Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Antioxidant Activity and Allelopathic Potential of *Lippia nodiflora* (L.) Michx on Germination and Growth of *Neptunia javanica* Miq. seeds

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Articleinfo

Article history:
Received: 3 April 2020
Revised: 6 August 2020
Accepted: 17 August 2020

Keywords:
Lippia nodiflora (L.) Michx., *Neptunia javanica* Miq., Antioxidant activity, Allelopathic potential

Abstract

The research objective was to evaluate the antioxidant activity, the allelopathic potential on germination growth of *Neptunia javanica* Miq. seeds and the total phenolic content (TPC) of crude extracts and fractions from *Lippia nodiflora* (L.) Michx. In this research, the ethanol crude extracts of leaves (LE), stems (SE) and roots (RE) from *L. nodiflora* were subjected to study the antioxidant activity by using DPPH free radical scavenging assay and the allelopathic potential on germination seeds of *N. javanica.* The RE extract had the highest antioxidant activity with IC_{50} value of 136.87 ppm. The allelopathic effect of LE extract at a concentration of 20 mg/mL completely inhibited the seed germination of *N. javanica.* The LE fractions were separated by liquid-liquid partition with hexane and ethyl acetate system to afford the leaf hexane fraction (LH), leaf ethyl acetate fraction (LEtOAc) and leaf hydroethanolic fraction (LW). The antioxidant, TPC and allelopathic activities of all fractions were investigated. The antioxidant activity of LW (158.21) and LEtOAc (175.45) fractions were better than those of LE (296.87) extracts, while LH fraction showed the lowest antioxidant activity at 722.08 ppm. The active allelopathic compounds in LEtOAc at a concentration of 10 mg/mL showed complete inhibition of radicle and hypocotyl on germination seed growth and 50% of seed growth inhibition (IC_{50}) was 3.14 mg/mL. Crude extracts and fractions showed more inhibited radicle growth than hypocotyl growth of *N. javanica* seeds. The TPC of leaves crude extracts and fractions were the highest. Interestingly, the total phenolic content of LEtOAc fraction, was the highest (0.072 mg GAE/g) that was related to the good allelopathic properties, compared with other fractions and crude extracts. Our results indicate that the ethanolic crude extracts and an ethyl acetate fraction of *L. nodiflora* leaves can be applied to weed control, especially for *N. javanica.*

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Introduction

Nowadays, human beings have more awareness of health and the environment. The chemical residue is one of the factors affecting health and the environment. Due to synthetic chemicals used for weeding, some will remain in the soil which affects the environment and human health. There are many ways to reduce chemical pollution; one of them is the natural plant extracts replacement. The effect chemicals from some plants have been released into the environment affected to another plant by the positive or negative is called allelochemical or allelopathy (Rice, 1984; Al-Samarai et al., 2018). Allelochemicals was found in many parts of plant such as leaf, stem, flower, pollen, root and seed, might be useful in agriculture systems (Iqbal et al., 2019). Some important allelochemicals may be flavonoids, alkaloids, terpenoids, steroids, tannins and phenolic compounds (Anwesa & Sanjib, 2015). These compounds have a various biological activity, including antioxidant, antimicrobial and allelopathic activities (Fonseca et al., 2017; Abd El-Gawad et al., 2015; Dacoreggio et al., 2019). The previous researches, the allelochemical from the plant could be extracted and fractioned by using various polarities of a solvent such as water and organic solvent (methanol, ethanol, ethyl acetate, dichloromethane and hexane). Fonseca et al. (2017) research -the ethanol extract and fraction (hexane, dichloromethane, ethyl acetate and hydroethanol) from dry powder leaves *Smilax brasiliensis* Sprengel on the antioxidant and allelopathic activities- showed higher antioxidant activity than BHT (2,6-di-tert-butyl-4-methylphenol) and the allelopathic effect on the growth of onion showed inhibition of hypocotyl and radicle at the lower concentration tested, *S. brasiliensis* Sprengel could be used as a natural antioxidant and herbicide. In another report, Dacoreggio et al. (2019) found that the leaves aqueous extracts of *Psidium cattleianum* Sabine, showed antibacterial, antioxidant and against on germination *Lactuca sativa* seed. The all tested extract show considerable on allelopathic activity, with growth inhibition on seed more than 50%. Teixeira de Oliveira et al. (2019) studied the allelopathic effect of the ethanol extract and dichloromethane fractions of the aerial parts of *Lippia alba*. The dichloromethane fraction extract from the aerial part of *L. alba* showed inhibiting the growth of *L. sativa* (lettuce) and *Allium cepa* (onion) seeds.

