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Original Article

Phytochemical Studies of *Phyllanthus emblica, Ananas comosus, Momordica charantia* Extracts

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ABSTRACT

The present study was carried out to screen phyto constituents and evaluate the antioxidant activity of *Phyllanthus emblica, Ananas comosus, Momordica charantia* extracts and its potential as preservative. The antioxidant effects were evaluated for radical scavenging activity using FRAP with certain modifications. The chloloform extract of *Momordica charantia* revealed highest free radical scavenging activity. Similarly the ethanolic extracts of *Phyllanthus emblica* has also possess significant scavenging effect. Total phenolic content of the extracts *Phyllanthus emblica, Ananas comosus, Momordica charantia* were determined by Follins Ciocalteau method with certain modifications. Positive correlations were found between Total Phenolic Content of the extracts and Antioxidant activity. The Phytochemical screening suggests that phenols and flavonoids of these extracts might provide a considerable antioxidant potential. Quantification of ethanol extract of *Phyllanthus emblica* by HPLC method revealed the Vitamin C content in them, which serve as a Preservative agent. Addition of Vitamin C content of *Phyllanthus emblica, Ananas comosus, Momordicacharantia* in commercial foods will increase the Antioxidant content and may have a potential as a natural preservative.

Keywords: Antioxidants,Free radical scavenging activity,Vitamin C, Preservatives

1. INTRODUCTION

Food preservation is to prevent the growth of bacteria, fungi (such as yeasts), or other micro-organisms (although some methods work by introducing benign bacteria or fungi to the food), as well as slowing the oxidation of fats that cause rancidity. Ascorbic acid neutralizes oxygen when it comes into contact with it. Oxygen allows foods to continue to ripen, an aging process similar to the one people go through that ends in death.

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Oxygen is also vital for many microorganisms to thrive, some of which cause decay. Ascorbic acid slows or neutralizes these events. The substance blocks cured meat's propensity to form carcinogens called nitrosamines, for example. In the process, the vitamin also preserves the color in addition, ascorbic acid preserves flavor.

Natural substances such as (Abdulmumeen et al., 2012) [1] salt, sugar, vinegar, alcohol, and diatomaceous earth are also used as traditional preservatives. Certain processes such as freezing, pickling, smoking and salting can also be used to preserve food. Another group of preservatives targets enzymes in fruits and vegetables that continue to metabolize after they are cut. For instance, citric and ascorbic acids from lemon or other citrus juice can inhibit the action of the enzyme phenolase which turns surfaces of cut apples and potatoes brown. Most foods contain enzymes or natural chemicals, such as acids or alcohols that cause them to begin to lose desirable characteristics almost immediately after harvest or preparation (Shazia khanum mirza etal., 2017). [2]

Ascorbic acid is found in nature in many fruits and vegetables (especially citrus fruits and peppers) and is also produced by the kidney of some animals. Humans are not able to produce ascorbic acid and must obtain it from the diet, or else they will develop a deficiency and, in more severe cases, scurvy. Industrially, ascorbic acid is produced through a multistep process involving bacteria that reduce glucose and produce ascorbic acid as a by product widely used as preservative.(Abubakar EI Ishaq etal., 2015) [3]

The low pH of ascorbic acid can help prevent microbial growth, thereby preventing spoilage and preserving freshness. Ascorbic acid is a vitamer of Vitamin C, which means it is a compound that provides the same vitamin activity as Vitamin C.For these reasons, ascorbic acid is a popular natural ingredient preservative. It can be used as a preservative in a vast array of food products, including bread, cured meats, jams and jellies, and other sauces and spreads.

The Vitamin C properties of ascorbic acid make it an excellent ingredient for vitamin supplementation. Simply adding ascorbic acid to food increases the Vitamin C content. Since naturally occurring Vitamin C is easily destroyed, many foods are fortified with ascorbic acid to replenish the Vitamin C content. Ascorbic acid is often added to fruit juices, dried fruit, cereal, and other snack foods for this purpose.

The flavor of ascorbic acid shouldn't be overlooked. Like any acid, it provides a nice tart flavor that enhances many food products. Candies, jams, jellies, and fruit juices often benefit from this burst of acidity that gives the consumer the distinct impression of fresh fruit.

