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Discovering Biochemical, Anti-Oxidant and Anti-Leukemic Profiling of Punica *granatum*, Malus *domestica*, Vitis *vinifera* and Citrus *sinensis*

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Abstract

This research evaluates the extracts of Punica *granatum*, Malus *domestica*, Vitis *vinifera*, and Citrus *sinensis* for their total phenol content, antioxidant capacity, and *in-vitro* anti-leukemic activity. On the basis of phytochemical analysis, it was found that P. *granatum* extract has the highest level of total phenolics, flavonoids, and tannins. From the FTIR spectroscopy, all the extracts contained different functional groups. DPPH, ABTS, superoxide, and nitric oxide scavenging actions holistically showed P.*granatum* extract as the most potent radical scavenging, followed by V.*vinifera*, M.*domestica*, and C.*sinensis*. These studies have demonstrated significant associations between the antioxidant activity and phytochemical concentration. THP-1 and HL-60 anti-leukemic activities showed that all fruits inhibited the cell growth in varying degrees; the highest significant percentage was provided by P.*granatum*. These outcomes present the perspective of these fruit extracts, primarily P.*granatum*, as sources of organic antioxidant and anti-leukemic components. Most of these plants have not been fully explored on their utilisation in medication and the way they work.

Keywords: *Phytochemical, Phenolic Content, Antioxidant activity, Anti-cancer activity*

Introduction

Fruits have been considered one of the vital sources in counters that provide vitamins, minerals, and bioactive compounds. This research focuses on four frequently eaten fruits: Some of the acknowledged fruits are orange (Citrus *sinensis*), grape (Vitis *vinifera*), pomegranate (Punica *granatum*), and apple (Malus *domestica*), and they are known for their different phytochemical composition profiles and possible healthy effects. Punica granatum, also called pomegranate, is a fruit that has attracted major interest among researchers in the recent past due to the high anthem antioxidant and possible anti-cancer properties [1]. It contains polyphenols, which include ellagitannins and anthocyanins besides ellagic acid, and has been linked with so many health qualities, including anticancer qualities and cardiovascular characteristics (Lansky and Newman, 2007). According to a recent scientific analysis, pomegranate possesses antiproliferative and pro-apoptotic properties against leukemia and other malignancies.(Dahlawi et al. 2013). [2]

The apple, scientifically known as Malus *domestica*, is one of the world's most eaten fruits and has many health benefits. Quercetin, catechin, and chlorogenic acid are the polyphenols in apples that are rich in flavonoids and have an antioxidant effect (Boyer and Liu, 2004) [3]. Analysing the available epidemiological data, it is possible to point out that the frequent consumption of apples can guard against numerous chronic diseases, like some types of cancer (Fabiani et al. 2016) [4]. Scientific literature proves that Vitis *vinifera*, nicknamed grape, has the highest polyphenols among the fruits containing resveratrol, anthocyanins, and proanthocyanidins. According to Xia et al. (2010) [5]. It influences the antioxidant properties and probable uses, for instance, increased immunity and cancer-preventing abilities (Chen et al. 2017) [6]. Going by the findings of the present study, the citrus fruits, as much as they did not reveal as many health-related uses as the other fruits did, have been beneficial in many ways.

It is therefore important to have a look at the antioxidant potential of these fruits because of the increased information associated with oxidative imbalance with several different diseases, with an emphasis on cancer. ROS, or reactive oxygen species, and free radicals can also damage biological systems' components through oxidative processes, resulting in mutations and physiological dysfunction. These toxic compounds could possibly be countered by antioxidants, possibly preventing or lessening the undesirable effects (Lobo et al. 2010) [7].

Also, the possibilities of fruit extracts as anti-leukemic agents have been in focus in the present years only. Hematological malignancy adorned as leukemia: the growth of immature white blood cells or stoppage of their maturity is a severe health concern worldwide. Although conventional medicines are said to have enhanced outcomes in several patients, they are still in a constant search for new treatments with fewer complications. Plant-derived compounds, particularly fruit extracts, have opened up a can of interesting chemicals with possibilities of anti-leukemic effects (Mokhtari et al. 2017) [8]. But due to the fact that fruit extracts contain numerous components, which often exhibit synergistic effects, the research of the biochemical characteristics and biological activity of such extracts should be comprehensive. Evaluating the character of numerous chemical ingredients using a number of analytical techniques and bioassays, authors try to uncover the relationships between fruit extracts and their potential health-improving properties. [9,10]

Thus, the outcomes of this research would have some contribution to the understanding of the mechanisms that underpin the observed health benefits of fruit consumption and could potentially be applied to the creation of new functional foods or therapies. Furthermore, while comparing the activities of these four frequently consumed fruits, we try to provide important information that might be useful in further research and in the exploration of new guidelines as to how one might adjust diet to function better and prevent diseases.

Methods and Materials

Preparing Plant Material and Extract

All the four fruits were procured from local markets in Ahmedabad, Gujarat, India and it cost the sum of Indian rupees two thousand only. The fruits were first cleaned with distilled water, and then they were air dried, and finally the pulps were peeled off from the seeds. The M. *domestica* flesh was chopped into small pieces and then homogenized by using a laboratory mixer [11,12]. P. *granatum* and V. *vinifera* were lyophilized overnight prior to the extraction procedures [14] while C. *sinensis* peels were air dried for 15-20 days in the sun. As such, the extracted materials were passed through ‘Whatman No. 1’ filter paper [15]. The concentrated extracts were then refrigerated at 4°C for future use. For P. *granatum*, 5g lyophilized powder was dissolved in 250 ml methanol and was kept on shaker overnight at 600 rpm at room temperature and on the consecutive day, the solvent was allowed to evaporate whereas for V. *vinifera*, 5g lyophilized powder was added in 50 ml methanol and the mixture was subjected to ultrasound sonication at 100 hz at 25°C for 20 minutes and the resultant supernatant was stored while the residue was sonicated for the second time and finally the solvent was evaporated [16].