Lippia nodiflora (L.) Michx. synonym *Phyla nodiflora* (L.) Greene (Verbenaceae family) has a few common names such as lippie, frog fruit, bukkan, jaipapli (Hindi), busbusi, chachahan (Philippines) and vernacular names in Thai also known as ya kelt pla. (Sharma & Singh, 2013; Stuartxchange, 2018) or yaa riet pla (Al-Snafi, 2019). *L. nodiflora* is a small creeping perennial medicinal herb, small white to pink flowers, roots sprout from some stem nodes with growing rapidly to the ground covering on sandy loams, sand, gravel, clay loams soil in floodplains or wetlands together with capable on fixating sand and conserving earth and water (Fu et al., 2013). The deep rooting system of this plant is better to hold soil than grass. *L. nodiflora* is well known for applying to prevent sand or soil surface from the riverbank erosion. This plant is a fast-growing and resistant to sunlight so we can see it throughout India, Sri Lanka, Tropical Africa, Ceylon, Baluchistan, South and Central America, Taiwan and The Philippines (Elakovich & Stevens, 1985; Sharma & Singh, 2013; Sharma, 2018). In Thailand, *L. nodiflora* can be found in many provinces such as Nakhon Ratchasima, Kanchanaburi, Lampang, Mae Hong Son, Khon Kaen, Kanchanaburi, Nakhon Sawan and Phetchaburi (BGO Plant Databases, 2013a). *L. nodiflora* is a traditional medicinal plant boiled dried stems in water for a cough remedy, and when the patient is coughing up blood. A bandage using a poultice of stems is able to treat chronic ulcers and cure blister (Medthai, n.d.). Some researchers have described chemical constituents, antimicrobial, antioxidant activities, anticancer properties, including allelopathic activities of *P. nodiflora* L. or *L. nodiflora* L. The aerial part of *L. nodiflora* contains many bioactive components e.g. antioxidant (Ashokkumar et al., 2008). Lin et al. (2014) reported that the HPLC-fingerprint and antioxidant constituents of flavone pure compounds were isolated from dried aerial part *P. nodiflora* methanol extract. The pure compounds such as onopordin, cirsiliol and eupafolin displayed a strong antioxidant activity with DPPH scavenging assay. The aerial part of *P. nodiflora* was extracted with hexane, chloroform, ethyl acetate, and methanol extract by using a soxhlet extractor. Preliminary screening of phytochemicals was implemented for different extracts of *P. nodiflora.* The ethyl acetate extract from aerial parts showed dominant antimicrobial and antifungal activity (Priya & Ravindhran, 2015). Liau et al. (2017) studied on antioxidant and anticancer properties of solvent partitioned extracts from leaves and stems of *P. nodiflora* L. The extracts were obtained using methanol, ethyl acetate and water fractions contained

higher amount of TPC (total phenolic content) than hexane and chloroform fractions. The DPPH radical scavenging activity of this plant increased in a concentration level depends on leaves and stems extracts. The EC_{50} value, leaves methanol extract showed higher antioxidant activity compared to stems methanol extract. The allelopathic effectiveness of *L. nodiflora*, from some researches on various seeds inhibited germination. Fu et al. (2013) reported that the investigation on the allelopathic effect on three crops as wheat, radish and rape with 4% and 1% aqueous extract of fresh aerial part of *P. nodiflora*. The result of this research showed allelopathic effect of inactive aqueous extract on the three crops, so this plant will not be harmful on wheat, radish and rape growth. Ali et al. (2019) reported that allelopathy of leaves leachates *L. nodiflora* 10 mg in sandwich method showed non effect on growth of *Lactuca sativa* L. (lettuce) seed.

From the researches mentioned above, the antioxidant and allelopathy activities of *L. nodiflora* have not been reported. Thus, the main objective of this study is to determine the DPPH radical scavenging, total phenolic content and allelopathy capacity of crude extracts and fractions of *L. nodiflora*. This will be the potential way of allelopathy on weed *Neptunia javanica* Miq. seed.

Materials and methods

1. Chemical reagents

All chemical reagents used in this study are analytical grade (AR). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Gallic acid monohydrate of Sigma-Aldrich, Folin-Ciocalteu's RS reagent, L-ascorbic acid (Vitamin C) of CARLO ERBA, Hexane, Ethyl acetate, Ethanol of RCI Labscan, Sodium Carbonate (Na_2CO_3) of KEMAUS.

2. Instruments

The determination of DPPH activity and total phenolic content were measured by a Jasco V-650 series UV-Visible spectrophotometer (JASCO international Co., Ltd., Thailand). The preparation of crude extracts was removed solvent to dryness with vacuum rotary evaporator (Rotavapor R-210, Heating bath B491, Vacuum pump V-700, CTL911) of BÜCHI, Thailand.

3. Plant materials

Lippia nodiflora (L.) Michx. and seed of *Neptunia javanica* Miq. were collected from Bang Bo District in Samutprakarn Province. The *L. nodiflora* (L.) and *N. javanica* Miq. were identified by comparing with herbarium database of Botanical Garden Organization (BGO) Plant Database, The Botanical Garden Organization, Chiang Mai, Thailand. *L. nodiflora* (L.) Michx. in the other name *P. nodiflora* (L.) Greene is a herbarium database of QBG No. 62171 and *N. javanica* Miq. is QBG No. 112212 (BGO Plant Databases, 2013b). Voucher specimen of *L. nodiflora* (L.) Michx. (WM/LP 1/2019) and *N. javanica* Miq. (WM/NJ 1/2019) were kept at the Division of Chemistry, Rajamangala University of Technology Krungthep, Bangkok, Thailand.

