IJAMS
INTERNATIONAL JOURNAL OF ADVANCED MULTIDISCIPLINARY STUDIES
Volume II, Issue 7 July 2022, eISSN: 2799-0664

Antioxidant Activity of The Terpenoid – Phenol Extract of Crescentia Cujete, Family Bignoniaceae

MS. MARY ANNE V. GUTIERREZ, RPh, MSPh, PhD (with academic units)

Abstract — This study focused on the investigation of the antioxidant activity of the terpenoid – phenol extract from the pulp of Crescentia cujete, a new plant in the Philippines which has not been extensively studied for its phytochemical and pharmacological properties. Cellular damage caused by reactive oxygen species (ROS) has been implicated in several diseases, and hence natural antioxidants have significant importance in human health. One of the major sources of antioxidants are plants and among the different constituents with antioxidant property includes terpenoids and polyphenols. These substances are widely distributed in many families of plants including the plants belonging to the Bignoniaceae family. Crescentia cujete is one of the plants belonging to this family. This plant has not well been investigated in the past though have been used in many countries as treatment for many diseases. Thus, this study will try to scientifically prove claims that the plant has a good antioxidant activity. The study focused on the phytochemical screening of the pulp of Crescentia cujete. It also dealt with the extraction of the terpenoid – phenol components through successive solvent extraction and the determination of the physico-chemical and instrumental properties of the terpenoid-phenol extract. Anti-oxidant activity was determined by total phenolic and radical scavenging assay using three radicals DPPH and nitric oxide. The anti-oxidant activity of the extract was compared with standard ascorbic acid. After conducting the different tests, the following findings were obtained: (a) The Phytochemical screening carried out on the Chloroform extract of Crescentia cujete showed the presence of secondary metabolite reducing sugars flavonoids, Anthocyanins, glycosides, phenols, terpenes and triterpenoids (b) Terpenoid – Phenol extract of Crescentia cujete revealed the presence of reducing sugar, glycosides, flavonoids, tannins, phenols, terpenes and triterpenes. The Terpenoid – Phenol extract is soluble in organic solvents like chloroform. Infrared bond identified in the extract are mainly phenolics and terpenes. (c) The antioxidant activity of the terpenoid – phenol extract of Crescentia cujete was investigated using DPPH and nitric oxide scavenging assays. Both methods have proven the effectiveness of the terpenoid – phenol extract of Crescentia cujete as antioxidant. (d) The difference in the DPPH and Nitric Oxide radical scavenging activity of Crescentia cujete Terpenoid – Phenol extract and standard ascorbic acid is statistically significant. This research demonstrated that the Terpenoid – Phenol extract of Crescentia cujete possesses an important antioxidant effect.

I. Introduction

Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. Antioxidants therefore can be used to reverse the harmful and pathological effect of the free radicals. The antioxidants generally scavenge the free radicals and detoxify the physiological system (Gulcin, Oktay, Aslan, & Kufrevioglu, 2002).

Plants are potential sources of natural antioxidants and produce various antioxidative compounds to counteract reactive oxygen species (Rice-Evans, Miller, & Perez, 1996). Local communities and folklore healers throughout the world use a wide range of plants and their parts for their medicinal properties. A large population from folk and tribal communities still uses a variety of plants for medicinal purposes due to either lack of advanced health care facilities in their remote regions or their traditional belief along with the success rate of these traditional medicines in different disease conditions (Kataki & Murugamani, 2012). However, the natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds become important (Espin, Soler-Rivas, & Wichers, 2000) . Therefore, search into the isolation of natural antioxidant sources is important.

Among the family of plants that have been reported to contain several secondary metabolites which possess antioxidant activity the Bignoniaceae family. Several phytochemical studies revealed that the extracts from many species of Bignoniaceae contained secondary metabolites such as saponins, tannins, flavonoids, quinones, alkaloids, anthralene derivatives, reducing sugars, terpenoids, glycosides, carbohydrates, quercetin, kaempferol, alpha-sitosterol, terpenes, steroids, coumarins etc. (Choudhury, 2011) . One of the plants under this family is *Crescentia cujete*. This plant has been reported to possess several pharmacological properties due to the various constituents present on its pulp namely polyphenols like flavonoids, terpenoids like iridoid glucosides, tannins, several plant acids, etc. Phenolic compounds found in both edible and nonedible plants have a capacity to scavenge free radicals and exerted multiple biological effects, including antioxidant activity (Valenzuela, Sanhueza, & Nieto , 2003) .There are only few scientific studies conducted however to establish folkloric use of the plant. It is therefore due to this reasons that the antioxidant and blood glucose lowering activity of the terpenoid and phenolic extract from the juice obtained from the pulp was the focus of investigation in this study (Bartholomew & et.al, June 1, 2007) .

