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Original Research Article

Effect of solid state fermentation with *Trichoderma* spp. on phenolic content and antioxidant capacities of mature Assam tea leaves

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ABSTRACT

In this study, the mature leaves of Assam tea which were considered as waste were used as substrate for solid state fermentation. The effects of solid state fermentation on phenolic content and antioxidant capacities of Assam tea leaves using 4 different *Trichoderma* spp. including *Trichoderma harzianum* CB-Pin-01, *Trichoderma* sp. NTY 211, *Trichoderma* sp. PSUT 001 and *Trichoderma* sp. PSUT 002 were explored. The Assam tea leaves were fermented in erlenmeyer flask by inoculum with the spore suspension and incubated at 30˚C for 7 days, while a non-inoculum condition was served as unfermented sample. The unfermented and fermented tea leaves were further extracted with different solvents including water, 95% ethanol, 50% ethanol, 95% acetone and 50% acetone. The result revealed that the color shade and intensity of fermented tea leaves extracts were changed by types of fungi and solvents used. Evaluation of phenolic content, flavonoid content and antioxidant capacities demonstrated that the highest phenolic (221.95±39.79 mg GAE/g extract) and flavonoid (206.17±3.33 mg QE/g extract) contents were significantly represented in unfermented tea extract prepared by 95% acetone. In addition, It also gave the highest IC_{50} of 13.74 ± 0.22 and 5.76 ± 0.10 µg/ml which were assayed by DPPH and ABTS radical scavenging activity, respectively, while the highest ferric reducing antioxidant power (FRAP) value was represented in 50% acetone extract. On the other hand, fermentation of Assam tea leaves dramatically decreased phenolic and flavonoid content as well as antioxidant capacities. In all *Trichoderma* spp. tested, the isolate NTY 211 seemed to mostly retain phenolic and flavonoid contents of 125.71 ± 3.61 mg GAE/g extract and 126.64 ± 3.14 mg QE/g extract, whereas IC_{50} values assayed by DPPH and ABTS radical scavenging activity were equal to 27.98 ± 0.17 and 11.05 ± 0.11 µg/ml, respectively. This indicates that solid state fermentation by *Trichoderma* spp. had an effect on reduction of phenolic antioxidants that could be not proper for bioactivity improvement of Assam tea leaves.

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INTRODUCTION

Camellia sinensis var. *assamica* or Assam tea is tea plant which originated from India and has been usually found in the northern of Thailand. Leaves of Assam tea are rich in antioxidant activity due to the presence of polyphenol especially flavonols. The major types of flavonols are included (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC), (-)-gallocatechin gallate (GCG) and (+)-catechin (C) (Komes et al., 2010; Kosinska and Andlauer, 2014; Sae-Lee et al., 2009; Zhen et al., 2002). In addition, it has been reported that these flavonols shows anti-carcinogenic, anti-microbial, anti-viral, anti-inflamatory and anti-diabetic properties (Ananingsih et al., 2013; Sueoka et al., 2006). Assam tea has commonly consumed as beverage and Miang (pickle tea) (Khanongnuch et al., 2017). In the tea harvesting process, buds and the first two to three leaves of Assam teas are normally collected to produce oolong, black and pu-erh tea, while the young leaves (3^{rd} – 6^{th} leaf from shoot tips) are mostly selected for Miang production (Khanongnuch et al., 2017; Senanayake, 2013; Kosinska and Andlauer, 2014). Conversely, the mature one is rarely used and unfavourable due to its acerbity taste and unpleasing scent (Wang et al., 2000). So, the mature leaves are useless and considered as waste. The mature Assam tea leaves showed amount of polyphenol and antioxidant activity but was lower than the shoot and young leaves (Dorkbuakaew et al., 2016; Chan et al., 2007). An attempt to make the mature leaves more interesting could be conducted.

