



Original article

Effect of drying methods on the free radicals scavenging activity of *Vernonia amygdalina* growing in Malaysia

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ABSTRACT

The extracts from *Vernonia amygdalina* leaves dried using different modes of drying were examined for their antioxidant activities. The component identification was carried out on extract with the highest antioxidant property using GC–MS analysis. Using DPPH, ABTS⁺ and H₂O₂ radical scavenging assays, the shade, the sun, oven at 40, 50, and 60 °C dried sample extracts were screened. The obtained scavenging activities were in the order: shade drying > sun drying > oven drying at 40 °C > oven drying at 50 °C > oven drying at 60 °C. The extract from shade-dried sample exhibited the highest activity by scavenging 63.21–92.25% of DPPH, 75.32–93.15% of ABTS⁺, and 82.53–95.78% of H₂O₂ radicals, which were higher than those of ascorbic and gallic acid scavenging activities. In addition, the effects of drying methods and extract concentrations on the antioxidant activity of *V. amygdalina* leaf were significant ($p < 0.05$). A total number of 11 major compounds were identified from the shade-dried extract viz: phytol (43.69%), (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid (17.60%), 2-methyl-3-hexanol (6.83%), ethyl ester-9,12-octadecadienoic acid (6.07%), ethyl ester linoleic acid (5.19%), ethyl ester hexadecanoic acid (5.06%), heneicosane (4.15%), heptacosane (3.25%), and others are less than 3%. Therefore, it can be deduced that an increase in drying temperature may deteriorate the antioxidant activity of *Vernonia amygdalina* leaf.

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1. Introduction

Free radicals had been reported to be responsible for the pathophysiological of several diseases like cancer, diabetes, and cardiovascular diseases (Ramkumar et al., 2007). Antioxidant has capacity to dissolve the free radicals cells in human bodies (Erasto et al., 2007; Thaipong et al., 2006). In recent years, several plants had been studied to contain phytochemicals that can protect a human system against different diseases. However, the structures of these phytochemicals made them scavenge free radical cells (Alara et al., 2017, 2016; Ayoola et al., 2008; Erasto et al., 2007; Farombi and Owoeye, 2011; Igile et al., 1994; Olalere et al., 2017). The World Health Organization had highlighted that many plants in the world possesses high medicinal values and

recommended the use of natural products in place of synthetic drugs because of their adverse effects (Qi, 2015). Therefore, studies are being done on novel types of antioxidants from different plant matrices.

Vernonia amygdalina, known as “bitter leaf” in English is one of the commonly consumed plants in Africa (West Africa) as food and medicine (Egharevba et al., 2014; Igile et al., 1994; Nwaoguikpe, 2010; Oduah, 2012). It is a shrub that grows up to 3 m above the sea level. This plant had been studied to possess several pharmacological properties such as antioxidant, anti-diabetes, anti-inflammatory, anti-cancer, anti-malaria, anti-leukaemia, anti-fungi, anti-microbial, and among others (Alam et al., 2013; Atangwho et al., 2013; Erasto et al., 2007; Ilondu, 2010; Iwalokun, 2008; Jisaka et al., 1993; Ngatu et al., 2012; Owoeye et al., 2010). Many bioactive compounds like flavonoids, sesquiterpenes, terpenes, edotides, xanthenes and alkaloids had been isolated from the extracts (Farombi and Owoeye, 2011; Igile et al., 1994; Jisaka et al., 1993; Luo et al., 2017).

Studies had revealed that phenolic compounds are responsible for the antioxidative activities of edible and non-edible plant products by providing protection against harmful free radicals and minimize the risk of diseases associated with oxidative stress (Vashisth et al., 2011). Different assays have been frequently used to

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determine the antioxidant activities in vegetables and fruits including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and hydrogen peroxide (H₂O₂) assays. [Thaipong et al. \(2006\)](#) observed the high correlation between ABTS, DPPH, FRAP, and ORAC for guava fruit extracts with ascorbic acid.

