A Phytochemical Analysis and Antioxidant Activity From Ethanolic Extract of Punica Granatum

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Abstract- ***"****Punica granatum" L.(Punicaceae) commoly called pomegranate."punica granatum"has large number of chemical compounds. Leaves of this plant were collected from Coimbatore. The present study detect the different Phytochemicals,such as Phenols,Flavonoids,Alkaloids,Tannins,glycosides from the ethanolic extract of "punica granatum'.Antioxidant play an specific role for some diseases.and Antioxidant is its ability to trap free radicals.The study of Antioxidant activity acts in one method. Hydrogen Peroxide Scavenging activity.H2 O2 is an important reactive oxygen species .Conclude from this that these extract produce proper Phytochemical,Antioxidant properties.*

Keywords- "punica granatum",Phytochemical Test, Antioxidant activity.

I. INTRODUCTION

Pomegranate is a usual name of *punica granatum* it is a family of punicaceae[1]. The rich amount of Tannins is present in this plant. Now a days these phytochemicals has countless medicinal uses.Phytochemicals acts a role against the number of disease.astma,Arthritis,Cancer etc[2].All the selected medicinal plants were found to contain chemical constituents such as Tannins,flavonoids, moreover tepenoids.its acts to make for a drug design[3].Antioxidant is a molecule. it inhibit the oxidation of other molecule or it against Reactive Oxygen Specious(ROS). Different colour, varieties of the plant have various Antioxidant Acitivity and that composition is also Different.

Fig.NO:1: Pomegranate leave

II. MATERIALS AND METHODS

SAMPLE COLLECTION AND PLANT EXTRACT PREPARATION:

Fresh leaves of pomegranate was collected from coimbatore and it washed with tap water and then with sterilized water.Leaves were Homozinized to fine powder using electric blender.The fine powder mixed with different solvent like ethanol,or methanol.The soxhlet metod was used for plant extract preparation. 30 Grams of plant was added in 175 ml of ethanol is added to a bottom flask. The process should run total of 16 hours[4].

PHYTOCHEMICAL SCREENING:

Phytocemicals are naturally occurring in the medicinal plants, leaves and roots that have defense mechanism and protect from various diseases[5].Qualitative screening for the presence of various phytochemical compounds was perfomed using the methanolic extract.

III. QUALITATIVE PHYTOCHEMICAL SCREENING

TEST FOR PHENOL:

To 1 ml of the plant extract added 20µl of 1% ferric chloride. The appearance of bluish black precipitate indicates the presence of phenol.

TEST FOR FLAVONOIDS:

To 1 ml of the extract added few drops of 1% sodium hydroxide solution. The appearance of yellow colour indicates the presence of flavonoids.

TEST FOR TERPENOIDS:

To 0.5ml of plant extract added 2 ml of chloroform and 3 ml of concentrated sulphuric acid along the sides of the test tube. The appearance of reddish brown colour at the interface indicated the presence of Terpenoids.

TEST FOR QUINONES:

To 1ml of the plant extract added few drops of concentrated HCl acid .the presence of yellow precipitate indicated the presence of quinines.

TEST FOR GLYCOSIDES:

To 2ml of the plant extract added 1 ml of glacial acetic acid. To that added 1% ferric chloride solution drop by drop and ten added concentrated sulphuric acid. Alone the sides of the test tube. The appearance of greenish blue colour indicates the presence of glycosides.

TEST FOR TANNINS:

To 1 ml of the extract added 10 ml of the distilled water. The solution was ten filtered and then added few drops of 0.1% ferric chloride slowly to the filtrate. The appearance of brownish green colour indicated the presence of tannins.

TEST FOR SAPONINS:

To 1 ml of the plant sample added 2 ml of distilled water. The solution was Shaken and then added three drops of coconut oil. The solution was shaken again and then observed for formation of Emultion. The formation of Emultion indicated the presence of saponins.

TEST FOR STEROIDS:

To 1 ml of the plant sample added few drops of chloroform, acetic anhydride and concentrated sulphuric acid. The appearance of dark pink of red colour indicates the presence of steroids.

TEST FOR ALKALOIDS:

To 1 ml of plant sample added 1 ml of saturated picric acid solution.the appearance of yellow precipitate indicates the presence of alkaloids.

TEST FOR REDUCING SUGAR:

To 1 ml of plant sample added equal volume of benedicts reagent and allowed to stand in a water bath for 10 minutes. The appearance of brownish red precipitate indicates the presence of reducing sugars.