Solid state fermentation (SSF) is the one types of fermentation process in which the microorganism grow on moist solid substrate in the absence or near absence of free water. It has several advantages, for example high productivities, low production costs, eco-friendly conditions and reduction a risk of contamination by bacteria and yeast (Mienda et al., 2011; Farinas, 2015; Thomas et al., 2013; Couto and Sanroman., 2006). The most suitable microorganism to culture in SSF process is several fungi and yeasts. Previously, several studies used SSF with fungal microorganisms for improvement phenolic antioxidants in plant materials (Karimi et al., 2010; Oliveira et al., 2012; Xiao et al., 2014; Xiao et al., 2015). Fermentation of soybean using SSF with *Trichoderma harzianum* NBRI-1055 (Singh et al., 2010) and fermentation of turmeric using SSF with *Trichoderma* spp. (Mohamed et al., 2016) can enhance phenolic compounds and antioxidant activity. In addition, antioxidant phenolic compounds in wheat were improved by SSF by *Rhizopus oryzae* RCK2012 (Dey and Kuhad, 2014).

Trichoderma species are saprophytic fungi in the rhizosphere. This species have rapid growth by using wide range of compound as carbon and nitrogen source by secrete extracellular such as cellulase and hemicellulase to break down plant polymers into simple sugars for energy and growth. These organisms have been found in all climate zones ranging from Antarctica to the tropics (Nevalainen and Neethling, 2002; Kubicek et al., 2003; Yedidia et al., 1999). The beneficial action of *Trichoderma* is fight plant diseases caused by pathogenic fungi, produced chemical characteristic coconut like aroma (Schuster and Schmoll, 2010), biofungicide agents (Verma et al., 2007), extracellular enzyme production such as cellulase (Peciulyte et al., 2014), xylannase (He et al., 2014), protease (Deng et al., 2018), plant growth promotion (Saba et al., 2012). The fungi *Trichoderma* could be considered non-pathogenic for a healthy human under normal exposure this organism (Nevalainen and Neethling, 2002). However, an application of *Trichoderma* spp. in food and cosmetic industries has been rarely reported. Therefore, the aim of this work was to study the role of solid state fermentation of mature tea leaves using *Trichoderma* spp. The possibility on enhance and change of phenolic content and antioxidant capacity was explored.

MATERIALS AND METHODS

Microorganism and inoculum

Trichoderma harzianum CB-PIN-01 (CB) was purchased from school of agricultural technology, Walailak University, Nakhon Sri Thammarat, Thailand. *Trichoderma* sp. NTY211 (NT), was supported from department of Science, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya Nakhon Sri Thammarat Saiyai Campus, Nakhon Sri Thammarat, Thalland. *Trichoderma* sp. PSUT 001 (P1) and *Trichoderma* sp. PSUT 002 (P2) were supported from department of Pest Management, Faculty of Natural Resources, Prince of Songkhla University, Songkhla, Thailand. These strains were grown on potato dextrose agar (PDA) medium at 28°C. The inoculums were prepared from 7 days by suspending the fungal spores in sterile water and the concentration was adjusted to 1×10^7 spore / ml.

Plant tea sample preparation

The fresh mature Assam tea leaves were collected from tea plantation at Teung District, Chiang Rai. They were dried in tray dryer at 50±1°C until the weight was constant. The dried leaves were ground by hammer mill through filter diameter size 0.5 cm. The dry powder was stored at 5°C until used.

Solid state fermentation of Assam tea

Assam tea powder (5 g) was taken in 250 ml Erlenmeyer flasks and mixed with 13 ml of water (70% moisture content). The mixture was then; autoclaved (121°C, 15 min) and then cooled down to ambient temperature. A 0.5 ml of fungal spore suspensions was inoculated onto the surface of the sterile substrate and mixed properly. The inoculated tea sample was incubated at 30°C for 7 days. The unfermented Assam tea (control) was also prepared without spore suspension inoculum.

Preparation of unfermented and fermented Assam tea extracts

The unfermented and fermented Assam tea samples were extracted using conventional shaking method with 150 rpm at room temperature for 3 hours. Different solvents (deionized water, 50% acetone, 50% ethanol, 95% acetone and 95% ethanol) were employed with a ratio of initial tea sample per solvent of ratio 1:20 w/v. The mixtures were filtrated by cheese cloth and further centrifuged at 8500 rpm for 30 min. The all extracts were finally evaporated by rotary evaporator and/or freeze-drier.

