Research Journal of Biological Sciences 6 (6): 275-280, 2011
ISSN: 1815-8846

Anti-Bacterial and Toxicological Assessment of *Lawsonia innermis* Linn. (Henna) Leaves on Rats

[1]Emad M. Abdallah and [2]Mona A. Aldamegh
[1]Department of Science, College of Science and Arts, Al-Rass,
P.O. Box 53, Qassim University, Saudi Arabia
[2]Department of Biology, College of Science and Arts, Onaizah Qassim University, Saudi Arabia

Abstract: The anti-microbial activity of methanol extracts of the leaves of Henna (20 and 40 mg mL^{-1}) was investigated against four pathogenic clinical isolates, namely; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. *S. aureus* was the most susceptible bacteria followed by *Pseudomonas aeruginosa* and *Escherichia coli*. While *Klebsiella pneumoniae* was the least susceptible micro-organism. The inhibition zone was correlated with the plant extract concentration. Chloramphincol (5 mg mL^{-1}) was used as standard anti-biotic. The MIC of the tested organisms at 40 mg mL^{-1} of Henna leaves methanol extract were ranged between 2.5-20 mg mL^{-1}, correlated with the inhibition zones. The toxicological effects of aqueous suspensions of Henna administered to males of Wister albino rats were evaluated using 6 weeks (42 days) treatment. Mean body weights, hematological and biochemical analysis were evaluated. The treatment did not produce any mortality and no significant changes in body weight observed with doses 200 and 1000 mg/kg/day compared to the control group. Blood parameters showed no significant clinical changes either except some variations in mean counts of WBC, RBC and PLT. Biochemical parameters of rat's serum revealed some significant differences. BUN, CRE, GLU and T. LIP for the test groups were in normal values and found statistically non-significant while ALT, AST, ALP, URIC, T. PROT and ALB were significantly decreased, particularly at dose 1000 mg/kg/day. Here, more investigations on sero-biochemical parameters are required. However, consumption of Henna leaves at least at level 200 mg/kg/day could be of no serious safety concern.

Key words: Lawsonia innermis, henna, agar well diffusion, MIC, anti-bacterial, toxicity, rats, hematological and biochemical parameters

INTRODUCTION

Now-a-days, the global interest is changing towards the use of non-toxic plant products of traditional medicinal application. Natural products derived from medicinal plants represent >50% of all the drugs in clinical use in the world. Higher plants contribute no <25% of the total. During the last 40 years at least a dozen potent drugs have been derived from flowering plants (Gurib-Fakim, 2006).

Lawsonia innermis linn. (Henna) is the sole species in the genus Lawsonia which belonging to family Lythraceae. It is a biennial dicotyledonous herbaceous shrub (Chaudhary *et al*., 2010). Henna is a potential and popular medicinal plant, it has been used in cosmetics and medicine for >9,000 years particularly in the Middle East, India and Pakistan. All Henna parts, leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments (Chaudhary *et al*., 2010; Abdelgadir *et al*., 2010). It was found to have anti-diabetic activity (Symsudin and Winarno, 2008; Arayne *et al*., 2007), immunomodulatory effect (Mikhaeil *et al*., 2004), hepatoprotective activity (Hemalatha *et al*., 2004), anti-oxidant effect (Endrini *et al*., 2007), anti-bacterial activity (Ali *et al*., 2001), anti-fungal activity (Singh and Pandley, 1989), anti-viral activity (Khan *et al*., 1991), anti-trypanosomal (Wurochekke *et al*., 2004), anti-parasitic activity (Okpekon *et al*., 2004) and anti-dermatophytic activity (Natarajan *et al*., 2000).

