Evaluation on bioactivities of total flavonoids from *Lavandula angustifolia*

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Abstract: *Lavandula angustifolia* was used to treat flus and fevers, joint swelling and pain in Uighur medicine. This study aimed to investigate antioxidant, antit anti-inflammatory and antalgic noids content (530.1mg/g rutin/g dry extract) with stronger DPPH scavenging abilities and reduciactivities of total flavonoids from *Lavandula angustifolia* (LTF). Results indicated that LTF possesses the highest total flavong power. Some flavonoids separated from LTF, and their DPPH scavenging abilities as follows: rosmarinic acid (2, near to Vit C) >luteolin (3) >apigenin (4) >luteolin 7-*O*-β-D-glucoside (5) >apigenin 7-*O*-β-D-glucoside (6) >luteolin 7-*O*-β-D-glucuronide (7). LTF significantly decreased malondialdehyde (MDA) level in D-galactose induced aging model compared to the control group (P<0.05), as well as significantly increased plasma superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (P<0.05). Moreover, 17.4, 34.8 and 69.6 mg/kg doses of LTF were exhibited significant analgesic and anti-inflammatory activities in a dose dependent manner (P<0.05). Cytotoxicity of LTF on Bel-7402 and Hela cell lines were showed by MTT assay also. These results verified traditional usage of this plant and suggested also that LTF is worth developing and studying further.

Keywords: *Lavandula angustifolia,* total flavonoids, antioxidant, anti-inflammatory, antalgic, antitumor.

INTRODUCTION

Lavender is a perennial sub-shrub, native to the Mediterranean coast at the southern foot of the Alps, is now cultivated commonly grown around the world (Ibrahim *et al*., 1999). Lavender is one of the most fashionable aromatic medicinal plants in the world, and its essential oil is widely applied in perfumery and cosmetics industry (Barocellia *et al*., 2004). The extracts from various Lavandula spp. are traditionally used to treat diseases such as epilepsy, migraine and to reduce spasms in colic pain in folk over the world (Gilani *et al*., 2000). At present, pharmaceutical research of Lavender mainly focused on essential oil, and its non-essiential oil pats have been little bioactive studied though it was extensively studied phytochemically. In addition to volatile compounds, lavender flowers contain also other bioactive compounds such as triterpenes and phenolic substances including cinnamic acid derivatives and flavonoids (Torras-Claveria *et al*., 2007; Papanov *et al*., 1992). Polyphenols and flavonoids in general are believed to possess antioxidative properties, and the activities of these compounds are closely related with their antioxidant properties. Flavonoids have been reported to exert multiple biological effects including anti-inflammatory, antiallergic, antiviral, and anticancer activities (Czaplinska *et al*., 2012) and are used for he treatment of several diseases (Romagnolo *et al*., 2012).

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As specie of Lavender, *Lavandula angustifolia* is cultivated at lli region in Xinjiang of China, and the annual output is far above 1000 t (Kamiyama *et al*., 2012). *Lavandula angustifolia* was used as a remedy in Uighur medicine for the treatment of abdominal fullness, cold cough, dizziness, headache, palpitations, and joint bone pain (Liu, 1999). Flavonoids are mainly characteristic compounds of *Lavandula angustifolia*, and we obtained total flavonoids active fraction and its some constituents by various chromatographic technique. In this study, we aim to investigate antioxidant, antitumor and anti-inflammatory activities of total flavonoids from *Lavandula angustifolia* (LTF) on the basement of researching results of compounds.

MATERIALS AND METHODS

Plant materials

Lavandula angustifolia were collected from lli region of Xinjiang, in China, in May 2011. The plant material was identified by associate researcher Jiang He, Xinjiang Institute of Material Medica. A voucher specimen was deposited at Xinjiang Institute of Material Medica in China.

Cells and animals

Human hepatoma carcinoma cell lines Bel 7402 and cervical carcinoma cell lines Hela were provided by Institute of Materia Medica, Chinese Academy of Medical

Sciences, and maintained with RPMI 1640 medium containing 10% fetal bovine serum, 100ng/mL penicillin and streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Kunming mice weighting 18±22g were obtained from the Experimental Animal Center in Xinjiang, China, They were allowed free access to food and water under a 12h/12h light/ dark cycle with the room temperature maintained at 27°C and relative humidity of 50-70%. The study protocols were approved by the Ethics Committee on Animal Experiment, Xinjiang Material Medica, China.

