STUDIES OF DIFFERENT SOLVENTS ON TOTAL PHENOLIC, FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITIES OF PEEL EXTRACTS OF PINEAPPLE PEEL VAR.JALDHUP

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Abstract
The pineapple (Ananas comosus L.) contains several bioactive and volatile components, the repercussions of oxidative stress are mitigated by natural antioxidants also found in a Pineapple peels var. Jaldhup. This study aims to quantify the total phenolic and flavonoid contents (TPC and TFC) and antioxidant properties of Pineapple peel extracted in various polar and non polar solvents using Microwave assisted extraction (MAE). These peels contain polyphenols and flavonoids that act as free radical scavengers, reduce oxidative stress, and may be an alternative remedy to cure various detrimental human diseases. After investigating several solvents, the highest TPC value was found in methanolic extract at 15.25 mg GAE/g, while the lowest was found in ethyl acetate extract at 2.83 mg GAE/g. The highest TFC value was found in water at 11.18 mg QE/g, while the lowest was found in ethyl acetate at 2.9 mg QE/g. The peel had remarkable antioxidant properties. With methanol, pineapple peel has the highest In vitro antioxidant activity (76.25 µg/mL,), whereas acetone has the lowest In vitro antioxidant activity (61.04 µg/mL). Since pineapple peel has exceptional phytoconstituents, it can be exploited in phytotherapy and pharmacology as a source of natural antioxidants.

Keywords: Microwave assisted extraction, TPC, TFC, antioxidant activity, Ananas comosus L., and phytoconstituents.

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INTRODUCTION

Pineapples (*Ananas comosus*) are an edible tropical fruit high in vitamins, enzymes, and antioxidants. They are economically important tropical fruit crops in tropical and subtropical climates. The pineapple contains water, carbs, sugars, vitamins A, C, and beta-carotene, protein, fat, ash, and fibre in addition to citric and ascorbic acids. Because of its amazing fragrance and flavour, it is also recognized as an exotic fruit (Da Silva, 2013). The crown, outer peel, and inner core of pineapple fruits are often discarded as pineapple trash after they have been canned or eaten, accounting for around 50% of the weight of the entire pineapple fruit (Orodu and Akpedi, 2021[a]). Most fruits' peels are left behind after eating, contributing to agricultural waste. Fruit peels are removed after the edible portion has been consumed and placed in municipal landfills, causing severe pollution and solid-waste management difficulties (Pathak, 2017). The exploitation of agro-industrial waste through conversion into value-added goods is an innovative approach to the issue of environmental waste (Orodu and Akpedi, 2021[b]). The waste consists mostly of seeds, skin, rind (peels), and pomace, which are rich in bioactive components that may be beneficial, such as carotenoids, polyphenols, dietary fibres, vitamins, enzymes, and oils. Plants are generally acknowledged for their medicinal effects, particularly their anti-oxidant action against the majority of ROS-induced diseases. These features have been related to the phytochemical components of the plant, specifically flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins, due to their well-researched antioxidant benefits (Sagar et al., 2018), (Aqil et al., 2006), (Abovwe et al., 2010).

In this research, the influence of various solvents (differ in polarity) employed for extraction on % yield, TPC, TFC, and free radical scavenging activity is examined.

MATERIAL AND METHODS

Chemicals

All the chemicals and reagents used for the research were of analytical grade and procured from authentic supplier.

Raw material collection and Preparation of extract

Pineapple peel were air-dried at room temperature and pulverized into powder for extraction. The powder (100 g) was extracted by Microwave (Make Microsynth) at 750 W in different solvents (1:10 Solid-Solvent ratio) at 60 degree C and for 20 min. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure to get an orange-brown semi-solid extract, then the extract was stored at 4 degree C till further analysis. Using dry weight, the % yield was calculated as %Yield of extract (g/100 g) = (weight of the extract residue after solvent removal× 100) / weight of dried powder (Adam 2019).

Extraction yield

Using dry weight, the % yield was calculated by formula (Adam 2019).

$$\%\, Yield\ of\ extract\ (g/100\ g) = \frac{weight\ of\ the\ extract\ residue\ after\ solvent\ removal}{weight\ of\ dried\ powder} x100$$

Preliminary phytochemical analysis

Phytochemical analysis of the Pineapple peel extract was performed to detect the presence of different classes of secondary compounds, including alkaloids, phenolics, flavonoids, tannins, saponins, terpenes (Khandelwal, 2008) and (Wagner, et al. 1984).