Background of the Study

Cellular damage caused by reactive oxygen species (ROS) has been implicated in several diseases, and hence natural antioxidants have significant importance in human health. One of the major sources of antioxidants are plants and among the different constituents with antioxidant property includes terpenoids and polyphenols. These substances are widely distributed in many families of plants including the plants belonging to the Bignoniaceae family. *Crescentia cujete* is one of the plants belonging to this family. This plant has not well been investigated in the past though have been used in many countries as treatment for many diseases. Thus, this study will try to scientifically prove claims that the plant is a good antioxidant and blood glucose lowering activity.

Theoretical Framework

Oxidative stress is currently suggested as a mechanism underlying different diseases (Morhan & al.) . Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environment stimuli and when a radical-generating system outweighs radical scavenging system oxidative stress results. These are all retarded if not prevented by anti-oxidants which may either be coming from natural and synthetic sources.

Often natural sources include polyphenolic and isoprene containing compounds like triterpenes, which includes iridoid glycosides which are present in *Crescentia cujete*. As such if proven that this plant possesses antioxidant activities it may be a valuable source for the treatment of different diseases.

Statement of the Problem

This study aimed to determine the antioxidant property of the terpenoid-phenol extract from the pulp of Crescentia cujete.

It specifically sought to answers the following questions:

1. What is the total phenol content of the terpenoid-phenol extract from *Crescentia cujete*?
2. Does the terpenoid-phenol extract from *Crescentia cujete* has the ability to scavenge radicals namely 1,1,diphenyl-2 picryl hydrazine (DPPH) and nitric oxide?
3. Is there a significant difference in the radical scavenging activity of the terpenoid-phenol extract from that of ascorbic acid?
4. What is the approximate lethal dose (ALD) and Median Lethal dose (LD50) of the terpenoid – phenol extract of *Crescentia cujete*?

Hypothesis

H1: There is significant difference in the anti-oxidant activity of the terpenoid – phenol extract from the pulp *Crescentia cujete from* that of ascorbic acid.

H0: There is no significant difference in the anti-oxidant activity of the terpenoid – phenol extract from the pulp *Crescentia cujete from* that of ascorbic acid.

Significance of the Study

This study focused on the investigation of the antioxidant and blood glucose lowering activities of the terpenoid – phenol extract from the pulp of *Crescentia cujete*, a new plant in the Philippines which has not been extensively studied for its phytochemical and pharmacological properties. The results of this study offer benefit to the following:

The **health practitioners** who are on their way to address the health conditions of the country. The results of this study offer solutions to the ever growing incidence of the disease.

Future researchers in herbal medicines, who continuously search for new plants with medicinal values and who are on their way developing methods of isolation, characterization and identification of specific constituents with pharmacologic activities. The results may serve as a basis for their future investigations.

Scope and Limitations of the Study

The study focused on the phytochemical screening of the pulp of *Crescentia cujete*. It also dealt with the extraction of the terpenoid – phenol components through successive solvent extraction and the determination of the physico-chemical and instrumental properties of the terpenoid-phenol extract. Anti-oxidant activity was determined by total phenolic and radical scavenging assay using three radicals DPPH and nitric oxide. The anti-oxidant activity of the extract was compared with standard ascorbic acid. The study did not deal with the determination of the specific phenolic or terpenoid substance that possess anti-oxidant activity.

Definition of Terms

Approximate Lethal Dose (ALD) is a standard measure of the toxicity of a material that will kill half of the sample population of a specific test animal in a specified period through exposure via ingestion, skin contact, or injection (Feldman, 2019)

Bignoniaceae family of flowering plants in the order Cucurbitales. The Bignoniaceae consists of two genera: *Begonia,* with some 1,000 species, and *Hillebrandia,* with one species. The family is distributed throughout most tropical and warm temperate regions, with a large percentage of species being native to the Americas (Encyclopedia Britannica, 2011)

DPPH Radical Scavenging Assay. A.k.a. 1-1-diphenyl-2-picryl-hydrazil is a decolorization assay that measures the capacity of antioxidants to directly react with (scavenge) DPPH radicals by monitoring its absorbance at 517 nm with a spectrophotometer. The DPPH radical is a stable organic nitrogen centered free radical with a dark purple color that when reduced to its nonradical form by antioxidants becomes colorless (Ni, 2008).