Preparation of LTF

Five kilograms of *L. angustifolia* was extracted with 70% ethanol under reflux at 80◦C for 1h in three times, and the solvent was evaporated under vacuum to afford ethanol extract. Ethanol extract was separated and purified by AB-8 resin. After deionized water eluting and cleaning impurities, 50% ethanol eluent was collected to afford total flavonoids (LTF). LTF (10g) was chromatographed over ODS RP-18 with a gradient solvent system of MeOH-H_2O (0:1-1:0). Fifty-one fractions were collected after combination by TLC guidance and repeated column chromatography over Sephadex LH-20 (MeOH). Finally, seven compounds were afforded: 1 (7.6mg), 2 (67.0mg), 3 (22.0mg), 4 (13.2mg), 5 (83.0mg), 6 (59.2mg) and 7 (41.0mg), respectively.

Determination of total flavonoids

Total flavonoid content was determined using a method described by Chinese pharmacopeia (ChP., 1999). Briefly, rutin standard solution (0.2-1.2mg/ml) and distilled water was mixed to 6.0 ml in a test tube, followed by addition of 1.0ml of a 5% (w/v) sodium nitrite solution. After 6 min, 1.0ml of a 10% (w/v) aluminium nitrate solution was added and the mixture was allowed to stand for a further 6 min before 10ml of 4%NaOH was added. The mixture was made up to 25.0ml with distilled water. The absorbance was measured at 509 nm after 15 min. Total flavonoid content was calculated as rutin (mg/g) using the following equation based on the calibration curve: y = 0.5251x-0.0223, R^2=0.9996, where y was the absorbance and x was the concentration.

Cytotoxicity Assays

Cytotoxicity of LTF on human hepatoma carcinoma cell lines Bel 7402 and cervical carcinoma cell lines Hela were determined using MTT assay (Mosmann T., 1983). Cells were added to 96-well culture plates at a density of 4.5×10^5 cell mL^{-1} and incubated at 37ºC for 24 h. Then the culture medium was removed and replaced with fresh medium supplemented with different concentrations of LTF. 20μL MTT reagent (5mg/mL) and 200 μL DMSO was added at 48 h, and plates were oscillated for 10 min in a balance oscillator after 4 h incubation at 37°C. The extent of the MTT reduction was measured by a plate reader at a wavelength of 570 nm. Inhibitory rate of cell proliferation was calculated as follows: inhibitory rate (%) = $(1\text{-}A_{\text{experimental group}}/A_{\text{control group}}) \times 100\%$.

Anti-oxidant activity

Reducing power

Reducing power of LTF was determined according to the reported method (Zubair *et al*., 2012). 1.0ml methanol solutions of LTF and Vit C (0.1-0.7mg, respectively) were mixed with 2.5mL phosphate buffer (0.2 M, pH 6.6) and 2.5mL 10mg/ml potassium ferricyanide [$K_3Fe(CN)_6$]; the mixture was incubated at 50°C for 20 min. 2.5ml 100 mg/ml trichloroacetic acid was added to the mixture, then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5mL) was mixed with 2.5ml distilled water and 0.5ml 1.0mg/ml $FeCl_3$ and the absorbance were measured at 700nm. Vit C was used as positive control. Increased absorbance of the reaction mixture indicated increased reducing power. All the measurements were made in triplicate and the results averaged.

