Nephroprotective property of *Cinnamomum zeylanicum* and its antibacterial activity in combination with gantamicin

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Abstract: *Cinnamomum zeylanicum* has strong antioxidant properties and has been presented to have nephroprotective effects. Present work was aimed to study the nephroprotective property of the plant extract through urinary enzymes excretion, to confirm its protective effects and to observe the antibacterial activities of gentamicin in combination with the plant extract. 200mg/kg/day of the plant extracts were administered alone and as co-therapy with gentamicin. Urinary lactate dehydrogenase (LDH) and Urinary alkaline phospatase (ALP) excretions were observed through reagents kits with the help of Power-Lab 300. Antibacterial activities were assessed for gentamicin alone and in combination with the extract. Present study showed that the plant extract have excess quantity of flavonoids, which may responsible for attenuating the excessive excretion of urinary LDH. However, Urinary ALP excretion was found remained same throughout the study period in all experimental groups; might be detected in acute damage. Further, the plant also proved to have no decreasing impact on the antibacterial activities of gentamicin.

Keywords: *Cinnamomum zeylanicum,* Lactate dehydrogenase, alkaline phosphatase, antibacterial activity.

INTRODUCTION

Cinnamomum zeylanicum (Lauraceae) is used as spices and preservatives in the food (Yu *et al*., 2007), and because of the sweet taste of bark, it is famous as sweet wood (Willis, 1973). It is commonly used as antioxidant plant (Mancini-Filho *et al*., 1998), and is effective in alleviating the symptoms of several diseases (Ranjbar *et al*., 2006; Senhaji *et al*., 2007). The plant extract is commonly used in the management of dyspepsia and gastritis (Wang *et al*., 2009), body pain, fever, cough, and allergic reaction (Singh *et al*., 2008).

The antimicrobial activity of the plant has also been reported, may due to the essential oil of the bark of the plant (Mastura *et al*., 1999). Further, antifungal activity has also been presented (Mishra *et al*., 2009). The antioxidant potentials of the plant, because of its phenolic contents (Tomaino *et al*., 2005) have useful role against free radical, which is responsible for the damage of cell membrane (Hasani-Ranjbar *et al*., 2009). The plant extract may be used for the healing of wounds (Kamath *et al*., 2003) may because of the stimulation of antioxidant enzymes (Dhuley, 1999).

From the above discussed literature, it can be assumed that *C. zeylanicum* plant have a number of medicinal properties, especially antioxidant which might due their phenolic fillings (Mancini-Filho *et al*., 1998; Ranjbar *et al*., 2006; Tomaino *et al*., 2005; Hasani-Ranjbar *et al*., 2009; Lee and Shibamoto, 2002; Dhuley, 1999). Further, this free radical scavenging properties is responsible for their nephroprotective properties by controlling renal functioning and histological changes associated with gentamicin (Ullah *et al*., 2013). The present study was aimed to confirm the nephro-protective role of *C. zeylanicum* with respect to their effects on urinary enzymes excretion and to explore their impact on the antibacterial activities gentamicin to precede a step towards their clinical implementation.

MATERIALS AND METHODS

Plant material

Sufficient quantity of plant *C. zeylanicum* (3Kg) was purchased after authentication by Professor Umar Farooq, Botanist Government College Abbottabad Pakistan. The voucher specimen (1024) was deposited to the herbarium of the same college.

Extraction

Plant material including bark of *C. zeylanicum* was dried; chopped and powdered. The powdered material was macerated with ethanol for about 20 days with stirring on alternate days. Filtered and evaporated with the help of Rotary evaporator (R-210, Germany) (Bhukya *et al*., 2009).

Preliminary phytochemical screening

The plant extracts were screened for the presence of flavonoids, alkaloids, carbohydrates, glycosides, saponins, tannins and terpenes by using simple qualitative

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method (Sofowora, 1993). Flavonoids were detected in each plant extract by following Shinoda test. Small pieces of magnesium fillings were added to the extract followed by concentrated hydrochloric acid. The appearance of pink or reddish color indicated the presence of flavonoids. For the detection of alkaloids few drops of Drangendorffs reagent and tannic acid solution (10%) was added to each extract separately in two test tubes. The presence of white precipitate in both of the tubes indicated the presence of alkaloids.

Carbohydrates were detected by the addition of few drops of Molisch's reagent and concentrated sulphuric acid to the extract. The appearance of a purple ring showed the presence of carbohydrates. For further confirmation the mixture was shaken and allowed to stand for some time. Formation of precipitate upon addition of water confirms the presence of carbohydrates.

