



Pharmacognostic specification, mutagenic and antimutagenic properties of *Cymbopogon nardus* roots in Thailand

Pravaree Phuneerub^a, Chanida Palanuvej^{a*} and Nijisiri Ruangrunsi^{a,b}

^aCollege of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

^bFaculty of Pharmacy, Rangsit University, Pathumthani, Thailand

ABSTRACT

Cymbopogon nardus Rendel (Family Gramineae) is widely distributed in tropical Asia. Its dried roots have been used in traditional Thai medicine for antipyretics, anti-inflammation, anti-analgesics and anticancer. The objectives of this study were to display the pharmacognostic specification, analyze the chemical constituents of essential oil and investigate the mutagenic and antimutagenic properties of *C. nardus* dried roots. Total ash, acid-insoluble ash, loss on drying and water content should be not more than 5.82, 3.97, 8.29 and 8.06 % of dry weight respectively; while volatile oils, ethanol and water soluble extractive values should not be less than 2.96, 4.89 and 8.12 % of dry weight respectively. The major constituents of volatile oil from dried roots of *C. nardus* analyzed by GC/MS were elemol (22.87%) and alpha-eudesmol (16.09%). The mutagenic testing demonstrated that the ethanol and fractionated water extract of *C. nardus* roots were no mutagenic to *S. typhimurium* strains TA98 and TA100. In addition, both ethanol and water extracts of *C. nardus* roots at 15 mg/plate demonstrated strong antimutagenic effect toward *S. typhimurium* strains TA98 and TA100.

Keywords: *Cymbopogon nardus*, dried root, quality specification, essential oil analysis, Ames *Salmonella* assay

INTRODUCTION

Cymbopogon nardus (L.) Rendle (Family Gramineae) is commonly known as citronella grass. The leaf oil of *C. nardus* is widely used in soap, perfumery, cosmetic and flavouring industries throughout the world [1]. In Ayurveda, *C. nardus* has been used in this ancient healing system for the treatment of toothaches, redness, irritation and inflammation of the skin, infectious diseases, digestive problems, headache, rheumatism and fatigue [2]. The roots of *C. nardus* are used for antipyretics, anti-inflammation, anti-analgesic and anticancer in traditional Thai medicine. This crude drug is an essential ingredient of Tree Phon Thad remedy which is a remedy notified in traditional Thai medicine textbook named Tumrapaadsard Song Khor.

The standardization and quality control of raw herbal materials are essential to herbal drug development [3]. To control the quality of raw herbal material, establishment of standardization parameter is needed. Several studies have been reported for biological efficacy such as antimicrobial, antifungi, antiviral activities and chemical constituents of *C. nardus* leaves [4-8]. However, there have been no reports about pharmacognostic properties and chemical constituents of *C. nardus* roots.

For a long period of traditional medicine, herbal drugs are often assumed to be safe. Nevertheless, previously research revealed that the water extracts from the root of *A. marmelos* which was one of plant material in Ben-Cha

Moon-Yai remedy exhibited direct mutagenicity on *S. typhimurium* strains TA98 and TA100 [9]. Therefore, toxicological study of herbal drugs is important, especially for the integration of traditional medicine into national health care system. This study was carried out to provide pharmacognostic standards of *C. nardus* dried root crude drug in Thailand including the chemical compositions of its essential oil and to evaluate the mutagenic and antimutagenic properties of ethanolic extract and fractionated water extract of *C. nardus* root toward *S. typhimurium* TA98 and TA100.

EXPERIMENTAL SECTION

Sample collection

The roots of *C. nardus* were collected from 12 different locations throughout Thailand and authenticated by Associate Professor Dr. Nijsiri Ruangrunsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

Chemicals

Methanol HPLC Grade was purchased from Labscan (Thailand). Agar-Agar, D (+)-glucose monohydrate and dimethylsulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). Oxoid nutrient broth No.2 was obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). L-Histidine monohydrochloride monohydrate and D-biotin were purchased from Sigma-Aldrich, (St. Louis, U.S.A.). Ammonium sulfamate and sodium ammonium hydrogen phosphate tetrahydrate were bought from Fluka AG (Buch, Switzerland). Ajax Finechem Pty Ltd. supplied sodium nitrite. 1-aminopyrene (Aldrich, St. Louis, U.S.A.) was used to interact with nitrite in acid solution to produce a standard direct mutagen of the Ames test.

