Research Article

www.ijrap.net

α-GLUCOSIDASE INHIBITOR AND ANTIOXIDANT ACTIVITY ASSAYS OF GUAVA LEAF, CASHEW LEAF AND THE COMBINATIONS AS ANTIDIABETIC AGENT

Asep Sukohar [1]*, Samsu Udayana N [2], Diana Mayasari [3], Arli Suryawinata [1]

[1]Department of Pharmacology and Therapy, Faculty of Medical, Lampung University, Bandar Lampung 35145, Indonesia

[2]Department of Agricultural Technology, Faculty of Agriculture, Lampung University, Bandar Lampung 35145, Indonesia

[3]Department of Community Medicine, Faculty of Medical, Lampung University, Bandar Lampung 35145, Indonesia

Received on: 19/01/17 Accepted on: 26/02/17

***Corresponding author**
E-mail: asepsukohar@gmail.com

DOI: 10.7897/2277-4343.08145

ABSTRACT

The leaves of guava and cashew have widely used as antidiabetic. Mechanism of antidiabetic effect on guava and cashew leaf caused by inhibition of α-glucosidase. More over antioxidant activity of guava and cashew leaf are expected decreased risk of DM complications by stop oxidative stress process. This study aimed to analyze the activity of α-glucosidase inhibitor and antioxidant of guava and cashew leaves This study use in vitro model with spectrophotometric method to analyze sample with λ= 517 nm for antioxidant analyze and λ= 405 nm for activity of α-glucosidase inhibitor. The data were analyzed by Analysis of Variance (ANOVA) and Least Significant Difference (LSD) on the real level of 1% and 5% The result showed that guava leaf extract has the highest α-glucosidase inhibitor activity by 97,006 % inhibition with IC_{50} = 2,16 mg/ml and antioxidant activity by 97,992 % on scavenging DPPH. The combination of guava leaf extract and cashew leaf extract did not give some effect on antioxidant activity or α-glucosidase inhibitor activity. The extract of guava and cashew leaves have α-glucosidase inhibitor activity and antioxidant activity. Combination of the extracts did not give some effect on antioxidant activity or α-glucosidase inhibitor activity.

Keywords: Antidiabetic, antioxidant, cashew leaf, guava leaf, *α-glucosidase inhibitor*

INTRODUCTION

Diabetes Mellitus (DM) refers to a group of chronic metabolic disorder characterized by hyperglycemia due to insulin insufficient, insulin resistance or both in glucose metabolism.[1] DM has become a serious health problem over the world with continuously increasing prevalence over years. In 2015, International Diabetes Federation (IDF) estimated the total of 415 million of people with DM from global population and projected to increased to 642 million in 2040.[2]

Diabetes Mellitus Type 2 (DMT2) or Non Insulin Dependent Diabetes Mellitus (NIDDM) is a type of DM with highest prevalence with 90-95% of all DM incident.[3] Patients with DMT2 are generally characterized by obesity leading to increase adiponectin cell which cause decreasing insulin sensitivity.[4]

To decrease blood glucose level is the aim of DM management. Non-pharmacology therapy, like diet and physical exercise, are the first line in DM management. If blood glucose levels fails to reach a normal level through non-pharmacological management, the second step is to use pharmacologic management by using oral antidiabetic agents or insulin substitution.[5]

The α-Glucosidase inhibitor is an oral antidiabetic agent used to prevent postprandial blood glucose increased. The mechanism of these drugs is by inhibiting α-glucosidase enzyme in the proximal small intestine which has function in the metabolization and absorption of carbohydrate complex. Acarbose is a α-glucosidase inhibitor drug used for patients with DM.[6]

The high incidence of complications in diabetic patients have also become a new problem in DM management procedures. Patients with DM have risk 4-10x more than normal people to have vascular diseases. This is due to high glucose level over blood leading to oxidative stress process. Therefore, the use of antioxidants should be able to play an important role to reduce risk of DM complications.[7]