Free radicals are compounds with an unpaired electron, which makes them extremely reactive (Jonas Dictionary, 2005).

Half Maximal Inhibitory Concentration (IC50) is a concentration of an inhibitor at which 50% inhibition of the response is seen; should only be used of in vitro test systems (encyclo.co.uk).

Median Lethal Dose is the quantity of an agent that will kill 50 per cent of the test subjects (medical-dictionary.com, n.d.)

Nitric Oxide Scavenging Assay was based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Umamaheswari & Chatterjee, October 2008).

Terpenes are large-sized group of unsaturated hydrocarbons with the empirical formula (C5H8)n. A large number of compounds found in plants comprise this type of functional group or its derivatives (Jonas Dictionary, 2005).

II. Methodology

This chapter presents the methods that were used to answer the problems of the study.

Crescentia cujete was collected during the fruiting season from Davao. Both experimental and descriptive methods of research were used in this study. Descriptive research is a fact finding method used to determine the properties of the Terpenoid – Phenol extract of *Crescentia cujete.* Experimental method using standard laboratory procedures was utilized to determine the antioxidant activity, ALD and the median lethal dose of the Terpenoid – Phenol extract of *Crescentia cujete* in Swiss mice.

1. Determination of Total Phenol Content of the Terpenoid-Phenol Extract from Crescentia *cujete*

1.1. Determination of Total Phenols

Total phenols were determined by Folin-Ciocalteu reagent. Gallic acid was used as a reference standard. In separate tubes, the standard and the aqueous solution of the terpenoid – phenol extract was mixed with Folin-Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) and 4 mL 1M sodium carbonate. The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm using spectrophotometer. The standard curve was prepared using 0, 50, 100, 150, 200 and 250 mg/L solutions of gallic acid in methanol and water (50:50, v/v) (Medina, 2011). Total phenol value was expressed in terms of gallic acid equivalents using the formula:

$$\text{mg of gallic acid/ g of sample} = \frac{(\text{mg of gallic acid})(\text{final volume of sample})}{\text{g of sample}}$$

2. In vitro test for antioxidant property of the terpenoid – phenol extract

The antioxidant activity of the terpenoid – phenol extract and natural antioxidant- ascorbic acid were assayed by determining their free radical scavenging activities following the procedures presented below:

2.1. 1,1-diphenyl-2-picryl-hydrazil (DPPH) scavenging assay

A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of different concentrations of the terpenoid – phenol extract. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using spectrophotometer. Controls were prepared and tested in the same manner (See plate 3) (Bakar & et al, 2009). The free radical scavenging activity was expressed as percentage DPPH scavenging activity or percentage inhibition using the formula:

$$\% \text{ DPPH scavenging effect (\%inhibition)} = \left(\frac{A_o - A_S}{A_o}\right) \times 100$$

Where Ao is the absorbance of the blank and as is the absorbance of the extract and Ac is the absorbance of the standard.

2.2 Nitric Oxide radical scavenging assay

In physiological pH Sodium Nitroprusside in aqueous solution spontaneously generates nitric oxide that interacts with oxygen to produce nitric ions and can be determined using greiss reagent.

The reaction mixture (3mL) containing sodium nitroprusside (10mM, 2 mL), phosphate buffer saline (0.5 mL) and extract or standard solution (20 – 100 µg, 0.5 mL), was incubated at 25ºC for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipette and mixed with 1 mL of sulphanilic acid reagent and allowed to stand for 5 min for complete diazotization. Then 1 mL of naphthylethylenediamine HCl (0.1%) was added, mixed and allowed to stand for 30 min. A pink-colored chromophore was formed (See plate 4). The absorbance of these solutions was measured at 540 nm against the corresponding black solutions (Marcoci, Maguire , & Droy, 1994) (Shukla, Mehta, Mehta, & Bajpaj, 2011)

The absorbance of standard solutions of ascorbic acid treated in the same way with Greiss reagent were the positive control. The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{Ao - At}{Ao} \times 100$$

Where Ao is the absorbance of the control and At is the absorbance of the extract in the presence of other reagents in the mixture. All the tests will be performed in triplicate and the graph will be plotted with the mean values. The positive control that will be used is Ascorbic acid.

