



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
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Antioxidant and Antidiabetic Potential of Malaysian *Uncaria*

¹R. Ahmad, ¹H.M. Hashim, ¹Z.M. Noor, ¹N.H. Ismail, ¹F. Salim, ²N.H. Lajis and ²K. Shaari

¹Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

²Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Corresponding Author: R. Ahmad, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia Tel: +60355444565 Fax: +60355444562

ABSTRACT

The aim of the study was to evaluate the antioxidant and antidiabetic potential of five Malaysian *Uncaria* species namely *U. lucida*, *U. acida*, *U. cordata*, *U. callophylla* and *U. longiflora* var. pteropoda, find any correlation between these two activities and relate them to their phytochemical content. Measurement of antioxidant activities employed ferric thiocyanate (FTC), thiobarbituric (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays while evaluation of total phenolic contents employed Folin Ciocalteu methods. Antidiabetic potential was evaluated by α -glucosidase inhibitory assays. All tested extracts exhibited very strong antioxidant potential in the FTC and TBA assays. *U. longiflora* v.p. (stems and leaves) and *U. callophylla* (stems) exhibited strong DPPH free radical scavenging activity with IC_{50} values of 8-20 $\mu\text{g mL}^{-1}$ compared to 8 $\mu\text{g mL}^{-1}$ for vitamin C and 7 $\mu\text{g mL}^{-1}$ BHT. In the α -glucosidase inhibitory assay, the stem extracts of the two plants showed strong α -glucosidase inhibition (>99%). The anti-diabetic activity exhibited by the two plants correlated well with its radical scavenging activities and its phytochemical content. This study has found Malaysian *Uncaria* to be potentially important sources of antioxidants and anti-diabetic agents, which may be used in prevention and control of type II diabetes.

Key words: *Uncaria*, antioxidant, total phenolic content, radical scavenging activities, antidiabetic, α -glucosidase inhibition

INTRODUCTION

The genus *Uncaria* (Rubiaceae) has been known for their medicinal and therapeutic properties. Among the *Uncaria* species, the most widely explored is the Peruvian *Uncaria tomentosa* or known as *una de gato* (cat's claw) which is believed to possess magical healing power. It has been extensively used for the treatment of asthma, cancer, cirrhosis, fevers, gastritis, diabetes, rheumatism, dysentery, inflammation of the urinary tract and many other diseases (Kepplinger *et al.*, 1999; Heitzman *et al.*, 2005; Kloucek *et al.*, 2005). In Asia, the medicinal plants *U. rhynchophylla*, *U. guianensis* and *U. hirsuta* are popularly used as an immune system stimulant, to reduce the risk of stroke and heart attack and lower cholesterol, to treat hypertension, for diabetes and for other medicinal purposes (Heitzman *et al.*, 2005; Stuart and Gulve, 2004; Suk *et al.*, 2002). In Malaysia, the more common representatives of *Uncaria* include *U. gambir*, *U. acida*, *U. cordata* and *U. longiflora* v.p. besides *U. lucida* and *U. callophylla*. The leaves and young shoots of *Uncaria* plants are used to cure diarrhea and dysentery, as gargle for sore throats or rubbed on the body to relieve pain from rheumatism while the juice is used for thrush

(Burkhill, 1966). However, apart from the study on the anti-hypertensive activity of the Malaysian *U. callophylla* (Goh *et al.*, 1986) not much has been published on the biological activities relating to the therapeutic properties of the plants. In a majority of the studies, the high biological activity of cat's claw and other *Uncaria* species has been attributed to unique tetracyclic and pentacyclic oxindole alkaloids (Pilarski *et al.*, 2010; Prado *et al.*, 2007; Heitzman *et al.*, 2005). However, due to the wide spectrum of the plants' activity, Reinhard (1999) suggested that synergistic participation of other chemical compounds in the healing process must be taken into account. These include phenolic constituents which consist of flavonoids, phenolic acids and tannins which are known for their high antioxidative activity (Balasundram *et al.*, 2006). Many investigations indicated that these compounds are of significant value in preventing the onset and/or progression of many human diseases if free radicals or other reactive species caused or significantly contributed to the progression of the disease. Evidence supported the view that increased free radical formation is usually a consequence of tissue damage by a disease or toxin. Thus, phenolic compounds influence the disease by diminishing damages in cellular structures (Gutteridge and Halliwell, 2010). Consequently, phenolic compounds show different biological activities such as antibacterial, anticarcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating agents (Pilarski *et al.*, 2006). This prompted us to search for antioxidant agents from Malaysian *Uncaria* species.

