# The Effect of Binahong (*Anredera cordifolia* (Ten.) Steenis) Medicinal Plant Extract Addition on Glucose Detection

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***Abstract*—The potential of binahong (*Anredera cordifolia* (Ten.) Steenis) medicinal plant extract as glucose biosensor have been analyzed using cyclic voltammetry (CV) method. Glucose biosensor was prepared by modified electropolymerization technique of polypyrrole (PPy) to the active materials, such as binahong (*Anredera cordifolia* (Ten.) Steenis on the surface of gold electrode. Modified pyrrole polymerization was conducted at potential -1.3 to +1.3 V using voltammetry method with sweep rate 50mV/s for 30 cycles at pH 6.8. The performance of the modified sensor was tested in samples: glucose, urea, ascorbic acid and uric acid at the same concentration 10 mM, respectively. All samples were analysed using cyclic voltammetry method from -1.3 to +1.3 V with sweep rate of 50 mV/s in 0.1 M phosphate buffer at neutral condition (pH 7) room temperature. The best response of polypyrrole-binahong (*Anredera cordifolia* (Ten.) Steenis)-modified gold electrode was obtained during glucose measurement. No response detected from urea, ascorbic acid and uric acid. The result was proved that the modified electrode has a good potential for selective electrochemical sensor in determination of glucose.***

***Keywords*—Binahong, Biosensor, Cyclic Voltammetry, Glucose, Gold Electrode, Medicinal Plant.**

## I. INTRODUCTION

As we know, glucose is a compound that functions as the center of all metabolism that occurs in the human body [1]. Glucose has a function as a carbon source for synthesis to produce energy. If glucose cannot be converted into energy, glucose will be in the blood. Increased glucose concentration in the blood is influenced by the amount of carbohydrates consumed by a person. When the level of glucose in the blood increases, the pancreas produces insulin in response to the amount of glucose to control the level of glucose in the blood. Insulin is an important hormone consisting of A and B polypeptide chains, which consist of 21 and 30 amino acids, respectively [2]. The inability of the pancreas to produce enough insulin to control the concentration of glucose in the blood is called diabetes. Diabetes is a chronic medical condition in which levels of glucose in the blood are highly raised from the normal range. Diabetes remains a national health problem and ranks the fourth in the priority of national research on degenerative diseases. The high levels of glucose in the blood will eventually cause damage to many tissues in the body, including heart, eyes, kidneys and nerves, leading to painful and life-threatening health complications.

According to the International Diabetes Federation (IDF), in 2014, there were 387 million cases of diabetes in the world, of which 9 million were in Indonesia. There were two types of diabetes, type 1 and type 2. When the pancreas in the human body cannot produce insulin at all, it is called type 1 diabetes. The second type is type 2 diabetes, which occurs when the pancreas can still produce insulin but the amount is not enough to control glucose in the blood at normal concentrations [3]. Therefore, to obtain information relating to the type of diabetes from patients, it is important to have a method or instrument to measure insulin directly in the blood before trying medical treatment for diabetic patients.

Determination of insulin concentration has been investigated intensively because it can be used as a tool to help treat diabetic patients. The demand for diagnostic equipment that can detect the appearance of symptoms of diabetes is increasing. The increasing demand has led to the development of simple, accurate and non-invasive detection techniques.

Biosensor technology is an analytical device that is sensitive to biochemical compounds based on the many needs of the community to detect biochemical compounds in the body with the aim of knowing and anticipating the emergence of diseases caused by abnormal levels of biochemical compounds in the body [4]. There are several studies using medicinal plants in order to their benefit to healing a disease, especially to treat a wound. Highly interests from many researchers using the plants as medicine is caused by the assumption that the plant medicine is safer than synthetic drugs.

People commonly use medicinal plants to heal various diseases due to its cost is relatively low and easily accessible. Some medicinal plants have been scientifically studied the mechanisms of the anti-diabetic activity, including activity as α-glucosidase inhibitor, induces insulin secretion and improve insulin function [5]. One of the plants commonly used in the medicinal in Indonesia as anti-diabetic is binahong. Binahong (*Anredera cordifolia* (Ten.) Steenis) is a medicinal plant that is most frequently used to cure various kinds of diseases, such as to treat diabetes mellitus, rheumatic, uric acid, asthma, hypertension, lowering blood pressure, postpartum recovery, wound healing and post-circumcision operating, gastritis and cancer [6].

