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Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.09.005

Chemical composition, antioxidant and antibacterial activities of essential oil and methanol extract of *Artemisia vulgaris* and *Gaultheria fragrantissima* collected from Nepal

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ARTICLE INFO

Article history:
Received 17 Jul 2017
Received in revised form 13 Aug 2017
Accepted 5 Sep 2017
Available online 18 Sep 2017

Keywords:
Artemisia vulgaris
Gaultheria fragrantissima
Flavonoids
Antioxidant
HPLC
Gas chromatography mass spectroscopy

ABSTRACT

Objective: To identify the chemical constituents and biological activities of essential oil and crude methanol extract of *Artemisia vulgaris* (*A. vulgaris*) and *Gaultheria fragrantissima* (*G. fragrantissima*).

Methods: Phytochemical screening, total phenolic and flavonoid content, antibacterial activities, anti-oxidant assay of the crude extract were carried out to identify the biological activities and phytonutrients present in the extract. Furthermore, the chemical constituents present in the essential oil and crude methanol extract were analyzed using gas chromatography mass spectroscopy and high performance liquid chromatography (HPLC) analysis.

Results: Gas chromatography mass spectroscopy analysis of essential oil from the aerial part of *A. vulgaris* revealed 24 different compounds in it. Sabinene (11.29%), β-thujone (19.19%), chrysanthenone (4.48%), camphor (11.89%), borneol (4.44%) and germacrene D (8.42%) were the major compounds. Similarly, leaves of *G. fragrantissima* contained methyl salicylate (95%) and asarone (4.64%). Furthermore, methanol extract of leaves of *A. vulgaris* and *G. fragrantissima* were found rich in the total flavonoids and phenolic content. HPLC analysis of the methanol extract of leaves *A. vulgaris* revealed the presence of morin and luteolin, whereas rutin was found as a major flavonoids compound in the leaves of *G. fragrantissima*. Further, methanol extract of the *A. vulgaris* and *G. fragrantissima* showed the highest antioxidant and antibacterial properties compared to the essential oil.

Conclusions: The HPLC analysis of the methanol extract of *A. vulgaris* shows the presence of luteolin and morin, whereas *G. fragrantissima* reveals the presence of rutin and a glycosylated flavonoids. Results reveal that *A. vulgaris* oil is the rich source of monoterpene and sesquiterpene compounds. Furthermore, *A. vulgaris* and *G. fragrantissima* are the rich source of the phenolic and flavonoids compounds and show good antioxidant and antibacterial activity.

1. Introduction

Artemisia vulgaris (*A. vulgaris*) and *Gaultheria fragrantissima* (*G. fragrantissima*) are the traditional medicinal plants used in Nepal for the treatment of the several diseases and belong to the family of Asteraceae and Ericaceae, respectively [1,2]. It has been reported that more than 400 species of *Artemisia* and 134 species of *Gaultheria* distribution worldwide [3,4]. In Nepal, both species inhabit wide-reaching topographical extent and engross ample thoughtfulness for their remarkable monetary values, in precise for their remedial arrays [5,6]. The health benefits of the essential oil of *A. vulgaris* have drawn the significant attention to scientific community. The essential oil and the plant extracts of *Artemisia* have been used for the treatment of several diseases, and ample scientific evidence supports its function as anti-epileptic, anti-hysteric, diuretic, digestive and stimulant [7,8].

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Peer review under responsibility of Hainan Medical University.
Foundation project: This research was supported by Academy Partnership Program Track 1-1 funded by KOICA (APP-Track 1-1 2015-0249).

In traditional medicine, plant leaves and the whole plant have been used for the treatment of diabetes, epilepsy, psychoneurosis, depression, irritability, insomnia, anxiety and stress [9,10]. Furthermore, it shows antispasmodic, antiseptic, antibacterial, antimalarial, antitumor, antirheumatic and hepatoprotective properties [11]. *G. fragrantissima* in Nepal is known as Dhasingre and its essential oil is one of the most exported oil from Nepal. It has been reported that *Gaultheria* species are used to treat inflammatory disorder, rheumatoid arthritis, reducing swelling, pain, chronic tracheitis, cold, acute and chronic prostatitis [12]. Further, analgesic and anti-inflammatory activities of the *Gaultheria* were supposed to be due to the presence of methyl salicylate. Hence, continuous identification of the value added compounds are very important, scientifically and commercially. Among the *Gaultheria* species, *Gaultheria yunnanensis* and *Gaultheria nummularioides* have been the most studied species till now since they are the rich source of flavonoids and steroids compounds [13], but little is known about the flavonoids contents of *G. fragrantissima* and *A. vulgaris*. Both species of *G. fragrantissima* and *A. vulgaris* have received increasing interest in the scientific community for their medicinal applications, especially with regards to the flavonoids. Flavonoids have been known for their high antioxidant activities and associated with several health benefits [14,15].

