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COMPARATIVE STUDY OF PHYTOCHEMICAL SCREENING AND ANTIOXIDANT CAPACITIES OF VINEGAR MADE FROM PEEL AND FRUIT OF PINEAPPLE (*ANANAS COMOSUS* L.)

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ABSTRACT

Pineapple peel is a by-product of the pineapple processing industry and it consists of residual pulp, peels, and skin. If pineapple peels are not utilized they can cause environmental pollution The study was carried out with the objective of producing vinegar from the peel and fruit of pineapple, sugar and starter culture. The phytochemical properties and antioxidant activity of pineapple peel vs fruit vinegar were assessed A light yellow colored vinegar was obtained from the pineapple fruit mixture and a light brown colored vinegar was obtained from pineapple peel mixture with mild fruity, acetic aroma through simultaneous fermentation by *Saccharomyces boulardii* and *Acetobacter.* Carbohydrates, saponins, flavanoids, tannins, quinones, terpenoids and coumarines were present in the both pineapple peel and fruit vinegar. The antioxidant content of peel vinegar (2077 mg acetate equivalence/100 ml) was found to be higher compared to that of fruit vinegar. Pineapple peel vinegar can be produced in large scale and marketed for its therapeutic effects and to exhibit environment friendly property over the synthetically produced chemical vinegars.

KEYWORDS: ***Ananas comosus,*** **Fruit peel, Phytochemical, Antioxidant, Vinegar**

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INTRODUCTION

The pineapple (*Ananas comosus* L.) is one of the most important fruits in the world and is the leading edible member of the family *Bromeliaceae*. This fruit juice is the third most preferred worldwide after orange and apple juices [1]. The increasing production of pineapple-processed items, results in massive waste generations. This is mainly due to the selection and elimination of components unsuitable for human consumption. Besides, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55% of product waste[2].Pineapple waste is a by-product of the pineapple processing industry and it consists of residual pulp, peels, and skin. These wastes can cause environmental pollution problems if not utilised. Recently there are investigations/studies carried out on how to utilise these wastes. Pineapple peel is rich in cellulose, hemicellulose, and other carbohydrates [3]. It contains high fiber content and soluble carbohydrates, as well as low protein content [4]. Bromelain is also present in pineapple wastes [5]. Bromelain contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium [6]. Vinegar can be made from any non-toxic material that has a sugar juice or can be made with sugar juice. Vinegar is the product obtained by acetic acid fermentation of alcohol-containing solutions. It is a clear aqueous liquid which is either colorless or has the color of the raw material. Vinegar is a product of two-stage fermentation[7]. Vinegar production is initiated by Saccharomyces spp, utilizing sugars to produce ethanol, which is further acted upon (oxidized) by Acetobacter spp, to yield acetic acid. The product must contain at least 4g acetic acid in 100 ml and pH value should fall between 2.0 and pH 3.5 [8]. The industrial vinegar making process increases supply of oxygen to the bacteria to speed up fermentation, reducing the time of fermentation from few months to 2-3 days or even less. Vinegar is normally produced by a two -stage fermentation process, the first one being the conversion of fermentable sugars to ethanol by yeasts, usually *Saccharomyces* species, and the second the oxidation of ethanol by bacteria, normally *Acetobacter* species [9]. As a result of previous research on vinegar production of various kinds of tropical fruit, pineapple was selected as being an interesting source [10]. Pineapple waste contains valuable nutrient components of simple sugar such as sucrose, glucose and fructose.The phytochemicals present in the final products depend on the source material, which will dictate their type, quantity and quality. Vinegar is a commonly used food ingredient, but also for its medicinal properties and for its physiological effects such as invigorating, a regulator of blood pressure, diabetes mellitus regulator, appetite stimulator, digestion and absorption of calcium[11]. Historically, vinegar has found use as a preservative and a condiment, but only recently has it been considered as a potential functional food ingredient.

MATERIALS AND METHODS

The study was carried out with the objective of producing vinegar from the peel and fruit of pineapple, sugar, and starter culture. The phytochemical properties and antioxidant, and activity of pineapple peel vs fruit vinegar were assessed.