Analytical methods

Determination of total phenolic content

Total phenolic content (TPC) was determined by using Folin-Ciocalteu reagent according to the method described by Jin et al. (2016) and gallic acid was used as standard. The reaction was developed and the colored mixture was measured at 765 nm using spectrophotometer. The result was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE / g extract).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined by aluminium colorimetric assay using quercetin as standard with slight modification (Heimler et al., 2005). The color of solution was measured at 510 nm using spectrophotometer. The result was expressed as milligrams of quercetin equivalent (QE) per gram of extract (mg QE / g extract).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The DPPH radical-scavenging activity of samples was determined according to the method of Thaipong et al., (2006) with some modification. An absorbance value was read in a spectrophotometer at 517 nm using spectrophotometer and the percentage of DPPH radical inhibition was calculated using the following equation; .

$$\text{Free radical inhibition (\%)} = ((Ac - As)/Ac) \times 100 \quad \textbf{(1)}$$

Where Ac = Absorbance of the control, As = Absorbance of the sample The IC_{50} value of DPPH scavenging activity was further interpreted from the percentage of inhibition.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity of samples was determined according to the method of Re et al., (1998) with slight modification. An absorbance was measured at 734 nm using spectrophotometer. The radical inhibition was calculated as described above (equation 1) and ABTS scavenging activity was finally expressed as IC_{50} value.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of extracts was determined by Fe (ll)-TPTZ complex method, according to Dey and Kuhad, (2014) using trolox as standard. The reaction was measured at 593 nm using spectrophotometer. The result was expressed as milligrams of trolox equivalent antioxidant capacity (TEAC) per gram of extract (mg TEAC / g extract).

Statistical analysis

Means and standard deviation (SD) were calculated from the data obtained from triplicate experiments. The data obtained from completely randomized design experiment was analyzed by one way analysis of variance (ANOVA), Duncan's multiple range test and correlation analysis using the SPSS software program (SPSS Inc., Chicago, IL, USA). A difference with p-value less than 0.05 ($p<0.05$) was considered significant.

RESULTS AND DISCUSSION

Solid state fermentation of mature Assam tea leaves

Various substrates can be used to grow fungi for producing biomass, enzymes and active substances. In the presence, agro-industrial and agricultural wastes have been more interesting and selected because they are cheaper substrate, cost effective for large-scale production and eco-friendly and available. *Trichoderma* spp. are mostly used to cultivated for biological control of plant disease and microbial enzyme production, especially cellulase and protease (Ahmed et al., 2017; Ellila et al., 2017; Zhang et al., 2018; Saravanakumar and Kathiresan, 2012). The grain and rice were considered as excellent substrates for their growth (Faruk et al., 2015; Rajput et al., 2014; Romero-Arenas et al., 2013). Up to date, many solid wastes, for example straws, manures, spent mushroom compost, sugarcane bagasse have been used in solid state fermentation (Kogo et al., 2017; Singh et al., 2014; Subash et al., 2014; Tewari and Bhanu, 2004). In our study, it can support a use of mature tea leaves which are considered as waste during tea harvesting process to act as an economic carbon substrate for *Trichoderma* spp. All *Trichoderma* spp. selected can grow well on substrate containing only Assam tea sample after cultivation for 7 days. They had similarly growth pattern in which there were appeared mycelium at 2nd day and full growth over the substrate for 3 days. The spore formation was observed at 4th day, except isolate P2 which had no spore production until the harvesting day (day 7).

