IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 6 Ser. III (Nov. – Dec. 2022), PP 01-06
www.Iosrjournals.Org

Anti-Inflammatory and Antioxidant Activity of *Scoparia dulcis* Extracts.

Olayinka Grace Okanlawon[1], Ruth Nzube Amaeze[2]
Saheed Adegbola Adeyanju[3], Chikaodili Edith Okonkwo[4]
Sherifdeen Abiola Okunlola[5], Alvan Chimere Okechukwu[6]
[1]*(Biochemistry, Ladoke Akintola University of Technology Ogbomoso, Nigeria)*
[2]*(Industrial Chemistry, Federal University of Technology, Owerri, Nigeria)*
[3]*(Bioinformatics,Teesside University, United Kingdom)*
[4]*(Medical Laboratory Science, Ebonyi State University, Nigeria)*
[5]*(Microbiology, Obafemi Awolowo University, Nigeria)*
[6]*(Industrial Chemistry, Federal University of Technology, Owerri, Nigeria)*

Abstract:
The anti-inflammatory activity of 70% ethanolic leaf extract of Scoparia dulcis was evaluated using the inhibition of albumin denaturation method, the percentage inhibition of protein denaturation was observed to increase with increasing concentration of the ethanolic extract with a maximum percentage inhibition of protein denaturation observed at 65% for 3.20mg/ml concentration of the ethanolic extract. The iron-reducing activity was also observed to increase with an increasing sample concentration of Scoparia dulcis extract and was evaluated by determining the reduction of ferricyanide to ferrocyanide by the extract, the amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussion blue at 700 nm after 10 minutes. An increase in absorbance of the reaction mixture indicated the reducing power. Ascorbic acid was used as standard.
Key Word*:Anti-Inflammatory Activity; Antioxidant Activity; Iron Reduction; Scoparia dulcis; Protein denaturation;*

Date of Submission: 24-11-2022 Date of Acceptance: 08-12-2022

I. Introduction

For the vast majority of Nigerians who live in the country's rural and urban areas, traditional herbal medicines continue to be the primary form of healthcare. The thorough scientific examination of both their therapeutic potential and the evaluation of safety concerns will be necessary for the complete integration of herbal medicine into primary healthcare programs that are currently used in society. Scoparia dulcis, popularly known as sweet broom weed or licorice weed, is also referred to by a number of ethnic names in Nigeria, including ohinohine-sesere (Ebira), ungungbuhi (Gwari), romafada (Hausa), aiya (Ibo), ufu-ija (Igede), and mesenmesengogoro (It is one of the significant medicinal herbs that has been linked to widespread traditional applications in Nigeria for treating and managing upper respiratory tract infections [1–5]. Other folkloric applications include the management of bronchitis in Brazil, the treatment of septicemia in Ecuador, and the use of antiseptic for wounds and mouthwash for newborns by the indigenous tribes of Guyana. According to ethnobotanical research, the plant is used to treat the signs and symptoms of numerous inflammatory and oxidative stress-related disorders, including arterial hypertension [6] and diabetismellitus[7-9]. Numerous medical benefits of S. dulcis have been proven in the past, including its effects on lipid peroxidation in the yolk of fowl eggs and its in vivo anti-diabetic, anti-inflammatory, and antioxidant activities [10–14]

The development of high resistance against antibiotics frequently used to treat upper respiratory infections and the need to look for other sources of novel antimicrobial agents, particularly from medicinal plants, based on ethnopharmacological information, have made the scientific examination of *S. dulcis* imperative.

A few phenolic and terpenic chemicals that were extracted from S. dulcis have also been studied for their potential biological effects.[15–20]. Lipid peroxidation[21], which can be mediated enzymatically or nonenzymatically, is a process whereby reactive oxygen radicals cause damage to phospholipids in biological membranes.[22] Lipid peroxidation is mediated by enzymes like lipoxygenase and xanthine oxidase, which are connected to inflammatory processes and produce lipid peroxidation products upon activation. [23] Lipid

peroxidation has been linked to aging and a number of ailments, including Alzheimer's disease, rheumatoid arthritis, cataracts, and atherosclerosis. [24-26]

However, it is becoming obvious that no single method can completely predict antioxidant efficacy for in vitro and in vivo evaluation of antioxidant propensities. Therefore, it is advised to use multiple methods, and extrapolating the in vitro findings should be done with greater caution. [27–30]

This study, employing the inhibition of albumin denaturation method and measuring the reduction of ferricyanide to ferrocyanide by the extract, reports the in vitro anti-inflammatory and antioxidant activities of scoparia dulcis leaf extracts.