Fig 3: Extraction of Apple [17]

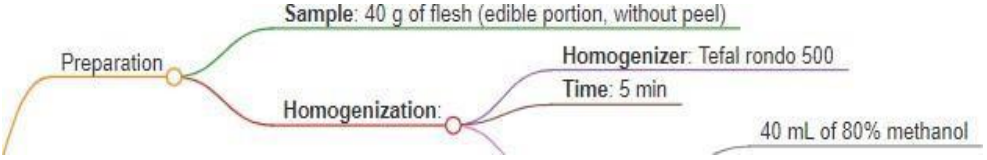
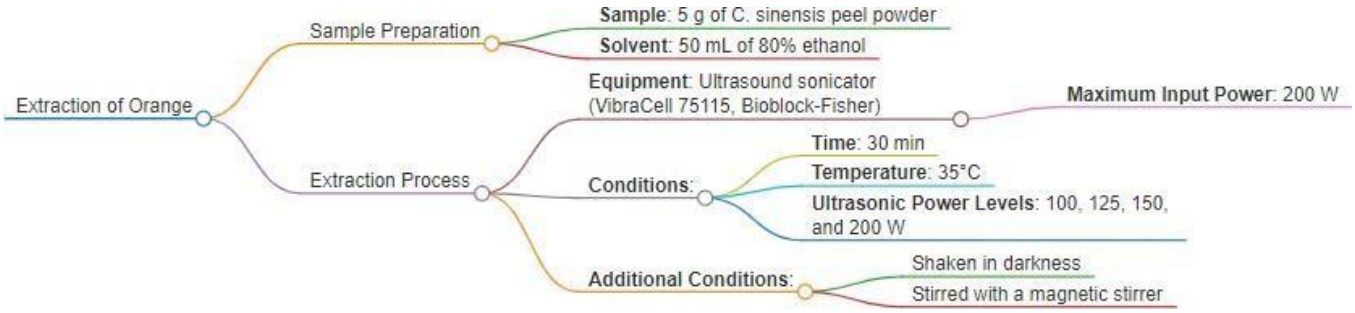


Fig 4: Extraction of Orange [18]



Analysis of Phytochemicals

Fig 5: TPC Methodology:

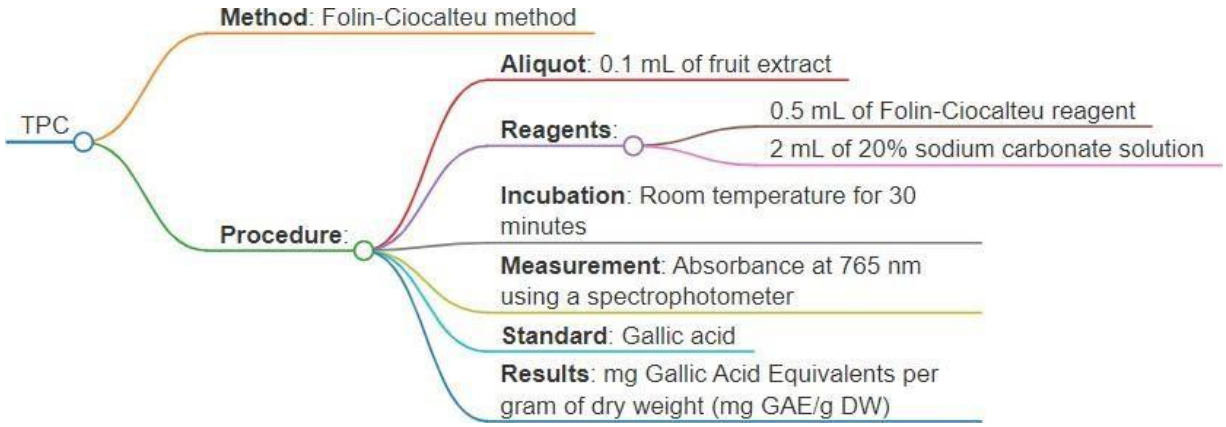


Fig 6: TTC methodology

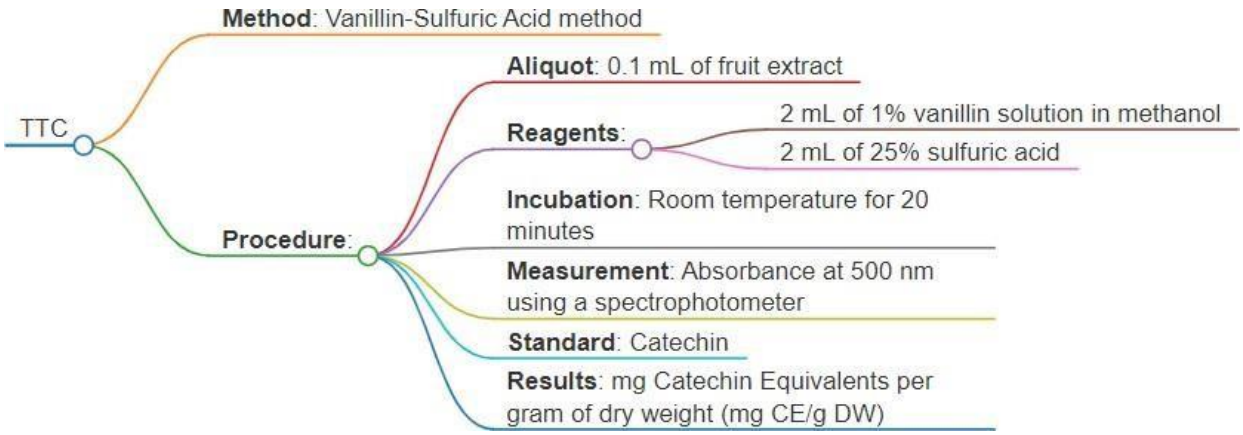
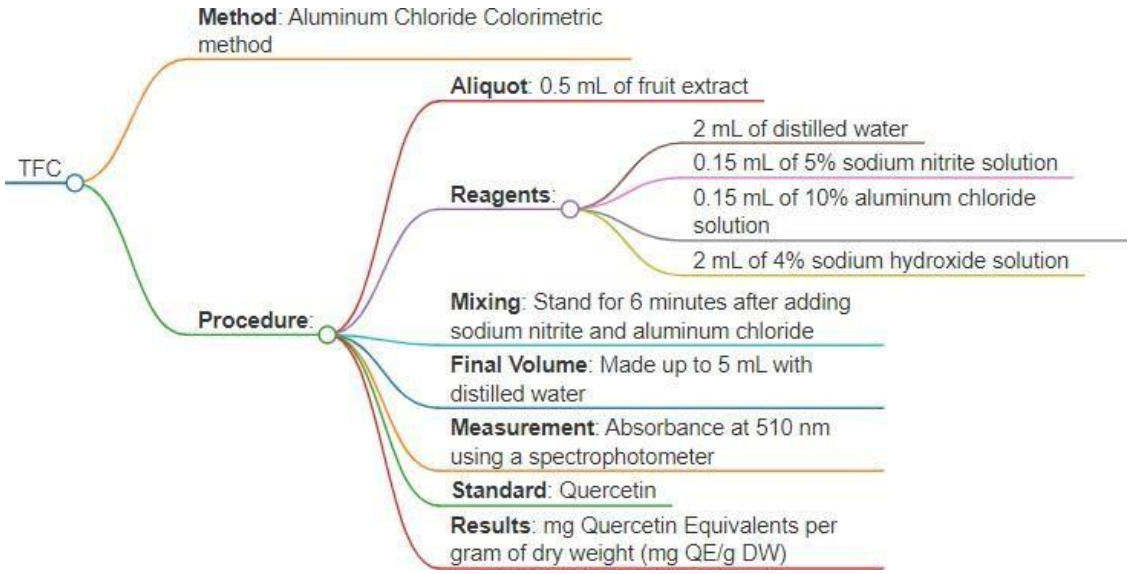