4. Preparation of plant materials

Plant material *L. nodifloa* (L.) Michx. (Fig. 1) was cleaned and dried under shade at room temperature and then separated into three parts of the plant as leaves, stems and roots. The stems and roots were made into small pieces approximately 2 cm each. Each part of plant was dried in a hot air oven at 50°C for 8 hrs. and ground by house blender.

Fig. 1 The morphological feature of *Lippia nodiflora* (L.) Michx

5. Extraction of plant

The fine leaves, stems and roots of *L. nodiflora* were soaked in ethanol at a ratio of dried plant per solvent (1:5 g/mL) at ambient temperature for 72 hrs. The ethanol extracts were filtrated with Whatman No.1 filter paper, the residue was repeatedly extracted for 4 times. The filtrates were combined and evaporated by using a vacuum rotary evaporator to afford sticky crude extracts of leaves ethanol (LE), stems ethanol (SE) and roots ethanol (RE) crude extracts. These crude extracts were reserved in a refrigerator at 4°C until further use.

6. Antioxidant activity of ethanol crude extracts

DPPH (2,2'-Diphenyl-1-picrylhydrazyl) radical scavenging assay: the DPPH radical scavenging activities of leaves ethanol crude extract (LE), stems ethanol crude extract (SE) and roots ethanol crude extract (RE) were tested according to Sudha & Srinivasan (2014) briefly 1 mL of each different concentration sample (62.5-1000

ppm) added to 3 mL of 0.1 mM of freshly prepared DPPH solution. The reaction mixtures were mixed and incubated in dark condition for 30 minutes at room temperature. The decrease of absorbance at 517 nm was determined by using a UV-Vis spectrophotometer (Jasco V-650 spectrophotometer). The IC_{50} (the concentration of specimen required to reduce the absorbance of DPPH by 50%) was calculated graphically. The percentage inhibition of the DPPH radical was calculated as following:

$$\text{DPPH radical scavenging activity (\%)} = 1 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

7. Allelopathic activity of ethanol crude extracts

The *Neptunia javanica* Miq. (Phak Kased Kok) seeds (Fig. 2) preparation for seed germination assay was prepared accordingly to the method of Yongvanich et al. (1997). In brief, the seeds were cleaned with distilled water and soaked in warm water (50-60°C) for 4 minutes, replaced the warm water with distilled water and kept at the room temperature for 24 hrs. The swollen seeds were selected for allelopathic assay.

Fig. 2 The botanical characteristic and seeds of *Neptunia javanica* Miq

This allelopathic activity of ethanolic extracts from leaves (LE), stems (SE), and roots (RE) were tested as described by Kalegari et al. (2012). The various concentrations of crude extracts (2.5, 5, 10 and 20 mg/mL) were dissolved in ethanol. The filter paper (Whatman No.1) was placed in the Petri dish (9 cm diameter, 2 cm height) and added 5 mL of crude extracts and fractions in each plate. After that, the solvent was let to evaporate within 24 hrs. at room temperature and each dish was added 5 mL of distilled water. The swollen *N. javanica* seeds were used for each experiment consisting of 10 germination seeds placed on filter paper in a Petri dish then kept in the dark place, triplicated for each treatment. Moreover, a control treatment was designed with distilled water and ethanol AR. The anti-germination activity could be calculated by using the following formula (Fonseca et al., 2017).

$$G\% = \frac{(M_a - M_c)}{M_C} \times 100$$

G%= Growth percentage, M_a = mean value of seeds, M_c = mean value of the control

$$R\% = \frac{(R_c - R_a)}{R_c} \times 100$$

R% = Radicle inhibition percentage, R_c= mean radicle length value of control, R_a= mean radicle length value of sample

$$H\% = \frac{(H_c - H_a)}{H_c} \times 100$$

H% = Hypocotyl inhibition percentage, H_c= mean hypocotyl length value of control, H_a = mean hypocotyl length value of sample

8. Liquid-liquid partition of leaves ethanol extract and bioactivity

In this study, we focused on leaves crude extract. Due to the effect of LE showed the completely inhibition of seed germination of *N. javanica*. The leaves crude extract for a liquid-liquid partition of ethanolic extract was performed according to Liau et al. (2017) method with different solvent modification. The dissolved dry crude extract 16.43 g in a mixture of ethanol:water (1:1) was added hexane (50 mL) 4 times to separate hexane extract and then added ethyl acetate (50 mL) 4 times to separate ethyl acetate extract. Each part of the solvent layer was removed by vacuum rotary evaporator to obtain hexane fraction extract (LH), ethyl acetate fraction extract (LEtOAc) and hydroethanolic fraction extract (LW), respectively. All fractions were tested antioxidant (62.5-1000 ppm) and allelochemicals (2.5, 5 and 10 mg/mL) potential germination on *N. javanica* seeds.