The antioxidant activity of *V. amygdalina* L. growing in different part of the world had been explored but to the best of our knowledge, the effect of drying methods on this activity has yet to be studied ([Atangwho et al., 2013](#); [Erasto et al., 2007](#); [Farombi and Owoeye, 2011](#); [Shon et al., 2003](#); [Qing et al., 2014](#)). Therefore, the antioxidant property of *Vernonia amygdalina* leaf growing in Malaysia as affected by different modes of drying (shade, sun, oven at 40, 50, and 60 °C) using three radical scavenging assays (DPPH, ABTS^{•+}, and H₂O₂) and the component identifications of the selected drying method extract were studied.

2. Materials and methods

2.1. Plant samples and reagents

The fresh leaves of *Vernonia amygdalina* were harvested from a garden at Gambang located in Malaysia (Latitude: 3° 42' 25.183"N and Longitude: 103°6' 8.982" E). Methanol, ethanol, 2,2-diphenylpicrylhydrazyl (DPPH), ascorbic acid, 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS^{•+}), sodium hydrogen phosphate monobasic anhydrous (NaH₂PO₄), potassium hydrogen phosphate dibasic anhydrous (K₂HPO₄), and potassium persulfate were purchased from Sigma–Aldrich (M) Sdn Bhd. The distilled water and 30% v/v hydrogen peroxide were obtained from Faculty of Chemical Engineering and Natural Resources Engineering laboratory. All the chemicals used were of analytical grade.

2.2. Soxhlet extraction

Fresh leaves of *Vernonia amygdalina* were washed and pre-treated using different methods of drying (open sun, shade, oven at 40, 50, and 60 °C) until no significant loss in weight were recorded. The dried samples were blended using a grinder (Panasonic Blender PANA-MX-801S HG, Malaysia). The crushed samples were sieved to 0.105 mm particles size. Thereafter, 100 g dried samples were subjected to soxhlet extraction for 2 h using 60% v/v ethanol solution. After the extraction process, the mixture was allowed to cool to room temperature, filtered through Whatman qualitative filter paper No. 1, and concentrated using rotary evaporator (Buchi Rotavapor R-200 coupled with Buchi Vac V-500 pump, Switzerland). The extracts were stored in the air-tight vial bottles and refrigerated at 4 °C until the time of analysis. The experiments were carried out in triplicate.

2.3. Free radical assays

2.3.1. DPPH free radical scavenging

The free radical scavenging capacities of *Vernonia amygdalina* leaf ethanolic extract were determined using DPPH assay according to the methods described by [Ayoola et al. \(2008\)](#) and [Alam et al. \(2013\)](#) with some modifications. Briefly, the stock solution of 1 mM DPPH was prepared in analytical grade methanol (99.95% purity) and stored at –20 °C until analysis. Fresh working DPPH solution (0.1 mM) was prepared by diluting 10 ml stock solution with 90 ml methanol. 0.2 ml of extract (or standard) at different concentrations (0.01–0.1 mg/ml) was dissolving in 2 ml of analytical grade methanol and 2 ml of DPPH solution was added. After 30 min of incubation in the dark, the absorbance at 517 nm using a UV–VIS Spectrophotometer (Hitachi U-1800, Japan) was

recorded. Methanol was used as the blank. The analysis was done in triplicate. Ascorbic and gallic acid were used as the standards. The percentage of DPPH radical inhibition was determined using Eq. (1).

$$\%DPPH\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} * 100\% \quad (1)$$

where A_{control} is the mixture of methanol and DPPH solution; and A_{sample} is the mixture of sample extract and DPPH solution.

2.3.2. ABTS radical scavenging assay

This assay was performed according to the procedure described by [Thaipong et al. \(2006\)](#) with some modifications. The ABTS^{•+} stock solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate solution in the ratio 1:1. Then, the mixture was incubated in the dark for 12 h at room temperature. The fresh working solution of ABTS radical cation was prepared by diluting 2 ml of ABTS^{•+} solution with 120 ml methanol to obtain an absorbance of 1.1 ± 0.02 at 734 nm using UV–VIS Spectrophotometer (Hitachi U-1800, Japan). 0.15 ml of *Vernonia amygdalina* leaves extract (or standard) was mixed with 2.85 ml of ABTS^{•+} solution and allowed to stand in the dark for 2 h at room temperature. Thereafter, the absorbance was measured at 734 nm using UV–VIS Spectrophotometer (Hitachi U-1800, Japan). Methanol was used as the blank. The percentage of ABTS^{•+} was determined using Eq. (2). Gallic acid was used as the standard. The analyses were carried out in triplicate.

$$\%ABTS^{•+}\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} * 100\% \quad (2)$$

where A_{control} is the mixture of methanol and ABTS^{•+} solution; and A_{sample} is the mixture of sample extract and ABTS^{•+} solution.

