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Full Length Research Paper

Examine the effect of hemagglutinin activity of lectin extract from selected green vegetables (*Vernonia amygdalina*, *Gnetum africanum, Petrocarpus soyauxil and Telifera occidentalis*)

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Lectins are carbohydrate binding proteins present in most plants and plant foods. They are toxic, inflammatory, and resistant to cooking and digestive enzymes. In this study, lectins were extracted from *Okazi* leaf (*Gnetum africanum*), Bitter leaf (*Vernonia amygdalina),* Fluted pumpkin leaf (*Telferia occidentalis),* and *Oha* leaf (*Pterocarpus soyauxii*). Partial purification of the lectin extracts was achieved by precipitation with ammonium sulphate and the extracts were tested for hemagglutinin activity using 4% suspension of human erythrocytes in lectin buffer. The blood specifity of the different blood groups was observed in the variation of lectins activities in a_0, a_1 and a_2. The hemagglutinin activities of the vegetable lectins decreased slightly at 60°C when exposed to processing (moist heating and oven drying) but at 100°C there was no agglutination activate in Onugbu and Okazi vegetables except in a_1 for blood group A and B in fluted pumpkin and Okazi leaves respectively. Loss in agglutinations activity suggest that lectins from these vegetables are heat labile and can be deactivated by moist or dry heating.

Key words: Agglutinations, hemagglutinin , lectins, *Vernonia amygdalina, Gnetum africanum, Petrocarpus soyauxil and Telifera occidentalis.*

INTRODUCTION

Lectins are proteins of non-immune origin that recognize and bind to carbohydrate without modifying them (Meagher *et al*., 2005; Sigh *et al., 2014*). Lectins can be found in plants, animal, microorganisms and viruses. Ingestion of lectins by animals may cause anti-nutritional effects, degeneration of cells membranes, inhibition of digestive enzymes and immune (allergic) reactions (Vasconcelos and Oliveira, 2004). The resulting interference with absorption of nutrients may give rise to serious physiological consequences (Silva *et al*., 2010). They can cause “food poisoning” by passing the gut wall and deposit themselves in the distant organs thereby causing real life disease (Puztai *et al*., 1989; Wang *et al*., *1998 Freed, 1999).* They are able to disrupt small intestine metabolism and damage small intestinal villi because of the ability of lectins to bind with brush border surfaces. Considering the vast effect of lectins, it is necessary to investigate the processing effect on haemagglutinin activity of lectin extract from selected green vegetables using human blood groups (A, AB, B and O).

Circumstantial argument in favour of the defense role of plant lectins is their marked stability under favourable conditions. Most lectins are stable over a wide range of pH and are able to withstand heat, insect proteases and resist animal attacks, to this respect they are believed to have strong defense-related proteins such as protease inhibitors, anti fungal proteins, alpha amylase

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inhibitor and thionins (Erdman and Foryce, 1999). Many plant lectins with diverse biological activities have been purified and characterized. Lectins activate cells and characterized several cytokines produced by immune cells (Yamamoto and Irimaru 2011).

The vegetable leaves contain anti-nutritive factors such as high level of tannic acid, saponin, lectin etc. Akwaowo *et al*. (2000) reported that young leaves often preferred for human consumption contain higher cyanide and tannin content than matured leaves. While some of the anti-nutrients in the leaves are above safety limit for human consumption, most are not harmful but rather have some health benefits to its consumers (Ladeji *et al*., 1995; Ajibade *et al*., 2006). Processing techniques can increase the nutritional quality of the plant foods by reducing specific anti-nutrients (Imaobong *et al*., 2013). The level of lectins decreased to a considerable extent during heat processing especially moist heating (cooking, extrusion, autoclaving and microwave) (Habiba, 2002; Wang *et al*., 2008, Embaby, 2010). Most lectins are known to have higher thermal stability (70^0C > 30 mins) and do not completely degrade with insufficient and low temperature cooking. While some lectins degrade others pass through the gut, about 1% - 5% absorbed into the blood stream of animals which is a significant amount sufficient to cause an immune response resulting in hemolytic anemia and jaundice (Breneman, 1984; Rabal and Akbar, 2009). This lectin toxicity is due to an inhibitory effect on plasma membrane repair (Miyake *et al.*, 2007).