Guava leaf has been used as therapeutic agents in the medicine. One of the utilization of guava leaf is as antidiabetic agent. The usage of 50-800 mg/KgBW guava leaf extract showing a decrease of blood glucose levels in mice that have been induced diabetes. The hypoglycemic effect of guava leaf extract is from biochemical compounds activity such as tannins, guiajaverin, quercetin and pentacyclic triterpenoids.[8] In addition, the content of bioactive compounds such as quercetin, quercetin-3-O-glucopyranoside and morin on guava leaf showing antioxidant activity with IC_{50} values 1,20 ± 0,02, 3,58 ± 0,05 and 5,41 ± 0,20 µg/ml.[9]

Cashew leaf has also been used in the medicine as antidiabetic. Guava leaf have biochemical compounds such as quercetin, kaempferol, and hydroxybenzoic acid which has the effect to decreased blood glucose levels by inhibiting α-glucosidase enzyme in small intestine.[10-11] Hypoglycemic effect on guava leaf extract was demonstrated with rats induced by alloxan and result showed decrease of 20.8% blood glucose levels in the first 4 hours.[12]

But various studies conducted on the efficacy of the antidiabetic had done by one material and not by mixing them. It certainly

does not describe the use of material in real life.[13] It is needed to test the effect of guava leaf and cashew leaf in single or mixture to determine synergistic or antagonistic effects.

MATERIALS AND METHOD

This study was conducted at Analysis Laboratory of Agricultural Products and Biochemistry Laboratory Faculty of Medicine, University of Lampung and held in October 2016. This study used a Randomized Complete Block Design (RCBD) with three repetitions.

Guava leaf are used in this study is dried in oven of 50 ˚ C until the water content of ± 12%. Furthermore, the dried material was crushed to obtain a coarse powder. The extraction process was based on research conducted by Dewi (2012)[14] with some modifications. Dry powder that was boiled in boiling water and stirred with a ratio of 1 g powder in 10 ml aquades for 20 minutes. The first extract filtered by the filter cloth to separate the dregs. Furthermore, the dregs of first process was treated in the same case getting second extract, then both the extraction mixed. The process of making guava leaf extract can be seen in Picture 1.

The α-glucosidase inhibitor activity was determined using the method of Rao et al. (2009)[15] with some modifications. A sample of 200 µL were incubated in 2 ml of α-glucosidase from Saccharomyces cerevisiae (0,1 U/ml) in 0.1 M buffer phosphate (pH 6,8) for 10 min at 37˚ C. Furthermore, it was added some 1 mL of substrate (5mM p-nitrophenyl-α-D glucopyranosida in 0.1 M phosphate buffer pH 6.8) to start the enzymatic reaction. The kinetics of the release of p-nitrophenyl-α-D glucopyranoside measured by using spectrophotometry continuously for 5 minutes with 30-second intervals with on wavelength 405 nm. IC_{50} value of α-glucosidase inhibitor activity was determined based on the curve of the observation by the formula y = ax + b, with y indicates the percentage of the activity of α-glucosidase inhibitor.

The antioxidant activity was determined by using the 2,2-diphenyl-1-picrylhydrazil (DPPH) as used by Bothon et al. (2013).[16] A total of 2 mL of DPPH solution (6.10^{-5} M in methanol) was mixed with 0,25 mL of sample and ethanol until 8 mL of total volume. Furthermore, it was incubated in the dark for 30 minutes at 37 ˚ C. The absorbance was determined using a wavelength of 517 nm. The antioxidant activity was calculated by formulation:

$$\% \text{ Antioxidant Activity} = (Ac - As) / Ac \times 100\ \%$$

The data were then carried out using Lilliefors Test, homogenity use Bartlett's test and addition by Tukey's test**.** Furthermore, the data were analyzed by analysis of variance (ANOVA) to get the difference between treatments. Furthermore, the test data used the Least Significant Difference (LSD) on the real level of 1% and 5%.

RESULT

The results of absorbance values test for α-glucosidase inhibitor activity of guava leaf extract, cashew leaf extract, and combinations presented in Table 1. Based on the absorbance value, guava leaf extract has the highest activity of α-glucosidase inhibitor by 97.922% inhibition percentage.

IC_{50} test performed to the highest result for α-glucosidase inhibitor activity. The results of IC_{50} test on guava leaf extract presented in Table 2 and Picture 2. Picture 2 show the IC_{50} formula y = 83,682 x – 3,602, so to get 50% of the activity of α-glucosidase inhibitors are needed as much as 2.16 mg/ml of guava leaf extract.

