Antinociceptive, antimicrobial potential and phytochemical screening of different solvent extracted samples from the stem of *Acer pentapomicum*

Shehla Khan[1], Jehan Bakht[1] and Mohammad Shafi[2]

[1]Institute of Biotechnology and Genetic Engineering, The University of Agriculture. Peshawar. KPK. Pakistan

[2]Department of Agronomy, The University of Agriculture. Peshawar. KPK. Pakistan

Abstract: The present study investigates the antinociceptive, antimicrobial activity and phytochemical assessment of samples from *A. pentapomicum.* Different microbes were tested using disc diffusion assay at three concentrations (1, 2 and 3 mg/disc). Antinociceptive activity was determined by acetic acid induced and hot plate methods. The tested plant extracts revealed significant antinociceptive activity at dose dependent manner when measured by acetic acid induced method. The growth of all the tested microbes was inhibited by methanol, butanol, and ethyl acetate extracted samples at all concentrations. Chloroform, n-hexane and aqueous extracts inhibited the growth of the different microbes at high concentration. The most susceptible microbe was *P. aeruginosa* measuring 94% and 79% zone of inhibition by butanol and ethyl acetate extracts at 3mg /disc. *C. fruendii,* however, was the most resistant bacterium followed by *S. aureus.* The presence of alkaloids, proteins, amino acids, carbohydrates, flavonoids, tannins, saponins, and fats were confirmed in phytochemical screening of different extracts.

Keywords: Antinociceptive activity, *Acer pentapomicum*, antimicrobial activity, phytochemical analysis, zone of inhibition.

INTRODUCTION

Mankind had used and continue to be using medicinal plants to cure various health issues due to its efficacy and universality (Mathews *et al*., 1999). Medicinally important plants are widely used in the developing world for good health and fitness. Various bio-active substances present in plants increase their medicinal value and are responsible for different physiological activities in the human body when consumed. The most important include alkaloids, carbohydrates, proteins, amino acids, flavonoids tannins and other phenolic compounds (Okwu, 2001). Medicinal herbs synthesized large amount of secondary metabolites possessing antimicrobial activity (Mari *et al.,* 2003).

Acer pentapomicum, a deciduous small tree with dark brownish-grey and smooth bark belongs to the family *Aceraceae*. *A. pentapomicum* is locally known as Tarkana and is native to the northern areas of Pakistan (Mehboob, 2012). The genus Acer also known as maple, is distributed in Asia, North America, and Europe and contains approximately 200 species (Van Gelderen *et al.,* 1994). Aceraceous plants contain polyphenols which have remarkable antibacterial and anti-tumor activity (Zhang *et al*., 2008).

MATERIALS AND METHODS

Collection and identification of plant materials

Stem of *A. pentapomicum* plant (6 Kg) was collected in spring season from the mountainous region of Swat Khyber Pakhtunkhwa and identified by plant taxonomists at Department of Botany, University of Peshawar. The stems were washed, shade dried and grinded into fine powder (Thomas Scientific, USA).

**Corresponding author:* e-mail: jehanbakht@yahoo.co.uk

Crude extract preparation

All chemicals used in the present study were obtained from Sigma Aldrich. One thousand grams of dried stem powder was soaked in 4000ml of analytical grade methanol in conical flask for 6 days and stirred daily. The sample was filtered, and fresh methanol was added to the residue. This procedure was followed thrice. The filtered methanolic solution was concentrated using rotary evaporator at 45 ºC. The crude methanol extract was divided into two parts. Out of total 170 g dried crude methanolic extracts, ten grams each were used for antibacterial activity and antinociceptive assays while 150 grams was used for fractionation. For antibacterial activity, the crude methanolic extract was dissolved in DMSO while for antinociceptive assays in distilled water.

Fractionation of crude extracts

One hundred and fifty grams was dissolved in 300ml distilled water for further fractionation with different solvents. Different solvents were added according to their polarity starting from low polarity. Two hundred and fifty ml n-hexane was added and mixed thoroughly for separation of two phases. The upper n-hexane phase was removed, and the lower phase was re-partitioned two times with fresh n-hexane. All fractions of n-hexane were dried to a semi-solid state through a rotary evaporator. The semisolid n-hexane fraction was dried at 45ºC and kept until used. A similar procedure was adopted for chloroform, ethyl acetate and butanol. The leftover lower aqueous phase was concentrated through rotary evaporator and water bath and used as an aqueous fraction. Thus, five fractions namely chloroform (0.6g), ethyl acetate (1.7g), butanol (2.5g) and aqueous fraction (0.8g) were obtained for further investigation. Fractionation was done in the order of n-hexane followed by chloroform, ethyl acetate, butanol and aqueous.