Experimental

Twenty-four rabbits have similar body weight and sex, were maintained on same diet fifteen days priers to the start of experiment after have approved by University Research Committee. Animals were divided into four groups, such as each group contains six rabbits; and were treated according to the dosage regimen given in table 1.

Estimation of urinary alkaline phosphatase (ALP)

Quantitative *in vitro* determination of ALP was performed according to the German Society of Clinical Chemistry (deutsche, 1972). Commercially available kits (DiaSys Diagnostic System, Germany) contained various reagents; di-ethanolamine (1.2mol/l), magnesium chloride (0.6 mmol/l) and p-Nitrophenylphosphate (50mmol/l) was used. Mixed the reagents and noted the absorbance at 405 nm by using Chemistry analyzer (Power Lab 300, Germany). Results were obtained by using the formula; ALP (U/l) = A (sample) ÷ A (calibrator) × concentration of calibrator (U/l)

Estimation of urinary lactate dehydrogenase (LDH)

Quantitative *in vitro* determination of LDH was performed according to German Society of Clinical Chemistry (deutsche, 1972). Commercially available kits (Dia Sys Diagnostic system, Germany) contained various reagents; phosphate buffer (64mmol/l), pyruvate (0.80mmol/l) and NADH (1.0mmol/l) was used. Mixed the reagents and noted the absorbance at 340 nm by using Chemistry analyzer (Power Lab 300, Germany). Results were obtained by using the formula;
LDH (U/l) = A (sample) ÷ A (calibrator) × concentration of calibrator (U/l)

Urinary examination

Urinary examination was performed both by reagents and microscopy. Multiple reagent strips (URS-10) (Teco diagnostic, USA) was used for the examination several urinary parameter. While some of the portion of urine was thoroughly mixed and centrifuged for 5 minutes at 2500 rpm. The supernatant of the sample was discarded and a drop was placed on the slide and covered with the help of cover slip and studied with the help of light microscope (Germany).

Impact of cinnamomum zeylanicum on antibacterial activities of gentamicin

Antibacterial activity of ethanolic extract of *C. zeylanicum* in combination with gentamicin was studied against five pathogenic micro-organisms including *Escherichia coli, Pseudomonas pickettii, Salmonella typhae, Proteus mirabilis* and *Micrococcus luteus*. Agar well diffusion method was used (Tortora *et al*., 2005). Media plates were sterilized with the help of autoclave (Hirayama) and loaded in laminar flow hood under aseptic environment. The bacterial species were cultured and incubated at 37°C for 24 hours. Few colonies were inoculated in Mueller-Hinton Broth and spread over the media plates by using cotton swab. The plates were dried in the incubator (Mammert, Germany) for 20 minutes. Wells were then cut down on Mueller-Hinton Agar by using standard cork borer. 500mg/ml of plant extract and 40mg/ml of gentamicin was used in combination to observe the impact of plant extract on antibacterial activity of gentamicin. Then inoculated plates were incubated at 37°C for 24 hours and the zone of inhibitions were measured by using measuring scale and Colony counter (Suntex 570, Taiwan).

STATISTICAL ANALYSIS

All the results were presented as mean ± SEM and compared with gentamicin treated animals by using one way analysis of variance (ANOVA) followed by Dunnett test with the help of Graph Pad Prism (version-5). Probability, *P*-value less than 0.05 were considered to be statistically significant.

RESULTS

Phytochemical analysis

Qualitative phytochemical study of *C. zeylanicum* revealed the presence of large quantity of Flavonoids, moderate quantity of carbohydrates, Tannins, Terpenes and Glycosides, and mild quantity of Saponins. However, no detectable amount of alkaloids was observed.

Measurement of urinary LDH excretion

Significant rise in the urinary excretion of LDH in group G, as 143.17±3.53 U/l in the mid of experimental period in comparison with group C as 91.33±1.86 U/l which was decreased to 103.17±4.28 U/l on last day of study period but was still significantly different from group C as 88.17 ±2.24 U/l. Group GCze (98±5.37 U/l) and group Cze (87 ±6.62 U/l) were found significantly different from group G in the mid, but group GCze on last day (98±3.27 U/l) was observed not statistically different from group G, as given in table 2.