The main objective of this study is to evaluate the processing effect (moist heating and oven drying) on haemagglutinin activities of lectin extracted from selected green vegetables using human erythrocytes (A, B, AB and O). Lectin extracted from processed (moist heated and oven dried) selected green vegetables will help to unveil the processing effect on haemagglutinin activities using human blood in vitro. Also this work will be useful in advising food processors, food chemist, food handlers and consumers on the effective method to adopt during food processing/preparation of these vegetables and which vegetables are to be avoided by various blood groups.

PREPARATION OF SAMPLES

Moist heated samples: The vegetables were blended fresh into slurry and 25g were measured into a beaker. It was then heated in a water-bath at two temperature regimes (60 and 100°C) for a period of 10 mins. The heated mash samples were completely washed and immersed in $1dm^3$ of distilled water contained in a conical flask. Thorough mixing was done by stirring at intervals for about 1hour. The homogenate was filtered through two layers of muslin cloth and the filtrate allowed to stand for 12hours at room temperature after which the supernatant were decanted and stored in an air tight container in a refrigerator for further analysis while the settled residue were discarded

Oven dried samples: The green leafy vegetables were dried for 10 hours each at 60°C and 100°C in an electric oven. The samples were then pulverized using the electric rotor blender (Emel, EM-242 Jumia). A 25g of the samples were completely immersed in $1dm^3$ of distilled water contained in a conical flask. Thorough mixing was done by stirring at intervals for about 1hour. The homogenate was filtered through two layers of muslin cloth and the filtrate allowed to stand for 12hours at room temperature after which the supernatant were decanted and stored in an air tight container in a refrigerator for further analysis while the settled residue was discarded

PREPARATION OF LECTIN BUFFER

Lectin buffer was prepared according to the method described by Brooks *et al*., (1997). A 6.057g of tris base, 8.70g of sodium chloride, 0.203g of magnesium chloride and 0.11g of calcium chloride were weighed out. The tri base and the salts were then mixed in a 1000ml volumetric flask and dissolved with 100mls of distilled water. Concentrated hydrochloric acid (HCl) was added to adjust the pH to 7.6 and the volume is made up to 1000mls.

PARTIAL PURIFICATION OF LECTIN

The method described by Brooks *et al*., (1997) was used in recovery of the protein (lectins) from the supernatant. The crude protein in the supernatant was precipitated by stirring ammonium sulphate ($(NH_4)_2SO_4$) into the liquid to give a 10%w/v solution. (1g of ammonium sulphate in 10mls). The mixture was allowed to equilibrate for 1hr at room temperature. The precipitate formed were separated at 2000rpm for 10mins using a bench top centrifuge and kept at 0^0C in ice bath. The recovered supernatant was further saturated with ammonium sulphate to give 20% weight per volume (w/v) solution with the same procedure. The supernatant was removed and the precipitate was put into a test tube and placed inside an ice bath. This process was repeated to precipitate the remaining proteins at 30%, 40% and 50% saturation. The precipitate obtained at different ammonium sulphate concentration were combined in 250ml measuring cylinder and dissolved in 50mls of lectin buffer, and was use for analysis the same day.

PREPARATION OF RED BLOOD CELLS (ERYTHROCYTES)

The red blood cells were prepared according to the method described by Diehl *et al*., (1998). A 5mls of fresh whole blood cells from human blood group A, B, AB, and O were collected from healthy donors at Daughters of Mary Private Hospital, Umuahia in Abia

State. The blood cells were obtained with 5ml syringe and transferred into sterile EDTA bottle. The Ethylenediaminetetracetic Acid (EDTA) in the bottle serving as anti-coagulant. The bottles were vigorously shaken to properly mix the blood with EDTA. The whole blood was transferred into a test tube to which 10mls of lectin buffer (pH 7.6) was added. It was then centrifuged at 2000rmp for 10 mins to enable sedimentation of the red blood cells at the bottom of the centrifuge with a pipette. Another 10mls of lectin buffer was reintroduced into the blood samples and the washing was repeated 3 times to obtain a clear supernatant. This was carefully removed with a pipette leaving behind the red blood cells. A 2mls of the red blood cells (washed) each from the blood types O, A, AB and B were placed into 100mls measuring cylinder and diluted with 50mls of lectin buffer to obtain 4% suspension of erythrocytes in lectin buffer solution. The supernatant (plasma and buffer) layer was carefully removed.

