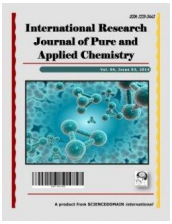


International Research Journal of Pure & Applied Chemistry
8(2): 73-80, 2015, Article no.IRJPAC.2015.075
ISSN: 2231-3443

SCIENCEDOMAIN *international*
www.sciencedomain.org

Identification of *Trans*-Cinnamic Acid in *Sinyo Nakal* (*Duranta repens*) Fruits' Methanol Extract

Sigit Eko Januar[1], Purwantiningsih Sugita[1*] and Budi Arifin[1]

[1]Department of Chemistry, Bogor Agricultural University, Bogor, West Java, Indonesia.

Authors' contributions

This work was carried out in collaboration between all authors. Authors PS and BA designed the study and author SEJ wrote the protocol. Authors PS and BA managed the literature search and author SEJ wrote the first draft of the manuscript with assistance from author PS. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2015/17616
Editor(s):
(1) Edmond Dik-Lung Ma, Department of Chemistry, Hong Kong Baptist University, Hong Kong, China.
Reviewers:
(1) Anonymous, Cumhuriyet University, Turkey.
(2) Weiting Wang, Tianjin Institute of Pharmaceutical Research, China.
(3) Anonymous, Universidade Lusófona de Humanidades e Tecnologias, Portugal.
(4) Anonymous, Universidade Federal do Paraná, Brazil.
(5) Anonymous, Vittal Mallya Scientific Research Foundation, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1050&id=7&aid=9302

Original Research Article

Received 20th March 2015
Accepted 6th May 2015
Published 19th May 2015

ABSTRACT

Aims: To isolate and to characterize the chemical constituents in *sinyo nakal* (*Duranta repens*) fruits' methanol extract. The fruits were collected from Jombang, East Java, Indonesia.
Methodology: The fruits have been extracted with *n*-hexane in previous study to separate the nonpolar components. Fractionation of the methanol extract was done by using liquid vacum chromatography (LVC) with step gradient elution from *n*-hexane to ethyl acetate, followed by methanol.
Results: Methanol fraction gave the highest yield. A yellowish white crystal was obtained in further fractionation of this fraction. The compound was still impure and could not be purified and characterized further because of limited amount of sample.
Conclusion: The compound has been identified as *trans*-cinnamic acid based on 1D and 2D-nuclear magnetic resonance (NMR) spectra analysis.

**Corresponding author: E-mail: atiek_ps@yahoo.com;*

Keywords: Trans-cinnamic acid; Duranta repens; fruits methanol extract.

1. INTRODUCTION

Duranta repens is a member of *Duranta* species which is widely spread in tropical and subtropical regions [1]. Most of this plants are cultivated as ornamental plants in West India, Pakistan, Middle and South America. In Indonesia, *D. repens* is generally known as *sinyo nakal.* Its fruits are traditionally used to treat malaria and worms, while its leaves are used as abscess medicine, antipyretic, diuretic, and antimalarial agents [2].

Several studies concerning the bioactivities of *D. repens* have been reported. The fruits and stem show antifungal and antibacterial activities, cytotoxicity, and larvicidal activity against *Culex quinquefascitatus* [3,4]. The leaves show antibacterial [2], antioxidant [5], and larvicidal activities against *C. quinquefascitatus* [6]. The whole plant also shows antioxidant, antiviral [7], and insecticidal activities against *Aedes aegypti* and *Attagenus piceus* [8], and inhibits alpha glycosidase enzyme [9].

Many active compounds have been isolated from the leaves and the whole plants of *D. repens*, including terpenoids and steroids [1,3,4,7,10–14], flavonoids [1,7,9,12], iridoid glycosides [10], coumarins and coumarinolignoids [8,13], and phenylethanoid glycosides [1,2]. Still few studies on *D. repens* fruits have been conducted. Moreover, different growing place can influence the amount and type of secondary metabolites produced by a plant. Therefore, this study aims to isolate and to characterize the chemical constituents of *D. repens* fruits from Indonesia. The isolation and characterization are focused on the polar fraction of the methanol extract.