The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, carotene.However numerous studies have conclusivelyshown that the majority of the anti-oxidant activity may be from compounds such as flavonoids, isoflavone, flavones, -carotene (Wang et al., 1996;βanthocyanin, catechin and isocatechin rather than from Vitamin C, E and Kahkonen et al., 1999) [4, 5]

Phyllanthusemblica, also known as emblica, emblicamyrobalan,myrobalan,Indiangooseberry,Malaccatree or amla from Sanskrit amalika is a deciduous tree of the family Phyllanthaceae. It is known for its edible fruit of the same name.

Medicinal uses

Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. Preclinical studies have shown that amla possesses antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, cardioprotective, gastroprotective, antianemia, antihypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, hepatoprotective, nephroprotective, and neuroprotective properties. In addition, experimental studies have shown that amla and some of its phytochemicals such as gallic acid, ellagic acid, pyrogallol, some norsesquiterpenoids, corilagin, geraniin, elaeocarpusin, and prodelphinidins B1 and B2 also possess antineoplastic effects. Amla is also reported to possess radiomodulatory, chemomodulatory, chemopreventive effects, free radical scavenging, antioxidant, anti-inflammatory, antimutagenic and immunomodulatory activities, properties that are efficacious in the treatment and prevention of cancer..

Dietary constituents are shown to play an important role in the development of diabetes fruits of *Emblica officinalis* or *Phyllanthusemblica Linn* possess anti-diabetic effects through their antioxidant and free radical scavenging properties. Amla has also been reported to prevent/reduce hyperglycemia, cardiac complications, diabetic nephropathy, neuropathy, cataractogenesis and protein wasting. However, clinical trial data with human subjects are limited and preliminary. (Tasduq et al., 2005,; Jayaweera, 1982). [6, 7]

Momordica charantia, known as bitter melon, bitter gourd, bitter squash, or balsam-pear, is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. Its manyvarieties differ substantially in the shape and bitterness of the fruit.

Medicinal Uses

It has been used as a folk remedy for a variety of ailments, particularly stomach complaints. In traditionalmedicine of India different parts of the plant are used as claimed treatments for diabetes (particularly Polypeptide-p, anti insulin analogue),and as a stomachic, laxative, antibilious, emetic, anthelmintic agent, for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcer, gout, and rheumatism.

*Ananascomosus*is a tropical plant with an edible multiple fruit consisting of coalesced berries, also called pineapples, and the most economically significant plant in the Bromeliaceae family.

2. MATERIALS AND METHODS

Sample collection

The vegetable and fruit sample was collected from S.L.N fruits and vegetable stall, K.Narayanpura, Bangalore.

Crude Extraction

Fresh plant material was collected, shade dried and powdered in a mixer grinder.10g of each plant material(*Phyllanthusemblica, Ananascomosus, Momordicacharantia)*were extracted with 50ml of different solvents such as Ethanol, Methanol and Chloroform for 48 hours at room temperature. The solvent was removed from the sample by evaporating at 65°C using a water bath. Then 50ml of the respective solvents were added into each extract in the beaker and filtered using sterile cotton gauze. The extract was stored in a air tight container and used for further studies. (SusyTjahjani *et al*.,2014). [6]

The extracts of different plant materials were subjected to Phytochemical studies using the method developed (Trease& Evans in 1989) [8]

A) Test for Terpenoids (Salkowski Test):

To 0.5ml of the each extract, add 2ml of chloroform. Then 3ml of Concentrated $H_2SO_4$was carefully added to form a layer .A reddish brown coloration of the interface indicates the presence of terpenoids.

B) Test for Flavonoids:

5ml of dilute ammonia was added to 0.5ml of the extracts .To that 1ml of concentrated sulphuric acid was added. A yellow coloration that disappeared on standing indicates the presence of flavonoids.

C) Test for Saponins:

To 0.5ml of extracts, 5ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion, presence of an emulsion indicates the presence of saponins.

D)Test for Tannins:

About 0.5ml of the different extract were boiled in 10ml of water in a test tube. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue or black coloration .This indicates the presence of tannins.

E)Test for Alkaloids:

0.5ml of the extracts were diluted to 10ml with acidified alcohol and boiled . To 5ml of this diluted extract, add 2ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10ml of acetic acid. To this , Mayer's reagent was added. The formation of a cream precipitate was regarded as positive for the presence of alkaloids.