HAEMAGGLUTININ TEST FOR AGGLUTINATING ACTIVITY

The lectin activity of the crude extracts was monitored using the method described by Brooks *et al*., (1997). The agglutination was carried out with serial dilutions of the lectin solution in improved wells of microtitre plates using human erythrocytes. About 0.1ml of lectin buffer was placed in each of the wells of multi well titre plates, and then 0.1ml of lectin solution (crude extract in lectin buffer) was added to first well. About 0.1ml of the first dilution was removed from the first well and added to the next well to obtain the second dilution. To each of the dilution in the well, 0.1ml of 4% suspension of the erythrocytes was carefully layered on top of the lectin solution. After the addition of the red blood cells suspension in all the cells, the mixture was agitated and allowed to rest for one hour at room temperature and was observed for agglutination. The result was monitored visually. A positive agglutination test was indicated by the formation of a uniform layer of solution over the surface of the well while a negative test was indicated by the formation of a discrete bottom at the bottom of the well.

RESULTS AND DISCUSSION

The results show that both moist heating and oven drying affected the agglutinating activities of the vegetable extracts. From Table 1 moderate agglutination was observed in a_0 (concentrated lectin extract) at 60^0C for blood groups B and AB (in Pumpkin) A, B and AB (in Oha) but Onugbu and Okazi showed slight agglutination at the same temperature. One fold dilution (a_1) at 60^0C showed a slight agglutination for all the blood Groups in Pumpkin and Group O, Groups A,B,O for Oha and Onugbu respectively. Okazi showed no agglutination for blood Group A and B.

In two fold dilution at 60^0C there were no agglutination except in AB for Pumpkin. At 100^0C for a_1 and a_2 no agglutination in all the vegetables except for blood Group A in Pumpkin. The negative agglutination in some of the blood groups might be as a result of agglutinating inhibitors. Some lectins can bind to cell but would not cause agglutination. Agglutinating inhibitors as described by Lis and Sharon (1986) are known for their hydrophobic and hydrophilic force which is able to alter the carbohydrate-lectin interaction.

The carbohydrate lectin complex is stabilized by intermolecular hydrogen bonds and *van der waal* forces. The destabilization of such forces by inhibitors hinders agglutination. It also indicates that as the dilution progresses, there is loss of activity of the lectin as observed in loss of hemagglutinin activity at a_1 and a_2 dilutions. According to Lis and Sharon (1986), erythroagglutination by lectin is affected by the molecular properties of lectin, cell surface properties, metabolic state of cells and conditions of assay such as temperature, cell concentration and mixing. Agglutinating activity was devoid at a_2 for all blood groups in all the vegetables such loss in activity may be due to the presence of agglutination inhibitors.

The drastic reduction of hemagglutinin activities of the selected vegetables at 100^0C (moist heating) in almost all blood Groups shows that hemagglutinin activities of the studied vegetables are heat-labile. Heat labile hemagglutinin is growth depressant at minute level in foods and tends to be toxic at high concentration (Liener, 1994).

Table 2. shows Hemagglutinating activities of oven dried vegetables. Fluted pumpkin Oven dried at 60^0C gave scanty agglutination. In blood group O, agglutinating activity was lost and at 100^0C there was total destruction of lectins except for blood group AB that showed slight agglutination at a_0.

In *Oha* leaf oven drying at 60^0C had slight agglutination as observed at a_0 for blood group A and AB and loss of activity at a_1 and a_2. Also weak agglutination was shown by blood group B and O at a_0 and a_1 and loss of activity was equally observed at a_2. At 100^0C, there was loss of activity for blood group A and O. Blood group B and AB showed weak agglutination at a_0.

In bitter leaf oven dried at 60^0C, weak agglutination was observed at a_0 for blood group A and O and at AB there was total loss of activity. Also at a_0 and a_1 slight or weak agglutination was observed by blood group B. At 100^0C there was total destruction of lectin activity in all blood groups except for blood group B that shows slight agglutination at a_0.

