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Research Article

Effects of *trans*-Resveratrol Enrichment Mediated Anti-Microbial Effects in Red Grapes and Peanuts

Vasanth K Bhaskara*, Aardra RS, Bhumika S, Syed Umar, Arya Anilkumar, Varun NM, Sheetal V Rao, S Rameen Taj, Ashwini BM and Aniruddha BS

Department of Chemistry and Biochemistry, MS Ramaiah College of Arts, Science and Commerce, MSR Nagar, Mathikere, Bengaluru, India

***Corresponding Author:** Vasanth K Bhaskara, Department of Chemistry and Biochemistry, MS Ramaiah College of Arts, Science and Commerce, MSR Nagar, Mathikere, Bengaluru, India

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Abstract

Resveratrol (RSV) has been evidenced with multiple ways of beneficial effects in cardiac protection and due to its anti-carcinogenic properties. Though its presence was reported abundantly in red grapes, in the recent past many edibles and its processed derivatives are attracting researchers for enriched RSV activities. Indiscriminate use of pesticides is another critical issue having wide variety of impacts. In this present work, authors have tested the enrichment of RSV and its mediated anti-microbial effects in peel (exocarp) and pulp (mesocarp) of red grapes and in steam cooked peanuts. Buffer extracts of red grape peel, pulp, steam cooked peanuts versus raw peanuts were evaluated for anti-oxidant and anti-microbial potentials with respect to their RSV contents. Further, effects of pesticide on anti-oxidant potential of RSV were evaluated under *in vitro* conditions. All the samples were subjected to estimations of moisture content, ash value and total phenols. Organic extracts of respective samples were used to estimate *trans*-RSV by using reverse phase HPLC technique. Reducing potential assay was done to estimate the reduction abilities of buffer extracts of all samples with and without the presence of organophosphorus pesticide in the reaction. Our data shown that, enriched RSV of about 48.2 µg/ml in peel (over 20.36 µg/ml in pulp) and 38.27 µg/ml in steamed peanuts (over 25.89 µg/ml in raw peanuts). Peel and processed peanuts have shown higher amounts of polyphenol contents in their buffer extracts. Zone of inhibition assays revealed effective anti-microbial properties against *Escherichia coli* and *Staphylococcus aureus* for the grape peel and steam-cooked peanut buffer extracts. These results could indicate possible potential effects of peel and processed peanuts associated with enriched RSV in respective samples. However, specific roles of RSV in these effects needs to be further evidenced.

Keywords: Resveratrol; Anti-Microbial Effects; Pesticide; Reducing Power Assay

Introduction

Resveratrol (RSV) has been well established phyto-estrogen reported to have health enhancing effects [1]. However, its therapeutic efficacies and role in lead modifications at present has been under intensive research [2]. RSV was reported as widely distributed in the variety of edibles with its peak levels in red grapes, Japanese knotweed polygonum japonicum, peanuts, mulberries etc [3]. In a study, boiled peanuts have been evidenced with efficient processing that altered phytochemical composition with enriched RSV fraction compared to other methods of processing like dry roasting or oil frying [4]. RSV was considered as a potential therapeutic molecule in clinical trials of diseases including cancer, neurological disorders, cardiovascular diseases, diabetes, non-alcoholic fatty liver disease (NAFLD) and obesity [5,6]. It is also used as a lead molecule in drug discovery, further subjected to lead modifications and screening the betterment effects of RSV derived molecules [7,8].

Resveratrol effects include anti-oxidant, anti-inflammatory, cardioprotective, vasodilatory, hypoglycaemic, anti-angiogenic, immunomodulatory, neuroprotective, anti-carcinogenic and anti-microbial properties [9]. Though RSV is present in wide variety of edibles, up on consumption its effects in the body are limited due to its bioavailability and are subjected to rapid phase-II metabolism [10]. Anti-microbial effects of resveratrol have been mainly emphasized in the treatment to overcome the multi-drug resistance [11]. Though RSV is a non-enzymatic anti-oxidant, pesticides or its remnants could impact RSV's anti-oxidant potential. Indiscriminate use of pesticide has been a challenging problem in the present times with detrimental effects on human health upon its consumption. Thus, it's important to understand the pesticide induced toxicity to estimate further possible and eventual ill effects [12].

In the present study, RSV enriched sample extracts of red grape peel and steam cooked peanuts were checked for anti-oxidant potentials with and without pesticide. This work gives an indication for the toxic effects of pesticides through attenuating the bioactive molecules present in the plants.