2. MATERIALS AND METHODS

2.1 Materials

Materials used include *D. repens* (collected from Jombang, East Java); twice distilled technical grade methanol, ethyl acetate, *n*-hexane, and dichloromethane; Wagner, Mayer, Dragendorf, and Liebermann-Burchard reagents; 10% NaOH (technical); 1% $FeCl_3$ (technical); H_2SO_4 (p.a); silica gel Merck 60 G for thin layer chromatography (TLC); silica gel Merck 60 (0.2–0.5 mm and 0.063–0.200 mm) for column chromatography; and silica gel Merck 60 GF_{254} for TLC.

2.2 Instrumentation

Basic instruments used were TLC plates (silica gel 60 F_{254}); liquid vacuum chromatography (LVC), gravitational column chromatography, and radial chromatography apparatus; rotary evaporator; and a set of common glassware. The nuclear magnetic resonance (NMR) spectra were obtained with an Agilent spectrometer working at a frequency of 500 MHz (^1H) and 125 MHz (^{13}C) at Bandung Institute of Technology.

2.3 Preparation and Extraction

Milled dried sample (2.28 kg) containing 12.0% of water was macerated in *n*-hexane for 24 hours. The filtrate was then saponified by liquid-liquid extraction with several portions of 0.5 N NaOH. The unsaponified *n*-hexane extract (the upper phase) was collected and evaporated under reduced pressure. The sample residue, which is now free of lipid and other non-polar components, was re-macerated in methanol (6 L) for 24 hours. This maceration was done in three replications. Phytochemical tests were conducted on the *n*-hexane and methanol extracts based on standard procedures [15].

2.4 Secondary Metabolites Compounds Isolation from Methanol Extract

The first fractionation step was tannin removal. Tannin was precipitated by pouring acetone (100 mL) into the methanol extract, and the precipitate was separated by filtration. This step was repeated until visually no more precipitate was formed. The tannin-free methanol extract was then concentrated under reduced pressure.

Brief fractionation was done by using LVC with step gradient elution of two eluents. The nonpolar eluent retarded the spots near the start line of the TLC plate, whereas the more polar one ran the spots near to the finish line. The separation pattern of the eluates was checked by using the best eluent, which was a mixture of two solvents giving the most well-separated spots on the TLC plate. Eluates with the same separation pattern were combined into fractions.

Fraction with the highest yield was fractionated further by using gravitational column chromatography with the same step gradient elution system. Fractions which produced single spot on the TLC plate were elucidated by using NMR spectrometer. Fractions which still contained several components were purified further by using radial chromatography, before being elucidated. The best eluent was determined again, and used as the mobile phase in an isocratic elution system.

3. RESULTS AND DISCUSSION

From 2.28 kg of *D. repens* fruits, 45.2 g of *n*-hexane extract was obtained, 10.0 g of which was unsaponifiable. Maceration of the residue in methanol resulted in 466 g of methanol extract, or 23.2% of yield based on dried weight (12.0% of water content). Based on phytochemical studies, the unsaponifiable *n*-hexane extract only contained steroids, whereas the methanol extract also contained tannins, saponins, alkaloids, and flavonoids. Steroids were evidenced by green colour formation in Liebermann-Burchard test. Tannins produced blue colour with 1% $FeCl_3$. Saponins formed stable foam after being shaked. Alkaloids were proved by orange, yellowish white, and brown precipitate with Dragendorf, Mayer, and Wagner reagents, respectively. Flavonoids produced red colour with 10% NaOH and pink colour with concentrated H_2SO_4. Jayalaksmi et al. [2] reported that *D. repens* leaves' methanol extract contains tannins, saponins, flavonoids, steroids, and terpenoids. Serena et al. [6] reported tannins, saponins, alkaloids, flavonoids, and terpenoids in the same extract. These results showed that both *D. repens* leaves and fruits contained approximately the same group of compounds, except terpenoids, which was only found in *D. repens* leaves.

Tannin removal from 49.1 g of the methanol extract left 28.1 g of tannin-free methanol extract. This extract was soluble in acetone. The best eluent composition for this extract was *n*-hexane-ethyl acetate (4:6). The step gradient elution in LVC apparatus was carried out on this extract (20.0 g) by using *n*-hexane (1×), *n*-hexane-ethyl acetate 8:2 (2×), 6:4 (2×), 5:5 (4×), 4:6 (2×), 3:7 (2×), ethyl acetate (2×), and methanol (10×), respectively. Nine fractions were collected based on TLC checking using the best eluent (Table 1).