DPPH radical scavenging activity

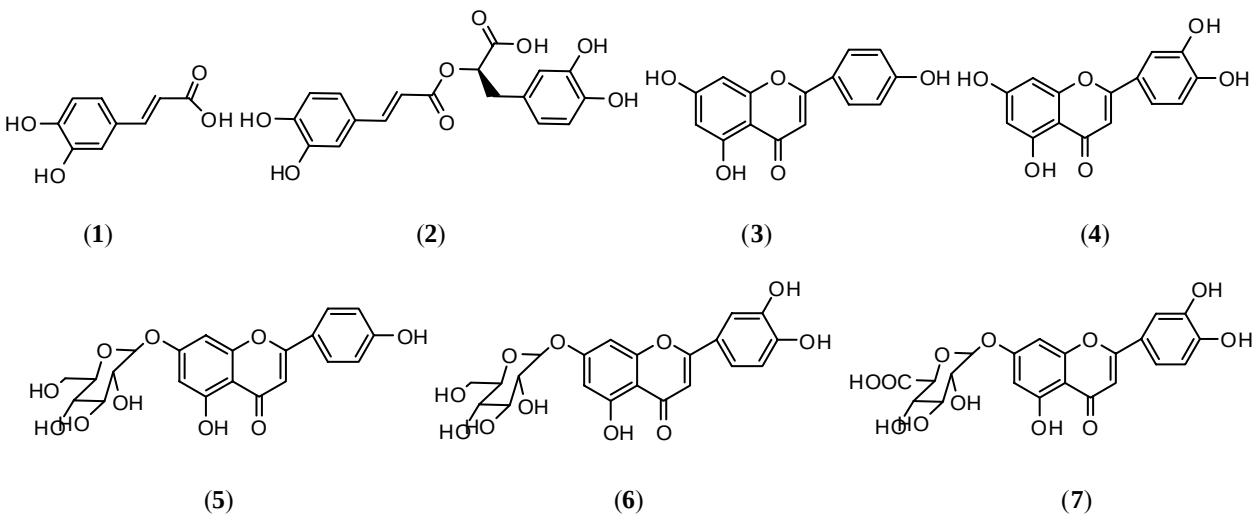
DPPH free radical scavenging activity was determined according to the previously described procedure (Zhao *et al*., 2012). Different concentrations of LTF ethanol solutions (0.5ml) were mixed with 2.5ml 6.5×10^{-5} M DPPH, and absorbance was measured at 517 nm after keeping the tubes in dark for 30min. The scavenging activity was determined by comparing the absorbance with that of control containing equal volumes of DPPH solution and ethanol. The percentage inhibition of radicals was calculated using the following formula: inhibition (%)=$(A_{\text{control}}\text{-}A_{\text{sample}})/A_{\text{control}}\times100$. Vit C was used as positive control.

Superoxide radical scavenging assay

Superoxide radical scavenging ability of the extract was determined with the reported methods (Xu *et al*., 2003). Briefly, the mixture of LTF (0.5mL) and Tris-HCl buffer (4.5mL, 50mM, pH 8.2) were incubated at 25°C for 20 min, and then 0.3mL of pyrogallol solution (80mM, 10 mM HCl) was added. The absorbance at 329nm was recorded immediately and every 30s until the reaction time reached 4min. Vit C was the reference. The percentage inhibition of radicals was obtained as followed: inhibition (%) = $[1\text{-}(A_1\text{-}A_2)/A_0]\times100$, where A_0 was the absorbance of without LTF, A_1 was the absorbance of with LTF andA_2 was the absorbance of without pyrogallol.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging ability was determined according to the reported methods (Liu *et al*., 2009). 1.5mL 1, 10-phenanthroline (1.0mM) and 0.5mL $FeSO_4$ (2.5mM) were dissolved in phosphate buffer (4.0mL pH 7.4) and mixed thoroughly. A total of 1.0mL H_2O_2 (0.2%) and 2.5mL of various concentrations of LTF were dissolved in ethanol. Subsequently, the volume was adjusted to 10mL. The mixture was incubated for 1 h at 37°C in the dark; the absorbance was measured at 536 nm. The percentage inhibition of radicals was calculated using



(**1**) (**2**) (**3**) (**4**)

(**5**) (**6**) (**7**)

Fig. 1: Structure of compounds 1-7 from LTF

the following equation: inhibition (%)=(As-A_1)/ (A_0-A_1)×100. As, the absorbance of the sample; A_1, the absorbance of control solution containing 2.0mL 1, 10-phenanthroling, $FeSO_4$, and 1.0mL H_2O_2; A_0, the absorbance of blank solution containing 1, 10-phenanthroline and $FeSO_4$.

Ageing model

After 1 week of acclimatisation, the mice were randomly divided into five groups for 12 mice per group and i.p. injected with 0.1ml/10g of 5% D-galactose once daily for 6 weeks. LTF treatment group mice were p.o. administered with different dosage (17.4, 34.8 and 69.6 mg/kg). The normal control group mice were i.p. injected with 0.3 ml of physiological saline and p.o. administered with 0.1mL/10g of 20% arabic gum each (Liu *et al*., 2003).