Oven dried *okazi* leaf at 60^0C has agglutination activity at a_0 for blood group A, O and AB. In blood Group B there was total loss of activity at 100^0C, the lectins lost agglutinating activity as negative agglutination was observed.

These losses of activity may be due to the presence of agglutinating inhibitors and it varies with the blood groups which agrees with the previous findings on lectin. Okamoto *et al*., (1990) who reported that heat

stability of these proteins differ from lectin to lectin. Hernandez-Infante *et al*., (1998) reported also that microwave heat treatment significantly reduced hemagglutinin lectin activity in beans and legumes.

CONCLUSION

In this work moist heating at 60^0C and 100^0C and oven drying at 60^0C and 100^0C used in determining efficiency of heat processing in reducing this anti-nutrient (lectin) reveals that at a high heat treatment the level of lectin activity

was reduced or eliminated. The degree of lectin binding activity differed from one blood type to the other and also with difference in temperature. From the study vegetables should be processed by boiling at 100^0C and also oven drying at 100^0C to ensure adequate removal of these anti-nutrients from the leaves.

RECOMMENDATION

It is recommended that further research should be done to evaluate the effect of other types of heat treatments on the hemagglutinin activity and their specificity in regards to different blood types in human as well as other animals. This will facilitate the knowledge on the prevailing processing method best suitable for these vegetables thereby helping the food industries, food processors and consumers to be aware of the best time and heat treatment required for effective processing of the above selected green leafy vegetables.

REFERENCES

Ajibade SR, Balogun MD, Afolabi OO, Kupolati MD (2006). Sex differences in the biochemical contents of Teltaira occidentalis. J. Food Agric Environ. 4:155-156.

Akwaowo EU, Ndon BA, Etuk EU (2000). Minerals and anti-nutrients in fluted pumpkin (Telfaria occidentalis Hook F). Food Chem.., 70:235-240.

Breneman (1984). Basics of Food Allergy. Spring field, III, Thomas, P 10-29 Brooks S.A., Leathem A.J.C., Schumacher U. (1997). Lectin histochemistry – A concise practical hand book. BIOS, Oxford, Uk. 1st Edition pp.16-152.

Brooks S.A., Leathem AJC, Schumacher U. (1997). Lectin histochemistry – a concise practical hand book. BIOS, Oxford, Uk 1st Ed. Pp 16-152

Diehl JA, Chend M. Roussel MF, Sherr CJ (1998). Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and sub cellular localization. Genes Dev 12(22): 3499-3511.

Embaby HE (2010). Effect of soaking, dehulling and cooking methods on certain anti-nutrients and in vitro protein digestibility of bitter and sweet lupin seeds. Food Sci. Biotechnol., 19, 1055-1062.

Erdman JW, Foryce (1999). Characterization of a Novel, antifungal, Chitin-binding protein. 181 (24): 7421- 7429.

Freed, D.J. (1999) Allergist. British Medical Journal 318 (7190):1023-1024.

Habiba RA (2002). Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods. Food Chem., 77, 187-192.

Hernandez-Infante M, Sousa V, Montalvo I, Tena E (1998). Impact of microwave heating in hemagglutinins, trypsin inhibitors and protein quality of selected legume seeds. Plant Foods Hum. Nutr., 52: 199 -208.

Imaobong, Iyang Udousoro, Roland, U., Ekop, Efiok Johnson Udo (2013). Effect of thermal processing on anti-nutrients in common edible green leafy vegetables. J. Nutr. 12 (2) 162-167.

Ladeji O, Okoye ZS, Ojobe T (1995). Chemical evaluation of the nutritive value of leaf (fluted pumkin Telferia occidentalis) Food Chem 53:353-355.

Liener IE (1994). Implication of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nut., 34, 31-67.

Lis H, Sharon N (1986). Biological properties of lectins. In: The “Lectins: Properties, Functions and Applications in Biology and Medicine” ed. by I.E. Liener, N. Sharon and I.J. Goldstein. Academic Press, Orlando, pp 265-291.

Meagher JL, Winter HC, Ezell P, Goldstein IJ (2005). Crystal structure of banana lectin reveals a novel second sugar binding life. Glycobiology is 1033-1042.

