International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 5, p. 17-24, 2013

RESEARCH PAPER OPEN ACCESS

Study on the comparative antibacterial activity of *Polyalthia longifolia* (Debdaru) leaf extracts to some selective pathogenic bacterial strains

Anzana Parvin[1], Jesmin Akter[1], Md. Mehedi Hassan[1, 3], Nirupam Biswas[1, 2*]

[1]*Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia 7003, Bangladesh*

[2]*Department of Nutrition and Food Sciences, University of Huddersfield, Huddersfield, United Kingdom*

[3]*Department of Microbiology and Genetics and Institute for Medical Science, Chonbuk National University Medical School, Jeonju 561-712, South Korea*

Key words: Bacteria, medicinal plant, MIC, disc diffusion method, *Polyalthia longifolia*.

doi: http://dx.doi.org/10.12692/ijb/3.5.17-24 Article published on May 20, 2013

Abstract

The increasing social and economic implications caused by pathogenic bacteria means there is a constant striving to develop near antibacterial agents. The present study was carried out *in vitro* to determine antibacterial activity of *Polyalthia longiflia* (Debdaru) leaf extracts with hexane, methanol and chloroform against six tested pathogenic bacteria viz. *Bacillus subtilis*, *Sarcina lutea*, *Xanthomonas compestris*, *Escherichia coli*, *Klebsiella pneumonia and Pseudomonas* sp. Using agar disc diffusion method and MIC determination test. The zone of inhibition against the tested bacteria was found ranging from 21.00 to 44.20mm. The highest zone of inhibition produced by the hexane, methanol and chloroform extracts of *Polyalthia longiflia* at a concentration of 500µh/10µl against pathogenic bacteria i.e. *Sarcina lutea* were found 41.80mm, 44.20mm and 43.50mm respectively. The MIC values of all extracts against six tested bacteria were almost 15.625 µg/ 10µl. The plant leaf extracts can be used in the treatment of infectious diseases caused by them. Extensive investigation is needed to isolate the secondary metabolites from the extracts in order to test specific compounds for antimicrobial activity and the underlying mechanisms.

* **Corresponding Author:** Nirupam Biswas ✉ nirupamgene@gmail.com

Introduction

Antimicrobial activity of medicinal plant has become a global concern nowadays. This problem is of great issue especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. There is a continuous and urgent need to discover new antimicrobial compounds for new infectious diseases. Therefore, researchers are increasingly turning their attention to traditional medicine and searching for new leads to develop better drugs against microbial infections (Parekh and Chanda, 2007). Although hundreds of plants species have been tested for antimicrobial properties, the vast majority have not yet been adequately examined (Balandrin *et al.*, 1985).

*Polyalthia longifolia*is a member of the Annonaceae family and is large genus of shrubs and trees distributed in many tropical countries around the world. It is commonly known as False Ashoka, the Buddha tree, Indian Fir tree,*Ashoka or Devadaru* in sanskrit, *Debdaru* in Bengali, *Deodar* in Hindi, *Asopalav* (Gujarati), *Glodogantiang* (Indonesian),devdar in Marathi and *Nettilinkam* in Tamil(Singh and Karthikeyan, 2000; Kirtikar and Basum, 1995).Traditionally the plant has been used in Bangladesh and India for several medicinal purposes. Literature survey revealed that various parts of the plant possess different biological activities. The similar variety of the plant i.e. *Polyalthia longifolia var. Pedula* has been used in traditional system of medicinthe treatment of fever, skin diseases, mouth ulcers, hypertension, helminthiasis, gonorrhea, uterine ailments, leucorrhoea and menorrhagia (Wu *et al.*, 1990; Rosakutty *et al.*, 2000;Sing and Pandey, 1998).The plants containing clerodane diterpenoids and alkaloids have found to be active against a wide variety of microorganisms (Faizi *et al.*, 2008).