F) Test for Anthraquinones:

0.5ml of the extracts were boiled with 10ml of sulphuric acid. 5ml of chloroform was added and shaken well. The chloroform layer was pipette into another test tube and 1ml of 10% dilute ammonia was added. The resulting solution was observed for colour changes as an indication for the presence of Anthraquinones.

G) Test for Cardiac Glycosides (Keller-Killiani Test):

To 0.5ml of extracts were diluted with 5ml of distilled water, add 2ml of glacial acetic acid containing one drop of 0.5% ferric chloride solution. This was mixed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. The formation of a violet ring below the brown ring and in the acetic acid layer a greenish ring formation just above the brown ring, and gradually spreading throughout this layer indicated the presence of cardiac glycosides.

H)Test for Steroids:

2ml of acetic anhydride was added to 0.5ml of the extracts. To this , 2ml of concentrated sulphuric acid was added. The colour changed from violet to blue or green indicated the presence of steroids.

I)Test for Phenols (Ferric Chloride test):

0.5ml of extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

J)Test for Carbohydrates:

0.5ml of extracts were dissolved individually in 5ml of distilled water and 2% anthrone reagent was added followed by concentrated sulphuric acid. A dark green colour indicated the presence of carbohydrates.

K)Tests for Oils and Resins:

The extract was applied on a whatsmann filter paper. The development of a transparent appearance on the filter paper indicated the presence of oils and resins.

Determination of Total Phenolic content

The ethanol, chloroform and methanol extract of *Phyllanthus emblica, Ananas comosus, Momordica charantia* was subjected to the estimation of total phenolics using Folin-ciocalteau reagent.0.2ml to 1ml of Working standard Resorcinol(100µg/ml) standard was pipetted out into clean dry test tubes.0.5ml and 1ml of the extract was pipette out into two different clean test tubes for each fruit respectively.The volume in each tube was made upto 3ml with distilled water.0.5ml of Folin-ciocalteau reagent was added to all the test tubes.After 3minutes, 2ml of 20% Na_2CO_3 solution was added to each tube.The tubes were mixed well and placed in the boiling water bath exactly for 1minute and cooled. The absorbance was measured at 650nm against a blank containing distilled water .The standard curve was plotted by taking absorbance against the concentration of the standard. Using the standard graph, the concentration of phenols present in the test samples were determined.

Evaluation of Antioxidant potential

i)FRAP ASSAY(Ferric Reducing Antioxidant Power)

0.2ml to 1ml of the standard was pipetted out into clean dry test tubes.0.2ml of extract was added to test tubes labeled as Test. Then 3.8ml of FRAP reagent [83.3ml of 0.1mM acetate buffer pH 3.6, 8.3ml of 0.3mM of 2,4,6-tripyridyl-s-triazine(TPTZ) solution and 8.3ml of 10mM of $FeCl_3.6H_20$] was added to all the tubes. The above reaction mixture was incubated for 30minutes at 37°C .After incubation, the absorbance was measured at 570nm against a blank using ascorbic acid as standard.

Quantification of vitamin C from Gooseberry extract by HPLC

The quantification of preservative compounds from gooseberry ethanol extract was carried out based on chromatography separation. Quantification was done based n peak height and peak area obtained. The presence of preservative compound in gooseberry ethanol extract were quantified by HPLC technique at 250nm using peak area by comparison to a calibration curve of suitable standard (Ascorbic acid)

Preparation of standard and sample solution

Standard Vitamin C was weighed separately (0.0252gms in 50ml buffer). T he ethanol extracts of gooseberry was accurately weighed. Extract is also taken as that of the standard concentration and filtered through whatsmann filter paper and the filtrate was used for analysis.

Chromatography equipment and conditions

The chromatography analysis was performed on HPLC 1200 series E2 chrome software with 100% buffer and trifluroacetic acid as mobile phase at the flow rate of 0.6ml/min.The column effluent was monitored at 250nm with 1200 series multi wavelength detector.

3. RESULTS AND DISCUSSION

Percentage yield of the samples

The percentage yield of *Phyllanthus emblica, Ananascomosus,* and *Momordica__charantia*extracts are shown in the Table No.2.

