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# Quantitative and Qualitative Phytochemicals Analysis of *Muntingia calabura*

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**Abstract**
*Muntingia calabura* is widely cultivated and has become one of the common roadside trees in Indonesia. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of plant *Muntingia calabura* in some solvents (water, methanol, ethanol, chloroform, ether, and citrid acid). Phytochemical screening method was used to identify qualitative analysis of bioactive component. While the quantitative analysis was analysed by UHLPC. The results showed the highest percentage extract concentration was resulted by methanol followed by water, ethanol, chloroform, ether and citric acid 13.15%, 11.93%, 9.57%, 9.04%, 4.52% and 3.69% respectively. The bioactive component ie., saponins, flavonoids and tannin were found in all those solvent. The higher concentration of flavonoid, saponin and tannin were resulted from polar solvent ie., water, methanol and ethanol. While non polar solvent has lower in concentration. Epigallocatechin Gallate (EGCG) and Genistein content of extract *Muntingia calabura* from methanol solvent were 135.15 µg/g and 136.29 µg/g respectively.
**Keywords:** Phytochemical constituents, *Muntingia calabura,* qualitative and quantitative analysis.

## 1. Introduction

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Okwu, 2001). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Prusti, 2008). Discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action becomes an urgent attention currently. The development of resistant strains of bacteria has increased the need for new antibiotics (Eloff, 1998). Higher plants, which are able to produce photosynthesis, produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms (Harborne, 1990). These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Sarac and Ugur, 2007). There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of those extracts that yielded active principles (Rabe and Van Staden, 2000; Palombo and Semple, 2001; Portillo et al., 2001; Srinivasan et al., 2001; El-Seedi et al., 2002).
*Muntingia calabura* is widely cultivated and has become one of the common roadside trees in Indonesia. It is known locally in Indonesia as 'Kersen. It is native to the American continent and is widely cultivated in warm areas of Asian region (Chin, 1989). Various parts of this tree have several documented medicinal uses in both Southeast Asia and tropical America (Kaneda et al., 1991; Nshimo et al., 1993). The roots have been employed as an emmenogogue in Vietnam and as an abortifacient in Malaysia. In the Philippines, the flowers of this species have been used to treat headaches, and as an antidyspeptic, antispasmodic and diaphoretic. Infusions of the flowers of this plant are drunk as a tranquillizer and tonic in Colombia (Kaneda et al.,1991). Therefore, the main objective of this study is to find the bioactive constituents of plant *Muntingia calabura* in some solvents which could serve as a good candidate for the development of new antimicrobial agents.

## 2. Material and Method

### *2.1 Collection of plant*

Fresh plant leaves of *Muntingia calabura* were collected from Sawiran Village, Tutur Sub District, Pasuruan Regency, East Java Province, Indonesia. The leaves was cleaned thoroughly with normal tap water followed by sterile distilled water. The leaves were dried under shaded condition at room temperature, then the leaves were dried in an oven at 60°C for 24 hrs. Leaves were crushed to powder using grinding machine. Powder were storage at 4°C in sealed bottle.

 

*2.2 Extraction*

50 g of the powdered material were extracted first with water and then the residues were further extracted with rotary evaporator. Same procedure was repeated for methanol, ethanol, chloroform, ether, and citric acid with same type of repeated residues. The extracts were evaporated to dryness on a water-bath. The plant extracts were distilled off with distillation apparatus and yielded quantities of leaves extracts in different solvents were obtained and were further taken to evaluate the phytochemical studies.

*2.3 Phytochemical analysis*

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Harborne (1998).

*2.4 Quantitative analysis of phytochemical constituents*

*Tannins*

1 gr of extract sample of *Muntingia calabura* is boiled in 20 ml of distilled water in a test tube and few drops of 10% NaCl and FeCl3 were added to the filtered samples. Formation of brownish green or a blue black colouration was indication presence of tannins.

*Saponins*

5 gr of extract sample of *Muntingia calabura* was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

*Flavonoids*

1 gr of extract sample of *Muntingia calabura* was shaken vigorously with 2 ml of methanol 50% in a test tube then few drops of Mg and 0.5 ml HCl were added. A yellow coloration is observed if flavonoids compound are present. The formation of red or purple coloration was taken as a positive test for flavonoids

*2.5 Determination of Epigallocatechin gallate (EGCG) and Genistein by UHLPC*

UHPLC was used to identify the constituent of epigallocatechin gallate (EGCG) and genistein of *Muntingia calabura.* Genistein and EGCG standards were purchased from Sigma-Aldrid (St.Louis, ISA). Calibration standard at 200, 400, 600 and 800 mg/L were prepared by serial dilution of the stock solution. Extract *Muntingia calabura* from water solvent was examines for EGCG and genistein content. 0.1525 gr of extract was placed in 20 mL of methanol, then it was sonificated for 5 minute. Added 25 mL of 1 M formic buffer combined with 1 ml aquabidest and analyzed directly by UHPLC.