Crude extract preparation

Roots of *C. nardus* were shade-dried and ground to coarse powders. The powders were continuously macerated with ethanol and water respectively. The ethanol extract was filtered and evaporated until dryness under reduced pressure at $\leq 50^{\circ}\text{C}$, whereas the water extract was lyophilized to dryness. The extract yields were weighed, recorded and stored at -20°C to decrease the possibility of degradation of active compounds.

Morphological identification

The characters of root of *C. nardus* were carried out by macroscopic and microscopic examinations. The physical properties such as shape, size, color, odor, texture and other visual inspection of crude drug were observed during the macroscopic evaluation. The transverse section and powder of *C. nardus* root were studied under microscopic with 10X, 20X, and 40X objective lens magnifications and 10X eyepiece lens. The results were displayed by hand drawing in proportional scale related to the original size.

Physico-chemical identification

The pharmacognostic characters including loss on drying, total ash, acid insoluble ash, moisture, volatile oil content and extractive values parameters were investigated according to the WHO Guideline for Quality Control Methods for Medicinal Plant Materials [10]. In brief, placed 3 g of the powdered dried *C. nardus* roots in pre-weighed crucible, dried the sample at 105°C until constant weight and recorded the constant of loss on drying. Total ash was carried out by incineration 3 g of powdered drug at 500°C until white (5 hours) to observe the carbonless ash in a muffle furnace (Carbolite AAF 110). Then, the ash was gently boiled with 25 ml of 2N HCl and the insoluble matter was incinerated at 500°C to obtain the acid insoluble ash. The moisture content was investigated by distillation with water-saturated toluene using Azeotropic apparatus. The volatile oil was hydrodistilled using Clevenger apparatus. Determinations of extractive values were carried out with 95% ethanol and water as solvent. All the experiments were done in triplicates and calculated for grand average and pooled standard deviation.

GC/MS analysis of *C. nardus* roots volatile oil

Cymbopogon nardus dried root volatile oils were analyzed on a Finnigan Trace GC ultra with Finnigan Trace DSQ mass spectrometry and ZB-5 capillary column (30 m x 0.25 mm, 0.25 μm film thicknesses). The oven temperature was 60°C for 1 min. then ramped to 240°C with the rate of $3^{\circ}\text{C}/\text{min}$. Auto injector (Finnigan AI 3000) was set at 180°C and split ratio of 100:1. Helium was used as carrier gas. The oil was diluted with HPLC grade methanol (1:100) and injection volume was 1 μl . The constituents of the volatile oils were identified by matching their mass spectra and retention indices with Adams Essential Oil Mass Spectral library and NIST02 Mass Spectral library.

Mutagenic testing

Salmonella typhimurium strain for frame-shift mutation (TA98) and strain for base-pair substitution mutation (TA100) were kindly provided by the Biochemistry and Chemical Carcinogenesis Section, Research Division, National Cancer Institute, Bangkok, Thailand. The mutant strains were confirmed for the genotypes of histidine/biotin dependence, *rfa* marker, *uvrB* deletion gene mutations and presence of plasmid *pMK101* [11]. The pre-incubation method of Ames test was performed to determine the mutagenic effect of *C. nardus* root extracts on both TA98 and TA100 *Salmonella typhimurium* strains without enzyme activating system [12]. Briefly, the ethanol extract was dissolved in DMSO, the fractionated water extract was dissolved in water to the concentration of 25, 50, 100 and 200 mg/ml. Added 200 μ l of each solution to the tube containing 550 μ l of 0.2 N hydrochloric acid to acidify the reaction mixture to pH 3.0-3.5 and added 250 μ l of solvent (DMSO or water) to obtain the final volume is 1,000 μ l. The reaction tube was shaken at 37 °C for 4 hr. Mixed 100 μ l of preparation sample with 500 μ l of 0.5 M phosphate buffer (pH 7.4), added 100 μ l of each tester strains that overnight cultured and incubated at 37 °C in shaking water bath for 20 min, after that, added to 2 ml top agar containing histidine–biotin, mixed well and poured over the surface of a minimal agar plate. The plates were incubated at 37 °C for 48 h and the numbers of his+ revertant colonies on each plate were counted. DMSO or water was used as a negative control to determine the spontaneous reversion activity. All tests were performed in triplicate. The mutagenic index (MI) was calculated from the number of revertant colonies of the sample treatment divided by the number of spontaneous revertant colonies. Positive mutagenic effect was considered when the number of induced revertant colonies increases in a dose response relationship manner, at least two doses were higher than spontaneous revertant ($MI \geq 1$) and at least one dose gave rise to twice over the spontaneous revertant ($MI > 2$) [13].