Fraction-H had the highest yield (62.4%). This fraction was eluted in methanol. Tannins were still detected in this fraction and removed by precipitation in acetone to avoid spot tailing during further fractionation. From 12.5 g of fraction-H, 6.45 g of tannin-free fraction-H was obtained, and 3.95 g of which was fractionated by using a gravitational column chromatography. Step gradient elution system of *n*-hexane-ethyl acetate gave 15 fractions. Two fractions (H_6 and H_9) produced only one spot each in the TLC test, with R_f value of 0.83 and 0.65, respectively, using the best eluent. Fraction H_6 was obtained only in small amounts (1.7 mg), so that only fraction H_9 (13.3 mg) was elucidated with ^1H NMR. However, its spectrum still showed mixture of compounds and could not yet be determined.

The most abundant fraction, H_7 (51.05 mg), produced only two components in TLC test with R_f value of 0.83 and 0.60. These components were then purified by using radial chromatography. The best eluent composition was dichloromethane-ethyl acetate (3:7), The first fraction, H_{71} (11.4 mg), was a yellowish white crystal with the same R_f value as fraction H_6. Its chemical structure was then elucidated from the NMR spectra.

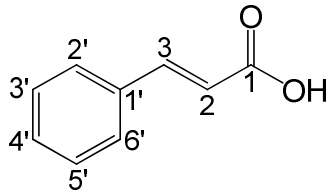
Table 1. LVC fractions from tannin-free methanol extract of *D. repens* fruit

Fraction	Eluent	Weight (g)	Yield (%)
A	*n*-Hexane + *n*-hexane-ethyl acetate 8:2 (elution 1)	0.0105	0.05
	n-Hexane-ethyl acetate:		
B	8:2 (elution 2) + 6:4 (elution 1)	0.113	0.57
C	6:4 (elution 2) + 5:5 (elution 1)	0.194	0.97
D	5:5 (elution 2–4)	0.289	1.44
E	4:6	0.089	0.44
F	3:7	0.115	0.57
G	Ethyl acetate	0.224	1.12
H	Methanol (elution 1–2)	12.5	62.4
I	Methanol (elution 3–10)	4.82	24.1

The ^{13}C NMR spectrum (Fig. 1) showed 7 carbon signals from 9 carbon atoms in 117.2, 128.3, 128.9, 130.7, 134.0, 146.9, and 171.7 ppm. Signal at 171.7 ppm showed a carbonyl group of a carboxylic acid. Two signals at 117.2 and 146.9 ppm were sp^2 carbon signals with different chemical environment. The existence of benzene aromatic ring was proved by 4 signals in 128.3, 128.9, 130.7, and 134.0 ppm. Each of two signals in 128.3 and 128.9 ppm rose from two equivalent carbon atoms, based on their higher intensities than the two other signals. This chemical shift pattern matched with a monosubstituted benzene structural unit.

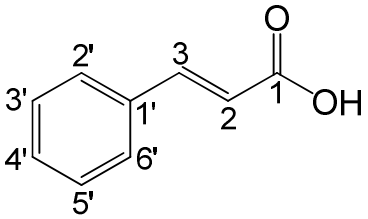
The ^1H NMR spectrum (Fig. 2) showed 4 proton signals in 6.4, 7.4, 7.6, and 7.8 ppm. Signals in 6.4 ppm (1H, *d*) and 7.8 ppm (1H, *d*) showed a *trans*-alkene group with coupling constant of 16 Hz. This result was supported by the total correlation spectroscopy (TOCSY) spectrum. When the proton in 6.4 ppm was irradiated, only peak in 7.8 ppm appeared. It suggested a direct correlation between the two adjacent protons. One signal was more downfield (δ_H 7.8 ppm) because of deshielding effect. The double bond resonated due to the electron-withdrawing effect of the carbonyl group leaving partial positive charge at the β-carbon. The α-carbon, which was not deshielded provided lower chemical shift. Aromatic protons of the monosubstituted benzene were evidenced from the absorption peaks in 7.4 ppm (3H, *t*) and 7.6 ppm (2H, *m*). However, the exchangeable proton of –COOH did not appear in the ^1H NMR spectrum. It is possibly because of a very rapid proton exchange between the acidic proton and protons from water residue which was still left during the preparation of the sample. The summary of ^1H and ^{13}C NMR analysis and their comparison with the literature [16] are shown in Tables 2 and 3.