Anti-inflammatory and analgesic activity

Acetic acid-induced writhing response in mice

The study was carried out as described by reported methods (Muhammad *et al*., 2012). Fifty male mice were used and randomly divided into four groups including normal control group (5% tween-80), LTF groups (17.4, 34.8 or 69.6mg/kg body wt). Each mouse was given an injection of 0.7% acetic acid aqueous solution in a volume of 0.1ml/10g body weight into the peritoneal cavity, and then was placed in a transparent plastic box. The number of writhes was counted for 15min beginning from 5min after the acetic acid injection. Test drugs and control vehicle were administered 1h before acetic acid injection. Percentage of inhibition of writhing response was calculated.

Xylene-induced ear edema in mice

Anti-acute inflammatory activity was determined by xylene-induced mice ear edema (Hossein *et al*., 2000). Fifty mice were equally divided into four groups including normal control group (5% tween-80), LTF groups (17.4, 34.8 or 69.6mg/kg body wt). The vehicle and LTF were administered orally once per day for 3 days, respectively. One and half-hour after the last administration of LTF, inflammatory response was induced on the inner and external surface of the right ear (surface: about 1cm^2) by application of 20µl xylene. 30 min later, mice were sacrificed by cervical dislocation and a section (Ø 6 mm) of ears were removed from both the treated (right) and the untreated (left) ears. Edema rate was measured as percentage of the weight difference between the two ear discs compared to the untreated (left) ears. The anti-inflammatory activity was expressed as percentage of inhibition in treated mice compared to the normal control mice.

STATISTICAL ANALYSIS

The data obtained were computed using SPSS 13.0 software and later analyzed using ANOVA of variance. The Duncan test with significance level of 0.05 between means was used.

RESULTS

Phytochemical analysis

Flavonoids were the major characteristic components in LTF whose content amounted to 53.01%. Seven flavonoids were isolated from LTF and elucidated as caffeic acid (1), rosmarinic acid (2), luteolin (3), apigenin (4), luteolin 7-*O*-β-D-glucoside (5), apigenin 7-*O*-β-D-glucoside (6), luteolin 7-*O*-β-D-glucuronide (7) by various spectroscopic methods including NMR experiments and physicochemical characteristic as well as by comparison of the data with literature values. The structure of the compounds 1-7 from LTF are shown in fig. 1.

Reducing power

As an indicator of electron-donating activity, Fe^{3+} reduction is closely related with antioxidant properties and often used in research of antioxidant mechanism. fig. 2 shows the dose-response curves of LTF. The reducing power of LTF increased from 0.340±0.072 at 0.1mg/ml to 1.319±0.061 at 0.7mg/ml.

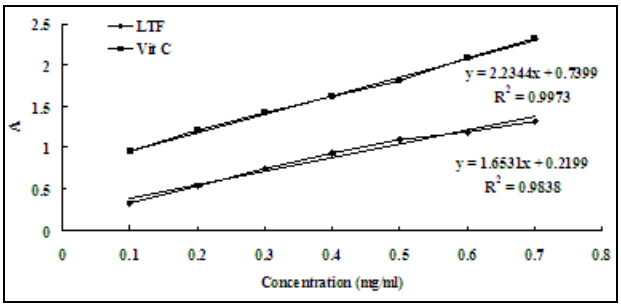


Fig. 2: Reducing power of LTF

DPPH radical scavenging activity

Fig.3 shows the DPPH free radical scavenging activities of LTF and its IC_{50} value was 6.48mg/ml. Furthermore, some compounds from LTF also showed stronger antioxidant activties, and their orders as follows: Vit C (IC_{50}, 1.62μg/ml) >rosmarinic acid (2; IC_{50}, 7.66μg/ml) >luteolin (3; IC_{50}, 14.62μg/ml) >apigenin (4; IC_{50}, 34.88 μg/ml)>luteolin 7-*O*-β-D-glucoside (5; IC_{50}, 64.04 μg/ml) >luteolin 7-*O*-β-D-glucuronide (7; IC_{50}, 71.4μg/ml)> apigenin 7-*O*-β-D-glucoside (6; IC_{50}, 103.42μg/ml).