II. Material And Methods

2.1 Reagents

0.2% w/v aqueous solution of BSA
Stock extract preparation (10mg/ml)
Stock Diclofenac (10mg/ml)
Phosphate buffered saline
0.2M phosphate buffer
1% potassium ferrocyanide ($K_3Fe(CN)_6$)
10% Trichloroacetic acid
0.1% Ferric chloride
Ascorbic acid (5mg/ml)
Folin–Ciocalteu's phenol reagent (10% v/v)
7.5% w/v Na_2CO_3

2.2 Preparation of extracts

The whole plant of fresh of Scoparia dulcis were collected, cleaned and air-dried for a period of three weeks. After air-drying, the plant was pulverized using blender. 100g of ground sample was soaked in a conical flask containing 420ml of ethanol and 180ml of water (70% of ethanol in ratio 1:6 of weight to volume) for 24 hours. Extracts were filtered and residues were re-extracted twice under same conditions using same solvent. The filtrate was concentrated, freeze dried and stored in a freezer for further use.

2.3 Assessment of invitro anti-inflammatory activity

The anti-inflammatory activity was assessed according to Chandra et al. 2012 [35] and Sangeetha et al. 2011 [36] using the inhibition of albumin denaturation method with slight modification and the results were analyzed in triplicate. The reaction mixture consisted of test extracts and 0.2% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37 ºC for 20 min and then heated to 70 º C for 5 min, after cooling the samples the turbidity was measured at 660nm. (UV-Visible Spectrophotometer). The percentage inhibition of protein denaturation was calculated by the following formula:

% of Inhibition = [(Abs Sample −Abs Control) / Abs Control] * 100

2.4 Iron-reducing activity (Fe (III) to Fe (II))

Iron reducing power of extract was determined by the method of Oyaizu (1986)[37]. Different studies have indicated that the antioxidant effect is related to the presence of reductones (Yen and Duh 1993)[38]. Reductones are reported to be terminators of free radical chain reactions (Gordon 1990)[39], thus, the antioxidant activity of a methanolic extract may be related to its reductive activity. This was done by determining the reduction of ferricyanide to ferrocyanide by the extract. Different concentrations of Scoparia dulcis extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (K3Fe (CN)6) (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min and 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%). The amount of iron (II)- ferricyanide complex formed was determined by measuring the formation of Perl's Prussion blue at 700 nm after 10 minutes. An increase in absorbance of the reaction mixture indicated the reducing power. Ascorbic acid was used as standard.

III. Results.

3.1 Total phenolic content (TPC)

The total phenolic content (TPC) of extracts was measured using the Folin–Ciocalteu method as described by Chew, Lim, Omar, and Khoo (2008)[31]

Table 1: Protocol for standard TPC curve

Test Tube	Blank	50µL	100µL	150µL	200µL	250µL	300µL
gallic acid	-----	50	100	150	200	250	300
H_2O	300	250	200	150	100	50	-----
Na_2CO_3	1200	1200	1200	1200	1200	1200	1200
Folin C	1500	1500	1500	1500	1500	1500	1500
Mix thoroughly and incubated in the dark for 30 min. Read @ 765nm							

Table 2: sample

Test Tube	Blank	100µL	200µL	300µL
sample	-----	100	200	300
H_2O	300	200	100	-----
Na_2CO_3	1200	1200	1200	1200
Folin C	1500	1500	1500	1500
Mix thoroughly and incubated in the dark for 30 min. Read @ 765nm				

3.2 In vitro anti-inflammatory activity

The anti-inflammatory activity was assessed according to Chandra et al. 2012 [32] and Sangeetha et al. 2011[33] using the inhibition of albumin denaturation method with slight modification and the results were analyzed in triplicate.

