RESEARCH ARTICLE

ISSN: 2348-8948

Vol: 2; Issue: 5

Biological Screening of some Chinese and Herbal drugs that are commercially available in Mauritius

MINU GUPTA BHOWON*, SABINA JHAUMEER LAULLOO, PRISCICA PARRIANEN, AHIDA OUMMEH DHURMEA, MARIE SOPHIE FERRY

Department of Chemistry, Faculty of Science, University of Mauritius, Reduit, Mauritius

Date Received:	Date of Accepted:	Date Published:
15th Apr2014	24th Apr2014	12th May2014

Abstract:

Introduction: In the past few years traditional medicines have become hot topics for life science researchers as there is a worldwide emergence of herbal drugs for treating various diseases affecting mankind. These herbal drugs are being increasingly used as drugs or dietary supplement for fighting or prevent common diseases such as colds, diarrhea, diabetic and so on. **Objective**: The phytochemmical screening, antioxidant, antiglycation and antibacterial properties of four Ayurvedic drugs (Diabecon, Pilex, Septilin and Rumalaya) and seven Chinese herbal drugs (Leung PuiKee, Jin Quian Chao, Po Chai, Cheng Sie Lung, EngAun Tong, Tin Hee, Bi Yan Pian) commercially available in Mauritius were evaluated. Powdered drugs or methanolic extracts of the drugs were analyzed for the phytochemical constituents. Antibacterial activity was evaluated by Disc diffusion assay with two gram positive and two gram negative bacteria. The free radical scavenging activity of the herbal drugs were assessed using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay and antiglycation activity of the drugs was also evaluated. **Results**: Most of the drugs showed the presence of alkaloids, saponins, tannins and polyphenols. The drugs showed higher activity for gram positive bacteria compared to gram negative bacteria. The Ayurvedic drugs showed higher activity as compare to the Chinese drugs against the different stains tested. Septilin showed highest activity with an MIC value of 1 mg/mL and 5 mg/mL against *Staphylococcus aureus* and *Bacillus cereus* respectively. The drugs were found to possess significant scavenging activity with SC_{50} values in the range of (1.3 × 10-4 – 200× 10-4) µg/ml and inhibit the AGE formation with IC50 values ranging 2.0-7.2 µg/ml. Among the drugs tested, Diabecon was found to be the most potent anti-oxidant and anti-glycation drug with SC50 and IC50 values of 1.3 × 10-4 and 4.2 µg/ml. Pearson analysis showed a good correlation between phenolic content and radical scavenging or anti-glycation activities while the tannin content was found to influence the anti-bacterial activity. **Conclusions**: The present study conclusively demonstrates that the presence of phenolic compounds influence positively the radical scavenging and antiglycation activities. In addition the results also indicated that tannin was the main metabolite responsible for the antibacterial activity.

Keywords: Chinese herbal drugs, ayurvedic herbal drugs, phytochemical, antimicrobial, antioxidant, anti-glycation, pearson analysis

Introduction

Traditional medicine (TM) is increasingly becoming popular as an effective alternative or complementary to modern medicine. TM are derived from products of plant roots, stems, leaves, bark, seeds, fruits and flowers which can be used to support general health and treat diseases [1]. These different products of the plants can be used in a prescription formula or processed into various ready-to-use products [2]. Indian Ayurvedic and Traditional Chinese systems are considered as great 'living traditions' and have a thorough organized databases of medicinal plants and herbs which can be use for bioprospecting new medicines and thus have become interesting for researchers.

These TM play an important role as alternative medicine due to their less side effects, economic viability and theurapeutic performance [3]. The plant extracts present in these herbal drugs have been reported to possess various biological activities including antibacterial, anti-diabetic and anti-oxidant [4-12]. The aim of this study were to screen some selected Chinese and Ayurvedic medicines for their phytochemical components and to evaluate their anti-microbial, anti-oxidant and anti-glycation activities. The relative influence of bioactive components (phenolics, saponins, tannins, alkaloids and flavonoids) with their biological properties was also investigated.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid (GA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and quercetin were obtained from Sigma-Aldrich (UK). Tannin acid (TA) was purchased from BDH (England) while glucose D and Bovine Serum Albumin (BSA) were obtained from Alpha Chemika (India). Sabourad Dextrose Agar and Mueller Hinton Broth have been purchased from HiMedia Laboratories, India. All other reagents used were of analytical grade.

2.1.2 Equipment

The absorbance of the compounds for antioxidant activity was recorded using Labsystems Multiskan Ms EIA Reader and the electronic spectra of the samples were obtained from the Biochrom Libra S22 UV-vis spectrophotometer. The intensity for antiglycation activity was recorded using a Perkin Elmer LS55 Fluorimeter. Centrifugation was performed using a Hermlt Z320 apparatus.

2.1.3 Herbal drugs

Seven Chinese and four ayurvedic drugs were purchased from the local markets, Port-Louis, Mauritius. The drugs used were sold in the form of pills except for Cheng Sie Lung which was in powdered form. The different plant species present in each drug are reported in Table 1.

2.2 Preparation of drug extracts

The coating of the drugs (Bi Yan Pian, Diabecon, Jin Qian Chao, Pilex, Rumalaya, Septilin) were removed by a scalpel and the drugs were then crushed into fine powder using a mortar pestle. The masses of the drugs with and without coating were recorded (Table 1). The powdered drug (10 mg) was dissolved in methanol (10 mL) and the solution was filtered. The methanolic extract was used to determine the quantity of tannin and total phenolic contents.