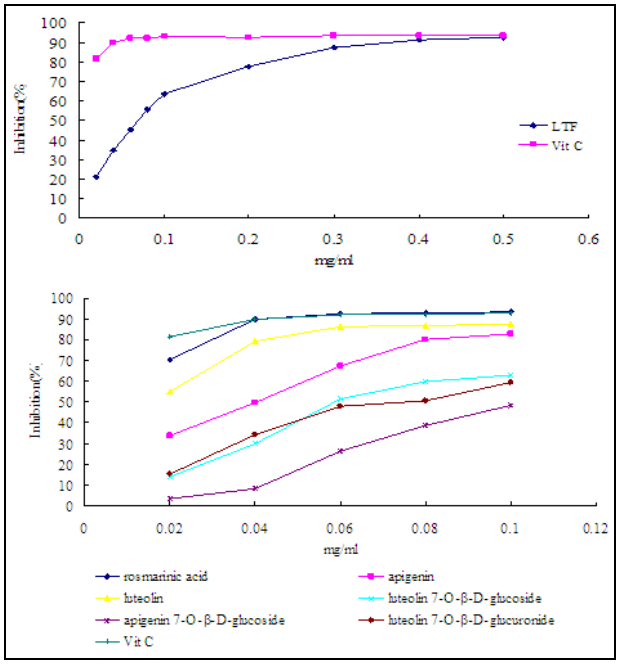


Fig. 3: DPPH radical scavenging capacity of LTF and its compounds

Scavenging activities on superoxide anion and hydroxyl radicals

The scavenging effects of LTF on superoxide anion radicals and hydroxyl radicals are shown in fig. 4. LTF showed lower activity on superoxide anion and hydroxyl radicals, and its scavenging activity on two radicals were 33.53±1.08% at 0.14mg/ml and 49.15±1.54% at 0.5 mg/ml respectively.

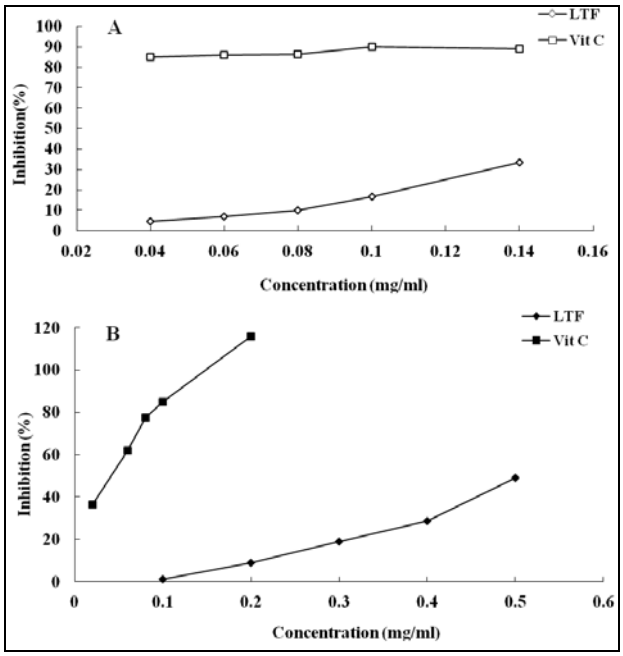


Fig. 4: Scavenging activities of LFT on superoxide anion radicals (A) and hydroxyl radicals (B)

Cytotoxicity activity

Human hepatoma carcinoma cell lines Bel 7402 and cervical carcinoma cell lines Hela were used to test effect of LTF on cell proliferation. LTF showed significant inhibitory effects on these two cell lines in a dose-dependant manner. LTF at the dose of 10mg/mL showed 80.68% and 71.64% inhibition of the growth of Bel-7402 and Hela cells, respectively (fig. 5).