Binahong plant contains saponins, alkaloids, polyphenols, flavonoid and mono polysaccharide including L-arabinose, D-galactose, L-rhamnose, and D-glucose are among the most common components of the attached chains [7]. These plant also have high compound of flavonoid from leaves, stems, tubers and flowers may be efficacious as anti-microbe. As flavonoid has direct roles as antibiotic function have a broadspectrum target. Binahong leaves has antioxidant activity, ascorbic acid


Figure 1: (A) binahong (*Anredera cordifolia* (Ten.) Steenis) leaves; (B) binahong (*Anredera cordifolia* (Ten.) Steenis) leaves extract.

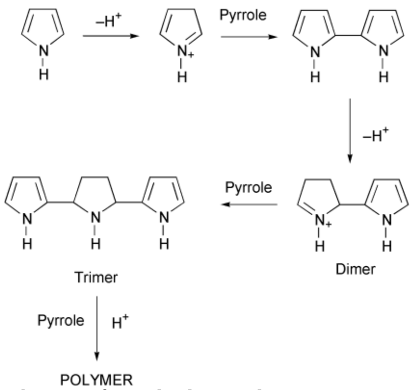


Figure 2. Mechanism of pyrrole electropolymerization.

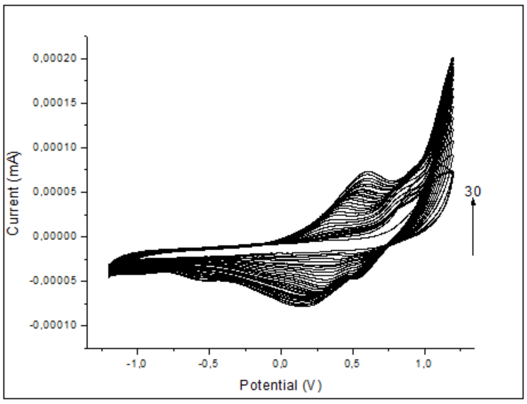


Figure 3. Voltammogram of pyrrole electropolymerization on the surface gold electrode.

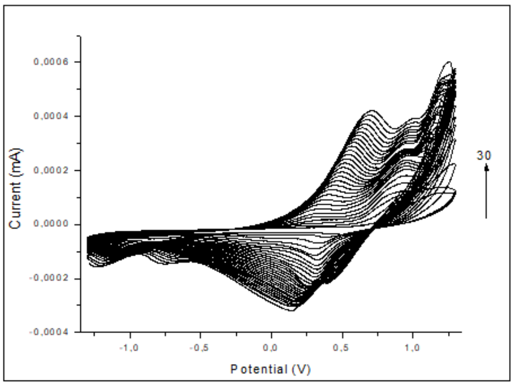


Figure 4. Voltammogram of polypyrrole-binahong leaves extract modified gold electrode.

and the highly of phenolic compound. The flavonoid in the leaves of binahong has an anti-inflammation effect, while saponin works as an antiseptic that can terminate or prevent the growth of microorganism in the wound to avoid an infection, increase the number of fibroblast cells, and stimulate the formation of collagen. A research conducted by [8] found that methanolic extracts of binahong (*Anredera cordifolia* (Ten.) Steenis) leaves at dosages of 50, 100, and 200 mg/kg BW were found to be able to decrease blood glucose. Saponin function as cleaners and antiseptics to prevent the growth of microorganisms that occur in the wound so that the wound did not experience severe infections other than the saponin is able to stimulate the formation of collagen I, which is a protein that plays a role in wound healing. Flavonoids contain lipofilik protein that will damage the membrane cell because flavonoid will damage the microbial cell wall membrane so the metabolites within the cell will be out, resulting in the death of microbes. Triterpenoids strengthen the endurance has the function of cells to infection and repair the damaged cells so the cells can regenerate properly. Alkaloids have the ability as an antibacterial by interfering the bacterial cell constituent components (peptidoglycan), so the layer of the cell wall are not fully formed and it caused the death of these cells. Vitamin C (ascorbic acid) in binahong (*Anredera cordifolia* (Ten.) Steenis) has a function to maintain the cell membrane, increase resistance to infection, and accelerate wound healing. Moreover, vitamin C acts as an antioxidant to activate prolil hydroxylase enzymes that support hydroxylation step in the formation of collagen in wound healing.

