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Study of estimation of L-ascorbic acid, phytochemical analysis and antibacterial activity in fruit juices

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Abstract
The fruits of lemon, pomegranate and black grapes were subjected to phytochemical analysis, vitamin C estimation and antibacterial activity. Amla juice was found to contain large amount of vitamin C compared to orange, lemon, pomegranate and black grape juices. Theproteins, carbohydrates, flavonoids, glycosides, alkaloids, steroids, saponins and tannins were present in juices of pomegranate and black grapes while the fruit juices of orange indicated the presence of flavonoids, steroids, glycosides, saponins and tannins. The alkaloids, proteins, carbohydrates, flavonoids and tanninswere found in the juice of amla.The aqueous extract of pomegranate showedanti bacterial activity against *B.cereus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa* and *S.aureus.*

Keywords: vitamin C, antibacterial activity, phytochemical analysis etc.

Introduction
Vitamin C is also known as ascorbic acid, is an organic acid with antioxidant properties. This miracle vitamin cannot be synthesized by the body. The vitamin C deficiency disease, scurvy has been known for many centuries and was described in the Ebers papyrus of 1500 BC and by Hippocrates. The Crusaders are said to have lost more men through scurvy than were killed in battle, while in some of the long voyages of exploration of the 14th and 15th centuries up to 90% of the crew died from scurvy. Cartier's expenditation to Quebec in 1935 was stuck by scurvy; the local Indians taught him to use an infusion of swamp spruce leaves to prevent or cure the condition.
Vitamin C is a water soluble, antioxidant vitamin, while the deficiency of vitamin C causes scurvy. Citrus fruits (lime, lemon, orange, and grapefruit) and tomatoes are good common sources of vitamin C. Good sources of vitamin C are amla, guava, orange, lime, papaya etc.
Citrus is a promising source of vitamin C, folate, and flavonoids due to which citrus is used as a cancer preventing agent[13].Citrus sinensis is a rich source of sugars, acids, polysaccharides, and many other phytochemicals such as vitamin C and carotenoids, which provided health benefits against various diseases including cardiovascular and cancer diseases [3,4].The antibacterial activity of orange peel (*Citrus sinensis*) in petroleum ether and aqueous extract of peel were screened against *E.coli, S.aureus, S.typhi, B.subtilis, K.pneumoniae,* the excellent antibacterial activity was noted[1]. The antimicrobial activity of orange peel extract in different solvents cold water, ethyl acetate, acetone and ethanolic extract of peel showed more inhibitory against *S. typhimurium, P. aeruginosa, E. coli, S.aureus, S. typhi, B. subtilis, K.pneumoniae* [7].

2. Materials and Methods
2.1. To determine the concentration of Ascorbic acid (Vitamin C)
Only vitamin C reduces indophenols dye. The other constituents of citrus fruit have no action on this dye.

Chemicals
Dye solution: Dissolve sodium salt of the dye (0.125g) in water (approximately 200 ml) and make up the volume to 250 ml in a measuring flask with phosphate buffer (equivalent to

0.0165 g of K_2HPO_4 and 0.0202 g of KH_2PO_4 per 250 ml of the solution).

Standard Ascorbic acid solution: A fresh solution (just before use) was prepared. Dissolve pure 100 mg ascorbic acid in distilled water in a 100 ml volumetric flask.

Fruit juice: It was obtained in the usual way by using a fruit juicer; the fresh juice is filtered and diluted with water so that approximately one ml of the diluted fruit juice contains at least 0.1 mg of ascorbic acid.

Procedure: In a 100 ml conical flask, pipette out 5 ml of the fruit juice with 1 ml glacial acetic acid followed by addition of 1 ml of chloroform. The solution was titrated against the dye solution taken in a burette till pink color was obtained. In case, the fruit juice was colored then observed the end point in chloro form layer. The titration is repeated till a concordant reading was obtained.
Similarly, perform titration with the standard ascorbic acid solutions of 5 ml each time until concordant readings were obtaine and a blank titration using 5 ml of water in the same manner.