3. Statistical Analysis of the Antioxidant activity

Means of absorbance and half maximal inhibition concentration (IC50) were computed in the antioxidant assay. Test Significant differences of the Terpenoid – Phenol Extract of *Crescentia cujete* against DPPH and Nitric Oxide assay were determined using Analysis of Variance (ANOVA) through t-test.

4. Biological Assay

The safety of the Terpenoid – Phenol Extract of *Crescentia cujete* was evaluated using swiss mice. The toxicological and pharmacological testing were conducted under the provision of official assistant from an accredited laboratory facility in accordance with the guidelines of the Philippine Association for Laboratory Animals Science (PALAS).

4.1 Toxicity test (Williamson, Okpako, & Evans, 1996)

Healthy 6-7 weeks old of swiss mice, weighing 18-25 grams, bred at the University of the Philippines, Department of Pharmacology were obtained and used as subjects of this study. The mice were fed pigeon pellets and water. They were acclimatized for a period of three days prior to toxicological testing. Different doses of the terpenoid-phenol extract were determined using the logarithmic method with 0.6 increments. The administration of all treatments was done via intraperitoneal injection.

4.1.1 Approximate Lethal Dose (ALD) Test

The approximate lethal dose of the *Crescentia cujete* Terpenoid-phenol extract was evaluated using 16 Swiss mice (equally numbered to male and female) and was grouped into 8. They were kept in individual observation cages and were acclimatized 7 days prior to the conduct of the study. Physiological changes and deaths were observed in all groups and were rated based on the standard central nervous system monitoring parameters.

4.1.2 Median Lethal Dose (LD50) Test

Post Approximate lethal dose test further acute toxicity study – median lethal dose or LD50 was carried out using sixty Swiss mice (30 males and 30 females). Ten Swiss mice, (5 males and 5 females), were assigned to each group: Negative control (NSS) and 5 *Crescentia cujete* Terpenoid – Phenol extract dose groups. The mice were fasted overnight before the administration of the treatments.

Starting dose for the *Crescentia cujete* Terpenoid – Phenol extract for LD50 was observed based on ALD results for any physiological changes.

The mortality rates were observed for a period of seven days and the median lethal dose was computed using the regression method.

III. Results and Discussion

Data presented here consists of the results in the tests stated in the previous chapter.

1. Total Phenol Content of the Terpenoid – Phenol Extract from *Crescentia cujete*

Phytochemical components, especially phenolic compounds are very important components for the free radical scavenging and antioxidant activities of plants.

Table 1: Total Phenol Content of the Terpenoid-Phenol Extract from the pulp of Crescentia cujete, L

	Absorbance at 765 nm			mg Gallic Acid Equivalents per g sample				Standard Deviation
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Mean conc.	
Terpenoid – Phenol extract	0.345	0.335	0.302	0.116	0.111	0.095	0.107	0.008957

Total phenolic content of *Crescentia cujete* was measured by employing the method involving Folin – Ciocalteu reagent as an oxidizing agent and gallic acid as a standard. The result, given in Table 1, showed that the total phenolic content is 0.107 expressed as mg gallic acid equivalent per gram of sample. Furthermore, as standard deviation is applied it revealed 0.008957 supporting that the total phenolic content of the *Crescentia cujete* terpenoid – phenol is comparable to gallic acid.

2. Antioxidant Property of the Terpenoid – Phenol extract of *Crescentia cujete, L*

The efficiency of antioxidant activity can be assessed by IC50 of the extract. The lower the IC50 extract have, the better the antioxidant property the extract contain.

2.1. Radical Scavenging activity of Ascorbic acid and Terpenoid – Phenol extract of *Crescentia cujete* against 1,1-diphenyl-2-picryl-hydrazil (DPPH) at 517 nm

A method based on the scavenging of the stable radical DPPH has been used extensively to predict the antioxidant activities of extracts of plants because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. The mean IC50 values for DPPH radical is shown in table 2.

Table 2: Half Maximal Inhibitory Concentration (IC50) value of Ascorbic acid and Terpenoid – Phenol extract of Crescentia cujete, L against DPPH

Antioxidant	IC50 value
Calabash extract	**36.91**
Ascorbic acid	**0.1519**

The DPPH scavenging activity of ascorbic acid was more pronounced than that of terpenoid – phenol extract but it was evident that the extract has potential antioxidant activity. Statistical analysis was performed by t – test. $p < 0.01$ was considered significant.