Determination of glucose is essential due to its clinical and industrial importance as well as in biosensor applications. Rapid determination of blood sugar is very important for treatment and control of diabetes. In particular, glucose is of special importance because of its involvement in human metabolic process. Numerous efforts were devoted to develop a glucose biosensor with fast and accurate response. Many researcher reported the electrochemical glucose sensors both in enzymatic or non-enzymatic form. Another promising material for development of nonenzymatic electrochemical sensors for glucose is gold. Another approach for development of gold-based glucose sensors is the immobilization of gold on a conductive carrying substrate. First attempts for using this approach were recently reported. The mechanism of electrochemical oxidation of glucose on gold electrodes was studied in. But this problem can be successfully hurdled by the enzyme immobilization technique which normally have a short-life due to properties of the enzyme, very easy to break.

Conducting polymers are being widely used in biosensor applications because they provide stable and porous matrices for biocomponent immobilization and they also facilitate the electron transfer process. The popular conducting polymers for immobilizing enzyme are polypyrrole [9], polyaniline [10], polythiophene [11], and their advantages for fabricating the glucose biosensor. The conducting polymers performed through electropolymerization are today one of the green alternatives to the protection of metals against corrosion. Polypyrrole (PPy) is one of the most commonly investigated polyconjungated conducting polymers due to lower oxidation potential of its monomer and high stability. The oxidation of pyrrole has been carried out by: (a) electropolymerization at a conductive material by the application of potential or (b) chemical polymerization in

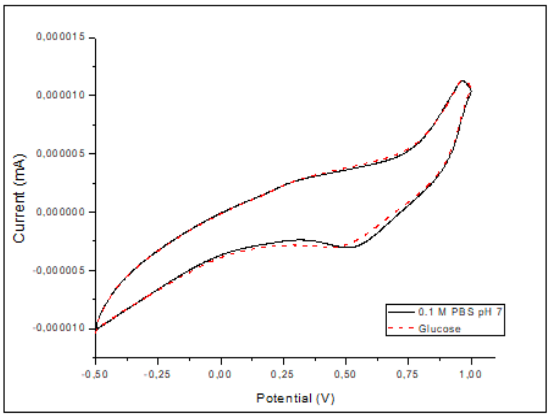


Figure 5. Voltammogram of gold electrode in the presence of glucose.

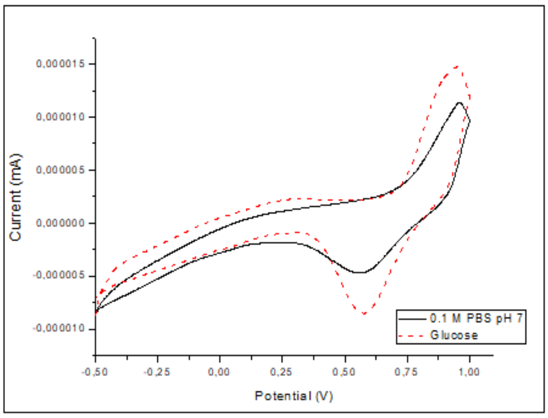


Figure 6. Voltammogram of modified gold electrode/Ppy in the presence of glucose.

solution by the use of a chemical oxidant [12]. The reasons for this intense focus on polypyrrole certainly lie in the fact that the monomer (pyrrole) is easily oxidized, water soluble and commercially available. Hence, polypyrrole presents several advantages including environmental stability, good redox properties and the ability to give high electrical conductivities

Electrochemical sensors for the measurement of analytes both in medical and industry analysis are ideally suited for these new application, due to their high sensitivity and selectivity, portable field-based size, rapid response time and low-cost. In the present study, we develop and use the extract of binahong (*Anredera cordifolia* (Ten.) Steenis) extract to modify the gold electrode as glucose biosensor. The addition of active material, such binahong (*Anredera cordifolia* (Ten.) Steenis) extract aims to improve the perfomance of the modified electrode. The performance of modified electrode was investigated in glucose, urea, ascorbic acid and uric acid. All measurement was analyzed using cyclic voltammetry method under neutral condition, in 0.1 M phosphate buffer.