Test Tube	PBS	sample	0.2% BSA	Diclofenac	
Blank	2mls	--	--	--	assay mixture and incubate at 37 °C for 20 min
Test Control	2mls	------	100µL	--	
Extract (10µL)	1990	10	100µL	--	incubate at 70 °C for another 5 min.
Extract (20µL)	1980	20	100µL	--	
Extract (30µL)	1970	30	100µL	--	cool and read the absorbance at 660 nm
Extract (40µL)	1960	40	100µL	--	
Extract (50µL)	1950	50	100µL	--	
Standard		--	100µL		

The percentage inhibition of protein denaturation was calculated by the following formula:
% of Inhibition = (Abs Control −Abs sample)/Abs Control * 100

3.3 Iron reducing activity

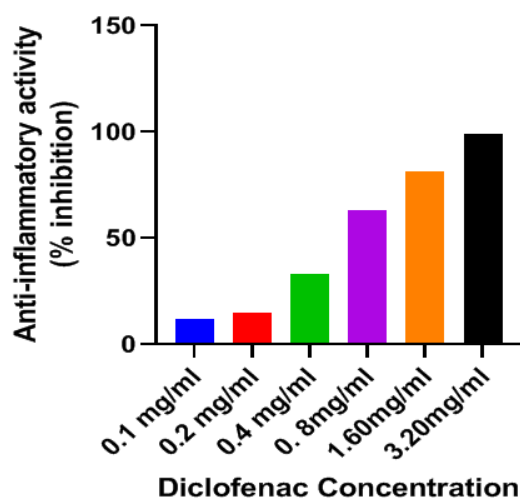
Iron reducing power of extract was determined by the method of Oyaizu (1986)[34].
N.B: The standard (ascorbic acid) is used for standard curve.

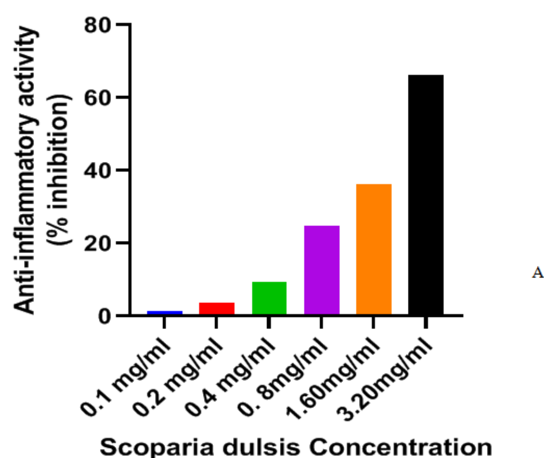
Test Tube	Phosphate buffer (µL)	1% $K_3Fe(CN)_6$ (µL)	Sample (µL)	Ascorbic Acid (µL)	dH_2O (µL)		10% TCA (µL)		dH_20 (µL)	0.1 $FeCl_3$ (µL)	
Blank	2,500	2,500	--	--	1000	Incubate at 50°C for 20 min	2500	Centrifuged for 10 min at 1000 g. Pick 2500µL of the upper layer into another test tube	2500	500	The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussion blue at 700 nm after 10 minutes.
Std (20)	2,500	2,500	--	20	980		2500		2500	500	
Std (40)	2,500	2,500	--	40	960		2500		2500	500	
Std (60)	2,500	2,500	--	60	940		2500		2500	500	
Std (80)	2,500	2,500	--	80	920		2500		2500	500	
Std (100)	2,500	2,500	--	100	900		2500		2500	500	

Sample (10)	2,500	2,500	10	--	990		2500		2500	500	
Sample (20)	2,500	2,500	20	--	980		2500		2500	500	
Sample (30)	2,500	2,500	40	--	960		2500		2500	500	
Sample (40)	2,500	2,500	80	--	920		2500		2500	500	
Sample (50)	2,500	2,500	160	--	840		2500		2500	500	