## 3. Result and Discussion

The results for extraction process of *Muntingia calabura* from various solvent showed in Table 1. Base on the results in Table 1, polar solvent (water, methanol and ethanol) has higher in the extract and % rendement compare to non polar solvent (cloroform, ether, citric acid). The highest value was resulted by methanol, following by water, ethanol, chloroform, ether and citric acid. The phytocemical characteristics of *Muntingia calabura* are summarize in Table 2.

**Table 1.** Extract *Muntingia calabura* from various solvent

| No | Solvent | Powder (gr) | Extract (gr) | Rendement (%) |
|---|---|---|---|---|
| 1 | Water | 50 | 5.964 | 11.928 |
| 2 | Methanol | 50 | 6.575 | 13.150 |
| 3 | Ethanol | 50 | 4.787 | 9.572 |
| 4 | Chloroform | 50 | 4.518 | 9.036 |
| 5 | Ether | 50 | 2.261 | 4.522 |
| 6 | Citric acid | 50 | 1.845 | 3.690 |

The bioactive component ie., saponins, flavonoids and tannin were found in all solvent. The higher concentration of flavonoid, saponin and tannin were resulted from polar solvent i.e., water, methanol and ethanol. While non polar solvent has lower in concentration. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari et al., 2011). The results also showed that flavonoid has higher concentration compare to saponin and tannin in all solvent. The results reveal the presence of bioactive constituent like saponin, tannin and flavonoid were present in this plant although they have different at concentration. Flavonoids are well documented for the biological effects including antimicrobial and anticancer. They have been found in vitro to be effective antimicrobial and anticancer compounds against a wide array of microorganism and cancer cell (Harbone and Williams, 2000; Ren et al., 2003). Bioactive constituent have been reported to be responsible for medical herbs in Chinese and Japanese (Njoku and Oby, 2009). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds have also been confirmed to have antimicrobial activity (Alagesaboopathi et al, 2011; Kevit et al., 2012). Hence it could be concluded that the plant extracts

 

could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

**Table 2.** Phytocemical analysis of extract *Muntingia calabura* leaves from various solvent

| No | Solvent | Bioactive constituents of *Muntingia calabura* | | |
|---|---|---|---|---|
| | | Saponin | Tannin | Flavonoid |
| 1 | Water | ++ | ++++ | ++++ |
| 2 | Methanol | ++ | ++++ | ++++ |
| 3 | Ethanol | ++ | ++++ | ++++ |
| 4 | Chloroform | + | ++ | +++ |
| 5 | Ether | + | ++ | +++ |
| 6 | Citric acid | + | ++ | +++ |

The bioactive constituent that has been found in *Muntingia calabura* leaves are epigallocatechin gallate (EGCG) and genistein. Both of those constituents are part component of catechin. Catechin are powerful antioxidants founds in tea that are thought to provide several of these health benefits. The most abundant catechins in tea products include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG). Based on the research was conducted in 2001, EGCG became the most abundant constituent of cathechin (Dionex, 2001).

Analysis of EGCG and genistein performed with a standard solution preparation stages, i.e., used a standard solution of epigallocatechin gallate (EGCG) and genistein, absorbance determination of EGCG and genistein and the calibration of measurement results with a standard has been made. Standard solution used was a compound epigallocatechin gallate (EGCG) and genistein at concentrations of 200, 400, 600 and 800 mg.L-1 respectively. Each concentration was added 25 mL of 1 M formic buffer combined with 1 ml aquabidest. The measurement results will be showed by absorbance area. Based on the absorbance area, the calibration curve can be made (Figure 1 and 2).

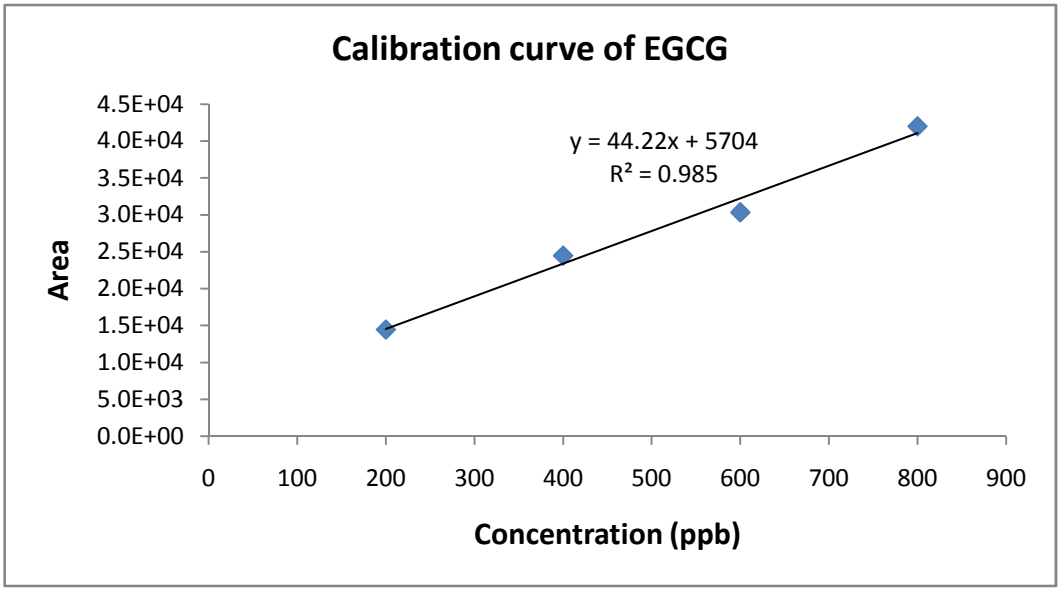


**Figure 1.** Calibration curve of epigallocatechin gallate (EGCG)

 