9. Total phenolic content of crude extracts and leaves fractions

The determination of total phenolic compounds in the samples was performed by using the Folin-Ciocalteu method according to the methodology described by Liau et al. (2017). Briefly, 100 µl (1 mg/mL) each of leaves, stems and roots crude extracts and leaves fractions or standard gallic acid solution was mixed with 4.5 mL of distilled water and 100 µl of Folin-Ciocalteu reagent. After 3 minutes, 300 µl of sodium carbonate solution

(2%w/v in water) was added and mixed. The mixture was kept for 2 hrs. in the dark at room temperature. The absorbance of each sample was measured by the UV-Vis spectrophotometer (Jasco V750) at 760 nm. Total phenolic content (TPC) was expressed as milligram gallic acid equivalents (GAE) per gram extract (mg GAE/g extract) and calculated by a standard curve of gallic acid solution (0, 15.625, 31.25, 62.5, 125, 250, 500, 1000 µg/mL). Each sample was analyzed 3 times.

10. Statistical analysis

The data were analyzed using One-way Analysis of Variance (ANOVA – SPSS Version 17) with Tukey's test and $p<0.05$ was utilized to evaluate significance between samples.

Results and Discussion

1. Extraction of plant

The fine leaves, stems and roots of *L. nodiflora* were soaked in ethanol and repeatedly extracted 4 times to obtain crude extracts as shown in Table 1. The dried powder of leaves (90.00 g), stems (100.00 g) and roots (18.40 g) were extracted by maceration (ratio of dried plant: ethanol; 1:5) for 3 days and repeated 4 times. After the ethanol extractions of three parts were filtered and evaporated under reduced pressure, the extract yields of leaf (LE), stem (SE) and root (RE) were 11.97, 12.22 and 7.02%w/w, sticky black-brown, respectively, as shown in Table 1.

Table 1 Percentage yield of leaves, stems and roots ethanolic crude extracts *L. nodiflora*

Extracts	Percentage yield (%w/w)	Color of crude extract
LE	11.97	Semisolid black green
SE	12.22	Semisolid black green
RE	7.02	Sticky black-brown

2. Antioxidant activity of ethanolic crude extracts

The antioxidant activity of leaves, stems and roots ethanol crude extracts (62.5-1000 ppm) were determined by DPPH radical scavenging activities. The data activity showed in Table 2, the vitamin C (3.125-25 ppm) was represented as a standard. The linear regression standard equation of vitamin C was y=3.8707x-3.9422 with $r^2 = 0.9963$. The IC_{50} of vitamin C was 13.94 ppm. The effect of ethanol solvent extracted from different parts of *L. nodiflora* showed significance ($p<0.05$) on DPPH radical scavenging activity. The IC_{50} value defined as the concentration of an antioxidant that caused a 50% decrease in the DPPH absorbance. The antioxidant activity of RE extracts was higher than those of LE and SE extracts as shown in Table 2. To compare with vitamin C, the antioxidant activity of RE extracts was lower than vitamin C to 9.82 times. The LE extracts had slightly higher antioxidant activity than SE extracts which was correlated to the report of Liau et al. (2017) who studied DPPH scavenging activities from methanol extracts of leaves and stems, and found that antioxidant activity of leaf extracts was higher, compared to stem extracts.

Table 2 DPPH radical scavenging activity in the percentage of ethanolic crude extracts *L. nodiflora*

Conc. (ppm)	DPPH radical scavenging activity (%)		
	Ethanolic crude extracts		
	LE	SE	RE
62.5	11.71±2.83^{e}	11.16±2.83^{e}	27.40±1.97^{d}
125	22.66±0.75^{d}	22.69±1.49^{d}	47.28±2.81^{c}
250	45.52±2.41^{c}	41.75±0.70^{c}	83.30±0.23^{b}
500	80.78±0.43^{b}	78.23±0.60^{b}	86.91±0.20^{ab}
1000	84.91±0.54^{a}	90.60±0.08^{a}	90.81±0.27^{a}
IC_{50} (ppm)	**296.87**	**310.41**	**136.87**

Remark: Values with different letters superscripts are in each column indicate values with significant variation at $P<0.05$ and obtained after round off to two decimal points

3. Allelopathic activity of ethanolic crude extracts

The allelopathic effect of LE, SE and RE extracts on germination of *N. javanica* seeds comparing to the water control for 7 days. The allelochemical activity of LE, SE and RE extracts tested on the growth of *N. javanica* seeds as shown in Table 3. The LE extracts at a concentration of 20 mg/mL showed no growth of seeds (Fig. 3c), while SE and RE extracts showed 80.00% (Fig. 3d) and 33.33% (Fig. 3e) of seed germination, compared to the water and ethanol as controls (Fig. 3a and 3b). The increasing concentrations of crude extracts could significantly more inhibit on seed germination ($p<0.05$). LE, SE and RE extracts also reduced the length of radicle and hypocotyl (Table 3). The radicle inhibition of LE, SE and RE extracts were 50-100, 28-61 and 76-90%, respectively, while hypocotyl inhibition of LE, SE and RE extracts were 13-100, 15-42 and 34-61%, respectively. The LE, SE and RE extracts had more effective on radicle than hypocotyl length growth.

