Antioxidant Activity of Polyherbal Extract of *Citrus Sinensis, Selenicereus Undatus* and *Punica Granatum* Peels

Akshata Patil[1], Rajat Rajendra Tamadaddi[2], Akshata Mashyal[3], Rashmitha D[4], Shridhar S Mangali[5], Vinaya Rudrayya Hiremath[6]

[1,2] *Assistant Professor, Department of Pharmacology, Agm College of Pharmacy, Navgrahteerth Kshetra, Varur, Hubballi-581207.*

[3,4,5,6] *B. Pharmacy Final Year Students, Agm College of Pharmacy, Navgrahteerth Kshetra, Varur, Hubballi-581207.*

Abstract*—Background and Objective: To evaluate antioxidant activity of polyherbal extract of *Citrus sinensis, Selenicereus undatus* and *Punica granatum.

To find out total phenolic content, flavonoids, nitric oxide free radical scavenging activity and hydrogen peroxide scavenging activity

Material and Methods: -Chemicals and instruments- 5% of ($NANO_2$) Sodium nitrite, 10% ($AlCl_3.6H_2O$) aluminium chloride hexahydrate, (NAOH) Sodium hydroxide, sodium nitroprusside, Griess reagent, phosphate buffer saline, gallic acid, folin-ciocalteus, sodium carbonate (Na_2CO_3) hydrogen peroxide (H_2O_2) refrigerator centrifuge UV spectrophotometer, test tubes, beakers, glass rod, measuring cylinder.

Result: "In all tests- flavonoid, phenolic, and nitric oxide scavenging- the ethanolic extract showed better antioxidant activity compared to the control and was comparable to the standard. This proves that the extract of *Citrus sinensis, Selenicereus undatus, Punica granatum* has strong antioxidant properties"

Conclusion: The study showed that the combined peels of *Citrus sinensis, Selenicereus undatus,* and *Punica granatum* have strong antioxidant activity due to high phenolic and flavonoid contents. The extract effectively neutralized reactive oxygen species, suggesting that these natural and widely available fruit wastes can be used to develop cost-effective, eco-friendly antioxidant formulations.

I. INTRODUCTION

1. IMPORTANCE AND CAUSES OF OXIDATION:

The importance of oxidation in the body and in foodstuffs has been widely recognized. Oxidative metabolism is essential for the survival of cells. The side effect of this dependence is the production of free radicles and other reactive oxygen species that cause oxidative changes. There is increasing evidence for the involvement of such species in a variety of normal in vivo regulatory systems. When an excess of free radicals is formed, they can overwhelm protective enzymes such as super oxide dismutase, catalase and peroxidase and cause destructive and lethal cellular effects by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. Furthermore, reactive oxygen species seen to influence cell signaling pathways in ways that are only now being unravalled[1].

2. DEFINITION OF ANTIOXIDANT AND MECHANISM:

A. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. There are two basic categories of antioxidant, namely, synthetic, and natural[2].

B. Antioxidant activity denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing oxidative damage.[3]

3. USES:

Antioxidant-based drug formulation is used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease,

and cancer, anti-cancers, antiinflammation, and anti-aging.

4. SOURCE:

A deep study of natural antioxidants, such as those from fruits and vegetables is of great importance to human health. The antioxidant property is presence the plant materials due to many active photochemical which include the vitamins, flavonoids, terpenoids, carotenoids, coumarins, lignin, saponin, plant sterols etc[4]

II. PLANT PROFILE (5,6,7,8,9,10,11,12, 13)

SI.NO	DISCRIPTION	PLANT-I Citrus sinensis	PLANT – II Selenicereus undatus	PLANT- III Punica granatum
01	SYNONYM	Orange	Dragon fruit	pomegranate, dalimba
02	BIOLOGICAL SOURCE	a flowering plant in the Rutaceae family	Hylocereus (a genus of cacti)	Punica granatum Tree
03	HABITAITE	Brazil, India & China	Malaysia, Thailand & India	Middle East India, California
04	CHEMICAL CONSTITUENTS	flavone glycosides, Terpenoids, & Vitamins	Vitamin B1, B2, B3, carbohydrate, flavonoids,	Anthocyanin and flavonoids, alkaloids, Tannis, triterpenes and phytosterol
05	USES	Vitamin C, fibre, and antioxidants, boosting immunity, skin health	smoothies, salads, yogurts desserts, & Antioxidant	Juicing, cooking eating fresh, Antioxidant