Materials and Methods

Preparation of organic extract

Red grapes after purchase, washed and separated for its peel and pulp. Further, peanuts were steam cooked along with its outer shell to boiling temperature until the seeds are soft enough. Then the samples were air/sun dried and homogenized to fine powder. Each of these sample was weighed and taken 10 g into soxhlet apparatus (Borosil) and organic extraction was carried out for ten cycles in methanol solvent on a heating mantle at 65°C. The extracted samples were centrifuged to remove the debris and supernatants were collected and stored at 4°C in the refrigerator.

Preparation of buffer extract

One gram of red grape peel, pulp, raw and steamed peanuts were soaked overnight in 0.2 M Tris HCl buffer, pH 7.2). Homogenization was done separately in 20 ml of pre-chilled 0.2 M Tris HCl buffer, pH 7.2 using cold mortar and pestle. The homogenates were squeezed through cheese cloth and centrifuged at 16,000 rpm for 15 min at 4°C. The clear supernatants were aliquoted and stored at -20°C.

Total phenolic content (TPC) estimation

In all the samples total phenolic anti-oxidants were measured calorimetrically. Total phenolic content was determined as per the method of Singleton and Rossi 1965, using Catechol as standard [13]. Various concentrations of catechol (0.2–1.0 mg/ml in ethanol) of the working solutions were pipetted into test tubes and made up to 1 ml with distilled water. To each tube of standard and samples, 0.3 ml of Folin-Ciocalteau reagent was added. The tubes were mixed and allowed to stand for another 3 min. After adding 1 ml of saturated sodium carbonate (3.5%) reagent to the tubes, they were allowed to stand for 10 min and then the intensity of the blue-color complex was measured at 765 nm. Total phenolic content was calculated as mg/ml catechol equivalents of respective buffer extract samples from the standard graph.

HPLC analysis of *trans*-resveratrol

The mobile phase for RP-HPLC was a mixture of acetonitrile: methanol (4:6) at a flow rate of 1 ml/min. The column used was C18-5µ (4.5 mm x 150 mm). The injection volume was 20 µL, and detection was at 310 nm. Standard solutions of resveratrol (Cat#CAS-501-36-0 CDH, India) (200 µL in 1 ml mobile phase) were prepared, and the sample was prepared in a concentration of 10 mg/25 ml mobile phase. The amount of resveratrol present in the samples were estimated by calculating the area of sample peaks versus standard peak in chromatogram (Azyme Biosciences Pvt Ltd., Bengaluru).

Determination of reducing power

The buffer extracts with reductive potential reacts with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}) in which solution changes to blue, based on the reducing power of the sample. Protein concentration normalized sample extracts were mixed with 1 ml of potassium phosphate and 1ml of 0.01 g potassium ferricyanide to test tubes. Other set of respective sample tubes were estimated for reducing potential by adding organophosphorus pesticide at different concentrations (0.25 M, 0.5 M and 1 M) to the tubes containing respective buffer extracts. Ascorbic acid was used as standard to measure the reducing potential in unknown sample from the standard plot. The tubes were incubated for 20 min at 50°C. After incubation, 1 ml of 0.1 g of trichloro-acetic acid in distilled water was added and the mixtures were centrifuged at

5000 rpm for 10 min. Distilled water (1 ml) and 0.2 ml of 0.001 g ferric chloride added to the upper layer and the absorbance was measured at 700 nm [14]. Higher the absorbance values, higher will be reducing abilities of the extracts. The results are expressed as ascorbic acid standard equivalents in mg/ml per gram of the sample extract protein.

Anti-microbial assay by Zone of inhibition assay

LB agar was used to detect antimicrobial potentials of sample buffer extracts. Agar well diffusion method was used to evaluate the antimicrobial activity of sample buffer extracts. The agar plate surface was inoculated by spreading a volume of the gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterial inoculums over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer, and a buffer extract volume normalized to 100 µg, 200 µg, 300 µg and 400 µg were loaded in the well. Then, agar plates were incubated for 24 hrs to observe the zone of inhibitions. Diameters of concentric rings were measured to assess the anti-microbial potentials of respective samples as per the methodology reported earlier [15].

Results and Discussion

Determination of total phenolic estimations

Total phenolic contents of buffer extract was done by using Folin-ciocalteu (FC) assay. The total phenolics were found to be present at higher levels of 0.06 ± 0.01 mg/ml in peel extracts and in steam cooked peanuts of about 0.07 ± 0.009 mg/ml catechol equivalents. Our results indicate the total polyphenol enrichment in bright colored peel extracts and more bioavailability in the steam cook processed peanuts extracts. The enriched phenolic content may include the resveratrol components as substantiated by our HPLC analyses in grape-peel and peanut-steamed samples.