2.3 Phytochemical screening

2.3.1 *Determination of alkaloids*

The quantitative test for alkaloids was determined by the method adapted from Edeoga *et al*. [13]. 40 mL of CH3CO2H (10% in C2H5OH) was added to the powdered drug (1 g). The mixture was allowed to stand for 4 h, filtered and concentrated to ¼ of its original volume. Concentrated NH4OH was then added until complete precipitation. The precipitate was collected, washed with dilute ammonia, dried and weighed to constant mass.

2.3.2. Saponins

The quantitative test for saponins was determined by the method adapted from Edeoga *et al.* [13]. 2 g of the powdered drug was added to 10 mL of 20 % C2H5OH. The solution was heated for 4 h on a waterbath and then filtered. The obtained residue was extracted with 20 mL of 20% C2H5OH. The combined extracts were reduced to 10 mL and washed with diethyl ether (5 mL). The aqueous layer was diluted with 6 mL of *n*-C4H9OH and washed with 2 mL of 5% NaCl. The organic layer was evaporated to dryness until constant weight was obtained.

2.3.2. Tannins

1 mL of the methanolic extract of the herbal drug (1mg/mL) was heated for 40 minutes at 95°C with 6 mL of HCl (70 % in butanol) and 0.2 mL ferric reagent (2% w/v NH4Fe(SO4)2.2H2O). The resulting solution was vortex and the yellow colouration developed was read at 520 nm against a blank standard (acidified ferric reagent). Results are expressed in mg of tannic acid equivalents per gram of drug tested (mg TA/g).

2.3.3. Total phenol content.

The total phenolic content of the different herbal drugs was determined using the Folin-Ciocalteu assay [14]. Sample of the methanolic extract of the drug (0.25 mL) and 0.25 mL Folin-Ciocalteu (diluted with 3.75 mL distilled water) was mixed with 1 mL of 20% sodium carbonate solution. The resulting mixture was vortex and heated for 40 minutes and absorbance of the blue colouration developed was read at 670 nm. The results are expressed as mg of gallic acid equivalent per g of herbal drug.

2.4. Antimicrobial

For anti-microbial activity, one pill/tablet/ packet of powdered drug was dissolved in DMSO (1 mL). The drugs which were insoluble in DMSO were tested as a suspension. The herbal drugs (100 mg/mL - 0.1 mg/mL) were screened against gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus* and gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* of clinical isolates using the disc diffusion method [15]. DMSO was used as negative control and

Ciprofloxacin (Ciprozol) was used as positive control. The plates were incubated for 24 h in sterile conditions at 40 °C.

The herbal drug extracts (100 mg/mL) were tested on the fungus *Aspergillus niger*. The fungus was cultivated on Sabourad Dextrose agar (65 g/dm3, HiMedia) for 72 hours at 37□C.

The diameter of the growth inhibition zones were then measured to the nearest millimeter. The tests were carried out in triplicate.

2.5 Anti-oxidant activity

The drugs were dissolved in DMSO at a concentration of 0.1g/ml and centrifuged at 4000 rpm for 4 min and the filtrate (supernatant) was used. The free radical scavenging activity of the herbal drugs were assessed using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay according to the method described in literature [16]. Absorbance at wavelength 492 nm was measured after 30 min. Positive anti-oxidant activity was detected through a change in color from purple to yellow. Ascorbic acid was used as the positive control and DMSO was used as negative control.

The percentage radical scavenging activity of the tested compounds, expressed as percentage inhibition DPPH, was calculated according to the formula:

% Inhibition = [(A0 – At) / A0] × 100

A0 is the absorbance of the blank sample.
At: the absorbance value of the drugs

The 50 % scavenging concentration (SC50) based on the amount of compound required to decrease the initial DPPH radical concentration by 50% was calculated using Microsoft Excel.

2.6 Anti-glycation activity

The anti-AGEs formation was determined according to Chen *et al.* [17] with some modifications. The crushed drug sample (0.25 g) was dissolved in DMSO (25 mL) and the solution was filtered and diluted to obtain the concentrations (10000 - 100 µg/mL). The drug extracts (1 mL) with 0.1 g/mL BSA, glucose (1.5 mL, 35 mM) and sodium azide (1.0 mL, 0.05 M) in phospahate buffer (0.2 M) were incubated under aseptic conditions at 37□C for 5, 10, 15, 30 60 and 90 days. The total AGEs formation in BSA-glucose system was measured with excitation and emission wavelength at 370 and 440 nm using the modified equation:

$$\%\text{Inhibition} = \left[1-\frac{(F_{BSA+glucose+herbaldrugextract}-F_{BSA+herbaldrugextract})}{(F_{BSA+glucose}-F_{BSA})}\right]\times 100\%$$

FBSA + glucose + herbal extract - FBSA + herbal extract : difference fluorescent intensity of drug sample incubated with and without glucose

FBSA + glucose - FBSA : difference fluorescent intensity of BSA with and without glucose.

Aminoguanidine was used as a positive control (1.5 mL) while DMSO was used as blank. The IC50 values, that is, the concentration which causes 50 % inhibition, were estimated by a non-linear algorithm using Microsoft Excel.

