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Phytochemical screening and influence of extraction solvents on antioxidant and antimicrobial activities of *Asparagus racemosus*willd. Root

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Abstract

The bioactive components present in the *Asparagus racemosus* root are known to be responsible for its medicinal properties. However, the solvent extraction for these bioactive components had effect on bioactive activities. The present study was aimed to compare the effect of using different extraction solvents to extract the active components, antioxidant and antimicrobial activities from *Asparagus racemosus* root. Aqueous (DW and NW) extracts showed the efficiency of *Asparagus racemosus* root as antioxidant agent and broad spectrum antibacterial agent against both Gram-positive and Gram-negative. 95% Ethanolic extract had the most potential in the Ferric Reducing Antioxidant Power (FRAP) of 14.400±0.001 mg AAE/100g FW. But aqueous (DW and NW) extracts showed the lowest IC_{50} value of 4.716±0.002 - 4.757±0.001 mg/ml (IC_{50} ascorbic acid of 3.422±0.001 mg/ml). Moreover, Natural distilled water extract had also the bactericidal effect on *P. aeruginasa* ATCC 27853 and *K. pneumoniae* and *E. faecalis* DMST 4736 at 3-6 h intervals after incubation. These biological activity may be due partly to the presence of various phytochemical compounds; phenolics, flavonoids saponins, steroids, terpenoids and cardiac glycosides. It also suggests that *Asparagus racemosus* root was a potential candidate in antioxidant and antimicrobial agents. It was useful in applications with modern medicine in the therapy or prevention of disease as well as the adoption in commercial various health products in the future. In addition to the ethanol and water were suitable solvent to extract the substance, natural distilled water was also the alternative solvent for bioactive compounds extraction.

Keywords:*Asparagusracemosus*, Phytochemical compounds,Antioxidant activities, Antimicrobial activities, extraction solvent.

Introduction

Plants have long been used traditionally for treatment of various diseases and ailments. The search of new antioxidants and antimicrobial activity from herbal source has taken very large attention in last decade. Secondary metabolites from plants, mainly phenolics having antioxidants, antimicrobial, antitumor, antiviral, enzyme inhibiting and radical scavenging properties.Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Isolation of antioxidant and antimicrobial compounds from plant is possible through extraction with different solvents and it depends on the nature of extracting solvents [1].

*Asparagus racemosus*Willd., is a perennial shrub, with a tuberous rootstock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers which is sweet-scented appears in October. It contains adventitious root system with tuberous roots. These tuberous roots after proper processing and drying are used as ayurveda medicine, with the name of Shatavari [2]. All parts of the plant in general and roots in particular areused by different traditional systems of medicine for the treatment of various humanailments [3, 4]. A lot of chemical analysis has been carried out on the roots of *Asparagusracemosus*. The major reported constituents includes steroidal saponins, shatavarin I, shatavarin II, shatavarin IV alkaloids, proteins, starch and tannins [4]. *Asparagus racemosus*is used to treat various diseases such as ulcer, dyspepsia and debility [5]. In Indian medicine it is well known as an antispasmodic, aphrodisiac, demulcent, diuretic, galactogogue, nervinetonic and refrigerant. It is also used in the treatment of diarrhea , rheumatism, diabetes, , urinary disorders, blood diseases, cough and bronchitis [6, 7, 8].

Therefore, the aim of this study was to evaluate *Asparagus racemosus* root extracts as potential sources of natural antioxidants and antimicrobials. In this study, the effects of extraction solvents (75%

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acetone, 95% ethanol, distilled water and natural distilled water) on the extract yield, antioxidant capacity and antibacterial activity of crude extracts were investigated. Information on the phytochemical compounds, total phenolic and flavonoid content, antioxidant capacity and antibacterial activity of *A. racemosus*rootextracts could be helpful in the selection of suitable solvents for extracting bioactive compounds from other similar plant materials.

Materials and Methods

Sample Preparation and Extraction

The roots of *Asparagus racemosus* used for the study were obtained from the Sri Chawna Garden, Prachantakham District, PrachinBuri Province, Thailand. The collectedroots were properly cleaned with water and dried in shade. Dried roots were cut into small pieces about 2-3 cm. All root samples then were separately maceratedwith 95% ethanol (ratio 1:12), 75% acetone (ratio 11:2) for 5 d and boiled into distilled water (DW: ratio 1:20) or natural distilled water (NW; ratio 1:20) at 60ºC for 30 min. The mixture was filtered through a filter paper (Whatman No. 1) and centrifuged at 8,000 rpm for 15 min. Then, the filtrate obtained was subsequently concentrated under vacuum on a rotary evaporator. The concentrated extracts were kept at -20ºC under dark condition until further analysis.