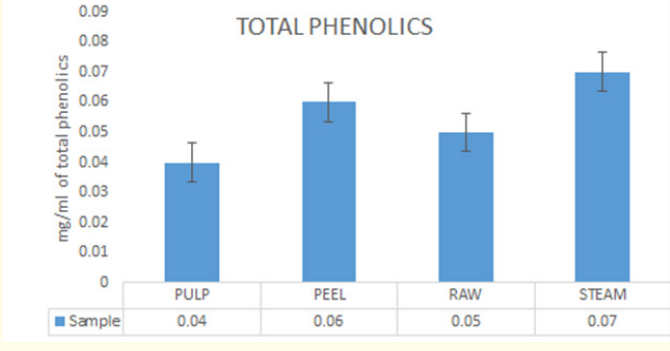
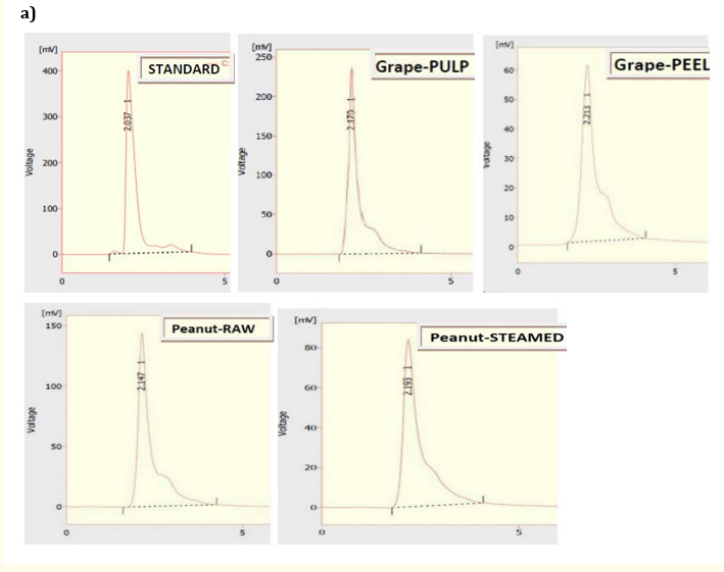


Figure 1: Bar graph showing total phenol content in peel, pulp of grapes and raw and steam cooked peanuts buffer extracts done for three individual experiments. The final calculated values of total phenol in each extract is represented as mean ± S.D. **P < 0.01 for triplicate experimental value variation of respective buffer extract samples.

trans-resveratrol estimations by HPLC

The analysis was made in isocratic mode with the mobile phase acetonitrile and methanol in the ratio 4:6 with the RP-HPLC C-18 column at a flow rate of 1ml/min. The standard resveratrol (2.5 µg/ml) and sample (3.33 µg/ml) were dissolved in 1ml of mobile phase and 20 µl was injected and the elution was monitored at 310 nm. On analysis, peel of grape had maximum content of 48.2 µg/ml compared to pulp and raw peanuts which showed 38.27 µg/ml.

HPLC analyses indicated the enriched resveratrol in peanut-peel and could be more bioavailability due to processing (steam cooking) of peanuts. It was reported that effect of different cooking methods can directly impact on the total phenol content and related anti-oxidant potentials [16].



Sample Name	Sample Area	Standard Area	Amount present in the sample (µg/mL)
Standard	-	8027.423	
Pulp	2043.756	-	20.36
Peel	4836.633	-	48.2
Raw	2598.665	-	25.89
Steamed	3840.931	-	38.27

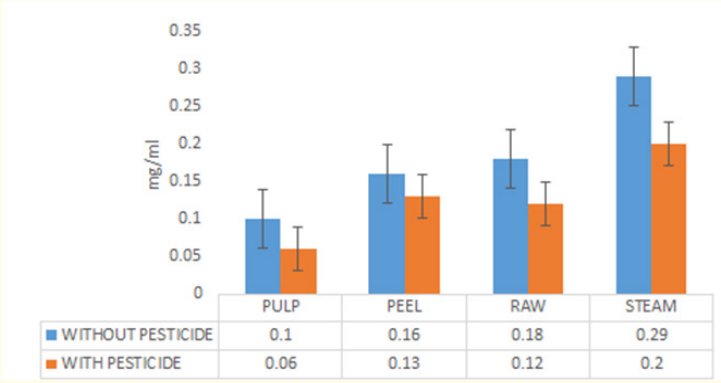
Figure 2: HPLC reverse phase analyses of organic extracts of pulp, peel samples of red grapes and raw, steam cooked peanuts. a) Peak areas of organic extracts with respect to pure resveratrol standard. b) Calculated peak areas corresponding to the volumes of respective sample analytes.

Reducing power estimation without and with organophosphorus pesticides

Reducing power assay of buffer extracts will give an understanding of overall reducing abilities of samples. This method is used to assess the antioxidant potentials of the total compounds present in the samples. Our results are in consistent with reported results of processed peanuts with enriched reducing power abilities [17]. In the literature there is no ample evidence for the effect of pesticides and its remnants on the anti-oxidant potentials of vital plant molecules. In this work, we report that the presence of pesticides can inhibit reducing power of the extract.

