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# Research Article
# *In vitro* Antioxidant Profiles and Phytochemical Content of Different Organs of Strawberry (*Fragaria ananassa* Duchesne)

Irda Fidrianny, Fadhila Syifa and Muhamad Insanu

Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Indonesia

## Abstract

**Background and Objectives:** Antioxidants are able to stabilize or eliminate free radicals before they attack the cells. Antioxidant compounds such as flavonoid substances are essential for maintaining optimum cellular work. Phenol and flavonoid compounds are widely contained in plants, included in strawberry. The purposes of this research were to compare antioxidant profiles from different organs of strawberry using two antioxidant testing methods 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Cupric reducing antioxidant capacities (CUPRAC) and also its phytochemical content. **Materials and Methods:** Antioxidant activities, total phenolic content (TPC) and total flavonoid content (TFC) were conducted by UV-Vis spectrophotometry. Correlation of TPC and TFC with their $IC_{50}$ of DPPH and $EC_{50}$ CUPRAC were analyzed by Pearson's method. **Results:** All different organs extracts of strawberry exposed $IC_{50}$ of DPPH varied from 0.22-10.14 μg $mL^{-1}$ and $EC_{50}$ of CUPRAC from 130.42-250.14 μg $mL^{-1}$. Ethanol stem extract gave the highest TPC 18.62 g gallic acid equivalent (GAE) 100 $g^{-1}$, while ethyl acetate leaves extract showed the highest TFC 7.40 g quercetin equivalent (QE) 100 $g^{-1}$. The TPC in fruit and leaves extract were significantly negative correlation with their $IC_{50}$ DPPH ($r = -0.865$; $r = -0.970$, $p<0.01$). **Conclusion:** All different organs extracts of strawberry were very strong antioxidant by DPPH method. Waste products of strawberry (leaves and stem) had antioxidant potential. The major contributor in antioxidant activities of fruit and leaves extracts by DPPH assay were phenolic compounds. Only strawberry fruits extract showed linear results in DPPH and CUPRAC assays.

Key words: Antioxidant potential, strawberry organs, stem extract, waste product, DPPH, CUPRAC

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Corresponding Author: Irda Fidrianny, Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Indonesia



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Production of reactive oxygen species (ROS) increases simultaneously with lifestyle the society. The excessive of free radical is closely related with many degenerative diseases such as diabetic, hypercholesterolemia, atherosclerosis cardiovascular[1].

Consumption of fruits and vegetables rich phenolic and flavonoid compounds had been suggested to prevent development in degenerative diseases such as cancer and heart diseases[2,3]. Exogenous antioxidant namely as natural antioxidant which can be from foods, vegetables and fruits, included strawberry can prevent the excessive of free radical.

Strawberry (*Fragaria ananassa* Duchesne) belong to Rosaceae family contained many phenolic and flavonoid compounds such as anthocyanin, hydroxycinnamic acid, flavonol, flavan-3-ol, ellagic acid, ellagitannin, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, cyanidin-3-O-glucoside, quercetin, quercetin-3-O-glucoside, kaempferol-3-O-glucoside[4-7]. Many previous researches published that flavonoid and phenolic compounds had anti-cancer[8], antimicrobial[9], anti-inflammatory[10] and antioxidant activity[11-15].

Previous researches stated that phenolic compounds linked with its antioxidant capacities of fruits[16-18]. Several studies revealed that strawberry have antioxidant activity which was related with its phenolic content[19,20] and its antioxidant activity around 2 to 11-fold of antioxidant activities of grapes, apples and oranges[21-22]. Research regarding comparison between maceration using hydromethanol solvent, infusion and decoction of strawberry roots have been done by Dias *et al.*[23], but comparison between three organs of strawberry using three polarities solvent have not been reported yet. Leaves and stem were the waste products of strawberry, which might have similar antioxidant potential with their fruit.

The goals of this study were to compare antioxidant activity by DPPH and CUPRAC methods, phenolic and flavonoid content of among three polarities extracts of different organs of strawberry, then analyze a correlation of their chemical content and antioxidant activities.