Antinociceptive assay

Acetic acid induced writhing

Albino mice of either sex (18-22 g body weights) were divided into three groups comprising five animals each. The group I received normal saline (10 ml/kg body weight; negative control) and group II (positive control) were subjected to standard drug (Diclofenac sodium) at 10 mg/kg body weight). Group III were injected plant extracts (100, 200 and 300 mg/kg doses body weight). After thirty minutes of the treatment, the animal received acetic acid (1%) through intra-peritoneal route. The abdominal writhings were initiated 5 minutes after acetic acid injection and counted for the following 10 minutes. Analgesic activity (%) was calculated with the formula given below.

$$\%\ \text{Analgesic effect} = 100 - \frac{\text{No of writhing in tested animals}}{\text{No of writhing in control animals}} \text{X}\ 100$$

Hot plate test (thermal) in mice

For this study, albino mice (18-22 g body weight) of either sex were used. The animals were randomly allotted into 3 groups, each group with five animals. Group I (control) received saline (intraperitoneal (I.P), group II was treated with standard drug (Tramadol, 5mg/kg I.P) and group III was injected with the plant (100, 200, 300mg/kg I.P). The response of the animals was recorded after 30 minutes of the treatment on the hot plate maintained at a temperature of 54.0±0.1°C. Hot plate reaction time (latency to response in seconds) was observed by noting licking, flicking of hind limb or jumping. A cut-off time of 30 seconds was fixed for their initial response.

Disc diffusion susceptibility assay

Antimicrobial activity was determined by disc diffusion susceptibility assay (Bauer *et al.,* 1966). Different bacterial strains used included *Citorbacter freundii* (ATCC # 8090), *Escherichia coli* (ATCC # 25922), *Klebsiella pneumonia* (*Clinical isolates), Bacillus subtilis (Clinical isolates), Pseudomonas aeruginosa* (ATCC # 9721), *Staphylococcus aureus* (ATCC # 6538) and *Xanthomonas compestris* (ATCC # 33913)

Phytochemical analysis

Phytochemical screening of the crude methanolic stem extract and its different fractions was carried out for proteins, alkaloids, carbohydrates, flavanoids, terpenoid (Siddiqui and Ali, 1997), phytosterols, oils, fats, tannins (Iyengar, 1995), saponins and glycoside (Harborne, 1988).

STATISTICAL ANALYSIS

The experiment was replicated thrice and for the analysis of the data, MSTAT computer software was used. ANOVA followed by Dunnett's post hoc analysis was used. For comparison of significant differences, LSD test was used (Steel *et al*., 1997).

RESULTS

Antinociceptive activity of A. pentapomicum by acetic acid induced method

ANOVA and Dunnett's post-hoc analysis revealed that the crude extract from the stem of *A. pentapomicum* at dose level 100,200 and 300 mg/kg body weight decreased writhing significantly ($P<0.01$) at dose depending manner when compared with diclofenac sodium (standard at a dose level of 25 mg/Kg). Highest inhibition was measured at 300mg/kg. Lowest antinociceptive activity was measured at 100mg/kg (fig. 1).

Antinociceptive activity of A. pentapomicum by hot plate method

The antinociceptive effect produced by the crude extract of *A. pentapomicum* was non-significant ($P>0.05$) at the tested doses compared with the standard drug (tramadol at 5 mg/kg) tested by hot plate method. However, maximum non-significant antinociceptive activity was observed at the highest dose of 300 mg/kg after 30 minutes of the treatment compared with other concentrations and the standard drug (fig. 2).

Antibacterial activity

B. subtilis showed that highest growth inhibition of 54.5%, 47% and 39.4% was confirmed by butanol extracted fraction at 3, 2, 1mg/disc respectively (fig. 3). n-hexane fraction was effective against *B. subtilis* at 2 and 3mg/disc concentration only. *C. freundii* was the most resistant showing inhibitory trend at high concentration of 3 mg/disc only (fig. 4). Ethyl acetate was active against *C. freundii* at all concentrations (76% ZI at 3 mg/disc).