Table 1: Percentage of yield extract of *Phyllanthus emblica, Ananascomosus,* and *Momordica charantia*

Sl No	Solvents	Yield (%) Sample		
		Gooseberry	Pineapple	Bittergourd
1	Ethanol	66	60	64
2	Methanol	65	64	60
3	Chloroform	64	64	64

Amongst all the samples ethanol extract of *Phyllanthus emblica*has the highest yield percentage of 66% and then followed by methanol extract of *Phyllanthus emblica*with 65%.Ethanol extract of *Ananascomosus* has the least yield percentage of 60%.

Phytochemical screening of the samples

The Phytochemical studies were performed to screen the presence of different phytochemical constituents in *Phyllanthus emblica, Ananascomosus,* and *Momordica charantia* extracts. The results revealed that the presence of ten Phytochemical is included. The result of Phytochemical screening of *Phyllanthus emblica, Ananascomosus,* and *Momordica charantia* extracts are shown in Table no.3.

Table 2: Phytochemical screening of *Phyllanthus emblica, Ananas comosus,* and *Momordica charantia*

Sample	Gooseberry			Pineapple			Bittergourd		
Phytochemicals	E	M	C	E	M	C	E	M	C
Terpenoids	+	+	-	+	+	-	+	+	-
Flavonoids	+	+	-	+	+	-	-	-	-
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	-	-	+	+	+	+	-
Alkaloids	+	+	-	+	+	-	+	+	+
Anthraquinones	+	+	+	+	+	+	+	+	-
Carbohydrates	-	+	-	+	+	-	+	+	-
Steroids	-	-	-	+	+	+	-	-	+
Phenols	+	+	-	-	-	-	-	-	-
Oils & Resins	+	+	-	+	+	-	+	+	-

\+ Presence Absence-

According to the Phytochemical screening study, highest number of Phytochemical is present in the methanol extract of *Ananas comosus* and *Phyllanthus emblica*. The least number of Phytochemical is found in chloroform extracts of *Phyllanthus emblica.*

Total phenol content

Total phenol content varies significantly between the extracts of *Phyllanthus emblica, Ananascomosus,* and *Momordica charantia*. The total phenol content was found to be higher in the ethanol extract of *Phyllanthus emblica*.The result of total phenol content that reported in the Table No.4.

Table 3: Total phenol content of *Phyllanthus emblica, Ananas comosus,* and *Momordica charantia*

Sl.no	Solvent	Test samples	Total Phenolic content («g)
1.	Ethanol	*Phyllanthus emblica*	20
2.	Ethanol	*Ananas comosus*	15
3.	Ethanol	*Momordica charantia*	5.4
4.	Methanol	*Phyllanthus emblica*	11.5
5.	Methanol	*Ananas comosus*	6.1
6.	Methanol	*Momordica charantia*	8.5
7.	Chloroform	*Phyllanthus emblica*	21.5
8.	Chloroform	*Ananas comosus*	9.1
9.	Chloroform	*Momordica charantia*	15

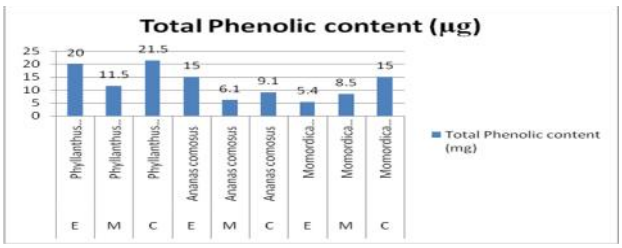

Fig 1: Total phenolic content

Free radical scavenging activity percentage showed high for chloroform extract of *Momordica charantia* (53.06%) and least for chloroform extract of *Ananas comosus* (2.04%).

Table 4: Scavenging activity percentage of *Phyllanthus emblica, Ananascomosus,* and *Momordica charantia*

Sl no	Sample	Scavenging activity %
1	*Phyllanthus emblica*(ethanol)	38.77
2	*Phyllanthus emblica* (methanol)	28.57
3	*Phyllanthus emblica* (chloroform)	44.80
4	*Ananas comosus*(ethanol)	26.54
5	*Ananas comosus*(methanol)	36.73
6	*Ananas comosus*(chloroform)	2.04
7	*Momordica charantia*(ethanol)	14.28
8	*Momordica charantia*(methanol)	16.32
9	*Momordica charantia*(chloroform)	53.06