Table 3 The effect of allelopathic activity of ethanolic crude extracts *L. nodiflora* on germination and growth of *N. javanica* seed in day 7

Sample concentration (mg/mL)	Percentage (%) of seed germination (G%)	Radicle length (mm)	Hypocotyl length (mm)	Percentage inhibition (%)*	
				Radicle inhibition (R%)	Hypocotyl inhibition (H%)
Water control	100.00±0.00^{a}	58.86±2.47^{a}	74.75±1.31^{a}	0.00±0.00^{f}	0.00±0.00^{h}
Ethanol control	100.00±0.00^{a}	57.36±2.07^{a}	72.88±1.64^{a}	2.54±3.52^{f}	2.50±2.05^{h}
Leaves extract					
2.5	100.00±0.00^{a}	27.38±2.00^{c}	64.71±1.53^{b}	53.48±3.40^{d}	13.43±2.05^{g}
5	80.00±0.00^{c}	23.80±1.45^{c}	56.45±1.60^{c}	59.56±2.47^{d}	24.48±2.14^{f}
10	73.33±5.77^{c}	22.83±1.80^{c}	50.22±2.92^{de}	61.21±3.06^{d}	32.81±3.91^{de}
20	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{h}	100.00±0.00^{a}	100.00±0.00^{a}
Stems extract					
2.5	96.67±5.77^{a}	42.37±1.76^{b}	63.28±2.07^{b}	28.01±2.99^{e}	15.34±2.78^{g}
5	93.33±5.77^{ab}	40.10±2.22^{b}	55.81±1.28^{c}	31.87±3.78^{e}	25.34±1.72^{f}
10	83.33±5.77^{bc}	24.33±3.39^{c}	53.26±2.66^{cd}	58.66±5.76^{d}	28.75±3.56^{ef}
20	80.00±0.00^{c}	22.46±2.58^{c}	43.07±2.55^{f}	61.84±4.39^{d}	42.38±3.41^{c}
Roots extract					
2.5	100.00±0.00^{a}	14.09±1.33^{d}	48.74±1.14^{de}	76.06±2.26^{c}	34.79±1.53^{de}
5	100.00±0.00^{a}	11.97±1.65^{d}	47.78±2.27^{ef}	79.66±2.82^{c}	36.08±3.04^{cd}
10	100.00±0.00^{a}	10.93±1.42^{de}	46.93±1.43^{ef}	81.43±2.42^{bc}	37.22±1.91^{cd}
20	33.33±5.77^{d}	5.82±1.94^{ef}	28.52±0.96^{g}	90.11±3.30^{b}	61.85±1.29^{b}

Remark: *Comparison to water control. R%=radicle length percentage inhibition, H%=hypocotyl length percentage inhibition. Values represented mean ± S.D. and obtained after round off to two decimal points. Superscripts with different letters are in each column indicate values with significant variation at P<0.05

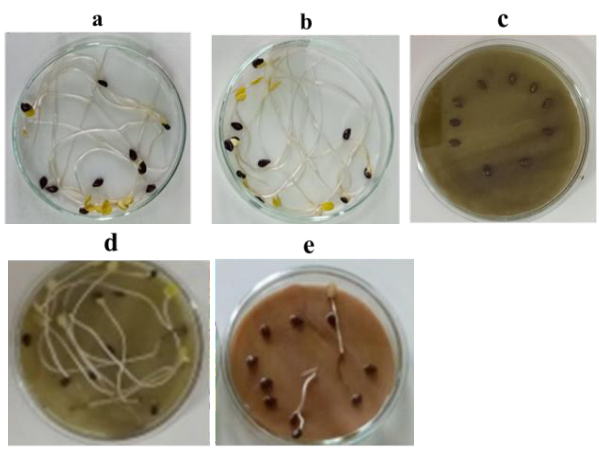

Fig. 3 Germination of *N. javanica* in control and crude extracts of *L. nodiflora* at 20 mg/mL, after 7 day. a=water control, b=ethanol control, c=Leaves ethanol extract (LE), d=Stems ethanol extract (SE), e=Roots ethanol extract (RE)

4. Liquid-liquid partition of leaves ethanol extract and bioactivities

In this study, the effect of LE showed completely inhibited seed germination of *N. javanica.* The further study, the crude extract of leaves (16.43 g) was separated by a liquid-liquid partition with hexane and ethyl acetate to obtain three fractions (hexane fraction; LH, ethyl acetate fraction; LEtOAc and hydroethanolic fraction; LW). The LW (69.22%w/w) had the highest percentage of yields, compared with LH (26.18%) and LEtOAc fractions (2.45%w/w). The physical property of hexane and ethyl acetate fractions were semisolid black green, while hydroethanolic fraction was sticky black-brown (Table 4).