The principal colouring matter of Henna is lawsone, 2-hydroxy-1:4 napthaquinone. Besides Lawsone, other constituents present are gallic acid, glucose, mannitol, fats, resin (2%), mucilage and traces of alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol (Chaudhary *et al*., 2010). For medicinal plants to be used alongside modern medicine,

Corresponding Author: Emad M. Abdallah, Department of Science, College of Science and Arts, Al-Rass, P.O. Box 53, Qassim University, Saudi Arabia

careful safety and toxicological standardization must be instituted (Verpoorte, 2005). Relatively few medicinal herbs have been investigated in clinical trials or post marketing surveillance therefore, information on adverse effects are mostly depends on spontaneous or anecdotal reports (Mills and Bone, 2005). Although, Henna is a famous medicinal plant of numerous applications and employed in traditional medicine in many parts of the world, scanty pharmacological studies have been reported about Henna cultivated in Al-Madinah district, Saudi Arabia. The aim of this study was to evaluate the anti-bacterial activity *Lawsonia innermis* leaves.

MATERIALS AND METHODS

Plant material and sampling: Leaves of *Lawsonia innermis* (Henna) were collected from Al-Madinah Al-Munawarah district, it was authenticated by G.T. B. El-Ghazali (Taxonomist), College of Science and Arts, Qassim University (Voucher herbarium specimen no.: MEDP 26). Leaves was washed with distilled water, dried in shade and crushed into fine powder using crushing machine. The plant powder was then kept in dark well tighten bottle for further investigation.

For anti-bacterial tests, plant powder was macerated in 95% methanol for 3 days and filtered twice. The filtrate was pooled and evaporated to dryness under reduced pressure on a rotary evaporator (337 mbar at 40°C) yielded 29.1%. For toxicological study, an aqueous suspensions from Henna powder was made according to the required doses (simulating traditional medicine prescription).

Anti-bacterial activity test: Four pathogenic clinical isolates (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) were provided from Al-Rass General Hospital, Department of Microbiology. Agar Well Diffusion Method was employed as described by El-Mahmood *et al*. (2010) with minor modification.

Briefly, 1 mL of fresh nutrient broth culture (18 h) was adjusted to 0.5 McFarland standard corresponding to approximately 1.0×10^{8} cfu mL^{-1} and loaded into sterile petri dish then 19 mL of sterile nutrient agar at 40°C was added. Plate was set to solidify. Four wells were punched on the agar plate using sterile 9 mm diameter cork borer, 0.5 mL of each concentration of *Lawsonia innermis* methanolic extract (20 and 40 mg mL^{-1}) was loaded into the first and second holes, 0.5 mL of 5 mg mL^{-1} of chloramphenicol solution was loaded into the third hole (as positive control) and 0.5 mL of pure methanol solvent was loaded into the fourth hole (as negative control). Plate was incubated for 24 h at 37°C. Test was repeated 3 times and mean zone of inhibition was recorded.

Minimum Inhibitory Concentration (MIC): The MIC of Henna was determined using doubling dilution as described by El-Mahmood *et al*. (2010) with minor modification. Briefly, 1 mL of Henna methanol extract from the concentration which showed highest inhibition zone (40 mg mL^{-1}) was added to 1 mL of Nutrient broth in a test tube.

Then, 2-folds serial dilutions were made, giving extract concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg mL^{-1}. About 1 mL of bacteria (adjusted to 0.5 McFarland) was loaded to each concentration. To another two test tubes containing nutrient broth, 1 mL of pure solvent was loaded to the first tube to serve as negative control and 1 mL of 5 mg mL^{-1} of chloramphenicol was loaded to the second tube to serve as positive control. MIC is recorded as The test tube with the lowest concentration of the extract that did not show any detectable growth.

Experimental design: Experiments were performed on Wister albino males (*Rattus norvegicus*) of 120-200 g body weight and of 2-3 months old. Rats were supplied from the Animal House, Faculty of Pharmacology, King Saud University, Saudi Arabia. The experimental protocols were approved by the Animal Care and Use Committee and were according to the recommendations in King Saud University Guide for the Care and Use of Laboratory Animals. Rats were housed in a well ventilated hygienic laboratory under constant environmental conditions. Rats were kept in cages and soft wood shavings were employed as bedding in the cages. The animals given free access to food and drinking water *ad libitum* throughout the experiment at natural day night cycle.

Treatment: Rats were divided into ten individuals for 3 groups. Group 1 is control group (no treatment), group 2 received Henna solution (200 mg/kg/day), group 3 received Henna solution (1000 mg/kg/day). Rat groups were administered the above mentioned doses for 6 weeks (42 days). Clinical signs were observed at least once a day through the 42 days of dosing. Body weights were measured once a week.

