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Antioxidant and Antibacterial Activities of Indian Marsh Fleabane (*Pluchea indica* (L.) Less)

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Abstract

Ethanolic extracts of various parts of *Pluchea indica* (L.) Less were analyzed for DPPH radical scavenging capacities, total phenolic contents and antibacterial activities. The results showed that fresh root extract had the most antioxidant activities (0.16±0.001 mg/mL EC_{50}, 20.02±0.177 mg/g TEAC and 15.79±0.008 mg GAE/g total phenolic content). Antioxidative activities of most fresh samples were significantly higher than that of dry samples (P<0.05). The low antioxidative activities of dry samples might be due to the effect of drying (60^{o}C for 2 days). In contrast, tea leaves extract exhibited the high antioxidant capacities (0.28±0.012 mg/mL EC_{50}, 11.86±0.519 mg/g TEAC and 3.18±0.012 mg GAE/g total phenolic content) due to the formation of Maillard reaction products during heating at lower temperature for a shorter period (50^{o}C for 2 hrs). Antibacterial activities assessed by the disc diffusion method showed that all of investigated bacteria were inhibited by the extract of fresh root, fresh twig, dry stem and tea leaves, while the extract of fresh and dry flower, fresh and dry leaves, and dry root showed the less inhibition potential. The minimal inhibitory concentrations (MICs) of extracts using agar microdilution and disc diffusion method showed significant inhibition activities even at 2-16 fold dilutions, with the most effective result in fresh root extract as low as 64 fold dilution. Tea leaves extract also had high inhibitory capacities when the concentrations were 4-16 fold dilution. Fresh root extract had the most excellent inhibition potential against *Bacillus cereus*, *Pseudomonas fluorescens* and *Salmonella typhimurium* (0.16, 0.16 and 0.32 mg/mL MICs, respectively). Inhibitory activities against *Escherichia coli* were very low. The increase in antioxidant activities did increase antibacterial abilities.
***Keywords**: Pluchea indica (L.) Less, Antioxidant, Antibacterial activities*

1. Introduction

Pluchea indica (L.) Less, commonly known as Indian Marsh Fleabane, is in the family of Asteraceae. It is the shrub plant that naturally grows in littoral areas of many Asian and Pacific islands countries. The plant is a source of phytochemicals and

antioxidants, which can protect and prevent cell damage from oxidative stress due to free radicals (1). Main antioxidants are tannins, terpenes, lignin glycosides, triterpenoids, polyphenol including some flavonoids; quercetin and quinic acid; and eudesmane derivatives (2, 3). It was demonstrated that extracts of *P. indica* had the DPPH, ABTS and ferric cyanide free radical scavenging activities with the highest contents in leaves (4). *P. indica* has been used in traditional medicines for treating respiratory disease, fever, rheumatism, anti-ulcer, anti-tuberculosis and also potential antiophidian principles (5, 6, 7). Biswas *et al.* (8) reported that methanolic extracts of root and leaves of this plant showed anti-amoebic against the HM1 strain of *Entamoeba histolytica* property. In addition, extracts of *P. indica* tea leaves had good antioxidant activity and potentially inhibited lipopolysaccharide-induced nitric oxide and prostaglandin E_2 production in RAW 264.7 macrophages (9). However, no study has been carried out to compare antioxidant and antimicrobial activities among the parts of *P. indica*, and also between fresh and dry samples. Thus, the objectives of this study were to investigate antioxidant activities, total phenolic contents and antibacterial activities against some gastrointestinal pathogenic bacteria, and to compare those activities among fresh and dry parts of *P. indica* of the same samples. Furthermore, tea leaves from *P. indica* were also observed due to the powerful antioxidant found in wide variety of herbal tea products. The results of this study would be useful to understand the health benefits of *P. indica* for its antioxidant activities and antibacterial properties.