Table 4 Percentage yield and physical property of leaves fractions *L. nodiflora*

Sample	Percentage yield (%w/w)	Physical property of fractions
Leaves fractions		
Hexane (LH)	26.18	Semisolid black green
Ethyl acetate (LEtOAc)	2.45	Semisolid black green
Hydroethanolic (LW)	69.22	Sticky black-brown

5. DPPH radical scavenging activity of leaves fractions

Three fractions (LH, LEtOAc and LW) tested antioxidant activity by DPPH scavenging assay and IC_{50} showed in Table 5. The results showed that the antioxidant activity of LW, LEtOAc and LH fractions (at concentration of 500 ppm) was 92.65, 83.13 and 40.75%, respectively. The effect of high polarity solvent was significantly increased antioxidant activity of extracts ($p<0.05$). The IC_{50} values of LW, LEtOAc and LH fractions were 158.21 ppm, 175.45 ppm and 722.08 ppm, respectively. The IC_{50} of LEtOAc and LW displayed lower value that means higher antioxidant activity than LH. The crude extracts and fractions of *L. nodiflora* had less antioxidant activity than vitamin C (IC_{50} = 13.94 ppm). The Fig. 4 showed IC_{50} DPPH scavenging activity of crude extracts (LE, SE, RE) and leaves fractions (LH, LEtOAc and LW) in comparison with vitamin C as a standard. The degrees of DPPH scavenging activity from highest to lowest were vitamin C>RE> LW>LEtOAc>LE>SE>LH. The LEtOAc (175.45 ppm) fractions showed antioxidant activity more than LE (296.87 ppm), which is similar to the report of Sudha & Srinivasan (2014) who studied DPPH scavenging activities of ethyl acetate fraction (26.06 µg/mL) and found higher antioxidant activity of LEtOAc than methanol extracts of aerial part (24.66 µg/mL) of *L. nodiflora.*

6. Allelopathic activity of leaves fractions

From the 100% of anti-germination of LE (20 mg/mL of concentration) as shown in Fig. 3, LE was fractionated to obtain hexane fraction (LH), ethyl acetate fraction (LEtOAc) and hydroethanolic fraction (LW). Three fractions of LH, LEtOAc and LW at various concentrations

Table 5 DPPH radical scavenging activity in percentage of leaves fractions *L. nodiflora*

Conc. (ppm)	DPPH radical scavenging activity (%)		
	Fraction extracts of leaves		
	LH	LEtOAc	LW
62.5	3.12±0.27^{e}	22.07±0.65^{d}	24.84±1.58^{d}
125	12.02±0.76^{d}	39.62±0.78^{c}	42.72±0.82^{c}
250	22.29±0.53^{c}	67.17±2.10^{b}	72.99±0.53^{b}
500	40.75±1.03^{b}	83.13±1.35^{a}	92.65±0.29^{a}
1000	65.01±0.26^{a}	-	-
IC_{50} (ppm)	**722.08**	**175.45**	**158.21**

Remark: Values with different letters superscripts are in each column indicate values with significant variation at P<0.05 and obtained after round off to two decimal points

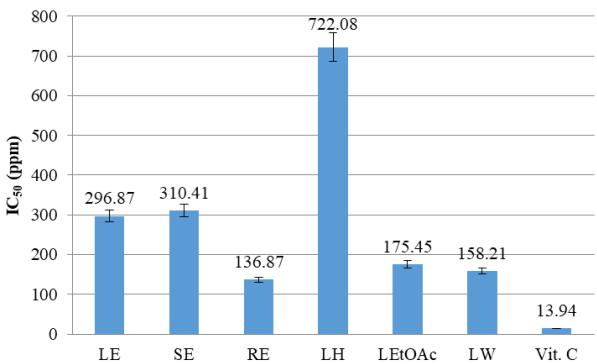

Fig. 4 IC_{50} values (ppm) of the DPPH radical scavenging activity of crude extracts, leaves fractions *L. nodiflora* and the vitamin C (standard)

of 2.5, 5.0 and 10 mg/mL were tested germination on *N. javanica* seeds for 7 days. The results showed the different level of inhibitory activity to growth of *N. javanica* radicle and hypocotyl (Fig. 5). The LH and LW fractions did not effect seed germination, while 10, 5 and 2.5 mg/mL of LEtOAc could significantly reduce seed germination to 0%, 33.33% and 60%, respectively (Fig. 6B), compared to the water control ($p<0.05$). The inhibiting effect of LH, LEtOAc and LW fraction concentration display in Table 6 on length of radicle and hypocotyl growth in percentage. The LH fraction presented 69-73% of radicle inhibition (R%) and 36-40% of hypocotyl inhibition (H%), R% and H% activities of LW fraction were 75-78 and 27-30%, respectively, in comparison to the water control (Table 6). All concentrations of leaf fractions showed inhibitory activity on the growth of radicle more than those of hypocotyl. The inhibition activity on seed germination, length of radicle and hypocotyl growth of LH, LEtOAc and LW fractions at 10 mg/mL concentration were compared to water control is shown in Table 6 and Fig. 5. From the assumption, the LEtOAc may have alkaloid, flavonoid, terpenoids and glycosides in leaves of *L. nodiflora* (Tamilselvi et al., 2018) and these compounds had allelopathic activity. From this research, LEtOAc significantly inhibited *N. javanica* weed. The LEtOAc showed completely inhibited seed germination at lower concentration (10 mg/mL showed Fig. 6B) than LE (20 mg/mL showed Fig. 6A). The LEtOAc displayed inhibitory activities on radicle length (1.63 times showed in Fig. 7A) and hypocotyl growth (3.05 times showed in Fig. 7B) than LE at 10 mg/mL. The active ingredient of leaves ethanol crude extract increased allelopathic activity after partition with ethyl acetate.