Table 1: Dosage schedule for a period of three weeks experimental period

S. No.	Animals Group	Treatments
1	C	2ml/kg 0.9% saline solution
2	G	80mg/kg gentamicin
3	GCze	80mg/kg gentamicin and 200mg/kg *C. zeylanicum*
4	Cze	200mg/kg *C. zeylanicum*

Table 2: Urinary enzymes excretion

	Lactate dehydrogenase (U/l)			Alkaline phosphatase (U/l)		
Group	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
C	91.17±1.58	91.33±1.86***	88.17±2.24*	12.6±1.08	14.2±0.92	14.1±1.29
G	86±2.92	143.17±3.53	103.17±4.28	11.9±1.20	11.9±1.15	12.4±0.67
GC-ze	93±5.93	98±5.37***	98±3.27	12.1±0.78	13±0.75	13.3±0.91
C-ze	91±6.13	87±6.62***	82±6.06*	12±0.85	11.6±0.69	12.2±0.58

Results were expressed as Mean ± SEM, *** was considered Extremely significant, ** Very significant and * Significant

Table 3: Microscopic Examination of Urine on last day of study period

Group	RBC	Leukocytes	Epithelial cells	Casts	Crystal	Yeast cells	Amorphous phosphate
C	10	15	15	Nil	Nil	Nil	25
G	55**	45*	25	65***	25	05	75***
GC-ze	Nil	20	10	25	Nil	Nil	35*
C-ze	Nil	10	10	20	Nil	Nil	30*

Mild amount (+) was denoted with 5 marks for each animal, Moderate amount (++) was denoted with 10 marks for each animal, Excess amount (+++) was denoted with 15 marks for each animal. 30-45 marks were considered significant (*), 46-60 marks were considered very significant (**), 61-90 marks were considered extremely significant (***)

Measurement of urinary ALP

No significant change in ALP was observed in group G and all other treated groups including group C throughout experimental duration as shown in table 2.

Microscopic examination of urine: Group G animals showed significant amount of RBC, WBC, and casts, Significantly different from group C animals. Further group GCze and Cze animals were found to have no significant amount of such abnormalities as given in table 3.

Impact of C. zeylanicum on the antibacterial activities of gentamicin

The zone of inhibition of combine treatment of gentamicin and *C. zeylanicum* against *S. typhae* was 34mm while for gentamicin alone it was 31mm (fig. 1A and B). The zone of inhibition measured for gentamicin alone against *E. coli* was 20mm while their combined treatment showed 26mm zone of inhibition (fig. 2A and B).

The zone of inhibition of gentamicin against *P. pickettii* was noted as 34mm while their combined treatment showed 42mm (fig. 3A and B). The zone of inhibition of gentamicin against *P. mirabilis* was found 15mm while the combined treatment of gentamicin and *C. zeylanicum*, 23mm zone of inhibition was recorded (fig. 4A and B). Further, gentamicin showed 34mm, zone of inhibition against *M. luteus* while their combined treatment showed 44mm zone of inhibition against *M. luteus* (fig. 5A and B).

DISCUSSION

In the current study, *C. zeylanicum* was studied against renal damage induced by toxic doses of gentamicin with Respected to changes associated in urinary enzyme excretions. Gentamicin has beenreported with dose dependent nephrotoxic effects (Bennett *et al*., 1991).
Gentamicin has been used in various high doses to induce renal damage as in the current study daily dose of 80 mg/kg was employed to induce toxicity (Gilbert *et al*., 1989). Significant rise in serum creatinine, BUN and uric acid with a decrease in creatinine clearance was reported as an indicator for renal damage caused by gentamicin (Ullah *et al*., 2013). However, in the present work, leaking of urinary enzymes was taken as renal functioning parameters.

Extremely significant increase in the LDH excretion was observed in gentamicin treated animals on day 11 of experimental period, which was decreased on day 21. Some of the researchers used the estimation of tubular brush border enzyme including LDH to investigate the renal function (Langhendries *et al*., 1988) which may be

secreted due to ruptured cells. The detection of LDH in the urine has been reported due to proximal tubular injury (Frances, 1998). The rupturing of cell membrane and proximal tubular damage which is responsible for the leaking of cytosolic LDH may due to the activation of reactive oxygen species. Therefore animals treated with simultaneous administration of *C. zeylanicum* proved to control significant rise in urinary LDH by scavenging free radicals. Significant increase in the excretion of LDH has been reported as observed in the present study for gentamicin (Abd El-Fattah *et al*., 2012). Most importantly it has also been documented that some time excretion of enzymes increases for short period of time in renal damage as observed in the present findings. In the current study, urinary LDH increased on day 11 of study period followed by a decrease till day 21, showed that estimation of LDH is needful for early detection of nephrotoxicity (Frances, 1998).