2. Materials and methods

All reagents were analytical grade: DPPH (2,2-diphenyl-1-picryl hydrazyl; Sigma-Aldrich USA), Trolox (Sigma-Aldrich USA), Ethanol (Merck Germany), Methanol (Merck Germany), Folin-Ciocalteu phenol reagent (Loba Chemie India), Sodium carbonate (Na_2CO_3; Univar Ajax Finechem New Zealand), Gallic acid (Sigma - Aldrich USA), Penicillin G sodium salt (Sigma-Aldrich USA). Bacteria species were derived from Thailand Institute of Scientific and Technological Research (TISTR Culture Collection): *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 8739), *Pseudomonas fluorescens*, *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhimurium* (ATCC 13311 = NCTC 74).

2.1 Crude extraction

Pluchea indica (L.) Less samples were collected from Tambon Bang-Sa-Kao, Laem Sing District, Chanthaburi Province, Thailand. Samples were washed with water and cut into small pieces, then prepared as leaves, stem, twig, root and flower, both fresh and dry samples. Dry samples were prepared by drying at 60^{o}C for 2 days in hot air oven. Young fresh leaves were processed into tea leaves by pan firing at 50^{o}C for 2 hours. All samples were macerated two times with 70% ethanol (1:10 of w/v) overnight. The extracts were filtered and concentrated using rotary evaporator at 70^{o}C. Crude extracts were yielded and diluted with double-distilled water into the appropriated concentration.

2.2 Determination of DPPH free radical scavenging activities and total phenolic contents.

DPPH free radical scavenging activities were assayed in triplicate according

to Shimada *et al.* (10) with a little modification. An aliquot of 0.05-0.30 mL of crude extract was mixed with 4.5 mL of 0.04 mg/mL DPPH. The mixtures were diluted with double-distilled water into 5 mL. After standing for 20 minutes (obtained from the kinetic behavior of DPPH free radical scavenging activities of Trolox and samples), the absorbance was determined at 515 nm. Free radical scavenging activities were expressed in term of EC_{50} and Trolox equivalent antioxidant capacity (TEAC) calculated from linear regression analysis of the standard curve performed between 0.001-0.006 mg/mL of Trolox.

Total phenolic contents were determined in triplicate using a Folin-Ciocalteu phenol reagent according to Wong *et al*. (11) with a little modification. An aliquot of 2 mL of crude extract was mixed with 5 mL of 10% Folin-Ciocalteu phenol reagent. After the 3 minutes, 2 mL of 7.5% Na_2CO_3 was added, then left standing for an hour in dark at room temperature. The absorbance was measured at 765 nm. Total phenolic contents were expressed as Gallic acid equivalent per gram of extract (mg GAE/g extract).

2.3 Determination of antibacterial activities and minimal inhibitory concentration (MIC)

The tested bacteria; *Bacillus cereus*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Salmonella typhimurium* were cultured at 37°C overnight in Nutrient Agar (NA) medium. Antibacterial activities of extracts were performed in triplicate by using disc diffusion method with 5 mm diameter discs. The diameter of inhibition zone was measured after 24 hours of incubation. The minimal inhibitory concentration (MIC) of extracts was determined using the agar microdilution method and disc diffusion method (12, 13) with a minor modification. The lowest concentration without an inhibition zone was defined as the concentration that completely inhibited bacterial growth. The data were compared with the inhibition capacity of 1 mg/mL Penicillin G.

2.4 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean of triplicate±standard deviation. The differences among samples were determined by t- test at a level of $P<0.05$ of significance.

3. Results and discussion

3.1 DPPH free radical scavenging activities and total phenolic contents.

The results of DPPH free radical scavenging activities and total phenolic contents of ethanolic extracts of *Pluchea indica* (L.) Less are shown in Table 1. The antioxidant activities were expressed as EC_{50} (the concentration required to decrease free radicals by 50%) and Trolox equivalent per gram of fresh or dry samples (TEAC). The EC_{50} values of the crude extract varied from 0.16±0.001 mg/mL (fresh root extract) to 3.58±0.024 mg/mL (dry leaves extract), while that of tea leaves extract was 0.28±0.012 mg/mL, compared with 0.00335±0.000 mg/mL of Trolox. The TEAC values of the crude extract ranged from 0.92±0.006 mg/g (dry leaves extract) to 20.02±0.177 mg/g (fresh root extract). The lower EC_{50} value, the greater free radical scavenging capacity. The results found that fresh root extract had the lowest EC_{50} value, indicating that this extract was the most active in DPPH radical scavenging. Antioxidative activities of most

fresh samples were significantly higher than that of dry samples (P<0.05). It would be due to the decrease of some antioxidants, such as tannins and flavonoid quercetin, during drying at higher temperature for a long period (60°C for 2 days). These antioxidants were decreased easily by thermal treatment (14, 15).

