Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

IN VITRO ANTIOXIDANT AND ANTI INFLAMMATORY ACTIVITY OF *COCCINIA GRANDIS*

ASHWINI.M[1], NISHA LATHER[1], SHIVAJI BOLE[1]*, VEDAMUTRHY AB[1], SAM BALU[2]

[1] PG Department of Biotechnology, [2] PG Department of Applied Genetics, The Oxford College of Science, Bangalore.
Email: shivaji_bole@rediffmail.com

Received: 11 Feb 2012, Revised and Accepted: 13 Mar 2012

ABSTRACT

Fruit extracts of *Coccinia grandis* (Cucurbitaceae) are used in India for reducing oxidative stress and inflammation. The objective of this study is to investigate the phyto-chemical constituents, antioxidant and anti-inflammatory activities to justify the use of the plant in folkloric medicine. Phytochemical screening of *Coccinia grandis* fruit powder in different organic solvents revealed the presence of alkaloids, carbohydrates, phytosterols, resins, tannins, flavonoids, proteins and diterpenes. Antioxidant activities of different fractions from different extracts were evaluated by using *InVitro* antioxidant assay models like phosphomolybdenum and reducing power assay. Anti-inflammatory activity was evaluated using induced protein denaturation method. The percentage of antioxidant activity was in the order methanol>ethanol>petroleum ether>chloroform>n-hexane. Almost all the fractions showed anti-inflammatory activity but n-hexane and petroleum ether showed the highest activity in induced protein denaturation method. The results obtained in this study showed that the fruits of *Coccinia grandis* have antioxidant and anti-inflammatory properties which provide a basis for the traditional use of the plant. IC_{50} value was calculated for both antioxidant and anti-inflammatory activity and was found to be 140 µg/ml (methanolic extract) and 100µg/ml (n-hexane extract) respectively.

Keywords: Antioxidant, Anti-inflammatory, *Coccinia grandis*, Reducing power assay, Protein denaturation.

INTRODUCTION

If infectious diseases are troubling the general population on one hand, the pace of modern life is adding to the woes of the middle class. Stress and pollution are known to generate reactive oxygen species termed as free radicals which play havoc with the body. These are found to induce tissue damage resulting in inflammation and physical pain.

Coccinia grandis, the ivy gourd, also called as baby water melon belongs to the family Cucurbitaceae. In traditional medicine fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice[1]The fruit possesses mast cell stabilizing; anti anaphylactic and antihistaminic potential [2].There is some research to support that compounds in the plant inhibit the enzyme Glucose-6-phosphatase is one of the key liver enzymes involved in regulating sugar metabolism[3]. Therefore, Ivy Gourd is sometimes recommended for diabetic patients[4]. Although these claims have not been supported, there currently is a few amount of research focused on the medicinal properties of this plant focusing on its use as an antioxidant, anti-hypoglycemic agent, immune system modulator. Some countries in Asia like Thailand prepare traditional tonic like drinks for medicinal purposes.

Free radicals play an important role in a number of biological processes including intracellular killing of bacteria and certain cell signalling processes. Free radicals are derived from molecular oxygen under reducing conditions. Excess amount of these free radicals can lead to cell injury, which results in many diseases like cancer and diabetes [5]. Free radicals may be involved in Alzheimer's, Schizophrenia, Parkinson's and drug induced deafness [6]. Because free radicals are necessary for life, the body has numerous mechanisms to reduce free radical induced damage and to repair this occurred damage [7], antioxidants are required. The function of antioxidant is not to remove oxidants entirely, but instead to keep them at an optimum level. In addition to this antioxidants play a key role in defence mechanisms [8].

In recent years, many studies revealed that plants contain high antioxidant and anti-inflammatory activity. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India. The world health organization has also recommended the evaluation of plants effective in conditions were safe modern drugs are lacking.

MATERIALS AND METHODS

Plant material

Fruits of *Coccinia grandis* were collected from rural areas of Bangalore, Karnataka (India) during the month of November 2011. The plant was identified by Botanist, Bangalore University, Bangalore, Karnataka, India.

Chemicals and reagents

All the chemicals and reagents used were of analytical grade and are purchased from Lancaster Research Lab, Chennai, India and Himedia Lab, Mumbai, India.

Extraction of plant material

Extraction was carried out at room temperature under normal conditions. About 20g of shade dried powder of fruits of *Coccinia grandis* was successively extracted with methanol, ethanol, chloroform, n-hexane, petroleum ether. The extract obtained was filtered, concentrated by heating at 100^0C in a water bath and dried in a vacuum oven.