Based on the antioxidant activity test, guava leaf extract was known to have a percentage of 97.006% activity. The result of absorbance values for antioxidant test of guava leaf extract, cashew leaf extract, and the combinations presented in Table 3.

Analysis of variance and LSD 5% on the antioxidant activity show that the treatment had no significant effect on the antioxidant activity of all samples tested. But the result of α-glucosidase inhibitor activity test of analysis of variance and LSD 5% showed that the activity of α-glucosidase inhibitor in guava leaf extract significantly different with cashew leaf extract but there was not significantly different with a combination of both.

Table 1: The *α-glucosidase inhibitor* activity absorbance value

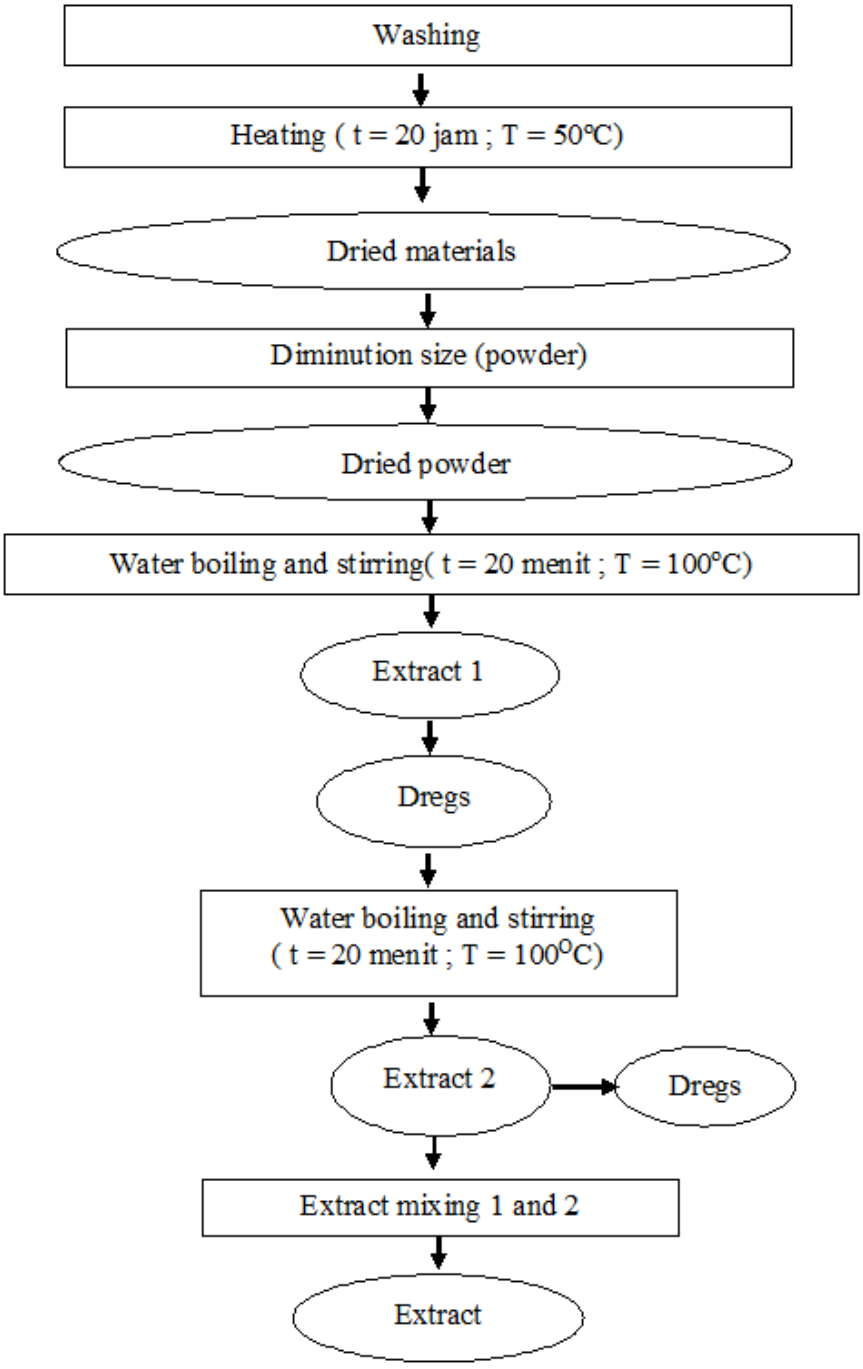
Materials	Repetition I	Repetition II	Repetition III
Control	2,028	2,032	1,991
Enzyme	0,071	0,081	0,066
Guava Leaf Extract	0,041	0,055	0,030
Cashe Leaf Extract	0,269	0,255	0,209
Combinations	0,030	0,037	0,062