Table 1. Antioxidant activities and total phenolic contents in *Pluchea indica* (L.) Less. Samples*. (n = 3)

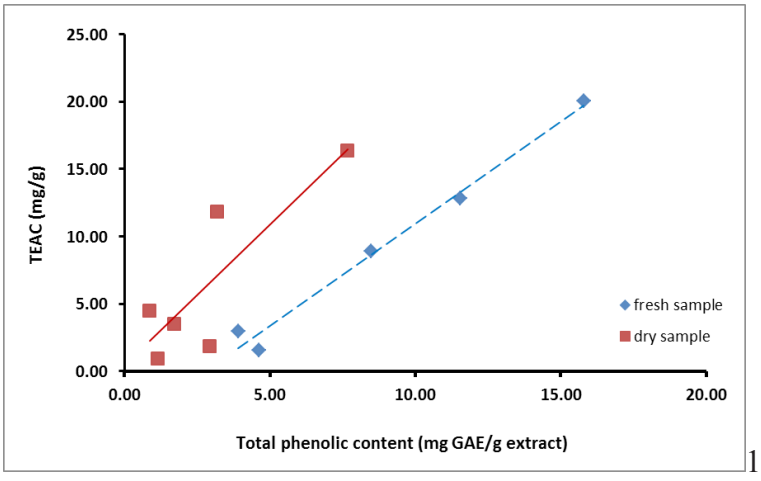
Sample	Type	Crude extract (mg ext/g samples)	DPPH radical scavenging activities		Total phenolic content (mg GAE/g)
			EC_{50} (mg/mL)	TEAC (mg/g)	
Leaves	fresh	44.42	1.12 ± 0.007[a]	2.94 ± 0.018[a]	3.91 ± 0.004[a]
	dry	270.06	3.58 ± 0.024[b]	0.92 ± 0.006[b]	1.13 ± 0.004[b]
Stem	fresh	22.41	0.26 ± 0.001[c]	12.85 ± 0.063[c]	11.55 ± 0.011[c]
	dry	30.63	0.20 ± 0.024[c]	16.37 ± 2.032[d]	7.68 ± 0.023[d]
Twig	fresh	32.16	0.37 ± 0.003[d]	8.89 ± 0.072[e]	8.47 ± 0.016[e]
	dry	146.79	0.73 ± 0.007[e]	4.52 ± 0.045[f]	0.87 ± 0.003[f]
Root	fresh	32.14	0.16 ± 0.001[f]	20.02 ± 0.177[g]	15.79 ± 0.008[g]
	dry	153.19	1.80 ± 0.174[g]	1.85 ± 0.171[h]	2.92 ± 0.009[h]
Flower	fresh	33.70	2.15 ± 0.125[h]	1.54 ± 0.087[i]	4.63 ± 0.013[i]
	dry	45.99	0.94 ± 0.084[i]	3.53 ± 0.304[j]	1.69 ± 0.013[j]
Tea leaves	-	331.72	0.28 ± 0.012	11.86 ± 0.519	3.18 ± 0.012

* Different superscript letters ([a], [b], [c],…) in the same columns represent significant differences (P<0.05).