Maximum activity of 79% was shown by butanol fraction at 3mg/disc against *E. coli* (fig. 5). Highest activity against *K. pneumonia* was noted by butanol (48%) at 3mg/disc concentration. At lowest concentration, n-hexane was ineffective against *K. pneumonia* while moderate inhibitory activity was exerted by the other extracts (fig. 6). The highest inhibitory activity (94%) was measured by butanol fraction at 3mg/disc (fig. 7). Ethyl acetate fraction was found to possess high inhibitory activity of 79% at 3 mg disc^{-1} respectively.

Maximum zone of inhibition (55%) was measured by ethyl acetate extracts against *S. aureus* at 3mg/ disc. At higher concentrations of 2 and 3mg/disc the same bacterium was also susceptible to n-hexane and water extracts (fig. 8). Suppression in the growth of *X. compestris* was observed by all extracts at the tested concentrations except n-hexane which was effective at 2 and 3 mg/disc concentration only. Maximum growth suppression was measured by ethyl acetate (58.5%) at 3 mg/disc. Moderate inhibitory activity was observed for n-hexane and methanol extracts at same the concentration compared with control (fig. 9).

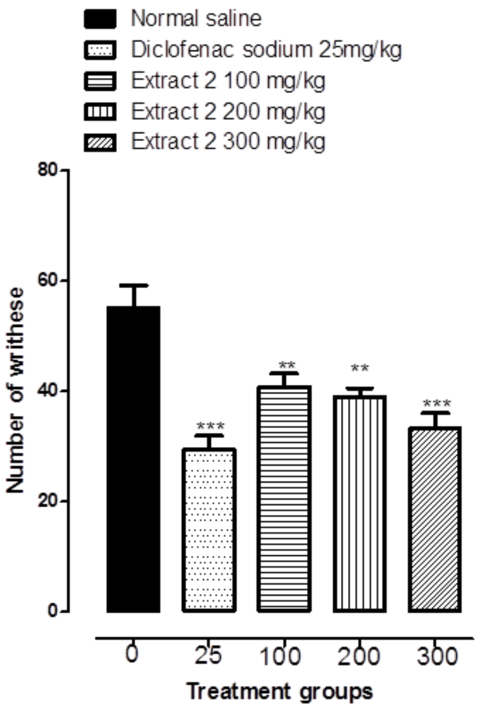

Fig. 1: Antinociceptive activity of stem extract of *A. pentapomicum* and diclofenac sodium (standard) in acetic acid induced writhing test in mice**.** (ANOVA followed by Dunnett's post-hoc analysis, *P<0.05; **P<0.01, ***P<0.01).

Qualitative analysis

Carbohydrates, flavonoids, proteins, amino acids, saponins, tannins and terpenoids were found in abundance in all tested solvent soluble fractions. The alkaloids were either found in moderate quantity in methanol, ethyl acetate, butanol while absent in other fractions (table 1).

DISCUSSION

Crude methanolic extract significantly decreased the writhing of the tested animals compared to the standard drug (diclofenac sodium) in dose depended manner.

Maximum decrease was noted for the highest dose of the crude extract (Coutaux *et al*., 2005). A non-significant antinociceptive effect was also observed at all the tested doses compared with the standard drug (tramadol). Acetic acid induced abdominal constriction is an easy and sensitive procedure commonly employed to measure peripheral analgesic effect (Gupta *et al*., 2005).

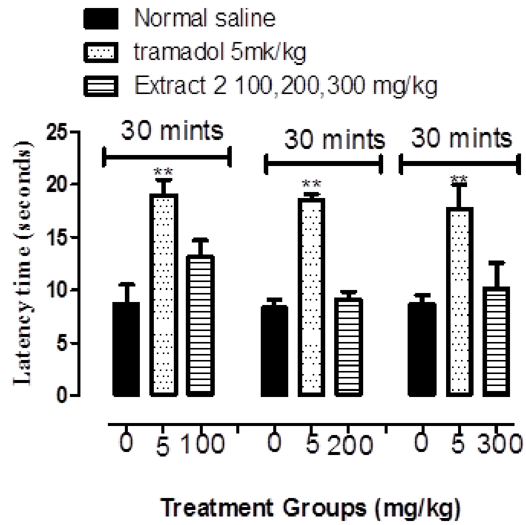

Fig. 2: Antinociceptive activity of stem extract of *A. pentapomicum* and tramadol (standard), in Hot plate test (Thermally induced) in mice**.** (ANOVA followed by Dunnett's post-hoc analysis, *P<0.05; **P<0.01).