Further, scientific evidence supports the fact that plants grown in different geographic locations and diverse climatic condition show the variation in the chemical constituents. It has been reported that the essential oil of *A. vulgaris* grown in France and Croatia differs significantly from each other [16]. The Croatian oil was found rich in hydrocarbon, whereas French oil was found absent in hydrocarbon. The fact is also supported by the variation in the major essential oil composition of *A. vulgaris* grown in French and North Lithuania. French oil is rich in oxygenated compounds, whereas, Lithuanian oil was rich in oxygenated monoterpenes and sesquiterpenes [17].

The aim of the present study is to investigate the chemical constituents of essential oils as well as the flavonoids contents in the methanol extract of *A. vulgaris* and *G. fragrantissima*, collected from the Dhulikhel, Nepal and their related biological activity. To our knowledge, this is the first report of the essential oil constituents and the flavonoids contained.

2. Materials and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagents, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, aluminum chloride and methanol were purchased from Sigma Aldrich Chemical Co. Ltd. (St. Louis, MO. USA). High performance liquid chromatography (HPLC) grade methanol, water and orthophosphoric acid were purchased from Fisher Scientific Co. Ltd. India. All other reagents and solvents were of analytical grade.

2.2. Collection of plant materials

The plant materials were collected from Dhulikhel (27.61°N, 85.55°E, 1 550 m above sea level). Dhulikhel is located in sub-locality, Dhulikhel locality, Bagmati district, central region state of Nepal and 30.5 km away from the capital. Plants were collected in the polythene bags during morning hours and placed in the ice box for preventing any contamination and preservation.

2.3. Essential oil extraction

The fresh plant materials were weighed (100 g) and oil was extracted from the leaves by hydro distillation method. The process was carried out in Clevenger apparatus model No (98-II-B) for 6 h reflux. The oil was dried over anhydrous sodium sulfate and stored at 4 °C until analysis.

2.4. Phytochemical analysis and determination of total phenol content

The phytochemical analysis of alkaloids, flavonoids, phenolic content, saponin, quinone, sterols, cardiac glycoside, tannin, terpenoid and reducing compound was performed with slight modification [18]. Total phenolic content was measured using Folin Ciocalteu's technique with slight alteration [19]. Aliquots of 1 mL and standard gallic acid (10, 20, 40, 60, 80 and 100 μg/mL) was placed into the test tubes and 0.5 mL of Folin Ciocalteu's reagent and 4.5 mL of distilled water were mixed and further shaken. In the same solution, 4 mL of 7% sodium carbonate was added after 5 min. The blue color mixture was shaken and incubated at 40 °C in water bath. UV visible spectrophotometer was used to measure absorbance at 760 nm. The experiments were performed in triplicates and results are expressed as gallic acid equivalent/g of dry weight.

2.5. Determination of total flavonoid content

Total flavonoid contents were determined using the aluminum chloride colorimetric assay as described by Park *et al.* [20]. The 1 mL standard quercetin solution (100, 200, 400, 600, 800 and 1 000) μg/mL and 1 mL of aliquots was positioned into test tubes and followed by the following steps: 0.3 mL of 5% sodium nitrite solution and 4 mL of distilled water were added into each sample, followed by 0.3 mL of 10% aluminum chloride was added. After incubation at another 5 min, 2 mL of 1 M sodium hydroxide was added. Final volume was adjusted to 10 mL with distilled water and was mixed well until yellowish color was developed. The absorbance was measured at 510 nm using UV–visible spectrophotometer. Distilled water was used to as a blank for control experiment. The experiments were carried out in triplicates. The total flavonoids of content are expressed as mg of quercetin equivalents/g of dry weight.

2.6. Antibacterial activity

The antibacterial screening of the plant extract was carried out against four pathogenic strains, *viz. Enterococcus* sp, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonia* by the disk-diffusion method [21,22]. The Mueller-Hinton agar plate dried surface was inoculated over the entire sterile agar surface by streaking the swab. The 10 μL of the plant extract (1 mg/mL) dissolved in 10% dimethylsulphoxide were loaded in sterile filter paper discs of 6 mm diameter. Methanol was used as negative control and ampicillin and kanamycin were used as positive control. The experiments were performed in triplicates under aseptic conditions. Plates were incubated for 18 h at 37 °C. The antibacterial activity was evaluated by

measuring the zones of inhibition of bacterial growth and results are expressed as mean values ± standard deviation (mean ± SD) of the triplicates sets of experiments.