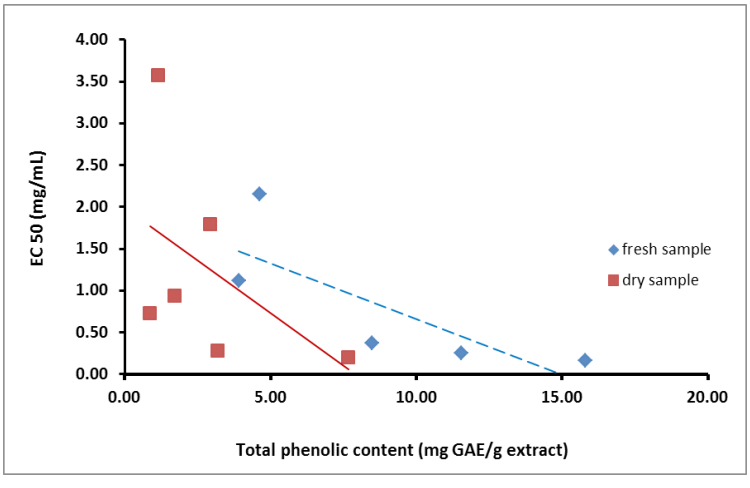
On the other hand, radical scavenging activities of tea leaves extract was higher than that of fresh and dry leaves significantly (P<0.05). It could be explained by the formation of Maillard reaction products that could promote the antioxidant activities (16) during heating at lower temperature for the shorter time (50°C for 2 hours).

The radical scavenging activities of the extracts were related to the electron or hydrogen donating ability of phenolic compounds (17). The amount of total phenolic compounds of *P. indica* extracts were determined according to the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE) per gram of plant extract. Table 1 shows the contents

of total phenolics ranging from 0.87± 0003 mg GAE/g (dry twig extract) to 15.99±0.008 mg GAE/g (fresh root extract). The amounts of total phenolic compounds of fresh samples were significantly different (P<0.05) when compared to that of dry samples. Phenolic compounds are highly effective free radical scavengers (18). In general, plant extract that has high content of phenolics also has high antioxidant activities. Previous studies indicated that *P. indica* had the high scavenging capacities not only due to hydroxyl groups existing in the phenolic compounds, such as tannins, saponins, flavonoid quercetin and proanthocyanins, but also non-phenolic compounds such as triterpenes and thiophene derivatives (5, 8, 9).



1A



1B

Figure 1. Relationships between total phenolic contents and TEAC (1A) and EC_{50} (1B) in fresh and dry samples among various parts of *Pluchea indica* (L.) Less. samples. (n = 3)

With reference to figure 1, the results showed excellent relationships for linear correlation between total phenolic contents and TEAC values (r^2=0.9849 and 0.7189 for fresh and dry samples, respectively; figure 1A) but fairly correlation with EC_{50} (r^2=0.6154 and 0.2458 for fresh and dry samples, respectively; figure 1B). It ensured that root extract was the most active in radical scavenging among other parts of plants and tea leaves. Andarwulan *et al*. (19) reported that crude aqueous extract of *P. indica* root was 78.9 ±0.6 mg GAE/g dry weight and many studies also reported in the same ways (2, 3).

Table 2. Antibacterial activities assessed by the Disc diffusion method of *Pluchea indica* (L.) Less extracts compared with 1 mg/mL Penicillin G (PG) *. (n = 3)

Sample \ Bacteria		Inhibition zone diameter (mm)				
		Bacillus cereus	***Escherichia coli***	***Pseudomonas fluorescens***	***Staphylococcus aureus***	***Salmonella typhimurium***
Leaves	fresh	0.20 ± 0.100	-	0.20 ± 0.100	-	-
	dry	-	-	-	0.93 ± 0.153	0.70 ± 0.100
Stem	fresh	4.37 ± 0.115^{a}	0.13 ± 0.058^{a}	0.77 ± 0.153^{a}	1.27 ± 0.115^{a}	1.67 ± 0.153^{a}
	dry	4.30 ± 0.100^{a}	0.14 ± 0.053^{a}	2.73 ± 0.208^{b}	1.10 ± 0.173^{b}	0.73 ± 0.058^{b}
Twig	fresh	2.80 ± 0.100^{b}	0.20 ± 0.000	1.93 ± 0.379^{c}	1.10 ± 0.100^{c}	1.23 ± 0.058^{c}
	dry	1.17 ± 0.153^{c}	-	1.97 ± 0.306^{c}	1.00 ± 0.100^{c}	1.33 ± 0.153^{c}
Root	fresh	9.53 ± 0.306^{d}	0.60 ± 0.173	6.67 ± 0.208^{d}	3.17 ± 0.058	3.80 ± 0.100^{d}
	dry	0.10 ± 0.100^{e}	-	0.40 ± 0.000^{e}	-	0.77 ± 0.058^{e}
Flower	fresh	0.83 ± 0.058	-	0.73 ± 0.208	-	0.83 ± 0.058^{f}
	dry	-	-	-	-	0.97 ± 0.058^{g}
Tea leaves	-	5.27 ± 0.208	0.17 ± 0.058	2.40 ± 0.300	1.00 ± 0.173	4.50 ± 0.100
PG	-	4.37 ± 0.252	0.23 ± 0.058	1.23 ± 0.153	5.03 ± 0.058	10.10 ± 0.100

