ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Anthelmintic and Antioxidant activity of Aqueous Ethanolic Extract of Erythrina subumbrans (Hassk.) Merr.

Ganesh S[1], Vijey Aanandhi M[*2]

[1]Research Scholar, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai – 600117, Tamil Nadu, India

[2]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai – 600117, Tamil Nadu, India

Article History:

Received on: 04 Nov 2020
Revised on: 09 Dec 2020
Accepted on: 13 Dec 2020

Keywords:

Erythrina subumbrans (Hassk.), Piperazine citrate, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Ascorbic acid

ABSTRACT

Aqueous Ethanolic extract of vegetative (leaf) part of herbal plant *Erythrina subumbrans (Hassk.)* was assessed for Anthelmintic and Antioxidant activity. The obtained crude extract was prepared in different concentration, i.e., 50 and 100 mg/mL, against the standard Piperazine citrate, i.e., 10mg/mL for anthelmintic activity. Pheretima posthuma test worms were used during the study and the anthelmintic activity was evaluated based on Paralysis and Death time. The average paralysis and death time of standard is 1.4 & 39.59 min (10mg/mL) compared to sample 5.3 & 65.09 min (50mg/mL); 2.5 & 54.08 min (100mg/mL). From the data, aqueous ethanolic extract possesses promising anthelmintic activity compared to piperazine citrate standard. Similarly, antioxidant activity was evaluated for sample at different concentration ranging from 5, 10, 20, 40, 80, 160, and 320 μg/ml premixed with 2.5mL of 0.0135mM DPPH solution. Ascorbic acid was used as a standard and treated in a similar way as that of a sample. Control samples were also prepared without standard and sample solutions. Prepared solutions were vortexed and kept at benchtop condition for 30 minutes and absorbance was measured at 517 nm in a UV-Vis spectrophotometer. The absorbance pattern of the sample is not comparable with that of the standard solution at the same concentration. Aqueous ethanolic extract with IC_{50} (half maximal inhibitory concentration) value is >320 μg/mL against standard Ascorbic acid with an IC_{50} value of 15.5 μg/ml. Which witness the crude extract possess less antioxidant activity.

*Corresponding Author

Name: Vijey Aanandhi M
Phone:
Email: hodpchemistry@velsuniv.ac.in

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4511

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Helminths a well-known parasitic worm infection affecting the wider portion of the world's population and it is one of the most common infections in humans. In well-developed and in developing countries like western countries, India, Africa etc., parasitic worm infection possesses a hefty threat to the health of the public and contributing the incidence of Morbidity, malnutrition, anemia, Pneumonia and eosinophilia. The majority of the infections due to parasitic worms were restricted to tropical zones (Bundy, 1994). The world health organiza-

tion discloses that the affected population due to parasitic infections is closer to 2 billion. Other than humans, crops and livestock are affected by parasitic worms resulting in a slowdown of economic growth, affecting the shortage of food production. These infections influence the majority of the social and economic consequences where the population is high in endemic areas. Although parasitic infection is it is limited to tropical zones, however, this can be developed for travellers who visited those countries, and some can develop in temperate condition. Despite this occurrence, the research of the anthelminthic drug is less significant (Tagboto and Townson, 2001).

Due to the increase in anthelmintic resistance and conventional impact on the environment, it is necessary to develop for alternative plan against the nematodes present in the gastrointestinal tract. Recently, it has been recognized that significant toxicity was observed in human beings that are present in foods that are derived from live stocks, posing a serious threat to living humans. The one common therapy to control these pathogens is phototherapy (Prakash and Mehrotra, 1987). Regardless of the situation, there is a steep increase in demand of herbal medicine compared to the past period worldwide. The majority of the Phyto plants were not assessed for their quality, efficacy and safety (Kirtikar *et al.*, 1999). The Discovery and progress of a new chemical entity for controlling parasitic worm infection is greatly needed. Several health agencies are promoting studies of conventional used plants, which are generally considered as a vital source for bioactive substances (Hammond *et al.*, 1997). The key cause for many ailments in humans is free radicals. These irregularities were generated between the neutralization and formation of prooxidants resulting in the condition called oxidative stress condition (Gangwar *et al.*, 2014). Nitrogen or oxygen species that are reactive (RNS, ROS) are vital free radicals, which makes our human body function complicated. Radicals like Hydroxy (OH^-), anion superoxide (O_2^-), peroxyl are the reactive oxygen species; Nitric oxide (NO^-), Peroxynitrite ($ONOO^-$), a free radical derived from nitrogen are the metabolites that affect physiologically. These were generated as a resultant of the aerobic organism during respiration; however, the unwarranted level has a direct link to the beginning of many diseases like diabetes, stroke, cancer and other immune deficiency (Kazeem and Ashafa, 2015).