Diabetes is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolisms, secondary to an absolute or relative lack of the hormone insulin. It has affected a considerable population and is expected to be a major disorder in the future (Alberti and Zimmert, 1998). The two major forms of diabetes are type I (insulin-dependent) and type II (noninsulin-dependent). Elevated blood sugar (glucose) levels characterize both types. In type I, specifically there is insufficient insulin. Type II diabetes is the most common form of diabetes and is usually characterized by an abnormal rise in blood sugar right after a meal, called post-prandial hyperglycemia.

α -Glucosidase Inhibitors (AGIs) are among the available glucose-lowering medications taken to decrease post-prandial hyperglycemia. AGIs treat diabetes by decreasing post-prandial hyperglycemia by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolysing enzymes α -glucosidase and α -amylase in the digestive tract (Krentz and Bailey, 2005). The α -glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrate to absorbable monosaccharides. The AGIs delay but do not prevent the absorption of ingested carbohydrates thus reducing the postprandial glucose and insulin (Stuart and Gulve, 2004; Andrade-Cetto *et al.*, 2007; Bhandari *et al.*, 2008). The therapy with synthetic oral hypoglycemic agents can produce serious side effects and may not be suitable for use during pregnancy (Langer, 2007). Thus, the search of natural antihyperglycemic agents devoid of adverse effect is important in controlling diabetes. Herbal remedies have been found to be effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared to oral synthetic hypoglycemic agents (Gupta *et al.*, 2005). This and the traditional use of Asian *Uncaria* species for diabetes have surged our interest in the search for anti hyperglycemic agents, and in particular, α -glucosidase inhibitors, from the genus.

We have previously reported on the biological activities of Malaysian plants (Ahmad *et al.*, 2005). In view of the medicinal and therapeutic properties of *U. tomentosa* and other *Uncaria* species, this study is now aimed at the evaluation of the antioxidant and antidiabetic potential of five Malaysian *Uncaria* and to find any correlation between these activities and to a certain extent, relate them to their phytochemical content.

MATERIALS AND METHODS

Plant materials and preparation of extracts: Plant materials were collected from Endau-Rompin Forest Reserves and various parts of Malaysia between 2005 and 2006. The voucher specimens were deposited at Institute of Bioscience, Universiti Putra Malaysia. Except for *Uncaria cordata* and *Uncaria acida*, the stems and leaves were separated and were soaked in methanol for 48 h and the solvent was evaporated off under reduced pressure. The plant extracts were stored at 4°C until tested.

Phytochemical screening: Alkaloids were detected by Mayer's reagent (Harborne, 1998) while the presence of flavonoids, saponins and tannins was detected according to Mojab *et al.* (2003).

Antioxidant assay: The FTC and TBA assays were carried out as previously described (Tagashira and Ohtake, 1998). In both methods, antioxidant activity was described by % inhibition:

$$\% \text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical-scavenging method: The method of Tagashira and Ohtake (1998) and Waterman and Mole (1994) was used with slight modification. A test-sample solution in methanol (200 µL) was added to 4.0 mL of 50 µM DPPH methanolic solution (to give final concentration of 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 µg mL⁻¹). After vortexing, the mixture was incubated for 30 min at room temperature. Absorbance at 517 nm was measured. The difference in absorbance between test sample and control expressed as % inhibition was taken as the activity.