Table 2. Comparison of ^1H NMR chemical shift of *trans*-cinnamic acid



H atom	This study (500 MHz, $CDCl_3$)		[16] (300 MHz, $CDCl_3$)	
	Σ H	δ_H (ppm) (multiplicity, *J* (Hz))	Σ H	δ_H (ppm) (multiplicity, *J* (Hz))
OH	-	-	-	-
2	1	6.4 (*d*, 16)	1	6.48 (*d*, 15.9)
3	1	7.8 (*d*, 16)	1	7.82 (*d*, 15.9)
2'/6'	2	7.6 (*m*)	2	7.65–7.54 (*m*)
3'/4'/5'	3	7.4 (*t*)	3	7.5–7.36 (*m*)

Table 3. Comparison of ^{13}C NMR chemical shift of *trans*-cinnamic acid



C atom	δ_C (ppm) (multiplicity, *J* (Hz))	
	This study (125 MHz, $CDCl_3$)	[16] (75 MHz, DMSO-d_6)
1	171.7	167.66
2	117.2	119.35
3	146.9	144.01
1'	134.0	134.32
2'/6'	128.3	128.24
3'/5'	128.9	128.96
4'	130.7	130.26

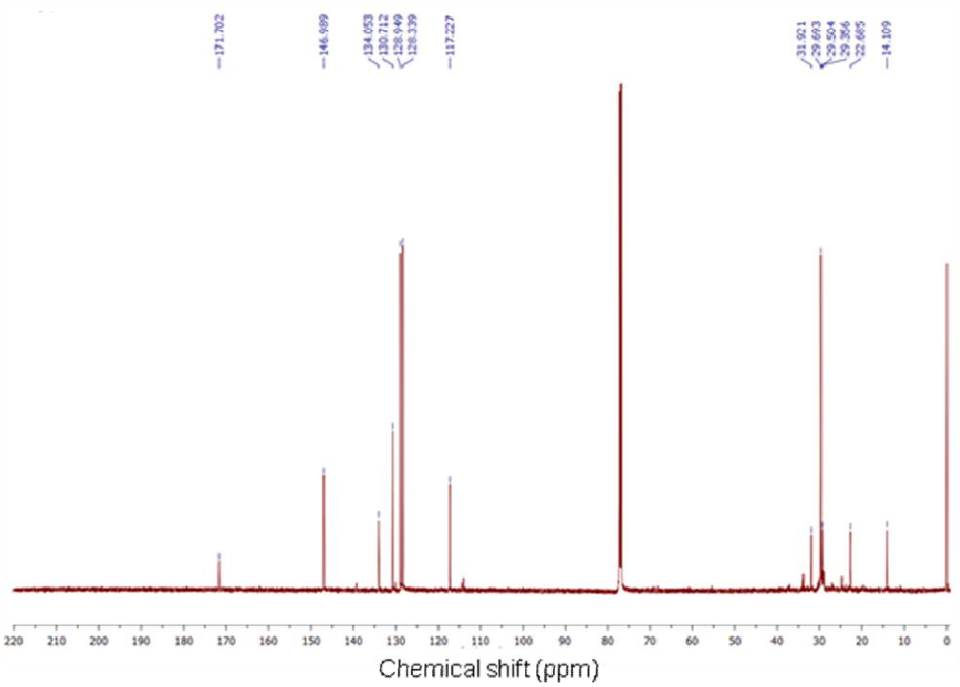


Fig. 1. ^{13}C NMR spectrum of H_{71} fraction (125 MHz, $CDCl_3$)