Phytochemical analysis

Qualitative phytochemical screening of *A. racemosus* roots extract were subjected to qualitative phytochemical screening for the presence and/or absence of different categories of chemical constituents, such as, alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, cardiac glycosides and terpenoids following prescribed methods [9, 10].

Total phenolic content assay

The total phenolic content (TPC) was determined byFolinCiocalteau (FC) coloimetricmethod following the procedure of Materska and Perucka(2005) with slight modification. Briefly, 100 µl of extracted solutionand 750 µl of fresh Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water)was mixed with thoroughly. After 5 min at room temperature, 750 µl of 6% (w/v) sodium carbonate (Na_2CO_3) was added to the mixtures and allowed to completely react for 90 min at room temperature under the dark condition. The absorbance was measured at 725 nm by using a spectrophotometer (Hanon instruments, Japan). The phenolic content was calculated using calibration curve of the gallic acid (0.05-0.25 mg/ml). The result was expressed as mg of gallic acid equivalent / 100g of fresh weight(mg GAE/100g FW) [11].

Total flavonoid content assay

Total flavonoid content (TFC) of tested extracts was determined by using the aluminum chloride colorimetric assay according to method described by Marinova*et al.* (2005). Briefly, 200 µl of crude extracts (of appropriate concentration) were mixed with 2.3 ml of in 30% of methanol solution, followed by the addition of 100 µl of 0.5 M $NaNO_2$ and 100 µl of 0.3 M $AlCl_3$, respectively. The mixture solution was thoroughly mixed with vortex and left to stand for 5 minutes at room temperature in the dark condition. Finally, the absorbance was taken against a blank without the $AlCl_3$ with same mixture at 506 nm using UV-spectrophotometer. The total flavonoid content was calculated using calibration curve of the rutin (0.25-4.00 mg/ml). The result was expressed as mg of rutin equivalent / 100g of fresh weight (mg RE/100g FW) [12].

Free radical scavenging activity by DPPH method

Free radical scavenging activity in sample extracts were evaluated which based on the method proposed by Akowuah*et al.* (2005). Briefly, 100 µl of different solvent of plantextracts dissolved with methanol were mixed with 900 µl of 0.1 mMDPPH in methanolic solution under vigorous shaking. The resultantmixtures wereincubated at room temperature in dark for 15-30 min. At the end of this period, the absorbance of mixture was measured at 517 nm. The control was prepared as mixing 900 µl of DPPH with 100 µl of methanol. Percentage of DPPH scavenging activity was calculated as %inhibition of DPPH used to evaluate the antioxidant activity of compounds [13]. The antioxidant activity of the sample expressed as IC_{50} value defined as concentration (in mg/ml) of sample that inhibits the DPPH radicals formation by 50%

Ferric reducing/ antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) was determined according to the method described by Benzie and Strain (1996). The freshly prepared FRAP reagent in acetate buffer (1.6 g of sodium acetate and 8 ml of acetic acid make up to 500 ml; pH 3.6) was warmed to 37 C in oven until used. A total of 300µl of tested extracts was mixed with 2.7 ml of the FRAP reagent. After 30 min, the absorbance was measured at 596 nm using spectrophotometer. Standard curve of ascorbic acid (0.01-0.08 mg/ml) was prepared. Results were expressed as mg of ascorbic acid equivalents / g fresh weight (mg AAE/100g FW) of the plant materials [14].

Microorganisms and Culture Condition

The antimicrobial activities of all crude extracts were evaluated against nine pathogenic strains including Gram-positive bacteria (*Bacillus cereus* DMST 5040, *Enterococcus faecalis* DMST 4736, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus*

epidermidis) and Gram-Negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* DMST 8212 and *Salmonella typhimurium* ATCC 13311). The bacteria strains were obtained from the laboratory of the Department of Biotechnology, King Mongkut's University of Technology North Bangkok, Thailand. All tested strains were maintained on brain heart infusion (BHI, Difco) agar medium at 37ºC.