3.0. Results and Discussion:

3.1. Total phenolic, alkaloids, tannins and saponins contents

The total phenolic, tannins, alkaloids and saponins contents were determined in the eleven different herbal drugs (ayurvedic and chinese) and the results are summarized in Table 2. The highest level of alkaloid was found in Jin Quian Chao, saponin and tannin in Bi Yan Pian while highest total phenolic content was found in Diabecon.

3.3. Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

Phenolic compounds, alkaloids and saponins [7, 18] have been reported to possess anti-oxidative properties. It is well known that the antioxidant activity of extracts containing phenolic compounds is due to their capacity to be donors of hydrogen atoms or electrons to capture free radicals. The free radical scavenging activity of the herbal drugs was evaluated on the ability to scavenge the synthetic DPPH. This assay is one of the tests to prove the ability of the components of the herbal drugs to act as donor of hydrogen atoms. The percentage of the radical scavenging activity of the different herbal drugs was carried out with varying concentrations (100 – 0.39) × 10^3 µg/mL. From Figure 1, the magnitude of the antioxidative potency varies with the different drugs tested and they were found to be dose dependent.

The five highest order of effectiveness for radical scavenging activity was Diabecon > Chen Sie Lung > Septilin > Rumalaya > Eng Aun Tong (Table 4). Diabecon showed highest anti-oxidant activity with even lower SC50 value (1.3 × 10-4) than the positive control ascorbic acid.

3.4 Anti-AGEs formation capacity 9

The reaction of glycation between reducing sugars and proteins results in the formation of Advanced Glycation End products (AGE), which is believed to play important roles in pathogenesis of diabetic and aging complications. Thus, agents that inhibit the formation of AGEs are purported to have therapeutic potentials in patients with diabetes or age related diseases. Traditional plant therapies are known to inhibit the formations of AGE's. AGE's are flurophores having an excitation and emission wavelength of 370 and 440 nm repectively [19].

Aminoguanidine hydrochloride (AG) is a known inhibitor of AGEs and it is used as a positive control. The Aryuvedic drugs (Diabecon, Pilex, Septilin and Rumalaya) and Chinese Drugs (Ubat Po Chai, Cheng Sie Lung and Eng Aun Tong) in different concentrations (100 - 10000 □g/mL) were evaluated as inhibitors of AGEs formation at different time intervals (Table 3). In the absence of inhibitor, a rapid increase in intensity from 5 to 30 days was observed. As the number of days increases, the intensity increases with AGEs formation till it reaches a maximum at 60 days. It has been reported that the AGEs formation normally occurs within 60-90 days [20]. In the presence of positive control, the intensities of the sample decreases as it hinders the formation of AGE's.

Plant extracts containing natural products such as phycobiliprotein possess inherent fluoresce properties affecting the detection of AGE formation [21]. In this study, the herbal drugs investigated for anti-AGEs formation also contain different amount of natural products which showed significant background fluorescent intensity and the levels were further increased when the extracts were mixed with BSA. The increase in fluorescence intensity may be due to the direct binding of auto-fluorescent material to BSA, hence the modified equation [17] was used to calculate the % inhibition.

Increasing the concentration of the drugs causes the fluorescent intensities to decrease due to the inhibition of AGEs formation. The % inhibitions of the different drugs at different concentrations and at different time intervals are summarized in Table 3.

The SC 50, value concentration which caused 50% Scavenging Activity and the IC50 value, the concentration at which 50% inhibition occurs, for the different drugs were calculated using the forecast formula and results are tabulated in **Table 4**

In general, the ayurvedic drugs showed better anti-glycation activity than TCM except for Eng Aun Tong. Diabecon showed the highest activity among the drugs tested.

3.2. Antimicrobial Screening of the herbal drugs

It has been reported that the plant extracts present in the herbal drugs exhibited antifungal activity against *Aspergillus niger* and /or *Candida albicans* [22-28] however, none of the drugs tested under this study were found to possess antifungal activity.

The DMSO extracts of both the Ayurvedic and Chinese drugs exhibited moderate inhibitory effects against the gram-positive as compared to the gram-negative bacteria. Septilin and Rumalaya showed the highest inhibitory effect against the gram-positive bacteria followed by Diabecon and Cheng Sie Lung. These drugs contain moderate concentration of the flavanoids alkaloids, saponins and phenolics which may be associated with anti-bacterial activities. Except for the Chinese drugs, Eng AunTong, all the other drugs tested showed no inhibition against *E. Coli* and our results are consistent for those obtained by [29, 30] who have tested the different plants extracts present in these drugs (Table 5).

Statistical analysis

The different assays were performed in triplicate and the results are reported as a mean ± standard deviation. Several previous studies have shown a close positive relationship between total phenolic content and anti-oxidative activities [17, 31]. The correlation among the phytochemicals present with the biological properties screened was studied using Pearson analysis. Pearson's correlation coefficients were calculated between each mean variable computed for the Ayurvedic and Chinese drugs and summarized in Table 7.