## II. METHOD

### *A. Chemical and materials*

Binahong leaves was obtained Surabaya, East Java, Indonesia. L(+)-Ascorbic acid [$C_6H_8O_6$], D(+)-Glucose [$C_6H_{12}O_6$], Urea [CO(NH)2), 99.5%], ethanol [$C_2H_5OH$], di-sodium hydrogen phosphate [$Na_2HPO_4$], and sodium dihydrogen phosphate monohydrate [$NaH_2PO_4.H_2O$] were purchased from Merck KGaA (Darmstadt, Germany) and used without any purification. Uric acid

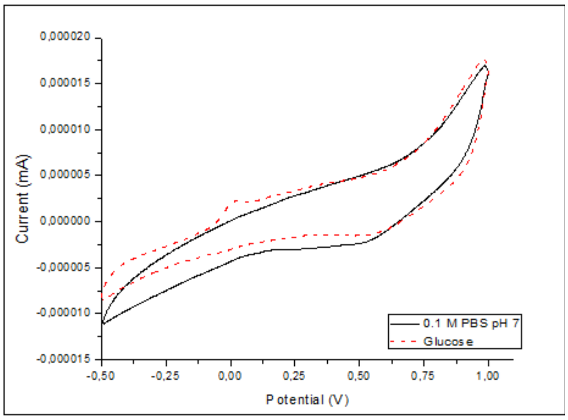


Figure 7. Voltammogram of modified gold electrode/Ppy/binahong leaves extract in the presence of glucose.

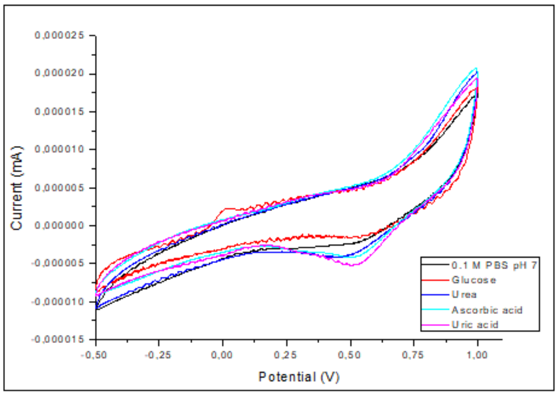


Figure 8. Voltammogram of modified gold electrode/Ppy/binahong leaves extract modified gold electrode in the presence of glucose, urea, ascorbic acid (AA) and uric acid (UA) in 0.1 M phosphate buffer (PBS) pH 7.

[$C_5H_4N_4O_3$, 99.0%] and potassium chloride were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and used without any purification. Pyrrole was purchased from Sigma Aldrich and purified prior to use by distillation technique. Sandpaper grade 1500, aluminum foil, Whatman 41 filter paper and shrinkage cable were bought from local market Surabaya, Indonesia. Gold 24K was purchased from Antam, Indonesia. All chemicals were used as purchased without further purification unless mentioned. Demineralized water was used for chemical preparation and cleaning.

### *B. Instrumentation*

Heat gun was used to heat the shrinkage cable for fabrication of gold electrode. Ultrasonic bath was used for preparing solution. All the electrochemical experiments were performed using eDAQ (potentiostat E161 and e-corder 410, equipped with e-chem software version 2.0.1). Three-electrode cell system with platinum as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference electrode (RE) and working electrodes (WE) were: gold, modified gold electrode/ and modified gold/Ppy/binahong leaf extract. Experiments were employed under voltammetry cyclic method. General laboratory glasswares were used during all chemical preparation.

### *C. Preparation of Binahong (Anredera cordifolia (Ten.) Steenis) Leaves Extract*

Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) as shown in Figure 1A was obtained from Surabaya, East Java, Indonesia. Binahong leaves was washed and dried at air for 5 days then left in the open air and protected from sunlight. Furthermore, the dry leaves were ground gently using a blender. A total of 10 grams of simplicia powder from binahong (Anredera cordifolia) leaves weighed and put into a maceration container. 200 mL of ethanol was added and then closed tightly until homogeneous. The solution was then macerated at 7 days, as described by [13]. After a certain time, the solution was filtered using Whatman 41 filter paper to obtain the binahong leaves extract (Figure 1B). The extract solution was evaporated using a rotary evaporator at a temperature of 40°C to obtain a condensed binahong leaves extract. Furthermore, the extract was kept in refrigerator prior to use as the electrode activde material for further experiments.

*D. Preparation of gold electrode*

Gold electrode with diameter of 1 mm and 5 cm in length was used as working electrode. The electrode then washed and dried using ethanol and demineralized water respectively. The clean gold electrode was prepared by as from our previous work.

*E. Preparation of Modified Gold electrode/Ppy/binahong Leaves Extract*

The modified gold electrode was prepared by adding of active material (binahong leaves extract) and pyrrole in 0.1 M KCl as electrolyte solution. All materials with composition 1:1 were mixed until homogeneous under sonication condition. The mixed material was attached to the surface of the gold electrode by electropolymerization technique.