Table 6 The effect of allelochemical activity of hexane, ethyl acetate and hydroethanolic leaves fractions *L. nodiflora* on germination and growth of *N. javanica* seed in the day 7

Sample concentration (mg/mL)	Percentage (%) of seed germination	Radicle length (mm)	Hypocotyl length (mm)	Percentage (%) inhibition*	
				Radicle inhibition (R%)	Hypocotyl inhibition (H%)
Water control	100.00±0.00^{a}	58.86±2.47^{a}	74.75±1.31^{a}	0.00±0.00^{e}	0.00±0.00^{g}
Ethanol control	100.00±0.00^{a}	57.36±2.07^{a}	72.88±1.64^{a}	2.54±3.52^{e}	2.50±2.20^{g}
LH					
2.5	100.00±0.00^{a}	17.94±0.00^{b}	47.57±0.91^{cd}	69.52±0.07^{d}	36.37±1.23^{de}
5.0	100.00±0.00^{a}	16.55±2.11^{bc}	44.45±1.68^{de}	71.88±3.59^{cd}	40.54±2.25^{cd}
10.0	96.67±5.77^{a}	15.49±1.87^{bc}	44.20±1.50^{de}	73.68±3.19^{cd}	40.86±2.01^{cd}
LEtOAc					
2.5	60.00±0.00^{b}	7.22±2.21^{d}	39.10±2.84^{e}	87.73±3.7^{b}	47.69±3.79^{c}
5.0	33.33±5.77^{c}	1.39±0.46^{e}	14.29±2.46^{f}	97.64±0.79^{a}	80.88±3.29^{b}
10.0	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{g}	100.00±0.00^{a}	100.00±0.00^{a}
LW					
2.5	100.00±0.00^{a}	16.76±2.54^{bc}	54.30±2.80^{b}	71.58±4.32^{cd}	27.36±3.75^{f}
5.0	100.00±0.00^{a}	13.39±0.56^{bc}	52.55±2.38^{bc}	77.23±0.96^{c}	29.69±3.19^{ef}
10.0	100.00±0.00^{a}	12.80±2.08^{c}	51.96±1.47^{bc}	78.25±3.54^{c}	30.49±1.97^{ef}

Remark: *Comparison to water control. LH=Leaves hexane fraction, LEtOAc=Leaves ethyl acetate fraction, LW=Leaves hydroethanolic fraction, R%= radicle length percentage, H%= hypocotyl length percentage. Values represented mean ± S.D. and obtained after round off to two decimal points. Superscripts with different letters are in each column indicate values with significant variation at P<0.05

7. Total phenolic content of crude extracts and leaves fractions

The total phenolic contents (TPC) of extracts (leaves, stems, roots) and leaves fractions (hexane, ethyl acetate and hydroethanolic) from the *L. nodiflora* expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g extract) and showed various significant ($p<0.05$) in different extracts and fractions, and ranged from 0.011±0.000 to 0.072 mg GAE/g. The regression equation of standard curve of gallic acid was $y = 1.0045x - 0.0015$ with $r^2 = 0.9971$. The results of TPC of extracts and fractions were showed in Table 7. In the present study,

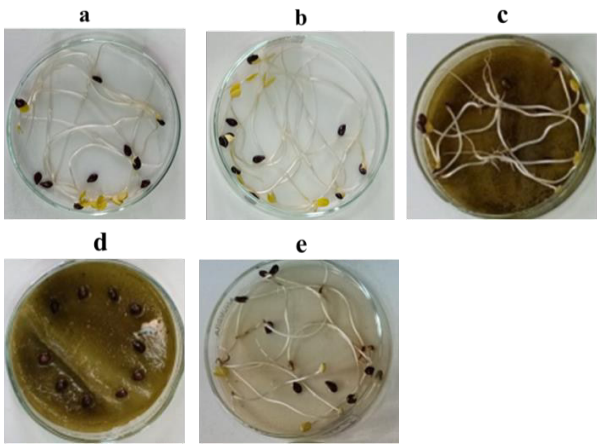

Fig. 5 Germination of *N. javanica* seeds in control and fractions of *L. nodiflora* at 10 mg/mL, a=water control, b=ethanol control, c=Leaves hexane fraction (LH), d=Leaves ethyl acetate fraction (LEtOAc), e=Leaves hydroethanolic fraction (LW)

the ethyl acetate fraction (LEtOAc) and hexane fraction (LH) had levels of TPC at 0.072 and 0.011 mg GAE/g, respectively (Table 7), which similar to the report of Liau et al. (2017) who studied TPC. The ethyl acetate fraction and hexane fraction had the total phenolic contents at 0.081 and 0.020 mg GAE/g, respectively.