## MATERIALS AND METHODS

**Chemicals:** 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproine, gallic acid and quercetin were purchased from Sigma-Aldrich (MO, USA). Other chemicals used were analytical grade.

**Collection of sample:** Three organs of strawberry were fruits namely as FRT, leaves as LEV and stem as STM. Strawberry was collected from Lembang, West Java-Indonesia, identified in Herbarium Bandungense-School of Life Science and Technology-Bandung Institute of Technology and stated as strawberry (*Fragaria ananassa* Duchesne). Sample was selected, washed, dried and grinded into powder.

**Preparation of extraction:** Different polarity solvents were used to extract each sample. Extraction was done triplicate by reflux for each solvent. Sample 300 g was extracted using n-hexane, ethyl acetate and ethanol, consecutively. There were three n-hexane extracts (namely FRT1, LEV1 and STM1), three ethyl acetate extracts (FRT2, LEV2 and STM2) and three ethanol extracts (FRT3, LEV3 and STM3).

***In vitro* antioxidant activities by DPPH assay:** Each extract was prepared in various concentrations. Ascorbic acid was used as standard, methanol as a blank and DPPH 50 μg $mL^{-1}$ as control. Antioxidant activity by DPPH assay was conducted using modification of Blois's method[24]. Extract 2 mL was added into 2 mL DPPH 50 μg $mL^{-1}$. After incubation 30 min, the absorbance was read at wavelength 515 nm by UV-Vis spectrophotometer. The $IC_{50}$ (inhibitory concentration 50%) of DPPH scavenging activity exposed its antioxidant activity which can be calculated using its calibration curve.

***In vitro* antioxidant activities by CUPRAC assay:** The CUPRAC solution was prepared in ammonium acetate buffer pH 7 using modified Apak's method[25]. Each extract was prepared in various concentrations and pipetted 2 mL extract into 2 mL CUPRAC 100 μg $mL^{-1}$. After incubation 30 min, the absorbance was observed at wavelength 450 nm. Ammonium acetate buffer was used as a blank, CUPRAC 100 μg $mL^{-1}$ as control and ascorbic acid as standard. Concentration of sample or standard that can exhibit 50% of CUPRAC capacity is $EC_{50}$ (exhibitory concentration 50%) of CUPRAC capacity. The $EC_{50}$ of CUPRAC capacity presented its antioxidant capacity which was determined using its calibration curve.

**Total phenolic content (TPC):** Folin-ciocalteu reagent was used to investigate total phenolic content. Gallic acid 40-120 μg $mL^{-1}$ was used as standard. Gallic acid 0.5 mL was added by 5 mL Folin-Ciocalteu reagent (which diluted 1:10 with aquadest) and 4 mL sodium carbonate 1 M. Keep solution 15 min at room temperature, then absorbance was seen at wavelength 765 nm. The same procedure was carried out for sample. The TPC of sample was calculated using calibration curve of gallic acid and reported as gallic acid equivalent (GAE) per 100 g extract (g GAE 100 $g^{-1}$)[26].

**Total flavonoid content (TFC):** Quercetin 36-104 μg $mL^{-1}$ was used as standard. Modified Chang's method was performed to determine TFC. Quercetin solution 0.5 mL was diluted by adding 1.5 mL methanol, 0.1 mL aluminium (III) chloride 10%, 0.1 mL sodium acetate 1M and 2.8 mL aquadest. Sample was conducted by the same procedure. Absorbance was read at wavelength 415 nm after incubation 30 min. Quercetin equivalent (QE) expressed TFC per 100 g extract (g QE100 $g^{-1}$)[27].

**Statistical analysis:** All of results are means±standard deviation at least triplicate experiments. Statistical analysis was investigated using one way ANOVA *post hoc* Tukey ($p<0.05$) by SPSS 16 for Windows. Meanwhile the correlations between TFC, TPC and their antioxidant activities and also between two antioxidant testing methods were analyzed by Pearson's method.