Calculations
Weight of ascorbic acid dissolved in 100 ml of solution = W g
Volume of dye solution consumed with 5 ml fruit juice = V_1 ml
Volume of dye solution consumed with standard Ascorbic acid (5 ml) =V_2 ml
Volume of dye solution consumed in blank (5 ml water) =V_3 ml
Concentration (mg/100 ml) of ascorbic acid

$$\text{In the fruit juice} = \frac{V_1 - V_3}{V_2 - V_3} \times W \times \text{dilution of the fruit juice}$$

Note: If 50 ml of original fruit juice was diluted to 100 ml, the dilution will be 2.

2.2. Photochemical analysis in Fruit juices
Preparation of Extract
The fresh fruits were taken (100 g) and crushed using mixer grinder, the extracts of fruits were filtered through What man filter paper No.1. The clear filtrate was obtained and was stored in the refrigerator for experimental purpose.

Phytochemical analysis
The analysis was carried out by using the standard methods of Sofowora and Kepam [15, 6]

2.3. Antibacterial activity of fruit extract
Preparation of extract
Fruits should be washed with distilled water, air dried in oven and ground properly to get powder form. Weighed 10g of fruit powder with 100ml of water and the extract was mixed well using a magnetic stirrer and filtered through a What man filter paper. The filtrate was evaporated in a water bath to obtain the solid extract.

Method
Muller Hinton agar was prepared and sterilized for 15 min. at 121° C. Then 20ml of medium was poured into a sterilepetridishes. After solidification the microbial pathogens were swabbed on the agar surface. After 10 min the well was made by sterile cork borers and the plant or fruit extracts were poured on the well in known concentration (10 & 20 µg/ml). After 24hrs incubation the zone of inhibition will be measured and expressed as millimeter (mm).

3. Results and Discussion
In this present estimation the concentration of ascorbic acid in lemon juice, orange juice, amla juice, pomegranate juice, and black grape juice was determined by titrating against a solution of a dye viz., 2, 6-dichlorophenol indophenols dye. The concentration of ascorbic acid in juices like lemon, orange, amla, pomegranate, black grape was studied using 2, 6-dichloro phenol indophenol dye in the following way. In a 100 ml conical flask, pipette out 5 ml of the fruit juices with 1 ml of glacial acetic acid followed by addition of 1 ml of chloroform. The solution was titrated against the dye solution taken in a burette till a pink colour was obtained. The concentration of fruit juices per 100 ml of lemon, orange, amla, pomegranate and black grape were estimated at 170.52 mg, 127.36 mg, 232.63 mg, 48.42 mg, 16.84 respectively (Fig. I).
From the result we concluded that the amla juice has larger amount of vitamin C content compared to lemon, orange, pomegranate, and black grape. The concentration of vitamin C content in black grape was minimum when compared with the other fruit juices. Fig.1**.** Compare the ascorbic acid content in the fruit juices (lemon, orange, amla, pomegranate, black grapes) in 100ml.

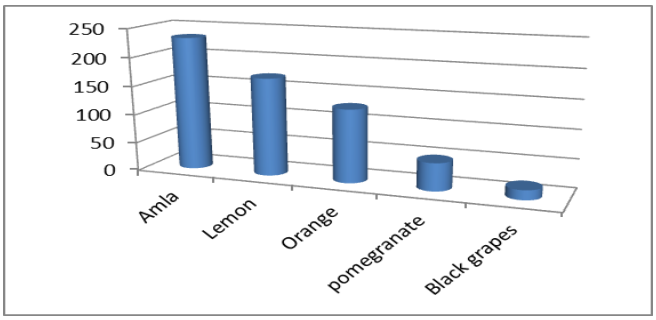

Table 1: Qualitative analysis of various fruit juices for phytochemical composition

Components	Lemon	Orange	Pomegranate	Black grape	Amla
Proteins	+	-	+	+	+
Carbohydrates	+	+	+	+	+
Tannins	+	+	+	+	+
Saponins	+	+	+	+	-
Glycosides	+	-	+	+	-
Steroids	-	+	+	+	-
Flavonoids	+	+	+	+	+
Alkaloids	+	-	+	+	+

+ = present - = absent

In the present study, the fruit juices of lemon, orange, amla, pomegranate, black grape were phytochemically analysed (Table 1). The alkaloids, proteins, flavonoids, glycosides, steroids, carbohydrates, saponins and tannins were present in juices of pomrgranate and black grapes while the fruit juices of orange indicated the presence of flavonoids, carbohydrates glycosides, steroids, and tannins. The alkaloids, proteins, flavonoids, carbohydrates andtannins were found in the juice of amla.
The alkaloids, flavonoids, saponins, glycosides, proteins, carbohydrates and reducing sugars were present in juices of lemon. phytochemical analysis on the fruit of C. sinensis was reported [11] the presence ofphenols, flavonoids, reducing

sugars, steroids, terpenoids and tannins.