*F. Electropolymerization of Modified Gold electrode/Ppy/ binahong Leaves Extract*

Electropolymerization on the surface of gold electrode was carried out using Pt and Ag/AgCl cyclic voltammetry method at potential -1.3 V to +1.3 V and scan rate 50 mV/s for 30 cycles. The pH was adjusted at 6.8 before electropolymerization performed. Trapping of active material, binahong (*Anredera cordifolia* (Ten.) Steenis) in polypyrrole on the surface of modified gold electrode was performed using the same technique and condition as the pyrrole polymerization that was described before.

*G. Electrochemical Measurement*

All electrochemical measurements were performed using three-electrode cell system which platinum as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference electrode (RE), polypyrrole modified gold electrode, polypyrrole/binahong leaves extract modified gold electrodes as working electrodes (WE). Voltammetry technique was done at potential range -1.3 V to +1.3 V using scan rate 50 mV/s for 10 cycles to all samples. Glucose, urea, ascorbic acid and uric acid were prepared in 10 mM under neutral pH condition (0.1 M of PBS, phospate buffer), respectively. All samples were measured to analyze the selectivity of the modified electrode obtained.

## III. Result and Discussion

*A. Electropolimerization of Pyrrole*

Electropolimerization is a convenient method for the synthesis of conductive polymer films on electrode and is applicable to the preparation of electronic devices such as electrochromic displays, rechargeable batteries, transistors and capacitors. Pyrrole is known to be easily electropolymerized to yield a polypyrrole (PPy) film with high conductivity in electrolyte solution, such as potassium chloride.

Figure 3 shows successive cyclic voltammograms corresponding to pyrrole in potassium chloride electrolyte solution (0.1 M) solution and recorded in a potential range between -1.3 to 1.3 V with scan rate of 50 mV/s and 30 cycles. The current is increased gradually for further cycles. It shows that the polypyrrole layers have formed on the surface of gold electrode successfully. There are two peaks observed, both anodic and cathodic sweep. Anodic peak observed at +0.6 V with current responses from 0.00003 to 0.00008 mA The cathodic peak was found at +0.15 V with current responses from -0.00008 to -0.00007 mA, respectively. The anodic and cathodic waves correspond respectively to oxidation and reduction of the polypyrrole.

The oxidation wave is seen to shift slowly towards higher voltages with repeated scans, whereas the reduction wave is observed to shift to even lower negative voltages. These shifts are accompanied by an increase in the current intensity. It is worth nothing the increase in the voltage difference range with increasing the number of cycles, which is a clear indication of the deposition of polypyrrole film on the electrode surface. A slight decrease of the current intensities of the anodic and cathodic peaks was observed. This is probably due to the inhibition of the surface by an insulating polymeric film which was carried out on the electrode. A study by [12] also suggested that the current intensity of the anodic peak decreases during cycling and it stabilizes after many cycles when the electrode surface is coated by an insulating film. However, the anodic and cathodic peak potentials were observed to remain the same, indicating that the kinetic process of both the oxidation and the reduction reactions remains unchanged during cycling [14].

The electrochemical signal obtained is relatively low, and there is difference of electrochemical signal in the presence and in the absence of active material from binahong leaves extract. Electropolymerization was carried out at pH 6.8 in order to obtain conductive polymers. Polypyrrole has to form head to tail coupling, where this is only occurred in low acidic conditions. In more basic condition head to head coupling will take place, and as the result will form non-conjugated polymer which has nonconductive properties. The mechanism electropolymerization of pyrrole shown in Figure 3.

Voltammogram of polypyrrole modified gold electrode in the presence of binahong leaves extract in 0.1 M potassium chloride electrolyte solution shown at Figure 4. Voltammogram shows the current decrease for further cycles. Anodic peak observed at +0.71 V with current responses from 0.0001 to 0.0004 mA The cathodic peak was found at +0.17 V with current responses from -0.0003 to -0.00018 mA, respectively. The anodic and cathodic waves correspond respectively to oxidation and reduction of the modified electrode with binahong leaves extract addition. Modification was continued by trapping of binahong leaves extract on the surface of polypyrrole modified gold electrode. Trapping was done in binahong leaves extract solution which is described in experimental section. A small amount of pyrrole which remains in the surface of polypyrrole modified gold electrode from previous polymerization process will be polymerized.