Total Phenolic Content (TPC)

Folin-Ciocalteu colorimetric analysis was used to determine pineapple peel extracts (Chun et al., 2003). The extracts were produced at different concentrations (25, 50, 75, and 100 µg/mL). At 760 nm, absorbance for each concentration of the extracts was recorded against a blank using the standard gallic acid. For each examination, the samples were made in triplicate, and the average absorbance value was used to plot the calibration curve and ascertain the concentration of phenolics in the extracts. The extracts' total phenolic content was quantified as mg gallic acid equivalents (GAE) per g dry sample weight (mg/g).

Total Flavonoid Content (TFC)

The amount of total flavonoid was measured using a colorimetric technique. Qurecitin served as the reference standard for the calibration curve. It was estimated that the extract contained mg/g of Qurecitin equivalents for all flavonoids (Zhishen et al., 1999). Sample preparation for total flavonoid content. The extracts were made as stock solutions at a concentration of 4 mg/mL in methanol, and they were serially diluted to create solutions at various concentrations (0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL). Using a UV spectrophotometer, the absorbance was determined at 510 nm.
The testing of samples for total flavonoid content were analysed in triplicate.

Radical Scavenging Activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl).

The extracts' in vitro antioxidant properties were assessed using the DPPH free radical scavenging test reported by (Gogavekar et al., 2012). In methanol, DPPH in oxidised state produces a rich violet colour. An antioxidant chemical transfers an electron to DPPH, causing it to be reduced, and its colour changes from deep violet to yellow when reduced. DPPH solutions have a high absorbance at 517 nm, resulting in a deep violet colour. The ability of a test sample to scavenge DPPH free radicals indicates its free radical scavenging capacity or antioxidants potential, which demonstrates its effectiveness, avoidance, detection, and repair mechanism against injury in a biological system.

DPPH solution (0.1 M) was formed by dissolving 0.39 mg of DPPH in a volumetric flask, then diluting it with methanol to a final volume of 100 mL. By soaking the needed amount of each extract in the required volume of methanol, a stock solution of 1 mg/mL extracts was created. Each extract was made in 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL solutions from the sample stock solution. 1 mL of DPPH solution was added to the sample solutions of various concentrations and incubated at room temperature for 30 minutes in the dark. A control solution was made by combining 1 mL methanol and 1 mL DPPH solution. Finally, the absorbance of the solutions was measured at 517 nm with a spectrophotometer. Ascorbic acid was taken as the reference.

Statistical analysis

All experimental results were obtained in triplicate and the average values of the results were mentioned as mean ±SD

RESULTS AND DISCUSSION

Extraction yield

Extraction in highly polar solvents resulted in high extract yield but low phenolic and flavonoid content as compared to non-polar ones. The polarity-dependent increase in total antioxidant activity and reducing properties indicates the extraction of strong antioxidant compounds in polar solvents (Nawaz et al., 2020). Methanolic extract showed highest extraction yield 11.95±0.06, while acetone exerted low extraction yield 6.95±0.04, as illustrated in Table 2.

Phytochemical screening

As shown in Table 1, the presence of alkaloids, flavonoids, tannins, phenols, steroids, and terpenoids in water was revealed by phytochemical screening of different pineapple peel extracts, with methanolic and ethanolic extracts being the most polar solvents and acetone and ethyl acetate being the least polar solvents.

Table 1: Preliminary screening of *Pineapple peel with different solvents*

Sr No.	*Phytoconstituents*	*Test*	*Results*				
			Water	*Ethanol*	*Methanol*	*Acetone*	*Ethyl Acetate*
1	Alkaloids	Dragendorff ’s test	+	+	+	+	+
		Mayer’s test	+	+	+	+	+
		Hager’s test	+	+	+	+	+
2	Flavonoids	Alkaline reagent test	+	+	+	+	-
		Shinod’s test	+	+	+	+	+
3	Phenolic compounds and tannins	Ferric chloride test	+	+	+	+	+
		Lead tetra acetic acid test	+	+	+	-	-
4	Glycosides	Keller Killiani test	+	+	+	+	+
5	Saponins	Foam Test	-	-	-	-	-
6	Terpenoids	Horizon test	-	+	+	+	-
7	Steroids	Salkowski test.	-	+	+	+	-

Table 2: Extraction Yield of *Pineapple peel with different solvents*

Sr. no.	*Solvent used in extraction*	*% Extraction Yield*
1	Water	8.84±0.04
2	Ethanol	9.14±0.05
3	Methanol	11.95±0.06
4	Acetone	6.60±0.03
5	Ethyl Acetate	6.61±0.04

(Results are mean ± SD of 3 determinations)

Total Phenolic content

The total phenolic contents of pineapple peel extracts in different solvents were determined using the Folin-Ciocalteu (F-C) technique using gallic acid as the standard. The absorbance values obtained at various gallic acid concentrations were utilized to generate a calibration curve. The total phenolic content of the extracts was estimated using the calibration curve regression equation ($Y = 0.0108x$; $R^2 = 0.993$) and represented as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). TPC values were higher in methanolic extracts than in the other extracts (Methanol >Water >Ethanol >Acetone >Ethyl Acetate), with ethyl acetate extracts having the lowest. The TPC value of the methanolic extract was 15.25±0.296 mg GAE/g, while Ethyl acetate had a value of 2.83±0.438 mg GAE/g (Table 3). The antioxidant capabilities of plants are closely connected to their phenolic content. Phenolic chemicals operate as reducing agents, hydrogen donors, and free radical scavengers (Wojdyło et al., 2007).