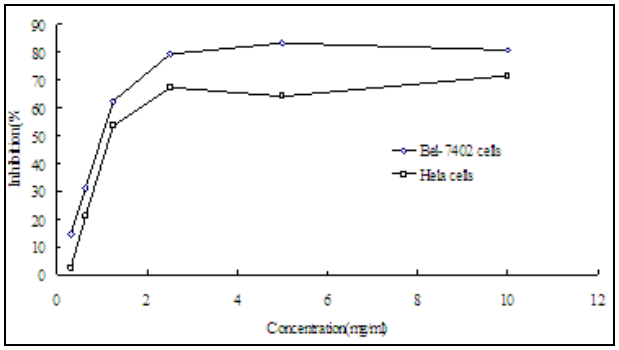


Fig. 5: Influence of LTF on Bel-7402 and Hela cells growth

Antioxidative effects for ageing mice

The results of antioxidant enzyme (SOD and GSH-Px) activities and level of MDA in serum are listed in fig. 6. After treatments, different dose group of LTF (17.4, 34.8 and 69.6mg/kg) inhibited significantly the formation of

MDA in serum and markedly raised the activities of SOD and GSH-Px in a dose-dependent manner (P<0.05).

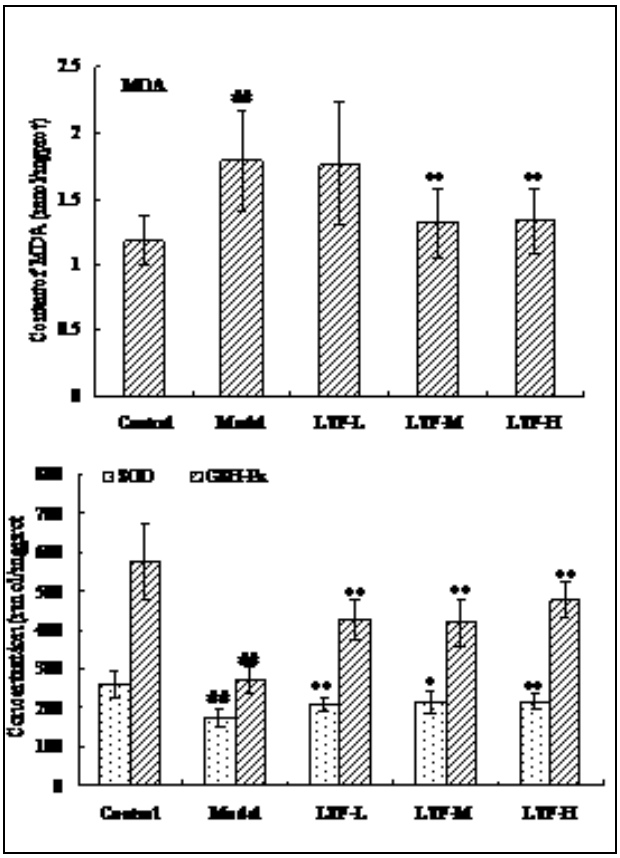


Fig. 6: Antioxidant effect of LFT on MDA, SOD and GSH-Px in liver in D-galcose induced aging mice. Values are the mean ± S.D. $^{\#\#}P$<0.01, compared with normal group; *P<0.05, **P<0.01, compared with model group.

Anti-inflammatory and analgesic activities

The oedema inhibitory rates of LTF were 13.68%, 18.09% and 21.65% at doses of 17.4, 34.8 and 69.6 mg/kg, respectively. Peripheral analgesic activity was assessed by acetic acid-induced writhing test, which showed significant (P<0.01 and P<0.05) suppression of writhes (table 1). The oral administration of LTF induced a dose dependent analgesic activity and the values ± SD for the extract are shown in table 1. Injection of acetic acid into the control mice resulted in 70.2±16.2 writhes. Pretreatment with methanol extract of LTF at doses of 17.4, 34.8 and 69.6 mg/kg reduced the number of writhes to 23.8±15.7 (40.99% inhibition), 22.8±13.1 (43.27% inhibition) and 14.3±12.3 (64.39% inhibition) respectively.

DISCUSSION

In this study, total flavonoids from *L. angustifolia* (LTF) were enriched and purified by AB-8 macro porous resins, and the content of flavonoids in LTF is 53.01%. Furthermore, we evaluated on bioactivities of LTF by determination of radicals scavenging, xylene induced ear edema and acetic acid-induced writhing test as well as MTT assay.

