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Research Article

COMPARATIVE PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING ACTIVIY IN DIFFERENT EXTRACTS OF PEEL AND PULP OF *ACTINIDIA CHINENSIS*

INDHUMATHI .T*[1], SRUTHI .G[1], DR.MOHANDASS .S[2]

[1]PG and Research Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore- 641048, [2]Principal, Kamadenu Arts and Science College, Sathiyamangalam, Erode, Tamilnadu.

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ABSTRACT

Actinidia chinensis is also called as kiwi fruit in some parts of the world, is the edible berry and hybrids between this and other species in the genus *Actinidia.* It has antioxidant, antibacterial, and antiviral effects. This study was designed with objective to assess the Comparative phytochemical and Free radical scavenging analysis in different extracts of peel and pulp of *Actinidia chinensis*. The phytochemical analysis in aqueous, ethanolic and petroleum ether extracts of pulp shows the presence of saponins , flavonoids, protein, alkaloids and steroids and the aqueous, ethanolic and Petroleum ether extracts of peel shows the presence of alkaloids, phenol and tannins. From the present study it could be concluded that, the aqueous, ethanolic and petroleum ether extracts of peel and the aqueous extracts of pulp shows significant level of inhibition in free radical scavenging activity assay.

Key Words: *Actinidia chinensis,* Phytochemical, Free radical scavenging.

INTRODUCTION

Fruits and vegetables are a rich source of phenolic acids and flavonoids, called phytochemicals, which also serve as antioxidants to counteract the prooxidant load of the body. These phytochemicals can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of the immune system, regulation of cellular signal transduction pathway, reduction of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure[1]. In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases, and extensive lysis. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines. Recently, many natural antioxidants have been isolated from different plants and fruits[2].

MATERIALS AND METHODS

COLLECTION OF THE FRUIT

The fruit sample was collected from the Saravana fruit stall, Avarampalayam, Coimbatore. Fresh fruits are obtained, fresh pulp and shade dried, powered peel extracts were used. The aqueous, ethanolic and petroleum ether extracts of peel and pulp were prepared and used for the analysis of various parameters.

PREPARATION OF AQUEOUS, ETHANOLIC AND PETROLEUM ETHER EXTRACTS OF PEEL AND PULP OF *ACTINIDIA CHINENSIS*

Preparation of aqueous extract of peel

10gms of shade dried, powdered peel of *Actinidia chinensis* was weighed and transferred to a sterile beaker. 100ml of sterile distilled water (1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

Preparation of aqueous extract of pulp

10gms of fresh fruit pulp of *Actinidia chinensis* was weighed and grind using mortar and pestle and transferred to a sterile beaker. 100ml of sterile distilled water (1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

Preparation of ethanolic extract of peel

10gms of shade dried, powdered peel of *Actinidia chinensis* was weighed and transferred to a sterile beaker. 100ml of sterile ethanol

(1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

Preparation of ethanolic extract of pulp

10gms of fresh fruit pulp of *Actinidia chinensis* was weighed and grind using mortar and pestle and transferred to a sterile beaker. 100ml of ethanol (1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

Preparation of petroleum ether extract of peel

10gms of shade dried, powdered peel of *Actinidia chinensis* was weighed and transferred to a sterile beaker. 100ml of petroleum ether (1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

Preparation of petroleum ether extract of pulp

10gms of fresh fruit pulp of *Actinidia chinensis* was weighed and grind using mortar and pestle and transferred to a sterile beaker. 100ml of petroleum ether (1:10) was added to it and mixed well and kept in the shaker overnight and filtered through What Mann No: 1 filter paper. Then the filtered solution was used as a test extract.