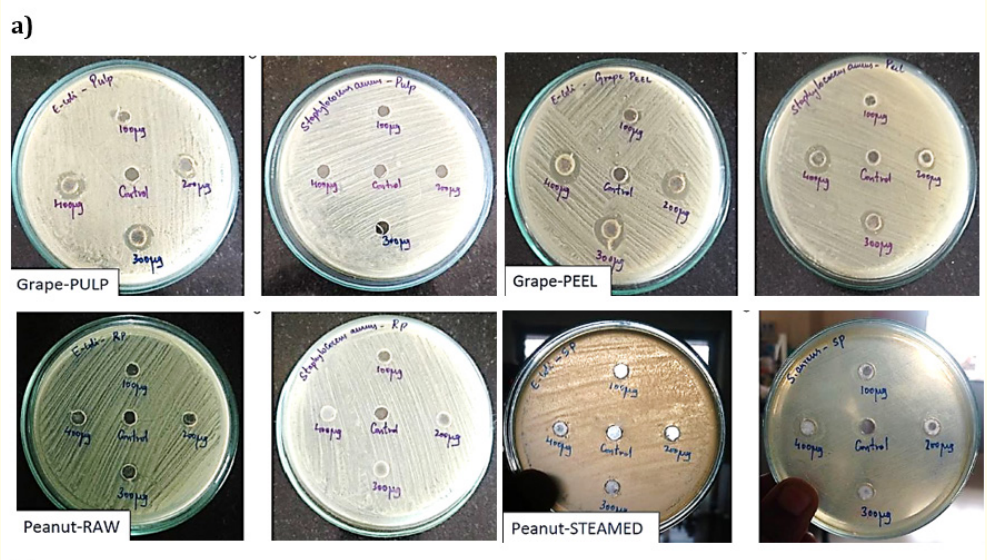
Zone of inhibition assays with buffer extracts of respective samples

Zone of inhibition assay for all the sample extracts has depicted effect of grape peel extract significantly on both the cultures of *E.coli* and *S. aureus*. Whereas in the case of peanut samples, steam cooked peanut buffer extracts have shown inhibitory zone areas in *S. aureus* culture plates. Raw peanut samples have not shown any zones of inhibition at any of the sample concentrations used. This may indicate the enrichment of resveratrol as evidenced by our HPLC analysis and its potential on anti-microbial tendencies. However, this work is limited by the effects of resveratrol alone in the samples.



	PULP	PEEL	RAW	STEAM
WITHOUT PESTICIDE	0.1	0.16	0.18	0.29
WITH PESTICIDE	0.06	0.13	0.12	0.2

Figure 3: Representative bar graph of reducing power estimations of pulp, peel of red grapes and raw, steam cooked peanut buffer extracts without and with pesticide presence. Calculated values of each sample represent three independent repetitions of the experimental values expressed as mg/ml per gram of sample protein ascorbic acid equivalents. Data represent three independent experimental repetitions presented as mean ± SD. **P < 0.01 for triplicate experimental value variation of respective buffer extract samples.



b)

Samples	Bacterial strains	Sample concentrations loaded in the wells			
		100 µg	200 µg	300 µg	400 µg
		Zone of inhibition in mm			
Grape - PULP	*E. coli*	-	12	14	15
	S. aureus	-	-	-	-
Grape - PEEL	*E. coli*	10	14	15	16
	S. aureus	-	11	13	14
Peanut - RAW	*E. coli*	-	-	-	-
	S. aureus	-	-	-	-
Peanut - STEEMED	*E. coli*	-	-	-	-
	S. aureus	-	12	13	15

Figure 4: Zone inhibition assays of buffer extracts. a) Agar plates with inhibitory zones around the sample wells b) Table showing measurements of inhibitory zones measured in millimetres

Conclusion

Resveratrol is one of the potential therapeutic bioactive molecules widely distributed in edibles. Though its cardioprotective and anti-oxidant effects have been well established, its anti-cancer cell and particularly anti-cancer stem cell properties have been under intensive study at present [18,19]. In the present study we could observe enrichment of resveratrol in certain parts of fruit or due to the effects of processing like steaming. Though our study is limited due to the lack of evidencing the effects of resveratrol alone in buffer extracts, its enrichment mediated reducing potentials and further impact of pesticides on same along with respective anti-microbial efficiencies could indicate the possible role of resveratrol role. However, it requires further experimentation which is currently in progress.

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Conflicts of Interest

All the authors declare that there are no conflicts of interest.

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