Antimutagenic testing

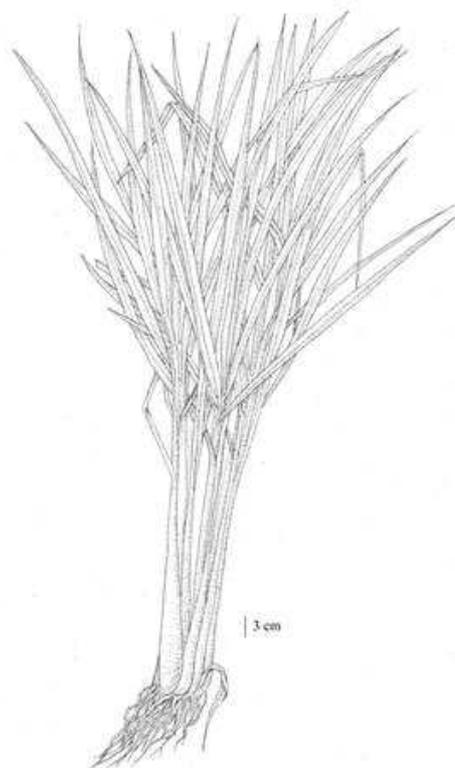
The antimutagenic effect of extracts against 1-aminopyrene treated with sodium nitrite was determined by the pre-incubation method of Ames test similar to the mutagenic testing. Ten microliters (tested on TA98) or 20 μ l (tested on TA100) of 0.075 mg/ml 1-aminopyrene was transferred into the sterile test tube. Then, 740 μ l or 730 μ l of 0.2 N hydrochloric acid and 250 μ l of 2M sodium nitrite were added to obtain the total volume at 1 ml. The mixtures were shaken at 37 C for 4 h. Stopped reaction for 1 min in an ice bath, then added 250 μ l of 2 M ammonium sulfamate and allowed the test tube to stand in an ice bath for 10 min before Ames test. Mixed 25 μ l of nitrite treated 1-aminopyrene with 500 μ l of 0.5 M phosphate buffer (pH 7.4), added 100 μ l of each tester strains that overnight cultured. An aliquot (0, 25, 50 and 75 μ l) of both extracts of *C. nardus* roots (200 mg/ml in DMSO or water) were added and the final volume was adjusted to 700 μ l with DMSO or water. The mixture was incubated at 37 °C in shaking water bath for 20 min, after that, added to 2 ml top agar containing histidine–biotin. The mixture was poured over the surface of a minimal agar plate and incubated for 48 h at 37 °C. After incubation, revertant (mutant) colonies were counted. All tests were performed in triplicate. The percent inhibition was calculated by the following formula [14].

$$\% \text{ Inhibition} = [(A-B)/(A-C)] \times 100$$

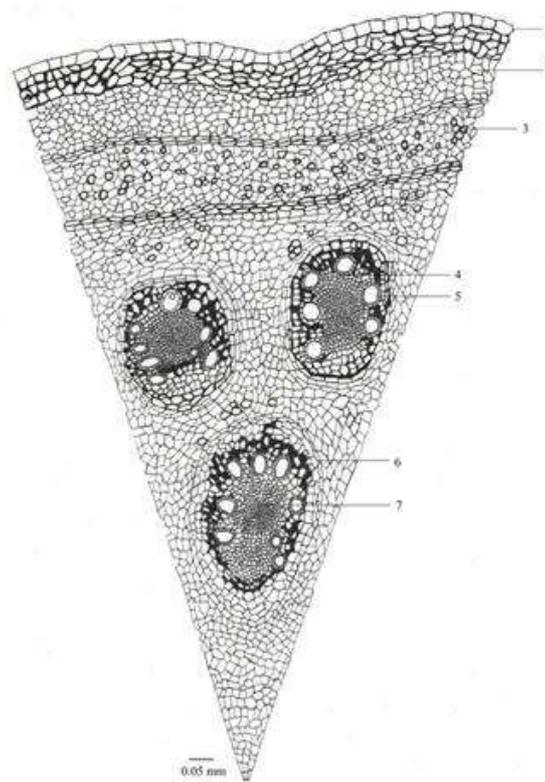
Where A was the number of histidine revertant colonies per plate induced by nitrite treated 1-aminopyrene, B was the number of histidine revertant colonies per plate by nitrite treated 1-aminopyrene in the present of each extract and C was the number of spontaneous revertant colonies per plate. The percentage of inhibition was classified as strong when it is higher than 60%, moderate ranged from 60-41%, weak ranged from 40-21% and negligible effect when it was less than 20% [15].