## RESULTS AND DISCUSSION

**Extraction:** Crude drug was separated using three different polarities solvents such as n-hexane, ethyl acetate and ethanol, consecutively, to extract most nonpolar compound by n-hexane, most semi polar compound by ethyl acetate and most polar compound using ethanol.

**Density of extracts:** The similarity density among extracts was important point. The higher density extract may give higher activity and or phytochemical content compared to extract with lower density. It was difficult to put 100% concentrated (thick extract) into pycnometer, so it can be presented by 1 and 5% or other concentration. In the present study, density of each extract of strawberry organs were determined as density 1% extract and showed similarity density around 0.66-0.9 g $mL^{-1}$ for all extracts.

**Antioxidant activities:** Inhibitory concentration 50% ($IC_{50}$) of DPPH scavenging activities of different extracts of strawberry organs presented their antioxidant activities by DPPH assay, ranged from 0.22-10.14 μg $mL^{-1}$ (Table 1). The highest antioxidant activity was demonstrated by the lowest of $IC_{50}$ compared to $IC_{50}$ of DPPH of ascorbic acid as standard.

The present study found the ethanol leaves extract of strawberry had the highest antioxidant activity by DPPH assay ($IC_{50}$ 0.22 μg $mL^{-1}$) compared to its fruit and stem extracts. It was similar to the other research reported that methanol leaves extract of Malus Sparkler cultivar of crabapples which was belong to the same family with strawberry (Rosaceae) found the highest antioxidant activity by DPPH assay [277.76 mmol Trolox equivalent (TE) $g^{-1}$] among seven cultivars of crabapples and compared to its flower and fruit extracts[28]. The $IC_{50}$ DPPH of ethanol leaves extract of strawberry was lower than $IC_{50}$ DPPH of ascorbic acid (0.56 μg $mL^{-1}$). Ascorbic acid was used as standard to verify DPPH assay that conducted in the present study. All extracts (n-hexane, ethyl acetate and ethanol) of different organs of strawberry (fruit, leaves and stem) varied from 0.22-10.14 μg $mL^{-1}$ ($IC_{50}$ DPPH <50 μg $mL^{-1}$), therefore it can be classified as very strong antioxidant[24]. Dias *et al.*[23] studied regarding antioxidant activities of roots extract of commercial and wild strawberry which were extracted by infusion, decoction and maceration methods. The results exhibited that hydromethanolic roots extract of wild strawberry by maceration gave the lowest $EC_{50}$ of DPPH (50.03 μg $mL^{-1}$) compared to commercial strawberry and the others extraction methods.

Research by Chaves *et al.*[4] presented that acidified methanol strawberry fruit extract of Camarosa cultivar showed the highest antioxidant activity by DPPH assay ($EC_{50}$ 76.73 mg $mL^{-1}$) compared to Albion, Aromas, Camino real, Monterey, Portola, San Andreas cultivars. Two cultivars of strawberry (Sweet Charlie and Camarosa) with four maturation stages (green, greenish white, whitish red and red) were evaluated by Mandave *et al.*[29]. Each sample was extracted using ethanol and 0.2% acetic acid, then each extract was evaluated its antioxidant activity by DPPH and reducing power assays. The result demonstrated that 0.2% acetic acid extract of Sweet Charlie strawberry cultivar with red maturation stages had the highest antioxidant activity by DPPH assay which exposed the lowest $EC_{50}$ DPPH (9.71 mg $mL^{-1}$) compared to other maturation stages and Camarosa cultivar.