Phytochemical analysis

The extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner's and Meyer's tests), Carbohydrates (Molish's, Benedict's and Fehling's test),saponins (foam and froth tests), phytosterols (Salkowski's test), fats (Stain test), resins (Acetone-water test), Phenols ($FeCl_3$ test), tannins (gelatin test), flavonoids (Alkaline reagent and Lead acetate tests),proteins (Xanthoproteic and Biuret tests), diterpenes (Copper acetate test). The screening was done as per the standard method[9].

Antioxidant Activity

Determination of phospho- molybdenum scavenging assay

The antioxidant activity of all the extract was determined by the phosphor-molybdenum Method as described by Prieto et al[9]. 0.3 ml of extract of different concentrations (100 to 500µg/ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95^0C for 90 min and cooled to room temperature [10]. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam) against blank. Distilled water (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid (AE).

Reducing Power Activity (Iron (III) to iron (II) reduction)

The ferric reducing power method was applied with slight modifications of the method in the Literature [11]. 2.5 mL of extract solution of different concentrations (100 to 500 µg/mL was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). This was incubated at 50 C° for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added. 2.5 mL of the reaction mixture was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The solution absorbance was measured at 700 nm. Increasing absorbance of the reaction mixture indicates increasing reducingpower. The same procedure was applied for ascorbic acid which acts as the standard. Increase in the absorbance indicates increase in reducing power [12].

Anti-Inflammatory Activity

Inhibition of protein denaturation

0.5ml of test solution having different concentrations (100 to 500µg/mL) of extract was taken with 1.5ml of bovine serum albumin (BSA). The mixture was incubated at 27±1°C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added [13]. Percentage of anti-inflammatory activity of the extract is given by,

$$\% \text{ AI} = \frac{(A_c - A_t) \times 100}{A_c}$$

Where A_c is the absorbance of the control

A_t is the absorbance of the test.

RESULTS AND DISCUSSION

In this study *In Vitro* antioxidant activity and anti-inflammatory activity of different fractions of fruit extracts of *Coccinia grandis* was evaluated by different in-vitro screening methods.

Preliminary phytochemical screening of the different fractions of dried fruit extracts revealed the presence of alkaloids, carbohydrates, phytosterols, tannins, flavonoids, proteins and diterpenes. The type of phyto-constituents of methanol, ethanol, chloroform, n-hexane and petroleum ether are given in Table 1.

Table 1: Phytoconstituents present in different extracts of *Coccinia* grandis

Phytochemical Constituent	Test	Extract of *Coccinia grandis* in				
		Methanol	Ethanol	Chloroform	n-hexane	Pet.Ether
Alkaloids	Meyers	+	+	-	-	-
	Wagner's	+	+	-	-	-
Carbohydrates	Molish's	+	+	-	-	-
	Benedict's	+	-	+	-	-
	Fehling's	-	-	-	-	-
Saponins	Froth	-	-	-	-	-
	Foam	-	-	-	-	-
Phytosterols	Salkowski's	-	+	+	+	-
Fixed Oil and Fat	Stain	-	-	-	-	-
Resin	Acetone-water	-	-	-	-	-
Phenol	Ferric chloride	-	-	-	-	-
Tannins	Gelatin	+	+	-	-	-
Flavonoids	Alkaline Reagent	+	+	+	-	-
	Lead Acetate	+	+	+	+	-
Proteins	Biuret	-	-	-	+	+
	Xanthoproteic	-	+	-	-	-
Diterpenes	Copper Acetate	+	+	-	-	-

The results of the free radical scavenging potentials of different fractions tested by phospho- molybdenum assay method are depicted in Figure [1] and table [2].The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as ascorbic acid equivalents.The percentage of antioxidant activity was in the order methanol>ethanol> petroleum ether>chloroform>n-hexane. The IC_{50} value of methanol fraction was found to be 140 µg/mL, whereas the IC_{50} value of ethanol 200, chloroform and n-hexane and petroleum ether was found to be, >400 µg/mL.

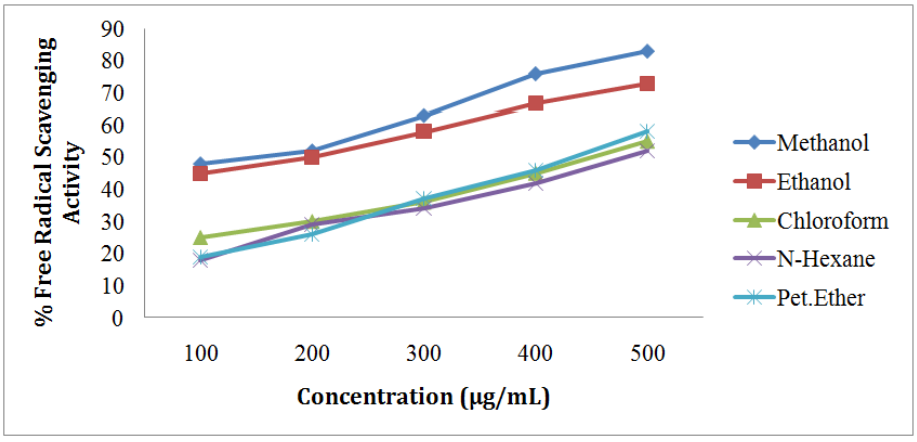

Fig. 1: Free radical scavenging potential of different fractions by phospho- molybdenum scavenging assay at different concentrations (µg/mL)