Reduction of DPPH radical was determined by the decrease of its absorbance at 517nm induced by antioxidants. DPPH reacts with antioxidants and gets converted into 1, 1-diphenyl-2-picrylhydrazine because of rapid hydrogen accepting ability and hence a quick decrease in absorbance is showed (Kai *et al*., 2007). The degree of decrease in absorbance indicates the scavenging potential of the anti-oxidant extract (Philip *et al*., 2004). LTF and its compounds (rosmarinic acid, luteolin, apigenin, luteolin 7-*O*-β-D-glucoside, apigenin 7-*O*-β-D-glucoside and luteolin 7-*O*-β-D-glucuronide) all show better DPPH radicals scavenging activities, of which IC_{50} value of LTF was 6.48mg/ml, and IC_{50} value of rosmarinic acid was 7.66μg/ml. However, LTF exhibited weak scavenging effect on superoxide anion and hydroxyl radicals compared to reference. The antioxidant system in the body comprises several important enzymes such as SOD and GSH-Px (Kasapoglu *et al*., 2001; Warner *et al*., 1996). Subacute mice ageing were induced by injection of D-galactose (Ke *et al*., 2009), and the biological redox substance in mice can be disturbed by long-term injection of D-galactose, such as decreasing of SOD and GSH-px activities. LTF can inhibit significantly the formation of MDA in serum and markedly raise the activities of SOD and GSH-Px in a dose-dependent manner.

One of central feature of inflammation is the activation of macrophage cells that synthesize and release large amounts of reactive oxygen species (ROS) causing cell and tissue injury (Ansari *et al*., 1996; Uma *et al*., 2008). ROS, such as superoxide, hydrogen peroxide and hydroxyl radical, are important mediators that provoke or sustain inflammatory processes. Therefore the usage of antioxidants and radical scavengers can attenuate inflammation, and the importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses (Vendemiale *et al*., 1999). Anti-inflammatory effect of LTF was evaluated by xylene induced ear edema. This model can reflect the oedematization during the early stages of acute inflammation, which was probably related with the release and inhibition of the inflammation factors (Okoli *et al*., 2004). The oral administration of LTF suppressed significantly ear oedema in mice.

CONCLUSION

In summary, our results suggest that LTF shows antioxidant, antitumor, anti-inflammatory and analgesic activities. The results may provide preliminary scientific evidence to support the folk medicinal utilization of *L. angustifoli*a and pharmacodynamics in-depth shall be further studied.

Table 1: Anti-inflammatory and analgesic activity of LTF in the different tests

Group	Concentration (mg/kg)	Xylene-induced ear edema		Acetic acid-induced writhing	
		Auricular swelling (mg)	Inhibition (%)	Number of writhings	Inhibition (%)
Model	-	70.2±16.2		40.2±20.0	-
LTF	17.4	60.6±24.7	13.68	23.8±15.7*	40.99
	34.8	57.5±25.3	18.09	22.8±13.1*	43.27
	69.6	55.0±11.2*	21.65	14.3±12.3**	64.39

*P<0.05, compared with model group.

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REFERENCES

Ansari KU (1996). Free radical induced diseases. *J. Indian Med. Assoc.,* **94**(6): 238-239.

Barocellia E, Calcinaa F, Chiavarinia M, Impicciatorea M, Brunib R, Bianchib A and Ballabenia V (2004). Antinociceptive and gastro protective effects of inhaled and orally administered Lavandula hybrida Reverchon "Grosso" essential oil. *Life Sci*., **76**(2): 213-223.

Chinese Pharmacopoeia Commission of Sanitary Ministry of People's Republic of China (1999). Chinese Pharmacopoeia, part one. Chemical Industry Publishing House, Beijing, China, p.247.

Czaplinska M, Czepas J and Gwozdzinsi K (2012). Structure, antioxidative and anticancer properties of flavonoids. *Postepy Biochem*., **58**(3): 235-240.

Gilani AH, Aziz N, Khan MA, Shaheen F, Jabeen Q, Siddiqui BS and Herzig JW (2000). Ethnopharmacological evaluation of the anti-convulsant, sedative and antispasmodic activities of Lavandula stoechas L. *J. Ethnopharmacol.*, **71**(1-2): 161-167.

Hossein H and Mohammad R (2000). Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats ss Salmani. *J. Ethnopharmacol.,* **73**(3): 379-385.