2.7. Radical scavenging activity and reducing power assay

DPPH radical was used to determine the free radical scavenging capacity of the extract [23,24]. The solution was prepared in methanol at different concentration ranging from 20 μg/mL to 100 μg/mL. The various concentrations of extracts (0.3 mL) were assorted with freshly prepared methanol solution comprising DPPH concentration [0.004% (w/v), 2.7 mL]. The mixture was vigorously shaken and left for 30 min in the dark. The range of reduction of the DPPH radical was measured at 517 nm. As a reference standard ascorbic acid was used and DPPH solution without extract was used as the control. Total reducing power of selected medicinal plants was analyzed following standard method with some modifications [25]. Various concentration of the sample aliquot was prepared and mixed with 2.5 mL of sodium phosphate buffer (pH 6.6, 0.2 mol/L) which was followed by the addition of 2.5 mL of 1% potassium ferricyanide and incubated at 50 °C for 20 min. The mixture was then supplemented with trichloroacetic acid (10%, 2.5 mL) and centrifuged at 1000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionized water and ferric chloride solution (0.1%, 0.5 mL) and absorbance was measured at 700 nm; higher absorbance indicates higher reducing power. The above assays were carried out in triplicate and the results are expressed as mean ± SD. The results were expressed as effective concentration (EC_{50}) when the absorbance was 0.5 mL at 700 nm and ascorbic acid was used as a standard.

2.8. Analytical method

The analysis of the essential oil was performed using Shimadzu GCMSQP2010 plus. And the analysis Rtx5 MS (30 m length × 0.25 mm diameter × 0.25 mm μm thickness) was used. The carrier gas was helium at 1.3 mL/min in constant flow mode. The injector temperature was 250 °C, the injection volume was 1 μL, and the split ratio was 1:30. The initial oven temperature of 50 °C was held for 2 min, then increased at a rate of 3 °C/min up to 200 °C and finally increased at 15 °C/min up to 250 °C and was hold for 3 min. Further, the methanol extracts of *A. vulgaris* and *G. fragrantissima* were analyzed using a SHIMDZU Prominence – i LC2030 equipped with an UV detector and C18 column (dimension 4.6 mm × 150 mm). The flow rate was 1 mL/min and the injection volume was 5 μL. The solvents used in the mobile phase were 0.25% orthophosphoric acid in water (solvent A) and methanol (solvent B) with gradient system of 40% B for 5 min, 55% B for 5–10 min, 65% B for 10–15 min, 50% B for 15–20 min, and 30% B for 25–30 min. The detection was carried out at 254 nm and 280 nm.

2.9. Statistical analysis

The analysis of the data was carried out using the SPSS version 15 and the graph were plotted using Origin 7.5 software. All analysis was performed in triplicate, and the results are expressed as mean ± SD. Significant difference of the data among the parameter was calculated by performing one way anova analysis followed by multiple comparisons using Tukey's test at level 0.05.

3. Results

3.1. Phytochemical screening, total phenolic content and total flavonoid content

The qualitative chemical analysis of phytoconstituents in the methanol extract of the leaves of *A. vulgaris* and *G. fragrantissima* was analyzed following standard methods as described in material and methods. Results showed the presence of high amount of alkaloids, flavonoids and terpenoids, steroids and tannin along with several other phytonutrients (Table 1). The authors next studied the amount of total phenolic content and total flavonoids content in the methanol extract of *A. vulgaris* and *G. fragrantissima* using gallic acid and quercetin as a standard. The *A. vulgaris* contain (86.62 ± 0.04) mg GAE/g DW and (81.31 ± 0.53) mg Quercetin/g DW of total phenol and total flavonoids, respectively. Whereas, *G. fragrantisimma* revealed (77.06 ± 0.12) mg GAE/g DW and (70.79 ± 0.01) mg Quercetin/g DW of total phenol and total flavonoids, respectively. The results presented here demonstrate that the *A. vulgaris* contain higher amounts of phenolic and flavonoids contents than that of *G. fragrantissima*.

Table 1
Phytochemical analysis of methanol extract of *A. vulgaris* and *G. fragrantissima*.