Total phenolic content (TPC): Total phenolic content of plant extracts was estimated by the Folin-Ciocalteu assay (Waterman and Mole, 1994).

In-vitro α-Glucosidase inhibitory assay: The assay was performed according to Khan *et al.* (2002) with slight modifications. Inhibitory activities of plant extracts against α-glucosidase were studied spectrophotometrically at pH 6.8 and 37°C using 7 mM. p-nitrophenyl-α-D-glucopyranoside (PNP-G) as a substrate and 0.0073 units/mL enzyme in 0.05 M sodium phosphate buffer containing 100 mM NaCl. Fifteen microliter of sample extracts (1 mg mL⁻¹) and 140 µL of 0.05-M sodium phosphate buffer containing 20 µL α-glucosidase solution was incubated in 96-well plates at 37°C for 15 min. After preincubation, 25 µL of 7 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.05 M sodium phosphate buffer (pH 6.8) was added to each well. The increment in absorption at 405 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with a spectrophotometer (BioTek EL800, USA). The results were evaluated as the percentage inhibition of sample at a final concentration of 75 µg mL⁻¹. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\Delta A_{405}^{\text{Control}} - \Delta A_{405}^{\text{Extract}}}{\Delta A_{405}^{\text{Control}}} \times 100$$

RESULTS AND DISCUSSION

Antioxidant activity: Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant activities. Table 1 shows the total phenolic content of five Malaysian *Uncaria* methanolic extracts. In general, for all five species (excluding *U. acida*) the stems showed relatively higher phenolic content than the leaves. The total phenolic contents were expressed as mg Gallic acid equivalents (GAE)/g Plant Extract (PE). The TPC of the stems range from 17.37 to 43.57 mg GAE/g PE while the TPC for the leaves range from 9.61 to 25.37 mg g⁻¹ GAE mg GAE/g PE. *U. longiflora* v.p stems possessed the highest amount of phenolics. In a study on total phenolic content of *U. tomentosa*, Pilarski *et al.* (2006) found the bark of the plant to be twice higher in ethanol than in aqueous extract (292 and 111 measured in mg D-catechin equivalents/g of fresh weight of the plants, respectively). The difference in the TPC units, the different objectives of Pilarski's study and the use of ethanolic instead of methanolic extract makes comparison of TPC values rather difficult. Nevertheless, the presence of tannins, as found in the phytochemical screening of the methanolic extracts of our plant samples (Table 2) to a certain extent, supports Pilarski's findings of high tannin content in the alcoholic extract of *U. tomentosa* which could contribute significantly to the antioxidant activity observed.

In the evaluation of antioxidant activity, the ferric thiocyanate (FTC) method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. In contrast, the thiobarbituric acid (TBA) assay measures the total peroxide content

Table 1: α -Glucosidase inhibition, DPPH free radical scavenging activity and total phenolic content of five Malaysian *Uncaria* species

Plant extracts	% α -glucosidase inhibition	IC ₅₀ (μ g mL ⁻¹)	Total phenolic content (mg GAE/g PE)
<i>U. lucida</i> (L)	95.9 $\pm$ 0.6	58	14.70 $\pm$ 0.6
<i>U. lucida</i> (S)	96.5 $\pm$ 0.5	65	17.37 $\pm$ 0.6
<i>U. longiflora</i> v.p (L)	48.9 $\pm$ 0.3	8	9.61 $\pm$ 0.4
<i>U. longiflora</i> v.p (S)	99.1 $\pm$ 0.6	10	28.30 $\pm$ 0.1
<i>U. calophylla</i> (L)	55.3 $\pm$ 0.7	35	17.69 $\pm$ 0.3
<i>U. calophylla</i> (S)	99.4 $\pm$ 0.1	20	25.11 $\pm$ 0.3
<i>U. cordata</i> (L)	96.7 $\pm$ 0.1	200	25.37 $\pm$ 0.2
<i>U. cordata</i> (S)	89.1 $\pm$ 0.3	80	43.57 $\pm$ 0.2
<i>U. acida</i> (L)	54.8 $\pm$ 0.4	65	17.62 $\pm$ 0.2
Vitamin C	NA	8	NA
BHT	NA	7	NA
1-Deoxyjinorimycin	38.4 $\pm$ 0.7	NA	NA