3.5 Anti-inflammatory activity of Scoparia dulcis

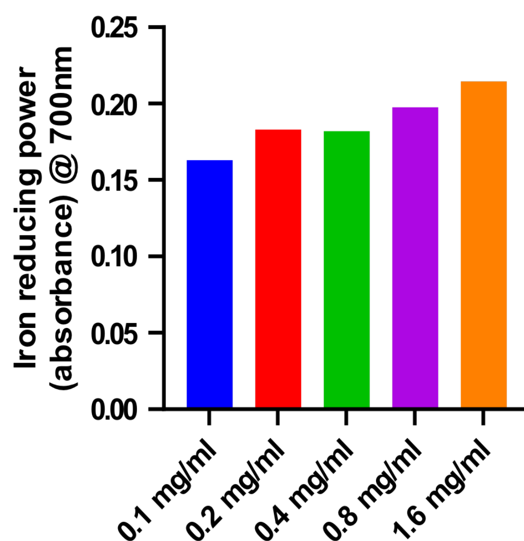
Ethanolic extracts of Scoparia dulcis was able to inhibit protein denaturation in a concentration-dependent manner. A known anti-inflammatory drug (Diclofenac) was used as the standard. The inhibitory effect of different concentrations of standard drug and sample extract on protein inhibition are shown below.





3.6 Iron reducing activity of Scoparia dulcis

The iron reducing ability of scoparia dulcis increased with increased sample concentration.



Iron reducin power of Scoparia dulsis

IV. Discussion

Using the inhibition of albumin denaturation method with a minor modification, the anti-inflammatory activity was evaluated in accordance with Chandra et al. 2012 [35] and Sangeetha et al. 2011 [36], and the findings were analyzed in triplicate. Test extracts and a 0.2% aqueous solution of bovine albumin fraction made up the reaction mixture. The turbidity was measured at 660 nm after the sample extracts had been cooled for 20 minutes at 37ºC and then heated for 5 minutes at 70 º C. (UV-Visible Spectrophotometer). The following equation was used to determine the % inhibition of protein denaturation:

((Abs Sample Abs Control) / Abs Control) * 100 = % of Inhibition

The Oyaizu (1986)[37] approach was used to calculate the extract's iron-reducing capacity. Reductones are thought to have an antioxidant impact, according to various research (Yen and Duh 1993)[38]. Reductones are thought to be chain reaction breakers for free radicals (Gordon 1990)[39]; as a result, a methanolic extract's antioxidant effect may be linked to its reductive activity. This was accomplished by measuring the extract's ability to reduce ferricyanide to ferrocyanide. Different Scoparia dulcis extract concentrations were combined with potassium ferricyanide (K3Fe (CN)6) (2.5 ml, 1%) and phosphate buffer (2.5 ml, 0.2 M, pH 6.6) in 1 ml of distilled water. 2.5 ml of trichloroacetic acid (10%) was added to the mixture after it had been incubated at 50°C for 20 minutes. The mixture was then centrifuged at 1000 g for 10 minutes. FeCl3 (0.5 ml, 0.1%) and distilled water (2.5 ml) were combined with the upper layer of the solution (2.5 ml). After 10 minutes, the development of Perl's Prussion blue at 700 nm was measured to quantify the amount of iron (II)- ferricyanide complex that had formed. The reaction mixture's increased absorbance was a sign of the reducing power. Ascorbic acid served as the benchmark.

References

[1]. Dalziel JM: The Useful Plants of West Tropical Africa. London. Grown Agents for Oversea Governments and Administrations, 1955, p. 415.
[2]. Nacoulma OG: Plantes me´ dicinales et pratiques me´ dicalestra- ditionnelles au Burkina Faso. Tome II. Cas du plateau central. The`sed'e´tat. Universite´ de Ouagadougou, 1996.
[3]. Satyanarayana K. Chemical examination of Scoparia dulcis (Linn): Part I. J Ind Chem Soc 1969;46:765–766.
[4]. Briones AM, Touyz RM: Oxidative stress and hypertension: current concepts. CurrHypertens Rep 2010;12:135–142.
[5]. Forbes JM, Coughlan MT, Cooper ME: Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 2008;57: 1446–1454.
[6]. Adaikpoh MA, Orhue N EJ, Igbe I: The protective role of Sco- paria dulcis on tissue antioxidant defense system of rats exposed to cadmium. Afr J Biotechnol2007;6:1192–1196.
[7]. Freire SMF, Emim JAS, Lapa AJ, Souccar C, Torres LMB: Analgesic and anti-inflammatory properties of Scoparia dulcis L. extracts and glutinol in rodents. Phytother Res 1993;7:408–414.
[8]. Pari L, Latha M: Antidibetic effect of Scoparia dulcis: effect on lipid peroxidation in streptozotocin diabetes. Gen Physiol Bio- phys2005;24:13–26.
[9]. Babincova´ M, Sourivong P: Free radical scavenging activity of Scoparia dulcis extract. J Med Food 2001;4:179–181.