IV. ANTIOXIDANT ACTIVITY

Determination of Antioxidant activity by Hydrogen Peroxide Scavenging Assay:

The ability of " *punica granatum"* leaf extract to scavenge Hydrogen Peroxide by the method of (**Ruch** *et al.,***1989).[6]** The 40Mm Hydrogen Peroxide solution was prepared in Phosphate Buffer.The range of Plant extract (100ul-500ul) were added to 0.6ml of Hydrogen Peroxide solution(H2 O2). And the total volume was made upto 3 ml. The absorbance of the solution was measured at 230 nm in a spectrophotometer.A blank solution contain only Phosphate Buffer without H2 O2 was added.

Determination of antioxidant Activity by Ferric Reducing Antioxidant Power Assay:

The sample was scavenged with Ferric chloride.**(Oyaizu***et al.,***1986)[7].** Varying concentration of standard (20,40,60,80 and 100µg) in double distilled water was mixed with 2.5 ml of phosphate buffer and 2.5 ml of Fe3+. The mixture was incubated at 50°C for 20 minutes after which 1.5ml of TCA was added and centrifuged at 3000rpm for 10 minutes. From all the tubes,0.5 ml of supernatant was mixed with 1ml of distilled water and 0.5 ml of ferric chloride. The absorbance was measured at 595nm in a spectrophotometer.Ascorbic acid ware used as standards for comparison. Increased absorbance of the reaction mixture indicated increasing reducing power, Incubation with water in place if additives was used as the blank.

V. RESULTS AND DISCUSSION

PHYTOCEMICAL SCREENING:

The table:1 shows the data of qualitative analysis of aqueous and ethanolic extract of"*punica granatum"* leafs.These tests revealed the presence of various bioactive

components such as,phenol,flavanoid,,glycosides,tannins,saponins,,alkaloids,and reducing sugar[7].

Qualitative analysis of Phytochemicals

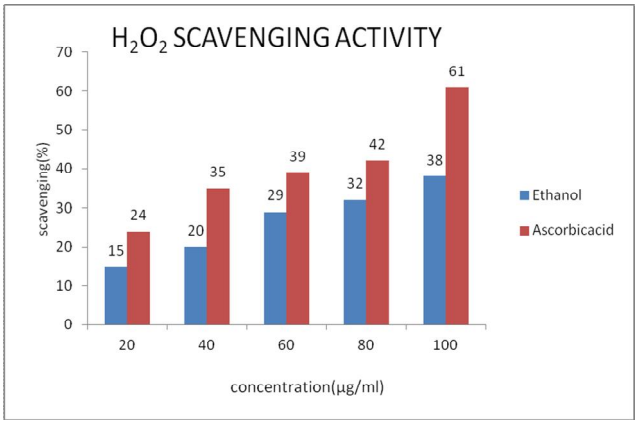
S.No	Phytoconstituents	Water	Ethanol
1.	Phenol	+	+
2.	Flavonoids	+	+
3.	Terpenoids	-	-
4.	Quinones	+	-
5.	Glycosides	-	-
6.	Tannins	-	-
7.	Saponins	-	+
8.	Steroids	+	-
9.	Alkaloids	-	+

VI. ANTIOXIDANT ACTIVITY

Hydrogen peroxide scavenging activity(H2 O2):

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membrane.hydrogen peroxide itself reacts with ethanolic extract of punica granatum.Hydrogen peroxide scavenged with plant extract it may be produced by the phenolic ,flavonoids contents.This plant have a ability to reduceH2 O2 into water and it is donate an electron.H2O2 reacts with ethanolic leaf extract of punica granatum and it is compared with concentration(20,40,60,80,100µg).it gets neutralize H2O2 indifferent concentration.The result showed in the higher concentration(100µg) in higher values(61%) from the plant extract.The compounds which were present in the plant extract.[8].