* Different superscript letters (^{a}, ^{b}, ^{c},…) in the same columns represent significant differences (P<0.05).

Table 3. Minimal inhibitory concentrations (MICs) assessed by Agar microdilution and Disc diffusion method of *Pluchea indica* (L.) Less extracts compared with 1 mg/mL Penicillin G (PG)*. (n = 3)

Sample \ Bacteria		Minimal inhibitory concentrations (mg/mL)				
		Bacillus cereus	***Escherichia coli***	***Pseudomonas fluorescens***	***Staphylococcus aureus***	***Salmonella typhimurium***
Leaves	fresh	33.07	-	16.54	33.07[a]	-
	dry	-	-	-	83.04[b]	41.52
Stem	fresh	0.94[a]	-	7.51[a]	1.88[c]	1.88[a]
	dry	1.54[b]	24.56	1.54[b]	6.14[d]	12.28[b]
Twig	fresh	2.70[c]	21.59[a]	2.70[c]	2.70[e]	2.70[c]
	dry	6.19[d]	24.75[b]	3.09[d]	6.19[f]	12.38[d]
Root	fresh	0.16[e]	5.12	0.16[e]	0.32	0.32[e]
	dry	40.15[f]	-	40.15[f]	-	20.08[f]
Flower	fresh	8.43	-	8.43	16.86[g]	8.43[g]
	dry	-	-	-	18.77[h]	9.39[h]
Tea leaves	-	0.91	58.35	7.29	14.59	3.65
PG	-	0.02	1.00	0.02	0.13	0.03

* Different superscript letters ([a], [b], [c],…) in the same columns represent significant differences ($P < 0.05$).

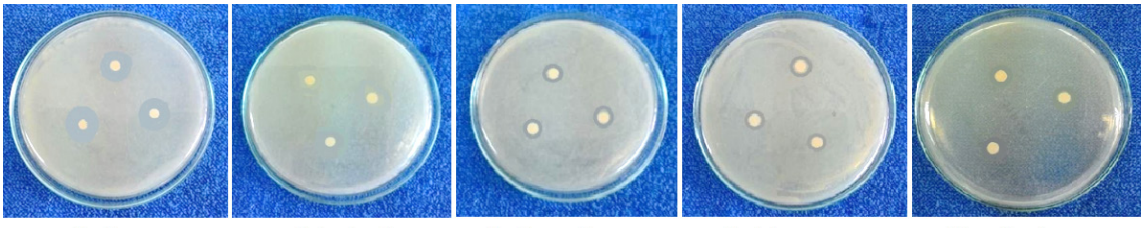


Figure 2. Inhibition zones of fresh root extract.

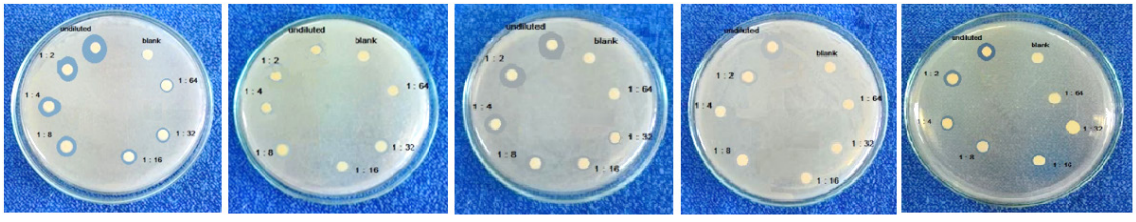


Figure 3. MICs test of fresh root extract.