Agar Well Diffusion Method

In vitro antibacterial activity was carried out by agar well diffusion method [15]against these nine pathogenic strains. The crude extracts of *A.racemosus* root were diluted with sterile distilled water to obtain a concentration of 25, 50, 100, 200 and400 mg/ml. Overnight bacterial cultures of tested strains were adjusted the OD_{600} to 0.2 (10^8CFU/ml) by spectrophotometer. Briefly, 25 ml of BHI agar was poured into each petri plate. Once the agar solidified, the microorganisms were mixed into 0.75% BHI agar and poured on the surface of the plates. On the surface of the agar plate, 6 mm-diameter wells were created using a sterile cork borer. Each well was filled with 80 µl of each crude extracts at different concentrations. Simultaneously, Ampicillin (Amp, 5 µg/ml) and Norfloxacin (CIP, 5 µg/ml) were used as positive control. After 1, 3 and 5 day incubation at 37º C, all plates were observed the inhibition zones, and the diameter of these zones was measured in millimeters.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assay

The estimation of MIC values of the extracts were carried out by two-fold serial dilution method to quantify inhibitory activity of the sample extracts by using two-fold broth microdilutions in 96 well microtiter plates. All crude extracts and oils were first dissolved in sterile distilled water at the highest concentration of 3,200 mg/ml. Concentration of crude extracts range from 3,200 to 6.25 mg/ml were used. Then, 5 µl of bacterial suspension 10^8CFU/ml (OD_{600} = 0.2) was addedto each wells. The microtiter plates were incubated at 37 C for 24 h. Similar tests were performed simultaneously for growth control (BHI + inoculums) and sterility control (BHI + test sample). The MIC value was determined as the lowest sample concentration. After MIC determination,subcultures were made by spreading visually clear broth dilution MIC well to HBI agar (Difco) at 37 C for 24 h. The lowest concentration which showed the complete absence of bacterialgrowth, was considered as MBC values.

Time-killing Curves Assay

In-vitro bactericidal ability of crude extracts with significant antimicrobial activity was determined by using time-killing curves according to the protocol of NCCLS [16]. Crude extracts of *A.racemosus* rootand tested bacterial strains were mixed and incubated at 37ºC. The viable counts were conducted at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h by plate count method. Cultures with an initial cell density of 1.5-4.0 10^8 CFU/ml were exposed to the crude extract. Plate counts were made after 24 h of incubation and only plates containing between 30 and 300 counts for each series of dilution were counted. Curves were plotted as the viable cells (log10 CFU/ml) versus time. A bactericidal effect is defined as 3 log10 decrease in the CFU/ml or a 99.9% kill over a specified time. The definition of kill for this study was as described by May *et al.* (2000) with modification [17]. Antimicrobial experimental positive controls used in this protocol were specific by the microorganisms that presented susceptibility to *A.racemosus* root extracts.

Statistical analysis

Results were reported as mean ± SD of triplicate measurements. Significance differences for multiple comparisons were determined by One-way ANOVA followed by t-tests. The confidence limits used in this study were based on 95% ($P<0.05$).

Results and Discussion

Preliminary phytochemical screening

After obtaining the extracts in the crude form, the percentage yield of the extracts was calculated (Table 1). Our results show that maximum percent yield is obtained in 75% acetone extract (22.84%), followed by 95% ethanol (16.29%), distilled water (7.17%) and natural distilled water (4.52%). The factors investigated were the types and amount of solvent used and solids-to-liquid ratio. Generally, the reliability of herbal plant for its usage is evaluated by correlating the phytochemical compounds with their biological activities. The different solvent extracts of *A.racemosus* root were tested for the screening of phytochemical constituents, antibacterial activity and antioxidant activity. Phytochemicals present in different solvent extracts weredisplayed in the Table 1. From a single test we cannot conclude the nature of secondary metabolites. The phytochemical screening of crude extracts of *A.racemosus* root revealed the presence of some secondary metabolites such as saponins, flavonoids, steroids, terpenoids, cardiac glycosides and alkaloids.

Ethanolic and aqueous (DW and NW) extracts of *A. racemosus* root were positive for most of the phyto-constituents, which contain such as saponins, flavonoids, steroids, terpenoids and cardiac glycosides. These five compounds are responsible for antimicrobial and antioxidant activities in this plant. Saponin,

steroids, terpenoid and cardiac glycosides were present in all solvent extraction (aqueous, 95% ethanol and 75% acetone). All the extracts tested for the tannins and anthraquinone were negative. Between DW and NW extract, the phytochemical screening of NW extract had a better extraction potential than that of DW (Table 1) and it has been observed that most of the secondary metabolites were identified in the polar extracts.