Table 2: Free radical scavenging activity of different fractions at different concentrations by phospho- molybdenum scavenging assay

Concentration µg/ml	Methanol	Ethanol	Chloroform	n-hexane	Pet. Ether
100	48	45	25	18	19
200	52	50	30	29	26
300	63	58	36	34	37
400	76	67	45	42	46
500	83	73	55	52	58
IC_{50}(µg/mL)	140	200	450	475	430

In reducing power assay, ethanol fraction seemed to have quite high reducing activity when compared to methanol, chloroform, n-hexane and petroleum ether. The reducing power of different extracts is given in Table [3].

The *In Vitro* anti-inflammatory activity was estimated by inhibiting protein denaturation. The inhibitory effect on protein denaturation by different extracts is shown in Table [4].and fig [4]

n-hexane (100 to 500µg/mL) showed significant (93%) inhibition of denaturation of bovine serum albumin (BSA) in concentration dependent manner. Petroleum ether also showed significant (92%) inhibition of denaturation of BSA, which is slightly lesser than n-hexane. Ethanol, methanol and chloroform also showed significant (60 to 85%) inhibition of denaturation of BSA when compared with the control.

Table 3: Reducing power assay of different extracts at different concentrations

Concentration µg/mL	Methanol	Ethanol	Chloroform	n-hexane	Pet. Ether
100	48	45	25	18	19
200	52	50	30	29	26
300	63	58	36	34	37
400	76	67	45	42	46
500	83	73	55	52	58

Table 4: The inhibitory effect on protein denaturation by different extracts and standard drug at different concentration

Concentration (µg/ml)	Methanol	Ethanol	Chloroform	n-hexane	Pet.Ether	Sodium Diclofenac
100	46	45	38	50	49	89
200	59	57	43	69	65	89.5
300	70	69	49	82	77	91
400	83	80	56	90	89	92
500	85	81	62	93	92	94
IC_{50}(µg/mL)	130	140	310	100	105	<50

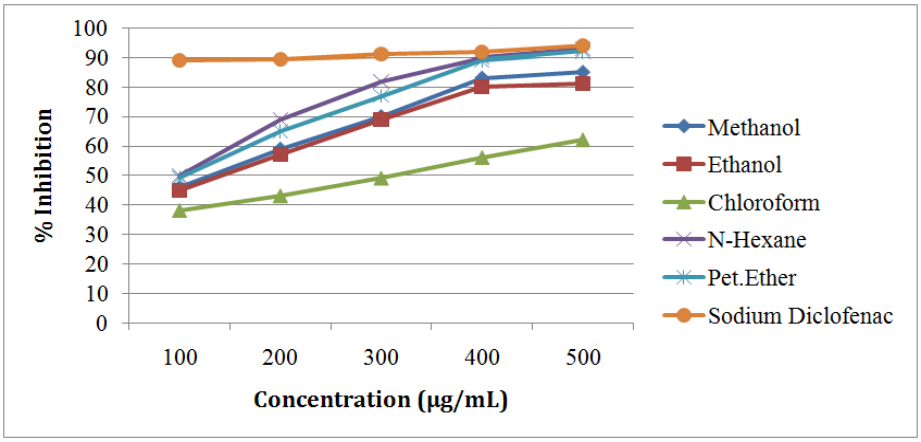

Fig. 2: Percentage inhibition on protein denaturation by different extracts and standard drug at different concentration.

CONCLUSION

Two methods were used to estimate the amount of antioxidant activity in the fruits of *Coccinia grandis*. Among the two methods, antioxidant activity was more when performed with phosphomolybdate assay. Ethanol and methanol extracts seemed to have more antioxidant activity than any other extracts.

Denaturation of proteins is well documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of n-hexane and pet-ether extracts to bring down thermal denaturation of protein is possibly a contributing factor with the mechanism of action.

The presence of alkaloids, carbohydrates, phytosterols, tannins, flavonoids, proteins and diterpenes in the fruits of *Coccinia grandis* has been observed by general observation tests. Ethanol and methanol extracts were found to have significant antioxidant property which may be due to the presence of phytochemicals. The presence of proteins and amino acids in the extracts of n-hexane and

petroleum ether may be responsible for the anti-inflammatory activity possessed by them.