L = Leaves, S = Stems, Values are Means $\pm$ SEM, n = 12, NA = Not Applicable

Table 2: Phytochemical content of five *Uncaria* species

<i>Uncaria</i> species	Alkaloids	Flavonoids	Tannins	Saponins (cm)
<i>Uncaria lucida</i> (L)	-	++	+++	<1
<i>Uncaria lucida</i> (S)	-	-	+++	<1
<i>Uncaria longiflora</i> v.p. (L)	++	+++	++	1-2
<i>Uncaria longiflora</i> v.p. (S)	+++	+++	++	>2
<i>Uncaria calophylla</i> (L)	-	+++	+++	1-2
<i>Uncaria calophylla</i> (S)	-	+	++	<1
<i>Uncaria cordata</i> (L)	-	-	+++	>2
<i>Uncaria cordata</i> (S)	-	-	++	1-2
<i>Uncaria acida</i> (L)	++	+++	+++	<1

L = Leaves, S = Stems For alkaloids, flavonoids and tannins, + = Low amount, ++ = Moderate amount, +++ = High amount, For saponins, Froth <1 cm = Weakly positive, froth 1-2 cm = positive, froth >2 cm = strongly positive

at a later stage of lipid oxidation involving the quantitation of the secondary products formed from oxidation. Thus, the trend of antioxidant potential of the plant extracts in the two assays should be the same. In the FTC method, all tested extracts exhibited strong antioxidant activity when compared to vitamin E (α -tocopherol). The absorbance values on the last day ranging from 0.1480-0.0165 corresponded to a % inhibition of 91.26-99.02% compared to 65.37% inhibition for vitamin E. In this study a good agreement between the results of FTC and TBA assay was indeed found, as shown in Fig. 1. Although the inhibition of lipid peroxidation as measured by the FTC method has been reported to be an easy, rapid, sensitive and complete measure of hydroperoxidation of lipids (Mihaljevic *et al.*, 1996) it does not measure the radical-scavenging properties of the plant samples.

For the evaluation of the free radical-scavenging effect of specific compounds, the DPPH method largely used in plant or food biochemistry was employed. This stable free radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In its radical form, DPPH has a broad absorption band with a maximum at 517 nm and loses this property upon protonation by an antiradical compound (Goncalves *et al.*, 2005) to form the yellow-coloured diphenylpicrylhydrazine. In the DPPH radical scavenging assay as shown in Table 1, *U. longiflora* v.p. (stems and leaves) extracts showed strong activity with IC_{50} value comparable to the standards vitamin C and BHT while *U. calophylla* also active with IC_{50} value of $20 \mu\text{g mL}^{-1}$. Other extracts showed IC_{50} values of $35\text{-}65 \mu\text{g mL}^{-1}$ with the exception of *U. cordata* with an IC_{50} value of $200 \mu\text{g mL}^{-1}$. The strong radical scavenging activities of the extracts tested exhibited by their IC_{50} values (except for *U. cordata*) indicate their strong antioxidant potential. It is interesting to note that *U. cordata* did not test positive for the presence of alkaloids or flavonoids in our phytochemical screening which could explain the relatively higher IC_{50} value observed. Among the extracts tested, *U. longiflora* v.p. (leaves and stems) was found to possess IC_{50} values ($8\text{-}10 \mu\text{g mL}^{-1}$) comparable to *U. tomentosa* (ethanol extract) whose reported value was $7.23 \mu\text{g mL}^{-1}$ (Lo Scalzo, 2008) and comparable to standards vitamin C ($8 \mu\text{g mL}^{-1}$ and BHT ($7 \mu\text{g mL}^{-1}$). Phytochemical screening of both leaves and stems extracts of *U. longiflora* v.p. (Table 2) indicated the presence of significant