Design of the study

The experimental design was carried out in the following phases

Phase I: Collection of fruit and formulation of Vinegar

A pineapple of 3kg was purchased from the local market. Healthy and ripe pineapples were selected; the pineapple was peeled with well-sterilized knife. The peel was thoroughly rinsed with distilled water and cut into thin strips.The fruit was cut into thin strips.

Preparation of pineapple fruit vinegar

About 125g of chopped fruit was weighed and put into a sterile glass bottle(1000ml). To the fruit 20.0g of sugar, 800ml of distilled water and 3.0g of yeast- *Saccharomyces boulardii* were added and mixed well.The lid was tightly closed and the mixture was allowed to ferment for 4-5 days at 25-28°c with a periodic check of pH.After five days the residues were strained out by filtering through cheese cloth.Two ml of acetobacter mother inoculum was added to the solution and allowed to undergo aerobic fermentation for 15 days by covering the lid with a cheese cloth with periodic shaking

Preparation of pineapple peel vinegar

About 125g of chopped peel was weighed and put into a sterile glass bottle (1000ml), to the peel 20.0g of sugar, 800ml of distilled water and 3.0g of yeast- *Saccharomyces boulardii* were added and mixed well. The lid was tightly closed and the mixture was allowed to ferment for 4-5 days at 25-28°c with a periodic check of pH. After five days the residues were strained out by filtering through a cheese cloth. Two ml of acetobacter mother inoculum was added to the solution and allowed to undergo aerobic fermentation for 15 days by covering the lid with a cheese cloth with periodic shaking.

Phase II: Evaluation of Selected Chemical Constituents

Proximate Principles: Proximated principles like carbohydrate, fat and protein in the vinegar was evaluated using standard AOAC procedures. Minor Nutrients: Quantative analysis of vitamin C, potassium, and phosphorus were carried out.

Phase III: Qualitative phytochemical analysis

The phytochemical tests were carried out using standard methods of analysis of carbohydrates,tannins,saponins, flavanoids, alkaloids,quinines,glycosides,cardiacglycosides,terpenoids,triterpenoids,coumarins,oids,phytosteroids,phlobataninsanthroquinones[12.]

a. ***Test for Carbohydrates***
   To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.
b. ***Test for Tannins***
   To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.
c. ***Test for Saponins***
   To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.
d. ***Test for Flavonoids***
   To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.
e. ***Test for Alkaloids***
   To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.
f. ***Test for Quinones***
   To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.
g. ***Test for Glycosides***
   To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.
h. ***Test for Cardiac Glycosides***
   To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.
i. ***Test for Terpenoids***
   To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.
j. ***Test for Phenols***
   To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.
k. ***Test for Coumarins***
   To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.
l. ***Tests for Steroids and Phytosteroids***
   To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of

bluish brown ring indicates the presence of phytosteroids.

m. Tests for Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

n. Tests for Anthraquinones:

To 1ml of plant extract few drops of 10% ammonia solution were added, appearance pink color precipitate indicates the presence of anthraquinones.

Phase IV: Determination of total phenol content

The amount of total phenol content of different solvent extracts was determined by Folin-Ciocalteu's reagent method [13]. The various concentrations of the extract were made up with 900µl of distilled water and 0.5 ml of Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 minutes. Then, 4 ml of saturated sodium carbonate solution (0.7 N) was added and further incubated for 30 min at room temperature and the absorbance was measured at 765 nm using a digital spectrophotometer , against a blank sample. The calibration curve was made by preparing gallic acid solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g-1 of extracted compounds).

Phase V: Ferric reducing antioxidant power assay (FRAP)

The antioxidant activity of the pineapple vinegar was evaluated by FRAP assay. The reducing ability of different solvent extracts was determined by FRAP assay **.** FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of TPTZ, forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 750 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6- tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM FeCl3] and the reaction mixture is kept in a water bath at 50°C for 20 min and then the absorbance was measured at 750 nm. FeSO4 (100 to 1000 µM ml-1) was used as a positive control [14]**.** The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M FeSO4 equivalents per gram of extracted compound.