The plant extract and isolated compounds were studied for various biological activities like antibacterial activity, antileishmanial activity, antiinflammatory activity, antineurotoxicity, hepatoprotective activity, antiulcer activity, hypotensive activity, cytotoxicity, antifungal activity (Marthanda *et al.*, 2005; Misra *et al.*, 2010; Tanna *et al.*, 2009; Shih *et al.*, 2009; Malairajan *et al.*, 2008; Saleem *et al.*, 2005; Stevigny *et al.*, 2005; Nair and Chanda, 2006a, b).The methanol extract of *Polyalthia longifolia* exhibited noncytotoxic and antibacterial (Vijaya *et al.*, 1995). It has been reported that the chloroform extract of *Polyalthia longifolia* leaf part was tested for antibacterial activity (Santos*et al.*, 1995; Annapurna *et al.*, 1983). Further screening of this medicinal plant may result in the discovery of novel effective compounds. By considering the medicinal importance for microbial infections, the leaf of *Polyalthia longifoilia* was screened against some pathogenic bacterial strains. The present study was an attempt to investigate the antibacterial activity of *Polyalthia longifoilia* leaf extracts against gram positive bacteria *Bacillus subtilis*, *Sarcina lutea*, and gram negative bacteria *Xanthomonas campestris*, *Escherichia coli*, *Klebsiella pneumonia and Pseudomonas* sp. However, widespread exploration is desired to isolate the secondary metabolites from the extracts in order to test specific compounds for antimicrobial activity.

Materials and methods

Plant materials

Healthy, disease free, mature *Polyalthia longifolia* leaf was collected directly from local region of Kushtia and Jhenidah in Bangladesh. The leaves were cleaned with rinsed water. After cutting into small pieces, they were air dried in room temperature. After 7 days, dried leaves were pulverized into a fine powder by blender machine.

Solvent extraction

30gm powdered sample of *Polyalthia longifolia* leaf was sequentially extracted with solvents namely hexane, methanol and chloroform and also with water by Soxhlet apparatus for 36 hours (Singh *et al.*, 2012).The crude extracts were then filtered through Whatman No.1 filter paper and all the crude extracts were concentrated under reduced pressure. All the extracts were stored in refrigerator until use.

Test microorganisms

Antibacterial activity of *polyalthia longifolia* leaf extracts was investigated against four gram negative (*Xanthomonas campestris*, *Pseudomonas sp.*, *Escherichia coli* and *Klebsiella pneumonia*) and two gram positive (*Bacillus subtilis* and *Sarcinalutea*) registered bacterial isolates, which were obtained from the microbial type culture collection of Microbiology Laboratory of the Biotechnology and Genetic Engineering Department, Islamic University, Kushtia, Bangladesh.

Screening the extracts for antimicrobial activity

The disc diffusion method was used to determine the growth inhibition of bacteria by plant extracts (Nostro *et al.*, 2000). In the disc diffusion method, the discs were placed aseptically over the bacterial culture on nutrients agar plates and incubated at 37^0C for 24hrs. After inoculation for 24 hrs, the zones of inhibition around the discs were measured by millimetre scale. The experiment replicated three times to confirm the reproducible results. Sterile blank paper discs were impregnated with only sterile solvent (hexane, methanol and chloroform) used as negative control each time. In this study, six bacterial strains were inoculated into nutrient broth and shaken on a shaker machine about 24hrs at 37^0C for incubation. After 24hrs, 25µl of inoculums from the prepared culture was transferred to plate count agar media containing petri dishes. The inoculums were spread on the surface of the media with a sterile spreader in laminar air flow. Paper discs embedded within a plant extract were placed on previously inoculated plates and were incubated at 35 ± 0.1^0C for 24hrs. After incubation the zones of growth inhibition around disks were measured in mm. Antibacterial activity studies were carried out for each test strains in triplicate and average measurement were calculated. Six microorganisms were tested in the study to determine the antibacterial effect of crude extracts (hexane, methanol and chloroform) of *Polyalthia longifolia* leaf. In antibacterial screening, plate count agar was used as a culture media. Standard amoxicillin, tetracycline and ciprofloxacin were used as positive control for comparison of the antibacterial activity.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined according to methods described by Shahidi, 2004 and Kabir *et al.*, 2005 by broth dilution assay. Hexane, methanol and chloroform extracts were serially diluted to concentrations ranging from 500µg/10µl to15.625 µg/10µl in hexane, methanol and chloroform solvent against six tested pathogenic microorganisms. For preparing 500 µg / 10µl to 15.625 µg / 10µl, 1ml of the solvent was added to each of the six screw capped test tube. 1ml of the having 500 µg / 10µl extracts was added to the first test tube containing 1ml of respective solvent and mixed well and then 1ml of this solution was transferred to the second test tube containing 1ml of the same solvent. After mixing well, 1ml of this solution was transferred to the third test tube. This process of serial dilution was continued up to the last test tube. Finally, the concentration of the last test tube was 15.625 µg / 10µl.The plates were incubated at 37 °C for 24 hour. The lowest concentration of all extracts that produced no visible bacterial growth was recorded as the MIC.