2.3.3. Hydrogen peroxide scavenging assay

This assay was carried out followed the procedure described by [Keser et al. \(2012\)](#). Briefly, 50 mM of phosphate buffer (pH 7.4) was prepared. 40 mM hydrogen peroxide solution was prepared from 30% concentration of hydrogen peroxide solution. 0.1 mg/ml extract (or standard) was mixed with 0.6 ml of hydrogen peroxide solution (A_{sample}) and 3.6 ml of phosphate buffer was mixed with 0.6 ml of hydrogen peroxide solution (A_{control}). The mixture was incubated in the dark for 10 min and the absorbance was measured at 230 nm using UV–VIS Spectrophotometer (Hitachi U-1800, Japan). Phosphate buffer (pH 7.4) was used as the blank. Gallic acid was used as the standard. The analyses were repeated in triplicate. The percentage of hydrogen peroxide inhibition was calculated using Eq. (3).

$$\%H_2O_2\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} * 100\% \quad (3)$$

where A_{control} is the mixture of phosphate buffer and H₂O₂ solution; and A_{sample} is the mixture of sample extract and H₂O₂ solution.

2.4. Gas chromatography–mass spectrometry analysis

The components identification and their percentage of abundance in *Vernonia amygdalina* leaf extracts were evaluated using gas chromatography–mass spectrometric analysis. [Atangwho et al. \(2013\)](#) procedure with some modifications was employed. An Agilent GC 7890A (USA) coupled with MS 597C, equipped with a DB-1MS capillary column (30 m tubular column length × 0.25 mm i.d. × 0.25 μm film thickness) and autosampler was used. The operating conditions for the analysis were as follows: initial temperature of 35 °C was raised to 95 °C at the rate of 3 °C/min for 10 min, then raised to 270 °C at the rate of 10 °C/min and finally to 300 °C at the rate of 3 °C/min which was

maintained for 3 min; column flow rate of 0.8 ml/min; carrier helium at a flow rate of 1.0 ml/min; split ratio 10:1; the ionization voltage of 70 eV; run time of 67.5 min; and sample injection volume was 1 μ l solution of extract (5 mg/ml). The components were identified by relating the spectra obtained with those on NIST 05a library database, and the percentages of abundance were calculated with the total ion chromatogram. 1 ml sample was prepared by diluting the extract with analytical absolute ethanol at a ratio of 1:20 (w/v).

2.5. Statistical analysis

The experiments and analyses were carried out in triplicate and expressed as mean \pm SD. The calculations and curves were done in MS Office Excel[®], 2013. The effects of drying methods and extract concentrations on the antioxidant assays were carried out using two-way ANOVA ($p < 0.05$).