Hematological analysis: Immediately after dissection, blood samples were collected by direct heart puncture using BD Vacutainer System (Belliver Industrial Estate, UK) in EDTA and analyzed for White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Platelets (PLT), Neutrophils (Neut), Lymphocytes (Lymp), Monocytes (Mono), Basophils (Baso) and Eosinophils (Eos) using Coulter counter (Electronic Ltd., USA).

Sero-biochemical analysis: The collected blood (without anti-coagulant) was centrifuged at 3000 rpm for 5 min and the supernatant (serum) was collected and analyzed for the activities of Alanine aminotransferase (ALT), Asparate aminotransferase (AST), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN), Creatinine (CRE), Uric acid (URIC), Glucose (GLU), Total Protein (T. PROT), Albumin (ALB) and Total lipids (T. LIP) using Chemistry automatic analyzer 911 with the appropriate reagents purchased from Hitachi Ltd., Japan.

Statistical analysis: The group data are expressed as mean±SEM (Standard Error of the Mean). SPSS (Statistical Package for Social Science) for windows (Ver. 11.5) was applied for the Analysis of data. One way Analysis of Variance (ANOVA) was used to determine significance between treated groups and control. Students t-test was performed to compare the difference among means. $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Lawsonia innermis (Henna) is a popular multipurpose plant cultivated and employed in Saudi traditional medicine for treatment of various disorders. Its applications in treating wounds and stomach ache inspired studying its anti-bacterial activity. In this study, methanol was used as solvent because it is a better solvent for more consistent extraction of anti-microbial substances from medicinal plants compared to other solvents (Karaman *et al*., 2003).

The results of the anti-bacterial activity of Henna leaves methanol extracts (20 and 40 mg mL^{-1}) are shown in Table 1 and Fig. 1. Generally, inhibition zone was correlated with the plant extract concentration. As to Philip *et al*. (2009), an inhibition zone of 14 mm or greater was considered as high anti-bacterial activity. Accordingly, the anti-bacterial test results showed that the most obvious activities with inhibition zone >14 mm was recorded by the concentration 40 mg mL^{-1} where the highest susceptible bacteria was *Staphylococcus aureus* (22.5±0.7 mm) followed by *Pseudomonas aeruginosa* (19.6±0.6 mm) and *Escherichia coli* (18.3±0.8 mm). While, *Klebsiella pneumoniae* was the least susceptible microorganism towards the methanol extract of Henna (12.8±0.1). The standard anti-biotic chloramphincol (5 mg mL^{-1}) had the highest zone of inhibition. This is due to its presence in a pure form while the impurity of methanol extract may affect on the zone of inhibition. However, methanol extracts from some plants (e.g., *Commiphora molmol*) showed anti-bacterial activity higher than penicillin (Abdallah *et al*., 2009a).

Though, further investigations using fractionation and separation of the active compounds of the methanol extracts of Henna are required. Generally, results of this investigation is in harmony with some previous studies which reported that Henna (from different localities in the world) has anti-bacterial activities against some pathogens at different concentrations (Dama *et al*., 1998; Ali *et al*., 2001; Habbal *et al*., 2005; Nayak *et al*., 2007).

The MIC values obtained in this study from methanol extract of Henna ranged from 2.5-20 mg mL^{-1} (Table 2). It is known that very low MIC values signal high anti-bacterial activity (Rios *et al*., 1988). According to MIC results, maximum anti-bacterial activity amongst methanol extract of Henna was shown by *S. aureus* (2.5 mg mL^{-1}) followed by *E. coli* and *Ps. aeruginosa* (5 mg mL^{-1}) while the least one was *K. pneumoniae* (20 mg mL^{-1}) these are confirming the results of Agar well diffusion test.

The toxicological study of medicinal plants is of great interest because there is still a lack of reliable information on the safety of most medicinal plants (Ernst, 1998). In general, little is known about toxicity of Henna, particularly Saudi Henna. However, Henna is recommended as a prospective source of natural metabolites which could be effective drug against heavy metal toxicity (Guha *et al*., 2009).

