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Anti-inflammatory and antioxidant properties of six common fruit extracts: An *in vitro* study

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Abstract:

Fruits like *Ananas comosus*, *Malus pumila* and *Manilkara zapota* are known to have antioxidant activity *in vitro*, while *Mangifera indica* and *M. pumila* have moderate anti-inflammatory effects. Extracts were tested at $10-50\,\mu$ L and compared with standard drugs. Antioxidant activity was concentration-dependent and statistically significant (p \leq 0.05). Anti-inflammatory activity, though notable, was less than diclofenac. Thus, the potential of fruit extracts in managing oxidative stress and inflammation is reported.

Key words: Oxidative stress, fruit extract, glycaemic index, anti-inflammatory, anti-oxidant, standard drug

Background:

The human body relies on various defense mechanisms to maintain health and respond to external threats. One such essential mechanism is inflammation, which serves as an involuntary yet vital physiological response of the immune system to stimuli such as pathogens, toxins, or tissue injury. This response is marked by redness, swelling, pain, heat and functional loss, aiming to eliminate harmful agents and initiate healing [1]. However, chronic inflammation is a major contributor to the onset and progression of numerous diseases, including cancer, diabetes, arthritis, cardiovascular disorders and neurological conditions [2]. Therefore, effective management of inflammation is crucial in reducing the burden of chronic diseases. Oxidative stress, defined as an imbalance favoring oxidants over antioxidants, leads to molecular damage and disruption of redox signaling and control [3]. Oxidative stress and inflammation are closely interlinked, often exacerbating each other and fueling a vicious cycle that underpins the pathogenesis of many chronic diseases [4]. Oxidative stress results from excessive production of reactive oxygen species (ROS) and inadequate antioxidant defenses, leading to cellular and tissue injury [5,6]. Chronic oral inflammation has systemic implications, as pro-inflammatory cytokines and oxidative stress mediators may enter the bloodstream and contribute to conditions such as diabetes, rheumatoid arthritis and cardiovascular disease [7]. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, are commonly used for managing inflammation by inhibiting cyclooxygenase enzymes responsible for prostaglandin synthesis [8,9]. Likewise, synthetic antioxidants like ascorbic acid are employed to neutralize free radicals and alleviate oxidative stress. However, these agents often suffer from limitations such as poor bioavailability, degradation during storage and reduced efficacy when used alone [10]. In recent years, natural alternatives like fruits have garnered considerable interest due to their rich content of bioactive compounds with dual antioxidant and anti-inflammatory potential. Fruits contain phytochemicals such as flavonoids, polyphenols, carotenoids and vitamins that help modulate inflammatory pathways, scavenge free radicals and enhance endogenous antioxidant enzyme activity [11]. Studies have shown that fruits with high polyphenol contentsuch as pomegranates and berries-exhibit beneficial effects in mitigating inflammation and oxidative stress [12]. Furthermore, fruits are associated with fewer side effects than synthetic agents, making them a safer and more holistic therapeutic option [13]. Another important consideration is the glycaemic index

(GI) of fruits. The GI reflects how quickly carbohydrate-rich foods raise blood glucose levels. Low-GI foods contribute to more stable blood sugar levels, potentially reducing inflammation and oxidative damage [14]. Therefore, evaluating the GI of fruits is essential in assessing their therapeutic benefits [15]. Despite the known benefits of fruits, limited research has compared their antioxidant and anti-inflammatory efficacy against standard agents such as diclofenac and ascorbic acid. Moreover, comparative evaluations of fruits with varying glycaemic indices-such as watermelon, apple, sapodilla, pineapple, mango and orange-remain scarce. Filling this gap can provide valuable insights into the therapeutic value of these fruits and inform dietary or adjunctive treatment strategies. Therefore, it is of interest to assess the anti-inflammatory and antioxidant properties of fruits categorized by glycaemic index and compare their effectiveness with diclofenac and ascorbic

Materials and Methodology: Study setting:

The study was conducted in the laboratory of the department of biochemistry assessing the anti-inflammatory and antioxidant properties of commonly consumed fruits: mango (Mangifera indica), apple (Malus pumila), sapodilla (Manilkara zapota), orange (citrus sinensis), pineapple (ananas comosus) and watermelon (citrus lanatus). Standardized protocols were followed to minimize external variability and ensure accurate results.