Table1: Preliminary Phytochemical analysis of *A.racemosus* root extracts

Phytochemical Compounds		Distilled water	Natural distilled Water	95% Ethanol	75% Acetone
Saponin		+	++	+++	++
Tannin		-	-	-	-
Flavonoid	Shinoda test	-	+	-	-
	10% Lead (IV) acetate	+	+	++	-
Antraquinone		-	-	-	-
Steroids		++	+++	++	++
Terpenoids		+++	+++	+	++
Cardiac Glycoside	Liebermann test	+	++	++	+
	Keddy reagent	-	-	-	-
	Keller-kiliani test	+	+++	-	+
Allaloids	28% NH_4OH	-	-	-	-
	Wagner' reagent	-	-	-	-
	Dragendoff's reagent	+	+++	-	-

+ = Presence; - = Absence

The phytochemical compounds detected are known to have medicinal importance. Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [18]. Alkaloids have been reported as biological activities like, anti-inflammatory [19] antimalarial [20], antimicrobial [21], cytotoxicity, antispasmodic and pharmacological effects [22, 23]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [24]. Other phytochemicals called cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia [25].

Total Phenolic and Flavonoid Contents

Phenolic compound of plant acts as primary antioxidants or free radical scavenger [26]. The result revealed that the highest amount of phenolic content was found in ethanolic extract of *A.racemosus* root followed by acetonic and aqueous (DW and NW) extracts. Distilled water extract with phenolic content 164.000±0.001 GAE/100g FWshowed lowest antioxidant activity. Plant polyphenols are the significant group of compounds acting as free radical scavenging or primary antioxidants. Among polyphenols, flavonoids are of great importance because they help human body to fight against diseases. The ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. They are abundantly found in plants as their glycoside [27]. Our results for total flavonoid content for *A.racemosus* root extracts was range from 9.800±0.005 - 90.800±0.002 mgRE/100g FW (Table 2)

Based on the total phenolic content in the plant extracts, the selected parts can be divided into three ranges of GAE values. The lower, middle and higher ranges of total phenolic compounds were below 1000, 1000–2000 and higher than 4000 mg GAE/100g FW, respectively [28]. The results in the Table 2 show that all the investigated extracts have low phenolic and flavonoid contents. The total phenolic and flavonoid contents values significantly differ for aqueous (DW and NW), ethanolic and acetonic extracts. However, the natural distilled waterextract had more phenolic and flavonoid phytochemicals than distilled water(table 2). Although this plant is not rich in phenolic compounds, as shown by the low total phenolic content, it may contain other phytochemicals.95% ethanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, while the higher molecular weight flavonoids are better extracted with distilled water.

Antioxidant Activities

The antioxidant ability using DPPH and radical scavenging properties (FRAP assay) of plants is widely used to evaluate the antioxidant capacity of extracts produced from the medicinal plants in which higher inhibition level is an indicator of a strong antioxidant. In this study, the antioxidant activity of *A.racemosus* root extracts was measured using two different assays, namely FRAP and DPPH. Performing a single assay to evaluate the antioxidant properties would not give the correct result because antioxidant activity of plant extract is influenced by many factors, for example the test system and composition of extract. Therefore it is important to carry out more than one type of antioxidant capacity measurement to cover the various mechanisms of

antioxidant action [29]. Ferric reducing antioxidant power (FRAP) assay depends on the reduction of ferric ion into ferrous ion [14]. DPPH is nitrogen centered free radical having an odd electron which gives a strong absorption at 517 nm, its color changes from purple to yellow when DPPHradical dot odd electron paired off in the presence of radical scavenger to form the reduced DPPH-H [30].

In the present study, the antioxidant capacity of *A. racemosus* root extract with various solvents was evaluated by using DPPH radical scavenging method by comparing with the activity of the ascorbic acid as a known antioxidant activity. The result showed that aqueous extracts extract of *A. racemosus* root had the highest amount of free radical scavenging activity and reducing capacity as expressed as ascorbic acid equivalents. It was followed by acetonic and ethanolic extracts (Table 2). High DPPH inhibition activity in *A. racemosus* root extract with NW could be attributed to its higher contents of flavonoid and phenolic compared to other extraction solvents (95% ethanol and 75% acetone).