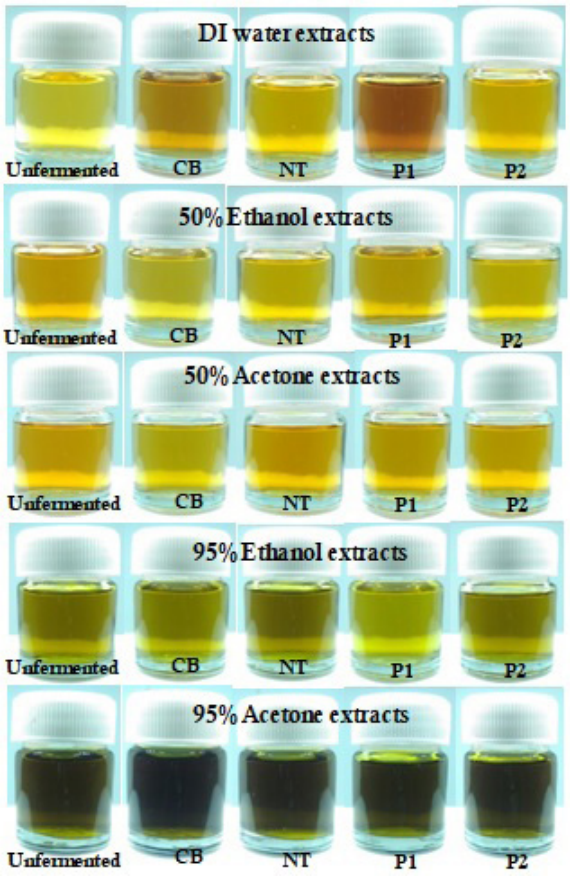

Figure 1. Appearance of unfermented and fermented Assam tea extracts with different solvent. CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

The unfermented and fermented Assam tea extracts can be prepared by using different solvents (Figure 1). It can be observed that the color shade and intensity of extracts were different depending on types of fungi and solvents used. In addition, fermentation process also had an effect on this change when comparing with unfermented sample. This may be possible due to biotransformation or bioconversion of tea leaves components during solid state fermentation.

Table 1. Total phenolic content of unfermented and fermented Assam tea extracts

Sample	Phenolic content (mg GAE / g extract)				
	Water**	50% Ethanol	50% Acetone	95% Ethanol	95% Acetone
Unfermented	89.20 ± 1.05^{Ad***}	185.17 ± 10.58^{Ab}	173.77 ± 9.10^{Ab}	143.81 ± 5.79^{Ac}	221.95 ± 14.07^{Aa}
CB*	39.79 ± 0.89^{Dd}	59.87 ± 5.78^{Dbc}	57.57 ± 5.56^{Dc}	67.02 ± 1.61^{Cb}	91.29 ± 4.97^{Ca}
NT	58.78 ± 0.52^{Cc}	87.96 ± 2.65^{Bb}	84.64 ± 2.55^{Bb}	87.09 ± 3.66^{Bb}	125.71 ± 3.61^{Ba}
P1	71.49 ± 1.78^{Bab}	69.71 ± 5.44^{CDab}	68.34 ± 5.34^{Cab}	65.24 ± 1.12^{Cb}	75.55 ± 6.13^{Da}
P2	60.53 ± 2.11^{Cc}	80.25 ± 2.99^{BCb}	78.71 ± 2.93^{Bb}	67.79 ± 3.51^{Cc}	106.41 ± 10.00^{Ca}

* CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

** Value is expressed as means ± SD (n = 3).

*** $^{a-d}$ means the row followed by different letters are significantly different ($p < 0.05$) and $^{A-D}$ means the column followed by different letters are significantly different ($p < 0.05$).

Table 2. Total flavonoid content of unfermented and fermented Assam tea extracts

Sample	Flavonoid content (mg QE / g extract)				
	Water**	50% Ethanol	50% Acetone	95% Ethanol	95% Acetone
Unfermented	77.43 ± 0.67^{Ae***}	138.01 ± 8.11^{Ad}	186.91 ± 1.24^{Ab}	148.46 ± 4.79^{Ac}	206.17 ± 3.33^{Aa}
CB*	19.06 ± 0.23^{De}	30.99 ± 0.72^{Cd}	59.07 ± 2.29^{Eb}	45.24 ± 0.89^{Dc}	64.85 ± 1.18^{Ea}
NT	38.61 ± 0.82^{Cd}	68.74 ± 2.36^{Bc}	130.30 ± 5.05^{Ba}	79.76 ± 2.59^{Bb}	126.64 ± 3.14^{Ba}
P1	39.74 ± 0.23^{Bd}	37.52 ± 0.63^{Ce}	69.54 ± 0.62^{Db}	46.73 ± 1.27^{Dc}	78.23 ± 1.46^{Da}
P2	40.14 ± 0.62^{Bd}	69.14 ± 1.06^{Bc}	114.92 ± 7.11^{Ca}	66.93 ± 1.13^{Cc}	107.16 ± 1.41^{Cb}

* CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

** Value is expressed as means ± SD (n = 3).

*** $^{a-d}$ means the row followed by different letters are significantly different ($p < 0.05$) and $^{A-D}$ means the column followed by different letters are significantly different ($p < 0.05$).