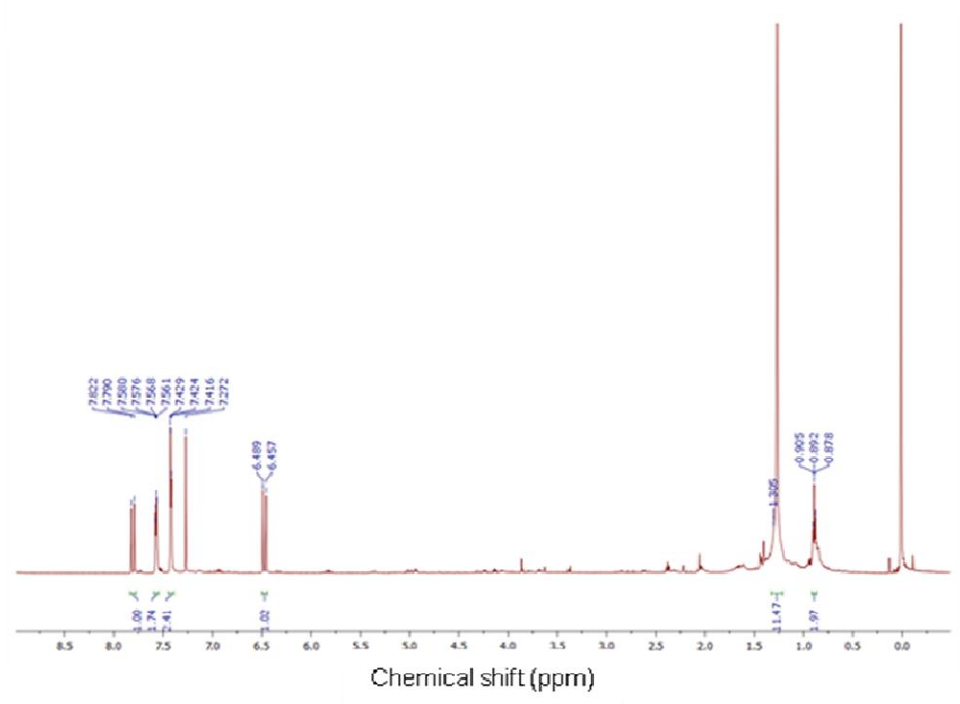


Fig. 2. ^{1}H NMR spectrum of H_{71} fraction (500 MHz, $CDCl_3$)

The heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) spectra gave further evidence of the *trans*-cinnamic acid structure. The HSQC spectrum showed correlation between proton and carbon through a single bond. Signal correlations between δ_C 117.2 ppm and δ_H 6.4 ppm and also δ_C 146.9 ppm and δ_H 7.8 ppm proved the existence of a disubstituted alkenes, RCH=CHR' (Fig. 3). The evidence of a monosubstituted benzene was explained as follows. Two *ortho* protons in δ_H 7.6 ppm were correlated with δ_C 128.3 ppm, two *meta* protons in δ_H 7.4 ppm were correlated with δ_C 128.9 ppm, and a *para* proton in δ_H 7.4 ppm was correlated with δ_C 130.7 ppm. The carbon signal in δ_C 134.0 ppm was not correlated with any signal in ^{1}H NMR spectrum, suggesting that it was a quaternary *ipso* carbon.

The HMBC spectrum showed correlation between proton and carbon atoms separated by two or three bonds. Conjugation of the carbonyl group with the double bond was evidenced from correlation between carbonyl signal (δ_C 171.7 ppm) and 2 proton signals (δ_H 6.4 and 7.8 ppm) (Fig. 4). The C-2 atom (δ_C 117.2 ppm) was correlated with H-3 atom (7.8 ppm), and the C-3 atom (δ_C 146.9 ppm) was correlated with H-2'/6' signals (δ_H 7.6 ppm). This results also proved that C-3 atom was directly attached to the benzene unit.

Overall correlations observed in the HMBC spectrum are given in Table 4. An unpredicted correlation was still observed between C-2 atom and the *orto* hydrogens. As shown in Figs. 1 and 2, fraction H_{71} and H_6 still contain aliphatic impurities. An aromatic compound with similar structure as *trans*-cinnamic acid, but with a C-2 atom of the aliphatic moieties directly attached to a monosubstituted benzene ring was predicted to be an impurity accounts for this correlation. However, from NMR analysis explained above, the chemical compound in fraction H_{71} and H_6 could be predicted as crude *trans*-cinnamic acid, but still needed further purification.

trans-Cinnamic acid and *trans*-*p*-methoxycinnamic acid have been reported by Kuo et al. [10] in the ethanol extract of *D. repens* leaves grown in Taiwan. Ester of cinnamic acid with an iridoid glucoside moiety, namely durantoside I, has also been isolated from *D. erecta* leaves [17]. *trans*-Cinnamic acid itself is a common chemical compound found in plants. Therefore, investigation on other chemical constituents in polar as well as nonpolar fractions of the methanol extract, which is characteristic of *D. repens* fruits in Indonesia, needs to be carried out in immediate study.