Table 1: Antioxidant activities of strawberry organs by DPPH methods

| | $IC_{50}$ DPPH (μg $mL^{-1}$) | | |
|---|---|---|---|
| Samples | n-hexane extract | Ethyl acetate extract | Ethanol extract |
| Fruit | 7.71±0.44[a] | 2.15±0.12[a] | 5.67±0.54[a] |
| Leaves | 10.14±0.83[b] | 1.41±0.11[b] | 0.22±0.02[b] |
| Stem | 2.52±0.21[c] | 0.68±0.04[c] | 0.80±0.03[c] |
| Ascorbic acid | 0.56±0.03[d] | 0.56±0.03[c] | 0.56±0.03[d] |

[a-d]Different letter in the same column means significant different ($p<0.05$)

Table 2: Antioxidant capacities of strawberry organs by CUPRAC methods

| | $EC_{50}$ CUPRAC (μg $mL^{-1}$) | | |
|---|---|---|---|
| Samples | n-hexane extract | Ethyl acetate extract | Ethanol extract |
| Fruit | 212.05±15.93[a] | 130.42±6.28[a] | 245.69±2.96[a] |
| Leaves | 245.60±0.97[b] | 186.05±0.47[b] | 250.14±9.30[a] |
| Stem | 199.66±2.35[a] | 201.43±1.77[c] | 417.28±26.70[b] |
| Ascorbic acid | 12.58±0.34[c] | 12.58±0.34[d] | 12.58±0.34[c] |

[a-d]Different letter in the same column means significant different (p<0.05), CUPRAC: Cupric reducing antioxidant capacities

Research by Dyduch-Sieminska *et al.*[30] was recorded antioxidant activity of water fruit extract of wild strawberry by percentage DPPH scavenging activity, which shown dried fruit of Regina cultivar gave the highest percentage DPPH scavenging activity (24.60%). The higher percentage of DPPH scavenging activity was not always given by higher concentration of sample, otherwise the lower percentage of DPPH scavenging activity was not always given by the lower concentration. It was presented by previous study[31], which exposed that methanol pineapple peel extract 100 μg $mL^{-1}$ had higher percentage of DPPH scavenging activity (95.74%) compared to 200 μg $mL^{-1}$ (95.17%) and 400 μg $mL^{-1}$ (94.96%). It might be only some compounds in extract had antioxidant activities, while the other compounds act as antagonist of antioxidant. In methanol peel extract 100 μg $mL^{-1}$ the antagonist antioxidant compounds have not reach their effective minimum concentration yet, meanwhile in 200 μg $mL^{-1}$ they reached the effective minimum concentration, then reduced the percentage of DPPH.

The CUPRAC was the other method that carried out in the present study. The exhibitory concentration 50% ($EC_{50}$) of CUPRAC capacity in the range of 130.42-417.28 μg $mL^{-1}$ (Table 2), which FRT2 gave the lowest $EC_{50}$ (130.42 μg $mL^{-1}$) and showed the highest antioxidant activity by CUPRAC method compared to the other extracts. The $EC_{50}$ CUPRAC of ascorbic acid standard was 12.58 μg $mL^{-1}$. It means that antioxidant capacity of ascorbic acid around 10-fold of FRT2 by CUPRAC method. The CUPRAC reagent was prepared in ammonium acetate buffer pH 7 by mixing Cupric (II) chloride and neocuproine. Sample will act as antioxidant in CUPRAC assay if it had reduction potential lower than reduction potential of Cu (II)/Cu (I) which was 0.159 V. Complex Cu (I)-neocuproine gives yellow color and shows characteristic absorption at wavelength 450 nm[25]. Amount of Cu (II) that can be reduced to Cu (I), related with the intensity of yellow color and amount of antioxidant compounds in sample.

Liu *et al.*[28] presented that methanol leaves extract of Malus Sparkler, one of crabapples cultivar (Rosaceae) showed the highest reducing power (119.05 mg vitamin C equivalent (VE) 100 $g^{-1}$) compared its fruits and flowers, also compared to the others cultivars. Hydromethanolic roots of wild strawberry which was extracted by maceration had the highest antioxidant activity by reducing power method ($EC_{50}$ 40.98 μg $mL^{-1}$) compared to the other extracts[23]. It was contrary with its antioxidant activity using beta- carotene bleaching (BCB) method which revealed that infusion of commercial strawberry had the highest antioxidant ($EC_{50}$ 23.44 μg $mL^{-1}$) by BCB method. The other research exposed that Camarosa strawberry cultivar with red maturation stages had the highest antioxidant activity ($EC_{50}$ 24.16 mg $mL^{-1}$) compared to other maturation stages and Sweet Charlie cultivar by reducing power assay[29].