Table 2: Total phenolic and flavonoid contents and Antioxidant activity of *A.racemosus* root extracts

Tests	Solvent Extraction			
	Distilled water	Natural distilled Water	95% Ethanol	75% Acetone
Total Phenolic Content (mg GAE/100g FW;±SD)	164.000±0.001	400.000±0.001*	340.000±0.005	191.000±0.004
Total flavonoids Content (mg RE/100g FW; ±SD)	37.000±0.001	90.800±0.002*	28.300±0.003	9.800±0.005
Antioxidant Capacity				
- FRAP assay (mg AAE/100 g FW; ±SD)	11.200±0.004	13.700±0.002*	14.400±0.001	11.400±0.003
- DPPH assay (mg AAE/100g FW±SD)	91.400±0.001*	62.600±0.003	42.300±0.001	54.600±0.001
IC_{50} (Sample); mg/ml	4.716±0.002*	4.757±0.001	5.218±0.001	5.818±0.002
$IC_{50Ascorbic\ acid}$; mg/ml	3.422±0.001			

GAE = Gallic Acid Equivalent; RE = Rutin Equivalent; AAE = Ascorbic acid Equivalent; IC_{50} = 50% Inhibitory Concentration; * = P<0.05, Ascorbic = Standard Control

A lower IC_{50} value in plant extracts indicates a greater ability to neutralize free radicals. High variation in antioxidant activity was observed, with IC_{50} values ranging from 4.716±0.002– 0.81 - 5.818±0.002 mg/ml. Aqueous extracts of *A. racemosus* root including distilled water and natural distilled water showed the best IC_{50} values of 4.716±0.002, and 4.757±0.001 mg/ml, respectively, when comparing with ascorbic acid as standard of 3.422±0.001 mg/ml. But lower activity was presented in ethanolic (5.218±0.001 mg/ml) and acetonic (5.818±0.002 mg/ml) extracts.

The antioxidant properties of the plant extracts were evaluated through FRAP assays. It must also be noted that the antioxidant activities assessed are direct relation with the polyphenolic content of the extract. The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. A higher absorbance indicates a higher ferric reducing power. In this study, it was found that ethanolic extract of *A.racemosus* root showed the highest amount of ferric reducing power expressed as ascorbic acid (14.400±0.001 mg AAE/100 g FW), followed by natural distilled water extracts (13.700±0.002 mg AAE/100 g FW). Moreover, the natural distilled water extract (13.700±0.002 AAE/100 g FW) had higher antioxidant capacity than distilled water (11.200±0.004 AAE/100 g FW) by FRAP assay. There was no correlation between antioxidant activity and total phenolic and flavonoid content among the various crude extracts. In spite of the lower phenol content, ethanolic and distilled water extract showed higher antioxidant activity. The antioxidant capacities of the extracts have a strong relationship with the solvent employed, mainly due to the different antioxidant potential of compounds with different polarities [31].

Plant extracts obtained using organic solvents have limitations. The use of water as the extracting solvents is more desirable than the use of organic solvents due its environmentally friendly and non-toxic characteristics. From the results shown in table 2, it is evident that water (DW and NW) is a good solvent in extracting a sizable quantity of phenolic and flavonoid compounds along with antioxidant activities (DPPH and FRAP assay) from *A.racemosus* root. Water extracts containing phenols and flavonoids with high activities can safely be exploited in numerous food applications.

Antimicrobial activity

It is well known that phenolic antioxidants act as inhibitors for radical chain reactions on autoxidation of organic substrates. Moreover, some phenolic compounds were known to show antimicrobial activities in addition to their antioxidant effects. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The antibacterial activity of aqueous (DW and NW), ethanolic and acetonic extracts of *A.racemosus* root was tested against 9 bacterial strains using agar well diffusion method. The antibacterial activity, measured in terms of the diameter of the zone of inhibition is shown in Table3.

Table 3: Antibacterial activity of *A.racemosus* root extracts against some pathogenic stains

Concentration (mg/ml)		Average Inhibition Zone (AIZ: ∅ = 6 mm); mm±SD								
		Gram positive strains					Gram negative strains			
		BC	EF	SA	SE	EC	KP	PA	PM	ST
Distilled Water	25	-	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-	-
	100	-	-	14.00±1.00	-	-	7.00±0.00	-	-	-
	200	-	-	-	-	-	19.00±6.00	-	-	-
	400	-	-	22.00±6.24	-	-	-	-	-	-
Natural distilled water	25	7.00±0.00	7.00±0.00	-	-	-	7.00±0.00	15.00±1.00	-	8.00±1.00
	50	9.33±2.52	9.67±0.58	-	-	13.00±1.73	8.00±0.00	9.33±0.58	-	20.00±2.00
	100	15.67±1.53	7.33±0.58	-	-	-	7.00±0.00	11.67±1.15	-	11.00±1.00
	200	-	14.33±3.21	-	-	-	19.00±6.00	8.67±0.58	-	18.67±3.21
	400	18.33±5.51	15.67±4.93	-	-	32.00±2.00		7.33±0.58	-	24.67±5.03
95% Ethanol	25	-	8.33±1.15	-	-	-	-	-	-	-
	50	-	9.33±1.15	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-
	200	-	9.00±1.00	-	-	-	9.00±1.73	-	-	-
	400	-	9.33±0.58	-	-	-	8.00±1.00	-	-	-
75% Acetone	25	-	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-	-	-

