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Department of Chemistry, Easwari Engineering College (SRM Group of Institutions), Chennai-600089, India.

A Laborious Invitro Analysis of Primary Phytochemical Screening and Antioxidant Activities in *Punica Granatum*

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ABSTRACT: The human body has a pH balance, a measure of range between acidic and alkaline. The body's pH balance depends mostly on what we eat and how our bodies process. The food. Acidity is the major problem in human being. It leads to various disorders such as ulcer, cancer and digestive problem. The chemical compounds that have health promoting, disease preventing or medicinal properties. The medicinal plants posses to prevent the ulcer. The anti- ulcer and acidity properties of pomegranate is reported that a photochemical compound with anti – ulcer activity includes tannin. Our bodies should be more alkaline than acidic. The tannin is the best remedy for the acidity. The aim of the study is to analyze the effect of pomegranate for acidity and stomach problems. In the present investigation the fruit and skin of the pomegranate was taken and the pH level, the amount of bicarbonate, tannin, antioxidant activity, were measured.

KEY WORDS: Pomegranate, acidity, antioxidant. Tannin

I. INTRODUCTION

Pomegranate produces anticancer effects in experimental models of lung, prostate and skin cancer. More recently, pomegranate has been found to be anti-carcinogenic in the colon. (seher a. Khan). Pomegranate juice can offer a wide protection against cardiovascular diseases which could be related to its inhibitory effect on oxidative stress and on serum ACE activity (Michael Aviram et al.). (Huang, Tom H. et al.,) indicate that Punica granatum flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats, at least in part, by the diabetic heart shows increased fibrosis, which impairs cardiac function. The photochemistry and pharmacological actions of all Punica granatum components suggest a wide range of clinical applications for the treatment and prevention of cancer, as well as other diseases where chronic inflammation is believed to play an essential etiologic role (Ephraim P. Lansky et al.,). Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity (Julie Jurenka, MT (ASCP) et al.,)

II. MATERIALS AND METHODS

DETERMINATION OF pH

A small amount of sample is taken in clean watch glass place the pH paper from wide range book-let is dipped into a sample with the help of a forceps and the colour attained on the paper is compared with that of the colour on the pH chart. The approximate pH value is thus determined. Another suitable narrow range pH paper is taken and the procedure is repeated for accurate value of the same sample. The same procedure is taken and the procedure for the sample band pH values is recorded.

ESTIMATION OF CARBONATE AND BICARBONATE

Carbonate and bicarbonate are generally determined in extract by titration with 0.01 N H₂SO₄ to pH 8.3 and 4.5, respectively (Richards, 1954). Pipette 10 - 15 ml sample saturation extract into a wide-mouthed porcelain crucible

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or a 150-ml Erlenmeyer flask.. Added 1 drop phenolphthalein indicator. If pink color developed, added 0.01N sulfuric acid by a burette, drop by drop, until the color disappears.. Take the reading, y.. Continue the titration with 0.01N sulfuric acid after adding 2 drops 0.1% methyl orange indicator until the color turns to orange.. Take the reading, t.

Calculations

For Carbonate and Bicarbonate in sample: CO $_3$ (meq/L) = $-2y \times N \times R \times 1000$ Wt

 $HCO_3 \text{ (meq/L)} = (t - 2y) \times N \times R \times 1000$

Wt

Where: R = Ratio between total volume of the extract and extract volume used for titration. $N = Normality \ of \ H_2SO_4$ solution.

Tannin

The tannin present can be analysed by thin layer chromatography. The samples of *pomegranate* were wetted with a half diluted NH₄OH and with EtOAc for 24 hours at room temperature. The organic phase is separated from the acidified filtrate and basified with NH₄OH (pH 11-12). It is extracted with chloroform (3 xs), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture Acetone, Toluene, Formic acid, Ethyl Acetate in the ratio of 60:60:10:1. The color and R_f value of the separated tannin were recorded visible light after spraying with Prussian blue reagent.

REDUCING POWER SCAVENGING ACTIVITY

To determine the reducing power assay of Plant Sample by Yildrim et al., Method, 2001.

1 ml of plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferric chloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

Calculation

Percentage scavenging activity =
$$\frac{A_{control} A_{test}}{A_{control}} = \times 100$$

Where $A_{control}$ is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

III. RESULT AND DISCUSSION

TEST	SAMPLE1(SKIN)	SAMPLE2(FRUIT)
pН	2	2
Carbonate and bicarbonate		
Anti oxidant activity	96.4%	98.3%
Tannin	Rf =0.3133	Rf =0.4217

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pH is essential for the skin. The stomach is acid base nature. More acid compounds leads to the ulcer. The same pH are 2 in both skin and fruit. Small amounts of sodium bicarbonate have been shown to be useful as a supplement for athletes in speed-based events, like middle distance running, lasting from about one to seven minutes. But overdose is a serious risk because sodium bicarbonate is slightly toxic and in particular gastrointestinal irritation is of concern. Here the bicarbonate are absent in the sample. The sample pomegranate contains the tannin and the Rf value is 0.42 and 0.31 in fruit and skin. The most abundant of pomegranate tannins are called <u>punicalagins</u>. Punicalagins have a molecular weight of 1038 and are the largest molecule found intact in rat plasma after oral ingestion and were found to show no toxic effects in rats who were given a 6% diet of punicalagins for 37 days. Punicalagins are also found to be the major component responsible for pomegranate juice's <u>antioxidant</u> and health benefits. Anti-oxidant activities of the samples were estimated by power reducing assay. The fruit extract of the sample shows higher activity than the skin. In fruit extract 98.3% of activity was present in where as the skin possess the 96.4% respectively.

EXTRACT PREPARATION



DETERMINATION OF pH TEST



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ANTI OXIDANT ACTIVITY





ISOLATION OF TANNIN





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