Total phenolic content (TPC) and total flavonoid content (TFC)

TPC and TFC of the extracts from unfermented and fermented Assam tea using different solvents are present in Table 1 and Table 2, respectively. The types of solvent had significant effect on phenolic and flavonoid extraction ($p<0.05$) in which 95% acetone provided the highest content of phenolic and flavonoid (221.95±14.07 mg GAE/g extract and 206.17±3.33 mg QE/g extract, respectively), while water was a poor solvent for extraction (89.20±1.05 mg GAE/g extract and 77.43±0.67 mg QE/g extract, respectively). Similarly with Doekbuakaew et al. (2016), 70% acetone also showed the best solvent for extraction phenolic and flavonoid compounds from mature Assam tea leaves. In addition, use of 50% acetone extract of black tea also gave the highest polyphenol content of 114.01±1.13 mg GAE/g dw tea (Turkman et al., 2007). When the Assam tea leaves were fermented with fungi, *Trichoderma* spp., it was found that TPC and TFC values were significantly decreased ($p < 0.05$) as compared to unfermented extracts which were similar in all solvents used. This indicates phenolic compounds represented in Assam tea leaved may be transform or degraded during fermentation by *Trichoderma* spp. According to studied of Xu et al. (2011) and Peterson et al. (2005), polyphenol and flavonoid content were decreased after fermentation of tea leaves with fungi and anaerobic bacterial. During fermentation, the fungi can secrete extracellular enzyme such as polyphenol oxidase and tannase to react with tea polyphenol like oxidation, degradation and polymerization, that had an effect to transformation and degradation of tea polyphenol (Zhao et al., 2015; Lv et al., 2013). As represented in the Table 1 and 2, the phenolic and flavonoid content of Assam tea leaves, which were fermented with *Trichoderma* sp. NTY211 seemed to be mostly remained at 125.71± 3.61 mg GAE / g extract and 126.64±3.14 mg QE / g extract when 95% acetone was employed as solvent.

Antioxidant capacities

In this study, ability to be antioxidant was evaluated by 3 analytical methods including DPPH free radical scavenging activity, ABTS cation radical scavenging activity and ferric reducing antioxidant power (FRAP). The values of free radical inhibition of the extracts was expressed as IC_{50} values (µg/ml), while the ability to reduce ferric ion was represented as trolox equivalent. The DPPH free radical inhibition represented in Table 3 revealed that the unfermented extract significantly showed the lowest IC_{50} value of 13.74±0.22 µg/ml when 95% acetone was employed as a solvent. This demonstrated that the extract shows the highest antioxidant activity. However, it seemed to be lower than trolox and ascorbic acid standard which was equal to 4.34±0.01 and 3.23±0.01 µg/ml, respectively. Similar to ABTS assay, the lowest IC_{50} value of 5.76±0.01 µg/ml were obtained when the Assam tea leaves were extracted with 95% acetone, (IC_{50} value of trolox = 3.26±0.02 µg/ml and ascorbic acid = 3.26±0.03 µg/ml), but the highest FRAP value of 712.69±17.82 mg TEAC/g extract were obtained from 50% acetone (Table 4 and 5). So, it can be implied that acetone is an excellent solvent for providing antioxidant activity from Assam tea leaves.