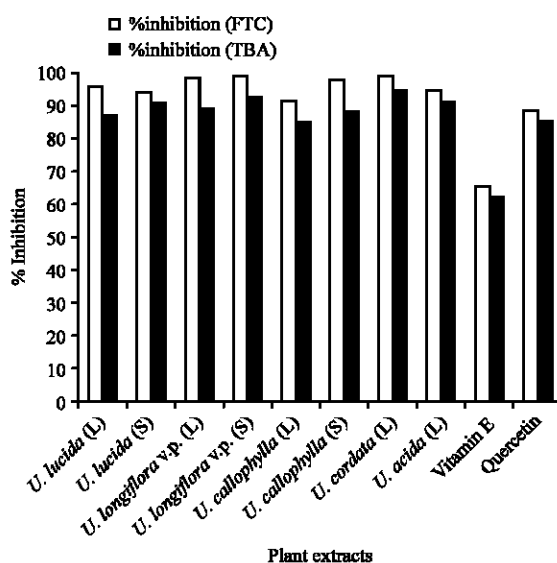


Fig. 1: Antioxidant activity of five Malaysian *Uncaria* as measured by the FTC and TBA methods

amount of flavonoids, alkaloids and saponins with moderate amounts of tannins. In a related study, Sandoval *et al.* (2002) also reported the strong anti-inflammatory and antioxidant activity of *U. tomentosa* and *U. guianensis*.

In-vitro α -glucosidase inhibitory activity: The effective treatment of diabetes is increasingly dependent on active constituents of medicinal plants capable of controlling hyperglycemia as well as its secondary complications (Maurya *et al.*, 2008). The evaluation of antidiabetic potential via α -glucosidase inhibition is expected to lead to the discovery of new, effective and safe therapeutic agents for the treatment of diabetes. All five *Uncaria* species tested showed higher percentage of α -glucosidase inhibition compared to the potent α -glucosidase inhibitor 1-deoxynojirimycin hydrochloride. Figure 2 shows the α -glucosidase inhibitory activities of the methanolic extracts of five Malaysian *Uncaria* where strong activities are indicated by high % inhibition. Extracts of *U. cordata* (leaves), *U. lucida* (leaves), *U. callophylla* (stems) and *U. longiflora* v.p (stems) all exhibited strong inhibitory activities indicated by high percentage of inhibition of more than 95% against the enzyme. However, the leaf extracts of *U. acida*, *U. longiflora* v.p. and *U. callophylla* showed only moderate inhibition of 49-55%. Interestingly, for *U. callophylla* and *U. longiflora* v.p., both stem extracts showed stronger inhibition than the leaves indicating that α -glucosidase inhibitors may be more abundant in the stems than in the leaves.

As shown in Table 1, for *U. longiflora* v.p., strong α -glucosidase inhibition was paralleled by its strong antioxidant potential as evaluated by the FTC and TBA antioxidant assays and DPPH radical-scavenging activity. Oxindole alkaloids have been reported to be the bioactive constituents of *Uncaria* for the treatment of various diseases including gastric ulcers, diarrhea, arthritis, rheumatism and cancers (Pilarski *et al.*, 2010). Interestingly, Sandoval *et al.* (2002) earlier reported that the anti-inflammatory and antioxidant activities of cat's claw (*U. tomentosa* and *U. guianensis*) are independent of their oxindole alkaloid content. This was based on a significantly higher content of oxindole alkaloids in the former. The higher potency of the latter consequently suggested that the activities observed are independent of their alkaloid content. Following this argument and