Table 1: Anti-bacterial activity of Lawsonia innermis methanol extract*

	Methanol extract (mg mL^{-1})		
Micro-organisms	20	40	Chloramphincol 5 mg mL^{-1}
S. aureus	17.6±0.3	22.5±0.7	35.3±0.6
E. coli	15.6±0.6	18.3±0.8	32.6±0.3
K. pneumoniae	11.1±0.1	12.8±0.1	29.3±0.3
Ps. Aerugenosa	17.0±0.0	19.6±0.6	31.6±1.6

*Mean of 3 replicates±SEM

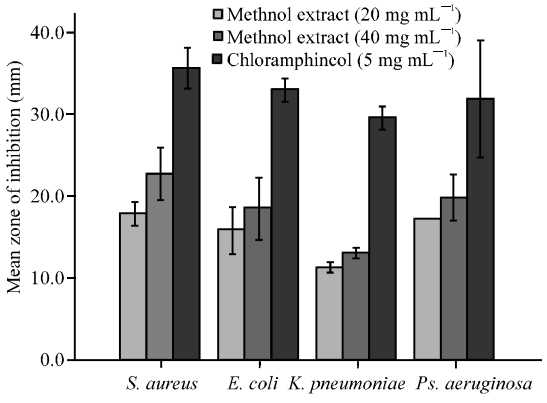

Fig. 1: Anti-bacterial activity of *Lawsonia innermis* methanol extract

Table 2: Minimum Inhibitory Concentration (MIC) of *Lawsonia innermis* leaves

Micro-organisms	MIC (mg mL^{-1})
S. aureus	2.5
E. coli	5.0
K. pneumoniae	20.0
Ps. aerugenosa	5.0

Toxicological results of this investigation on Henna leaves revealed that no deaths among tested rat groups observed and no significant clinically relevant changes were observed in general behavior of rats understudy. As shown in Table 3 and Fig. 2, there were no significant differences in mean body weight for all rat groups administered Henna leaves suspension compared to the control. Normal body weighting is a good indicator for non-toxicity and safety of plant (Abdallah *et al*., 2009b). Hematological analysis of rat groups treated with Henna suspensions are shown in Table 4. In general, results showed no significant ($p>0.05$) clinical changes in blood parameters of rats either at dose 200 mg/kg/day or at dose 1000 mg/kg/day except some variations in mean counts of WBC, RBC and PLT.

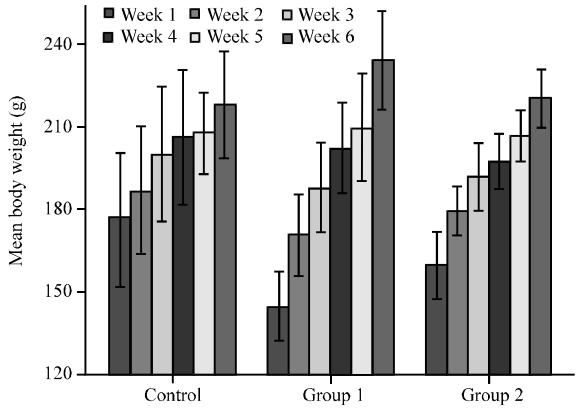

Fig. 2: Mean body weight (in grams) of rats after 42 days treatment with *Lawsonia innermis*

Mean WBC was significantly decreased only at dose 200 mg/kg/day, this symptom may be temporary even though, the mean WBC at dose 1000 mg/kg/day was statistically non-significant. Mean RBC was within the normal values as compared to control at the low dose (200 mg/kg/day) but it was significantly ($p>0.05$) decreased at the high dose (1000 mg/kg/day). This may be related to the high dose treatment which might exist some kind of inhibition for the active ingredients of Henna on hematopoiesis. It is known that a decrease in RBC counts is an indication of anemia (Cheesbrough, 2004). The mean PLT counts were significantly increased at doses 200 and 1000 mg/kg/day when compared to the control, this is considered as thrombocytopenia, increase in PLT was found to be associated with drug exposure, e.g., cimetidine (Guida *et al*., 2003).