RESULTS AND DISCUSSION**Pharmacognostic specification**

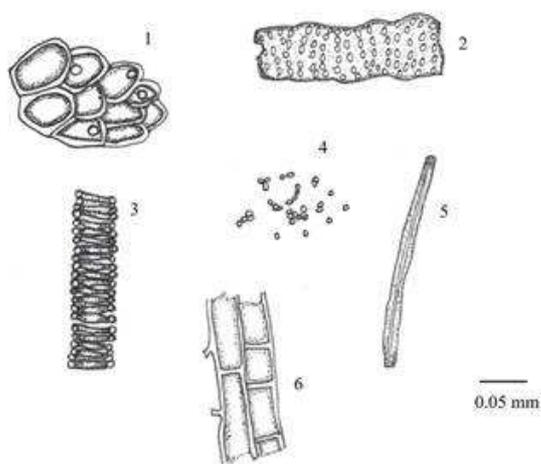
Macroscopic characters of *Cymbopogon nardus* and its dried roots were illustrated. The root was about 2-8 cm in length. The surface dried root has brown in color, but inside it was cream in color. The anatomical character of root transverse section revealed that epidermis, parenchyma, oil gland, phloem, xylem vessel, endodermis and pith. Histological characters of root powder showed parenchyma containing oleoresin, parenchyma in longitudinal view, reticulate vessel, annular vessel, starch granules and fragment of fiber (Figure 1).



The Whole plant of *Cymbopogon nardus* Rendle Roots



Transverse section of the root of *Cymbopogon nardus* Rendle; 1. Epidermis 2. Parenchyma 3. Oil gland 4. Phloem 5. Xylem vessel 6. Endodermis 7. Pith



Powder of the root of *Cymbopogon nardus* Rendle ; 1. Parenchyma containing oleoresin 2. Reticulate vessel 3. Annular vessel 4. Starch granules 5. Fragment of fiber 6. Parenchyma in longitudinal view



Cymbopogon nardus Rendle dried roots

Figure 1. Macroscopic and microscopic characteristics of *Cymbopogon nardus* Rendle

Plant description

Perennial from a stout rootstock, culms tufted, robust, up to 2.5 m tall, 1–2 cm in diam. Leaf sheaths reddish purple at base, smooth, glabrous; leaf blades dark green or dark brown when dry, drooping for 1/3 of their length, 30–100 × 1–2 cm, glabrous, abaxial surface scabrid, adaxial surface smooth, base narrow, apex long acuminate; ligule 2–3 mm. Spathe panicle large, narrow, congested, interrupted, 60–90 cm; spatheoles reddish brown, 1.2–2.5 cm; racemes 1–1.5 cm; rachis internodes and pedicels ciliate on margins; pedicel of homogamous pair not swollen. Sessile spikelet oblong-lanceolate, 3–4.5 × 1–1.2 mm; lower glume flat or slightly concave, reddish brown or purplish upward, sharply 2-keeled, keels narrowly winged, obscurely 0–3-veined between keels; upper lemma linear, entire or slightly 2-lobed, mucronate or very shortly awned. Pedicelled spikelet 3.5–7 mm [16].