**Phytochemical content:** The STM3 gave the highest TPC 18.62 g GAE 100 $g^{-1}$, followed by LEV3 (17.11 g GAE 100 $g^{-1}$), while the lowest was shown by FRT 1 (1.45 g GAE 100 $g^{-1}$) (Fig. 1).

The TFC among different extracts of strawberry organs were exposed in term of quercetin equivalent per 100 g varied from 0.48-7.40 g QE 100 $g^{-1}$. The STM3 demonstrated the lowest TFC (0.48 g QE 100 $g^{-1}$), while the highest given by LEV2 (7.40 g QE 100 $g^{-1}$) (Fig. 2).

Research by Dias *et al.*[23] expressed that root infusion of wild strawberry gave the highest total phenolic content (TPC) 253.42 mg $g^{-1}$ compared to commercial strawberry and other extraction methods. Dyduch-Sieminska *et al.*[30] reported that the highest total flavonoid content of water fruit extract of wild strawberry was found in Baron von Solemarcher cultivar (1.245 mg QE $g^{-1}$), meanwhile Regina cultivar had the highest total phenolic acid content (4.858 mg caffeic acid equivalent $g^{-1}$).

The TPC in STM3 (18.62 g GAE 100 $g^{-1}$) and TFC in LEV2 (7.40 g QE 100 $g^{-1}$) were the highest among all extracts, but STM3 and LEV2 did not show the highest antioxidant activities by DPPH method. It demonstrated that the highest TPC and or TFC in sample didn't always gave the higher antioxidant activities. Antioxidant activities of ethyl acetate and ethanol strawberry fruit extracts using DPPH assay were higher than their n-hexane extracts. It might be correlated with their phenolic content. The TPC in methanol leaves extract of Malus Sparkler cultivar of crabapples (Rosaceae)