Study design: An In vitro study

Sample selection:

Fruits were categorized based on their glycaemic index into high, medium and low groups. Two fruits were randomly selected from each category to evaluate their anti-inflammatory and antioxidant activity.

The six fruits included in the study were:

- [1] High glycaemic index: Citrullus lanatus, Mangifera indica
- [2] Moderate glycaemic index: Manilkara zapota, Citrus sinensis
- [3] Low glycaemic index: Ananas comosus, Malus pumila

These samples were ripe fruits purchased from a grocery store. The edible parts of each fruit were used for extraction.

Materials:

Chemicals and reagents were used, including bovine serum albumin (BSA), 1N Hydrochloric acid (HCL), Diclofenac sodium,

DPPH (2,2- diphenyl-1 -picrylhydrazyl), tris HCL buffer (50 Mm, PH 7.4), ascorbic acid and distilled water to ensure accurate biochemical analysis.

Extraction and preparation of samples:

Fruit samples were extracted using a standardized aqueous extraction method to obtain bioactive compounds from the selected fruits. Fresh ripe mango (Mangifera indica), apple (malus pumila), sapodilla (Manikara zapota), orange (Citrus sinesis), pineapple (Ananas cosmous) and watermelon (Citrullus lanatus) were purchased from a grocery store and stored at 4°C before processing to maintain freshness [12]. The edible portion (pulp) of each fruit was separated, ensuring the exclusion of seeds and peels unless otherwise required. (10g) of fruit pulp was weighed and crushed using a sterile mortar and pestle. The crushed pulp was mixed with 100 ml of distilled water and then stirred for 15 minutes using a glass rod to enhance solubility. The mixture was subjected to heat-assisted extraction without degrading heatsensitive compounds. After heating, the solution was filtered first using muslin cloth and then through Whatman No.1 filter paper for finer filtration, removing solid residues and obtaining a clear extract.

Anti-inflammatory activity using bovine serum albumin denaturation assay:

The anti-inflammatory activity of the fruit extract was evaluated using the Bovine serum albumin (BSA) denaturation assay, which measures the ability of the extract to inhibit protein denaturation, a key process in inflammation. In this assay, 0.45 mL of 1% BSA solution was mixed with 0.05 ml of fruit extract at different concentrations (10, 20, 30, 40 and $50\mu L$). The pH of the mixture was adjusted to 6.3 using 1N hydrochloric acid and the samples were incubated at room temperature for 20 minutes. After incubation, the mixture was heated at 55 °C for 30 minutes using a water bath and then allowed to cool to room temperature. The absorbance was measured at 660nm using UV-Vis spectrophotometer and diclofenac sodium was used as the standard anti-inflammatory drug for comparison. The percentage inhibition of protein denaturation was calculated using the formula:

%Inhibition = Absorbance of control- Absorbance of sample×100
Absorbance of control

Antioxidant activity using DPPH (2, 2- diphenyl- 1-picrylhydrazyl) assay:

The antioxidant activity of fruit extract was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, which measures the ability of the extracts to neutralize free radicals. In this assay, fruit extracts were tested at five different concentrations (10, 20, 30, 40 and $50\mu L$) to observe a dose-dependent response. A mixture containing 1 ml of 0.1 mm DPPH in methanol and $450\mu L$ of 50mM Tris -HCL buffer (pH 7.4) was prepared. The fruit extract was then added to the DPPH solution and the mixture was incubated at room temperature in the dark for 30 minutes to prevent auto-oxidation. After

incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with ascorbic acid used as the standard antioxidant for comparison. The percentage of free radical scavenging activity was calculated using the formula.