Table 1 Physico-chemical parameters (% by weight) of *C. nardus* root

| Content (% by weight) ^a | Mean | SD | Range (Mean ± 3SD) |
|------------------------------------|------|------|-----------------------|
| Total ash | 5.82 | 0.65 | 3.89 – 7.75 |
| Acid-insoluble ash | 3.97 | 0.64 | 2.05 – 5.89 |
| Ethanol-soluble extractive | 4.89 | 0.85 | 2.34 – 7.43 |
| Water-soluble extractive | 8.12 | 0.54 | 6.50 – 9.73 |
| Loss on drying | 8.29 | 0.22 | 7.62 – 8.95 |
| Volatile oil | 2.96 | 0.38 | 1.82 – 4.10 |
| Water | 8.06 | 0.45 | 6.70 – 9.41 |

^a The parameters were shown as grand mean ± pooled SD. Samples were from 12 different sources throughout Thailand. Each sample was performed in triplicate.

Physico-chemical identification

The physico-chemical specifications of *C. nardus* dried root crude drug evaluated from 12 different sources throughout Thailand were established (Table 1). Total ash, acid-insoluble ash, loss on drying and moisture content should not be more than 5.82, 3.97, 8.29 and 8.06 % respectively; while volatile oil, ethanol and water soluble extractive values should not be less than 2.96, 4.89 and 8.12 % respectively. Previous study on the physico-chemical specifications of *C. nardus* leaves showed that total ash, acid insoluble ash and moisture content should not be more than 6, 2 and 3, respectively. The volatile oil, ethanol and water soluble extractive values should not be less than 0.1, 6 and 8 %, respectively [17]. The roots were found to contain more acid insoluble ash, moisture and volatile oil than the leaves.

Table 2 The chemical composition of the oil *C. nardus* roots

| RT | Compound name | Area% ^a | KI |
|-------|------------------------------|--------------------|------|
| 24.84 | Elemene (beta) | 2.13±0.95 | 1390 |
| 28.43 | Germacrene D | 1.82±0.60 | 1485 |
| 28.63 | Selinene (beta) | 0.9±0.32 | 1490 |
| 28.98 | Selinene (alpha) | 0.68±0.23 | 1498 |
| 29.19 | Muurolene (alpha) | 0.80±0.35 | 1500 |
| 29.38 | Cuparene | 2.16±0.96 | 1504 |
| 29.71 | Cadinene (gamma) | 2.52±1.03 | 1513 |
| 30.06 | Cadinene (delta) | 2.44±1.02 | 1523 |
| 31.12 | Elemol | 22.87±3.11 | 1549 |
| 32.11 | Cadinene (alpha) | 1.69±0.72 | - |
| 32.40 | Caryophyllene oxide | 1.89±0.81 | 1583 |
| 33.23 | Eudesmol (5-epi-7-epi-alpha) | 2.00±0.88 | 1607 |
| 33.58 | Cubenol | 1.91±0.81 | 1645 |
| 33.75 | Unidentified | 6.75±1.69 | - |
| 34.20 | Eudesmol (gamma) | 11.72±2.23 | 1630 |
| 34.57 | Cadinol (epi-gamma) | 6.99±1.72 | 1640 |
| 34.74 | Torreyol | 1.73±0.82 | 1646 |
| 34.91 | Eudesmol (Beta) | 11.33±2.19 | 1650 |
| 35.05 | Eudesmol (alpha) | 16.09±2.61 | 1652 |
| 35.20 | Eudesm-7(11)-en-4-ol | 4.20±1.06 | 1700 |
| 35.55 | Eremophilone | 2.63±1.01 | 1736 |

^a The parameters were shown as mean ± SD. Samples were from 12 different sources throughout Thailand.

Chemical compositions of *C. nardus* dried root volatile oils

Previous studies of *C. nardus* dried leaf oils indicated monoterpenes as the major components which in Thailand were geraniol (35.7%), *trans*-citral (22.7%) and *cis*-citral (14.2%) [7]; in Togo were citronellal (35.5%), geraniol

(27.9%) and citronellol (10.7%) [8] while in Malaysia were citronellal (29.06%) [18]. The major components of volatile oil from dried roots of *C. nardus* were revealed in this study as sesquiterpenes which were elemol (22.87%) and eudesmols (-alpha 16.09%, -gamma 11.72% and -beta 11.33%). The chemical compositions of *C. nardus* dried root oil in Thailand were demonstrated in Table 2 and GC fingerprint was shown in Figure 2.

Various biological activities of plant extracts consisted of elemol and alpha-eudesmol were reported. The essential oil of *Schinus molle*, which elemol was one of the main compounds, had-biological activity as repellent and had to be applied as fumigant for controlling *Sitophilus oriza* [19].