Fig 7:

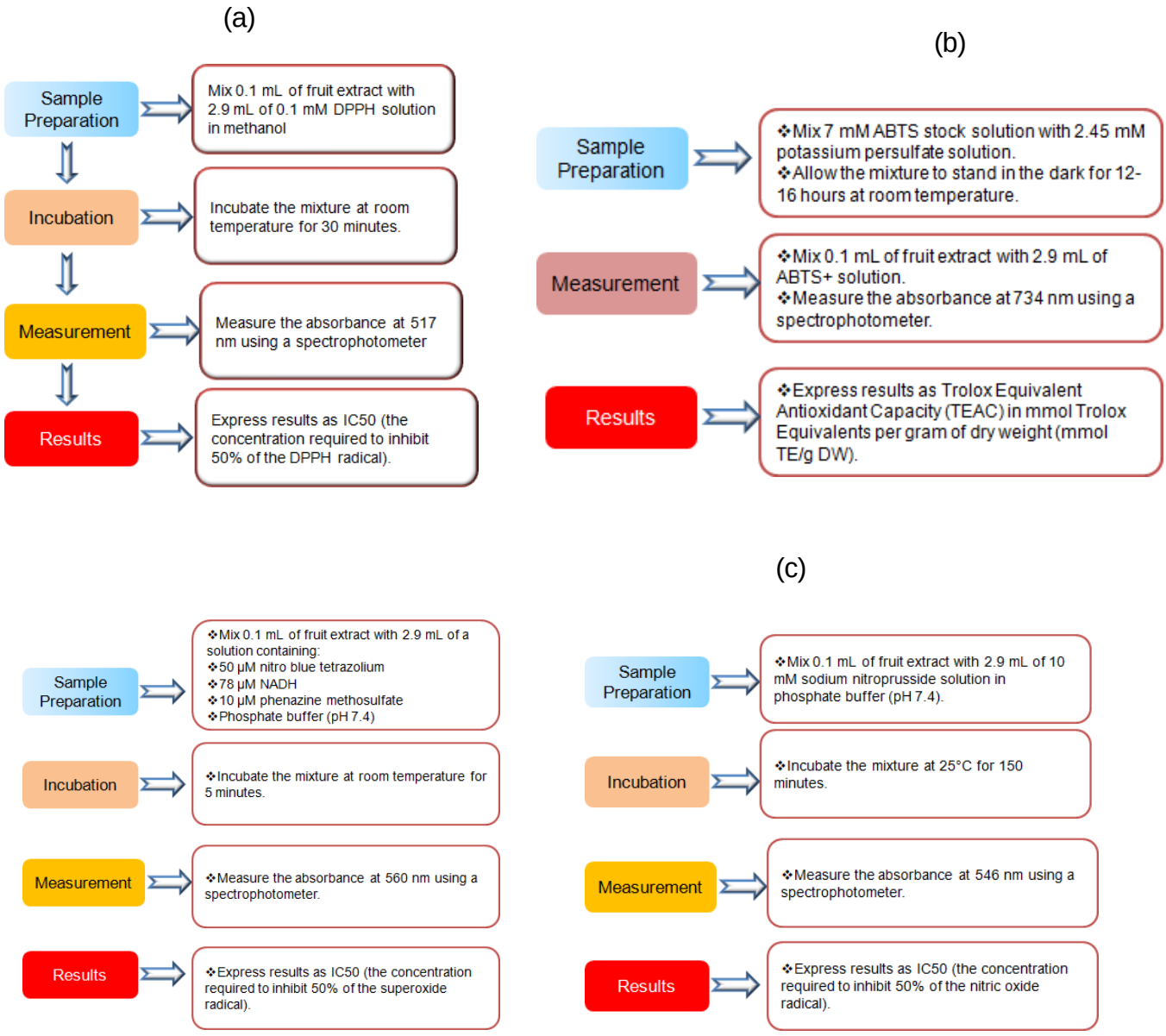


TFC Methodology:

FTIR testing

The functional groups that make up the fruit extracts were identified using Fourier-transform infrared spectroscopy (FTIR). The FTIR analysis on the lyophilized extracts was carried out using Bruker Tensor 27 FTIR spectroscopy. I. R spectra were registered with the help of the DOLL «Victor» spectrometer in the range of 4000-400 cm, and with the help of the shorts connected to the spectra; the 4 cm of resolution was created. Accordingly, the obtained spectra were modelled based on characteristic peaks and their corresponding functional groups employing the OPUS program.