3. Results and discussion

3.1. Antioxidant activity of *Vernonia amygdalina* leaf extracts using different drying methods

The free radical scavenging activities of *Vernonia amygdalina* leaf extracts from different drying methods were evaluated using the DPPH, ABTS⁺, and H₂O₂ assays at 519, 734, 230 nm absorbance, respectively. Variations in the percentage of inhibitions at concentrations 0.01, 0.02, 0.05, and 0.1 mg/ml in comparison with ascorbic and gallic acid are shown in Figs. 1–3. DPPH radical is a stable free radical that can donate hydrogen when reacts with antioxidant compounds and reduced to diphenyl picrylhydrazine (Thaipong et al., 2006). These showed the ability of extracts to neutralize free radicals which possess unpaired electrons (Atangwho et al., 2013; Erasto et al., 2007; Thaipong et al., 2006). The DPPH scavenging activities of the extracts were in the order: shade drying > sun drying > oven drying at 40 °C > oven drying at 50 °C > oven drying at 60 °C (Fig. 2). At different concentrations (0.01, 0.02, 0.04, 0.05, and 0.1 mg/ml), extracts obtained from shade-dried sample showed the highest activities of $63.21 \pm 2.11\%$, $69.70 \pm 3.27\%$, $87.99 \pm 1.10\%$, and $92.25 \pm 2.43\%$ of the DPPH radical scavenging, respectively (Fig. 2). An increase in the percentage of DPPH inhibition caused by antioxidant might be due to the scavenging ability of radicals by hydrogen donation. It can also be seen that the extracts were more active than that of ascorbic and gallic acid at the same concentrations. This showed that *Vernonia amygdalina* leaf extract possesses a stronger antioxidant activity. The DPPH radical scavenging for ascorbic

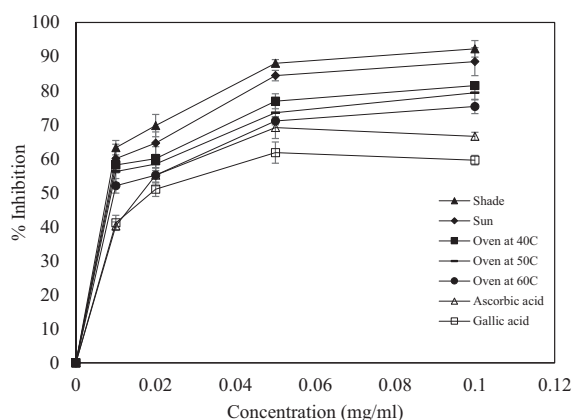


Fig. 1. DPPH radical scavenging activities at different drying methods.

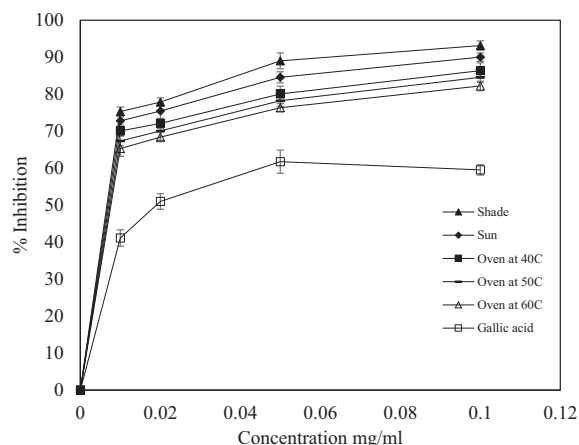


Fig. 2. ABTS scavenging activities at different drying methods.

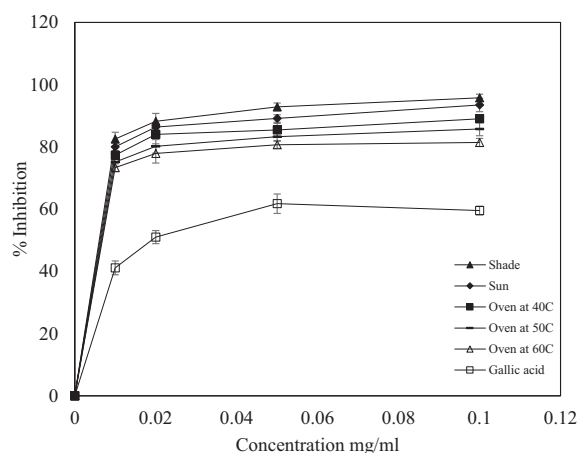


Fig. 3. H₂O₂ scavenging activities at different drying methods.

and gallic acid ranged from 40.21 to 66.55% and 41.12 to 59.54%, respectively. Therefore, it can be deduced that drying methods have significant effect on the antioxidant activity of the plant extracts resulting in different values of antioxidant inhibitions.