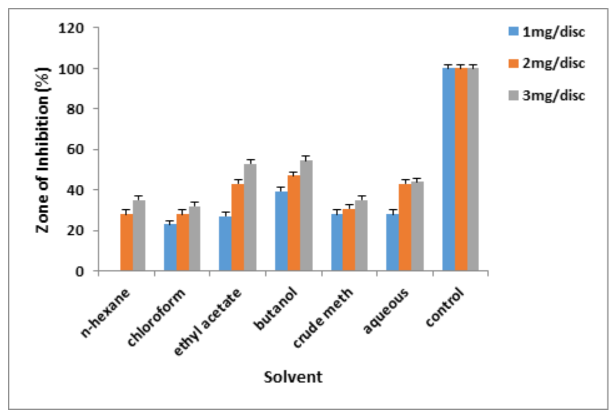

Fig. 3: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *B. subtilis* by disc diffusion assay (Bar represent LSD value at p<0.5).

B. subtilis, C. freundii, E. coli, K. pneumonia, S. aureus and X. compestris were susceptible to n-hexane extracts at higher concentrations showing varying degrees of zones of inhibition and completely resistant at 1 mg/disc concentration.

P. aeruginosa was susceptible to n-hexane extracts at all concentrations. *B. subtilis, E. coli, K. pneumonia, P. aeruginosa*, and *X. compestris* were susceptible to chloroform extract. *S. aureus* was completely resistant to chloroform extract. *C. freundii* showed minor growth at highest concentration only. Highest growth inhibition was measured against *E. coli* and *P. aeruginosa*. Butanol extracts were highly effective against all the tested pathogens and *P. aeruginosa* and *E. coli* were the most susceptible. The growth of *E. coli* was also suppressed by ethanol extracts of *A. palmatum* and *A. ginalla* (Danwu *et*

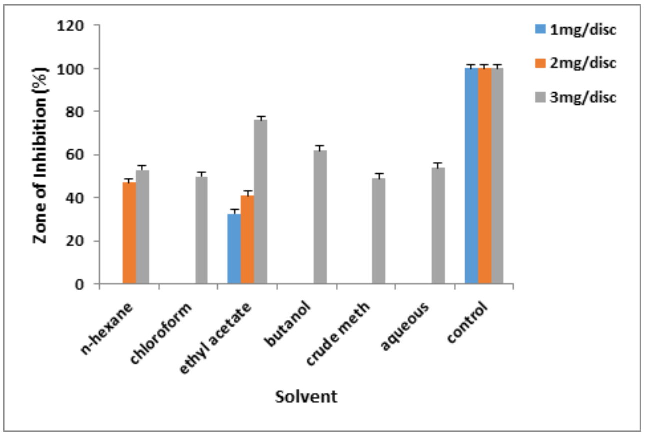

Fig. 4: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *C. freundii* by disc diffusion assay (Bar represent LSD value at p<0.5).

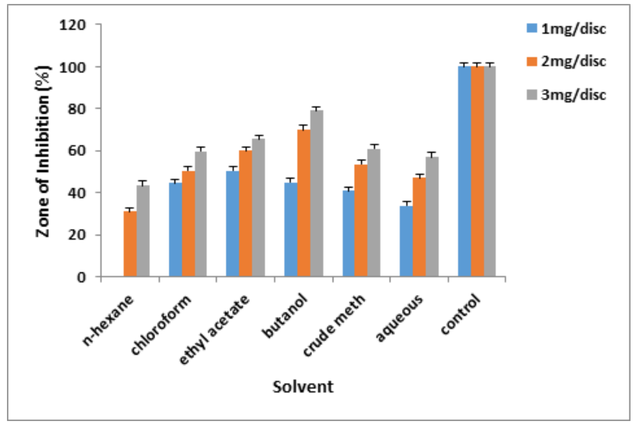

Fig. 5: Antibacterial activity of different solvent extracted samples from stems of *A. pentapomicum* against *E. coli* by disc diffusion assay (Bar represent LSD value at p<0.5).