(Absorbance of control - absorbance of the test)/Absorbance of control) $\times\,100$

Statistical analysis:

The statistical analysis in this study was conducted to determine the significance of differences in antioxidant and anti-inflammatory activities among fruit extract at various concentrations. Mean \pm standard deviation (SD) was used for descriptive statistics. One-way ANOVA was performed to compare the activities of different fruit extracts, with a significance level set at $p \le 0.05$. If significant differences were found, post hoc Tukey's test was used for pairwise comparisons between fruit extracts. To compare the effectiveness of fruit extracts with standard drugs (Ascorbic Acid for antioxidant activity and Diclofenac sodium for anti-inflammatory activity), an independent t-test was conducted. All statistical analyses were performed using SPSS version.20 IBM, USA software, ensuring accuracy and reliability in data interpretation.

Table 4: Pairwise comparison of anti-inflammatory activity

(I) Group	(J) Group	10	20	30	40	50
Manilkara zapota	Malus pumila	0.993	0.190	0.967	1.000	0.427
	Ananas comosus	0.138	0.975	0.643	0.948	0.999
	Citrullus lanatus	1.000	0.975	0.846	0.703	1.000
	Citrus sinensis	0.309	0.999	0.999	0.991	1.000
	Mangifera indica	0.056	1.000	0.967	0.991	0.257
Malus pumila	Ananas comosus	0.056	0.060	0.257	0.948	0.257
	Citrullus lanatus	0.993	0.060	0.427	0.703	0.427
	Citrus sinensis	0.138	0.319	0.999	0.991	0.427
	Mangifera indica	0.022*	0.190	1.000	0.991	0.999
Citrullus lanatus	Ananas comosus	0.138	1.000	0.999	0.991	0.999
	Citrus sinensis	0.993	0.879	0.427	0.703	0.999
	Mangifera indica	0.993	0.975	0.257	0.703	0.144
Ananas comosus	Citrus sinensis	0.309	0.879	0.643	0.385	1.000
	Mangifera indica	0.056	0.975	0.427	0.385	0.257
Citrus sinensis	Mangifera indica	0.874	0.999	0.999	1.000	0.257

Post hoc Tukey test; * indicates a significant difference at p≤0.05

Table 5: Comparison of antioxidant activity of fruits with standard drug

Fruit	Fruit		Standar	rd .	p-value	
	Mean	SD	Mean	SD		
10						
Manilkara zapota	62.95	0.45	66.43	1.27	0.011*	
Malus pumila	63.24	0.42	66.43	1.27	0.015*	
Ananas comosus	63.75	0.28	66.43	1.27	0.024*	
Citrullus lanatus	61.42	0.56	66.43	1.27	0.003*	
Citrus sinensis	63.60	0.52	66.43	1.27	0.024*	
Mangifera indica	62.62	0.58	66.43	1.27	0.009*	
20						
Manilkara zapota	75.99	0.30	78.66	0.73	0.004*	
Malus pumila	74.42	0.47	78.66	0.73	0.001*	
Ananas comosus	74.27	0.56	78.66	0.73	0.001*	
Citrullus lanatus	72.58	0.43	78.66	0.73	<0.001*	
Citrus sinensis	75.44	0.33	78.66	0.73	0.002*	
Mangifera indica	74.38	0.59	78.66	0.73	0.001*	
30						
Manilkara zapota	82.12	0.45	85.24	0.93	0.006*	
Malus pumila	81.95	0.36	85.24	0.93	0.005*	
Ananas comosus	82.55	0.47	85.24	0.93	0.011*	
Citrullus lanatus	81.33	0.58	85.24	0.93	0.004*	

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Citrus sinensis	82.19	0.24	85.24	0.93	0.005*
Mangifera indica	82.35	0.60	85.24	0.93	0.011*
40					
Manilkara zapota	86.60	0.31	88.45	0.70	0.014*
Malus pumila	85.34	0.37	88.45	0.70	0.002*
Ananas comosus	86.40	0.46	88.45	0.70	0.013*
Citrullus lanatus	82.55	0.49	88.45	0.70	<0.001*
Citrus sinensis	85.36	0.37	88.45	0.70	0.002*
Mangifera indica	86.15	0.16	88.45	0.70	0.005*
50					
Manilkara zapota	91.01	0.45	93.16	0.82	0.016*
Malus pumila	91.08	0.49	93.16	0.82	0.020*
Ananas comosus	90.62	0.61	93.16	0.82	0.013*
Citrullus lanatus	86.28	0.38	93.16	0.82	<0.001*
Citrus sinensis	89.13	0.51	93.16	0.82	0.002*
Mangifera indica	89.19	0.32	93.16	0.82	0.001*