This study has revealed the potential for antioxidant activity and also lent scientific justification to the traditional use of plant in anti-inflammatory conditions. However, the exact structures of the bioactive components and the mechanisms involved in both antioxidant and anti-inflammatory activity of different extracts are yet to be elucidated.

ACKNOWLEDGEMENT

Authors are thankful to the Oxford College of Science for providing laboratory facilities to carry out this research work.

REFERENCES

1. Kirthikar KR, and Basu BD. Indian Medicnal Plants 2nd ed. International Book Distributors, Dehradun; 1987. p. 1151-1154.
2. Taur DJ, Patil RY, Mast cell stabilizing, antianaphylactic and antihistaminic activity of Coccinia grandis fruits in asthma Chinese J of Natural Medicines 2011; 9 (5): 359-362.
3. Shibib BA, Khan LA, Rahman R. Hypoglycemic activity of Coccinia indica and Momordica charantia in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase Biochem J 1993; 292(Pt 1): 267-270.
4. Venkateswaran S. and Pari L. Effect of Coccinia indica leaves on antioxidant status in streptazotocininduced diabetic rats Ethnopharmacol J 2003; 84(2-3): 163-168.
5. Bandyopadhyay U, Das A. and Bannerjee RK. Reactive oxygen species Oxygen damage and pathogenesis Curr Sci 1999; 77(5): 658-666.
6. Floyd RA. Neuroinflammatory processes are important in neurodegenerative diseases: An hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development Free Radical Biology and Medicine 1998; 26(9-10):1346-1355.
7. Rajamani Karthikeyan, Manivasagam T, Anantharaman P, Balasubramanian T, Somasundaram ST. Chemopreventive effect of Padina boergesenii extracts on ferric nitrilotriacetate (Fe-NTA)-induced oxidative damage in Wistar rats J Appl. Phycol 2011; 23, Issue 2: 257–263.
8. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotecin D: A docosahexanoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress Proceedings of the National Academy of Sciences of the USA, 2004; 101(22): 8491-8496.
9. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. 2nd ed. Bristol Wright Science technical 1975; 81– 82.
10. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E Anal Biochem 1999; 269: 337–41.
11. Fejes S, Blazovics A, Lugasi A, Lemberkovics E, Petri G. and Kery A. In vitro antioxidant activity of Anthriscus cerefolium L. (Hoffm.) extracts. J. Ethnopharmacol 2000; 69: 259-265.
12. Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga Ecklonia cava. Food Chem. Toxicol 2006; 44: 1065-1074.
13. Elias G, Rao MN. Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. Indian J Exp Biol 1988; 26: 540-542.
14. Nagore DH, Ghosh VK, Patil MJ, Wahile AM. In vitro antioxidant and in-vitro-anti-inflammatory activity of *Cassia sophera Linn*. Int. J. Pharm and Pharmaceutical Sci 2010; 2(1): 113-121.
15. Shivaji B. Bole, Manju R, Nagaraj M, Sandhya V, Supriya G, Pranitha Kumari, Kiran B, Lalitha V. Comparative study of antibacterial and antioxidant activity of plant extract amla [*Phyllanthus emblica* L.] TULSI [*Ocimum tenuiflorum* L.] NEEM [*Azadirachta indica* A.JUSS] Pharmacophore 2010; 1 (3): 178-183
16. Santanu Sannigrahi1, Sambit ParidaV, Jagannath Patro, Uma Shankar Mishra, Ashish Pathak. Antioxidant and anti-inflammatory potential of *pterospermum acerifolium.* Int J of PharmSci Rev and Res 2010; 2: Issue 1: pp:44-49
17. Chanda S. and. Dave R. In vitro models for antioxidant activity evaluation of some Medicinal plants possessing antioxidant properties: An overview African J Microbio Research 2009; 3(13): 981-996.
18. Cook NC, Samman S. Flavonoids-Chemistry, metabolism, cardioprotective effects, and dietary sources Nutr Biochem 1996; 7: 66-76.
19. Czeczot H. Biological activities of flavonoids-A review Polish J. Food Nutr Sci. 2000; 950: 4: 3-13
20. Hollman PC, Hertog ML, Katan MB. Role of dietary falvonoids in protection against Cancer and coronary heart disease Biochem Soc Trans 1996; 24: 785-789.
21. Kuntz S, Wenzel U, Daniel H. Comparative analysis of the effects of flavonoids on Proliferation, cytotoxicity and apoptosis in human colon cancer cell lines Eur JNutr 1999; 38 (3): 133-142.
22. Haraguchi H. Antioxdative plant constituents. In: bioactive compounds from natural Sources first published. New York Taylor and Francis Inc 2001; 338-377.
23. Surai PF. Natural Antioxidants in Avian Nutrition and Reproduction Nottingham University Press 2002; 5-9.