Data analysis

All data were measured average value of three replicates and standard error (±). Results were subjected to ANOVA using Statistical Packages for Social Sciences (SPSS version 20.0) and the means separated using least significant differences (LSD) at 5% level of significance.

Results

In the present study, the antimicrobial activity of three extracts against two gram positive and four gram negative bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.

Antibacterial activity

All the extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. The antimicrobial activities of *Polyalthia longifolia* leaf extracts are compared with standard antibiotics such as amoxicillin, ciprofloxacin and tetracycline, which were used as positive controls. Results of the antimicrobial activity obtained using the disc diffusion assay is summarized in Table 1, 2 and 3 and Figure 1. Diameter of the inhibition zone of different organic extract include 6 mm disc were tested at a concentrations of 500 µg/10µl.

Table 1. Activity of Methanol extract of *Polyalthia longifolia* leaf against different bacterial strains.

Bacterial Strain	Zone of inhibition (mm)						
	Control			Methanol extract con. of *P. longifolia* leaf (µg/10µl)			
	A-10	C-5	T-30	500	250	125	62.5
B. subtilis	28.90±0.42	32.95±0.07	31.00±0.00	34.10±0.00	33.50±0.56	30.35±0.07	25.23±0.32
S. lutea	30.10±0.28	32.85±0.07	31.30±0.14	44.20±0.14*	39.00±0.14	35.75±0.35	33.50±0.56
X. campestris	16.85±0.07	28.15±0.07	21.15±0.21	31.30±0.14	29.00±0.00	28.66±0.47	23.00±0.00
E. coli	14.00±0.14	27.40±0.14	20.10±0.00	36.00±0.00	32.80±0.14	27.00±0.00	26.00±0.43
K.pneumoniae	21.05±0.07	32.00±0.00	25.20±0.28	30.00±0.00	28.15±0.07	27.10±0.14	25.70±0.49
Pseudomonas sp.	13.15±0.21	28.75±0.35	20.25±0.42	33.00±0.00	25.00±0.00	23.00±0.43	22.30±0.42

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc). (*) indicates significance value P<0.005

Table 2. Activity of Chloroform extract of *Polyalthia longifolia* leaf against different bacterial strains.

Bacterial Strain	Zone of inhibition (mm)						
	Control			Chloroform extract con. of *P. longifolia* leaf (µg/10µl)			
	A-10	C-5	T-30	500	250	125	62.5
B. subtilis	28.90±0.42	32.95±0.07	31.00±0.00	32.25±0.35	32.85±0.21	32.30±0.28	28.00±0.00
S. lutea	30.10±0.28	32.85±0.07	31.30±0.14	43.50±0.70	37.00±0.14	35.50±0.56	31.00±0.00
X. campestris	16.85±0.07	28.15±0.07	21.15±0.21	40.00±0.00	31.35±0.49	28.66±0.47	27.00±0.00
E. coli	14.00±0.14	27.40±0.14	20.10±0.00	35.75±0.35	30.00±0.00	24.16±0.23	23.00±0.43
K. pneumoniae	21.05±0.07	32.00±0.00	25.20±0.28	30.10±0.14	32.15±0.07	26.50±0.00	23.00±0.00
Pseudomonas sp.	13.15±0.21	28.75±0.35	20.25±0.42	27.40±0.56*	27.65±0.49	25.70±0.49	24.16±0.23

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc). (*) indicates significance value P<0.005