Independent t-test; * indicates a significant difference at p≤0.05

Results:

The antioxidant activity of six different fruits was evaluated at five concentration levels (10, 20, 30, 40 and 50) Table 1. Shows the antioxidant activity of the six fruit extracts at different concentrations. The results indicate a concentration-dependent increase in activity, with ananas comosus, malus pumila and Manilkara zapota exhibiting the highest antioxidant potential. Statistical analysis using one-way ANOVA indicated significant differences in antioxidant activity at most concentration levels (p ≤ 0.05), except for the 30% concentration, which was not significantly different across fruits. Table 2 presents the pairwise comparison of antioxidant activity among different fruits. The results indicate that Citrullus Lanatus had significantly lower antioxidant activity than most other fruits, particularly at lower concentrations. The antioxidant potential of, Ananas comosus, Manilkara zapota and malus pumila was comparable, suggesting a similar composition of active compounds. Table 3 shows the anti-inflammatory activity of the same six fruits was analysed across five concentrations. Unlike antioxidant activity, the differences in anti-inflammatory potential were less pronounced. At 10% concentration, statistical significance (p= 0.008) indicated variation among fruits, but at higher concentrations, differences were not statistically significant (p > 0.05). The result implies that while some fruits have superior antioxidant properties, their anti-inflammatory effects may be less distinct. Table 4 provides a pairwise comparison of the anti-inflammatory acts of Mangifera indica. At lower concentrations 10 and 20, it showed significantly different activity from other fruits, while at higher concentrations, all fruits exhibited similar anti-inflammatory potential. Table 5 compares the antioxidant activity of fruits with a standard, the results indicate that all fruits exhibited lower activity, with significant differences (p \leq 0.05) at all

concentrations. However, the antioxidant potential of *Ananas comosus*, *Malus pumila* and *Manilkara zapota* was relatively closer to the standard, especially at higher concentrations. **Table 6** presents the comparison of anti-inflammatory activity with the standard drug. The anti-inflammatory activity of the tested fruits was generally lower than the standard drug. While *Mangifera indica* and *malus pumila* showed significant differences at multiple concentrations others like *Citrullus lanatus* and *ananas comosus* had fewer significant differences. These results suggest that while fruits exhibit anti-inflammatory properties, they may not match the potency of standard pharmaceutical agents.

Table 6: Comparison of anti-inflammatory activity of fruits with standard drug

fruit	Fruit		Standa	rd	p-value
	Mean	SD	Mean	SD	_
10					
Manilkara zapota	45.67	0.58	46.67	1.53	0.349
Malus pumila	46.00	1.00	46.67	1.53	0.561
Citrullus lanatus	44.00	1.00	46.67	1.53	0.065
Ananas comosus	45.67	0.58	46.67	1.53	0.349
Citrus sinensis	44.33	0.58	46.67	1.53	0.069
Mangifera indica	43.67	0.58	46.67	1.53	0.033*
20					
Manilkara zapota	56.67	1.53	60.33	1.53	0.042*
Malus pumila	59.00	1.00	60.33	1.53	0.275
Citrullus lanatus	56.00	1.00	60.33	1.53	0.015*
Ananas comosus	56.00	1.00	60.33	1.53	0.015*
Citrus sinensis	57.00	1.00	60.33	1.53	0.034*
Mangifera indica	56.67	1.16	60.33	1.53	0.029*
30					
Manilkara zapota	69.33	1.53	71.67	1.53	0.135
Malus pumila	68.67	0.58	71.67	1.53	0.033*
Citrullus lanatus	70.67	0.58	71.67	1.53	0.349
Ananas comosus	70.33	1.53	71.67	1.53	0.345
Citrus sinensis	69.00	1.00	71.67	1.53	0.065
Mangifera indica	68.67	0.58	71.67	1.53	0.033*
40					
Manilkara zapota	74.00	2.00	77.67	1.53	0.065
Malus pumila	74.00	1.00	77.67	1.53	0.025*
Citrullus lanatus	75.00	0.00	77.67	1.53	0.094
Ananas comosus	75.67	1.53	77.67	1.53	0.184
Citrus sinensis	73.33	2.08	77.67	1.53	0.044*
Mangifera indica	73.33	0.58	77.67	1.53	0.010*
50					
Manilkara zapota	82.00	1.00	83.67	1.53	0.189
Malus pumila	80.33	0.58	83.67	1.53	0.024*
Citrullus lanatus	82.33	1.53	83.67	1.53	0.345
Ananas comosus	82.00	1.00	83.67	1.53	0.189
Citrus sinensis	82.00	1.00	83.67	1.53	0.189
Mangifera indica	80.00	1.00	83.67	1.53	0.025*