The antioxidant activity of the extract using ABTS radical assay was generated by potassium persulfate in order to determine its hydrogen donating ability. Likewise, hydrogen peroxide is a weak oxidizing agent that can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups (Keser et al., 2012). In the ABTS⁺ and H₂O₂ assays, similar trends of DPPH assay were obtained. Extract from the shade-dried sample exhibited the highest antioxidant activity in the ranges 75.32–93.15% and 82.53–95.45%, respectively (Figs. 3 and 4). Higher percentage inhibition might be due to its ability in donating hydrogen and terminating the oxidation process by converting free radicals into their stable form (Thaipong et al., 2006; Xiong et al., 2012). The oven-dried samples at 60 °C showed the lowest antioxidant activity but slightly higher than gallic acid. The scavenging activity at different concentrations followed the trend: 0.01 < 0.02 < 0.05 < 0.1 mg/ml (Figs. 3 and 4). The ability of *V. amygdalina* leaf to scavenge H₂O₂ may be attributed to the presence of phenols and tannins which could donate electrons, thereby neutralizing it into water (Babu et al., 2013). The obtained results confirmed the antioxidant activity of the *V. amygdalina* leaf.

The extracts from *Vernonia amygdalina* leaves dried at different conditions have demonstrated concentration-dependent radical

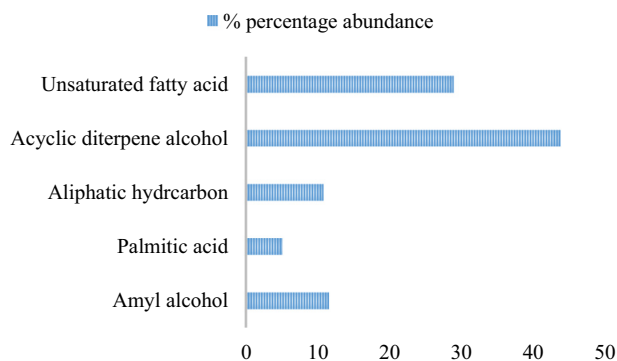


Fig. 4. Percentages of different classes of compounds extracted from shade-dried *Vernonia amygdalina* leaves.

scavenging activities. In general, the extracts were found to possess higher antioxidant activities even at different drying conditions whereby shade drying showed the best method of obtaining the highest antioxidant from *Vernonia amygdalina* leaf. The obtained results were similar to those in the literature for *Vitex negundo* and *Vitex trifolia* leaves (Chong and Lim, 2012), ginger species leaves (Chan et al., 2009), and Lamiaceae herbs (Adámková et al., 2015). However, drying process may cause changes in the percentage of antioxidant inhibition (Adámková et al., 2015; Chan et al., 2009; Vashisth et al., 2011). Therefore, it can be deduced that increase in drying temperature may lead to deterioration of antioxidant activity of the extracts.

Furthermore, two-way analysis of variance was conducted on the effect of drying methods and extract concentrations on the antioxidant activities of *V. amygdalina* L. extracts (Table 1). Drying methods included five levels (shade, sun, oven drying at 40, 50, and 60 °C) and extract concentrations consisted of five levels (0, 0.01, 0.02, 0.05 and 0.1 mg/ml). All the effects were statistically significant at 0.05 significance level. The main effect for drying methods yielded an F ratio of 383.18 and $p < 0.0001$, indicating a significant difference between shade, sun, oven drying at 40, 50 and 60 °C. Likewise, the main effect for extract concentrations yielded an

F ratio of 4.81, $p = 0.002$, indicating a significant difference between 0, 0.01, 0.02, 0.05, and 0.01 mg/ml of the extracts. In contrary, the interaction effect was insignificant with the F ratio of 0.34 and $p = 0.989$. Therefore, it can be deduced that drying methods and extract concentrations have a significant influence on the antioxidant activity of *V. amygdalina* leaves (Table 2).