BC = *B. cereus* DMST 5040, EF = *E. faecalis* DMST 4736, SA = *S. aureus* ATCC 25923, SE = *S. epidermidis*, EC = *E. coli* ATCC 25922, KP = *K. pneumoniae*, PA = *P. aeruginosa* ATCC 27853, PM = *P. mirabilis* DMST 8212 and ST = *S. typhimurium* ATCC 13311; (-) : indicates no inhibition

The growth inhibitory effects of the crude *A.racemosus* root extracts by broth micro-dilution method were presented in table 4. The result indicated that the *A.racemosus* root extracts were shown antibacterial activities at variable degree against all tested pathogenic strains, with MIC values varying from 1,600 to more than 3,200 mg/ml. The MIC analysis of plant extracts showed the optimum bacteriostatic concentration for aqueous(DW and NW) extracts of *A.racemosus* root.

Table 4: The MIC and MBC of *A.racemosus* root extracts against some pathogenic strains by broth microdilution method

Solvents Extraction			Microorganisms									
			Gram positive strains					Gram negative strains				
			BC	EF	SA	SE		EC	KP	PA	PM	ST
Distilled water		MIC	1,600	1,600	1,600	1,600		1,600	1,600	1,600	1,600	1,600
		MBC	-	-	-	-		-	-	-	-	-
Natural distilled water		MIC	1,600	1,600	1,600	1,600		1,600	1,600	1,600	1,600	1,600
		MBC	-	-	-	-		-	-	-	-	-
95% Ethanol		MIC	1,600	-	-	-		-	-	-	-	1,600
		MBC	-	-	-	-		-	-	-	-	-
75% Acetone		MIC	-	-	-	1,600		-	-	-	-	-
		MBC	-	-	-	-		-	-	-	-	-
Antibiotics	Amoxicillin	MIC	0.078	0.078	0.313	0.078		0.078	0.078	0.078	0.156	0.078
		MBC	0.156	0.078	2.500	0.156		0.078	0.078	0.156	0.156	0.078
	Norfloxacin	MIC	0.078	0.078	0.078	0.156		0.078	0.078	0.156	0.019	0.039
		MBC	0.078	0.078	0.078	0.156		0.078	0.078	0.156	0.156	0.078

BC = *B. cereus* DMST 5040, EF = *E. faecalis* DMST 4736, SA = *S. aureus* ATCC 25923, SE = *S. epidermidis*, EC = *E. coli* ATCC 25922, KP = *K. pneumoniae*, PA = *P. aeruginosa* ATCC 27853, PM = *P. mirabilis* DMST 8212 and ST = *S. typhimurium* ATCC 13311; (-) = >3,200 mg/ml

Time killing curve assay

The bactericidal ability of the *A. racemosus*root in with significant antimicrobial activity was determined by time-killing curves. *P. aeruginasa* ATCC 27853, *K. pneumoniae*, *S. typhimurium* ATCC 13311 and *B. cereus* DMST 5040 and *E. faecalis* DMST 4736 were selected for the assay depending on agar well diffusion and MIC values (Figure 1 and 2).

Among extraction solvent, the time killing curve of *A. racemosus* root extract with NW revealed a significant bactericidal effect, in which the strains of *P. aeruginasa* ATCC 27853 and *K. pneumoniae* and *E. faecalis* DMST 4736 were completely killed in 3, 3 and 6 h intervals after incubation, respectively when compared to control (2.0-3.0 log 7 CFU/ml). All of the sterilization effects of the extracts were maintained for 36 h, thus showing that the ethanolic extract of *A. racemosus* root had antibacterial activities at 9 h intervals after incubation. *A. racemosus* root extracts with natural distilled water not only possessed antibacterial activities, but also had bactericidal abilities at 3 h intervals after incubation.Although crude extract with distilled water had bactericidal effect on *S. typhimurium* ATCC 13311 at 3 h intervals after (3.33 log 4 CFU/ml), this strain is able to resist the crude extract at 9 h intervals after incubation (2.00 log 6 CFU/ml) when compared to control (1.33 log 8 CFU/ml) (Figure 1C). From the aqueous extracts, the result also showed that crude *A. racemosus* root extract in NW was significantly revealed the better potential bactericidal effect than that of DW especially on *S. typhimurium* ATCC 13311 strain (P<0.05) (Figure 2).