Table 3. DPPH radical scavenging activity (IC_{50}) of unfermented and fermented Assam tea extracts

Sample	IC_{50} of DPPH (µg/ml)				
	Water**	**50% Ethanol**	**50% Acetone**	**95% Ethanol**	**95% Acetone**
Unfermented	$38.88 \pm 0.24^{Ae***}$	19.58 ± 0.23^{Ac}	14.58 ± 0.05^{Ab}	21.79 ± 0.26^{Ad}	13.74 ± 0.22^{Aa}
CB*	583.74 ± 8.57^{De}	235.96 ± 3.41^{Dd}	66.78 ± 0.92^{Ea}	208.06 ± 2.32^{Ec}	92.58 ± 2.00^{Eb}
NT	207.42 ± 1.75^{Be}	66.00 ± 1.05^{Bc}	27.98 ± 0.17^{Ba}	79.88 ± 2.66^{Cd}	37.60 ± 1.44^{Bb}
P1	428.92 ± 8.10^{Ce}	194.87 ± 3.53^{Cd}	63.85 ± 1.15^{Da}	181.4 ± 0.71^{Dc}	88.95 ± 3.14^{Db}
P2	209.75 ± 2.46^{Bd}	65.92 ± 0.64^{Bc}	32.00 ± 0.21^{Ca}	67.45 ± 1.55^{Bc}	46.18 ± 0.73^{Cb}

* CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

** Value is expressed as means ± SD (n = 3).

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Table 4. ABTS radical cation scavenging (IC_{50}) of unfermented and fermented Assam tea extracts

Sample	IC_{50} of ABTS (µg/ml)				
	DI Water**	**50% Ethanol**	**50% Acetone**	**95% Ethanol**	**95% Acetone**
Unfermented	$10.70 \pm 1.62^{Ac***}$	8.21 ± 0.03^{Ab}	5.75 ± 0.10^{Aa}	7.44 ± 0.10^{Ab}	5.76 ± 0.10^{Aa}
CB*	32.32 ± 0.22^{De}	24.00 ± 0.49^{Ed}	14.68 ± 0.14^{Da}	23.15 ± 0.26^{Dc}	17.37 ± 0.31^{Eb}
NT	23.56 ± 0.14^{Ce}	18.40 ± 0.12^{Cd}	12.82 ± 0.06^{Bb}	16.11 ± 0.13^{Bc}	11.05 ± 0.11^{Ba}
P1	17.24 ± 0.19^{Bb}	21.23 ± 0.22^{Dd}	19.04 ± 0.07^{Ec}	23.95 ± 0.09^{Ee}	15.38 ± 0.16^{Da}
P2	22.78 ± 0.17^{Ce}	17.36 ± 0.16^{Bc}	13.34 ± 0.03^{Cb}	18.51 ± 0.10^{Cd}	12.53 ± 0.19^{Ca}

* CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

** Value is expressed as means ± SD (n = 3).

*** $^{a-d}$ means the row followed by different letters are significantly different ($p < 0.05$) and $^{A-D}$ means the column followed by different letters are significantly different ($p < 0.05$).

Table 5. Ferric reducing antioxidant power (FRAP) of unfermented and fermented Assam tea extracts

Sample	FRAP (mg TEAC / g extract)				
	Water**	**50% Ethanol**	**50% Acetone**	**95% Ethanol**	**95% Acetone**
Unfermented	$207.69 \pm 2.67^{Ae***}$	448.66 ± 13.15^{Ad}	795.46 ± 6.39^{Aa}	646.30 ± 18.75^{Ac}	712.69 ± 17.82^{Ab}
CB*	42.88 ± 1.39^{Ee}	77.81 ± 1.39^{Dd}	303.95 ± 9.56^{Eb}	205.86 ± 4.01^{Cc}	355.96 ± 38.68^{Ba}
NT	96.28 ± 1.04^{Ce}	171.37 ± 10.33^{Bd}	506.58 ± 26.49^{Ba}	305.86 ± 7.86^{Bc}	388.07 ± 15.03^{Bb}
P1	107.94 ± 1.05^{Bc}	96.28 ± 4.81^{Cc}	363.33 ± 10.90^{Da}	214.58 ± 4.34^{Cb}	351.50 ± 14.40^{Ba}
P2	90.63 ± 1.40^{De}	173.41 ± 9.25^{Bd}	452.79 ± 15.37^{Ca}	308.83 ± 7.57^{Bc}	353.53 ± 13.28^{Bb}

* CB : Assam tea fermented with *Trichoderma harzianum* CB-Pin-01, NT : Assam tea fermented with *Trichoderma* sp. NTY211, P1: Assam tea fermentation with *Trichoderma* sp. PSUT 001, P2 : Assam tea fermentation with *Trichoderma* sp. PSUT 002

** Value is expressed as means ± SD (n = 3).

*** $^{a-d}$ means the row followed by different letters are significantly different ($p < 0.05$) and $^{A-D}$ means the column followed by different letters are significantly different ($p < 0.05$).