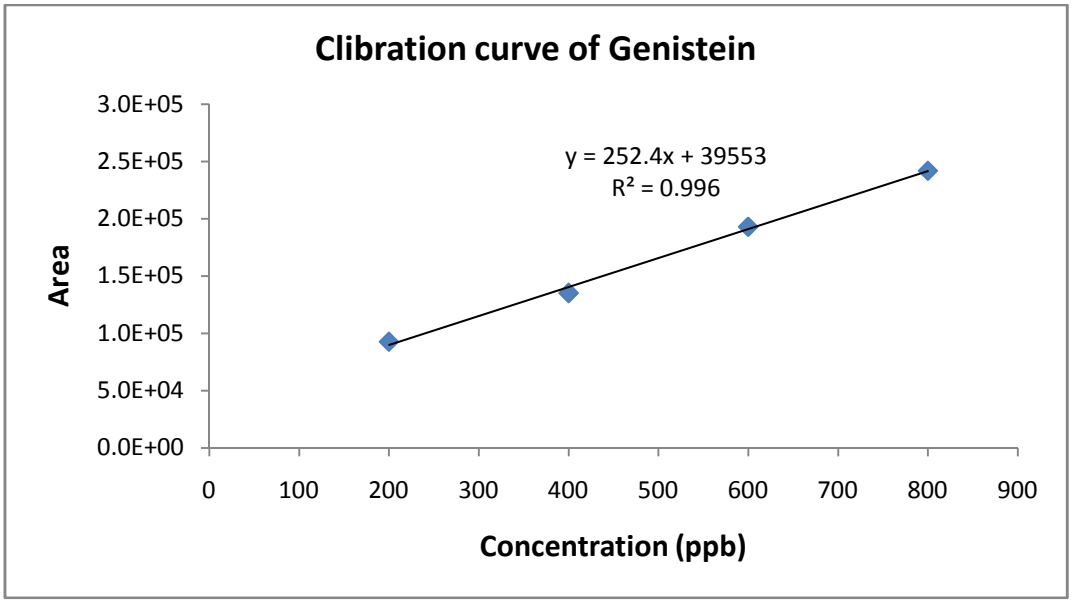


**Figure 2.** Calibration curve of genistein

The results showed that absorbance standard solution of EGCG and genistein has linear correlation with concentration, r = 0.99 and 0.98 respectively. The linear equation for those both EGCG and genistein are y = 252.4 x + 39553 and y = 44.2 x + 5704. The equation will be used to count the concentration of standard solution in each level. The results are shown in table 3 and 4.

**Table 3.** EGCG results from standard solution at different level concentration

| No | File name | Absorbance area | Concentration (ngr/gr) | Counted concentration (ppb) |
|---|---|---|---|---|
| 1 | Standard 1 | 14.463 | 200 | 197.99 |
| 2 | Standard 2 | 24.469 | 400 | 424.16 |
| 3 | Standard 3 | 30.340 | 600 | 556.87 |
| 4 | Standard 4 | 41.987 | 800 | 820.14 |

**Table 4.** Genistein results from standard solution at different level concentration

| No | File name | Absorbance area | Concentration (ngr/gr) | Counted concentration (ppb) |
|---|---|---|---|---|
| 1 | Standard 1 | 92.834 | 200 | 211.10 |
| 2 | Standard 2 | 135.347 | 400 | 379.53 |
| 3 | Standard 3 | 193.064 | 600 | 608.21 |
| 4 | Standard 4 | 241.909 | 800 | 801.71 |

Results for absorbance area of EGCG and genistein from extract *Muntingia calabura* were 52.668 and 52.558, while the counted concentration for both constituent were 136.29 µg/g and 135.15 µg/g. respectively. The amount of both constituent is lower than EGCG and genistein which is found in various tea like white tea, green tea, black tea, 42.6 mg/g, 30.7 mg/g and 12.3 mg/g respectively. This study also find out that EGCG as a part of catechin has higher value compare to genistein, this research is inline with (Dionex, 2001; Saito et al., 2006) which reported that EGCG in various tea has higher compare to other catechin component. The composition of catechin varies based on the species, season, horticultura condition and the most importantly, the degree of oxidation during the manufacturing. In addition, EGCG and genistein as composition of catechin are powerfull antioxidant, therefore it can be used as a one of antimicrobial agent.

## 4. Conclusion

The extract of leaves of *Muntingia calabura* provides evidence that *Muntingia calabura* contains important bioactive compounds and their potencial as a source of the use of plant species as antimicrobial activity.

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