The recent development on the usage of crude herbal drugs that contain free radical scavengers known for their healing activity (Hakiman and Maziah, 2009). The most common natural antioxidants include ascorbic acid, flavonoids, phenolic compounds and carotenoids are highly effective by inhibiting the peroxidation of lipids by lipoxygenase inactivation and to scavenge the oxygen species that are active and free radicals by proliferating the reaction mechanism and by heavy metal ion chelation (Sundararajan *et al.*, 2006). Traditional use of medicinal plants is considered safer in comparable antibiotics if the synthetic origin and are effective in clinical (Solanki, 2010). Carotenoids, tannins and flavonoids that are produced by plants have a very impressive range on avoiding susceptible substrate oxidation (Qusti *et al.*, 2010). Currently, Natural occurring antioxidants have a target for numerous research activities to find the basis drugs are effective, cheap and clinically safe (Mundhe *et al.*, 2011). Hence, the present research activity was carried out to search for anthelmintic and antioxidant activity in ethanolic extract of crude *Erythrina subumbrans (Hassk.)*.

MATERIALS AND METHODS

Material

A chemical such as Piperazine citrate, DPPH (2, 2-diphenyl-1-picrylhydrazyl), Ascorbic acid and were purchased from SISCO research laboratories Ltd Maharashtra, India. Other chemicals procured from local manufactures (Analytical reagent grade).

Plant material

The leaves of Erythrina subumbrans (Hassk.) Merr were collected from Western Ghats of India and authenticated at Siddha central research institute (Ministry of AYUSH, Government of India), Chennai by Research Officer and Head of the pharmacognosy department Dr K.N. Sunil Kumar and confirmed by Assistant Director in-charge Dr P. Sathiyarajeswaran (Authentication certificate 112.04011901 dated 04 Apr 2019). The harvested vegetative part was dried in a nominal condition and stored in a container made of Kraft paper.

Preparation of plant Extract

The plant material was washed with distilled water several times and was subjected to air-drying under the shade. After drying, they were ground by an electric mixer until they became a powder. Then the powdered samples were stored in a dark place and subjected to extraction method (Sai *et al.*, 2019). Extraction of powdered samples was done using aqueous ethanol (80%). Aliquots of 50 g of the powdered samples were soaked in 250 ml of the solvent for 72 hrs. Later the samples were filtered and concentrated under reduced pressure using a rotary evaporator and keep stored at room temperature

(25° C).

Test Organism

Pheretima posthuma (*P. posthuma*), a known Indian adult earthworm were collected from damp soil. The washing was made several times with raw water, followed by a saline solution (0.9% sodium chloride). The collection of earthworms for the study was made by considering the physical and structural resemblance with roundworms that are present in the human intestine. Length of 3-5 cm and width of 0.15-0.25 cm were selected for anthelmintic study (Thorn *et al.*, 1977).

In Vitro Anthelmintic Activity

The Anthelmintic activity of the aqueous ethanolic extract of Erythrina subumbrans (Hassk.) was carried out according to the method with minor modification (Dash *et al.*, 2003). The aqueous ethanolic crude extract was prepared in normal saline solution in two different concentration, i.e., 50 and 100 mg/mL. Piperazine citrate was used as a standard with a 10 mg/mL concentration. Standard drug solution and extract with different concentrations were prepared freshly on the day of use before initiating the experiment. A total of three groups with three earthworms in each group was placed into 10ml of the solution and evaluated for the paralysis and death time of the earthworm.