The Pearson's correlation analysis for ayurvedic drugs indicated a weak correlation between anti-oxidant/anti-glycation and total alkaloid/saponin/tannin contents but good correlation between anti-oxidant/anti-glycation (r = 0.82 & 0.92) and phenolic contents. The Chinese drugs showed good to moderate correlation (r = 1.00, 0.61 & 0.42) between the antioxidant and total tannin/alkaloid/phenolic contents while for antiglycation good correlation was obtained with saponin and phenolic constituents. The amount of tannins present was the key determinant for the bacterial activity against S. *aureus* with correlation coefficients 0.77 and 1.00 for Ayurvedic and Chinese drugs.

Table 1

The Chinese and Ayurvedric drugs

Name of Drug (Company)	plant species present	Weight With coating (g) Without coating (g)
Diabecon (The Himalaya Drug Co. India)	*Gymnema sylvestre, Pterocarpus marsupium, Glycyrrhiza glabra, Casearia esculenta, Eugenia jambolana, Asparagus racemosus, Sphaeranthus indicus, Tinospora cordifolia, Swertia chirata, Tribulus terrestris, Phyllanthus amarus, Gmelina arborea, Gossypium herbaceum, Aloe vera,* Berberis aristata, *Triphala,* Shilajeet, *Curcuma longa*, piper nigrum, Vidangadi lauham, Boerhaavia diffusa and *Ocimum sanctum*.	(tablet) 0.629 (0.533)
Pilex (The Himalaya Drug Co. India)	*Balsammodendron mukul, Shilajeet, Melia azadiracta seeds*, *Berberis aristata, Emblica officinalis, Terminalia bellerica, Terminalia chebula, Cassia fistula, Bauhinia variegate* and *Mesua ferrea*	tablet 0.302 (0.289)
Septilin (The Himalaya Drug Co. India)	*Balsammodendron mukul, Shankh bhasma, Maharasnadi quath, Tinospora cordifolia*, *Rubia cordifolia*, *Emblica officinalis*, *Moringa pterygosperma* and *Glycyrrhiza glabra*	tablet 0.675 (0.402)
Rumalaya (The Himalaya Drug Co. India)	*Mahayograj gugul, Shankh bhasma Shilajeet, Hisbiscus abelmoschus, Swarnamakshik gbhasma, Maharasnadi quath, Rubia cordifolia*, *Moringa pterygosperma*, *Tribulus terrestris and Tinospora cordifolia*	tablet 0.648 (0.451)
Jin Qian Chao (Guangdong Yihetang Pharmaceutical Co. Ltd, China)	*Herba lysimachiae, Herba Desmodii*, *Folium Pyrrosiae*, *Rhizoma Alismatis*, *Rhizoma corydalis*, *Spora Lygodii* and *Radix Glycyrrhizae*.	tablet 0.373
Ubat Po Chai (Tung Kin Factory Building, Hong Kong)	*Exocarpium Citri Grandis*, *Poriae Cocos*, *Rhizoma Atractylodis*, *Radix Angelicae Dahuricae*, *Pericarpium Citri Reticulatae*, *Fructus Hordei Germinatus*, *Herba Pogastemonis Semen coicis, Radix Puerariae Lobatae, Radix Trichosanthis, Herba Menthae, Flos Chrysanthemi* and *Radix Aucklandiae.*	0.487
Leung Pui Kee (Leung Pui Kee Medical Factory, China)	*Aster tataricus*, *Stemona Japonica*, *Gentianaca Scabra Bunge, Ephedra Simica, Tussilago Farfara, Platycodon, Verticalla wild var thundbergii Bak, Cyanchum Stauntoni, Citrus Reticulata Blanco, Schltrex levl*	Sachet 2.50
Cheng Sie Lung (Cheng Sie Lung Drug Co., Hong Kong)	*Borneo camphor, Liquorice root, Coptis root*, *Euchresta japonica*, Calcareous Spar and Indigo residues.	Greyish green powder in bottle 0.90
Eng Aun Tong (Hudson Malaysia SDN BHD Malaysia)	*Berat Bershish Sebungkus,* C*offeinum Citric,* acetyl salicylicum	sachet 0.35
Tin Hee (Tin Hee Tong Medicine Factory Ltd., Hong Kong)	*Fructus Amomi Rotundus, Rhizoma Zingiberis, Cortex Cinnamomi* and *Radix Glycyrrhizae*.	pill 0.31
Bi Yan Pian (WuHan Zhong Lian Pharmaceutical Group Co. Ltd. Works, (China)	*Fructus Xanthii, Flos Magnoliae*, *Radix Glycyrrhizae, Radix Platycodi*, *Fructus Schisandrae, Fructus Forsythiae, Radix Angelicae Dahuricae*, Rhizoma amemarrhenae, *Flos Chrysanthemi Indici*, , *Radix Sapshnikoviae* and *Herba Schizonepetae. Uralensis*	tablet 0.328 (0.298

Table 2

Phytochemical constituents (mg/g) in the herbal drugs	Pilex	Septilin	Rumalaya	Jin Quian Chao	Ubat Chai Diabecon	Po	Leung Pui Kee	Cheng Sie Lung	Eng Aun Tong	Tin Hee	Bi Yan Pian
Alkaloids	60.0 □ 9.2	62.0 □ 2.9	78.0 □ 10.5	28.5 □ 1.7	90.0 □ 2.1	40.0 □ 3.2	*	*	40.0 □ 10.8	*	36.0 □ 2.9
Saponins	27.2 □ 1.0	47.8 □ 1.1	78.0 □ 1.5	10.7 □ 1.0	64.0 □ 1.0	77.0 □ 2.0	*	69.2 □ 2.4	61.0 □ 1.3	*	84.0 □ 1.4
Tannins	52.0 □ 0.1	60.0 □ 0.1	**	**	**	48.0 □ 0.1	*	*	**	*	137.0 □ 0.2
Phenolic	181.7 □ 1.5	39.0 □ 1.5	155.0 □ 1.5	43.7 □ 3.2	7.0 □ 1.0	10.7 □ 0.6	92.3 □ 1.2	155.7 □ 2.2	106.7 □ 1.8	4.0 □ 1.0	12.0 □ 0.3