Conclusion

In this study, *Lippia nodiflora* collected from Bang Bo in Samutprakarn Province showed biological activity on antioxidant, allelopathy and total phenolic content (TPC). The roots extract (RE) showed higher antioxidant and TPC than leaf (LE) and stem extracts (SE). The RE and SE had no effect on germination of *N. javanica* seeds, while LE showed lower antioxidant activity and TPC than those of RE. However, LE exhibited allelopathic activity on germination and seed growth. The leaves ethyl acetate fraction (LEtOAc) of

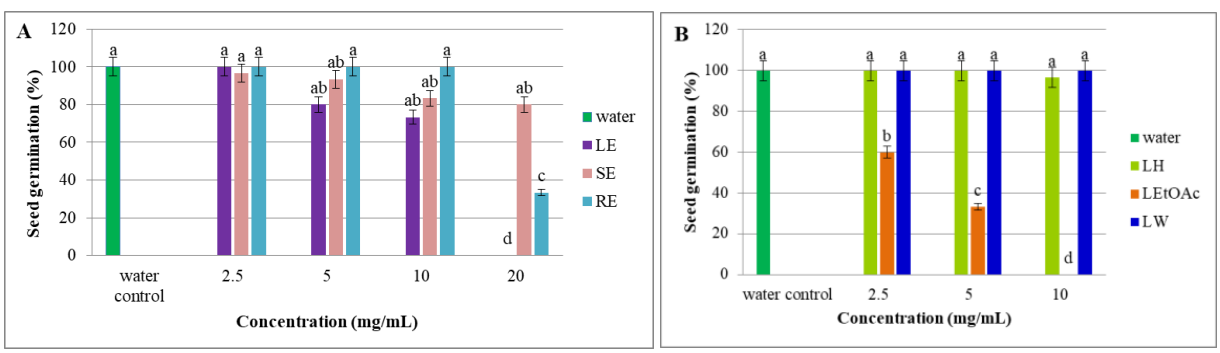

Fig. 6 Percentage of seeds germination on *N. javanica* of crude extracts and leaves fractions of *L. nodiflora.* (A) = Seed germination percentage of crude extracts, (B) Seed germination percentage of fraction extracts. Different letters indicate significant difference (Tukey's test, $p<0.05$)

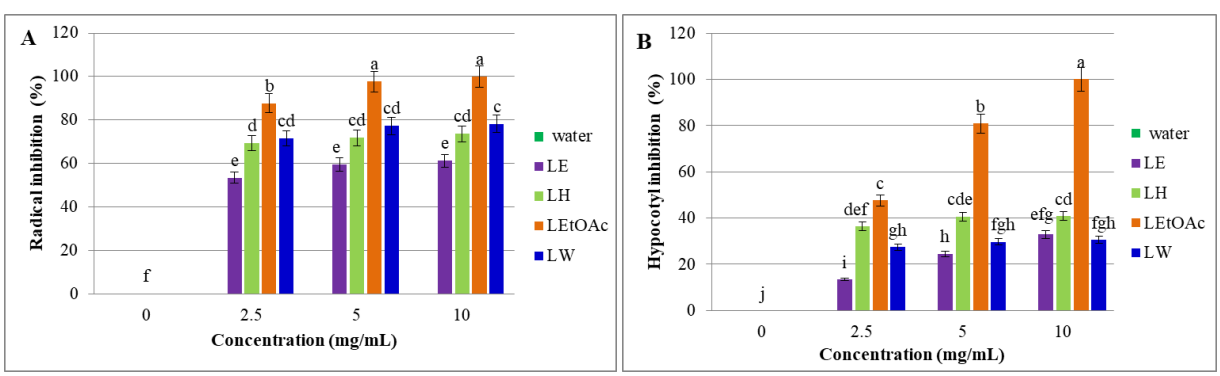

Fig. 7 Effect of four concentration of *L. nodiflora* leaves crude extract and fractions on germination of *N. javanica* seeds. (A) = Radicle growth inhibition percentage, (B) Hypocotyl growth inhibition percentage. Different letters indicate significant difference (Tukey's test, $p<0.05$)

Table 7 Total phenolic content of ethanolic extract from leaves, stems, roots and fractions from leaves consisting of hexane, ethyl acetate and hydroethanolic

Sample	TPC (mg GAE/g)
Leaves ethanol crude extract (LE)	0.032±0.001[d]
Leaves hexane fraction (LH)	0.011±0.000[f]
Leaves ethyl acetate fraction (LEtOAc)	0.072±0.001[a]
Leaves hydroethanolic fraction (LW)	0.046±0.001[c]
Stems ethanol crude extract (SE)	0.022±0.000[e]
Roots ethanol crude extract (RE)	0.053±0.000[b]

Remark: Values with different letters superscripts are in each column indicate values with significant variation at P<0.05 and obtained after round off to three decimal points

L. nodiflora had the highest total phenolic contents and antioxidant and allelopathic activities, compared with other fractions and crude extracts. The leaves crude extract (LE) at least 20 mg/mL and leaves ethyl acetate fraction (LEtOAc) at least 10 mg/mL will be recommended to reduce seed germination and inhibit radicle and hypocotyl growth of *Neptunia javanica* weed. Both LE and LEtOAc should be an optional application in control of *N. javanica* seed and other weeds. To further study, LEtOAc of *L. nodiflora* will be isolated and characterized allelochemicals to specify allelophatic activities on weeds.

Acknowledgments

The instruments and laboratory room for this work was supported by Department of Chemical Technology, Faculty of Science and Technology, Suan Dusit University and Division of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Krungthep.

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