Electropolymerization in a small amount pyrrole aims to trap binahong leaves extract only on the surface of polypyrrole modified gold electrode. Range pH 6.8-7 was chosen according to pH stability of pyrrole, because in the low pH will be denaturized. Even though the polypyrrole formed at pH 6 is non-conductive layer, but we already have a conductive layer from previous pyrrole polymerization. If we keep non-conductive layer as thin as possible, the electrode should work as glucose sensor. The voltammogram shows that the current is decreased gradually for further cycles because no more pyrrole available in the solution can be polymerized. The current is decreased gradually after 30 cycles. Last step, polypyrrole-binahong leaves extract modified gold electrode was attached in gold solution which was described at experimental section. Gold electrode were deposited using cyclic voltammetry technique. The technique was conducted in three times. The electrode was cleaned at room temperature before the next step to keep stability of deposited gold in the surface of polypyrrole-binahong leaves extract modified gold electrode. More immersion will give thicker gold, but the stability of the gold nanoparticle layer will be decrease. As the result, the gold layer will peel off from the surface of polypyrrole-binahong leaves extract modified gold electrode.

### *B. Characterization of electrode*

Voltammogram of polypyrrole/binahong leaves extract modified gold electrodes for 30 cycles in 0.1 M KCl solution using scan rate 50 mV/s can be seen at Figure 3. The current is increased gradually for further cycles. It means the polypyrrole layers have formed on the surface of gold electrode successfully. The electrochemical signal of polypyrrole/binahong leaves extract modified gold electrode is higher than in the absence of binahong leaves extract. Addition of active material, such binahong leaves extract gives higher signal.

The electrochemical signal of polypyrrole-binahong leaves extract modified gold electrode in the present of glucose is higher than in the absence of glucose. Addition of binahong leaves extract in polypyrrole has converted glucose to an electroactive species. Binahong leaves extract can be oxidized and the oxidized product also can be reduced again. Even though the redox reaction can take place, but the voltammogram shows a sloping shape without peak that indicates a slow redox reaction rate. The electrochemical signal is also relatively low in comparison to voltammogram of polypyrrole-binahong leaves extract gold modified gold electrode.

Different response from electrochemical sensor based on gold electrode, polypyrrole-modified gold electrode, polypyrrole-binahong leaves extract modified gold electrode was found during glucose measurement at pH 7. Oxidation and reduction reaction of glucose was disappeared as shown in Figure 5 and Figure 6, respectively. Glucose oxidation and reduction peak at polypyrrole-binahong leaves extract-gold modified gold electrode were observed at potential +0.53 V and -0.3 V respectively. The oxidation peak of polypyrrole-binahong leaves extract-gold modified gold electrode shifts to more positive and the reduction peak shifts to more negative with glucose concentration. Figure 7 is voltammogram of polypyrrole-binahong leaves extract-gold modified gold electrode in the presence of glucose after corrected by the blank. The voltammogram shows clear increment with glucose concentration. It means that polypyrrole-binahong leaves extract-gold modified gold electrode can be used for glucose detection. Electrochemical signal at all these potential can be used for glucose detection

### *C. Selectivity of the Polypyrrole Modified*

Glucose analysis in biological liquids is performed mainly in blood or urine. Most physiological components of these liquids are not electrochemically active in the potential range used in the glucose sensor. Selectivity of polypyrrole-binahong leaves extract-gold modified electrode for glucose detection was studied in the presence of urea, ascorbic acid and uric acid in. The voltammogram of polypyrrole-binahong leaves extract- gold modified gold electrode in the presence of 10 mM glucose, urea, ascorbic acid and uric acid as can be seen at Figure 8.
The electrochemical signal of urea, ascorbic acid and uric acid has usual monotonous shape; the current is almost zero. The concentrations of glucose in blood and urea are respectively 3 – 8 mM and below 0.5 mM while these values for urine are respectively below 0.65 mM and 1.2 – 6 mM [22]. Comparing these values with the experimental data (Fig. 9), we conclude that uric acid should not interfere the measurements in bloodOnly the glucose gives positive electrochemical signal. It indicates that polypyrrole-binahong leaves extract-gold modified gold electrode has good selectivity for glucose detection.

## IV. CONCLUSION

The performance of polypyrrole-binahong leaves extract modified gold electrode was successfully studied for glucose detection. The modified electrode gives the best response during glucose measurement under neutral condition (pH 7) in room temperature. Glucose was oxidised and reduced at potential of +0.53 V and -0.3 V respectively. This response not interfered by the presence of urea, ascorbic acid and uric acid.

## ACKNOWLEDGEMENTS

The authors would like to thank to all member of Pusat Kajian Halal LPPM – ITS for their immense help and support during the research.

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