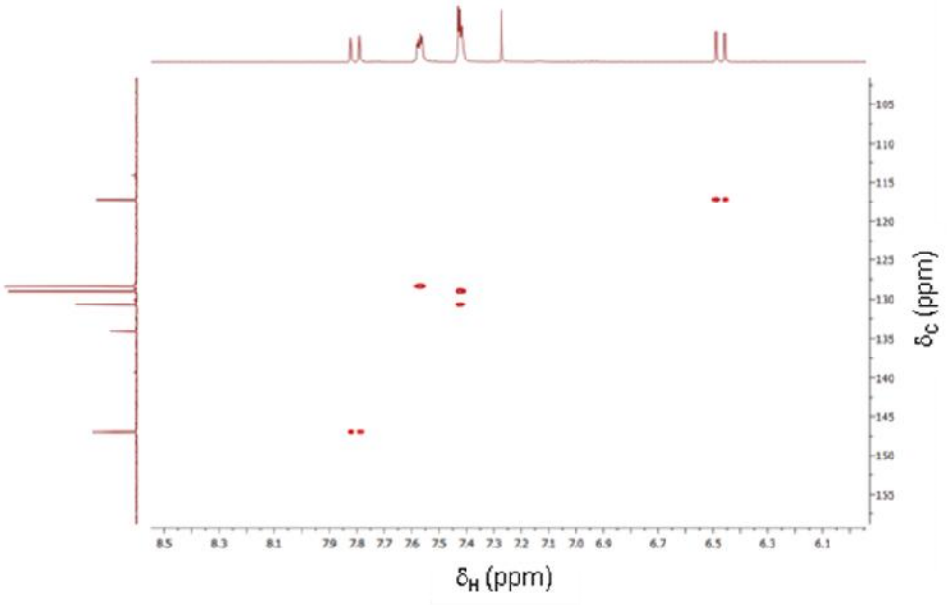


Fig. 3. HSQC spectrum of H_{71} fraction

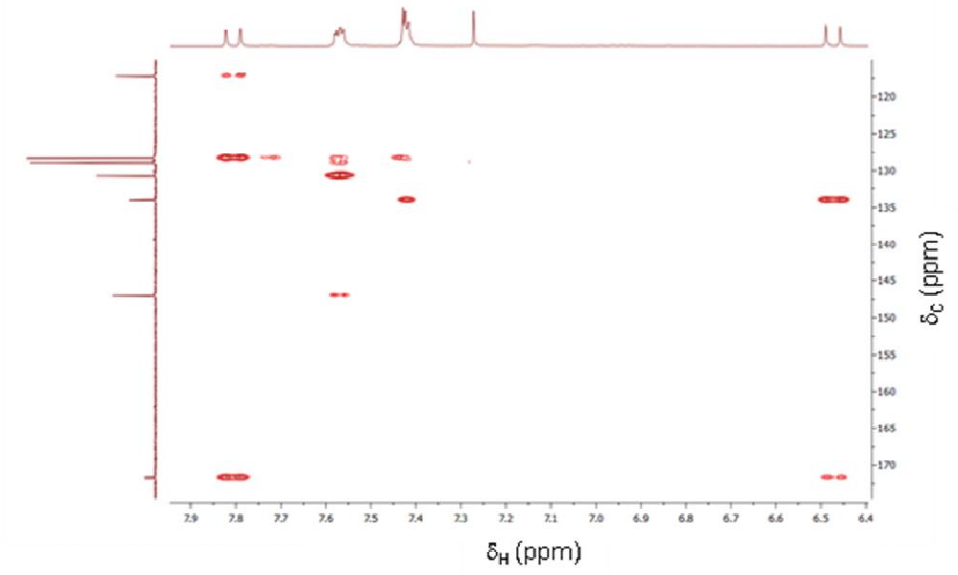


Fig. 4. HMBC spectrum of H_{71} fraction

Table 4. Two (α) and three (β) bonds correlation between ^{1}H and ^{13}C NMR peak in the HMBC spectrum

	^{13}C NMR	C-1	C-2	C-3	C-1'	C-2'/6'	C-3'/5'	C-4'
^{1}H NMR	**ppm**	171.7	146.9	117.2	134.0	128.3	128.9	130.7
H-2	6.4	α	DB*	α		**		
H-3	7.8	β		DB	α			
H-2'/6'	7.6		β			DB	α	β
H-3'/4'/5'	7.4				β	α/ β	DB	DB