3.2. GC–MS chemical compositions of shade-dried ethanolic *Vernonia amygdalina* leaf extract

The quantitative composition and percentage abundance of compounds in the shade-dried ethanolic extracts of *Vernonia amygdalina* leaf are shown in Table 1. A total number of 11 major compounds were identified viz: phytol (43.69%), (*z,z,z*)-methyl ester-9,12,15-octadecatrienoic acid (17.60%), 2-methyl-3-hexanol (6.83%), ethyl ester-9,12-octadecadienoic acid (6.07%), ethyl ester linoleic acid (5.19%), ethyl ester hexadecanoic acid (5.06), heneicosane (4.15%), heptacosane (3.25%), and others (less than 3%). Previous studies had reported that *V. amygdalina* leaf possesses several phytochemical including diterpenes, flavonoids, alkaloids, saponins and tannins (Farombi and Owoeye, 2011; Igile et al., 1994; Jisaka et al., 1993; Luo et al., 2017). Thus, the results obtained therein were in agreement with the observations from previous studies. However, the phytochemicals identified from the GC–MS analysis of the shade-dried extract were acyclic diterpene alcohol (43.69%), unsaturated fatty acid (28.86%), amyl alcohol (11.53%), aliphatic hydrocarbon (10.76%), and palmitic acid (5.06%) as illustrated in Fig. 4. The presence of the higher percentage of acyclic diterpene alcohol and unsaturated fatty acids reflected the antioxidant and anti-inflammatory potentials of *V. amygdalina* leaf (Akinmoladun et al., 2007; Atangwho et al., 2013; Erasto et al., 2007; Saravanan and Parimelazhagan, 2014). Moreover, unsaturated fatty acids had been reported as one of the predominant fatty acids that play important role in the infant brain development, reduction of coronary heart diseases and treatment of rheumatic symptoms in the traditional medical system (Erasto et al., 2007; Oliver, 1997). Therefore, this study showed that the crude extracts of *V. amygdalina* leaf have a higher potential in alternative medicine.

Table 1
Two-way analysis of variance for the effect of drying methods and extract concentrations on the antioxidant activities of *V. amygdalina* L.

Source of Variation	SS	df	MS	F	P-value	F critical
Drying methods	75165.99	4	18791.50	383.18	<0.0001	2.56
Extract concentrations	943.6985	4	235.92	4.81	0.002	2.56
Interaction	267.191	16	16.70	0.34	0.989	1.85
Within	2452.025	50	49.04			
Total	78828.9	74				

df: degree of freedom, SS: sum of square, MS: mean square.

Table 2
Qualitative and quantitative profile of shade-dried ethanolic *Vernonia amygdalina* leaf extracts.

S/N	Compound name	Retention time (min)	Molecular weight	Abundance (%)
1	2-Pentanol	7.617	C ₅ H ₁₂ O	2.28
2	2-Methyl-3-hexanol	8.029	C ₈ H ₁₆ O	6.83
3	2,4-Dimethyl-3-pentanol	8.029	C ₇ H ₁₆ O	2.42
4	Ethyl ester hexadecanoic acid	41.998	C ₁₈ H ₃₆ O ₂	5.06
5	Heptacosane	42.212	C ₂₇ H ₅₆	3.25
6	Phytol	43.140	C ₂₀ H ₄₀ O	43.69
7	Heneicosane	43.164	C ₂₁ H ₄₄	4.15
8	Ethyl ester-9,12-octadecadienoic acid	43.498	C ₂₀ H ₃₆ O ₂	6.07
9	Ethyl ester linoleic acid	43.501	C ₂₀ H ₃₆ O ₂	5.19
10	(<i>z,z,z</i>)-methyl ester-9,12,15-octadecatrienoic acid	43.523	C ₁₉ H ₃₂ O ₂	17.60
11	Eicosene	45.716	C ₂₀ H ₄₀	3.36

4. Conclusion

The obtained results showed that shade drying is more effective to other drying methods in preserving the antioxidant activity of *Vernonia amygdalina* leaf. Thermal drying (sun and oven) resulted in the deterioration of the antioxidant activity as confirmed using DPPH, ABTS*, and H₂O₂ radical scavengers. More so, the extracts showed promising antioxidant potential from the in vitro analysis. In addition, the GC–MS analysis of the shade-dried extract showed the presences of unsaturated fatty acids indicating that *Vernonia amygdalina* leaves could be utilized in pharmaceutical as well as food industries. It is therefore recommended that in vivo antioxidant activity of *V. amygdalina* L. be considered for further studies.

Conflict of interest

We declare no conflict of interest.

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