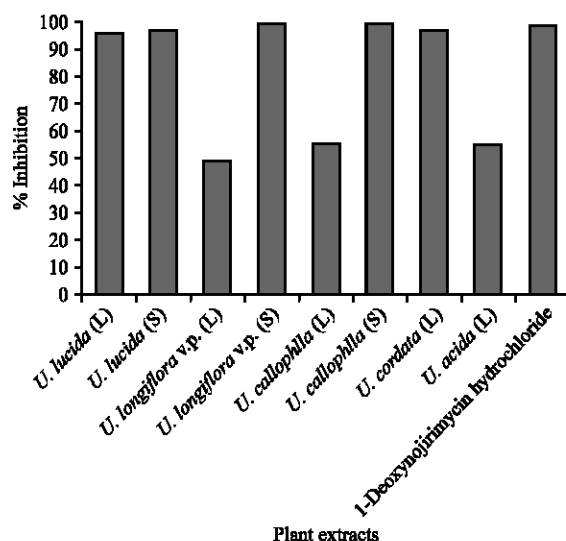


Fig. 2: Antidiabetic potential of five Malaysian *Uncaria* as measured by the α -glucosidase inhibitory activities

noting the well-known radical-scavenging activities of flavonoids, it could be deduced that the antioxidant activity and α -glucosidase inhibitory properties observed for *U. longiflora* in our study could be attributed to the flavonoids and other phytochemicals of the plant and not affected by their alkaloid content.

To date, there has not been much report on the *in vitro* or *in vivo* antidiabetic activity of oxindole alkaloids despite the ethnomedicinal claims for the use of many Asian *Uncaria* species for diabetes. In contrast, there have been numerous reports on the correlation between the high concentration of total phenolic and flavonoids contents and antidiabetic properties (Aslan *et al.*, 2007; Czinner *et al.*, 2000). Due to their phenolic structure, flavonoids are reported to be involved in the healing process of free radical-mediated diseases including diabetes. In other studies, plants with bitter taste have been said to contain steroid-saponins that possess antidiabetic potential (Jung *et al.*, 2006; Ugochukwu and Babady, 2003). Phytochemical screening on the *Uncaria* extracts, as mentioned earlier, indicated the presence of various phytochemicals including alkaloids, flavonoids, tannins and saponins with *U. longiflora* v.p. possessing the most diversified phytochemicals. The presence of these phytochemicals working in synergy may explain the stronger DPPH radical scavenging activity exhibited by the plant, which in turn would explain the relatively stronger antidiabetic potential. In a recent report, Pilarski *et al.* (2010) reported that alkaloid-rich ethanolic extract of *Uncaria tomentosa* resulted in low *in vivo* anti-cancer studies suggesting that alkaloids may not be associated with the activity and the pharmacological potency of the plant. Interestingly, in our *in-vitro* study, the alkaloid-rich and flavonoid-rich *U. longiflora* v.p. showed the strongest antioxidant and antidiabetic activity indicating that a synergistic effect of the phytochemicals may still account for the observed activities and the therapeutic properties of the active plants. On the other hand, the presence of the alkaloids, as argued earlier, may not affect the antioxidant and antidiabetic activities of the five *Uncaria* species tested. The evaluation of the activities of isolated alkaloids from the plant should be able to confirm this.

CONCLUSION

In conclusion, we have found that five Malaysian *Uncaria* species including *U. lucida*, *U. acida*, *U. cordata*, *U. callophylla* and *U. longiflora* var. *pteropoda* showed strong *in vitro* antioxidant and moderate to strong *in vitro* antidiabetic potential via α -glucosidase inhibition. *U. longiflora* v.p. (leaves and stems) and *U. callophylla* (stems) are found to be strong radical scavengers and similarly exhibited strong α -glucosidase inhibitory activities. These activities may be attributed to synergistic effects of various phytochemicals in both plants. It is anticipated that they can be potentially used in the prevention and control of type II diabetes. *In vivo* antidiabetic studies of the plants would be our next priority. We are also currently investigating the antioxidant and α -glucosidase inhibitory activities of pentacyclic oxindole alkaloids isolated from *U. longiflora* v.p. in order to ascertain whether they play a significant role in the antioxidant and antidiabetic potential of the plants.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for research grant 02-01-01-SF0057 under the E-Science fund.

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