Ibrahim EG, Renee JG, Geoffrey CK and Nigel CV (1999). Hypolaetin 8-O-glucuronide and related flavonoids from *Lavandula coronopifolia* and *L. pubescen*s. *Biol. Sys. Ecol.*, **27**(8): 843-846.

Kai M, Klaus HV, Sebastian L, Ralf H, Andreas R and Ulf-Peter H (2007). Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, **7**: 2080-2095.

Kamiyama M and Shibamoto T (2012). Flavonoids with potent antioxidant activity found in young green barley leaves. *J. Agric. Food Chem*., **60**(25): 6260-6267.

Kasapoglu M and Ozben T (2001). Alterations of antioxidant enzymes and oxidative stress markers in aging. *Exp. Gerontol.*, **36**(2): 209-220.

Ke CL, Qiao DL, Gan D, Sun Y, Ye H and Zeng XX (2009). Antioxidant acitivity *in vitro* and *in vivo* of the capsule polysaccharides from Streptococcus equi subsp. Zooepidemicus. *Carbohydrate Polymers*, **75**(4): 677-682.

Liu BJ, Guo YS, Diao PF, Wei LM and Yu LP (2009). Free radical scavenging effect of total flavonoids from red clover. *Nat. Prod. Res. Dev.,* **21**(1): 44-47.

Liu J, Ho S, Lai T, Liu T, Chi P and Wu R (2003). Protective effects of Chinese herbs on D-galactose-induced oxidative damage. *Methods Find Exp. Clin. Pharm.*, **25**(6): 447-449.

Liu YM (1999). Pharmacography of uighur, part one. Xinjiang Science & Technology & Hygiene Publishing House, Urumuqi, China, pp.412-415.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. *J. Immunol. Methods*, **65**: 55-63.

Muhammad A, Gaurav G, Imran K, Mahfoozur R, Obaid A, Jahangir A, Khalidur RH, Mohammad P, Ritu G and Firoz A (2012). Anti-inflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. *Fitoterapia*, **83**(5): 853-858.

Okoli CO and Akah PA (2004). Mechanisms of the anti-inflammatory activity of the leaf extracts of *Culcasia scandens* P. Beauv (Araceae). *Pharmacol. Biochem. Behav.,* **79**(3): 473-481.

Papanov G, Bozov P and Malakoy P (1992). Triterpenoids from *Lavandula spica*. *Phytochemistry,* **31**(4): 1424-1426.

Philip M (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Tech*., **26**(2): 211-219.

Romagnolo DF and Selmin OI (2012). Flavonoids and cancer prevention: A review of the evidence. *J. Nutr. Gerontol. Geriatr.*, **31**(3): 206-238.

Torras-Claveria L, Jauregui O, Bastida J, Codina C and Vlladomat F (2007). Antioxidant activity and phenolic composition of Lavandin (Lavandula x intermedia Emeric ex Loiseleur) waste. *J. Agric. Food Chem.*, **55**(21): 8436-8443.

Uma DP and Chinnaswamy P (2008). Age and gender related changes in free radical pathology and antioxidant defense in Schizophrenia. *Asian J. Biochem.*, **3**: 143-152.

Vendemiale G, Grattagliano I and Altomare E (1999). An update on the role of free radicals and antioxidant defense in human disease. *J. Clin. Lab. Res.*, **29**(2): 49-55.

Warner BB, Stuart L, Gebb S and Wispe JR (1996). Redox regulation of manganese superoxide dismutase. *Am. J. Physiol*., **271**(1): L150-158.

Xu SH., Hang H and Li Yunping (2001). Improvement of pyrogallol measurement of superoxide dismutase (SOD). *Chemistry,* **8**: 516-519.

Zhao J, Liu T, Ma L, Yan M, Gu ZY, Huang Y, Xu F and Zhao Y (2012). Antioxidant and preventive effects of extract from *Nymphaea candida* flower on *in vitro* immunological liver Injury of rat primary hepatocyte cultures. *Evi. Comp. Alt. Med.*, 497673. doi: 10.1093/ecam/nep003.,

Zubair M, Anwar F and Shahid SA (2012). Effect of extraction solvents on phenolics and antioxidant activity of selected varieties of Pakistani rice (*Oryza sativa* L.). *Int. J. Agric. Biol.*, **14**: 935-940.