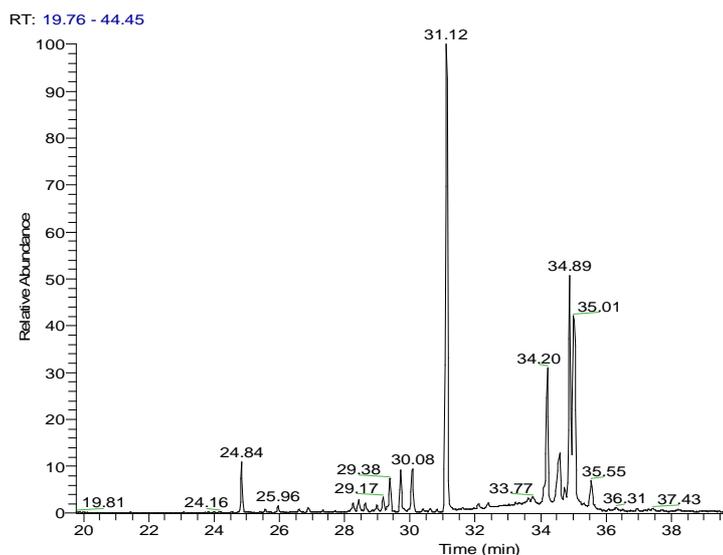


Figure 2 GC chromatogram of essential oil of *C. nardus* dried root

The essential oil of *Nepeta septemcrenata* which consisted of 13.8% elemol acted as antimicrobial against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* [20]. Moreover, elemol was identified as an active principle responsible for the larvicidal activity of *C. nardus* fractional distilled oil [21]. Alpha-eudesmol was shown for high voltage-gated calcium channel blocker activity which possibly affected on anti-migrain treatment [22]. Furthermore, alpha-eudesmol displayed an ability to attenuate post-ischemic brain injury in rats [23].

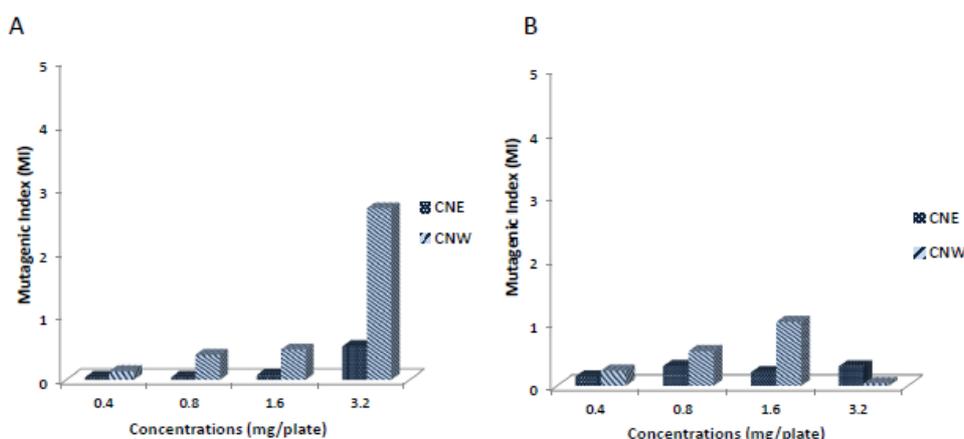


Figure 3 The mutagenic index (MI) induced by the ethanol and fractionated water from roots of *C. nardus* on *S. typhimurium* strains TA98 (A) and TA100 (B) using Ames test. CNE: ethanol extract of *C. nardus* and CNW: fractionated water extract of *C. nardus*

Mutagenic assay

The Ames *Salmonella* assay is highly efficient in detecting carcinogens and mutagens which is a short-term *in vitro* testing. It has been tested with a wide variety of carcinogens such as direct alkylating agents, nitrosamines, polycyclic hydrocarbons, fungal toxins, aromatic amines, nitrofurans, a variety of antineoplastic agents, and antibiotic carcinogens such as adriamycin, daunomycin, and mitomycin C [26]. MI values of ethanol and fractionated water extracts from roots of *C. nardus* obtained by the Ames test were shown in Figure 3. The result demonstrated that the ethanol and fractionated water extracts of roots of *C. nardus* was not mutagenic to strain TA98 and TA100. Positive control as 1-aminopyrene at 0.075 mg/ml induced 358.83 ± 31.188 revertant colonies to strain 98 (MI=15.07) and 641.333 ± 78.368 revertant colonies to strain 100 (MI=4.70).