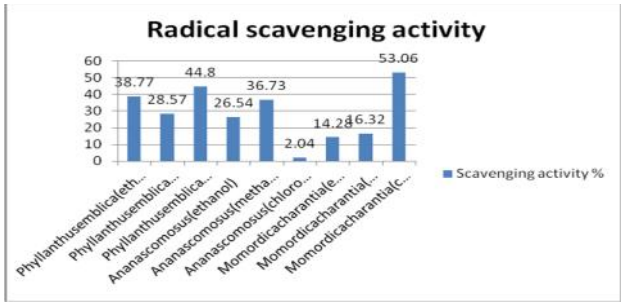

Fig 2: Free radical scavenging activity

QUANTIFICATION OF VITAMIN C FROM GOOSE BERRY EXTRACT BY HPLC

The ethanol extract of gooseberry fruit extract was analysed using HPLC showed the presence of food preservative compound (ascorbic acid).The food preservative compound was quantified at 254nm using peak area by comparsion of the caliberation curve of suitable standard ascorbic acid. The quantification of active food preservative compounds from gooseberry ethanolic fruit extract was carried out based on chromatographic separation.

The retention time was recorded for active preservative compound in gooseberry ethanol extracts was between 4.09 to 4.21 minutes shown in the table. Quantification was carried out using peak height and peak area obtained from the calibration graph ,the amount of vitamin c in the sample injected was calculated. Gooseberry fruit ethanol extract showed the presence of the significant quantity of vitamin c (0.56 gm used as food preservative).

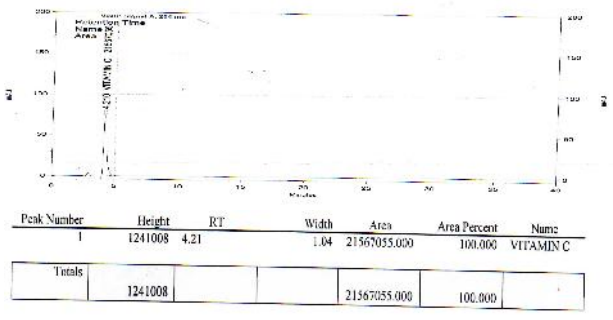

Fig 3.1: HPLC Chromatogram of Standard vitamin C

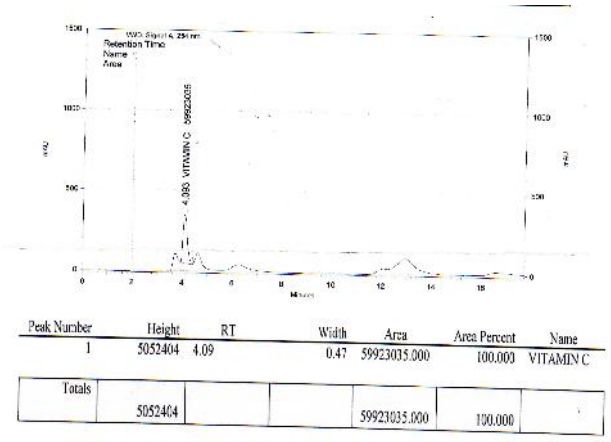

Fig 3.2: HPLC chromatogram of *Phyllanthusemblica* ethanol extracts

4. SUMMARY

Phytochemical analysis of *Phyllanthus emblica, Ananas comosus, Momordica charantia* fruit extracts revealed the presence of 11 phytochemicals (terpenoids,flavonoids,saponins,tannins,alkaloids,anthraquinones,carbohydrate,steroids,phenols ,oil and resins) in ethanol, methanol and chloroform extracts. These extracts were analysed for total phenol content,the results possess significant antioxidant activity .the fruit extracts of *Phyllanthus emblica, Ananascomosus, Momordica charantia* showed considerable antimicrobial activity against fungi (*Penicilliumsp, Aspergillussp and Rhizopussp*),bacteria (*E.Coli, Pseudomonas sp, Bacillus sp*),were these extracts may be used as source to develop drugs for bacterial and fungal infections. Activity steered fractionation of these fruits extracts by HPLC analysis showed the presence of bioactive compound (ascorbic acid). From the result of present study .It is concluded that *Phyllanthus emblica, Ananas comosus, Momordica charantia* fruit extracts are found to have antioxidant and antimicrobial potential and greater amount of bioactive compounds which could be used as sources of natural preservatives and drugs.

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