Phytonutrient	*A. vulgaris*	*G. fragrantissima*
Alkaloid	+++	+++
Saponin	++	+
Protein	+	+
Quinone	++	++
Sterols	++	+
Cardiac glycoside	+	+
Flavonoids	+++	+++
Tannin	++	++
Terpenoid	++	++
Reducing sugar	+	+

Where, +, ++ and +++ represents the relative higher activity towards the phytonutrients.

3.2. HPLC analysis of methanol extract

The analysis of the flavonoids in the methanol extract of *A. vulgaris* and *G. fragrantissima* was investigated using HPLC equipped with UV detector using gradient system as described in the part of materials and methods. The compounds were identified through the comparison of the chromatogram of authentic flavonoids compounds (Figure 1). The methanol extract of *A.*

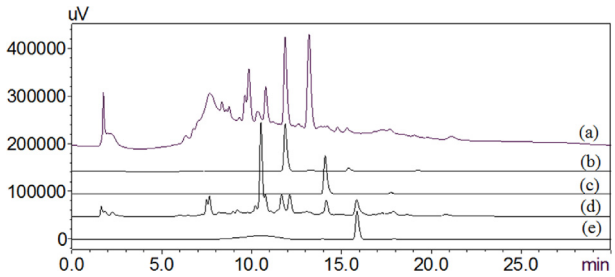

Figure 1. HPLC chromatogram.
(a) methanol extract of *G. fragrantissima*; (b) rutin; (c) morin; (d) methanol extract of *A. vulgaris*; (e) luteolin.

vulgaris revealed the presence of two different members of flavonoids luteolin and morin. Similarly, analysis of the methanol extract of *G. fragrantissima* showed the presence of rutin (quercetin 3-*O*-rutinose) and the member of flavonol.

3.3. Gas chromatography and mass spectroscopy (GC–MS) analysis of essential oil

GC–MS analysis of the essential oil derived from the aerial part of the *A. vulgaris* showed the presence of 24 compounds (Figure 2). The *A. vulgaris* oil was found rich in monoterpene and sesquiterpene compounds. The major components were sabinene (11.29%), beta-thujone (19.99%), chrysanthenone (4.48%), camphor (11.89%), borneol (4.44%) and germacrene D (8.42%) (Table 2). The fragmentation pattern of mass spectra of sabinene and beta-thujone was found to have *m/z* ratio of 136:152, 121:110, 93:95, 79:81 and 41:41. The compounds were identified based on the fragmentation pattern of mass spectra. Next, the authors investigated the essential oil from the leaves of *G. fragrantissima* obtained by using hydro-distillation. GC–MS analysis showed methyl salicylate (95%) as a major compound with small amount of asarone (4.64%). The mass fragmentation pattern of methyl salicylate and beta-asarone was 152, 121, 92, 65 and 208, 193, 165, 162, respectively. Due to the high amounts of methyl salicylate, G. *fragrantissima* oil is being used for making fragrance and it is one of the most exported oils from Nepal.

Table 2
GC–MS chemical composition of essential oil from *A. vulgaris* aerial part.

Compounds	Retention time (min)	Area (%)
alpha-pinene	8.817	0.34
Camphene	9.408	2.38
Sabinene	10.500	11.29
beta-pinene	10.592	1.63
Vinyl amyl carbinol	10.675	0.25
Cymene	12.625	0.85
Limonene	12.817	1.52
1,8-Cineole	12.933	2.49
gamma-terpinene	14.175	0.25
alpha-thujone	16.450	8.92
beta-thujone	17.050	19.19
Chrysanthenone	17.383	4.48
Camphor	18.308	11.89
Isoborneol	18.825	0.25
Pinocarvone	19.058	0.38
Borneol	19.267	4.44
Terpinen-4-ol	19.725	0.46
Myrtenal	20.617	1.05
Carveol	21.625	0.16
Germacrene D	33.108	8.42
Bicyclogermacrene	33.667	0.59
Caryophyllene oxide	37.075	0.64
Viridiflorol	39.308	0.15
alpha-cadinol	39.758	0.28

Compounds were identified using the NIST 11, FFNSC 1.3 library and the mass spectroscopy data analysis.