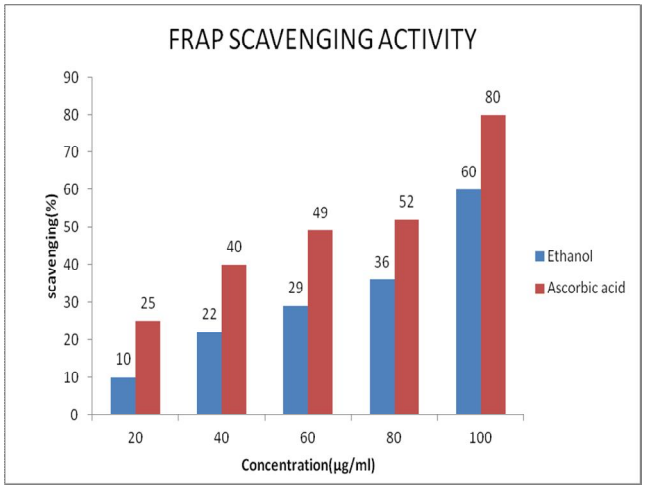
Fig 2:Hydrogen peroxide scavenging activity from ethanolic extract of *punica granatum*



FERRIC REDUCING ANTIOXIDANT POWER ASSAY:

The antioxidant capacity of ethanolic extract of *punicagranatum* is also determined by its reducing power. Reducing power (Fe III) Reduction is often used as an indicater of electron donating activity, Which is an important mechanism of phenolic antioxidant action

The yellow colour of the sample changed to different green and blue colour. Its depending on the Reducing power of plant extract. Ferric solution was reacts with ethanolic extracts of punica granatum to reduce their power. The results of the *punica granatum* leaves it Showes better antioxidant activity by this method. Which is mainly depends upon the Reducing potential with increasing concentration(20 to 100µg). The highest concentration (100µg) gives (80%) highest value of ethanolic extracts. In this method the sample gives good antioxidant activity compared standard of Ascorbic acid (60%).



Amount of Fe2+ Complex can be then be monitored by measuring the formation of blue colour at 595 nm. From this analysis it is Obsereved that the reducing power is directly

proportional to the concentration of *punica granatum* leaf extract [10]

VII. CONCLUSION

Phytochemical analysis of te Ethanolic extract of *punica granatum* leaves showed the presence of phenols, Flavonoids, alkaloids, terpenoids. Tannis, sapponins, Reducing sugar, Quinones, Glycosides compared with two solvents. The ethanolic extract of *Punica granatum* exhibited significant radical scavenging activity.Ferrous Reducing Antioxidant power assay is based on the absorbance.It increases the reducing power.

REFERENCE

[1] Rashida parveen and Naveed akhtar,2013,in Vitro Evalution of Antioxidant Activity of Phytochemical Extract from Different Varieties of Various plants.
[2] Sahira Banu ,Dr.L.Cathrine,April 2015,General techniques involved in phytochemical analysis,International Journal of Advanced Research in Chemical science?(IJARCS),PP 25-32.
[3] Ibrahim.s.Abaas and Mohammed.J.Hamzah,Ali.J.Ali,2014,Extraction,Identification and Antifungal studies of Leaves Extract of punica granatum L,World Journal of pharmacy and pharmaceutical sciences,ISSN 2278-4357.
[4] James Redfern,Malcolm kinninmonth,Daried Burdass,Joanna Verran,may 2014,Using Soxhlet Ethanolto produce and test plant material(Essential oils) for their Antimicrobial properties,page no:45-46.
[5] Nostro, A.Germano,M.P.Dangelo, V. Marino,A.Cannatelli,M.A., 2000,Extraction methods and bioautography for evalution of medicinal plant antimicrobial activity,Lett Appl Microbio,379-384.
[6] Radical Mandade,S.A. Sreenivas, Avijit Choudhury, 2011, Radical Scavengig and Antioxidant activity of carthamus tinctorius Extracts.
[7] Oyaizu M. Studies on product browning reaction prepared from glucose amine, Jpn. J. N. Nutr., 7, 1986, 307-315.
[8] Shahla najafi,Nima Sanadgol,Batool Sadeghi Nejad,Maryam Ashofteh Beiragi,and Ehsan Sanadgol,2010,Phytochemical screening and antibacterial activity of citrullus colocynthis(linn.) schrad against stapylococcus aureus.
[9] Vinothini S,Shri preethi M,Nusrath fathima,Shivani S kushwaha,Devi Rajeswari V,2016,Antioxidant and Free Radical Scavenging activity of punica granatum leaf extract,0974-2441.
[10] Gupta Daksha, Chandra shaker, Richard lobo, Yogendra and Gupta, Nilesh, 2012, Invitro antioxidant and antidiabetic activity of Bauhinia purpurea linn, Scolers research library, 614-619.