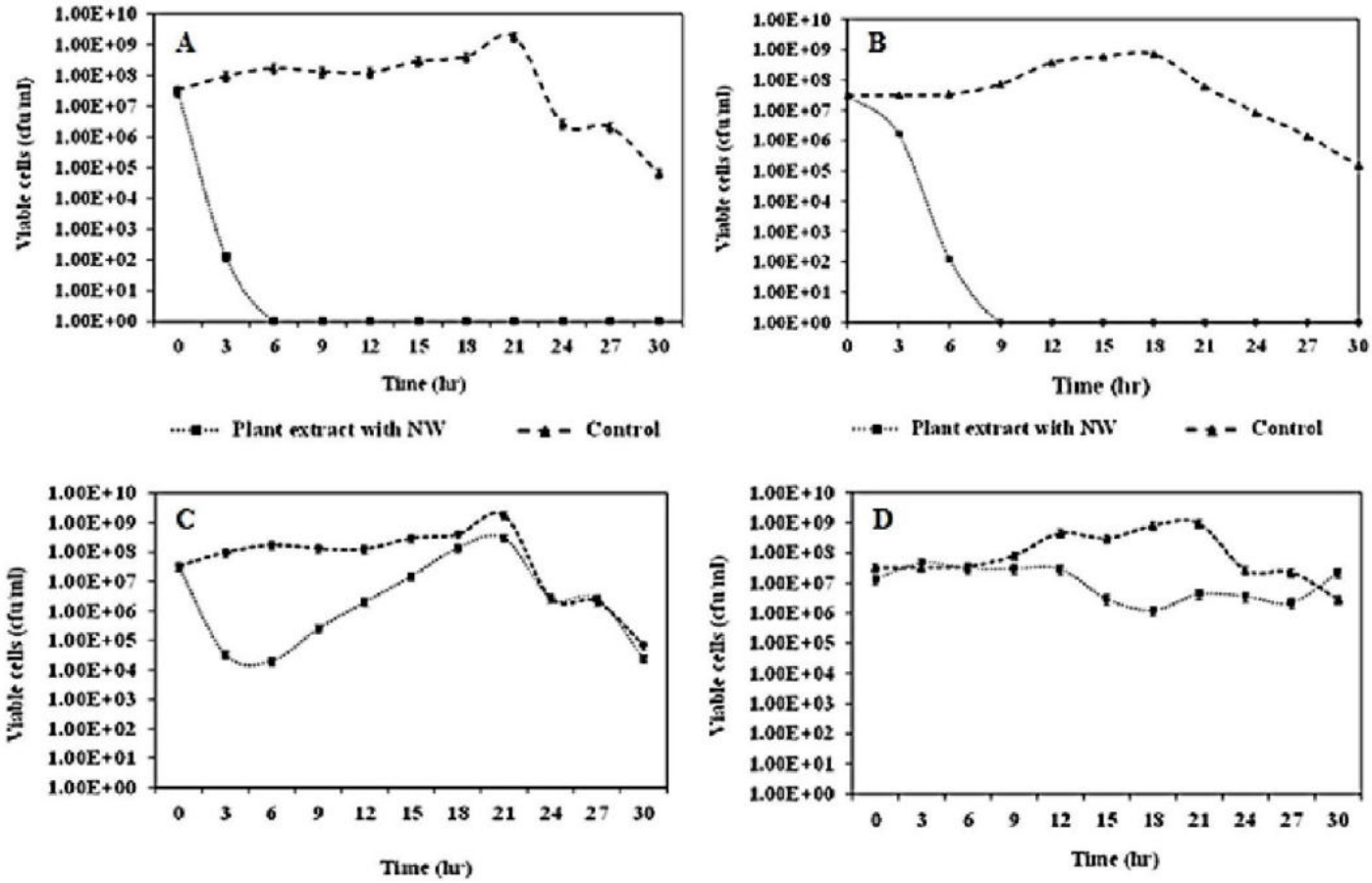

Figure 1: Time-killing curves of *Asparagus racemosus* root extract with NW on *P. aeruginosa* ATCC 9027 (A) and *E. faecalis* DMST 4736 (B) and with DW extracts on*S. typhimurium* ATCC 13311 (C) and *B. cereus* ATCC 11778 (D)