3.4. Antioxidant activity

Antioxidant properties of the essential oil and methanol extract of *A. vulgaris* and *G. fragrantissima* were measured using DPPH radical scavenging and reducing power activities. The crude methanol extract of *A. vulgaris* was found to be effective in scavenging the DPPH radicals. The DPPH radical scavenging properties were found to be concentration dependent. The *A. vulgaris* was found to have 33.12%, 43.47%, 56.40%, 68.27% and 82.80% inhibition at 20, 40, 60, 80 and 100 μg/mL of methanol extract, respectively. The percentage inhibition of this radical was found to increase with the increase in concentration of extract. At 20 μg/mL, the inhibition of methanol extract of *A. vulgaris* and *G. fragrantissima* was 33.12% and 25.34%, respectively, whereas ascorbic acid was 38.76% (Figure 3). The greater scavenging capacity means the higher antioxidant activity. The concentration required for 50% inhibition (IC_{50}) value of ascorbic acid was found to be

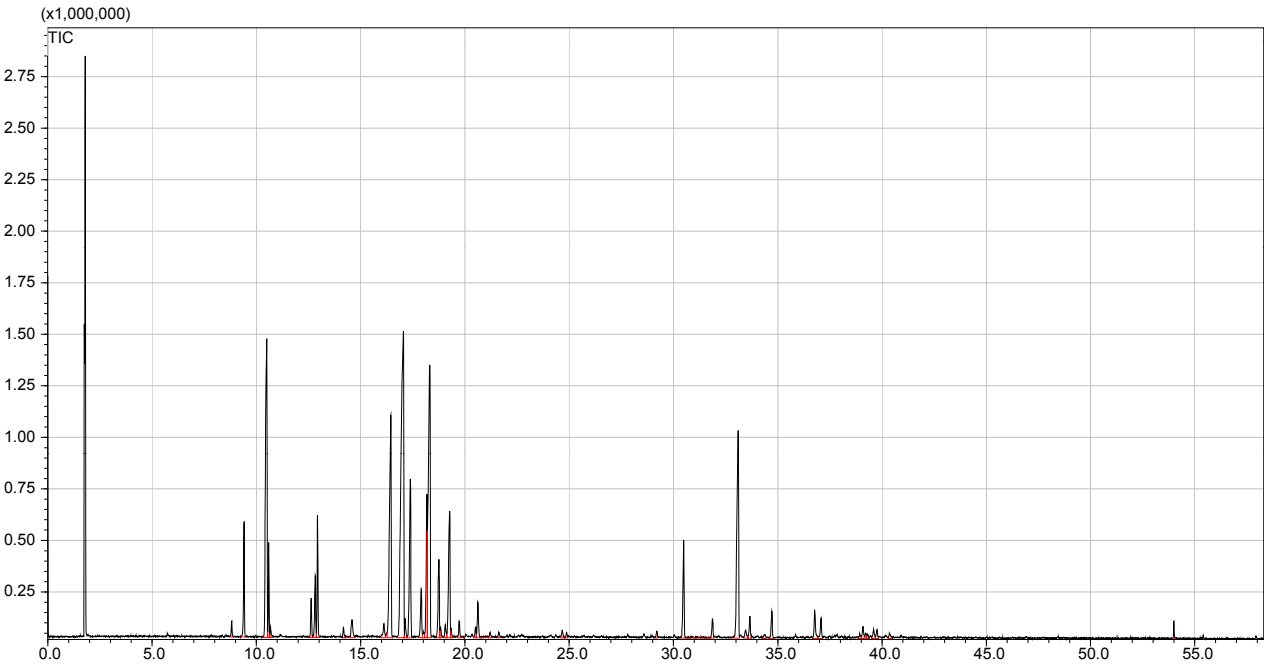

Figure 2. GC–MS spectrum of essential oil from *A. vulgaris*.
The compounds were identified using the NIST library and their corresponding mass spectra fragmentation pattern.

(35.05 ± 0.11) μg/mL, while that of *A. vulgaris* and *G. fragrantissima* methanolic extract was (48.77 ± 0.11) μg/mL and (65.02 ± 0.12) μg/mL, respectively. Results revealed that *A. vulgaris* methanol extract showed the higher antioxidant activities. Similarly, IC_{50} value of essential oil of *A. vulgaris* and *G. fragrantissima* was found to have (63.82 ± 0.08) μg/mL and (78.09 ± 0.13) μg/mL, respectively.