In Vitro Antioxidant Activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay of the aqueous ethanolic extract of Erythrina subumbrans (Hassk.) was carried out according to the method with minor modification (S and Perumal, 2018). In details, the DPPH solution was prepared in methanol at a concentration of 0.0135mM. 2.5mL of the prepared DPPH solution were mixed with different concentration of test sample (i.e., 5, 10, 20, 40, 80, 160 and 320 μg/ml), standard (Ascorbic acid), and control (without standard/test samples). Then, these sample standard mixtures were vortexed thoroughly and kept at the benchtop (i.e., room temperature) condition for 30 minutes. The absorbance of the mixture was measured at 517 nm by using a UV-visible spectrophotometer. The standard, sample and control sample final solutions were shown in Figure 1 and Figure 2. The percentage of Antioxidant activity was measured using the following expression: % DPPH Inhibition = [(OD of Control - OD of Test)/OD of control] x 100 Where OD of Test is the absorbance of the test and OD of control is the absorbance of the control. All experiments were performed in triplicate. From the data obtained, a curve to be plotted and the IC_{50} value will be calculated.

RESULTS

Plant Extract

The percentage yield of the ethanolic extracts was calculated by using the following equation: yield (%) = (weight of extract/weight of dried plant material) x 100 and the obtained yield was presented in Table 1.

Anthelmintic activity

Anthelmintic activity of Erythrina subumbrans (Hassk.) was evaluated by time, recorded during Paralysis and death of the earthworm. The paralysis time of the worms was concluded when there is no movement of worms and death time is concluded when there is a change in body texture and completely lost its motility (Girme *et al.*, 1970). Erythrina subumbrans (Hassk.) at the concentration of 50 mg/ml exhibits death and paralysis time of 65.09 and 5.30 minutes; 100 mg/mL concentration exhibits 54.08 and 2.50 minutes, respectively. Similarly, piperazine citrate exhibits death and paralysis time of 39.59 and 1.42 minutes at 10mg/ mL concentration. Details of the data were presented in Table 2 and Table 3.

DPPH radical scavenging Activity

The average control (i.e., without standard/test samples) absorbance value is 0.886 AU. The absorbance value and % assay of standard ascorbic acid was calculated using the formula and details of the calculation was summarized in Table 4 & Table 5. Similarly, absorbance value and % inhibition of aqueous ethanolic extract of Erythrina subumbrans (Hassk.) Merr sample preparation was calculated using the formula and the details of the calculation was summarized in Table 6 & Table 7. From the data, a curve was plotted, and the Assay concentration (IC_{50}) value was calculated. IC_{50} is defined as the concentration of the samples required for a 50% Assay of the enzyme. The IC_{50} value was calculated from the graph by plotting concentration on X-Axis and % Assay on Y-axis and the statistical curve was displayed in Figure 3. The IC_{50} value of the ethanolic extract of *Erythrina subumbrans (Hassk.) Merr* was found to be >320 μg/ml and the standard drug (Ascorbic acid) was 15.5 μg/ml, respectively.

DISCUSSION

Traditional medicinal plants possess active metabolites that are bioactive in nature and exhibits promising activity in anthelmintic and antioxidant activity in the Human system (Altemimi *et al.*, 2017). The most known compounds, like flavonoids and