I. Preliminary phytochemical analysis of different extracts of peel and pulp of

Actinidia chinensis

The preliminary phytochemical analysis of different extracts of peel and pulp of *Actinidia Chinensis* were done by the method of

II. *Invitro* Free radical scavenging activity in different extracts of peel and pulp of the *Actinidia chinensis.*

1. Superoxide Scavenging Activity (Riboflavin Phospho Reduction Method)

Super oxide scavenging activities of compounds were determined by the method of McCord and Fridovich,(1969), which depends on the light, induced super oxide generation by riboflavin and the corresponding reduction of Nitro blue terazoluim (NBT). The assay mixture contained different concentrations of the test compound together with EDTA (6.6 mM) containing 3 µg, NacN, riboflavin (2 µM), NBT (50 µm), and Phosphate buffer (0.06 M, pH 7.4) to give total volume of 3ml. The tubes were allowed to receive uniform

illumination for 15 min and optical density was measured at 560 nm. The percentage of inhibition of super oxide production by the compounds was evaluated by comparing the absorbance of the control and experimental tubes.

Percentage of inhibition – (C-T/C) X 100

2. Hydroxyl Radical Scavenging Activity (Halliwell *et al.,* 1987)

Hydroxyl radical scavenging activity was measured by studying the competition between deoxy ribose and test compounds for hydroxyl radicals generated from the Fe^{3+} / ascorbate / EDTA / H_2O_2 System. The hydroxyl radical attacks deoxy ribose, which eventually results in thiobarbituric acid (TBA) reacting substance formation. The reaction mixture contains deoxyribose (28 mM), $Fecl_3$ (0.1Mm), H2O2 (1 mM), ascorbic acid (0.1 mM). KH_2P0_4 – KOH buffer (20mM, pH 7.4) and various concentration of the extract in final volume of 1ml. The reaction mixture was incubated for one hour at 37°c. Deoxy ribose degradation was measured a TBA reacting substance and percentage of inhibition was calculated.

Percentage of Inhibition = (C- T/C) x 100

3.Nitric Oxide Radical Scavenging Activity (Govindarajan *et a*l., 2003)

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mM) in standard phosphate buffer saline solution (0.025M, pH 7.4) was incubated with different concentrations (50-250µg/mL) of the extracts dissolved in phosphate buffer saline (0.025 M. pH: 7.4) and the tubes were incubated at 25°C for 5 hours. Control experiment without the test compound but with equivalent amounts of buffer was conducted in an identical manner. After 5 hours 0.5mL of incubation solution was removed and diluted with 0.5mL of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrocloride). The absorbance of the chromophere formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546nm. The experiment was repeated in triplicate.

4. DPPH Radical Scavenging Activity (Glida *et al.*, 2001)

1-Diphenyl 1-2 Picrylhydrazyl (DPPH) Radical Scavenging Activity

Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 100, 50, 25, 10 and 5 µg/ml, in ethanol. One ml of a 0.3 mM DPPH ethanolic solution was added to 2.5 ml of sample solutions of different concentrations, and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula:

$AA\ \% = 100 - \{[(ABS_{SAMPLE} - ABS_{BLANK}) \times 100] / ABS_{CONTROL}\}$

Ethanol (1.0 ml) plus compound solution (2.5 ml) was used for blank. DPPH solution (1.0 ml; 0.3 mm) plus ethanol (2.5 ml) was used for negative control. The positive controls were those using the standard solutions. Average percent of antioxidant activity from three separate tests were calculated.

RESULT AND DISCUSSION

I. Preliminary Phytochemical analysis

From the table (1) the aqueous, ethanolic and petroleum ether extracts of pulp of *Actinidia chinensis* shows the presence of alkaloids, flavonoids, protein, steroids and saponins.

Table 1: Phytochemical analysis in different extracts of the pulp and peel of Actinidia *chinensis*

Test	Pulp			Peel		
	Aqueous	Ethanol	Petroleum ether	Aqueous	Ethanol	Petroleum ether
Alkaloids						
Dragendroff's test	+	+	+	-	+	-
Wagner's test	-	-	+	+	+	+
Meyer's test	+	-	-	+	+	-
Flavonoids						
Dil.HCl	+	+	+	+	+	-
Carbohydrates						
Fehling's test	+	+	-	+	-	-
Benedict's test	-	-	-	+	-	-
Mollisch's test	-	-	+	+	-	-
Protein						
Millon's test	+	+	+	+	-	-
Phenol						
Ferric chloride test	-	+	-	+	+	+
Lead acetate test	-	-	-	-	+	-
Libermann's test	-	-	-	+	-	-
Glysoides	-	-	-	-	+	-
Steroids						
Libermann Burchard's test	-	-	-	+	-	-
Salkowski test	+	+	+	+	-	-
Tannins						
Ferric chloride test	-	-	-	+	+	+
Lead acetate test	-	-	+	-	+	-
Saponins						
Sodium bicarbonate test	+	+	+	+	+	-
Thiols	-	-	-	-	-	-