In order to further confirm the antioxidant activities, the authors analyzed reducing power assay as described in materials and methods. It was observed that the absorbance at 700 nm increased with the increase in the concentration of crude extract. The increase in absorbance was mainly due to the conversion of ferric to ferrous ions in the presence of reducers in extracts. The absorbance of crude methanol extract of *A. vulgaris* was observed as 0.379, 0.639, 1.009, 1.452 and 1.787, while that of ascorbic acid was 0.466, 0.703, 1.206, 1.862 and 2.104 at concentration of (200, 400, 600, 800 and 1 000) μg/mL, respectively (Figure 4). Furthermore, *G. fragrantissima* methanol extract showed comparatively lower absorbance in all concentration, which signified the higher antioxidant activities of *A. vulgaris*. Furthermore, effective concentration for reducing ferric to ferrous ion, EC_{50} value (effective concentration at 0.5 absorbance) of crude extract and essential oil was measured and compared with ascorbic acid (255.38 ± 0.60) μg/mL. The EC_{50} value of crude methanol extract of *A. vulgaris* and *G. fragrantissima* was found to be (296.44 ± 0.50) μg/mL and (344.96 ± 0.30) μg/mL, respectively. In a similar way, EC_{50} value of essential oil of *A. vulgaris* and *G. fragrantissima* was found to be (380.43 ± 0.46) μg/mL and (468.74 ± 0.82) μg/mL, respectively. It was observed that methanol extract revealed higher antioxidant tendency in comparison with essential oil in both plant species.

3.5. Antibacterial activities

Antibacterial activities of *G. fragrantissima* and *A. vulgaris* were investigated with gram positive and gram negative pathogenic strains using the disc diffusion method as described in materials and methods. Both methanol extract and essential oil of *A. vulgaris* showed significant antibacterial activities against the test organism. Results revealed that methanol extract of *A. vulgaris* showed higher antibacterial activity against *Bacillus subtilis* and *Enterococcus* spp. with zone of inhibition (12.48 ± 0.04) mm and (12.06 ± 0.08) mm, respectively, comparable with standard antibiotics ampicillin and kanamycin (Table 3). The least antibacterial activity was observed with *G. fragrantissima* oil against gram positive and negative pathogenic strains. Although the oil of *G. fragrantissima* showed the least activity, methanol extract revealed comparatively higher antibacterial activities with zone of inhibition in the range of 11–14 mm.

4. Discussion

Phytochemical analysis of leaves of *A. vulgaris* and *G. fragrantissima* revealed the presence of alkaloids, flavonoids, saponin, quinone, strols, tannin, terpenoids and reducing sugar. Such phytonutrients have diverse biological activities, such as free radical scavenging, anti-carcinogenic, antimicrobial and anti-

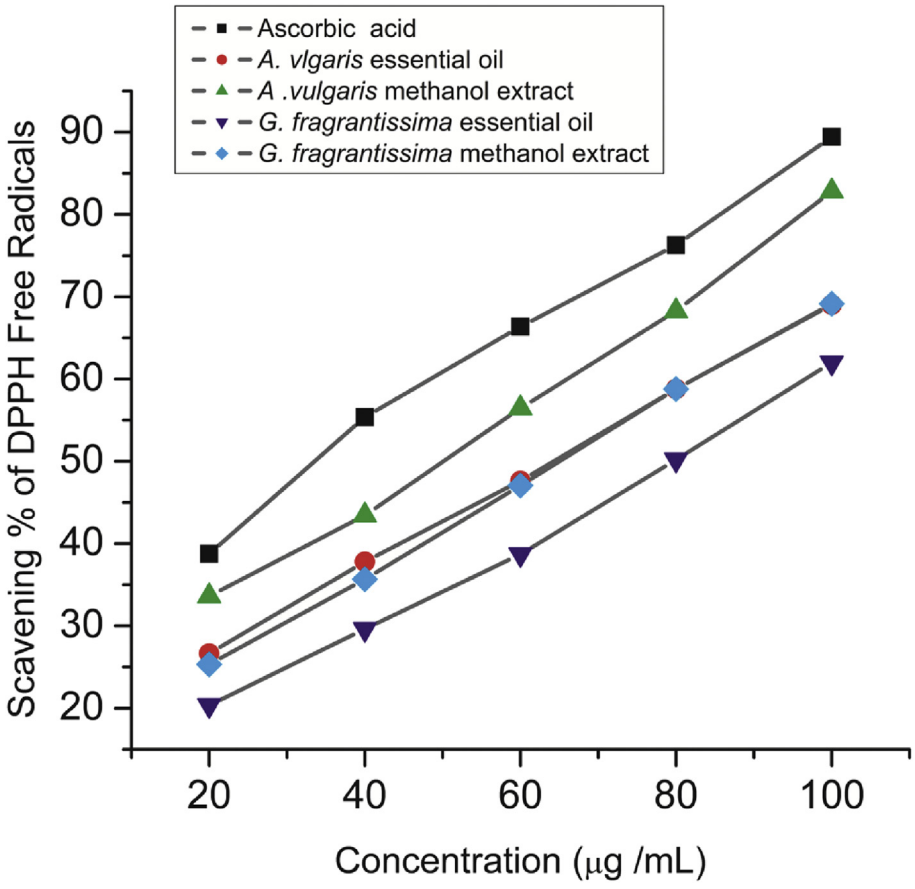

Figure 3. DPPH radical scavenging activity of the essential oil and methanol extract of *A. vulgaris* and *G. fragrantissima*.