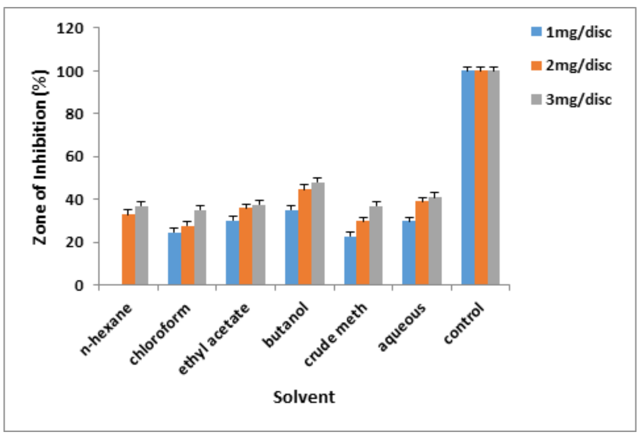

Fig. 6: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *K. pneumonia* by disc diffusion assay (Bar represent LSD value at p<0.5).

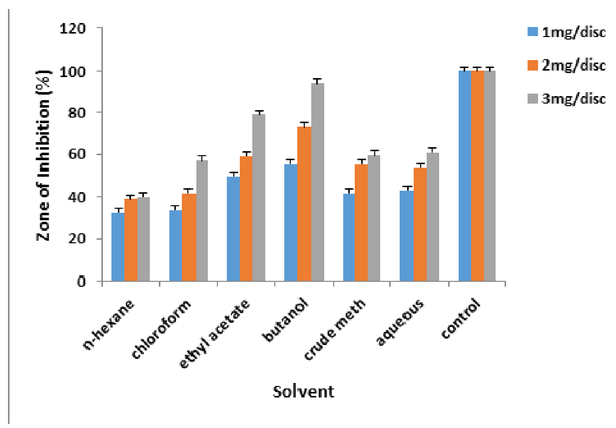

Fig. 7: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *P. aeruginosa* by disc diffusion assay (Bar represent LSD value at p<0.5).

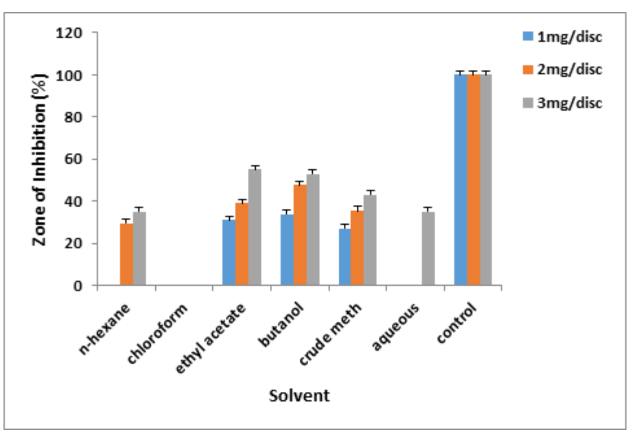

Fig. 8: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *S. aureus* by disc diffusion assay (Bar represent LSD value at p<0.5).

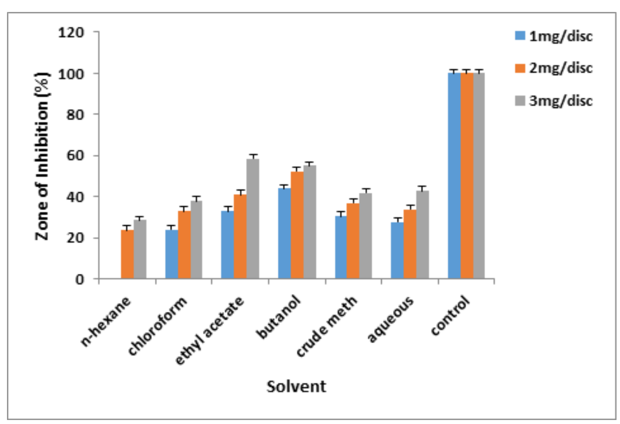

Fig. 9: Antibacterial activity of different solvent extracted samples from stem of *A. pentapomicum* against *X. compestris* by disc diffusion assay (Bar represent LSD value at p<0.5).