Antimutagenicity assay

The investigation for antimutagenicity of plant extract is important in the discovery of new effective anticarcinogenic therapeutic drug. The Ames test has been widely used to evaluate the antimutagenic and anticarcinogenic activity of various compounds [27]. This present study showed the ethanol and fractionated water extracts inhibited mutagenicity effect towards *S. typhimurium* strains TA98 and TA100 from the reaction of 1-aminopyrene treated with nitrite under acid condition pH 3-3.5 (Figure 4A-4B). The effects were ranged from negligible (0-20% inhibition) to strongly active (>60% inhibition). All doses of the ethanol extract from roots of *C. nardus* demonstrated strong antimutagenic effect toward strain TA98 and all doses of the water extract from roots of *C. nardus* demonstrated strong antimutagenic effect toward strain TA100. Ethanol extract at 15 mg/plate showed the highest inhibition effect to mutagenicity on both strains of *S. typhimurium*. The moderate antimutagenic activity was observed on 5 and 10 mg/plate of fractionated water extract on *S. typhimurium* strains TA98 and 10 mg/plate of ethanol extract towards TA100. Whereas, the concentration of 5 mg/plate of the ethanol extract exhibited the negligible effect toward strain TA100. All of the extracts had dose-related inhibition effect to their mutagenicity of nitrite treated 1-aminopyrene toward *S. typhimurium* strains TA98 and TA100 in the absence of enzyme activating system. Although Ames test uses prokaryotic cell as the model, which differs from mammalian cell in many factors such as metabolism, chromosome structure and DNA repair processes. Ames test has been used as a basic biological assay to assess the mutagenic potential of chemical compounds prior to *in vivo* animal model [27].

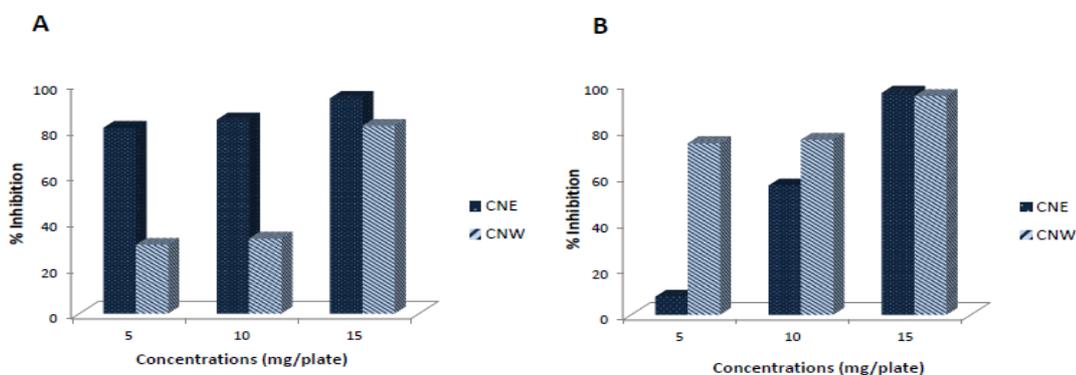


Figure 4 Inhibitory effect of the ethanol and fractionated water extracts from root of *C. nardus* on the mutagenicity of sodium nitrite-treated 1-aminopyrene on *Salmonella typhimurium* strains TA98 (A) and TA100 (B) using Ames test. CNE: ethanol extract of *C. nardus* and CNW: fractionated water extract of *C. nardus*

CONCLUSION

Quality specification of *C. nardus* dried root crude drug in Thailand was established. The root essential oil was rich in sesquiterpenes dominated by elemol and eudesmol. *In vitro* study indicated that the ethanol and fractionated water extracts of *C. nardus* roots were not mutagenic and, in addition, exhibited strong anti-mutagenicity on TA98 and TA100 strains of *S. typhimurium*.

Acknowledgements

P. Phuneerub are grateful to Mae Fah Luang University for the scholarship of Ph.D. programme and thankful to College of Public Health Sciences, Chulalongkorn University for providing facilities throughout the work.