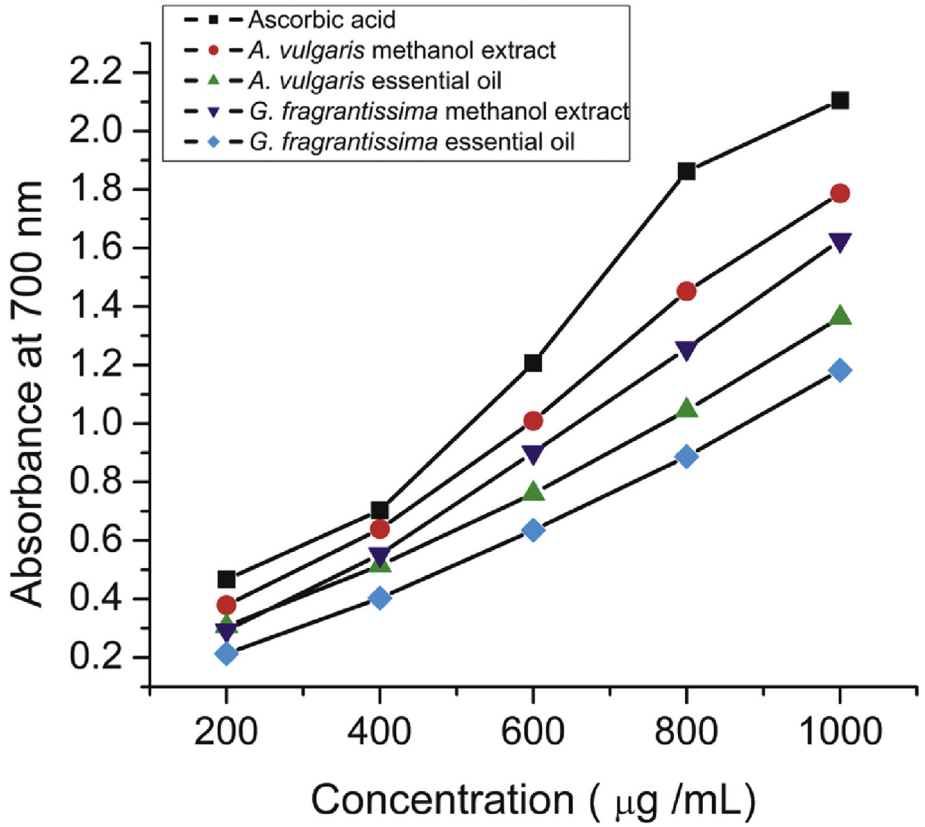

Figure 4. Reducing power of methanol extract and essential oil of *A. vulgaris* and *G. fragrantissima*.

inflammatory functions. Presence of such a phytonutrients ensures the medicinal uses of these plant species. Among these, phytonutrients flavonoids and polyphenol have gained significant scientific interest due to their reducing properties. Hence, finding out the flavonoids in these species helps to explain the health benefits of the plant species. Results revealed that *A. vulgaris* contained higher amount of polyphenol and flavonoids in comparison to *G. fragrantissima*. Flavonoids are a diverse group of phytonutrients that are produced by various plants species. Based on their skeleton, flavonoids are classified into eight groups: flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidines, chalcones and flavonolignans. Flavonoids play an important role in plant growth and development, and in defense of plants against microorganisms and pests serving as means of plant–animal warfare. The best-described property of almost every group of flavonoids there has capacity to act as antioxidants. The flavonoids seem to be the most powerful for protecting the body against reactive oxygen species.

HPLC analysis of the methanol extract of *A. vulgaris* showed the presence of luteolin and morin, whereas *G. fragrantissima* showed the presence of rutin, a glycosylated flavonoids. Although the authors identified three flavonoids molecules from two species, HPLC chromatogram of *A. vulgaris* and *G. fragrantissima* showed several unidentified peaks, suggesting the possibility of having diverse flavonoids molecules. It is expected that further analysis of metabolites might lead to the identification of the diverse flavonoids molecules in plant species. Morin belongs to the member of flavonol, and scientific evidence supports that it inhibits the amyloid formation and plays an important role in lowering the risk of Alzheimer's disease as well as type 2 diabetes [26]. Similarly, luteolin belongs to member of flavone and it has been reported to function lowering the risk of cardiovascular disease and also act as a anticancer agent [27]. Although Cong *et al.* have reported a dhasingreoside from the stems and leaves of *G. fragrantissima* along with several other glycosylated flavonoids [28]. To our knowledge, this is the first report of the presence of rutin in leaves of *G. fragrantissima*. The rutin possesses high antioxidant capacity and hence was reported to function for the treatment of the several diseases associated with metabolic syndrome, including diabetes as

Table 3

Antibacterial activity of the essential oil and methanol extract of *A. vulgaris* and *G. fragrantissima* (mean ± SD) (mm).