III. CHEMICAL AND INSTRUMENTS

5% of (NANO2) Sodium nitrite, 10% ($AlCl_3.6H_2O$) aluminium chloride hexahydrate, (NAOH) Sodium hydroxide, sodium nitroprusside, griess reagent, phosphate buffer saline, gallic acid, folin-ciocalteus, sodium carbonate (Na_2CO_3) hydrogen peroxide (H_2O_2) refrigerator centrifuge UV spectrophotometer, test tubes, beakers, glass rod, measuring cylinder.

IV. OBJECTIVES

Here we will Discussion about

- To evaluate antioxidant activity of polyherbal extract of *Citrus sinensis, Selenicereus undatus* and *Punica granatum.*
- To find out total phenolic content, flavonoids, nitric oxide free radical scavenging activity and hydrogen peroxide scavenging activity.

EXTRACTION PROCESS [14]

V. EVALUATION PARAMETERS

DETERMINATION OF FLAVONOIDS:

A slightly modified version of the method was used to determine flavonoid contents of samples. 1 ml of extract was placed in a 10 ml volumetric flask, and 5ml of distilled water and 0.3ml of 5% NANO2 (Merck)

were added and mixed. After 5 min, 0.6ml of 10% AlCl3.6H2O was added. 2ml of mol 1-1 NaOH (Merck) was added 5min later and then the volume was made up to 10 ml with distilled water. The solution was mixed well, and the absorbance was measured immediately at 510 nm. Flavonoid contents were calculated using a standard calibration curve, prepared from (+) catechin.[14]

NITRIC OXIDE FREE RADICAL SCAVENGING ACTIVITY

50µl of each of the concentrations of extracts previously dissolved in DMSO, as well as ascorbic acid (Standard compound) were taken in separate tubes, and the volume was uniformly made up to 150µl with methanol. To each tube 2.0ml of sodium nitroprusside (10mM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150min. The similar procedure was repeated with methanol as blank which served as controlled. After the incubation, 5ml of Griess reagent was added to each tube including control the absorbance of chromophore formed was measured at 546nm on UV-visible spectrometer Shimadzu, UV-1601, Japan, ascorbic acid was used as positive control. The IC50 value for each test compounds as well as standard preparation were calculated.[15]

DETERMINATION OF TOTAL PHENOLIC CONTENT:

To 0.5 ml of methanolic solution of extracts, 7ml of distilled water and 0.5 ml of FolinCiocalteu reagent were added and mixed well. After 3min, 2ml of 20% sodium carbonate was added and mixed well again. Absorbance of the resultant solution was read at 720nm, after 1h standing in water bath at 25ºC. The results were expressed as mg of catechin 1-1of fresh weight material using a standard calibration curve of (+)-catechin.[14]

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The ability of the extract to scavenge hydrogen peroxide was determined according to the method a solution of hydrogen peroxide was prepared in phosphate buffer. Extract in distilled water were added to a hydrogen peroxide solution absorbance of hydrogen peroxide at 230 nm was determined 10mins later against a blank solution containing the phosphate buffer without hydrogen peroxide. The % of hydrogen peroxide scavenging of *citrus sinensis, selenicereus undatus* and *Punica granatum* extract and standard compounds were calculated.[16]

% scavenged [H_2O_2] = [(Ac – As)/Ac] x 100

VI. RESULT AND DISCUTION

Table no 01: The Flavonoids content of ethanolic extract of*citrus sinensis, selenicereus undatus* and *Punica granatum.*