* not tested; ** non-detectable

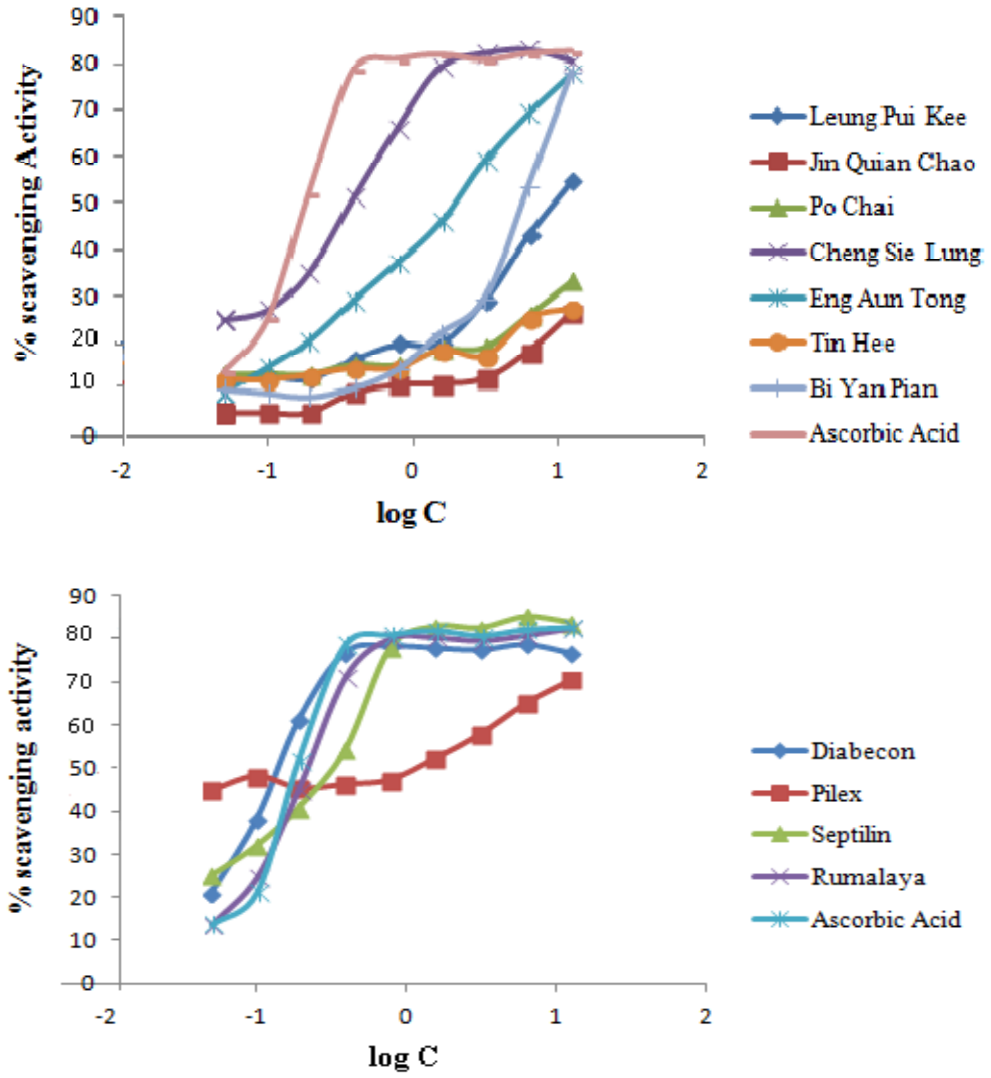

Figure 1: Antioxidant activities of (a) Ayurvedic Drugs (b) Chinese Drugs at different concentration using DPPH assay.

Percentage Inhibition of AGEs formation for the Ayurvedic and Chinese Herbal drugs