REFERENCES

- [1] P. Jayasinha. Medicinal and aromatic plant series: Citronella, Information services centre industrial technology institute, Colombo, **1999**; 1-2.
- [2] S. Luangnarumitchai; S. Lamlertthon; W. Tiyafoonchai. *Mahidol University Journal of Pharmaceutical Sciences*, **2009**, 34, 60-64.
- [3] W. J.H. Liu. Traditional Herbal Medicine Research Methods, John Wiley & Sons, Inc., New Jersey, **2011**, 7-8.
- [4] N. Kazuhiko; SA. Najeeb; Y. Tadashi; TTN. Huong; T Gassinee. *Japan International Research Center for Agricultural Sciences*, **2003**, 37, 249-252.
- [5] M.M.D.O. Maira; F.B. Danilo; D.G.C. Maria; A. Eduardo; H.P. Roberta. *Food control*, **2010**, 21, 549-553.
- [6] A.B. Adibah; I. Nazlina; I.B. Ahmad. *Malays. Appl. Biol.*, **2010**, 39/2, 19-23.
- [7] K. Koba; K. Sanda; C. Guyon; C. Raynaud; J.P. Chaumont; L. Nicod. *A Journal of the Bangladesh Pharmacological Society*, **2009**, 4, 29-34.
- [8] K Nakahara; NS Alzoreky; T Yoshihashi; HTT Nguyen; G Trakoontivakorn. *Japan International Research Center for Agricultural Sciences*, **2003**, 37(4), 249-252.
- [9] R Manohan; C Palanuvej; N Ruangrungsi. *Research Journal of Pharmaceutical, Biological and Chemical*, **2013**, 4(1), 1144-1155.
- [10] World Health Organization. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines, Regional Office for the Western Pacific, Manila, **1993**.
- [11] DE Levin; E Yamasaki; BN Ames. *Mutation Research*, **1982**, 94(2), 315-330.
- [12] D Maron; BN Ames. *Mutation Research*, **1983**, 113, 173-215.
- [13] EZ Kristien. *Mutation Research*, **2000**, 455, 29-60.
- [14] KA Reid; J Maes; A Maes; J van Staden; ND Kimpe; DA Mulholland; L Verschaeve. *Journal of Ethnopharmacology*, **2006**, 106(1), 44-50.
- [15] EP Springfiel; PKF Eagles; G Scot. *J Ethnopharmacol*, **2005**, 101, 75-83.
- [16] http://efloras.org/florataxon.aspx?flora_id=2&taxon_id=200025100
- [17] http://www.rlc.nrct.go.th/ewtadmin/ewt/nrct_expo/ebook/B0003/index.html
- [18] SW Lee; W Wendy. *Iranian Journal of microbiology*, **2013**, 5, 147-152.
- [19] V. Benzi; N. Stefanazzi; A.A. Ferrero. *Chilean journal of agricultural research*, **2009**, 69, 154-159.
- [20] H.I.A. El-Moaty. *Journal of Natural Products*, **2010**, 3, 103-111.
- [21] S.S. Ranaweera; K.R. Dayananda. *J. Natn. Sci. Coun. Sri Lanka*, **1996**, 24, 247-252.
- [22] K. Asakura; T. Kanemasa; K. Minagawa; K. Kagawa; T. Yagami; M. Nakajima; M. Ninomiya. *Brain Res*, **2000**, 873(1), 94-101.
- [23] K. Asakura; Y. Matsuo; T. Oshima; T. Kihara; K. Minagawa; Y. Araki; K. Kagawa; T. Kanemasa; M. Ninomiya. *Eur J Pharmaco*, **2000**, 394(1), 57-65.
- [24] A Anand. Essential oil-bearing grasses: The genus *Cymbopogon*, CRC Press, New York, **2010**; 123-128.
- [25] NA Bruce; M Joyce; Y Edith. *Mutation Research*, **1975**, 31, 347-364.
- [26] Y Ikken; P Morales; A Martinez; ML Marin; AI Haza; MI Cambero. *Journal of Agricultural Food Chemistry*, **1999**, 47, 3257-3264.
- [27] K Mortelmans; E Zeiger. *Mutat Res*, **2000**, 455(1-2), 29-60.