Miyake, Y; Asano, K; Kaise, H; emaru,M; Nakayama, M and Tanaka, M (2007). Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell associated antigens. Jornal of Clinical Investigation ISSN 0021-9738. 1558-8238 – online http://www.jci.org/articles/view/31990

Okamoto Y, Minamoto S, Shimizu K, Mogami H, Taniquchi T (1990). Interleukin 2 receptor beta chain expressed in an oligodendroglioma line bonds interleukin 2 and delivers growth signal. Proc. Natl. Acad. Sci. USA 87(17): 8684-6588.

Pusztai A, Greer F, Grant G (1989). Specific uptake of dietary lectins into the systemic circulation of rats. Biochem Soc Trans. 17:481–482.

Rabia H, Akbar M (2009). Dietary lectin as disease causing toxicants. Pak. J. Nutr. 8 (3) 293-3003.

Sigh SS, Devi SK, Ng TB (2014). Banana lectin- A Brief Review. Molecules 19:18817-18827

Silva MC, Angelita DC, Santos DD (2010). Flavis Cristina Almedia Marcus and Celester-Maria Patto de Abreu. Partial purification of leaf lectin from Manihot esculenta and screening its fungicidal activity. J. Agric. Biotec and Sustain. Dev. pp136-141.

Vasconcelos IM, Oliveira JT (2004). Antinutritional properties of plant lectins. Toxicology 44: 385-403.

Wang N, Hatcher DW, Gawalko EJ (2008). Effect of variety and processing on nutrients and certain anti-nutrients in field peas (Pisum sativum). Food Chem., 111: 132-138.

Wang Q, Yu L-G, Campbell BJ, Milton J, Rhodes JM (1998). Identification of intact peanut lectinin peripheral venous blood. Lancet. 352:1831–1832. [PubMed].

Appendix

Result on Effect of Moist Heating on Hemagglutinin Activity of Crude Lectin Extract of the selected Vegetables

Table 1. Hemagglutinin Activity of Crude Lectin Extract from moist heated Vegetables.

Blood group	DILUTION FOR HEATED FLUTED PUMPKIN						CONTROL	DILUTION FOR HEATED OHA LEAF						CONTROL	DILUTION FOR HEATED BITTER LEAF (Onugbu)						CONTROL	DILUTION FOR HEATED OKAZI LEAF						CONTROL
	60^0C			100^0C				60^0C			100^0C				60^0C			100^0C				60^0C			100^0C			
	a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2	
A	+	+	-	+	+	-	-	++	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-
B	++	+	-	+	-	-	-	++	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+	-	-
O	+	+	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-
AB	++	+	+	++	-	-	-	++	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-

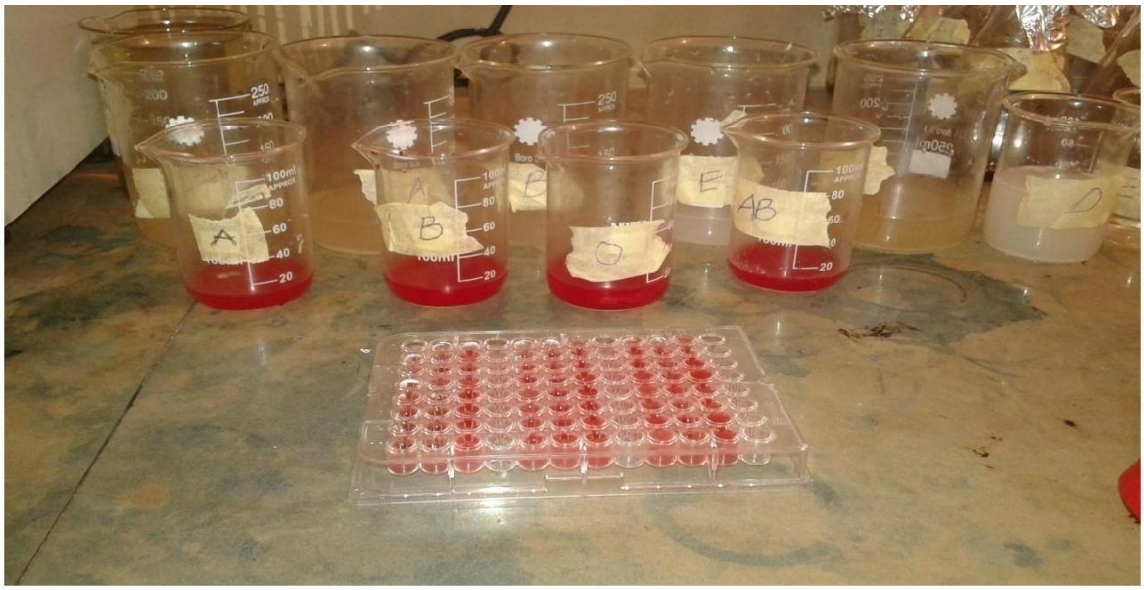
++ = Moderate Agglutination, + = Slightly Agglutination, - =No Agglutination, a_0=Concentrated Lectin Extract, a_1= 1 Fold Dilution, a_2 = 2 Fold Dilution, Control =Without Erythrocytes.