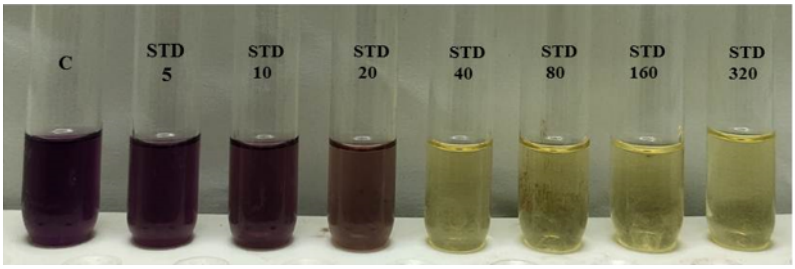

Figure 1: Standard solution

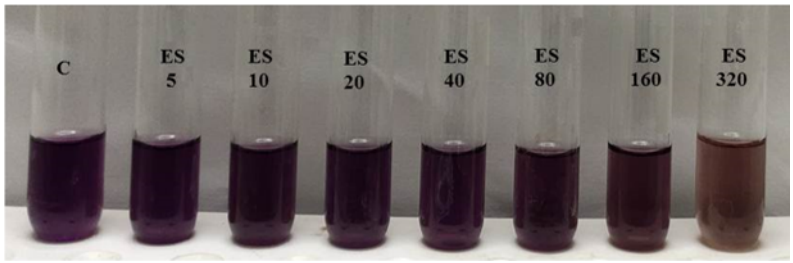

Figure 2: Sample solution

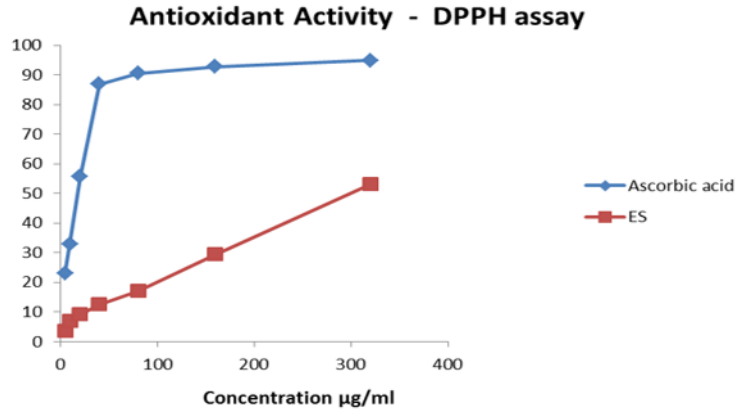

Figure 3: Statistical curve

Table 1: Extract Yield values

Solvent system	Powder weight (g)	Extract weight (g)	Extract Yield (%)
Aqueous ethanol (80%)	50 g	2.62 g	5.24

Table 2: Anthelmintic Activity-Paralysistime

Groups	Dose	Time of paralysis (min)			Avg. paralysis time in min
		G-1	G-2	G-3	
Piperazine citrate	10mg/ml	1.39	1.45	1.42	1.4
Ethanolic extract	50mg/ml	5.45	5.3	5.25	5.3
	100mg/ml	2.50	2.45	2.54	2.5

Table 3: Anthelmintic Activity-Deathtime

Groups	Dose	Death Time (min)			Avg. death time in min
		G-1	G-2	G-3	
Piperazine citrate	10mg/ml	40.12	39.45	39.30	39.59
Ethanolic extract	50mg/ml	65.12	64.45	64.50	65.09
	100mg/ml	54.25	53.45	54.55	54.08

Table 4: Antioxidant Activity-Standard (Absorbance)

Type	Control Absorbance	Conc. (μg/mL)	Absorbance Prp-1	Prp-2	Prp-3
Ascorbic acid	0.886	5	0.683	0.685	0.681
		10	0.597	0.591	0.595
		20	0.391	0.395	0.393
		40	0.113	0.118	0.119
		80	0.081	0.088	0.085
		160	0.062	0.065	0.067
		320	0.045	0.043	0.049

Prp.: Preparation, Conc.: Concentration

Table 5: Antioxidant Activity-Standard (% Assay)

Type	Conc. (μg/mL)	% Inhibition Prp-1	Prp-2	Prp-3	Mean	Std. Dev	IC_{50} value
Ascorbic acid	5	22.9699	22.7443	23.1954	22.9699	0.2255	15.5 μg/mL
	10	32.6691	33.3458	32.8947	32.9699	0.3445	
	20	55.9022	55.4511	55.6766	55.6766	0.2255	
	40	87.2556	86.6917	86.5789	86.8421	0.3625	
	80	90.8646	90.0751	90.4135	90.4511	0.3960	
	160	93.0075	92.6691	92.4436	92.7067	0.2838	
	320	94.9248	95.1503	94.4736	94.8496	0.3445	