As shown in Table 5, the biochemical parameters of rat's serum for the groups administered suspensions of Henna leaves (200 and 1000 mg/kg/day) revealed some significant differences for some parameters compared to the control group. The parameters of BUN, CRE, GLU and T. LIP for the test groups were in normal values and found statistically non-significant when compared to control. The normal values of BUN and CRE of tested groups indicating that there is no toxic effect of Henna on kidney functions at doses 200 and 1000 mg/kg/day.

Kidney functions are evaluating by means of serum urea and creatinine levels (Wingard *et al*., 2000). The normal levels of serum glucose indicating that Henna leaves does not affect carbohydrates metabolism (Konan *et al*., 2007).

Table 3: Mean body weight (g)* of rats after 42 days treatment with lawsonia innermis

Treated groups	Weeks					
(mg /kg/day)	1	2	3	4	5	6
Control	177.0±10.8	187.3±10.8	200.6±11.0	207.1±11.0	208.4±6.6	218.8±8.6
Group 1	144.7±5.60	171.1±6.60	188.5±7.30	202.9±7.40	210.5±8.7	235.1±7.9
Group 2	159.8±5.40	179.6±4.00	192.4±5.50	198.0±4.40	207.4±4.2	221.3±4.7

*Mean of 10 individuals±SE of mean

Table 4: Hematological analysis of rat groups after 6 weeks treatment*

Treated groups (mg/kg/day)	WBC ($\times10^3$ µL^{-1})	RBC ($\times10^6$ µL^{-1})	HGB (g dL^{-1})	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})	PLT ($\times10^3$ µL^{-1})	Neut ($\times10^3$ µL^{-1})	Lymp ($\times10^3$ µL^{-1})	Mono ($\times10^3$ µL^{-1})	Eos ($\times10^3$ µL^{-1})	Baso ($\times10^3$µL^{-1})
Control (no treatment)	12.1±0.4	9.2±0.3	13.3±0.3	45.8±1.9	49.9±0.3	20.8±0.4	29.3±0.1	502.8±51.3	17.5±1.0	79.5±0.9	2.0±0.2	1.0±0.0	0.0±0.0
Henna (200)	6.9±1.5	8.9±0.2	12.8±0.2	42.8±0.5	49.6±0.3	20.9±0.4	29.5±0.4	805.6±12.3	20.8±1.9	76.6±1.9	1.4±0.2	1.2±0.2	0.0±0.0
Henna (1000)	11.7±1.1	8.3±0.1	12.4±0.2	42.6±1.0	51.0±0.6	20.6±0.3	29.4±0.1	1157±39.0	18.8±2.0	78.4±1.9	1.6±0.4	1.2±0.2	0.0±0.0

Table 5: Serum analysis of rat groups after 6 weeks treatment*

Treated groups (mg/kg/day)	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	BUN (mmol L^{-1})	CRE (U mol L^{-1})	URIC (U mol L^{-1})	GLU (mmol L^{-1})	T.PROT (g dL^{-1})	ALB (g dL^{-1})	T. LIP (l mg dL^{-1})
Control (no treatment)	57.5±1.8	174.0±7.9	312.6±2.70	21.1±0.6	1.1±0.3	1.9±0.1	210.1±23.2	7.3±0.1	3.9±0.1	406.5±16.0
Henna (200)	43.8±1.2	93.0±3.1	197.8±11.9	19.5±0.8	1.2±0.1	1.8±0.1	187.1±29.3	6.6±0.1	3.2±0.0	395.3±14.8
Henna (1000)	46.6±1.6	106.3±1.8	212.3±8.40	19.3±0.8	1.1±0.1	2.5±0.2	213.3±8.90	6.9±0.1	3.5±0.1	402.3±2.10

*Data presented as mean±SEM; n = 10; Significantly different from control; $p<0.05$

Results presented that liver enzymes such as ALT, AST and ALP were significantly decreased at both tested doses ($p>0.05$). Significant changes in these enzymes suggest liver impairment or altered integrity of cellular membrane as well as cell lyses or death which may indicate some toxic effects of this plant on liver functions (Amida *et al*., 2007). An additional toxicological evaluation is recommended here as the serous toxic effects are mostly associated to increase in the levels of these enzymes rather than their decrease. In the point of view, the decrease in such enzymes might represents a type of inhibitory effects for henna extracts on the biosynthesis of these enzymes. URIC, T. PROT and ALB were found to be significantly decreased for all tested groups in comparison to control group. This could be related to poor nutrition or liver damage. Finally, the general view on results lead to conclude that the leaves of *Lawsonia innermis* (Henna) are relatively safe toxicologically when administered orally at dose up to 200 mg/kg/day. Nevertheless, not all parts of this plant previous study on the seeds of Henna reported that its seeds have a significant toxic effect on male Wister rats (Abdelgadir *et al*., 2010).