RESULTS AND DISCUSSION

Formulation of vinegar from pineapple peel and fruit

During the anaerobic fermentation, there is conversion of sugars to ethanol by the yeast *Saccharomyces boulardii*. Then during the aerobic fermentation, *Acetobacter* converted the ethanol into acetic acid in 15 days where yellow colored vinegar was obtained from pineapple fruit mixture and a light brown colored vinegar was obtained from pineapple peel vinegar with mild fruity acetic aroma. These results are in line with the study done by Yusuf (2012) where he has stated that the conversion of pineapple peels to vinegar, which is a useful product, will reduce the environmental pollution and in addition yield value added product [15].

Evaluation of Chemical constituents

Proximated principles like moisture, carbohydrate, protein, fat in Pineapple peel vinegar, and Pineapple fruit vinegar were evaluated using standard AOAC procedures is presented in Table 1**.**

Table 1
Chemical constituents of Pineapple vinegars

Content	Pineapple fruit vinegar	Pineapple peel vinegar
Moisture (%)	92.3	85.9
Acidity (%)	5.88	8.199
Carbohydrate (%)	1.049	7.69
Protein (%)	1.334	5.39
Fat (%)	0.04	0.039
Potassium (mg)	93.4	124.5
Phosphorus (mg)	12.33	15.98
Vitamin C (mg)	1.98	4.34

From the results obtained, it can be clearly seen that being a liquid the Pineapple fruit vinegar and Pineapple peel vinegar consists of 92.3 percent and 85.9 percent of moisture respectively. According to Walter[16], acetic acid and other organic acids determine the acidity of vinegar. The pineapple peel vinegar found to have high acidity (8.199%) compared to Pineapple fruit vinegar (5.88%). Also the Pineapple peel vinegar was found to possess high amount of carbohydrate (7.69%), protein (5.39%), potassium (124.5mg), phosphorus (15.98mg) and vitamin C (4.34mg) compared to Pineapple fruit vinegar that is composed of 1.049% carbohydrate, 5.39% protein, 93.4mg of potassium, 12.33 mg of phosphorus and 1.98mg of vitamin C. The pineapple skin waste had higher amount of total sugar (9.75%)and non-reducing sugar(8.8%)than juice, which is essential for growth of microorganism and also found to be higher protein content in waste (10mg). Vitamin C present in the pineapple is powerful antioxidant that supports the formation of collagen in bones, blood vessels, cartilage and muscle, as well as the absorption of iron. It also retards the development of urinary tract infections during pregnancy and reduces the risk of certain cancers, including colon, esophagus and stomach [17].

Phytochemical analysis of Pineapple peel and fruit vinegar

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity [18]. Phytochemicals could act as an antioxidant and anti-inflammatory. It plays vital role in detoxification of harmful and deleterious chemicals of the body.The phytochemical tests was carried out using standard methods of analysis of carbohydrates,tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones and is presented in Table 2.

Table 2
Qualitative phytochemical analysis

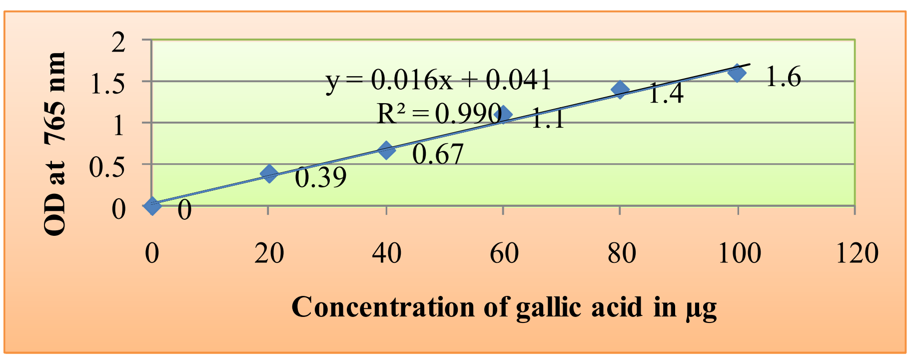
Phytoconstituents	Pineapple fruit vinegar	Pineapple peel vinegar
Carbohydrate	+	+
Tannin	+	+
Saponins	+	+
Flavanoids	+	+
Alkaloids	-	-
Quinines	+	+
Glycosides	-	-
Cardiac glycosides	-	-
Terpenoides	+	+
Phenol	-	-
Coumarins	+	+
Sterols and phytosterols	-	-
Phlobatannins	-	-
Anthoquinones	-	-