Citrus sinensis fruit wastes contain stores of potentially useful biological products [10]. The aqueous *Citrus limonum* peel extracts revealed the presence of carbohydrates, alkaloids, tannins, fixed oils, proteins, cardiac glycosides, steroids, phenols and flavonoids and amino acids and the ethanolic peel extracts contained carbohydrates, saponins, tannins, fixed oils, cardiac glycosides, steroids, phytosterols, phenols and flavonoids [8] that citrus fruits are rich sources of phytochemicals [3].

Steroids are known to be an important cardio tonic activities posses antimicrobial property and also used in herbal medicines and cosmetics. The presences of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na+ and K+ pump that increase the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. A large number of exo-heterocyclic steroids display a potential as inhibitors of human cytochrome $P450_{17\alpha}$ [14]. The group of phenolic compounds are flavanoids and tannins that act as primary antioxidants [12]. The herbs contain aconitine, diterpenoid esters and derivatives that activate sodium channels *in vivo*, causing dose-dependent cardiac and neurological toxicity [5]. Saponins which act as antimicrobial potential in plants [9].

Table 2: Antibacterial activity of five fruit extracts measured in (mm)

Bacterial Culture	Fruit Extracts				
	Lemon	pomegranate	Amla	Black grapes	Orange
B.cereus	-	18	-	-	-
E.coli	10	17	14	7	23
K.pneumoniae	30	25	-	15	7
P.aeruginosa	-	21	5	-	28
S.aureus	26	23	-	-	25

mm= millimeter (diameter of inhibition zone), - = No Inhibition

Five out of two bacteria used (*B.cereus* and *S.aureus*) are Gram-positive and three (*E. coli*, *K. pneumoniae*and *P. aeruginosa*) are Gram-negative. Aqueous extract of pomegranate was found to be most effective against the bacterial strain, *B. cereus*. The inhibitory concentration was calculated to be 18mm likewise. Orange aqueous extract was found to be most effective against *E.coli* as 23mm and *P.aeruginosa* at 28mm and no activity against *B. cereus*. Aqueous extract of lemon was found to be most effective against *K. pneumoniae* as 30mm and *S.aureus* as 26mm and no activity against *B.cereus* and *P.aeruginosa*. Aqueous extract of amla showed zone of inhibition of 14mm and 5mm against *E.coli* and *P.aeruginosa*. Aqueous extract of black grapes showed zone of inhibition of 7mm and 15mm against *E.coli* and *K.pneumoniae*.

The antibacterial activity of pomegranate juice against bacterial species in the order

K.pneumoniae>S.aureus>P.aeruginosa>B.cereus>E.coli

For orange juice, the order is *P.aeruginosa>S.aureus>E.coli> K.pneumoniae*

For lemon juice, the order is *K.pneumoniae>S.aureus>E. coli*

For amla juice, the order is *E.coli>P. aeruginosa*

For black grapes juice, the order is *K.pneumoniae>E.coli*

4. Conclusion

Amla juice was found to contain maximum of vitamin C content compared to other fruit juices which were taken for this study. Phytochemical analysis indicated that the presence of tannins, saponins, proteins, carbohydrates, glycosides, steroids, flavonoids and alkaloids were present in pomegranate and black grape. The alkaloid and glycoside components of the fruit possessing can be showed the anticancer activity which can be further used as drug supplement. The anti-bacterial activity were observed in the aqueous extract of pomegranate against *B.cereus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa* and *S.aureus.* The natural fruits have relevant advantages over synthetic compounds; they are easily available and comparatively cheaper can be used without any side effects.

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