Table 3

% Inhibition Drugs	**Conc .(µg/mL)**	**5 days**	**15 days**	**30 days**	**60 days**	**90 days**
Diabecon	10000	52.45 ± 1.93	55.20 ± 1.43	80.99 ± 1.69	84.69 ± 0.72	84.70 ± 0.53
	5000	21.37 ± 1.89	28.27 ± 1.62	68.59 ± 2.06	71.04 ± 0.75	71.03 ± 0.54
	1000	17.24 ± 1.97	24.88 ± 2.38	61.68 ± 2.11	68.83 ± 0.52	68.88 ± 0.95
	100	12.70 ± 0.79	20.55 ± 1.69	50.93 ± 2.21	59.52 ± 0.05	60.48 ± 0.06
Pilex	10000	50.89 ± 0.22	61.39 ± 1.94	70.23 ± 0.83	75.07 ± 0.55	76.30 ± 0.56
	5000	18.95 ± 0.95	58.29 ± 1.52	62.94 ± 0.92	69.20 ± 0.53	70.48 ± 0.93
	1000	12.32 ± 1.64	34.92 ± 0.96	45.02 ± 0.52	50.83 ± 0.03	51.94 ± 0.53
	100	09.84 ± 1.34	22.26 ± 1.04	30.97 ± 0.19	42.08 ± 0.30	45.28 ± 0.52
Septilin	10000	39.47 ± 1.27	74.31 ± 2.53	84.20 ± 1.34	89.30 ± 0.53	90.64 ± 0.82
	5000	36.73 ± 1.85	68.35 ± 3.63	72.62 ± 0.63	84.00 ± 0.75	84.96 ± 0.53
	1000	28.24 ± 2.25	49.24 ± 0.64	52.52 ± 0.73	60.83 ± 0.95	61.02 ± 0.24
	100	24.83 ± 2.53	35.92 ± 0.73	42.69 ± 0.46	51.08 ± 0.85	51.09 ± 0.83
Rumalaya	10000	42.96 ± 1.74	69.73 ± 1.53	77.73 ± 2.02	79.53 ± 0.75	79.99 ± 0.72
	5000	32.72 ± 1.72	53.83 ± 1.04	60.25 ± 1.83	65.02 ± 0.30	66.08 ± 0.19
	1000	19.73 ± 0.52	49.62 ± 0.84	51.65 ± 0.93	54.80 ± 0.92	55.92 ± 0.83
	100	17.36 ± 0.92	26.67 ± 0.52	39.43 ± 0.74	42.39 ± 0.54	45.29 ± 0.75
Ubat Po Chai	10000	32.74 ± 0.62	46.65 ± 0.34	64.63 ± 0.54	68.64 ± 0.52	69.43 ± 0.75
	5000	27.63 ± 0.96	30.00 ± 0.53	43.08 ± 0.53	45.03 ± 0.57	46.98 ± 0.57
	1000	20.62 ± 1.52	27.83 ± 0.34	38.36 ± 0.02	40.64 ± 0.35	40.64 ± 0.46
	100	11.73 ±1.74	19.04 ± 0.43	28.63 ± 0.53	32.27 ± 0.22	33.53 ± 0.68
Cheng Sie Lung	10000	42.53 ± 2.04	49.92 ± 0.57	79.92 ± 1.43	79.99 ± 0.29	80.31 ± 0.97
	5000	29.94 ± 1.42	34.10 ± 5.46	58.04 ± 1.91	61.52 ± 0.63	64.64 ± 0.46
	1000	21.92 ± 1.26	28.25 ± 4.29	42.69 ± 1.33	46.94 ± 0.63	48.98 ± 0.38
	100	10.54 ± 1.11	12.00 ± 0.37	49.94 ± 1.29	52.74 ± 0.28	38.65 ± 0.27
EngAun Tong	10000	30.68 ± 0.46	45.82 ± 0.63	74.03 ± 0.44	80.45 ± 0.41	81.42 ± 0.55
	5000	25.94 ± 1.85	39.97 ± 1.46	68.29 ± 1.86	72.52 ± 0.74	75.91 ± 0.33
	1000	19.09 ± 1.74	27.01 ± 1.47	40.62 ± 1.95	61.53 ± 0.92	68.03 ± 0.36

	100	15.92 ± 1.95	17.03 ± 1.87	38.25 ± 1.74	45.92 ± 0.52	49.83 ± 0.96
Amino Guanidine	10000	71.85 ± 4.68	90.92 ± 0.15	98.66 ± 0.15	99.02 ± 0.12	99.21 ± 0.11
	5000	61.18 ± 1.16	79.61 ± 0.31	96.86 ± 0.36	98.39 ± 0.45	99.00 ± 0.45
	1000	53.51 ± 1.99	71.89 ± 0.27	95.48 ± 0.21	97.84 ± 0.38	97.99 ± 0.40
	100	40.52 ± 0.36	63.11 ± 5.06	75.69 ± 0.65	82.04 ± 0.75	84.95 ± 0.75