Organism	Gram reaction	*A. vulgaris*		*G. fragrantissima*		Antibiotics	
		Methanol extract	Essential oil	Methanol extract	Essential oil	Amp^r	Kan^r
Staphylococcus aureus	$^+$ve	11.16 ± 0.16	11.20 ± 0.14	11.35 ± 0.07	10.36 ± 0.07	12.00 ± 0.05	11.50 ± 0.17
Bacillus substilis	$^+$ve	12.48 ± 0.04	11.35 ± 0.08	12.15 ± 0.05	8.50 ± 0.06	13.02 ± 0.14	12.60 ± 0.15
Klebsiella pneumoniae	$^-$ve	12.15 ± 0.05	14.03 ± 0.09	11.50 ± 0.14	11.03 ± 0.12	14.00 ± 0.10	13.30 ± 0.12
Enterococcus sp.	$^+$ve	12.06 ± 0.08	11.40 ± 0.12	11.03 ± 0.18	10.10 ± 0.11	12.50 ± 0.05	12.40 ± 0.14

The crude extract with concentration 1 mg/mL were used for analysis and ampicillin (Amp^r) and Kanamycin (Kan^r) were used as a positive control in a concentration of 50 μg/mL.

well [29,30]. Further, Park *et al*. have reported the therapeutic potential of rutin for treatment of neurodegenerative diseases associated with oxidative stress [31]. Several scientific reports support the fact that regular consumptions of flavonoids or polyphenols lower the risk of cardiovascular disease, diabetes and cancer [32].

Together with the flavonoids, the authors also identified 24 different compounds in the essential oil of aerial parts of *A. vulgaris* through the GC–MS analysis. Compared with the chemical constituents of *A. vulgaris* collected from Hetauda, low altitude of Nepal, the authors found the variation in the major chemical constituents and composition of essential oils. The compound sabinene and germacrene D found in higher amount in the study were least observed by Satyal *et al*. [33]. This clearly indicates that the climatic condition, harvesting time and environmental stress are responsible for the production of different compounds in the plant species. Among the identified compounds in oil of *A. vulgaris*, thujone was reported to have gamma-aminobutyric acid receptor antagonist [34]. Furthermore, compound borneol is used as a natural insect repellent and 1,8-cineole is used in the fragrance [35]. Presence of thujone and borneol makes the strong insect repellent properties of *A. vulgaris* leaves [36].

Furthermore, higher antibacterial and antioxidant activities were observed with *A. vulgaris* oil in comparison to the *G. fragrantissima* oil. Since *A. vulgaris* oil contained mono- and sesquiterpenes compounds such as sabinene, β-thujone, chrysanthenone, camphor, borneol and germacrene D. Whereas, *G. fragrantissima* contains 95% of methyl salicylate as a sole compounds. On the other hand, crude methanol extract revealed higher antioxidant when compared with essential oil. It is mainly due to the presence of higher amounts of polyphenolic and flavonoid compounds in the crude methanol extract. Although these plant species are in use for the treatment of several diseases in Nepal, little was known about its true nutritional values. This finding sheds light on the medicinal values of the *G. fragrantissima* and *A. vulgaris* leaves. Further, it also opens up the possibility in future to explore the diverse chemical constituents present in these plant species and their uses in cosmetic, pharmaceutical and chemical industries.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgements

This research was supported by Academy Partnership Program Track 1-1 funded by KOICA (APP-Track 1-1 2015-0249). We express our gratitude to Prof. Jae Kyung Shong, Sun Moon University and Prof. Byung Gee Kim, Seoul National University for providing the authentic flavonoids compounds. Similarly, we extent our thanks to Dr. Ramesh Pandey, Dr. Dipesh Dhakal, Sun Moon University and Mr. Prajjwal Rajbhandari, RIBB for technical support. Further we also like to extend our gratitude to Department of Microbiology, School of Medical Sciences, Kathmandu University.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.apjtm.2017.09.005.

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