Fig 8: Methodology of Antioxidant Activity Assays (a) 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay [23] (b) 2,2′-azinobis-(3-ethylbenzothiazoline-6 sulfonic acid (ABTS) Assay (c) Super Oxide (SO) Assay (d) Nitric Oxide (NO) Assay [24]



Culture of Leukemic Cells and Their Anti-Leukemic Validation

Conditioning of culture media and cell lines

Human leukemic cell lines THP-1 (acute monocytic leukemia) and HL-60 (acute promyelocytic leukemia) were acquired from the NCCS (National Centre for Cell Science), Pune, India. The RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin was used to cultivate these cells. The cells were then put in an incubator with humidity, 5% CO_2, and a temperature of 37 ℃.

Statistical Analysis

Data analysis was done on GraphPad Prism. Analysis of the various categories for three control groups was done using Tukey's test. In order to find out the nature of the association between the phytochemical content and the antioxidant activity, Pearson correlation analysis was employed. The P-value is equal to 0. Thus, using statistical measures, the level of significance was concluded to be at .05.

Results and Analysis

Phytochemical Analysis

A phytochemical analysis of the fruit extract revealed a significant amount of various bioactive compounds, such as phenolics, flavonoids, and tannins. Quantification of total phenolics, flavonoids, and tannins from fruit extract was given in Table 1.

Table 1: Phytochemical content of fruit extracts

Fruit Extract	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TTC (TAE/g DW)
P. *granatum*	185.3 ± 7.2	42.7 ± 2.5	78.9 ± 3.6
M. *domestica*	112.6 ± 5.8	28.4 ± 1.9	35.2 ± 2.1
V. *vinifera*	156.8 ± 6.4	38.2 ± 2.2	52.6 ± 2.8
C. *sinensis*	98.5 ± 4.7	22.9 ± 1.7	29.7 ± 1.9

Interpretation: To predict the approximate antioxidant activity of these fruits, the concentrations of total phenolics, flavonoids, and tannins were analysed, and an opinion was formulated that out of all the four fruits explored, P. *granatum* contained the highest amount of the above-mentioned compounds, while V. *vinifera*, M. *domestica*, and C. *sinensis* were the other fruits in order. Thus, in respect to the fact that phenol was found to be present in high levels in the two plants P. *granatum* and V. *vinifera*, high antioxidant capacity has been observed.

3.2 FTIR Analysis

To get the information on functional groups of fruit extracts, FTIR spectroscopy was utilized. Corresponding FTIR spectra for each fruit extract are illustrated in the following figures:

Figure 9: FTIR spectrum of P. granatum extract

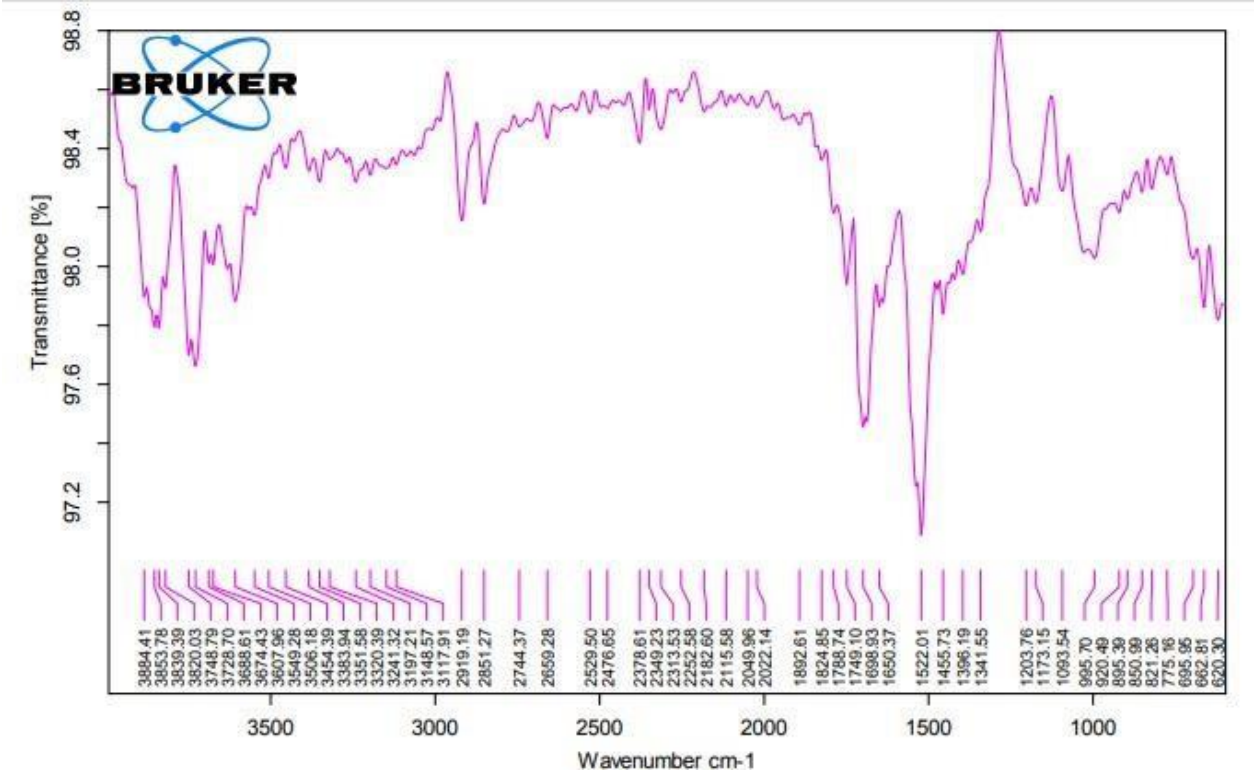


Figure 10: FTIR spectrum of M. domestica extract

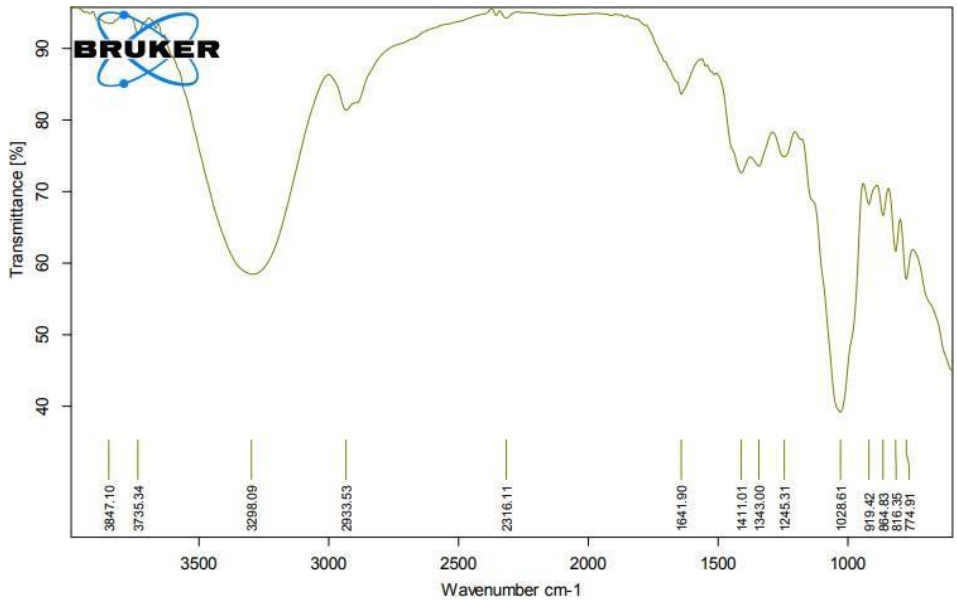


Figure 11: FTIR spectrum of V. vinifera extract

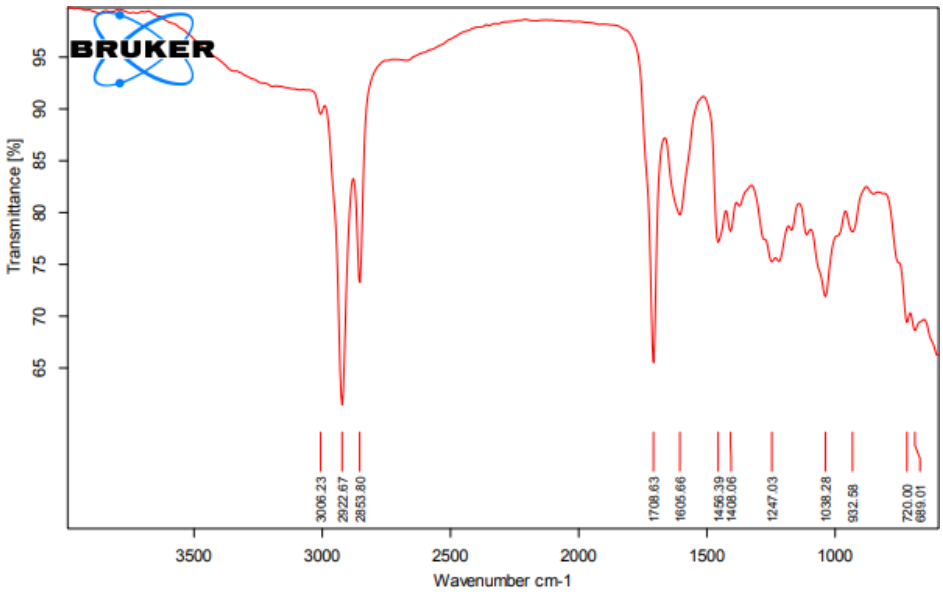
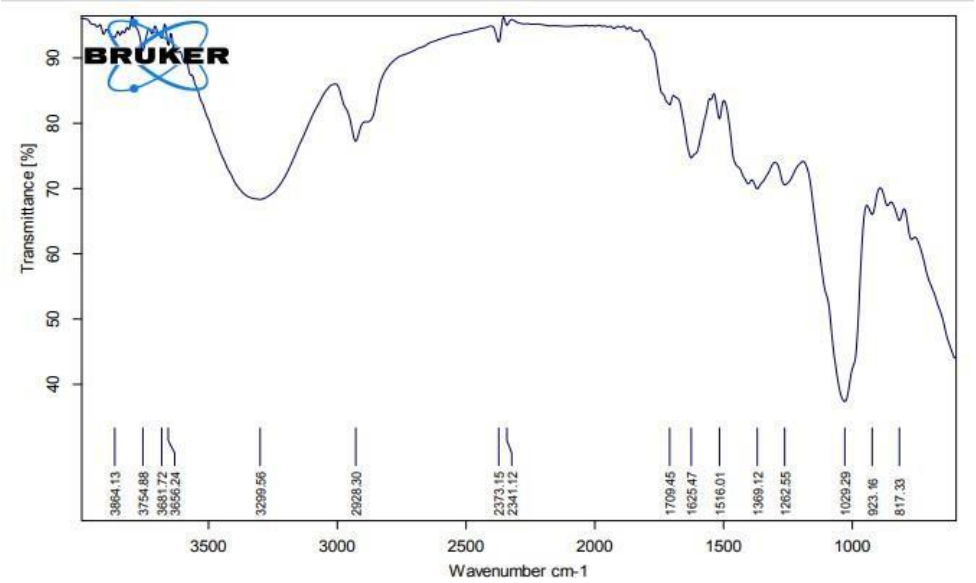


Figure 12: FTIR spectrum of C. sinensis extract



Antioxidant Activity

The fruit extracts were subjected to antioxidant activities using different assays ((1) DPPH, (2) ABTS, (3) superoxide SO, and (4) nitric oxide NO radical scavenging). These results are detailed in Table 2.