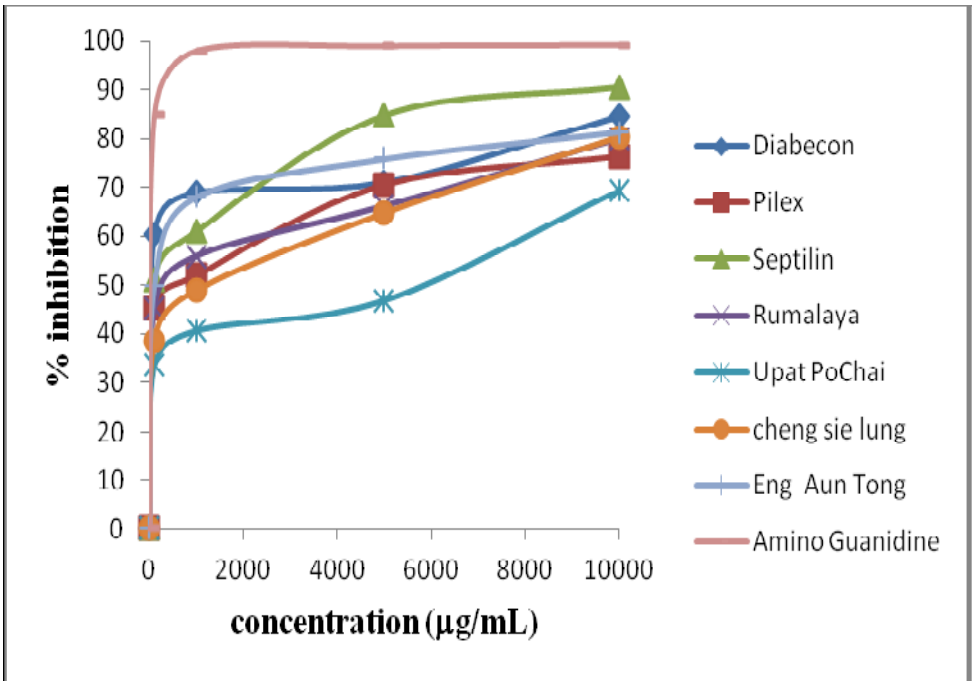

Figure2: % inhibition of drugs and aminoguanidine with various concentrations at 90 days

Table 4

The and of the Aryurvedic and Chinese drugs Drug name	□g/mL (× 10-4)	□g/mL
Diabecon	1.3	4.2
Pilex	9.6	5.6
Septilin	3.9	5.0
Rumalaya	4.8	5.5
Jin Qian Chao	200.0	*
Ubat Po Chai	191	7.2
Leung Pui Kee	83	*
Cheng Sie Lung	3.7	6.1
Eng Aun Tong	19.2	4.7
Tin Hee	2870	*
Bi Yan Pian	60.9	*
Ascorbic acid	3.3	
Amino guanidine	-	2.0

Table 5

MIC values in mg/ mL of the different herbal drugs

Herbal Drug	***E. coli***	***P. aeruginosa***	***S. aureus***	***B. cerus***
Diabecon	>492	<492	5	5
Pilex	>290	<290	25	25
Septilin	>380	<95	1	5
Rumalaya	>443	>443	1	5
Jin Qian Chao	<314	>314	235	157
Ubat Po Chai	>470	>470	5	10
Leung Pui Kee	-	-	-	-
Cheng Sie Lung	>250	>250	10	10
Eng Aun Tong	5	<80	50	50
Tin Hee	100	>300	100	100
Bi Yan Pian	100	>310	80	25

Table 7
The pearson correlation for Ayurvedic and Chinese drugs between different phytochemicals and the different assays

	Alkaloids	***Saponin***	***Tannin***	***Phenolic***
Ayurvedic drugs	Anti-oxidant	0.03	0.16	0.28	0.82
	Anti-glycation	0.34	0.00	0.22	0.92
	Anti-bacterial (S. *Aureus)*	0.18	0.11	0.77	0.50
Chinese drugs	Anti-oxidant	0.61	0.09	1.00	0.42
	Anti-glycation	0.00	1.00	0.00	0.60
	Anti-bacterial (S. *Aureus)*	0.93	0.31	1.00	0.50

Conclusion

It can be deduced that the phenolic content is main responsible factor for anti-oxidant and anti-glycation activity while the tannic content contributes mainly for their antibacterial properties. However, in most cases only one phytochemical may not be the sole factor responsible for the activity. In fact, a mixture/combination of different phytochemical components acts synergistically to enhance the biological properties. These biological properties could have contributed, at least partly, to the therapeutic benefits of the certain traditional benefits of the certain traditional claims. The outcome of such studies may be useful for the clinical applications of these drugs against various diseases and may open up new therapeutic avenue. In view of the use of herbal drugs in the health industry, the therapeutic benefits and bioactive warrant for further investigations.

References

[1] Tang SY. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radical Biology & Medicine* 2004; **36**: 1575-1587.

[2] Hyun YK, Takuya O, Takako Y. Beneficial effects of Chinese prescription kangen-karyu on diabetes associated with hyperlipidemia, advanced glycation endproducts and oxidative stress in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* 2009; **124**: 263-269.

[3] Kavishankar GB, Lakshmidevi N, Mahadeva MS, Prakash HS, Niranjana SR. Diabetes and medicinal plants-A review. International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; **2**: 65-80.

[4] Tang SY, Halliwell B. Medicinal plants and antioxidants: What do we learn from cell culture and *Caenorhabditis elegans* studies. *Biochemical and Biophysical Research Communications* 2010; **394**: 1-5.

[5] Li H-B, Wong C-C, Cheng K-W, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT* 2008; 41: 385-390.

[6] Kongkaneramit L, Witoonsaridsilp W, Peungvicha P, Ingkaninan K, Waranuch N, Sarisuta N. Antioxidant

Activity And Antiapoptotic Effect of *Asparagus Racemosus* Root Extracts In Human Lung Epithelial H460 Cells. *Experimental and Therapeutic Medicine* 2010; **2**: 143-148.

[7] Chakraborthy GS, Ghorpade PM. Free Radical Scavenging activity of *abutilon indicum* (LINN) sweet stem extracts. *International Journal of ChemTech Research* 2010; **2**: 526-531.

[8] Chakraborthy GS. Antioxidant Activity of *Abutilon Indicum* Leaves. *International Journal of PharmTech Research* 2009; **1**: 1314-1316.