**DB: directly bonded*
***unpredicted correlation*

4. CONCLUSION

Phytochemical investigations on *D. repens* fruits' methanol extract from Jombang, East Java showed the existence of tannin, saponin, alkaloid, flavonoid, and steroid. The *trans*-cinnamic acid was isolated from acetone-soluble fraction of the extract as confirmed by 1D and 2D-NMR spectra analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ahmed WS, Mohamed MA, El-Dib RA, Hamed MM. New triterpene saponins from *Duranta repens* Linn. and their cytotoxic activity. Molecules. 2009;14:1952-1965.
2. Jayalakshmi B, Raveesha KA, Amruthesh KN. Phytochemical investigations and antibacterial activity of some medicinal plants againts pathogenic bacteria. J. Appl. Pharm. Sci. 2011;1(5):124-128.
3. Nikkon F, Hasan S, Rahman MH, Hoque MA, Mosaddik MA, Haque ME. Biochemical, hematological and histiphatological effects of *Duranta repens* stem on rats. Asian J. Biochem. 2008;2: 366-372.
4. Nikkon F, Saud ZA, Hossain K, Parvin MS, Haque ME. Larvicidal effects of stem and fruits of *Duranta repens* against the mosquito *Culex quinquefasciatatus*. J. Pharm. Tech. Res. 2009;4:1709-1713.
5. Adu F, Gbedema SY, Brown P, Annan K, Boamah VE. Antibacterial and free radical scavenging activity of *Duranta plumieri* Linn. J. Pharm. Sci. Res. 2011;2:282−287.
6. Serena MM, Balasubraman M, Rajan K, Gerald IAJ. Evaluation of the larvicidal

activity of the leaf extracts of *Duranta erecta* Linn. (Verbenaceae) on the larvae of *Culex quinquefascitatus* (Say) (Culicidae). J. Biopesticides. 2010;3:582-585

7. Abou-Setta LM, Nazif NM, Shahat AA. Phytochemical investigation and activity of *Duranta repens*. J. Appl. Sci. Res. 2007.3: 1426-1433.
8. Ahmad N, Zeb F, Ahmad I, Wang F. Repenins A–D, four new antioxidative coumarinolignoids from *Duranta repens* Linn. Bioorg. Med. Chem. Lett. 2009;19: 3521-3524.
9. Iqbal K, Malik A, Mukhtar N, Anis I, Khan SN, Choudhary MI. α-Glucosidase inhibitory constituents from *Duranta repens*. Chem. Pharm. Bull. 2004;52:785-789.
10. Kuo YH, Chen ZS, Lin YL. Chemical components of the leaves of *Duranta repens* Linn. Chem. Pharm. Bull. 1996;44: 429-436.
11. Ahmad S, Nizami TA, Nawaz HR, Malik A, Afza N. A new steroid from *Duranta repens* [abstrak]. Fitoterapia. 1998;69:448-450.
12. Anis I, Ahmed S, Malik A, Yasin A, Choudary MI. Enzyme inhibitory constituents from *Duranta repens*. Chem. Pharm. Bull. 2002;50:515-518.
13. Shahat AA, Nazif NM, Abousetta LM, Ibrahim NA, Cos P, Miert SV, Pieter L, Vlietinck AJ. Phytochemical investigation and antioxidant activity of *Duranta repens*. Phytother. Res. 2005;19:1071-1073.
14. Nikkon F, Habib MR, Karim MR, Hossain MS, Mosaddik MA, Haque ME. Antishigellosis and cytotoxic potency of crude extracts and isolated constituents from *Duranta repens*. Mycobiology. 2008; 36:173-177.
15. Zulhipri Kartika IR, Sumaji I. Uji fitokimia dan aktivitas antidiabetes ekstrak biji rambutan (*Nephelium lappaceum* L.) dengan berbagai pelarut. Ebers Papyrus. 2007;13(3):89-98.
16. Kim SM, Kim YS, Kim DW, Yang JW. Transition metal-free, NaO^tBu-O_2-mediated one-pot cascade oxidation of allylic alcohols to α,β-unsaturated carboxylic acids. Green Chem. 2012;14:2996-2998.
17. Takeda Y, Morimoto Y, Matsumoto T, Ogimi C, Hirata E. Iridoid glucosides from the leaves and stems of *Duranta erecta*. Phytochemistry. 1995;39:829-833.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1050&id=7&aid=9302