2.2. Radical Scavenging activity of Ascorbic acid and Terpenoid – Phenol extract of *Crescentia cujete* against Nitric Oxide

Terpenoid – phenol extract of *Crescentia cujete* significantly inhibited nitrite formation in concentration dependent manner. Results exist in table 3.

Table 3: Half Maximal Inhibitory Concentration (IC50) value of Ascorbic acid and Terpenoid – Phenol extract of *Crescentia cujete, L* against Nitric Oxide

Antioxidant	IC50 value
Calabash extract	209.67
Ascorbic acid	360.31

Since, the lower the IC50 value the better the antioxidant activity therefore, the nitric oxide scavenging activity of Terpenoid – Phenol extract of *Crescentia cujete* at 209.67 showed strong antioxidant capacity compared to the standard ascorbic acid at 360.31. Both exhibited a significant dose dependent inhibition of Nitric oxide. Statistical analysis was performed by t – test. $p < 0.01$ was considered significant.

3. Statistical Analysis of the Antioxidant Activity

To test the null hypothesis, statistical analysis was performed. Statistical analysis using t-test of the data for antioxidant activity showed that the antioxidant activity of plant extract, compared to control was significant at the 0.01 level. Since, the computed value, 34.870402, is greater than the tabulated value, 4.6041, the null hypothesis is rejected. Hence, there is a significant difference in the anti-oxidant activity of terpenoid – phenol extract from the pulp of *Crescentia cujete* from that of ascorbic acid. The result clearly showed that the terpenoid – phenol extract from the pulp of *Crescentia cujete* had strong DPPH and Nitric Oxide radical scavenging activities as compared to the natural antioxidant, ascorbic acid. The high concentration of polyphenolics in terpenoid – phenol extract from the pulp of *Crescentia cujete* is the responsible for its high free radical scavenging activity due to presence of hydroxyl groups in the polyphenolics.

Table 4: t-Test Result of the Half Maximal Inhibitory Concentration (IC50) of Ascorbic Acid and *Crescentia cujete, L* against DPPH and Nitric Oxide Scavenging

DPPH			
Df	**Computed t**	**Tabular t**	**Interpretation**
4	34.870402	4.6041	Significant
Nitric Oxide			
Df	**Computed t**	**Tabular t**	**Interpretation**
4	132.795890	4.6041	Significant

t - tab α = 0.01

4. Results on Biological Assay

Table 5: Approximate Lethal Dose of the Terpenoid – Phenol Extract of *Crescentia cujete, L*

Log Dose	**Dose mg/kg**	**Deaths**	**Physiological Activities Monitored**								
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1.0	10	0 / 2	0	0	0	0	0	0	0	0	0
1.6	40	0 / 2	0	0	0	0	0	0	0	0	0
2.2	160	0 / 2	0	0	0	0	0	0	0	0	0
2.8	630	0 / 2	0	0	0	0	0	0	0	0	0
3	1 000	0 / 2	0	0	0	0	0	0	0	0	0
3.4	2 500	0 / 2	1	1	1	1	0	0	0	0	2
4.0	10 000	1 / 2	3	3	4	2	1	2	2	1	4
4.4	25 000	2/2	3	3	4	2	1	3	2	2	4

Approximate lethal dose test showed a relative safety dose of up to 1000mg/kg and did not demonstrate visible symptoms of toxicity in the test animals as reflected in Table 5. However, there were physiological changes observed starting at a dose of 2,500 mg/kg and significant changes were seen at 10,000 mg/kg.

Table 6: Median Lethal Dose (LD50) of the Terpenoid – Phenol Extract of *Crescentia cujete, L*

Log Dose	Dose mg/kg	Deaths	Percentage Death (%)
3.4	2 500	0 / 8	0
3.6	4 000	0 / 8	0
3.8	6 300	2 / 8	25
4.0	10 000	3 / 8	37.8
4.2	16 000	4 / 8	50
4.4	25 000	5 / 8	62.5
4.6	40 000	7 / 8	87.5
4.8	63 000	8 / 8	100

Please refer to Appendix N for interpretations of rating

Legend of Monitored Physiological Activity

1 – Decrease in motor activity
2 – Ataxia rating
3 – Loss of righting reflex
4 – Analgesia
5 – Anesthesia
6 – Respiratory rate and depth
7 – Corneal and pinnal reflex
8 – Paralysis of forelegs, hind legs and head
9 – Loss of screen grip

The Median Lethal Dose of the terpenoid – phenol extract of *Crescentia cujete* was calculated using percent mortality and established to be 16,000 mg/kg. The regression equations between log of doses and percentage death is Y = 75.14881 x + -262.798 and resulted in 14,454.40 mg/kg. The two values are equal confirming the validity of LD50. All swiss mice were died at a dose of 63,000 mg/kg.