Prp: Preparation, Conc.: Concentration

Table 6: Antioxidant Activity -Sample (Absorbance)

Type	Control Absorbance	Conc. (μg/mL)	Absorbance Prp-1	Prp-2	Prp-3
Erythrina subumbrans (ES)	0.886	5	0.856	0.854	0.855
		10	0.824	0.826	0.823
		20	0.801	0.808	0.805
		40	0.772	0.775	0.778
		80	0.732	0.738	0.735
		160	0.621	0.626	0.628
		320	0.413	0.416	0.418

Prp: Preparation, Conc.: Concentration

Table 7: Antioxidant Activity-Sample (% Assay)

Type	Conc. (μg/mL)	% Inhibition Prp-1	Prp-2	Prp-3	Mean	Std. Dev	IC_{50} value
Erythrina subum-brans (ES)	5	3.4586	3.6842	3.5714	3.5714	0.1127	> 320 μg/mL
	10	7.0676	6.8421	7.1804	7.0300	0.1722	
	20	9.6616	8.8721	9.2105	9.2481	0.3960	
	40	12.9323	12.5939	12.2556	12.5939	0.3383	
	80	17.4436	16.7669	17.1052	17.1052	0.3383	
	160	29.9624	29.3984	29.1729	29.5112	0.4066	
	320	53.4210	53.0827	52.8571	53.1203	0.2838	

Prp: Preparation, Conc.: Concentration

phenol, are widely dispersed in plants that are used as medicine. These compounds own significant Anthelmintic, antioxidant, Anti-ulcer, antidiabetic, antioxidant, hepatoprotective and anticarcinogen properties. Piperazine citrate, a known anthelmintic agent, works by blocking the response of muscle worms to acetylcholine by producing hyperpolarization of nerve endings, resulting in paralysis and removed from the human intestinal lumen (Balasundram *et al.*, 2006).

Radical scavenging activity (DPPH) a traditional mechanism used for the evaluation of antioxidant activity in the crude extract. The absorbance of the solution decreases when the color of the solution changes to yellow from purple. Traditional medicinal plants can able to lower the free radical level to yellow color 1,1 diphenyl -2-picrlhydrazyl (Rahman *et al.*, 2015). Hydrogen donation property is a key element for producing DPPH scavenging activity by antioxidants. When free radicals react with reducing agents, the solution becomes color less and losses it's color depending on the electrons used during the reaction mechanism (Sanchez-Moreno, 2002).

It was found that crude extract of *Erythrina subumbrans (Hassk.) Merr* possess promising anthelmintic activity with observed with a sample concentration of 50 & 100 mg/mL compared to standard piperazine citrate at the concentration of 10 mg/mL. The crude extract does not possess antioxidant activity with IC_{50} values of 320 μg/ml as compared to the positive control, Ascorbic acid (IC_{50}: 15.5 μg/ml). When there is a rise in absorbance value of the mixture, it an indicative of reducing inability, which serves as a substantial indication of antiscavenging/ oxidant activity. However, this was not observed in the sample during the analysis and the absorbance of the standard and sample does not possess a similar pattern which is evident that it has less antioxidant activity.

CONCLUSION

In this study, leaves of *Erythrina subumbrans (Hassk.) Merr* was collected from the Western Ghats of India and authenticated at Siddha central research institute, Chennai. Extraction was carried out using aqueous Ethanol (80%) and the obtained extract was subjected for anthelmintic and antioxidant activity. A promising anthelmintic activity was observed with a sample concentration of 50 and 100mg/ml against standard piperazine citrate 10mg/mL, whereas less antioxidant activity observed from crude extract (IC_{50}: >320g/ml) compared against standard Ascorbic acid (IC_{50}: 15.5mg/ml). Further activities to be carried out on isolation of bioactive compounds for the crude extract to evaluate its efficiency on the various disorder.

ACKNOWLEDGEMENT

The authors are thankful to the School of Pharmaceutical Sciences, Vels Institute of Science, Technology, and Advanced Studies, and its management for providing research facilities and encouragement.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., Lightfoot, D. 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6(4):42–42.