[10]. Ratnasooriya WD, Jayakody JR, Premakumara GAS, Ediriweera ER: Antioxidant activity of water extract of Scoparia dulcis. Fitoterapia2005;76:220–222.
[11]. Hayashi K, Niwayama S, Hayashi T, et al.: In vitro and in vivo antiviral activity of scopadulcic acid B from Scoparia dulcis, Scrophulariaceae against herpes simplex virus type 1. Antivir Res 1988;9:345–354.
[12]. Hayashi T, Kawasaki M, Miwa Y, Taga T, Morita N: Antiviral agents of plant origin III. Scopadulin, a novel tetracyclic diterpene from Scoparia dulcis L. Chem Pharm Bull 1990;38:945–947.
[13]. Hayashi T, Asano S, Mizutani M, et al.: Scopadulciol, an in- hibitor of gastric H+ , K+-ATPase from Scoparia dulcis and its structure activity relationships. J Nat Prod 1991;54:802– 809.
[14]. Nishino H, Hayashi T, Arisawa M, Satomi Y, Iwashima A: Antitumor promoting activity of scopadulcic acid B from me- dicinal plant Scoparia dulcis L. Oncology 1993;50:100–103.
[15]. Sarada SKS, Dipti P, Anju B, et al.: Antioxidant effect of beta- carotene on hypoxia induced oxidative stress in male albino rats. J Ethnopharmacol2002;79:149–153.
[16]. Niki E, Yoshida Y, Saito Y, Noguchi N: Lipid peroxidation: mechanisms, inhibition, and biological effects. BiochemBiophys Res Comm 2005;338:668–676.
[17]. Zhang R, Brennan ML, Shen Z, et al.: Myeloperoxidase func- tions as a major enzymatic catalyst for initiation of lipid perox- idation at sites of inflammation. J Biol Chem 2002;277:46116– 46122.
[18]. Pratico` D, Rokach J, Lawson J, FitzGerald GA: F2-isoprostanes as indices of lipid peroxidation in inflammatory diseases. Chem Phys Lipids 2004;128:165–171.
[19]. Aruoma OI: Assessment of potential prooxidant and antioxidant actions. J Am Oil Chem Soc 1996;73:1617–1625.
[20]. Aruoma OI, Cuppett SL: Antioxidant Methodology. In Vivo and in Vitro Concepts. Champaign, IL, USA, AOCS Press, 1997.
[21]. Frankel EN., Meyer AS: The problem of using one dimensional methods to evaluate multifunctional food and biological antiox- idants. J Sci Food Sci Agric 2000;80:1925–1941.
[22]. Singleton VL, Orthofer R, Lamuela-Raventos RM: Analysis of phenols and other oxidation substrates and oxidant by means of Folin-Ciocalteu reagent. Meth Enzymol1999;299:152–178.
[23]. Arvouet-Grant A, Vennat B, Pourrat A, Legret P: Standardisation d'un extrait de propolis et identification des principaux con- stituants. J Pharm Belgique 1994;49:462–468.
[24]. Alli, A. I., Ehinmidu, J.O. and Ibrahim, Y.K.E (2011). Preliminary Phytochemical Screening and Antimicrobial Activities of Some Medicinal Plants Used InEbiraland. Bayero Journal of Pure and Applied Sciences. Vol. 4(1): 10 – 16
[25]. Chidume, F.C., Kwanashie, H.O., Adekeye, J.O., Wambebe,C., Odama, L.E. and Gamanial, R.S. (2002). Evaluation of the methanolic extract of Cassia tora leaves for immunodulatory and antimicrobial activities. Journal of Phytomedicine and Therapeutics. Vol. 7: 46-55
[26]. Alli et al. /Nig J. Biotech. Vol. 26 (2013) 21 - 25
[27]. Darwish, S. K.A. and Hamouda, H. M. (2012). Histological and Bacteriological Studies on the Effect of Some Antibiotics on Klebsiella pneumoniae-infected rats. Journal of Applied Sciences Research. Vol. 8(2): 953-964