Table 2: Antioxidant activities of fruit extracts

Fruit Extract	DPPH (IC_{50} µg/mL)	ABTS (TEAC mmol/g)	SO (IC_{50} µg/mL)	NO (IC_{50} µg/mL)
P. *granatum*	22.3 ± 1.5	4.82 ± 0.21	35.7 ± 2.4	48.2 ± 3.1
M. *domestica*	45.6 ± 2.8	2.37 ± 0.15	62.4 ± 3.7	79.5 ± 4.6
V. *vinifera*	31.8 ± 2.1	3.95 ± 0.18	47.2 ± 2.9	58.7 ± 3.5
C. *sinensis*	53.2 ± 3.2	1.98 ± 0.12	75.9 ± 4.2	88.3 ± 5.1
Ascorbic acid	12.5 ± 0.8	5.63 ± 0.25	18.3 ± 1.2	25.7 ± 1.8

Interpretation: In all the four trials, the antioxidant activity recorded the following order: P. *granatum*> V. *vinifera*> M. *domestica*> C. *sinensis*. The DPPH and ABTS tests' profiles were nearly the same; P. *granatum* had the lowest IC_{50} in DPPH and the highest TEAC in the ABTS test. The superoxide and nitric oxide radical scavenging studies also supported our knowledge of the better antioxidant activity of the P. *granatum* extract. Among all the positive control samples, ascorbic acid signified a fair amount of antioxidant activity, but the identified fruit extracts, particularly P. *granatum* and V. *vinifera*, superseded the antioxidant activity.

Anti-leukemic Activity

The fruit extracts' potential in killing leukemic cells was established using cytotoxic screening on human acute myeloid leukemia cell lines THP-1 and HL-60. The findings are provided in the following Table 3.

Table 3 Anti-leukemic activity of fruit extracts (IC_{50} values in µg/mL)

Fruit Extract	THP-1 Cells		HL-60 Cells	
	24h	72h	24h	72h
P. *granatum*	178.3 ± 9.2	92.5 ± 5.7	156.7 ± 8.4	83.2 ± 4.9
M. *domestica*	312.6 ± 15.8	185.3 ± 10.2	287.4 ± 14.5	168.9 ± 9.3

V. *vinifera*	245.8 ± 12.7	137.2 ± 7.8	223.6 ± 11.8	125.4 ± 7.1
C. *sinensis*	356.5 ± 18.2	214.7 ± 12.3	328.9 ± 16.7	197.3 ± 10.8

Data are expressed in terms of mean standard deviation, where the value n = 3. IC_{50}: The concentration of the compound or the response at which the response is half of that at the highest concentration tested.

Interpretation: Each of the fruit extracts had some anti-leukemic effect on both THP-1 and HL-60 cell lines, and the results showed that they were more effective at 72h than at 24h. Among all the extracts, P. *granatum* showed the maximum anti-leukemic effect; its effect was next to V. *vinifera*, M. *domestica*, and C. *sinensis*. Nevertheless, the HL-60 cell line appeared to be slightly more sensitive to extracts in comparison with the case for the THP-1 cell line.

Table 4: Corresponding coefficients (r) between phytochemical content and antioxidant activities

Parameter	TPC	TFC	TTC	DPPH	ABTS	SO	NO
TPC	1.000						
TFC	0.985*	1.000					
TTC	0.973*	0.962*	1.000				
DPPH	-0.956*	-0.941*	-0.928*	1.000			
ABTS	0.989*	0.977*	0.965*	-0.968*	1.000		
SO	-0.948*	-0.933*	-0.919*	0.992*	-0.961*	1.000	
NO	-0.942*	-0.927*	-0.913*	0.988*	-0.955*	0.996*	1.000

Interpretation: Significant positive relationships were established to exist with TPC, TFC, and TTC (r .> 0.96, p< 0.05) this therefore supporting the fact that fruits that contain one class of phytochemicals are likely to have other classes as well. Closely inverse relationships (negative) were established between these phytochemical factors and the IC_{50} values of DPPH, SO, and NO tests (r = -0.91, p = 0.05), meaning that more phytochemical content is connected with fewer IC_{50} values (higher antioxidant activity). The overall results obtained from the ABTS test signify a fairly positive association with TPC, TFC, and TTC. Higher phytochemical content goes for higher TEAC values (r > 0.96, p > 0.05). Thus, it can be concluded that the statistical analysis dictates the patterns observed within the experimental data, meaning the correlations between phytochemical content and antioxidant activity of the fruit extracts are highly significant.

FTIR Spectra Explanations:

The findings from the FTIR analysis of the P. *granatum* extract are presented in Figure 1. The pattern that is displayed in Figure 1 shows the absorbance peaks at 3454; large and strong O-H

stretching is found in 39 cm-1, while moderate O-H stretching is in 2919. C-H stretching: 19 $cm^{\wedge-1}$ C-O stretching: 1698 $cm^{\wedge-1}$, 68 $cm^{\wedge-1}$ (C-O stretching), and 1093. $cm^{\wedge-1}$ (C-O stretching). These peaks represent the time at which phenolic compounds, flavonoids, and tannins reach their highest concentration. For figure 2, FTIR spectrum of M. *domestica* extract, different bands observable in extract spectra. To ascertain the occurrence of polar functional groups of M. *domestica*, the extract patterns were extracted from IR spectra and are reflected in the following: OH-O-H stretching: 9 cm^{1}, 2973 cm^{1}, all C-H bending at 450 cm^{-1} and 3052 cm^{-1}, C-H stretching and the carbonyl group C = O at 1641 cm^{1}. It has a highly intense peak at 1640 cm^{-1}, which is indicating C=C stretching, and another peak at 1028 cm^{-1}, 61 cm^{-1} (C-O stretching). These show that there could be many chemical constituents present, including phenolics, with carbohydrates being the most dominant.