Independent t test; * indicates a significant difference at p≤0.05

Table 1: Comparison of antioxidant activity

fruit	10		20	20 30		40	40		50	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Manilkara zapota	62.95	0.45	75.99	0.30	82.12	0.45	86.60	0.31	91.01	0.45
Malus pumila	63.24	0.42	74.42	0.47	81.95	0.36	85.34	0.37	91.08	0.49
Ananas comosus	63.75	0.28	74.27	0.56	82.55	0.47	86.40	0.46	90.62	0.61
Citrullus lanatus	61.42	0.56	72.58	0.43	81.33	0.58	82.55	0.49	86.28	0.38
Citrus sinensis	63.60	0.52	75.44	0.33	82.19	0.24	85.36	0.37	89.13	0.51
Mangifera indica	62.62	0.58	74.38	0.59	82.35	0.60	86.15	0.16	89.19	0.32
p-value	0.001*		<0.001*		0.094		<0.001*		<0.001*	

One-way ANOVA test; * indicates a significant difference at p≤0.05

Table 2: Pairwise comparison of antioxidant activity

(I) Group	(J) Group	10	20	30	40	50
Manilkara zapota	Malus pumila	0.972	0.012*	0.997	0.014*	1.000
	Ananas comosus	0.377	0.006*	0.860	0.985	0.903
	Citrullus lanatus	0.019*	<0.001*	0.356	<0.001*	<0.001*
	Citrus sinensis	0.572	0.693	1.000	0.016	0.004*
	Mangifera indica	0.955	0.010*	0.989	0.686	0.005*
Malus pumila	Citrullus lanatus	0.782	0.999	0.627	0.042*	0.832
	Ananas comosus	0.006*	0.004*	0.592	<0.001*	<0.001*
	Citrus sinensis	0.931	0.139	0.987	1.000	0.003*
	Mangifera indica	0.626	1.000	0.891	0.165	0.004*
Citrullus lanatus	Ananas comosus	0.001*	0.007*	0.064	<0.001*	<0.001*
	Citrus sinensis	0.999	0.075	0.924	0.048	0.020*
	Mangifera indica	0.111	1.000	0.994	0.955	0.026*
Ananas comosus	Citrus sinensis	0.001*	<0.001*	0.281	<0.001*	<0.001*
	Mangifera indica	0.078	0.005*	0.149	<0.001*	<0.001*
Citrus sinensis	Mangifera indica	0.196	0.117	0.998	0.185	1.000

Post hoc Tukey test; * indicates a significant difference at p≤0.05

Table 3: Comparison of Anti-inflammatory Activity

fruit	10		20	20 30		40		50		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Manilkara zapota	45.67a,b	0.6	56.67	1.5	69.33	1.5	74	2	82	1
Malus pumila	46.00a	1	59	1	68.67	0.6	74	1	80.33	0.6
Ananas comosus	44.00^{b}	1	56	1	70.67	0.6	75	0	82.33	1.5
Citrullus lanatus	45.67a,b	0.6	56	1	70.33	1.5	75.67	1.5	82	1
Citrus sinensis	44.33a,b	0.6	57	1	69	1	73.33	2.1	82	1
Mangifera indica	43.67a,b	0.6	56.67	1.2	68.67	0.6	73.33	0.6	80	1
p-value	0.008*		0.061		0.151		0.323		0.072	

