonies on initial day. The day at which it became 550 colonies were taken as doubling period. This design could easily interpret the rate at which microbial cells doubled thus accounting to the efficacy of preservative. The compounds and nisin + compound concentrations were added based on results obtained in 5 log reduction studies explained above. It is obvious for control fresh juices that the doubling time was 1 day, since it contained no preservative and were not pasteurised. Therefore, they should be consumed within few hours of preparation or within one day if refrigerated. Vasicine was found to significantly increase the life of uncontaminated Tropicana (orange) by 5 days (RT) and by 10 days at 4°C. nisin was found to preserve Tropicana and Dabur juices up to 5 days (RT) and 10 days (4°C). BDMS preserved pasteurised juices by 3-4 days whereas under refrigeration by 6-7 days. The best combinations which worked out in preserving the juices is nisin + vasicine combination which extended the doubling time up to 15 days (orange and grapes) under refrigerated conditions. An important fact to be considered is that even at day 15, the pasteurised juices still may contain only few amounts of microbes, which makes it still consumable compared to unpasteurised juices that contain significantly large amount of microbes at the day of doubling. For example, in control juices (pasteurised) the amount of microbes

present at day 10 (4°C) (which is regarded safe for drinking by the manufacturers) was around 10^2 cells. These counts were seen only on day 24 in juice containing nisin+ vasicine, which technically suggests that these juices could be consumed till 20-24 days under refrigeration conditions which is 2.5 times greater than initial shelf life. However, these conclusions should be proved with more similar research studies with other pathogenic strains. Since, the molecules studied in present study have been already reported to be less toxic through *in vivo* studies[25], they are therefore safe at concentrations (ppm) used in present study. Thus, it is suggested that the shelf life of commercially available pasteurised juices could be doubled under the similar storage conditions by adding meagre level of safe plant based nutraceutical food preservatives, thereby not only extending the shelf life of food products but also increasing the nutritional and therapeutic quality of foods with relation to increase in food industrial economics and market growth.

Conclusions

In the present study, two napthoquinones (β,β dimethylacryl shikonin and Acetyl shikonin) from *A. nobilis,* Ursolic acid from *C. buxifolia,* and vasicine from *A. vasica* were isolated and their dual role - antimicrobial activity against common food pathogens and food preservative property were determined. Minimum inhibitiory and bactericidal concentrations observed were moderate for individual compounds. Synergistic antimicrobial profiles of these compounds with commercial antibiotics depicted that ciprofloxacin followed by methicillin exhibited synergies in combating food pathogens, thus suggesting their use in managing food borne infections along with antibiotics. Combinations with nisin depicted synergistic profiles with Gram positive bacteria and one Gram negative bacteria (*Salmonella enterica*). Time kill curve analysis performed with synergistic combinations showed that all pathogens were killed within 24 h, however cidal effect was quick in Gram positive bacteria then in Gram negative bacteria. Food preservative challenge studies were performed in real fruit juices to find the concentrations of individual compounds and combinations which gave 5 log reduction of inoculated bacteria. Growth curve studies were performed at these concentrations over inoculated bacteria into juices to find the duration of inhibition. Final studies performed with fruit juices showed that nisin + vasicine combination best worked in extending the life of uncontaminated commercial fruit juices by 5 days at RT and up to 15 days under refrigeration. Thus, it is suggested that above nutraceuticals in combination with nisin could effectively act as food preservatives. However, further studies are required to apply these nutraceuticals to other food stuffs, in combination with other safe food preservatives and testing the antimicrobial action of these preservatives on more pathogenic and resistant food microbes.

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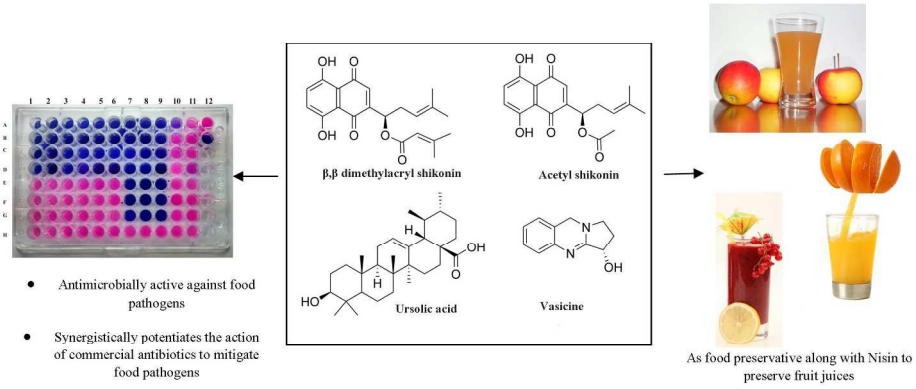

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