Group	Flavonoids content
Control	1.340± 0.0
Test	3.294± 0.0
Standard	3.948±0.0

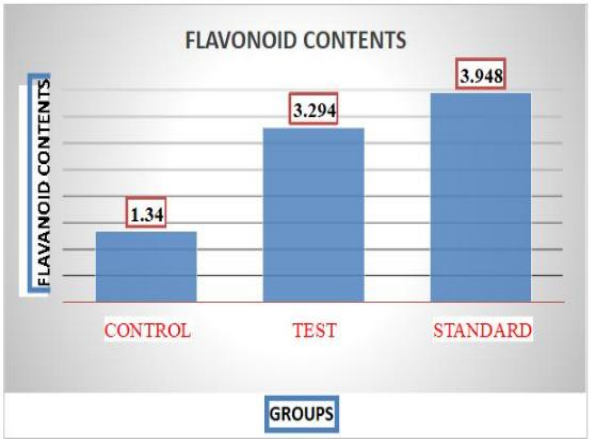


Table no 02: The Nitric oxide free radical scavenging activity of ethanolic extract of *citrus sinensis, selenicereus undatus* and *Punica granatum.*

Group	The Nitric oxide free radical scavenging activity
Control	0.6470±0.0
Test	0.9600±0.0
Standard	0.8580±0.0

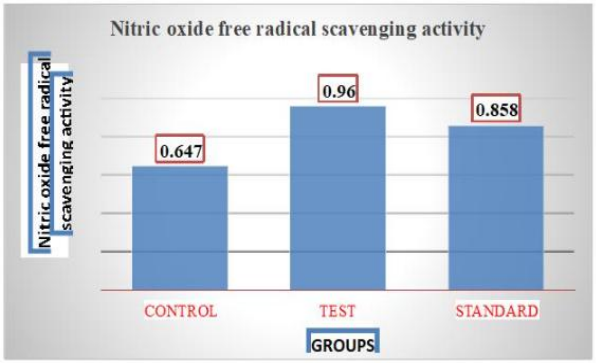


Table no 03: The total phenolic content of ethanolic extract of *citrus sinensis, selenicereus undatus* and *Punica granatum*

Group	The total phenolic content
Control	1.166±0.0
Test	3.458±0.0
Standard	3.458±0.0

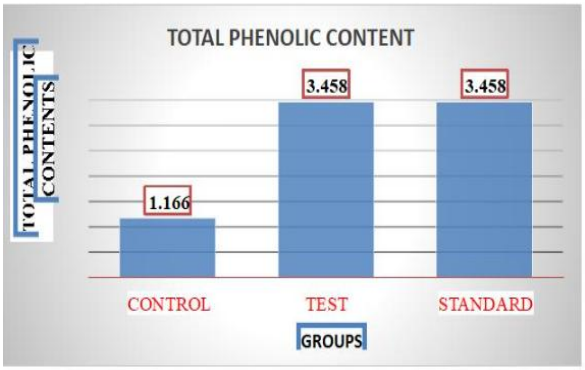
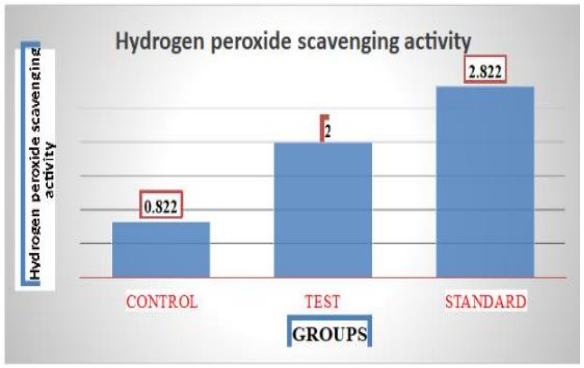


Table no 04: The Hydrogen peroxide scavenging activity of ethanolic extract of *citrus*

Group	The Hydrogen peroxide scavenging activity
Control	0.8220±0.0
Test	2.000±0.0
Standard	2.822±0.0



VII. DISCUSSION

The importance of oxidation in the body and in food stuffs has been widely recognized. Oxidative metabolism is essential for the survival of cells. A side effects of this dependence is the production of free radicles and other reactive oxygen species that cause oxidative changes. There is increase in evidence for the involvement of such species in a variety of normal in vivo regulatory systems.

When an excess of free radicles is formed, they can overwhelm protective enzymes such as superoxide dismutase, catalase and peroxidase and cause destructive and lethal cellular effects (e.g., apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. Furthermore, reactive oxygen species seem to influence cell signalling pathways in ways that are only now being unrevealed.