CONCLUSION

In this investigation, the presence of anti-bacterial activity in the leaves of *Lawsonia innermis* (Henna) cultivated in Saudi Arabia, Al-Madinah district supported its traditional application in treating wound and stomach ache. The toxicological study revealed the relative safety of this plant on rats, particularly at dose up to 200 mg/kg/day. Thus, the leaves of Henna are a potential natural source of novel anti-microbial agents with least side effects and toxicity symptoms. Further multidisciplinary studies are needed as to further fractionate and purify various active constituents in henna and use them separately to stud their anti-microbial and toxic effects.

ACKNOWLEDGEMENTS

This study was supported by a research fund from Qassim University (No. 1023). The researchers also thankful to the Animal House, Faculty of Pharmacology, King Saud University for providing rats and to Al-Rass General Hospital for providing bacterial isolates.

REFERENCES

Abdallah, E.M., H.E. Khalid and K.S. Al-Khalifa, 2009a. Toxicological assessment of the oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* in rats. J. Med. Plants Res., 3: 526-532.

Abdallah, E.M., S. Amna, A.S. Khalid and N. Ibrahim, 2009b. Anti-bacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against Methicillin Resistant *Staphylococcus aureus* (MRSA). Sci. Res. Essay, 4: 351-356.

Abdelgadir, E.H., R.H. Ahmed, S.I.Y. Adam and A.M. Husein, 2010. Evaluation of toxicological activity (Acute and sub-chronic toxicities) of the aqueous extract of *Lawsonia inermis* seeds on wistar rats. J. Pharmacol. Toxicol., 5: 324-333.

Ali, N.A.A., W.D. Julich, C. Kusnick and U. Lindequist, 2001. Screening of Yemeni medicinal plants for anti-bacterial and cytotoxic activities. J. Ethnopharmacol., 74: 173-179.

Amida, M.B., O.K.O.K. Yemitan and O.O. Adeyemi, 2007. Toxicological assessment of the aqueous root extract of *Sanseviera liberica* Gerome and Labroy (Agavaceae). J. Ethnopharmacol., 113: 171-175.

Arayne, M.S., N. Sultana, A.Z. Mirza, M.H. Zuberi and F.A. Siddiqui, 2007. *In vitro* hypoglycemic activity of methanolic extract of some indigenous plants. Pak. J. Pharm. Sci., 20: 268-273.

Chaudhary, G., S. Goyal and P. Poonia, 2010. *Lawsonia inermis* Linnaeus: A phytopharmacological review. Int. J. Pharm. Sci. Drug Res., 211: 91-98.

Cheesbrough, M., 2004. District Laboratory Practice in Tropical Countries. Cambridge University Press, Cambridge, UK., pp: 62-70.

Dama, L.B., B.N. Poul and B.V. Jadhav, 1998. The tuberculostatic activity of herb enna. J. Ecotoxicol. Environ. Monitoring, 8: 213-213.

El-Mahmood, A.M., O.B. Ogbonna and M. Raji, 2010. The anti-bacterial activity of *Azadarichta indica* (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. J. Med. Plants Res., 14: 1414-1421.

Endrini, S., A. Rahmat, P. Ismail and Y.H. Taufiq-Yap, 2007. Comparing of the cytotoxicity properties and mechanism of *Lawsonia inermis* and *Strobilanthes crispus* extract against several cancer cell lines. J. Med. Sci., 7: 1098-1102.

Ernst, E., 1998. Harmless herbs: A review of the recent literature. Am. J. Med., 104: 170-178.