[9] Thamilvaani M, Teng lL, Appleton D, Ming CH, Masilamani T, Palanisamy UD. Antioxidant and antiglycemic potential of *Peltophorum pterocarpun* plant parts. *Food Chemistry* 2011; **129**: 1355-1361.

[10] Suresh VN, Prathapan A, Cherian OL, Raghu KG, Venugopalan VV, Sundareson, A. *In vitro* antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against 16 LDL oxidation and key enzymes linked to type 2 diabetes. *Food and Chemical Toxicology* 2011; **49**: 125-131.

[11] Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry* 2007; **100**: 1409-1418.

[12] Gul M Z, Bhakshu L M, Farhan A, Kondapi A K, Insaf A Q, Ghazi I A. Evaluation of Abelmoschus moschatus extracts for antioxidant, Free radical scavenging antimicrobial and antiproliferative activities using in vitro assays. *BMC Complementary and Alternative Medicine* 2011; 11-64.

[13] Edeoga H, Okwu D, Mbaebie B. Phytochemical Constituents of some Nigerian medicinal plants, *African Journal of Biotechnology* 2005; **4**: 685-688.

[14] Deetae P, Parichanon P, Trakunleewatthana P, Chanseetis C. Antioxidant and antiglycation properties of Thai herbal teas in comparison with conventional teas. *Food Chemistry* 2012; **133**: 953-959.

[15] Narain Y, Jhaumeer-Laulloo S, Bhowon MG. Structure activity relationship of Schiff base derivatives of bis(aminophenyl)disulfide and *p*-vanillin as antimicrobial agents. *International Journal of Biological and Chemical Sciences* 2010; **4**: 69-74.

[16] Jhaumeer-Laulloo S, Bhowon MG, Mungur S, Mahomoodally F, Subratty H. *In vitro* antiglycation and antioxidant properties of synthesized Schiff bases. *Medicinal Chemistry* 2012; **8**: 409-414.

[17] Chen Y-F, Roan H-Y, Lii, C-K, Huang Y-C, Wang T-S. Relationship between antioxidant and antiglycation ability of saponins, polyphenols and polysaccharides in Chinese herbal medicines used to treat diabetes. *Journal of Medicinal Plants Research* 2011; **5**: 2322-2331.

[18] Wu J-W, Hsieh, C-L, Wang H-Y, Chen H-Y. Inhibitory effects of guava (*Psidium guajava L*) leaf extracts and its active compounds on the glycation process of protein. *Food Chemistry* 2009; 113: 78-84.

[19] Monnier VM, Kohn RR, Cerami A. Accelerated age-related browning of human collagen in diabetes mellitus. *Proc Natl Acad Sci U S A* 1984; 81(2): 583–587.

[20] Hatfield J, Mulfinger L. Advanced glycation end products in hyperglycemic patients. *Journal of Young Investigors* 2005; **13**: 3895-3903.

[21] Matsuura N, Aradate T, Sasaki C, Kojima H, Ohara M, Hasegawa J, Ubukata M. Screening system for the Maillard reaction inhibitor from natural product extracts. 2002; **48**(6): 520-526.

[22] Zhang J-D, Xu Z, Cao Y-B, Chen H-S, Yan L, An M-M. Antifungal activities and action mechanism of compound from *Tribulus terrestris L. Journal of Ethnopharmacology* 2006; **103**: 76-84.

[23] Helmy WA, Amer H, El-Shayed MA. Antimicrobial activities of the aqueous extracts from neem tree (*Azadirachta indica A*). *Journal of Applied Sciences Research* 20073; 1050-1055.

[24] Chan LW, Cheah LCE, Saw LLC, Weng W, Heng WSP. Antimicrobial and antioxidant activities of *Cortex magnoliae officinalis* and some other medicinal plants commonly used in South East Asia. *Chinese Medicine* 2008; **3**: 1-15.

[25] Irani M, Sarmadi M, Bernard F, Ebrahimi GH, Bazarnov HS. Leaves antimicrobial activity of *Glycyrrhiza Glabra L. Iranian Journal of Pharmaceutical Research* 2010; **9**: 425-428.

[26] Talreja, T. Screening of crude extract of flavonoids of *Moringa oleifera* against bacteria and fungal pathogen. *Journal of Phytology* 2010; **2**: 31-35.

[27] Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences* 2008; **4**: 839-843.

[28] Jagessar RC, Mars A, Gomes G. Selective antimicrobial properties of *Phyllanthus acidus* leaf extracts against *Candida albicans*, *E. coli* and *Staphylococcus aureus* using disc diffusion, well diffusion, streak plate and a dilution method. *Nature and Science* 2008; **6**(2): 24-38.

[29] Vimalraj RT, Kumar SS, Vadivel S, Ramesh S, Thejomoorthy P. Antibacterial effect of *Cassia fistula* extract on pathogenic bacteria of veterinary importance. *Tamilnadu Journal of Veterinary and Animal Sciences* 2009; **5**: 109-113.

[30] Maheswari P, Kumar A. Antimicrobial activity of Abelmoshus moschatus leaf extracts. *Current Trend in Biotechnology and Pharmacy* 2009; **3**: 260-266.

[31] Meng J, Fang Y, Zhang A, Chen S, Xu T, Ren Z et al. Phenolic content and antioxidant capacity of chinese raisins produced in Xinjiang province. *Food Research International* 2011; **44**: 2830-2836.