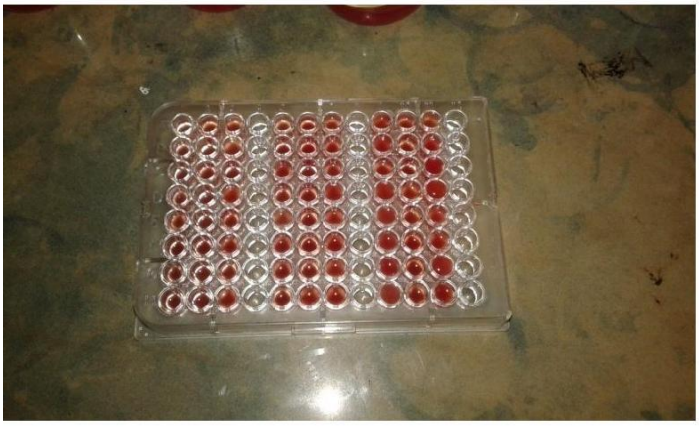
Result on Effect of oven drying on Hemagglutinin Activity of Crude Lectin Extract of the vegetables.

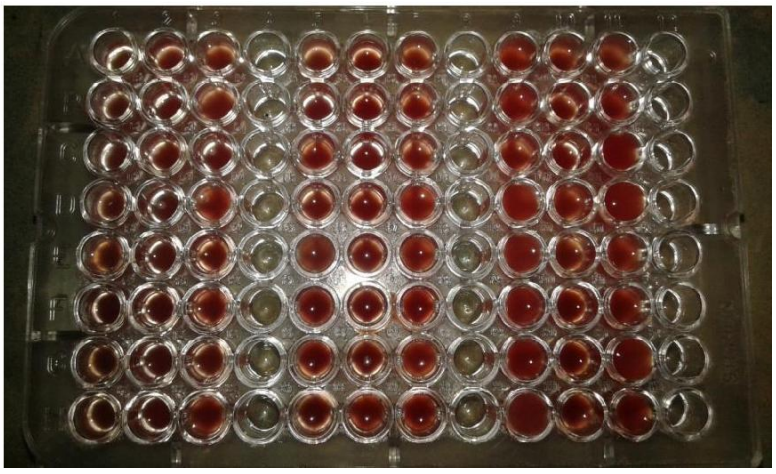
Table 2. Hemagglutinin activity of crude lectin extract from oven dried vegetables.

Blood group	DILUTION FOR OVEN DRIED FLUTED PUMPKIN						CONTROL	DILUTION FOR OVEN DRIED OHA LEAF						CONTROL	DILUTION FOR OVEN DRIED BITTER LEAF						CONTROL	DILUTION FOR OVEN DRIED OKAZI LEAF						CONTROL
	60 ^{0}C			100 ^{0}C				60 ^{0}C			100 ^{0}C				60 ^{0}C			100 ^{0}C				60 ^{0}C			100 ^{0}C			
	a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2		a_0	a_1	a_2	a_0	a_1	a_2	
A	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
B	+	+	-	-	-	-	-	+	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
O	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
AB	++	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

++ = Moderate Agglutination, + = Slightly Agglutination, - =No Agglutination, a_0= Concentrated Lectin Extract, a_1= 1 Fold Dilution, a_2 = 2 Fold Dilution, Control =Without Erythrocytes







MICRO TITRE PLATES CONTAINING HEMAGGLUTININ ASSAY