Balasundram, N., Sundram, K., Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1):191–203.

Bundy, D. A. P. 1994. The global burden of intestinal nematode disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88(3):259–261.

Dash, G. K., Mishra, S., Panda, A., Patro, C. P., Ganapathy, S. 2003. Anthelmintic activity of Evolvulus nummularius. *Indian Journal of Natural Products*, 19(3):24–25.

Gangwar, M., Gautam, M. K., Sharma, A. K., Tripathi, Y. B., Goel, R. K., Nath, G. 2014. Antioxidant Capacity and Radical Scavenging Effect of Polyphenol RichMallotus philippenensisFruit Extract on Human Erythrocytes: An In-Vitro Study. *The Scientific World Journal*, pages 1–12.

Girme, A., Bhalke, R., Ghogare, P., Tambe, V., Jadhav, R., Nirmal, S. 1970. Comparative In vitro Anthelmintic Activity of Mentha piperita and Lantana camara from Western India. *Dhaka University Journal of Pharmaceutical Sciences*, 5(1):5–7.

Hakiman, M., Maziah, M. 2009. Non-enzymatic and enzymatic antioxidant activities in aqueous extract of different Ficus deltoidea accessions. *Journal of Medicinal Plants Research*, 3(3):120–131.

Hammond, J. A., Fielding, D., Bishop, S. C. 1997. Prospects for plant anthelmintics in tropical vet-

erinary medicine. *Veterinary research communications*, 21(3):213–228.

Kazeem, M. I., Ashafa, A. O. T. 2015. In-vitro antioxidant and antidiabetic potentials of Dianthus basuticus Burtt Davy whole plant extracts. *Journal of Herbal Medicine*, 5(3):158–164.

Kirtikar, *et al.* 1999. Indian Medicinal Plants. *Plates*, 2. Publisher : M/s Bishen Singh Mahendra Pal Singh.

Mundhe, K. S., Kale, A. A., Gaikwad, S. A., Deshpande, N. R., Kashalkar, R. V. 2011. Evaluation of phenol, flavonoid contents and antioxidant activity of Polyalthia longifolia. *J Chem Pharm Res*, 3(1):764–769.

Prakash, V., Mehrotra, B. N. 1987. Anthelmintic plants in traditional remedies in India. *Indian Journal of history of Science*, 22(4):332–340.

Qusti, S. Y., Abo-Khatwa, A. N., Lahwa, M. B. 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the Holly Quran. *EJBS*, 2(1):40–51.

Rahman, M. M., Islam, M. B., Biswas, M., Alam, A. H. M. K. 2015. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Research Notes*, 8(1):621.

S, K., Perumal, P. 2018. Antidiabetic and antioxidant activities of ethanolic extract of piper betle l. Leaves in catfish, clarias gariepinus. *Asian Journal of Pharmaceutical and Clinical Research*, 11(3):194–194.

Sai, K., Thapa, R., Devkota, H. P., Joshi, K. R. 2019. Phytochemical Screening, Free Radical Scavenging and α-Amylase Inhibitory Activities of Selected Medicinal Plants from Western Nepal. *Medicines*, 6(2):70–70.

Sanchez-Moreno, C. 2002. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Science and Technology International*, 8(3):121–137.

Solanki, R. 2010. Some medicinal plants with antibacterial activity. *Int. J. Comp. Pharm*, 1(4):1–4.

Sundararajan, R., Haja, N. A., Venkatesan, K., Mukherjee, K., Saha, B. P., Bandyopadhyay, A., Mukherjee, P. K. 2006. Cytisus scoparius link - A natural antioxidant. *BMC Complementary and Alternative Medicine*, 6(1):8.

Tagboto, S., Townson, S. 2001. Antiparasitic properties of medicinal plants and other naturally occurring products. *Advances in Parasitology*, 50:199–295.

Thorn, G. W., Adams, R. D., Braunwald, E., Isselbacher, K. J., Petersdrof, R. G. 1977. Harrison's Principles of Internal Medicine. pages 1088–1089, New York. Mcgraw Hill Co.