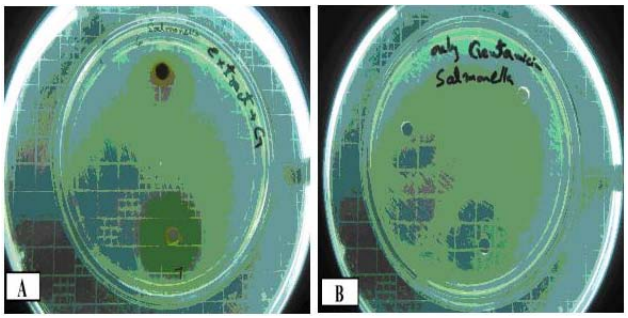

Fig. 1: (A) Zone of inhibition measured against *S. typhae* for gentamicin in combination with *C. zeylanicum* as 6 shown 34mm and (B) Zone of inhibition shown by gentamicin against *S. typhae* as 31mm

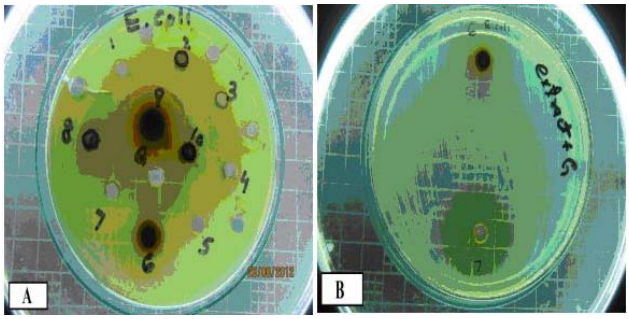

Fig. 2: (A) Zone of inhibition of gentamicin as G; shown 20mm of zone of inhibition and (B) Zone of inhibition measured against *E. coli* for gentamicin in combination with *C. zeylanicum* as 6 shown 26mm

Urinary ALP excretion was found statistically same in all studied groups both on day 11 and 21 of study period. However, previous reports showed significant alteration in alkaline phosphatase of gentamicin treated animals, showed acute renal damage (Knauss *et al*., 1983). ALP excretion is proposed to related with the acute proximal tubular necrosis which returns to normal level due to the repairing of renal damage; similarly in the current study due to prolong treatment of gentamicin the chronic renal damage has been established in which the ALP level was remained same, correlated with previous reports (Heiene *et al*., 1991). It has been reported that only acute renal damage is associated with the significant rise of urinary ALP; however, no increase in urinary ALP was observed in chronic renal damage (Heiene *et al*., 1991). Urinary excretion of enzymes has been reported to have potentials of determining the site of kidney damage because each site of nephron has a quality complement of enzymes. The role of antioxidant was not fully established because; due to chronic renal damage ALP was remained same in all studied groups. In contrast ALP excretion was also reported with a significant decline in gentamicin treated animals (Abd El-Fattah *et al*., 2012), may due to early detection of urinary ALP. It has also been reported that estimation of urinary enzymes provides information regards the progress and revival of nephrotoxicity by varying the concentration of enzymes with the severity of toxicity (Frances, 1998).

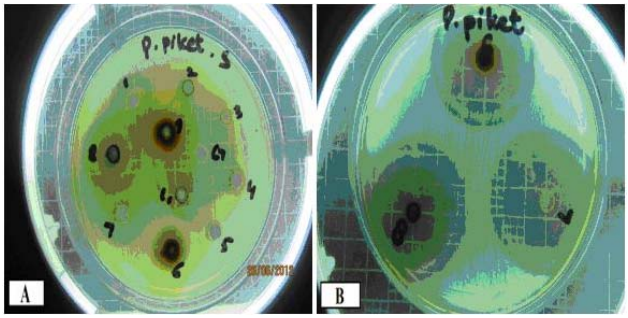

Fig. 3: (A) Zone of inhibition of gentamicin as G measured 34mm and (B) Zone of inhibition measured against *P. pickettii* for gentamicin in combination with *C. zeylanicum* as 6 shown 42mm