Table 2: The IC_{50} *α-glucosidase inhibitor* activity absorbance value

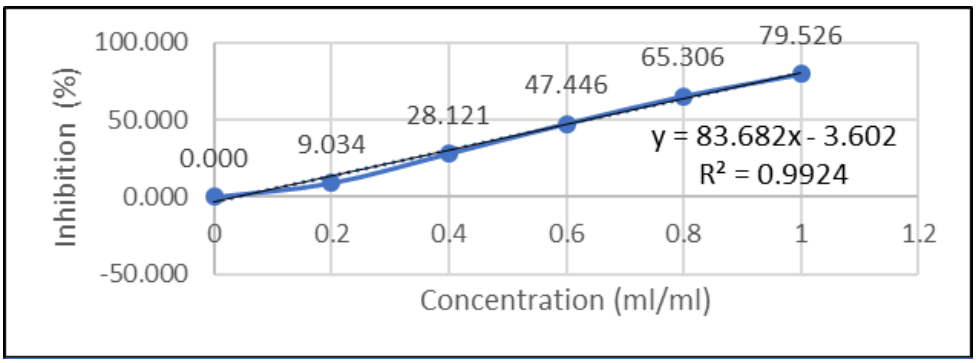
Concentration Acarbose (mg/ml)	Inhibition Acarbose (%)	Concentration Guava Leaf (ml/ml)	Inhibition Guava Leaf (%)
0	0	0	0
0,1	0,304	0,2	9,034
0,2	8,473	0,4	28,121
0,3	55,230	0,6	47,446
0,4	90,361	0,8	65,306
0,5	94,413	1	79,526

Tabel 3: Antioxidant activity absorbance value

Sample	Repetition I	Repetition II	Repetition III	Repetition IV
Control	0,652	0,734	0,726	0,752
Guava Leaf Extract	0,03	0,017	0,015	0,037
Cashew Leaf Extract	0,025	0,024	0,026	0,024
Combination	0,028	0,024	0,061	



Picture 1. Extraction Process



Picture 2: Value chart of IC_{50} *α-glucosidase inhibitor*

DISCUSSION

Diabetes Mellitus is a metabolic disorder with continuously increasing prevalence over years. Pharmacology therapy with minimum side effects still become a problem until today. Therefore, we should find drugs with minimum side effect, especially by using herbal as antidiabetic agent. One of them is the development of α-glucosidase inhibitor from guava and cashew leaf extract.[17]

In this study, the percentage of the activity of α-glucosidase inhibitor in guava leaf extract as 97.992% with 2.16 mg/ml IC_{50} values. The high activity of α-glucosidase inhibitors in this study are consistent with the results of antioxidant activity test that was also conducted with the percentage of 97.006% activity.

But the analysis of variance and LSD 5% test results of guava leaf extract and combinations showed no significant differences. This result showed that the usage of guava leaf extract and cashew leaf extract in combination did not cause synergistic or antagonistic effects.

The results obtained in this study are consistent with several studies that have been done before as the research conducted by Manikandan et al. (2013)[17] which analyzed the α-glucosidase inhibitor activity of guava leaf extract by in vitro method with the result 89,4% of percentage inhibition. Study for direct antidiabetic effects in rats using guava leaf extracts is supported by research conducted Wang et al. (2007)[18] to analyze the α-glucosidase inhibitor activity in small intestine of mice with the results of IC_{50} values of 1 g/L. The high activity of α-glucosidase inhibitors and antioxidants in guava leaf extract expected by quercetin effect. This is evidenced by Wang et al, (2010) [19] who has isolated the quercetin compound and analyzed the activity of α-glucosidase inhibitors with the results IC_{50} values by 3.5 mM. While the antioxidant activity of the extract of guava leaf evidenced by Lee et al. (2012)[20] using the essential oil of guava leaf that showed antioxidant activity with IC 50 values by 460,37 ± 1,33 µg/mL.