Solid state fermentation of Assam tea by *Trichoderma* spp. dramatically declined antioxidant activities assayed by the all three methods (Table 3-5). Moreover, similar trend of reduction can be observed in all types of *Trichoderma* spp. and solvent used. This meant that the fermented extract show lower antioxidant properties than unfermented extract. So, it can be emphasized that fermentation of Assam tea leaves by *Trichoderma* spp. cannot improve or enhance antioxidant capacity, but it strongly affect a reduction of the activity instead. As shown in Table 3-5, the most remaining antioxidant activities were observed in Assam tea leaves fermented with *Trichoderma* sp. NTY211. The highest remained values of IC_{50} assayed by DPPH was 27.98±0.17 µg/ml and FRAP value of 506.58±26.49 mg TEAC/g extract when 50% acetone was employed. While the highest IC_{50} assayed by ABTS was 11.05±0.11 µg/ml when 95% acetone was employed.

Correlation between antioxidant activities with phenolic and flavonoid of Assam tea leaves extract

Relationship between phenolic and flavonoid content and antioxidant activities was proven that the tendency of TPC and TFC were similar to the antioxidant activities. It can be observed that the high value of TPC and TFC indicated the presence of high value of DPPH, ABTS and FRAP. Analysis of correlation between phenolic and flavonoid content with antioxidant activities was further explored using Pearson correlation coefficient (r) and illustrated in Figure 2.

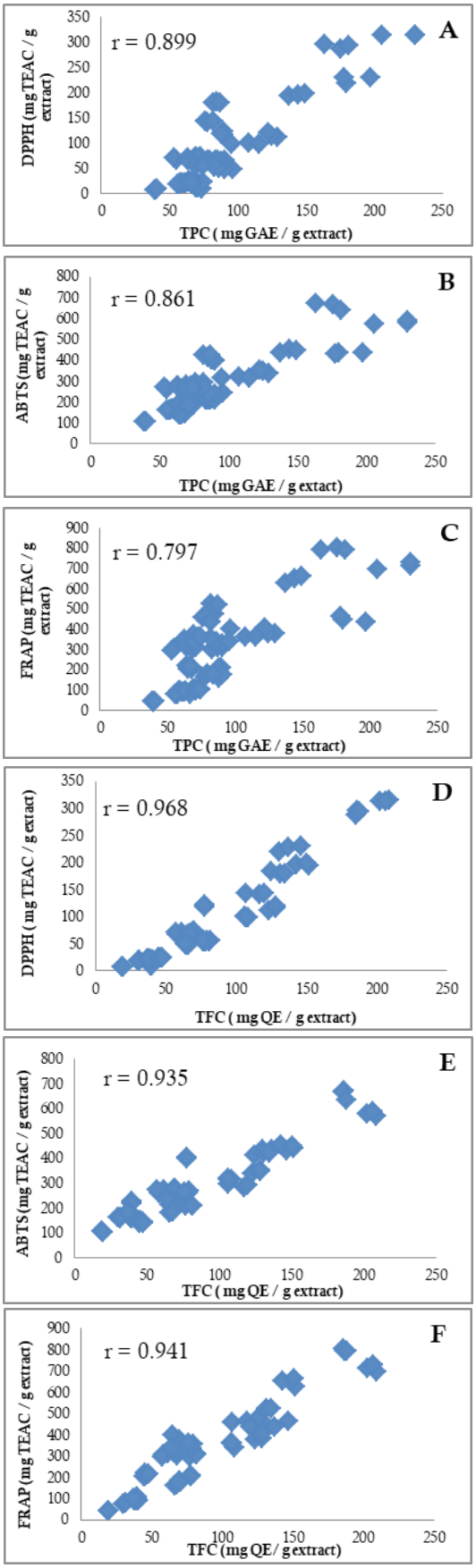

Figure 2. Correlation analysis of total phenolic content (A-C) and total flavonoid content (D-F) with antioxidant activities assayed by DPPH and ABTS radical scavenging activities and FRAP