Measurement of zone of inhibition

According to concentration (500 µg/10µl) the zone of inhibition for methanol extract was *B. subtilis* (34.10±0.00), *S. Lutea* (44.20±0.14), *X. campestris* (31.30±0.14), *E. Coli* (36.00±0.00), *K. pneumoniae* (30.00±0.00), *Pseudomonas* sp.(33.00±0.00); for chloroform extract was *B. subtilis* (32.25±0.35), *S. Lutea* (43.50±0.70), *X. campestris* (40.00±0.00), *E. Coli* (35.75±0.35), *K. pneumoniae* (30.10±0.14), *Pseudomonas* sp.(27.40±0.56); and for hexane extract was *B. subtilis* (34.85±0.21), *S. Lutea* (41.80±0.42), *X. campestris* (37.9±0.14), *E. Coli* (32.35±0.35), *K. pneumoniae* (35.00±0.00), *Pseudomonas* sp.(31.25±0.35) . The highest zone of inhibition to *Sarcina leutia* for methanol extract at concentration of 500 µg/10µl was 44.20±0.14 mm (p<0.005). The lowest zone of inhibition to *Pseudomonas sp.* for chloroform *Polyalthia*

longifoilia leaf extract at concentration of 500 µg/10µl was 27.40±0.56 mm ($p<0.005$).

Table 3. Activity of Hexane extract of *Polyalthia longifolia* leaf against different bacterial strains.

Bacterial Strain	Zone of inhibition (mm)						
	Control			Hexane extract con. of *P. longifolia* leaf (µg/10µl)			
	A-10	C-5	T-30	500	250	125	62.5
B. subtilis	28.90±0.42	32.95±0.07	31.00±0.00	34.85±0.21	31.15±0.07	30.25±0.21	28.75±0.21
S. lutea	30.10±0.28	32.85±0.07	31.30±0.14	41.80±0.42	42.20±0.14	38.85±0.21	30.00±0.00
X. campestris	16.85±0.07	28.15±0.07	21.15±0.21	37.9±0.14	28.45±0.63	24.35±0.49	22.40±0.42
E. coli	14.00±0.14	27.40±0.14	20.10±0.00	32.35±0.35	30.25±0.35	27.60±0.56	26.00±0.14
K. pneumoniae	21.05±0.07	32.00±0.00	25.20±0.28	35.00±0.00	29.00±0.00	26.15±0.21	25.10±0.28
Pseudomonas sp.	13.15±0.21	28.75±0.35	20.25±0.42	31.25±0.35	25.50±0.70	25.00±0.00	21.00±0.98

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc)

Table 4. Minimum inhibitory concentration (MIC) *Polyalthia longifolia* leaf in different solvents.

Bacterial strain	Minimum inhibitory concentration(MIC)		
	Organic extracts of *P. longifera* leaf (µg/10µl)		
	Methanol	Chloroform	Hexane
Bacillus subtilis	15.625	15.625	15.625
Sarcina lutea	15.625	15.625	15.625
X. campestris	31.250	15.625	15.625
Escherichia coli	15.625	15.625	15.625
K. pneumoniae	15.625	15.625	15.625
Pseudomonas sp.	15.625	15.625	31.250

MIC of different organic extracts (values in µg /10µl)

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was observed with all bacterial strains against three different leaf extracts of *Polyalthia longiflia* (Table 4). Almost all organic extracts showed MIC value at a concentration of 62.5µg/10µl.

Discussion

Our present work was designed to perform the study on comparative antibacterial activity of f different leaf extracts of *P. Longifolia* against some selective gram positive and gram negative pathogenic bacterial strains. All of the organic extracts of *P. Longifolia* exhibited greater extend of antibacterial activities.

The antibacterial activities of medicinal plants are attributed due to the presence of flavonoids, tannins and steroidal alkaloids (Fewell *et al.*, 1993; Barnabas *et al.*, 1988; Burapedjo*et al.*, 1995). These reports and presence of flavonoids, tannins and steroidal alkaloids in different extract of *Polyalthia longifolia* confirm its potential against all selected pathogens.

The plant extracts were justified with standard antibiotics i.e. amoxicillin (10 µg/disc), ciprofloxacin (5 µg/disc), and tetracycline (30 µg/disc) and negative control (only solvent absorbing disc). The negative control showed no activity against all tested bacteria. The standard antibiotics showed significant antimicrobial activity against all tested gram positive and gram negative bacterial strains.