IV. Summary and Conclusion

The study dealt with study of the Antioxidant and Blood Glucose lowering activity of the terpenoid – phenol extract from the pulp of Crescentia cujete Family Bignoniaceae (Calabash). This chapter also includes conclusions based on the findings and recommendations for possible action and resolution.

Summary of Findings

After conducting the different tests, the following findings were obtained:

1. The antioxidant activity of the terpenoid – phenol extract of *Crescentia cujete* was investigated using DPPH and nitric oxide scavenging assays. Both methods have proven the effectiveness of the terpenoid – phenol extract of *Crescentia cujete as* antioxidant.

2. The difference in the DPPH and Nitric Oxide radical scavenging activity of *Crescentia cujete* Terpenoid – Phenol extract and standard ascorbic acid is statistically significant

3. The toxicological test of the terpenoid – phenol extract of the pulp of *Crescentia cujete* revealed a non-toxic dose of up to 1000 mg/kg. The approximate lethal dose confirmed a physiological changed commencing at 2500mg/kg until 10, 000mg/kg. The LD50 was established to be 14,454.40 mg/kg.

Conclusion:

This research demonstrated that the Terpenoid – Phenol extract of *Crescentia cujete* possesses an important antioxidant effect

REFERENCES

[1] (n.d.). Retrieved from medical-dictionary.com.
[2] Bakar, M., & et al. (2009). Phytochemical and Antioxidant activity of different parts of Bambangan (Mangiferapajang) and Artocarpusodoratissimus. Food Chemistry, Vol. 113, 479-483.
[3] Bartholomew, I., & et.al. (June 1, 2007). Iridoids and Other Glycosides from Vietnamese Crescentia cujete. Journal of Medicinal Food.
[4] Choudhury, S. (2011). Phytochemical of the Family Bignoniaceae - A Review Department of Life Science and Bioinformatics. Assam University Journal of Science and Technology, Vol 7.
[5] Encyclopedia Britannica. (2011).
[6] Espin, J., Soler-Rivas, C., & Wichers, H. (2000). Characterization of the Total Free Radical Scavenger Acivity of vegetable oils and oils fractions using DPPH. J Agric Food Chem, 48, 648.
[7] Feldman, A. T. (2019). Bancroft's Theory and Practice of HIstological Techniques . Science Direct, 8th Edition.
[8] Gulcin, I., Oktay, M., Aslan, A., & Kufrevioglu, O. (2002). Determination of Antioxidant Activity of Lichen Cetraria islandica. Ethnopharmacol , 73, 325-329.
[9] Jonas Dictionary. (2005).
[10] Kataki, M., & Murugamani, V. (2012). Antioxidant, Hepatoprotective, and Anthelminthic Activities of Methanol Extract of Urtica dioica Leaves. Pharmaceutical Crops, 3, 38-46.
[11] Marcoci, L., Maguire , J., & Droy, M. (1994). The nitric oxide scavenging properties of Gingobiloba extract . EGb, 784-55.

[12] Medina, M. (2011). Determination of Total Phenolics in Juices and superfruits by a novel chemical method. Journal of Functional Foods, 79-87.
[13] Morhan, S. D., & al., e. (n.d.). Oxidative Stress and Diabetes, Laboratory of Cellular and Molecular Cerebral Ischemia. Wayne State University School.
[14] Ni, L. Y. (2008). Wheat Antioxidants. 120.
[15] Rice-Evans, C., Miller, N., & Perez, G. (1996). Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids. Free Radical Biol. Med., 933-956.
[16] Shukla, S., Mehta, A., Mehta, P., & Bajpaj, V. (2011). Antioxidant ability and total phenolic content of aqueous leaf extract of Stevia rehaudiana. Experimental and Toxicological Pharmacology.
[17] Umamaheswari, M., & Chatterjee, T. (October 2008). Complimentary and Alternative Medicines. African Journal of Traditional, 5.
[18] Valenzuela, A., Sanhueza, J., & Nieto , S. (2003). Cholesterol Oxidation: Health hazard and the role of Antioxidants in Prevention . Biol. Res, 36:291-302.
[19] Williamson, E., Okpako, D., & Evans, F. (1996). Selection and Preparation and Pharmacological Evaluation of Plant Material.