[28]. Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituent of some Nigerian medicinal plants. African Journal of Biotechnology. Vol. 4 (7): 685-688.
[29]. Ehinmidu JO (1993). Antifungal and novel food preservative potential of selected trioganotins. PhD dissertation, Ahmadu Bello University, Zaria, Nigeria.
[30]. Hasanat, A. et al (2011). Invitro antibacterial, antifungal and cytotoxicity of Scoparia dulcis L. International Journal of Pharmacy and Pharmaceutical Sciences. Vol. 3(2): 198-203
[31]. Haryani, Y., Noorzaleha, A.S., Fatimah, A.B., Noorjahan, B.A., Patrick, G.B., Shamsinar, A.T., Laila, R.A.S. and Son, R.(2007). Incidence of Klebsiella pneumonia in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD–PCR analysis. Food Control. Vol. 18(7): 847-853
[32]. Igoli, J.O,,Ogaji, O.G., Tor-Anyin, T.A. and Igoli, N.P. (2005).Traditional medicine amongst the Igede People of Nigeria. Part. II. African Journal of Traditional, Complementary and Alternative Medicine. Vol.2 (2):134-152.
[33]. Iroha, I.R., Ezeifeka, G.O. Amadi, E.S. and Umezurike, C.R. (2009).Occurrence of extended spectrum beta lactamase producings resistant Escherichia coli and Klebsiella pneumoniae in clinical isolates and associated risk factors. Resarch Journal of Biological Sciences. Vol.4: 588-592.
[34]. Iroha, I.R., Oji , A.E. and Ayogu, T.E. (2011). Analysis of antibiotic susceptibility of Klebsiella pneumouniae strains isolated from different clinical specimens in ENUGU state. International Journal of Current Research. Vol. 2(1): 008-014.
[35]. Latha, M., Ramkumar, K.M., Pari, L., Damodaran, P.N., Rajeshkannam, V. and Suresh, T. (2006). Phytochemical and Antimicrobial Study of an Antidiabetic Plant: Scoparia dulcis. Journal of Medicinal Food. Vol.9 (3):391-39.
[36]. Lorke, D. (1983). A new approach to practical acute toxicity testing. Archive of Toxicology. Vol.54: 275-287.
[37]. Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller, M. A. (ed.) (2007). Manual of Clinical Microbiology, 9th Edition. ASM Press, Washington, D. C.
[38]. National Committee for Clinical Laboratory Standards (NCCLS) (1993). Performance Standard for Antimicrobial Disc Susceptibility Tests. Villanova, PA, USA: Approved Standard NCCLS Publication.
[39]. National Committee for Clinical Laboratory Standards (NCCLS) (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That grow Aerobically: Approved standard-Fifth Edition. NCCLS Document M7-A5. Pennsylvania, U.S.A.
[40]. Saikia, K.K., Das, B.K., Bewal, R.K., Kapil, A., Arora, N. K. and Sood, S. (2012). Characterization of nasopharyngeal isolates of type b Haemophilus influenzae from Delhi. Indian Journal of Medicinal Research. Vol.136: 855-861
[41]. Yisa, J. (2009). Phytochemical analysis and Antimicrobial activity of Scoparia dulcis and Nymphaea lotus. Australian Journal of Basic and Applied Sciences. Vol. 3(4): 3975-3979.

Grace Olayinka Okanlawon, et. al. "Anti-Inflammatory and Antioxidant Activity of Scoparia dulcis Extracts." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(6), (2022): pp. 01-06.