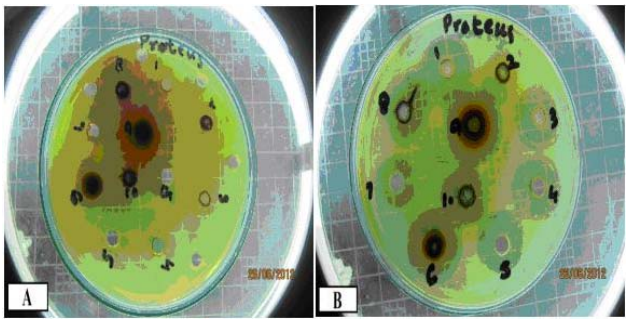

Fig. 4: (A) Zone of inhibition of gentamicin as G showed 15mm against *P. mirabilis* and (B) Zone of inhibition against *P. mirabilis* for gentamicin in combination with *C. zeylanicum* as 6 shown 23mm

The urinary examination revealed, significant amounts of leukocytes in gentamicin treated group showed the presence of infection or renal diseases, accompanied with moderate amount of red blood cells which may because of inflammation of kidney, ureter, bladder or urethra. Further, excess quantity of renal casts both hyaline and granular and amorphous phosphate was also observed on gentamicin treated animals significantly different from

control group animals, which are responsible for tubular damage. Further, animals treated with *C. zeylanicum* proved highly effective in combating renal toxicity associated with gentamicin due to the absence of urinary abnormalities.

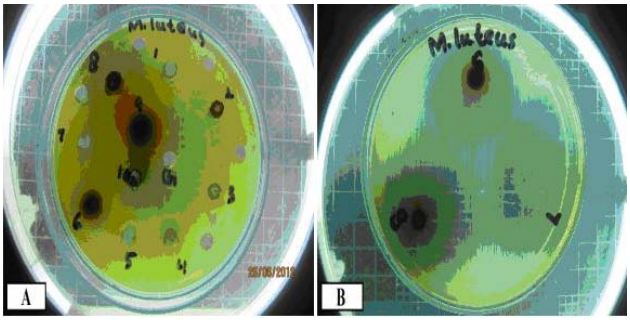

Fig. 5: (A) Zone of inhibition measured against *M. luteus* for gentamicin as G, shown 34mm and (B) Zone of inhibition measured against *M. luteus* for gentamicin in combination with *C. zeylanicum* as 6 shown 44mm

The antibacterial screening was performed against five bacterial pathogens including *S. typhi, E. coli, P. pickettii, P. mirabilis* and *M. luteus* to explore their impact on the antibacterial activities of gentamicin assessed by the presence or absence of inhibition zones. The possible mechanism for increase in the in vitro antibacterial activities of gentamicin in combination with *C. zeylanicum* extract; might, gentamicin triggers the antibacterial activities of these plants or these plants might be responsible to synergize the antibacterial activities of gentamicin.

The essential oil obtained from the bark *C. zeylanicum* has large quantity of cinnamaldehyde, which proved to have strong antimicrobial properties (Mastura *et al*., 1999), as exhibited in current study against *P. pickettii* and *M. luteus*. However, in contrast *C. zelyanicum* did not show any inhibitory activity against *S. typhae*, *E. coli* and *P. mirabilis*. Further, *C. zeylanicum* showed to have no decreasing effect on antibacterial activities of gentamicin.

CONCLUSION

The plant extract (*C. zeylanicum*) successfully proved to have renal protective properties clarified by attenuating the excessive excretion of urinary enzymes. Furthermore, it was also confirmed that *C. zeylanicum* have no significant decreasing impact on the antibacterial activities of gentamicin against *S. typhi, E. coli, P. pickettii, P. mirabilis* and *M. luteus*.

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REFERENCES

Abd El-Fattah HM and El-Sheikh NM (2012). Evaluation of chemo protective role of N-acetylcysteine and vitamin E on Gentamicin Induced Nephrotoxicity. *Aust. J. Basic Appl. Sci*., **6**: 263-270.

Bennett WM, Elzinga LW and Porter GA (1991). Tubulo-interstitial disease and toxic nephropathy. *In*: Brenner BM, Rector FC editors. The Kidney, 4th ed., WB Saunders Company, Philadelphia, pp.1430-1496.

Bhukya B, Anreddy RNR, William CM and Gottumukkala KM (2009). Analgesic and anti-inflammatory activities of leaf extract of *Kydia calycina* Roxb. *Bangladesh J. Pharmacol*., **4**: 101-104.

Deutsche GKC (1972). Standardisierung von Methoden zur Bestimmung von Enzymaktivitaten in biologischen flussigkeiten (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids) *Z. klin. Chem. Klin. Biochem.*, **10**: 182-192.