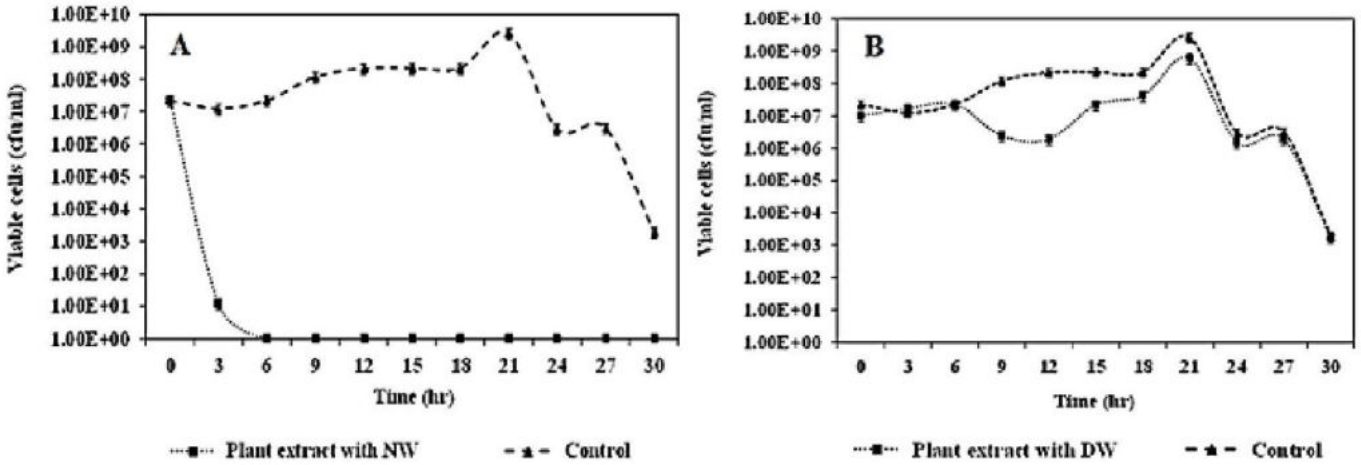

Figure2: Bactericidal effect of the NW (A) and DW (B) extracts of *A.racemosus* root on *K. pneumoniae* strain

Even if natural distilled water (NW) has some substances that are not good for consumption in the present. On the other hand, if we use to extract the bioactive compounds from plants, it may perform better than the distilled water (DW) or other solvents due to the presence of minerals or ions in the natural distilled water. They are able to support the extraction. However, the choice of natural distilled water in the study should be consider the source of water. It must so far from the factory that can release pollutants into the atmosphere as well as adequate cleanliness and safety to implement. In the way of life some Thais also use natural distilled water sources for consumption and consumption. In order to reach the actual principles of the use of medicinal plants to benefit the conditions of the Thai society thoroughly. In this study, natural distilled water was used as a solvent for extraction in biological studies compared with other solvents. The test results show that natural distilled water is good as a solvent for extraction.

Conclusion

On the basis of the results reported in this study, we conclude that the crude extracts of *A.racemosus* root exhibit significant antioxidant and antibacterial activities. Extraction with different solvents affect yield of total polyphenol content, antioxidant and antimicrobial activity of *A.racemosus* root. Regardless of other solvent used, the most efficient solvent for phenolic and flavonoid andscavenging activity were aqueous fractions including; natural distilled water and distilled water while in 75% acetone showed lowest phenolic and flavonoid content. 95% ethanol and natural distilled water extractsshowed highestferric ion reducing power. Aqueous extracts (DW and NW) showed the efficiency of *A.racemosus* root as antibacterial agent acting against both Gram positive and Gram negative bacteria. Moreover, natural distilled water extract had bactericidal effect on some pathogenic strains. The antioxidant potential with significant antimicrobial activity in *A.racemosus* root which may be due partly to the presence of phenolics, flavonoids saponins, steroids, terpenoids and cardiac glycosides. The present study provides additional information for supporting the use of natural distilled water to extract the bioactive compounds from herbal plant with significant levels of their activities. Thus, it also suggests that shows great potential to be used as a natural antioxidant and antimicrobial source and to be further explored for use in the food industry and pharmaceutical products.

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