From the Table 2, carbohydrate, saponins, flavanoids, tannins, quinones, terpenoids, coumarines are present in the Pineapple peel vinegar and Pineapple fruit vinegar whereas alkaloids, lycosides,phenol,sterols, phytosterols, phloba tannins and anthoquinones are absent in Pineapple peel vinegar and Pineapple fruit vinegar. Phytochemicals like saponin, flavonoids, glycosides and tannins from fruits and vegetables may play key roles in amelioration of diseases [19]. Pineapple contains antioxidant flavonoids and vitamin C. These antioxidants reduce the oxidative damage such as that caused by free radicals and chelating of metal. Studies have it that Saponin could possess antitumor and antimutagenic activities and can lower the risk of human cancer by preventing cancer cells from growing. Flavonoids are the most important plant pigments for flower colouration, producing yellow or red blue pigmentation in petals designed to attract pollinator animals. They have a putative role in inhibition of cancer and cardiovascular diseases[20]. They might induce mechanism that affects cancer cells and inhibit tumor invasion [18]. Tannins have been found to form reversible complexes with proline-rich proteins, resulting in the inhibition of the cell protein synthesis. Plants that have tannins as their main components, are astringent in nature and are used for treating of intestinal disorders such as diarrhoea and dysentery [21]. Phytochemicals in fruits and vegetables can have complementary and overlapping mechanisms of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism and antibacterial and antiviral effects [22].

Determination of total phenol content

Antioxidants are compounds which can delay or inhibit the oxidation of biomolecules by inhibiting chain reaction. Polyphenols are the most abundant antioxidants in our diets. The main classes of Polyphenols are phenolic acids (mainly caffeic acid) and flavonoids like flavonols (catechins plus proanthocyanidins), anthocyanin, and their oxidation products, which account for one- and two-thirds of Polyphenols, respectively[23].

Graph 1
Standard Calibration curve of gallic acid



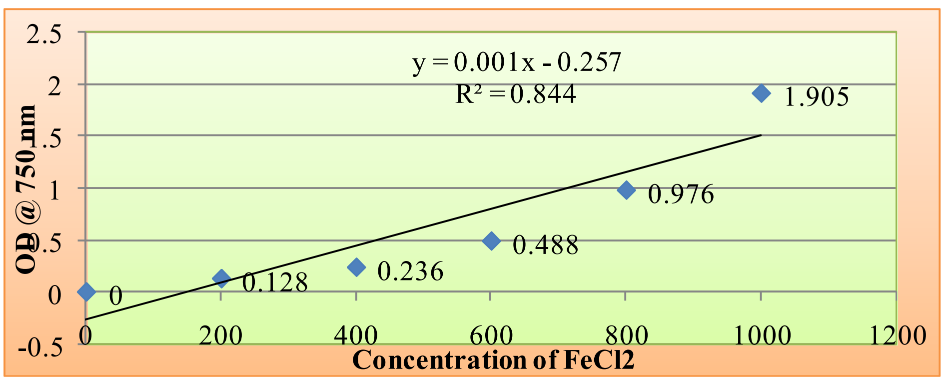
Total phenol compounds, as determined by the Folin-Ciocalteu method (Graph 1), are reported as gallic acid equivalents by reference to standard curve (y=0.016x + 0.041, R^2 = 0.990). The Total Polyphenol Content (TPC) of peel vinegar (93.06 mg GAE/100 ml) was found to be high compared to that of fruit vinegar (36.81 mg GAE/100 ml). This result in the present study is due to the presence of phytochemicals such as Phenolic compounds and flavonoids. These phytochemicals are a diversified group of secondary metabolites that are ubiquitous in the plant kingdom [24]. Phenolic compounds with health-promoting activity are found in dietary plants. Being so, fruits and vegetables represent remarkable sources of bioactive compounds that could play a role in preventing diseases caused as a result of oxidative processes, such as cardiovascular problems, auto-immune complications, type 2 diabetes and age-related dysfunctions [25]. Recent studies have shown that phenolic phytochemicals exert anti-diabetic activity through inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase. Natural alpha-amylase inhibitors offer an attractive approach to the management of postprandial hyperglycemia by decreasing glucose release from starch[26]. Several findings [27, 28] suggest that phenolic synergies may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of Type 2 diabetes. In addition, research results show that phenolic antioxidants have significantly *in vitro* antiglycation properties. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [29]. Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations [30].