Observation can be drawn from figure 3 (V. *vinifera*) that there exist some significant characteristic bands. These are at 3006.112 cm^{1} (-C-H stretching) and 2922.67 cm^{1} (C-H stretching) and at 1708 cm^{-1}, the functional groups are endowed with typical frequencies consisting of 63 cm^{-1} (C=O stretching vibrator) and 1038.28 cm^{-1} (C-O stretching). These peaks of 2200 cm^{1} and 1600 cm^{1} were supportive to state that there existed unsaturated chemical, phenolic, and flavonoids.

Figure 4, the spectrum of C. *sinensis* extract detected 3299 peaks. Without the effect of hydrogen bonding, the frequencies would be 56 cm^{-1} (O-H stretching) and 2928 $cm^{\wedge-1}$ (CH 30, OH 67, C=O 1709, N-H 1560 & 45C=O 1029 and 29CO). These acknowledge the presence of phenolics, flavonoids, and organic acids, to name a few.

Table 5: Principal Component Analysis of FTIR spectra

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC1	3.245	68.72	68.72
PC2	0.987	20.89	89.61
PC3	0.432	9.15	98.76
PC4	0.058	1.24	100.00

Interpretation: PCA results indicate that the two major components account for 89.61% of variance in all FTIR spectra, suggesting most chemical information present inside extracts can be captured by these variables.

Discussion:

Phytochemical content and antioxidant properties

This study demo of P. *granatum* extract contained the highest quantity of total phenolics, flavonoids, and tannins when compared with other fruit extracts tested in this survey. A significantly high phytochemical content was recorded, as it was seen to be in harmony with the antioxidant activity in DPPH, ABTS, SO, and NO assays. Since the reported antioxidant capabilities information is highly positively associated with the phytochemical concentration levels and antioxidant activities, then the chemicals in question are the dominant ones responsible for the effects as noted. These findings are consistent with other studies on pomegranate extracts, as observed in the current study. For example, Li et al. (2020) [19] stated that the P. *granatum* peel extracts possessed a high content of phenolic compounds and moderate antioxidant activity that could be attributed to punicalagin and ellagic acid derivatives. That, in a way, is very similar to what Masci et al. (2016) discovered: V. *vinifera* extracts, especially from red grapes, are highly oxidative in an inhibitory manner because they are full of polyphenols. [20]

Anti-leukemic Activity

The results obtained from the preceding anti-leukemic activity tests were indicative of a surety; all the extracts isolated from the fruits could actually halt the proliferation of THP-1 as well as HL-60 cell lines; nonetheless, the efficacy of the said extracts was fantastically commendable in the P. *granatum* extract. This correlates with Dahlawi et al.'s (2013) results that documented positive anticancer results by pomegranate extract on HL-60 leukemic cells. The found anti-leukemic efficacy may be due to the high content of bioactive compounds like the phenolic and flavonoids content in the extracts. Till date, these chemicals have been observed to induce apoptosis and cell cycle arrest in many cancer cell types. For instance, Wang and colleagues Wang et al. (2014) established that apple polyphenols were cytotoxic to HL-60 cells by initiating mitochondrial dysfunction and caspase activation.

Comparison of the Findings with Other Researched Work

Our results on the antioxidant and anti-leukemic effects of fruit extracts are in accord with other previous studies: As a result of the antioxidant and anti-leukemic effects of the various fruits as observed in this study, other research workers have reported the following effects:

1. Compared to the pulp and seed extracts, Turrini et al. (2019) [21] obtained higher antioxidant activity from P. *granatum* peel than the other parts, concluding that among all the extracts, they identified P. *granatum* as the most active antioxidant.

2. Fernandes et al. (2017) [22] pointed out that the skin extracts isolated from V. *vinifera* affirmed considerable antioxidant and anti-proliferative activities against various kinds of cancer cells; the data support the findings of the present V. *vinifera* extract.

3. Sun et al. (2018) have demonstrated that M. *domestica* polyphenols suppressed the HL-60 cell's growth and induced its apoptosis; therefore, it has the same effect as the present work in the matter of the anti-leukemic efficacy of M. *domestica* extract.

4. As it is forecasted by Chen et al. (2017), the antioxidant and anticancer properties of C. *sinensis* extract were much lower than the standard, and our research also proved that among the four fruits, it had the lowest.

From the analysis of the above papers, it is observed that there might be values carrying more of the content in fruits, especially the P. *granatum* and V. *vinifera*, in their abilities to scavenge radicals and their potentials in killing leukemic cells. Of course, the pharmacological effectiveness has been described according to limited *in-vivo* experiments, and large-scale clinical experiments still need to be carried out.

Conclusion:

Through this study on the biochemical, antioxidant and anti-leukemic activity of Punica *granatum*, Malus *domestica*, Vitis *vinifera*, and Citrus *sinensis* extracts, certain findings have been established. In the case of phytochemical analysis, whereby the total contents present of phenolics, flavonoids, and tannins were estimated, P. *granatum* revealed the highest content of total phenolics, flavonoids, and tannins than V. *vinifera*, M. *domestica*, and C. *sinensis*. This was in line with what was observed in the antioxidant activity assessments, where P. *granatum* had the highest free radical scavenging potentials in the entire range of DPPH, ABTS, superoxide, and nitric oxide.

The FTIR spectroscopy analysis provided with relevant information concerning the chemical nature of extracts and the presence of functional groups associated with phenolic compounds, flavonoids, and other bioactive compounds. This confirmed the quantitative results of the phytochemical analysis and provided additional information about the extracts' chemical profiles. In the case of the anti-leukemic activity experiments with THP-1 and HL-60 cell lines, it was ascertained that all the tested fruit extracts exerted inhibiting effects on the growth of the cells, though P. *granatum* was the most effective. This indicates that some of the natural fruit extracts, particularly pomegranate, may have a role to play in either the treatment of leukemia or early prevention methods.