This study suggests that the leaf extract of *Polyalthia longifolia* have a board spectrum of antibacterial activity, although the degree of susceptibility could differ between different organisms. The antibacterial activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually or in combination. Plant extracts obtained more activity than commercial antibiotics possibly because the plant active substances were soluble in organic solvents (Boer *et al*., 2005). Our results indicates the potential usefulness of *Polyalthia longifolia* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of bacterial diseases. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove costly in case of prolonged treatment. Therefore, there is needed to develop new antibacterial agents which can satisfy the present demand.

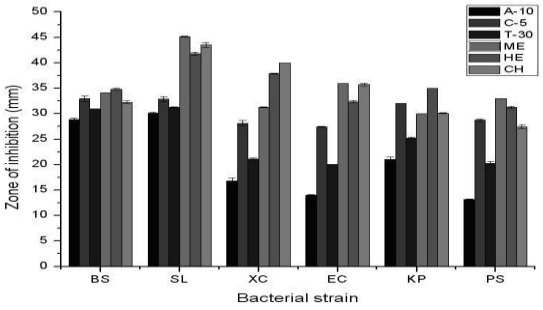

Fig. 1. The comparison of antibacterial potential of *p. Longifolia* leaf extracts and standard antibiotics against some pathogenic bacteria. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc), ME: Methanol, HE: Hexane, CH: Chloroform. The diagram of leaf extracts were revealed at the concentrations of 500 µg/10µl. BS: *B. subtilis,* Sl: *S. leutia,* XC*: X. campestris,* EC: *E. coli,* KP*: K. pheumoniae,* PS: *Pseudomonas* sp.

Conclusion

The extracts of *Polyalthia longifolia* (Debdaru) leaf were found to be effective antibacterial agent**.** This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity with the required minimum inhibitory concentration (MIC). Further studies should undertake to identify the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

Acknowledgements

The author would like to thank to the department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh for supporting the technical assistance.

References

Annapurna Y, Mitra S, Iyengar DS, Rao SN, Bhalerao UT. 1983. Antimicrobial Activity of Leaf Extracts of *Polyalthialongifolia*. Journal of Phytopathology **106**, 183–185. http://dx.doi.org/10.1111/j.1439-0434.1983.tb00041.x

Balandrin MF, Klocke JA, Wurtele ES,Bollinger WH. 1985. Natural plant chemicals: sources of industrial and medicinal materials. Science **228**, 1154–1160. http://dx.doi.org/10.1126/science.3890182.

Barnabas CG, Nagarajan S. 1988. Role of flavonoids and tannins in some medicinal plants- a review. Fitoterapia **3**, 508-510.

Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I,Levenfors JJ. 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology **96**, 461-9. http://dx.doi.org/10.1016/j.jep.2004.09.035

Burapedjo S, Bunchoo A. 1995. Antimicrobial activity of tannins from *Terminalia citrine*. Planta Medica **61**, 365-366. http://dx.doi.org/10.1055/s-2006-958103

Faizi S, Khan RA, Mughal NR, Malik MS, Sajjadi KE, Ahmad A. 2008. Antimicrobial activity of various parts of *Polyalthia longifolia* var.pendula: isolation of active principles from the leaves and the berries. Phytotherapy Research **22**, 907–12.
http://dx.doi.org/10.1002/ptr. 2414

Fewell A M, Roddick JG.1993.Interactive antifungal activity of the glycoalkaloids, solanine, and chaconine. Phytochemistry **33**, 323–328. DOI: http://dx.doi.org/10.1016/0031-9422(93)85511-O

Kirtikar KR, Basu BD. 1995. Indian Medicinal plants,Allahabad, India, Universal book distributors, p. 562.

Khabir AO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. 2005. Screening of crude extracts of six medicinal plants used in Southwest Nigerian orthodox medicine for anti-methicillin resistant *Staphlococcusaureus* activity.BMC Complementary and Alternative Medicine **5**, 6.

Marthanda MM, Subramanyam M, Hima BM, Annapurna J. 2005.Antimicrobial activity of clerodanediterpenoids from *Polyalthia longifolia* seeds. Fitoterapia **76(3-4)**, 336-9. http://dx.doi.org/10.1016/j.fitote.2005.02.005

Misra P,Sashidhara KV, Sing SP, Shukla PK 2010.Antimicrobial evaluation of clerodanediterpenes from *Polyalthialongifolia* var.pendula. Natural product communications 4 **(3)**, 327-30.

Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJ. 2008.Evaluation of anti ulcer activity of *Polyalthialongifolia* (Sonn.),Thwaites in experimental animals. Indian Journal of Pharmacology **40 (3)**,126-8. http://dx.doi.org/10.4103/0253-7613.42306

Nostro AM, Germano P, Angelo VD, Mariko A, Cannatelli MA. 2000.Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology **30**, 379-384. http://dx.doi.org/10.1046/j.1472-765x.2000.00731.x

Nair R, Chanda S. 2006a.Evaluation of *Polyalthialongifolia*(Sonn) Thw.leaf extract for antifungalactivity. J of Cell and Tissue Research **6**, 581–584.

Nair R, Chanda S. 2006b.Activity of some medicinalplants against certain pathogenic bacterial strains. Indian Journal of Pharmacology **38**, 142–144. http://dx.doi.org/10.4103/0253-7613.24625

Parekh J, Chanda SV. 2007. In vitroAntimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turkish Journal of Biology **31**, 53-58.

Reiner R. 1982. Antibiotica.An Introduction. F Hoffman, La Roche and Co. Ltd. Switzerland, 23-30. p.

Rosakutty PJ, Roslin AS, Ignacimuthu S. 2000. Ethanobotany and medicinal plants of Indian subcontinent.Jodhpur,India, Scientific Publishers, 369. p.

Santos SVK, Krishnakumar T, Manjula CB. 1995. Antimicrobial activity of nine common plants in Kerala, India. Phil. J. Science **127**, 65-71.

Sing V, Pandey RP.1998.Ethanobotany of rajasthan.Jodhpur, India, Scientific Publishers, p. 175.

Singh NP, Karthikeyan S. 2000. Flora of Maharashtra State Dicotyledones.Vol. 1. Botanical Survey of India, Kolkata, 175. p.

Singh R, Dar SA, Sharma P. 2012. Antibacterial activity and toxicological evaluation of semipurified hexane extract of *Urticadioica* leaves.Res. Journal of Medicinal Plants **6(2)**, 123-135.
http://dx . doi . org/10 . 392 3/rjmp . 2012 . 123 . 1 35

Shih YT,Hsu YY,Chang FR,Wu YC,Lo YC. 2010.6-Hydroxycleroda-**3,** 13-dien-15,16-olide protects neuronal cells from lipopolysaccaride induced neurotoxicity through the inhibition of microglia mediated inflammation. Planta Medica **76(2)**, 120-7. http://dx.doi.org/10.1055/s-0029-1186005

Saleem R,Ahmed M,Ahmed SI,Azeem M,Khan RA,Rasool N,Saleem H,Noor F,Faizi S. 2005.Hypotensive activity and toxicology of constituents from root bark of *Polyalthialongifolia* var. pendula. Phytotherapy Research **19(10)**, 881-4. http://dx.doi.org/10.1002/ptr.1732

Stevigny C, Bailly C, Quetin LJ. 2005. Cytotoxic and antitumor potentialities of Aporphinoid alkaloids. Current medicinal chemistry, Anti-cancer agents **5,** 173–182.

Shahidi GA. 2004. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus,Serratiamarceceans,Klebsiella pneumonia and Bordetellabranchoseptica*. Asian Journal Plant Sciences **3(1)**, 82-86.

Tanna A, Nair R, Chanda S. 2009.Assesment of anti inflammatory and hepatoprotective potency of *Polyalthialongifolia* var. pendula leaf in Wistar albino rates. Journal of Natural Medicines **63(1)**, 80-5.
http://dx.doi.org/0.1007/s11418-008-0288-2

Vijaya KS, Ananthan R,Nalini. 1995. Antibacterial effect of theaflavin, polyphenon 60 (Camellia sinensis) and Euphorbia hirta on *Shigella* spp a cell culture study. Journal of Ethnopharmacology **49 (2),** 115-8.
http://dx.doi.org/10.1016/0378-8741(95)90039-X

Wu YC, Duh CY, Wang SK, Chen KS, Yang TH. 1990. Two new natural azofluorene alkaloids and a cytotoxicaporphine alkaloids from *P. Longifolia*. Journal of Natural Products **53,** 1327–1331.