Test for glycated hemoglobin (HbA1c) levels used to determine glucose control in all types of DM. Glycated hemoglobin (HbA1c) describes the blood glucose level of patients over the past 4-8 weeks.21 The use of acarbose as α-glucosidase inhibitor have to reduce HbA1c levels by 0.5-0.8%. [3]

Oxidative stress plays an important role in the development of diabetes and its complications.22 Oxidative stress due to increase of free radical activity and significant decrease of antioxidant status in patients with diabetes. Antioxidant-rich foods can decrease oxidative stress and increase antioxidant status for patients with diabetics.23,24 Antioxidants, such as vitamin C (ascorbic acid) can prevent the accumulation of sorbitol in tissues and decreased production of AGEs so the numbers incidence risk of complications can be reduced. [25]

Based on the mechanism, the use of guava leaf extract as an antidiabetic agent so recommended because it has a percentage of the α-glucosidase inhibitors activity and antioxidants. So it does not only prevent the increase of blood glucose levels, but also reduce the risk complications in patients with DM.

CONCLUSION

Guava leaf extract has the activity of α-glucosidase inhibitor with by 97,992% percentage and IC50 values by 2.16 mg/ml and antioxidant activity amounted to 96,007%. There is no real synergestic or antagonist of guava leaf and cashew leaf in combinations.

REFERENCES

1. Powers AC. Diabetes Mellitus. In: Longo, Fauci, Kasper, Hauser, Jameson, Loscalzo, editors. Harrison's Principles of Internal Medicine. 18th ed. Jakarta: EGC; 2010. p. 2968–3001.
2. Achmad R, Agus Y, Alwi S, Asman M, Bowo P, Dharma L, et al. Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia. Jakarta: PB Perkeni 2015.
3. Soegondo S. Farmakoterapi pada Pengendalian Glikemia Diabetes Mellitus Tipe 2. In: Siti S, Idrus A, Aru WS, Marcellus SK, Bambang S, Ari FS, editors. Buku Ajar Ilmu Penyakit Dalam Edisi VI. 2nd ed. Jakarta: EGC; 2014. p. 2328–2335.
4. Kaku K. Etiopathogenesis and pathophysiology of type 2 diabetes. Japan Medical Association Journal 2010; 53(1):41–46. Hartono A. Terapi gizi dan Diet Rumah Sakit. 2nd ed. Jakarta: ECG; 2006.
5. Chisholm-Burns MA, Schwinghammer TL, Wells BG, Malone PM, Kolesar JM, DiPiro Jt, editors. Pharmacotherapy principles and practice. 3rd ed. New York: McGraw-Hill Companies; 2008. p. 649-657.
6. Ceriello A, Testa R. Antioxidant Anti-Inflamatory Treatment in Type 2 Diabetes. Diabetes Care 2009. 32 Suppl 2:232-6. DOI:10.2337/dc09S316.
7. Ojewole JA. Hypoglycemic and hypotensive effects of Psidium guajava Linn. (Myrtaceae) leaf aqueous extract. Methods Find Exp Clin Pharmacol (serial on the internet). 2005 Dec (cite 2016 Dec 20);27:689-695. Available from: https://journals.prous.com/journals/servlet/xmlxsl/pk_journals.xml_summaryn_pr?p_JournalId=6&p_RefId=948917.
8. Tachakittirungrod S, Ikegami F, Okonogi S. Antioxidant Active Principles Isolated from Psidium guajava Grown in