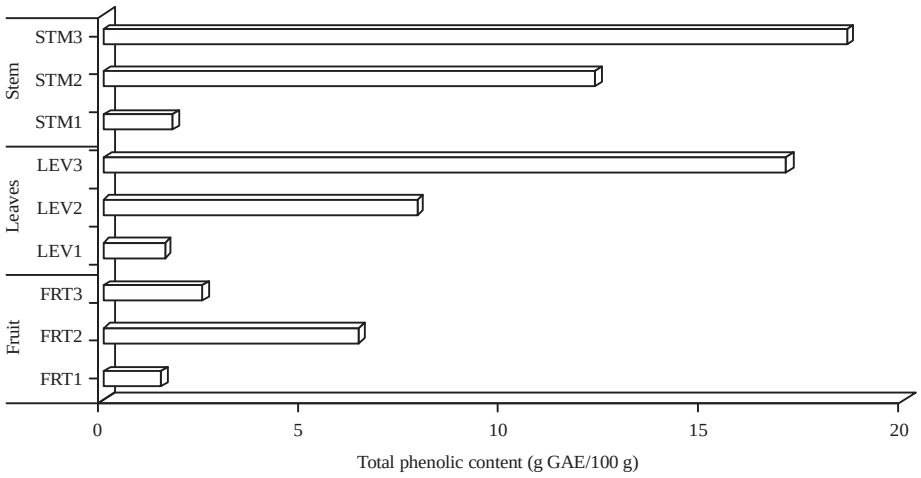


Fig. 1: Total phenolic content in different organs of strawberry, n = 3

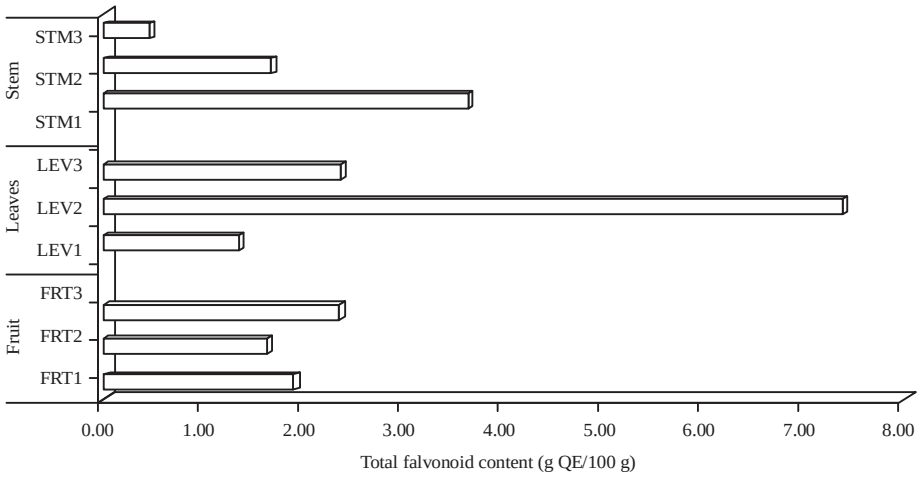


Fig. 2: Total flavonoid content in different organs of strawberry, n = 3

(3419 mg GAE 100 $g^{-1}$) was the highest compared to its fruits and flowers and also to the other cultivars[28]. The highest TFC (127.52 mg rutin equivalent (RE) 100 $g^{-1}$) was presented by methanol crabapples leaves extract of Malus Royalty cultivar, meanwhile the highest total anthocyanin was exposed by its flowers extract (374.87 mg catechin equivalent (CE) 100 $g^{-1}$). Similar to the present research which revealed that the highest TFC was shown by LEV2 (7.40 g QE 100 $g^{-1}$), but the highest TPC was demonstrated by STM3 (18.62 g GAE 100 $g^{-1}$). A study regarding seven cultivars of strawberry exhibited that total anthocyanin among all cultivars extracts in the range of 15.67-27.62 mg pelargonidin-3-O-glucoside per 100 g, which was the highest shown by Camarosa cultivars[4], while the highest total phenolic content using Folin-Ciocalteu reagent given by Monte Rey cultivars. Mandave *et al.*[29] revealed that chlorogenic acid was the most phenolic compound in strawberry fruit compared to catechin, rutin and quercetin. The fruit of Sweet Charlie strawberry cultivar with greenish white maturation which was extracted using ethanol gave the highest chlorogenic acid (843.4 mg $kg^{-1}$ fresh weight). The similar result was shown by ethanol extract of greenish white maturation of Camarosa strawberry cultivar. The other previous research compared major classes of phenylpropanoids/flavonoids between *Fragaria vesca* and *Fragaria* x *ananassa*[6]. The most compound in red and white genotype of *Fragaria vesca* and *Fragaria* x *ananassa* was ellagic acid, shown by earliglow (red genotype of *Fragaria* x *ananassa*) which had the highest ellagic acid (29.6 mg 100 $g^{-1}$ fruit fresh).

Roy *et al.*[6] reported that fruit of strawberry contained most of ellagic acid and flavan-3 ol compared to anthocyanin, hydroxycinnamic acid, flavonol and ellagitannin, which ellagic

Table 3: Correlation of TPC and TFC with its antioxidant activities

| Antioxidant parameters | Pearson's correlation coefficient® | |
|---|---|---|
| | TPC | TFC |
| $IC_{50}$ DPPH FRT | -0.970** | 0.483$^{ns}$ |
| $IC_{50}$ DPPH LEV | -0.865** | -0.528$^{ns}$ |
| $IC_{50}$ DPPH STM | -0.873** | -0.894** |
| $EC_{50}$ CUPRAC FRT | -0.867** | 0.879** |
| $EC_{50}$ CUPRAC LEV | -0.172$^{ns}$ | -0.953** |
| $EC_{50}$ CUPRAC STM | 0.790** | -0.772** |