Table 1: Preliminary qualitative analysis of different solvent extracted samples from the stem of *A. pentapomicum*

Qualitative Analysis	Crude methanolic	n-hexane	Chloroform	Ethyl acetate	Butanol	Aqueous
Alkaloids	++	-	-	++	A++	-
Carbohydrates	+++	+++	+++	+++	+++	+++
Flavonoids	+++	-	+++	+++	+++	+++
Fixed oil and fats	-	-	++	-	-	++
Proteins and amino acids	+++	-	+++	+++	+++	+++
Saponins	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	+++	+++	+++	+++
Terpenoids	+++	-	+++	+++	+++	+++

+++: shows the presence in abundance
++ : shows presence in moderate quantity
\+ : shows presence but in less amount
__ : shows complete absence of the compound

al., 2010). The growth of *C. freundii* and *S. aureus* was inhibited effectively by methanolic and aqueous extracts at high concentrations. The presence of different phytochemical compounds such as alkaloids, carbohydrates, proteins, tannins, terpenoids, saponin etc. in all solvent extracted samples of *A. pentapomicum* stems was also confirmed (Zhang *et al*., 2008).

CONCLUSION

Stem extracts of *A. pentapomicum* at different concentrations revealed significant antinociceptive activity in a dose-dependent manner. Extracts of *A. pentapomicum* were effective to inhibit the growth of both Gram positive and Gram-negative bacteria at different concentrations. Extracts of *A. pentapomicum* contain medicinally important bio-compounds which could have utility in the pharmaceutical industries.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the Higher Education Commission Islamabad Pakistan

REFERENCES

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by standardized single disk method. *Am. J. Clin. Pathol.*, **45**: 493-496.

Coutaux A, Adam F, Willer JC and Daniel LB (2005). Hyperalgesia and allodynia: Peripheral mechanisms. *Joint Bone Spine*., **72**: 359-371.

Dan Wu, Xiao-Dong Wu, Xue-Fu You, Xiao-Feng Ma and Wei-Xi Tian (2010). Inhibitory effects on bacterial growth and b-Ketoacyl-ACP reductase by different species of Maple leaf extracts and tannic acid. *Phytother. Res*., **24**: S35-S41.

Gupta M, Mazumder U, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti Y, Kakoti BB and Selven VT (2005). Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J. Ethnopharmacol.,* **98**: 267-273.

Harborne JB (1988). Flavonoids in the environment: structure-activity relationships. *Progr. Clin. Biol. Res.,* **280**: 17-27.

Iyengar MA (1995). Study of crude drugs. 8th edition, Manipal Power Press, Manipal, India, p.2.

Mari M, Bertolini P and Pratella GC (2003). Non-conventional methods for the control of post-harvest pear diseases. *J. Appl. Microbiol*., **94**: 761-766.

Mathews HB, Lucier GW and Fisher KD (1999). Medicinal herbs in the United states: Research needs. *Environ. Health Perspect*., **107**: 773-778

Mehboob R (2012). Wild plants of Swat, Pakistan. Department of Botany, Govt. Jehan Zeb College Swat, p.52.

Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavoring agents. *Global J. Pure and Appl. Sci*., **7**: 455-459.

Siddiqui AA and Ali M (1997). Practical pharmaceutical chemistry. 1st edition, CBS Publishers and Distributors, New Delhi, pp.126-131.

Steel RGD, Torrie JH and Dickey DA (1997). Principles and Procedures of Statistics. A Biometrical Approach, 3rd Ed. McGraw Hill Book Co. Inc. New York USA. pp.172-177.

Van gelderen DM, Dejng PC and Oterdoo HJ (1994). Maples of the world. Timber press, Portland, **1**: 15-20.

Zhang F, Luo SY, Ye YB, Zhao WH, Sun XG, Wang ZQ, Li R, Tian WX and Zhang YX (2008). The antibacterial efficacy of an aceraceous plant [Shantung maple (*Acer truncatum* Bunge)] may be related to inhibition of bacterial beta-oxoacyl-acyl carrier protein reductase (FabG). *Biotechnol. Appl. Biochemist*., **51**: 73-78.