Dhuley JN (1999). Antioxidant effects of cinnamon (*Cinnamomum verum*) bark and greater cardamom (*Amomum subulatum*) seeds in rats fed high fat diet. *Indian J. Exp. Biol*., **37**: 238-242.

Frances ASC (1998). Urinary enzyme evaluation of nephrotoxicity in the dog. *Toxicol. Pathol*., **26**: 29-32.

Gilbert DN, Wood CA, Kohlhepp SJ, Kohnen PW, Houghton DC, Finkbeiner HC, Lindsley J and Bennett WM (1989). Polyaspartic acid prevents experimental aminoglycoside nephrotoxicity. *J. Infect. Dis*., **159**: 945-953.

Hasani-Ranjbar S, Larijani B and Abdollahi M (2009). A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. *Inflamm. Allergy Drug Targets*, **8**: 2-10.

Heiene R, Biewenga WJ and Koeman JP (1991). Urinary alkaline phosphatase and gamma glutamyl transferase as indicators of acute renal damage in dogs. *Journal of Small Animal Practice*, **32**: 521-524.

Kamath JV, Rana AC and Chowdhury AR (2003). Pro-healing effect of *C. zeylanicum* bark. *Phytother. Res*., **17**: 970-972.

Knauss CT, Weinberg JW and Hume HD (1983). Alteration in renal cortical phospholipid contents induced by gentamicin in time and course specificity and sub cellular localization. *Am. J. Physiol*., **244**: 535-546.

Langhendries JP, Battisti O and Bertrand JM (1988). Amino glycoside nephrotoxicity and urinary excretion of NAG. *Biol. Neonate*., **53**: 253-259.

Lee KG and Shibamoto T (2002). Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J. Agric. Food Chem*., **50**: 4947-4952.

Mancini-Filho J, Van-Koiij A, Mancini DA, Cozzolino FF and Torres RP (1998). Antioxidant activity of cinnamon

(*C. zeylanicum*, Breyne) extracts. *Boll. Chim. Farm*., **37**: 443-447.

Mastura M, Nor Azah MA, Khozirah S, Mawardi R and Manaf AA (1999). Anticandidal and antidermatophytic activity of *Cinnamomum* species essential oils. *Cytobios.*, **98**: 17-23.

Mishra AK, Mishra A, Kehri HK, Sharma B and Pandey AK (2009). Inhibitory activity of Indian spice plant *C. zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata*, the pathogenic dematiaceous moulds. *Ann. Clin. Microbiol. Antimicrob*., **8**: 9.

Ranjbar A, Ghasmeinezhad S, Zamani H, Malekirad AA, Baiaty A, Mohammadirad A and Abdollahi M (2006). Antioxidative stress potential of *C. zeylanicum* in humans: a comparative cross-sectional clinical study. *Therapy*, **3**: 113-117.

Senhaji O, Faid M and Kalalou I (2007). Inactivation of *Escherichia coli* O157: H7 by essential oil from *C. zeylanicum*. *Braz. J. Infect. Dis*., **11**: 234-236.

Singh G, Kapoor IP, Singh P, De Heluani CS, De Lampasona MP and Catalan CA (2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food Chem. Toxicol*., **46**: 3295-3302.

Sofowora EA (1993). Phytochemical Assays. In: Medicinal Plants and Traditional Medicine in Africa. 3rd ed., Spectrum Books Limited, Nigeria, pp.150-153.

Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pusquale A and Saija A (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chem.*, **89**: 549-554.

Tortora GJ, Funke BR and Case GL (2005). Microbiology, An introduction. 7th ed., pp. 533.

Ullah N, Khan MA, Khan T and Ahmad W (2013). Bioactive traditional plant *Cinnamomum zeylanicum* successfully combat against nephrotoxic effects of amino glycosides. *Bangladesh J. Pharmacol*., **8**: 15-21.

Wang R, Wang R and Yang B (2009). Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innov. Food Sci. Emerg. Technol*., **10**: 289-292.

Willis JK (1973). A dictionary of the flowering plants and ferns. 8th ed., Cambridge University Press, Cambridge.

Yu HS, Lee SY and Jang CG (2007). Involvement of 5-HT1A and GABAA receptors in the anxiolytic-like effects of *Cinnamomum cassia* in mice. *Pharmacol. Biochem. Behav*., **87**: 164-170.