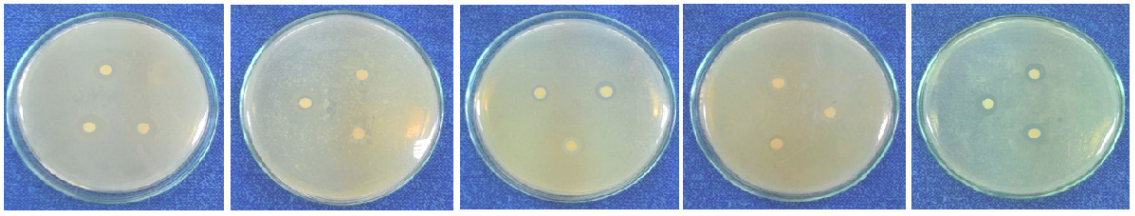


Figure 4. Inhibition zones of tea leaves extract.

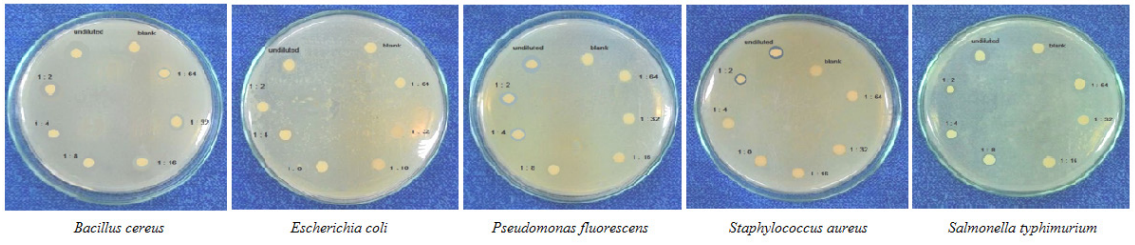
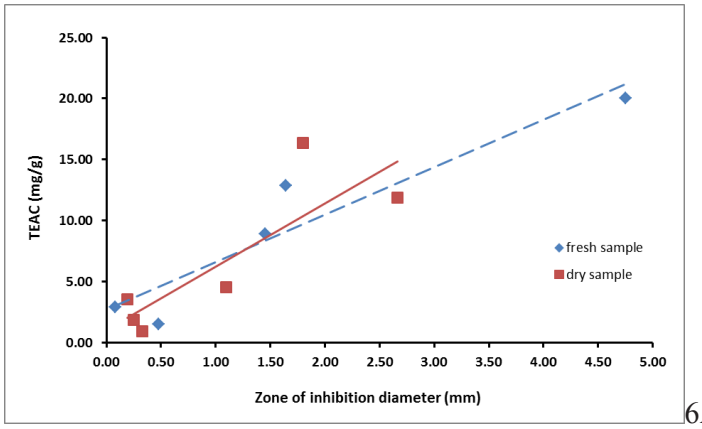


Figure 5. MICs test of tea leaves extract.

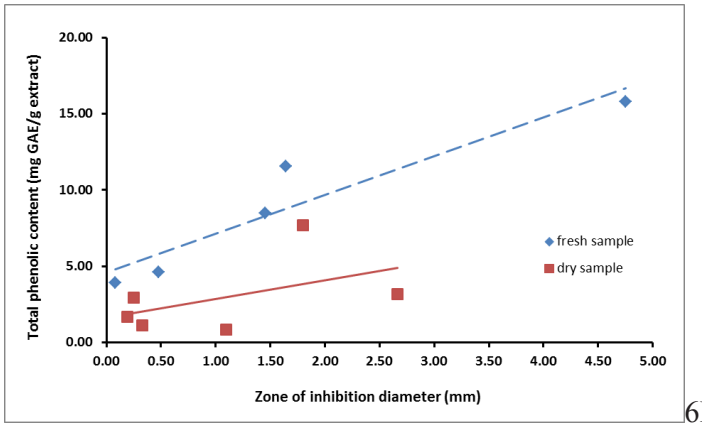
3.2 Antibacterial activities and minimal inhibitory concentration (MIC)