Guha, G., V. Rajkumar, R.A. Kumar and L. Mathew, 2009. Anti-oxidant activity of *Lawsonia inermis* extracts inhibits chromium(VI)-induced cellular and DNA toxicity. Evid Based Complement Altern. Med.,

Guida, J.D., A.M. Kunig, K.H. Leef, S.E. McKenzie and D.A. Paul, 2003. Platelet count and sepsis in very low birth weight neonates: Is there an organism-specific response. Pediatrics, 111: 1411-1415.

Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs tomorrow. Mol. Aspects Med., 27: 1-93.

Habbal, O.A., A.A. Aljabri, A.H. El-Hug, Z.H. Al-Mahroopi and N.A. Al-Hashmi, 2005. *In vitro* anti-microbial activity of *Lawsonia inermis* Linn. (henna). A pilot study on the *Omani henna*. Saudi Med. J., 26: 69-72.

Hemalatha, K., H.N. Natraj and A.S. Kiran, 2004. Hepatoprotective activity of leaves of *Lawsonia alba*. Indian J. Nat. Prod., 20: 14-17.

Karaman, I., F. Sahin, M. Gulluce, H. Oigutcu, M. Sengul and A. Adyguzel, 2003. Anti-microbial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231-235.

Khan, M.M., A. Ali, D.C. Jain, R.S. Bhakuni, M. Zaim and R.S. Thakur, 1991. Occurrence of some anti-viral sterols in *Artemisia annua*. Plant Sci., 75: 161-165.

Konan, N.A., E.M. Bacchia, N. Lincopan, S.D. Varelac and E.A. Varandac, 2007. Acute, subacute toxicity and genotoxic effects of a hydroethanolic extract of the Cashew (*Anacardium occidentale* L.). J. Ethnopharmacol., 110: 30-38.

Mikhaeil, B.R., F.A. Badria, G.T. Maatooq and M.M.A. Amer, 2004. Anti-oxidant and immunomodulatory constituents of henna leaves. J. Biosci., 59: 468-476.

Mills, S. and K. Bone, 2005. The Essential Guide to Herbal Safety. 1st Edn., Elsevier Churchill Livingstone Company, USA.

Natarajan, V., S. Mahendraraja and T. Menon, 2000. Anti-dermatophytic activities of *Lawsonia alba*. Biomedical, 20: 243-245.

Nayak, B.S., G. Isitor, E.M. Davis and G.K. Pillai, 2007. The evidence based wound healing activity of *Lawsonia inermis* Linn. Phytother. Res., 21: 827-831.

Okpekon, T., S. Yolou, C. Gleye, F. Roblot and P. Loiseau *et al*., 2004. Anti-parasitic activities of medicinal plants used in Ivory Coast. J. Ethnopharmacol., 90: 91-97.

Philip, K., S.N.A. Malek, W. Sani, S.K. Shin and S.Kumar *et al*., 2009. Anti-microbial activity of some medicinal plants from Malaysia. Am. J. Applied Sci., 6: 1047-1058.

Rios, J.L., M.C. Recio and A. Villar, 1988. Screening methods for natural products with anti-microbial activity: A review of the literature. J. Ethnopharmacol., 23: 127-149.

Singh, V.K. and U.K. Pandley, 1989. Fungitoxic studies on bark extract of *Lawsonia inermis* against ringworm fungi. Hindustan Anti-biot. Bull., 31: 32-35.

Symsudin, I. and H. Winarno, 2008. The effect of lnai (*Lawsonia inermis* L.) leaves extract on blood sugar level: An experimental study. Res. J. Phrmacol., 2: 20-23.

Verpoorte, R., 2005. Perspective of ethnopharmacology. J. Ethnopharmacol., 100: 1-2.

Wingard, J.R., M.H. White, E. Alaisie, J. Raffali, J. Goodman and A. Arrieta, 2000. A Randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. Clin. Infect. Dis. 31: 1155-1163.

Wurochekke, A.U., G. Chechet and A.J. Nok, 2004. *In vitro* and *in vivo* anti- trypanosomal activity of the leaf of *Lawsonia inermis* against *Trypanosoma brucei brucei* infection in mice. J. Medical Sci., 4: 236-239.