Resins	-	-	-	+	+	-

(+) ⟶ **Presence**

(-) ⟶ **Absence**

From the table (1) the aqueous, ethanolic and petroleum ether extracts of peel of *Actinidia chinensis* indicates the presence of alkaloids, phenol and tannins. Also the aqueous and ethanolic extracts contain flavonoids, saponins and resins, which is absent in the petroleum ether extracts of *Actinidia chinensis*. Flavonoids have been demonstrated to have anti inflammatory, anti allergic and anti viral activity. These broad therapeutic properties were widely used in pharmaceuticals. Due to the presence of alkaloids, flavonoids, proteins and phenols they have been used for therapeutic purposes.

II. *Invitro* Free radical scavenging activity

The *invitro* antioxidants analyzed in the present study are DPPH, Superoxide scavenging activity, Hydroxyl scavenging and nitric oxide scavenging activity.

1. DPPH Assay

1, 1-diphenyl-2-picryl hydrazyl (DPPH) a stable radical and investigated as reactive hydrogen acceptor, has been widely used for studying antioxidant properties of bioactive compounds isolated from the fruit and vegetable extracts.

Figure I shows the different extracts of pulp of *Actinidia chinensis* has DPPH radical scavenging activity. This reveals that the aqueous extract of *Actinidia chinensis* exhibit higher activity than ethanolic and petroleum ether extracts. Figure 2 shows the different extracts of peel of *Actinidia chinensis* has DPPH radical scavenging activity. This reveals that the ethanolic extract of *Actinidia chinensis* exhibit higher activity than aqueous and petroleum ether extracts.

Kumar *et al.,* 2009 reported that DPPH is widely used as a convenient method for antioxidant using for bioactive compounds isolated from fruit extracts.

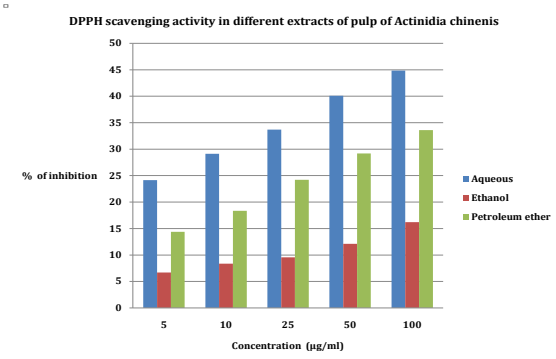

Fig 1: Percentage of inhibition in different extracts of pulp of *Actinidia chinensis*.

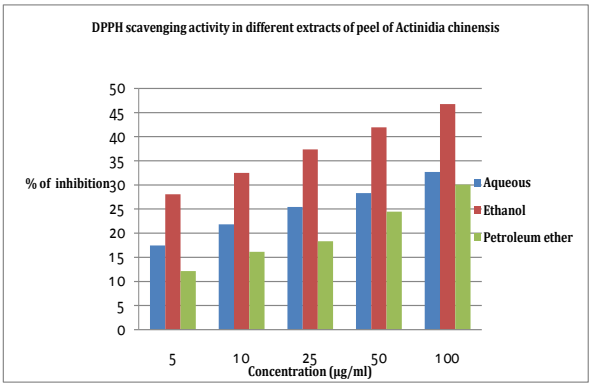

Fig 2: Percentage of inhibition of different extracts of peel of *Actinidia chinensis.*