Antibacterial activities of *Pluchea indica* (L.) Less extracts were expressed from the diameter of inhibition zone surrounding the disc. Table 2 shows the antibacterial activities assessed by the disc diffusion method. From the results, fresh root extract showed the most excellent inhibition potential against *Bacillus cereus*, *Pseudomonas fluorescens* and *Salmonella typhimurium* (figure 2). All of investigated bacteria were inhibited by the extract of fresh root, fresh twig, dry stem and tea leaves, while the extract of fresh and dry flower, fresh and dry leaves, and dry root showed less inhibition. Inhibitory activities against *Escherichia coli* were considerably low. The minimal inhibitory concentrations (MICs) of extracts shown in Table 3 revealed significant inhibition activities even at 2 – 16 fold dilutions, with the fresh root extract (64 fold dilutions) as the most effective. MICs of fresh root extract were low as 0.16, 5.12, 0.16, 0.32 and 0.32 mg/mL for *Bacillus cereus, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Salmonella typhimurium*, respectively (figure 3). Tea leaves extract also showed high inhibitory capacities (figure 4). MICs of tea leaves extract were 0.91, 58.35, 7.29, 14.59 and 3.65 mg/mL for *Bacillus cereus, Escherichia coli, Pseudomonas fluorescens, Staphylococcus*

aureus and *Salmonella typhimurium*, respectively (figure 5). It was clearly indicated that *P. indica* showed the high antibacterial activities even its concentrations were low.



6A



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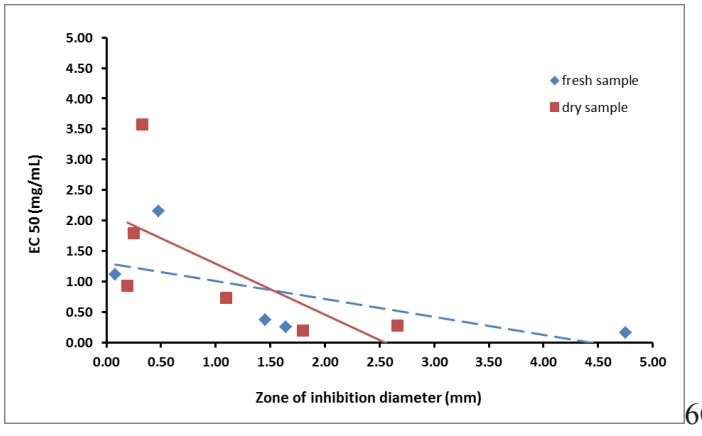

6C

Figure 6. Relationships between zone of inhibition diameter (mean values) and TEAC (6A), total phenolic contents (6B) and EC_{50} (6C) in fresh and dry samples among various parts of *Pluchea indica* (L.) Less. samples. (n = 3)

In addition, positive linear relationships between antibacterial and antioxidant activities were observed (Figure 6). The results showed good relationships between zone of inhibition diameter (mean values) and TEAC values (r^2=0.8904 and 0.7106 for fresh and dry samples, respectively; figure 6A), a fair correlation with total phenolic content (r^2=0.8898 and 0.2430 for fresh and dry samples, respectively; figure 6B) and a little correlation with EC_{50} (r^2=0.4154 and 0.4344 for fresh and dry samples, respectively; figure 6C).

4. Conclusion

In conclusion, the results of this study suggested that *Pluchea indica* (L.) Less extracts showed the high antioxidant and antibacterial activities, especially in root, stem and twig. Positive relationships between antioxidant and antibacterial activities were observed. Moreover, we found that most fresh samples had significantly higher bioactive activities than that of dry samples. But the formation of Maillard reaction products, which were confirmed previously increased the antioxidative properties, might be the reason why tea leaves extract had higher contents of radical scavenging and antibacterial activities than fresh and dry leaves. The antioxidant capacities of *Pluchea indica* (L.) less extracts might be due to the electron or hydrogen donating ability of phenolic compounds. However, even the results illustrated that fresh root extract of the plant had the most antioxidant and antibacterial activities, but consuming as an herbal tea was more pleasurable. It was possible an alternative potential functional food recommended to the consumers.

5. Acknowledgement

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