The presence study was undertaken to evaluate the antioxidant potential of polyherbal extracts prepared from the peels *of Citrus sinensis, Selenicereus undatus* and *Punica granatum*. Each of the plants has been reported individually to contain phenolic compounds, flavonoids, vitamins and other bioactive constituents with strong antioxidant and free radicals scavenging properties. In this work ethanol maceration of the fruit's peels provided concentrated extracts that were then assessed for total phenolic content, flavonoids, nitric oxide free radical scavenging activity.
The findings support that the polyherbal extract is rich in phenolic and flavonoid content. Since phenolic compounds and flavonoids are known for their ability to donate hydrogen atoms or electrons and chelate metal ions, their high concentration in the extract

explains the observed scavenging activities. The nitric oxide and hydrogen peroxide scavenging assays demonstrated that the extract can effectively neutralize reactive species, thereby preventing oxidative damage, such synergistic effects are expected from combining multiple fruit peels because different plant constituents act at different stages of free radicals and propagation.

The chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like tannins, reducing sugars and proteins present in the extract may be responsible for such activity.

Overall, the results indicate that the combined extract of *Citrus sinensis, Selenicereus undatus* and *Punica granatum* possesses significant antioxidant potential, which may contribute to the prevention of oxidative stress-related disorders. However further studies, including isolation of individual active compounds, dose standardization and in vivo evaluations, are recommended to substantiate and extend these findings.

VIII. CONCLUSION

The present study demonstrated that the polyherbal extract prepared from the peels of *Citrus sinensis*, *Selenicereus undatus* and *Punica granatum* possesses significant antioxidant activity. High levels of total phenolic and flavonoid contents, along with effective nitric oxide and hydrogen peroxide scavenging activities, indicate that the combined extract can efficiently neutralize reactive oxygen species and prevent oxidative damage. The synergistic effect of these three fruit peels highlights the potential of using natural, widely available plant materials especially agro-wastes as a valuable source of antioxidant.

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REFERENCE

[1] Antolovich M, Prenzler D P, Patsalides E, McDonald S at all., Methods for testing antioxidant activity., 2002, 127, 183-198.
[2] Y.S. Velioglu, G, Mazza, L, Gao and B, D. Oomah., Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products., 1998, 46, 4113-4117.
[3] Zhuo Zou, Wanpeng Xi, Yan Hu at all, Antioxidant activity of citrus fruits., 2016,196,885-896.
[4] Kaur.P, Arora M., Antimicrobial & Antioxidant Activity of Orange Pulp and Peel., 2013, 2, 2319-7064
[5] Ravi Mannan N, Nisansala A., study on antioxidant activity fruits and vegetables., 2017,4(3),93101.
[6] Ram Singh Meena, A comprehensive Review on phytochemical constituents, medicinal applications, and economic significance of orange peels., 2015,7,2455-5967.
[7] S Khandla, M Vala, B Maitreya, Phytochemical constituents, medicinal uses and economical value of orange peels., 2020,8,2320-2882.
[8] Lewis, Charlton T; Short, Charlies (1879). A Latin Dictionary. Perseus Digital Library
[9] Sunita N Yamini B, Madhuri M, Rakesh R at all., Complete novel review on Seleniferous Undatus., 2024,21(1),1596-1608.
[10] Bhadauria S, Mansi, Choudhary T, Chaurasia S., Exploring the therapeutic potential of dragon fruit., 2024,11(6),222-234.
[11] Kumari A, Dora J, Kumar A, Kumar A., pomegranate (Punica Grantum) overview.,2012,1(4),2227-5003.
[12] Ali T, Nafees M, Maqsood A, Shahzad U., Contribution of root anatomical characteristics in fruit profile of pomegranate.,2020,10 (6),810.
[13] Haque N, Sofi G, Ali W, Rashid M, Irat M., A comprehensive review on phytochemical and pharmacological profile of Anar (Punica granatum Linn)., 2015,1(1),22-26.

[14] Karadeniz. F, burdurlu. H, koca. N, soyer. Y., antioxidant activity of selected fruits and vegetables grown in turkey. 2005, 29,297-303.
[15] Patel Rajesh m and Patel Natvar., In vitro antioxidant activity of coumarins compounds by DDPH, super oxide and nitric oxide free radical scavenging methods., 2011, 1, 52-68.
[16] K Serhat, C Sait, T Semra at all., hydrogen peroxide radicals scavenging and total antioxidant activity of Hawthorn.,2012,2,9-12.