Thailand. Sci Pharm 2007; 75:179-93. DOI: 10.3797/scipharm.2007.75.179
9. Fagbohun TR dan Odufuwa KT. Hypoglycemic Effect of Methanolic Extract of Anacardium occidentale leaf in Alloxan-Induced Diabetic Rats. Nig J Physiology Sci 2010; 25:87-90.
10. Khare CP. Indian Medicinal Plants. India: Springer Science Business Media; 2007.
11. Sokeng S D, Lontsi D, Moundipa P F, Jatsa H B, Watcho, Kamtchoing P. Hypoglycemic Effect of Anacardium occidentale L . Methanol Extract and Fractions on Streptozotocin-induced Diabetic Rats. Global J Pharmacol 2007; 1(1):1–5.
12. Che CT, Wang ZJ, Chow MSS, Lam CWK. Herb-Herb Combination for Therapeutic Enhancement and Advancement: Theory, Practice and Future Perspectives. Molecules 2013; 18(5):5125-41. DOI: 10.3390/molecules18055125.
13. Dewi R. Aktivitas Antioksidan dan Sitotoksisitas Metabolit Sekunder Daun Salam (Syzygium polyanthum Wight.) dan Daun Jati Belanda (Guazuma ulmifolia Lamk.). Bogor: Institut Pertanian Bogor; 2012.
14. Ranga RR, Tiwari PP, Prabhakar RP, Suresh BK, Ali AZ, Madhusudana K dan Madhusudana RJ. New Furanoflavonoids, Intestinal α-glucosidase Inhibitory and Free Radical (DPPH) Scavenging, Activity from Antihyperglycemic Root Extract of Derris indica (Lam). Bioorg Med Chem 2009; 17(14):5170–5175. DOI**:** 10.1016/j.bmc.2009.05.051
15. Bothon FTD, Debiton E, Avlessi F, Forestler C, Teulade JC, Sohounhloue DKC. In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. BMC Complementary and Alternative Medicine 2013; 13:51. DOI**:** 10.1186/1472-6882-13-51
16. Manikandan R, Anand AV dan Muthumani GD. Phytochemical and in vitro anti-diabetic activity of methanolic extract of Psidium guajava leaf. International Journal of Current Microbiology and Applied Science 2013; 2(2):15-19.
17. Wang B, Liu HC, Hong JR, Li HG, Huang CY. Effect Of Psidium Guajava Leaf Extract On Alpha-Glucosidase Activity In Small Intestine Of Diabetic Mouse. Journal of Sichuan University Medical Science. 2007 Mar (cite 2016 Dec 21); 38(2):298-301. Available from: https://www.ncbi.nlm.nih.gov/pubmed/17441354.
18. Wang H, Du YJ, Song HC. α-Glucosidase and α-Amylase Inhibitory Activities of Guava Leaf. Food Chemistry. 2010 Nov (cite 2016 Dec 18); 123(1):6-13. Available from: http://www.sciencedirect.com/science/article/pii/S03088146 1000381X.
19. Lee WC, Mahmud R, Pillai S, Perumal S, Ismail S. Antioxidant Activities of Essential Oil of Psidium guajava L. Leaf. Elsevier: APCBEE Procedia 2012; 2:86-91. DOI: 10.1016/j.acpcbee.2012.06.016.
20. David E. Pankreas: Metabolisme Glukosa dan Diabetes Mellitus. In: Sylvia AP, Lorraine MW, editors. Patofisiologi Konsep Klinis Proses-proses Penyait Edisi VI Volume 2. 6th ed. Jakarta: EGC; 2014. p. 1264-25.
21. Giacco, Brownlee. Oxidative Stress and Diabetic Complications. Circulation Research 2010; 107: 1058-70. DOI: 10.1161/Circresaha.110.223545
22. Hajizadeh MR, Eftekhar E, Zal F, Jafarian A, Mostafavi-Pour Z. Mulberry leaf extract attenuates oxidative stress-mediated testosterone depletion in streptozotocin-induced diabetic rats. Iran J Med Sci 2014; 39(2):123-9. PMCID: PMC3957011.
23. Quilliot D, Walters E, Bonte JP, Fruchart JC, Duriez P, Ziegler O. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. Am J Clin Nutr 2005; 81(5):1117-25.
24. Fatmah AM, Siti BB, Zariyantey AH, Nasar A, Jamaludin M. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. Sultan Qaboos University Medical Journal 2012; 12(1): 5-18. PMCID: PMC3286717

Cite this article as:

Asep Sukohar *et al*. α-glucosidase inhibitor and antioxidant activity assays of guava leaf, cashew leaf and the combinations as antidiabetic agent. Int. J. Res. Ayurveda Pharm. 2017;8(Suppl 1):86-90 http://dx.doi.org/10.7897/2277-4343.08145

Source of support: Nil, Conflict of interest: None Declared