One-way ANOVA test; Post hoc Tukey test; * indicates a significant difference at p≤0.05

Discussion:

The last few decades have seen increased interest in the medicinal applications and food additive potential of naturally occurring anti-inflammatory and antioxidant agents, including herbs, spices, plants and fruits. The present study evaluated the antioxidant and anti-inflammatory activities of aqueous extracts from six commonly consumed fruits: Ananas comosus (pineapple), Malus pumila (apple), Manilkara zapota (sapodilla), Citrullus lanatus (watermelon), Citrus sinensis (orange) and Mangifera indica (mango). The results demonstrated variability in antioxidant capacity among the fruit extracts, with A. comosus, M. pumila and M. zapota showing the highest free radical scavenging activity. In contrast, M. indica and M. pumila exhibited notable but lower anti-inflammatory effects than diclofenac sodium, though they showed significance at various concentrations. The DPPH assay showed a concentrationdependent increase in antioxidant activity for all tested fruit extracts. A. comosus demonstrated the highest antioxidant activity, attributed to its rich content of ascorbic acid, flavonoids and polyphenols [13]. Bromelain, a proteolytic enzyme present in A. comosus, has known antioxidant and anti-inflammatory effects [14]. Antioxidant and cytotoxic effects of A. comosus extracts in breast cancer cell lines were also been seen [15]. In this study, M. pumila also exhibited strong antioxidant activity, aligning with findings by Sivapalan et al. who reported potent antioxidant properties attributed to flavonoids such as proanthocyanidin B1/B2, catechin, epicatechin, cyanidin-3-Ogalactoside and quercetin derivatives [16]. Similarly, M. zapota contains polyphenolic compounds contributing to its antioxidant potential. M. zapota pulp had higher antioxidant activity than peel and seed via DPPH and β -carotene bleaching assays [17]. High ORAC activity for M. zapota (without peel/seed) compared

to other fruits like strawberry and banana is also seen [18]. Although C. lanatus, C. sinensis and M. indica demonstrated antioxidant activity, it was lower than that of A. comosus, M. pumila and M. zapota. This may be due to differences in the concentration and bioavailability of antioxidant compounds. In this study, C. lanatus showed relatively lower antioxidant activity, contrasting prior findings [19]. This inconsistency may be due to variations in extraction methods, fruit ripeness, or environmental conditions. However, C. lanatus peel had higher antioxidant activity than its pulp, based on DPPH and ABTS assays, attributing this to differences in phytochemical composition [20]. Similarly, although M. indica is rich in carotenoids and polyphenols, it showed moderate antioxidant capacity, possibly due to differences in aqueous extraction efficiency [21]. Anti-inflammatory activity, evaluated using the bovine serum albumin (BSA) denaturation assay, was generally lower than that of diclofenac sodium. However, M. indica and M. pumila showed significant inhibition of protein denaturation at several concentrations, indicating noteworthy anti-inflammatory potential. M. indica's effects may be attributed to compounds like mangiferin, known to inhibit key inflammatory pathways [22]. Aqueous leaf extracts of M. indica significantly reduced inflammation in rat models, showing inhibition comparable to diclofenac at 10 mg/kg [23]. Topical and oral administration of M. indica extract reduced ear edema in mice and inhibited inflammatory mediators like TNF-α and PGE2 [24-27]. These findings support the therapeutic potential of fruit-derived antioxidants for use in functional foods and nutraceuticals. The strong antioxidant properties of A. comosus, M. pumila and M. zapota highlight their value in combating oxidative stress. The moderate anti-inflammatory activity of M. indica and M. pumila underscores their promise as natural anti-inflammatory agents. Future research should isolate and quantify specific bioactive compounds using techniques like high-performance liquid chromatography (HPLC) and mass spectrometry (MS) and validate these findings in in vivo and clinical trials.

Conclusion:

A. comosus, M. pumila and M. zapota had strong antioxidant activity M. indica and M. pumila showed notable anti-inflammatory effects. These fruits may serve as natural therapeutic agents in managing oxidative stress and inflammation. Further molecular-level studies are needed to confirm their clinical relevance.

Conflict of Interest: Nil

Sources of Support: Nil

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