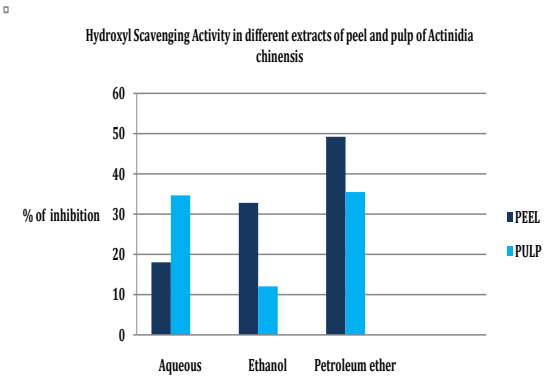

Fig 4: Hydroxyl radical scavenging in different extracts of peel and pulp of *Actinidia chinensis.*

2. SUPEROXIDE SCAVENGING ACTIVITY

Figure 3 shows that the aqueous and ethanolic extracts exhibit the same percentage of inhibition than petroleum ether extract of *Actinidia chinensis* pulp and peel.

Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include Superoxide dismutase (SOD) that catalyses the dismutation of superoxide to H2O2 and Catalase that breaks it down to water.

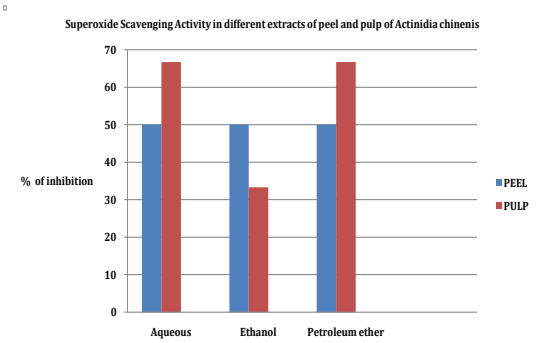

Fig 3: Superoxide radical scavenging in different extracts of peel and pulp of *Actinidia chinensis*

3. HYDROXYL RADICAL SCAVENGING ACTIVITY

Figure 4 shows that the petroleum extracts exhibit higher activity than aqueous and ethanol extracts of *Actinidia chinensis* pulp. It also show that that the aqueous, ethanolic and petroleum ether extracts of *Actinidia chinensis* peel exhibit the same level of inhibition.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [2].

4. NITRIC OXIDE SCAVENGING ACTIVITY

Figure 5 shows that the petroleum ether extracts of *Actinidia chinensis* pulp and peel exhibit higher activity than aqueous and ethanolic extracts.

The toxicity of nitric oxide increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extracts with fruits inhibit nitrite formation by directly competing with oxygen in the reaction with nitric oxide[2].

We can conclude that the result of the present study revealed that, in the *invitro* antioxidant activity study all the three extracts such as aqueous, ethanolic and petroleum ether extracts shows the same levels of percentage of inhibition.

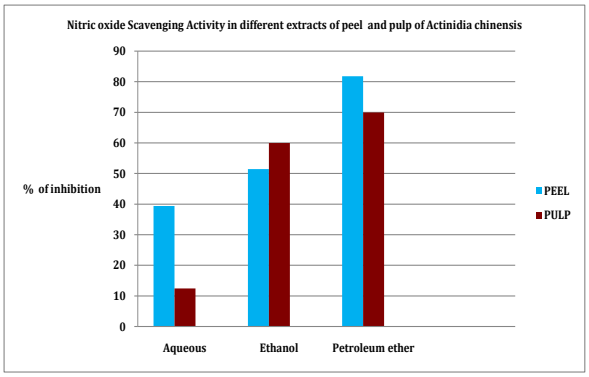

Fig 5: Nitric oxide radical scavenging in different extracts of peel and pulp of *Actinidia chinensis.*

CONCLUSION

The phytochemical analysis in aqueous, ethanolic and petroleum ether extracts of pulp shows the presence of saponins, flavonoids, protein, alkaloids and steroids and the aqueous, ethanolic and Petroleum ether extracts of peel shows the presence of alkaloids, phenol and tannins.

From the present study it could be concluded that, the aqueous, ethanolic and petroleum ether extracts of peel and the aqueous extracts of pulp shows significant level of inhibition in free radical scavenging activity assay.

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