Total Flavanoid content

The total flavonoid content of the extracts was estimated using the calibration curve's regression equation ($Y = 0.0011x$; $R2 = 0.992$) and represented as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). TFC values in water extracts were higher than in the other extracts (Water > Ethanol > Methanol >Acetone >Ethyl acetate), with ethyl acetate extracts having the lowest. The water extract showed a TFC value of 11.180.0826 mg QE/g, while ethyl acetate had a value of 2.920.168 mg QE/g (Table 4).

Table 3: TPC of *Pineapple peel with different solvents*

Solvent	*0.25mg/mL*	*0.5mg/mL*	*0.75mg/mL*	*1mg/mL*	*Mean*
Water	14.33	14.38	14.4	14.44	14.387 ±0.536
Ethanol	12.15	12.17	12.18	12.2	12.175±0.474
Methanol	15.22	15.23	15.26	15.29	15.25±0.296
Acetone	12.23	12.24	12.26	12.28	12.12±0.486
Ethyl Acetate	2.79	2.82	2.84	2.87	2.83±0.438

(Results are mean ± SD of 3 determinations)

Table 4: TFC of *Pineapple peel with different solvents*

Solvent	*0.25mg/mL*	*0.5mg/mL*	*0.75mg/mL*	*1mg/mL*	*Mean*
Water	11.15	11.17	11.18	11.22	11.18±0.0826
Ethanol	7.17	7.19	7.23	7.25	7.21±0.118
Methanol	9.84	9.86	9.9	9.92	9.88±0.359
Acetone	6.22	6.26	6.29	6.32	6.272±0.086
Ethyl Acetate	2.88	2.91	2.94	2.96	2.92±0.168

(Results are mean ± SD of 3 determinations)

Antioxidant activity

Methanol extracts exhibited higher DPPH values than the other extracts (Methanol > Ethanol > Ethyl acetate > Acetone >Water). The antioxidant activity of methanol extract was 76.255±0.113 µg/mL, while water was 59.19±0.227 µg/mL. (Table 5).

Antioxidants are hugely vital molecules that have the capacity to protect the organism from free radical-induced oxidative stress. Because of the hydrogen-donating characteristic of their hydroxyl groups, plant polyphenols operate as reducing agents and antioxidants (Aberoumand et al., 2007).

The presence of polyphenols, flavonoids, and phenolic chemicals in pineapple extracts may explain their radical scavenging activity, and phenols account for the majority of plant antioxidant activity. Natural antioxidants found in plants are responsible for reducing or preventing the negative effects of oxidative stress (Mansouri et al., 2007).

CONCLUSION

The total phenolic, flavonoid content, and antioxidant activities of pineapple peel extracts were all remarkably good. However, methanolic extracts performed significantly better in these criteria than the other extracts. The difference in TPC, TFC concentration, and antioxidant capabilities between solvent extracts could be explained by the polarity of the solvent used for phytochemical extraction. Because of its phenolic and flavonoid content, as well as its outstanding DPPH scavenging effects, the current study's findings revealed that pineapple peel could be a rich source of natural antioxidants. As an outcome, it may be more vital in reducing a variety of detrimental human diseases. Further efforts should be conducted toward the pineapple peels extensive in vivo antioxidant activities and the correlation of individual phenolic compounds to antioxidant

Table 5: Radical Scavenging Activity of DPPH of *Pineapple peel with different solvents*

Solvent	*25µg/mL*	*50µg/mL*	*75µg/mL*	*100µg/mL*	*Mean*
Water	59.15	59.18	59.2	59.23	59.19±0.227
Ethanol	64.47	64.5	65.54	65.59	65.025±0.623
Methanol	76.23	76.25	76.26	76.28	76.255±0.113
Acetone	60.98	61.1	61.04	61.07	61.04±0.227
Ethyl Acetate	63.54	63.57	63.59	63.62	63.58±0.108

(Results are mean ± SD of 3 determinations)

with different mechanisms, as well as the isolation, screening, and categorization of pure compounds responsible for antioxidant properties in order to authenticate their probable uses as sources of natural antioxidants and to validate their traditional uses in several treatment of various diseases.

Conflicts of interest

All authors declare that they have no conflicts of interest**.**

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