Antioxidant activity of Pineapple vinegars by FRAP method

The results showed that FRAP values were higher in Pineapple Peel vinegar compared to Pineapple Fruit vinegar. The Antioxidant activity as acetate as standard was determined by FRAP method are reported as $FeCl_2$ equivalents by reference to a standard curve (graph 2) (y=0.001x – 0257, R^2 = 0.844). The antioxidant content of peel vinegar (2077 mg $FeCl_2$ equivalence/100 ml) was found to be high compared to that of fruit vinegar (1497 mg acetate equivalence/100 ml).

Graph 2

Standard Calibration curve of FRAP assay



Antioxidants are radical scavengers that can protect the human body from free radicals that may cause many diseases, including cancer, and contribute to the aging process. The type of phenolic present could exert a crucial effect on its antioxidant activity of a substance [31]. According to Bocco *et al*. (1998)[32], it is also possible that non-phenolic substances could contribute to the antioxidant activity. Pineapple has been used as a medicinal plant in several native cultures and these medicinal qualities of pineapple are attributed to bromelain, which is a crude extract from the pineapple that contains, among other compounds, various closely related proteinases, exhibiting various fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities *in vitro* and *in vivo* [33]. A wide range of therapeutic benefitshas been claimed for bromelain, such as reversible inhibition of platelet aggregation, sinusitis, surgical traumas [34], thrombophlebitis, pyelonephriti angina pectoris, bronchitis, and enhanced absorption of drugs, particularly of antibiotics[35]. Recent studies have shown that bromelain has the capacity to modify key pathways that support malignancy. Presumably, the anticancerous activity of bromelain is due to its direct impact on cancer cells and their microenvironment, as well as on the modulation of immune, inflammatory, and haemostatic systems[36, 37,38,39].

CONCLUSION

In recent years, with the increasing production of pineapple, there is generation of massive waste products. These wastes can cause environmental pollution problems if not utilized properly. Hence, this study has enabled the conversion of the pineapple waste such as the peel into a value added product, thus reducing the wastage as well as producing healthy organic vinegar. Pineapple Vinegar may be used as an ingredient of sweet-and-sour sauces for meat and vegetable dishes, or as an ingredient in baking, as a part of the leavening process. Commercially and in the home it can be used in making salad dressings. Pineapple vinegar to preserve foods by the process of pickling , because the acidity of the vinegar keeps many microorganisms in check. It is used to preserve foods such as fruits, onions and walnuts. It is used as an ingredient in sauces such as ketchup, mustard and mayonnaise. Marinades often contain vinegar. Pineapple Vinegar can also be used as a condiment and as a substitute for lemon. On comparing the overall phytochemical and antioxidant activity between the obtained Pineapple fruit vinegar and Pineapple peel vinegar. Pineapple peel vinegar had comparatively high total phenol content and antioxidant activity. Hence, pineapple peel vinegar can be produced in large scale and marketed for its therapeutic effects and to exhibit environment friendly property over the synthetically produced chemical vinegars.

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