The correlation analysis is a useful tool for predictive relationship and checking the degree of linear between two sets of experiment results (De Oliveira et al. 2015).Pearson correlation coefficient of TPC with antioxidant activities assayed by DPPH, ABTS and FRAP assay had r = 0.899, 0.861 and 0.797, respectively, while Pearson correlation coefficient of TFC with antioxidant activities assayed by DPPH, ABTS and FRAP assay had r = 0.968, 0.935 and 0.941, respectively. The highest correlation was observed between TFC and DPPH, while the lowest correlation was found between TPC and FRAP. According to Dancey and Reidy (2006), it has been reported that the value of correlation coefficient from 0.1-0.3 means low correlation; from 0.4-0.6 means moderate correlation and from 0.7-1.0 are high correlation. So, this study showed high correlation between phenolic and flavonoid content with antioxidant activities. It can be implied that the antioxidant activities could be a result of an action of phenolic and flavonoid represented in the Assam tea extract. The result of relationship between TPC and TFC with antioxidant activities was been reported previously. Gonbad et al. (2015) reported high correlation between TPC (0.85) and TFC (0.88) with DPPH values of tea (*Camellia sinensis*) extract. Bunea et al. (2011) analyzed the correlation between antioxidant activity measured by DPPH, ABTS and FRAP with TPC and TFC of blueberries extract and found that TPC was significantly and highly correlated with DPPH (0.973), ABTS (0.966) and FRAP (0.975). For TFC, it also had significantly high correlation with DPPH (0.896), ABTS (0.853) and FRAP (0.95). These confirmed that phenolic content showed high correlation with bioactivities of plant extract.

The result of this work was similar with study of Karimi et al. (2010) in which phenolic content, flavonoid content and antioxidants activities of pistachio hulls were all decreased when they were fermented with different fungi by SSF. When the Assam teas undergo fermentation, polyphenol in tea was declined by polymerization. The small molecules of polyphenols were polymerized into polymer with higher molecular weight. Catechins are group of natural polyphenols in tea, which known as a powerful antioxidant. Catechins can be polymerized to theaflavins, thearubigin and theabrownins (Xu et al., 2011; Lv et al., 2013). Reduction of catechin can affect a reduction of antioxidant activities. In contrast, when fermentation times increase, microorganism might use compound in substrate for their growth (Karimi et al. 2010).

CONCLUSIONS

The mature Assam tea leaves which were considered as agricultural waste can be used as alternative solid substrate source for solid state fermentation with *Trichoderma* spp. that could be further beneficial for application in biomass and microbial enzyme production. The highest phenolic (221.95±39.79 mg GAE/g extract) and flavonoid (206.17±3.33 mg QE/g extract) contents were significantly represented in unfermented tea extract prepared by 95% acetone. In addition, It also gave the highest IC_{50} of 13.74 ± 0.22 and 5.76 ± 0.10 µg/ml which were assayed by DPPH and ABTS radical scavenging activity, respectively, while the highest ferric reducing antioxidant power (FRAP) value was represented in 50% acetone extract. The solid state fermentation had an effect on decrease phenolic and flavonoid content, as well as antioxidant activities of Assam tea leaf. Therefore, it is not recommended process to improve or enhance antioxidant activities of Assam tea leaves. In all *Trichoderma* spp. tested, the isolate NTY 211 seemed to mostly retain phenolic and flavonoid contents of 125.71 ± 3.61 mg GAE/g extract and 126.64 ± 3.14 mg QE/g extract, whereas IC_{50} values assayed by DPPH and ABTS radical scavenging activity were equal to

27.98±0.17 and 11.05 ± 0.11 µg/ml, respectively. However, finding a way or process to remain and enhance bioactivities could be further done and developed.

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