**Significant at p<0.01, ns: Not significant, TPC: Total phenolic content, TFC: Total flavonoid content

Table 4: Correlation pearson of DPPH and CUPRAC methods

| Antioxidant parameters | Pearson's correlation coefficient® | | |
|---|---|---|---|
| | $EC_{50}$ CUPRAC FRT | $EC_{50}$ CUPRAC LEV | $EC_{50}$ CUPRAC STM |
| $IC_{50}$ DPPH FRT | 0.786** | | |
| $IC_{50}$ DPPH LEV | | 0.399$^{ns}$ | |
| $IC_{50}$ DPPH STM | | | -0.466$^{ns}$ |

**Significant at p<0.01, ns: Not significant

and flavan-3-ol were soluble in ethyl acetate and ethanol. Ellagic acid have ortho di-OH in its structure, which may have related with its antioxidant capacity, while substitution OH in C3' and C4' in flavan-3-ol influence to give higher antioxidant activities[32].

In the present study exhibited that TFC in FRT 2 (1.65 g QE 100 $g^{-1}$) was similar to TFC in STM 2 (1.69 g QE 100 $g^{-1}$). The antioxidant capacities of FRT2 and STM2 by CUPRAC assay were not similar, which $EC_{50}$ CUPRAC FRT2 130.42 μg $mL^{-1}$ and STM2 201.43 μg $mL^{-1}$. It can be predicted that many of flavonoid compounds in FRT2 had lower redox potential than 1.59 V ($E^{o}$ Cu(II)/Cu(I)).

**Pearson's correlation:** Pearson's correlation coefficient (r) was significantly negative if $-0.61 \leq r \leq -0.97$ and significantly positive if $0.61 \leq r \leq 0.97$[33]. The higher TFC and TPC are often similar with the higher antioxidant activities, which exposed by lower $IC_{50}$ DPPH and $EC_{50}$ CUPRAC, therefore their correlation was significantly negative correlation[34].

Correlation between TPC and TFC in all extracts of (n-hexane, ethyl acetate, ethanol) of strawberry organs and their antioxidant activities by DPPH and CUPRAC methods can be found in Table 3, meanwhile correlation between two antioxidant testing methods in Table 4.

In the present research showed that TPC in different organs (fruit, leaves and stem) of strawberry were significant and negative correlation with their $IC_{50}$ of DPPH ($r = -0.970$, $r = -0.865$, $r = -0.873$, $p<0.01$) and only TFC in stem also had significantly negative correlation with its $IC_{50}$ of DPPH ($r = -0.894$, $p<0.01$). Research by Chaves *et al.*[4] expressed that total anthocyanin in seven cultivars of strawberry had significantly negative correlation with their $EC_{50}$ DPPH ($r = -0.94$, $p<0.01$), meanwhile its TPC showed no significant correlation ($r = -0.49$). Analyze of correlation between two antioxidant testing methods (Table 4) exposed that $IC_{50}$ of DPPH in strawberry leaves was linear with its $EC_{50}$ CUPRAC ($r = 0.399$, $p<0.05$).

## CONCLUSION

Waste product of strawberry (leaves and stem) had antioxidant activities. Two antioxidant testing methods (DPPH and CUPRAC) gave different results in antioxidant activities of different organs of strawberry. All extracts of fruits, leaves and stem of strawberry can be categorized as very strong antioxidant, using DPPH assay. The higher TPC and or TFC did not always show the higher antioxidant capacities.

Phenolic compounds in fruits and leaves extracts of strawberry were the major contributor in their antioxidant activities by DPPH method.

## SIGNIFICANCE STATEMENTS

This study discovered that the waste products of strawberry (leaves and stem) had antioxidant activities and potential to be recommended as sources of further natural antioxidant. The higher TPC and or TFC did not always show the higher antioxidant capacities.

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