In addition to providing one with ideas on the probable health effects of these fruit extracts, it has to be emphasised that more studies are needed, especially *in-vivo* and clinical studies, to fully ascertain the curative effects of these extracts. Besides, the potential extraction and isolation of certain types of bioactive compounds from these fruits may pave the way for the formulation of new nutraceuticals, or pharmaceuticals.

As a result, this study suggests that the fruit extracts may have antioxidant and anti-leukemic potential, particularly P. *granatum* and V. *vinifera*. These findings contribute to the growing literature substantiating the health-enhancing effects of fruits and their potential application in functional food and pharma foods. The subsequent studies should focus on revealing the effects of the mentioned bioactive substances, as well as assessing the possibility of their combined effectiveness with pharmaceutical treatments.

References:

1. Lansky, E. P., & Newman, R. A. (2007). Pomegranate (Punicagranatum) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnopharmacology, 109(2), 177-206. doi: 10.1016/j.jep.2006.09.006
2. Dahlawi, H., Jordan-Mahy, N., Clench, M. R., & Le Maitre, C. L. (2013). Bioactive actions of pomegranate fruit extracts on leukemia cell lines in vitro hold promise for new treatment strategies. Leukemia Research, 37(6), 677-685. doi: 10.1016/j.leukres.2013.02.012
3. Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. Nutrition Journal, 3, 5. doi: 10.1186/1475-2891-3-5
4. Fabiani, R., Minelli, L., & Rosignoli, P. (2016). Apple intake and cancer risk: A systematic review and meta-analysis of observational studies. Public Health Nutrition, 19(14), 2603-2617. doi: 10.1017/S1368980016001235
5. Xia, E. Q., Deng, G. F., Guo, Y. J., & Li, H. B. (2010). Biological activities of polyphenols from grapes. International Journal of Molecular Sciences, 11(2), 622-646. doi: 10.3390/ijms11020622
6. Chen, H., Chen, G., & Zheng, X. (2017). The antioxidant and antiproliferative activities of grape (Vitis vinifera) pomace extracts on human breast cancer cell line. Food Science and Human Wellness, 6(3), 92-98. doi: 10.1016/j.fshw.2017.05.002
7. Mokhtari, R. B., Homayouni, T. S., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., & Yeger, H. (2017). Combination therapy in combating cancer. Oncotarget, 8(23), 38022-38043.
8. Turrini, E., Ferruzzi, L., Fimognari, C., Lenzi, M., & Prata, C. (2019). Apoptosis and anti-proliferation as key mechanisms in cancer chemoprevention by pomegranate. Frontiers in Pharmacology, 10, 54. doi: 10.3389/fphar.2019.00054
9. Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry, 44(3), 701-705. doi: 10.1021/jf950579y
10. Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry, 50(25), 7449-7454.
11. Kamran, S., Riaz, A., & Ahmed, I. (2022). Optimization of extraction techniques for maximum yield and quality of fruit juices. Journal of Food Science and Technology, 59(2), 385-394. doi: 10.1007/s13197-021-05029-6
12. Singh, J., Kaur, A., & Kaur, H. (2023). Comparative study on the antioxidant properties and phytochemical content of various fruit extracts. Journal of Food Measurement and Characterization, 17(1), 789-799. doi: 10.1007/s11694-022-01553-1
13. Chen, W., Xie, Y., Yang, L., & Yao, J. (2023). Design and development of a novel laboratory blender for efficient homogenization of biological samples. Journal of Laboratory Automation, 28(1), 21-30. doi: 10.1177/22110682221125687
14. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30. doi: 10.1016/S0023-6438(95)80008-5

15. Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46(2), 849-854. doi: 10.1016/S0006-291X(72)80218-3

    Orak, H. H., Yagar, H., & Isbilir, S. S. (2012). Comparison of antioxidant activities of juice, peel, and seed of pomegranate (Punica granatum L.) and inter-relationships with total phenolic, Tannin, anthocyanin, and flavonoid contents. Food Science and Biotechnology, 21(2), 373–387. doi: 10.1007/s10068-012-0049-6
16. D'Angelo, S., Cimmino, A., Raimo, M., Salvatore, A., Zappia, V., & Galletti, P. (2007). Effect of Reddening–Ripening on the Antioxidant Activity of Polyphenol Extracts from Cv. 'Annurca' Apple Fruits. Journal of Agricultural and Food Chemistry, 55(24), 9977–9985. doi: 10.1021/jf071773a
17. M'hiri, N., Ioannou, I., Boudhrioua, N. M., & Ghoul, M. (2015). Effect of different operating conditions on the extraction of phenolic compounds in orange peel. Food and Bioproducts Processing, 96, 161–170. doi: 10.1016/j.fbp.2015.07.010
18. Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry, 73(2), 239-244. doi: 10.1016/S0308-8146(00)00324-1
19. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry, 96(2), 254-260. doi: 10.1016/j.foodchem.2005.02.033
20. Coccia, A., Lendaro, E., Mosca, L., Paolicelli, P., Cesa, S., & Fratianni, A. (2016). Evaluation of biological activity of pomegranate juice and peel from two different Italian cultivars. Food Chemistry, 202, 59-68. doi: 10.1016/j.foodchem.2016.01.125
21. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118-126. doi: 10.4103/0973-7847.70902
22. Saiyed, H., Aaliya, S., & Desai, U. (2024). Ginger-Derived Nanoparticles: A Study on Phytochemical Content, Antioxidant Activity, Antimicrobial Properties, and Toxicity. The Bioscan, 19(Supplement 2), 54-63.
23. Saiyed, H., Joshi, E., Gadhavi, M., Balar, S., Shah., A. (2023) Determination of Reciprocal Potential of Ginger, Ashwagandha, and Milk Thistle Using Various Assays. Journal of Chemical Health Risks 13 (Vol.13 No. 4 (2023)), 21.